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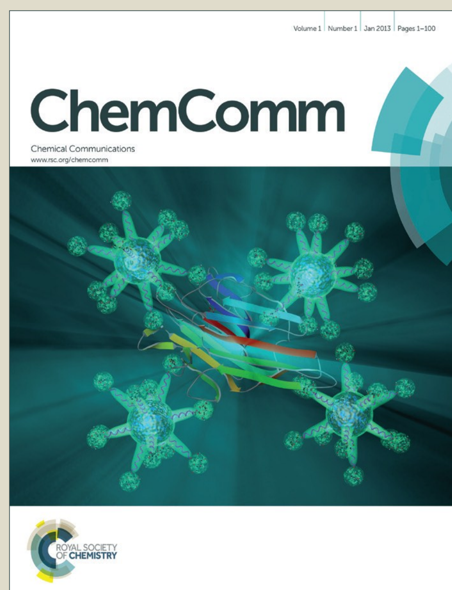

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ARTICLE TYPE

Nanoparticles Delivery of Sterically Hindered Platinum (IV) Prodrugs Show over 100 Times Potency as Cisplatin upon Light Activation

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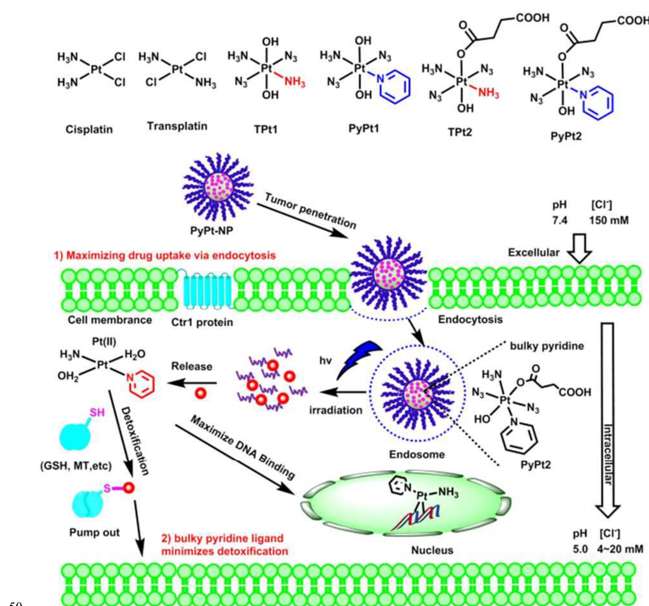
Introducing a sterically hindered pyridine ligand to a photosensitive platinum (IV) drug for drug delivery showed >100 times as effective as gold standard cisplatin.

The golden platinum agent cisplatin has been widely used for a variety of clinic regimens for solid tumor chemotherapy since its approval in 1978 [1,2]. However, great side effects arise from the premature aquation of cisplatin and rapid binding to biomolecules such as HSA (human serum albumin) while in blood circulation [3,4]. Another great drawback is drug resistance [5]. Systematic screen on thousands of Pt drugs revealed the major Pt drug resistance comes from reduced drug uptake and the increased detoxification by intracellular thiols such as glutathione (GSH) and metallothionein (MT) [6]. To reduce premature aquation by HSA binding and minimize the intracellular GSH detoxification, firstly, steric ligands could be introduced into Pt molecules such as picoplatin to increase steric hindrance [7]. To maximize drug uptake, the steric platinum drug can be further loaded into nanoparticles to circumvent the reduced uptake of conventional Pt based drugs. A series of photosensitive platinum(IV)-azide complexes were originally developed by Sadler et al [8-10]. It is generally believed that these peculiar platinum (IV) complexes could be triggered to release cytotoxic Pt(II) agents and to liberate N_2 concomitantly. However, their recent mechanistic study revealed that new platinum intermediates with one azide and one hydroxide ligand might be involved in this process [11]. However, none of them were functionalized for drug delivery. Herein, starting from PyPt1 (Scheme 1) [8], a functionalized sterically hindered photosensitive platinum(IV) drug (PyPt2, Scheme S1-S2, Figure S1-S3) with axial carboxyl group was conjugated to biodegradable polymers with pendent amine groups. The polymer conjugate of PyPt2 can self-assemble into nanoparticles(PyPt-NP) with Pt drugs and the hydrophobic chains in the core and PEG in the shell. PyPt-NP showed >100 times as much effective as the gold standard cisplatin upon photo-activation (Scheme 1). To our best knowledge, this is the first report on polymer based delivery of steric hindered photosensitive platinum(IV) prodrugs to inhibit premature binding and thiol detoxification(GSH or MT) of Pt drugs. The nanoparticle delivery and triggered release of the steric hindered Pt drugs on site led to dramatic increase in efficacy though there might be premature dissociation in the blood circulation and cause some side effects.



