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Anticancer agents

Anticancer Potencies of Pt^{II}- and Pd^{II}-linked M_2L_4 Coordination Capsules with Improved Selectivity

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Abstract: Pt^{II}- and Pd^{II}-linked M_2L_4 coordination capsules, providing a confined cavity encircled by polyaromatic frameworks, exhibit anticancer activities superior to cisplatin against two types of leukemic cells (HL-60 and SKW-3) and pronounced toxicity against cisplatin-resistant cells (HL-60/CDDP). Notably, the cytotoxic selectivities of the Pt^{II} and Pd^{II} capsules toward cancerous cells are up to 5.3-fold higher than that of cisplatin, as estimated through the non-malignant/malignant-cells toxicity ratio employing normal kidney cells (HEK-293). In addition, the anticancer activity of the coordination capsules can be easily altered upon encapsulation of organic guest molecules.

Coordination-driven supramolecular cages and capsules attract continuous scientific interest due to their fascinating structures, properties, and potential applications as molecular containers and reactors.[1,2] The biomedical applicability of such coordination hosts, however, remains unsolved, and most of the previous studies concentrate on host–guest interactions with biomolecules.[3] In 2008, Therrien et al. first reported the anticancer activity of a water-soluble Ru^{II}-linked coordination cage.[4] The $M_6L_2L'_3$ cage and the derivatives disassemble upon interactions with certain amino acids and generate half-sandwich Ru^{II} complexes showing high anticancer activities.[5] Several groups have actively investigated the anticancer activities of prism,[6] ring,[7] and box-shaped[8] coordination nanostructures including Ru, Os, Rh, or Ir complexes. However, the anticancer activities of Pt^{II}-linked coordination cages are still unexplored and the available data are limited to ring structures,[9] in spite of the rich chemistry and cytotoxicity of mono-nuclear Pt^{II}-complexes.[10] The known host capability of the coordination cages is a noteworthy property that opens additional ways to develop new functional anticancer reagents.[11] Very recently, cytotoxic properties against cancer cells have been shown for a water-soluble Pt^{II}-linked M_6L_4 coordination cage that also proved useful as a supramolecular container for a highly cytotoxic Pt^{IV} prodrug.[12] We expected that further studies on Pt^{II}-linked coordination hosts as well as Pd^{II} ones would contribute to the development of a new type of anticancer drugs displaying improved therapeutic properties.

Inspired by the biomedical potentials of the reported coordination cages, we initiated the anticancer activity evaluation of M_2L_4 coordination nanocapsules $\mathbf{1}^{M}$ (M = Pt or Pd; Figure 1) due to their well-established structural characteristics and host capability.[13] In contrast to common M_2L_4 cages with large openings,[14] the capsules provide a spherical cavity fully surrounded by multiple anthracene panels. The isolated cavity, with a diameter of ~1 nm and a volume of ~600 Å^3, can accommodate various organic molecules (e.g., cyclophane, pyrene, and triphenylene) through mainly hydrophobic effects. The capsular nanostructures can be handled in water under aerobic conditions at up to ~80 °C.

Herein, we present the cytotoxicity profiles of M_2L_4 coordination capsules $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ against human cancer cell lines (HL-

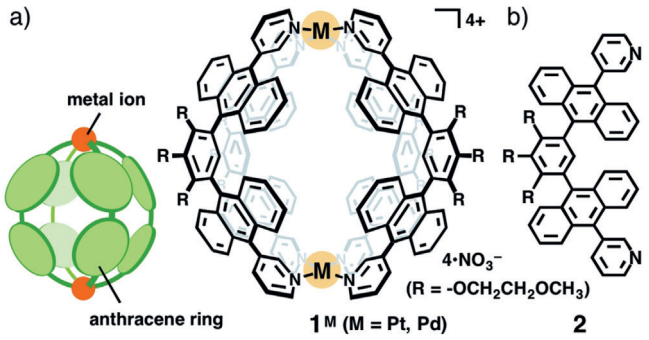

Figure 1. a) Cartoon and chemical structures of M_2L_4 coordination nanocapsules $\mathbf{1}^{M}$ (M = Pd or Pt) and b) chemical structure of bispyridyl ligand **2**.

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60 and SKW-3), non-malignant kidney cells (HEK-293), and cisplatin-resistant cells (HL-60/CDDP). The stability of the capsules is evaluated toward small biomolecules (e.g., amino acids and nucleobases). In addition, we reveal that the anticancer activities of the capsules can be altered upon encapsulation of organic molecules. The observed cytotoxicities of the Pt^{II}- and Pd^{II}-linked coordination capsules suggest their potential application as novel anticancer drugs with high selectivity and potency to overcome multidrug resistance.

For the sake of water solubility, we employed Pt^{II} and Pd^{II} capsules $\mathbf{1}^{M}$ (M = Pt and Pd) providing twelve methoxyethoxy pendants on the *exo*-surface (Figure 1). The stability of the capsules toward small biological molecules was revealed by NMR spectroscopy. As biomolecules, we selected amino acids bearing an aromatic ring (i.e., phenylalanine and tryptophan), a hydrophobic side chain (i.e., valine and proline), or a SH group (i.e., cysteine) (Figure 2a). In addition, DNA nucleobases such as adenine, thymine, guanine, and cytosine (Figure 2b) as well as glutathione (Figure 2c), which is a major antioxidant with a SH group present in cancer cells, were chosen.

The Pt^{II} or Pd^{II} capsule and each of the biomolecules (up to 100 equiv) were combined in aqueous solutions at room temperature, and the resultant mixtures were analyzed by ^{1}H NMR spectroscopy in the course of 1 to 3 days (Figures S3–S10, Supporting Information).[15] For example, ^{1}H NMR spectra of the 3:2 D_2O/CD_3OD solutions of capsule $\mathbf{1}^{Pt}$ containing valine or adenine (100 equiv each) showed simple signals derived from the capsule and the additives (Figure 3a,b). Although the tested biomolecules provide coordinative amino and carboxylic groups, no signal changes of capsules $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ were observed in the spectra. ESI-TOF MS analysis also indicated the absence of interactions between the capsule and the biomolecules. Interestingly, capsule $\mathbf{1}^{Pt}$ retained the assembled structure in the presence of cysteine (~100 equiv; Figure 3c), whereas capsule $\mathbf{1}^{Pd}$ quickly reacted with the SH group of cysteine under the same conditions, leading to the formation of precipitates due to the disassembly of the capsular structure. A similar reactivity was observed in the presence of a 10-fold excess of glutathione; while capsule $\mathbf{1}^{Pd}$ decomposed immediately upon addition of glutathione, capsule $\mathbf{1}^{Pt}$ was almost unchanged in the course of 72 h in aerobic conditions (Figure S10).[15] Thus, we revealed that the Pt^{II} and Pd^{II}-linked coordination capsules are stable in the presence of biomolecules with coordinative atoms, except for those with a SH group that react with the Pd^{II} capsule.

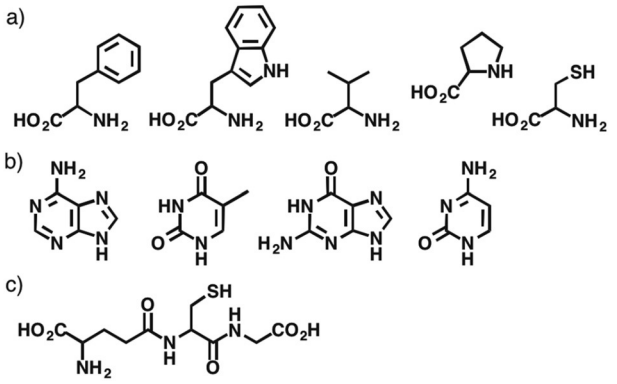

Figure 2. Chemical structures of a) amino acids, b) DNA nucleobases, and c) glutathione.

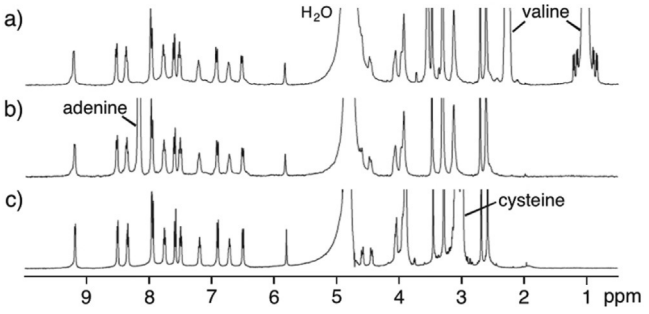

Figure 3. ^{1}H NMR spectra (400 or 500 MHz, D_2O/CD_3OD = 3:2, r.t.) of a) $\mathbf{1}^{Pt}$ + valine (100 equiv), b) $\mathbf{1}^{Pt}$ + adenine (100 equiv), and c) $\mathbf{1}^{Pt}$ + cysteine (100 equiv).

The MTT-bioassay data of in vitro cytotoxicity experiments showed that capsules $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ have anticancer activities superior to cisplatin against the chemosensitive human cancer cell lines, that is, acute myelocyte leukemia (HL-60) and T-cell leukemia (SKW-3) (Figure 4a and Table S1).[15] The cells were exposed to serial dilutions of compounds $\mathbf{1}^{Pt}$, $\mathbf{1}^{Pd}$, and cisplatin for 72 h at 37 °C. For each treatment group at least 8 wells were used, and the survival of the cells was determined by the MTT test.[16,17] The IC_{50} values (i.e., drug concentration necessary for 50% inhibition of cell proliferation) of capsules $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ were estimated to be 6.6 and 1.9 μM[16] on the HL-60 cells, respectively, whereas that of cisplatin was 8.1 μM under the

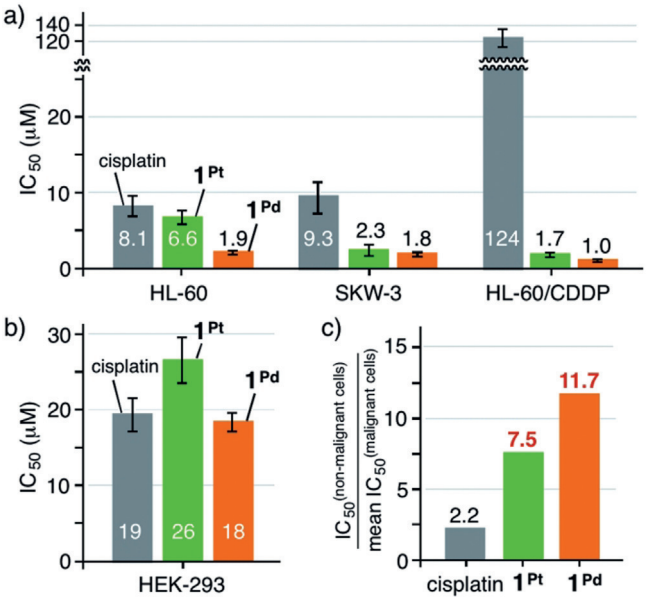

Figure 4. Comparison of the cytotoxicities of capsules $\mathbf{1}^{M}$ (M = Pt and Pd) and cisplatin against a) malignant cell lines (HL-60, SKW-3, HL-60/CDDP) and b) non-malignant cells (HEK-293) based on the IC_{50} values (μM).[16] c) Cytotoxic selectivities of capsules $\mathbf{1}^{M}$ (M = Pt and Pd) and cisplatin (in arbitrary units, obtained by dividing the IC_{50} for the non-malignant cells by the mean IC_{50} in malignant cell lines).[18]

same conditions. For SKW-3 cancer cells, the cytotoxicity data of $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ indicated more than 4-times enhanced activity as compared with that of cisplatin. In addition, both capsules demonstrated pronounced toxicity against the cisplatin-resistant cancer cells HL-60/CDDP (Figure 4a). The obtained IC_{50} values of the Pt^{II} and Pd^{II} capsules (1.7 and 1.0 μM, respectively) are 71 and 125-fold smaller than that of cisplatin, respectively. The calculated resistance indexes (RI) for the capsules, expressed as the IC_{50} (HL-60/CDDP) over IC_{50} (HL-60) ratio, were less than 0.5, which suggests higher cytotoxicity against the resistant cells than the chemosensitive parent cells. The organic ligand **2** of the capsules proved to be non-cytotoxic at concentrations of up to 80 μM. In all tested cases, the Pd^{II} capsule showed the highest activity. This behavior seems to be related to the observed decomposition of $\mathbf{1}^{Pd}$ by the addition of cysteine and glutathione. Fluorescence microscopy studies evidenced the internalization of the capsules inside the HL-60 cells and suggested that the intracellular disassembly of $\mathbf{1}^{Pd}$ occurs more efficiently than that of $\mathbf{1}^{Pt}$ (Figure S11).[15]

Most importantly, capsules $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ exhibited highly selective cytotoxicity against the tested cancer cells. We used human embryonic kidney 293 cells (HEK-293) as non-malignant cells for the utilized in vitro model for nephrotoxicity. The IC_{50} value of $\mathbf{1}^{Pt}$ (26.4 μM) for the cells turned out to be larger than that of cisplatin (19.3 μM) (Figure 4b). The Pd^{II} capsule provided comparable cytotoxicity to cisplatin against the HEK-293 cells. The cytotoxic selectivities were estimated through the ratio between the IC_{50} obtained in HEK-293 and the arithmetic mean of the IC_{50} values established in the malignant cell lines.[18] The obtained selectivity ratios of 7.5 and 11.7 for the $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$, respectively (Figure 4c), are up to 5.3-times higher than that of cisplatin (2.2). These results indicate that the studied nanocapsules are much more effective than cisplatin to selectively inhibit the proliferation of the cancer cells. It can be expected that the distinguishing structural characteristics of the studied capsules are some of the prerequisites for their strongly improved anticancer selectivity. In addition, the more acidic and hypoxic media inside cancer cells,[19] as compared with normal cells, may cause different reactivity (stability) of the capsules in the malignant and non-malignant cells. A similar cytotoxic selectivity has very recently been reported only for some Ru^{II}-linked coordination rings.[20]

Stimulated by the appropriate stability of the Pt^{II} and Pd^{II} capsules and the possessing large cavity, we attempted to estimate the role of the cavity within the capsules to modulate their anticancer activity.[21] In addition, study on the biological activity of host–guest complexes suggests their potential application as drug delivery system. When we treated the cancerous HL-60 and SKW-3 cells with host–guest composites $\mathbf{1}^{Pt}\cdot(Pyr)_2$ and $\mathbf{1}^{Pd}\cdot(Pyr)_2$, different cytotoxicities could be seen by comparing the IC_{50} data from the empty capsules and the host–guest composites (Table 1). The $\mathbf{1}^{M}\cdot(Pyr)_2$ composites were prepared by encapsulation of two molecules of pyrene (Pyr) within capsules $\mathbf{1}^{M}$ (M = Pt and Pd).[15] The obtained IC_{50} values of $\mathbf{1}^{Pt}\cdot(Pyr)_2$ (18.7 μM) and $\mathbf{1}^{Pd}\cdot(Pyr)_2$ (2.3 μM) against HL-60 cells are 2.8 and 1.2-times larger than those of empty $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$, respectively. A similar tendency was observed in the case of SKW-3 cells. As a relative estimation of the cavity effect on the capsule activities, we suggested the so-called cavity-modulated activity index, defined as a $IC_{50}^{[capsule\bullet(Pyr)2]}/IC_{50}^{[capsule]}$ ratio (Table 1). The index clearly reveals that the cytotoxic activities of Pt^{II} and Pd^{II} capsules can be tuned upon simple encapsulation of the guest molecules. These results probably stem from the increased stability of $\mathbf{1}^{Pt}$ and $\mathbf{1}^{Pd}$ in cancer cells due to the encapsulation of appropriate hydrophobic guests in the hydrophobic cavity that decrease the disassembly process of the capsular structure. Although further investigations are needed, the current observations lead to the conclusion that the biological activity of the coordination capsules can be readily altered through the encapsulation of various organic molecules.

Table 1. IC_{50} values of host–guest composites $\mathbf{1}^{Pt}\cdot(Pyr)_2$ and $\mathbf{1}^{Pd}\cdot(Pyr)_2$ against the chemosensitive cancer cell lines HL-60 and SKW-3, compared with the data for the empty capsules and ligand **2**.[16]

Compound	IC_{50} [μM] HL-60	SKW-3
capsule $\mathbf{1}^{Pt}$	6.6 ± 0.9	2.3 ± 0.8
$\mathbf{1}^{Pt}\cdot(Pyr)_2$	18.7 ± 2.5 (2.8)[a]	11.1 ± 1.7 (4.8)[a]
capsule $\mathbf{1}^{Pd}$	1.9 ± 0.2	1.8 ± 0.2
$\mathbf{1}^{Pd}\cdot(Pyr)_2$	2.3 ± 1.0 (1.2)[a]	7.3 ± 1.0 (4.1)[a]
ligand **2**	> 100	> 80

[a] Cavity-modulated activity, defined as $IC_{50}^{[capsule\cdot(Pyr)2]}/IC_{50}^{[capsule]}$.

In summary, we have estimated the cytotoxic potencies of Pt^{II}- and Pd^{II}-linked M_2L_4 coordination capsules against cancerous and non-malignant cell lines. The capsules showed higher anticancer activities (up to 5-fold) against human leukemic cells and even higher activities (up to 125-fold) against the cisplatin-resistant cells as compared with cisplatin. Moreover, the anticancer cytotoxicity of the Pt^{II} and Pd^{II} capsules is highly selective, that is, about 10-times more toxic to the cancer cells over the non-malignant cells. These findings strongly support the notion that the Pt^{II} and Pd^{II} capsules provide anticancer potencies with improved therapeutic effect through their higher anticancer selectivity and significant capability to overcome multidrug resistance. A decrease in the capsule's cytotoxicity could be achieved by encapsulation of hydrophobic guest molecules. Whereas the mechanistic aspects of the observed cytotoxicity and high selectivity of the capsules are unclear,[22,23] further biological studies on the coordination capsules, providing wide-ranging host capability, would develop novel supramolecular anticancer drugs with designed activity and selectivity.

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Keywords: anticancer activity · cancer · cisplatin · coordination capsules · encapsulation · platinum ion

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[15] See the Supporting Information. The stability experiments were carried out at 50–60 °C (or room temperature) in the pH value of ~7. The Pt^{II} and Pd^{II} capsules have no fluorescent properties due to the heavy atom effect but the free ligand emits very strong blue fluorescence.

[16] The tumor cell growth inhibitory effects of the tested compounds were evaluated using the MTT-dye reduction assay, as previously described,[17] with slight modifications.[17] In brief, exponentially proliferating cells were seeded in 96-well flat-bottomed microplates (100 μL well^{-1}; at a density of 1×10^5 cells mL^{-1} for the leukemic cells) and incubated for 24 h at 37 °C, in an incubator. Thereafter, the cells were exposed to serial dilutions of the tested compounds for 72 h. For each treatment group, a set of at least 8 wells was used. After the exposure period, 10 μL aliquots of MTT solution (10 mg mL^{-1} in PBS) were added in each well. Subsequently, the microplates were incubated for further 4 h at 37 °C, and the MTT-formazan crystals formed were dissolved through addition of 100 μL well^{-1} 5% formic acid solution in 2-propanol. The MTT-formazan absorption was determined using a multimode microplate reader (Beckman Coulter DTX-880) at 580 nm. The bioassay data were normalized as percentage of the untreated control (set as 100% viable) and fitted to sigmoidal dose response curves allowing the determination of the equieffective IC_{50} concentrations (concentrations inducing 50% suppression of cellular viability). In addition, the resistance indices as a relative merit for the level of resistance in HL-60/CDDP were determined as the ratio between the IC_{50} in the multi-drug resistant HL-60/CDDP and the corresponding IC_{50} in the sensitive parent line HL-60. All tests were run in triplicate. For the in vitro cytotoxicity experiments we used freshly prepared stock solutions of the capsules and their host–guest composites in water/ethanol (1:1) or pure ethanol for the free ligand. After the gradual dilution, the amount of ethanol is less than 0.1%. This solvent mixture is acceptable for the in vitro tests, based on our previous experience, and was used in order to ensure the stability of the host–guest composites as evidenced by ^{1}H NMR spectroscopic analysis. The IC_{50} values were calculated based on the molar concentration of the capsule.

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[23] DNA duplexes provide negatively charged phosphate groups and electron-deficient nucleobase rings. In contrast, the present capsules are composed of a positively charged (4+) framework (~1 nm in diameter) with embedded, electron-rich anthracene rings. Thus, we suppose that the capsules and/or their partial structures can effectively interact with DNA duplexes through electrostatic interactions.

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