Scheme 1. Delivery of steric hindered Pt drugs. (a) Structures of cisplatin, transplatin, TPt1, TPt2, PyPt1 and PyPt2. Amine ligand (red) in TPt2 was replaced with bulky pyridine ligand(red) in PyPt2

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which increases the steric hindrance.(b) Schematic illustration of intracellular pathway of PyPt-NP.

It is widely accepted that 24 h after *i.v* injection, 65% to 98% of cisplatin was reported to bind to human serum protein, which results in huge systemic toxicity [3,4]. Steric hinderance coming from the pyridine ligand in PyPt2 would reduce HSA binding. As shown in Figure 1a, via co-incubation of protein with Pt drugs from 6 h to 48 h, cisplatin rapidly bound to HSA while PyPt2 showed negligible binding (8.2 fold to 3.6 fold in difference). In a similar way, by incubating cisplatin and PyPt2 with GSH and monitoring the peak at 260 nm via UV-Vis (Pt-S bond formation) [8], cisplatin was found to undergo rapid deactivation by GSH(4 h,80%). However, this is not the case for PyPt2. When it is in oxidation state +4, PyPt2 has a characteristic peak at 286 nm which can be attributed to the N_3 to Pt LMCT(Ligand to metal charge transfer) band[8]. Monitoring the decrease in this peak of PyPt2 in the dark via UV-Vis can give reaction rate of GSH and PyPt2. As the reduced Pt(II) is supposed to bind GSH rapidly(detoxification process), the GSH-PyPt2 reaction rate can to some extent represent the detoxification rate. Up to 1000 min, only 6% of PyPt2 reacts with GSH (Figure 1b and Figure S4).However the detoxification rate of TPt2 without steric pyridine ligands seems faster(Figure 1b).

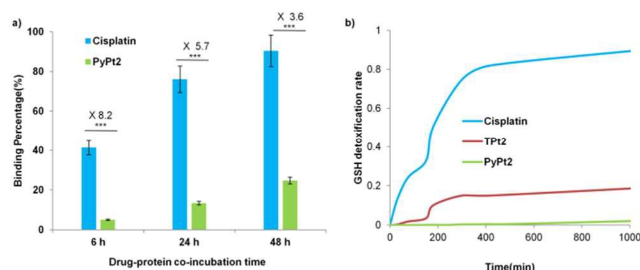

Figure 1. PyPt2 minimizes HSA binding and GSH detoxification. (a) Binding kinetics of HSA (5 μM) with cisplatin and PyPt2 at 5 μM.(b)Pt reaction kinetics(PyPt2 and TPt2 with GSH).

The reduced protein binding and minimized GSH detoxification makes PyPt2 as an excellent candidate for potential drug delivery. To further maximize its anticancer efficacy, PyPt2 was firstly conjugated with biodegradable polymer that self-assembled into nanoparticles, which would enhance its stability in circulation, accumulation and penetration in tumor tissue, and cell uptake [12-15]. The resultant nanoparticles had a diameter of ~150 nm and ~170 nm by TEM and DLS (Figure 2a-2c) with a Pt loading at 13.4 w/w% and zeta potential of +41 mV. PyPt-NP showed robust response to UVA light irradiation, with the peak at 286 nm dropping rapidly in a first order kinetics (Figure 2d-e), denoting the breakdown of Pt(IV) and release of active Pt(II) agents [9]. PyPt-NP was also found to show a Pt release rate at "pH5.0+ UVA> pH7.4+ UVA> pH5.0+ non-UVA> pH7.4+ non-UVA", denoting a greater UVA light dependence than pH values (Figure S5). Both the reaction products of 5'-GMP(guanosine 5'-monophosphate) with t,t-$Pt(NH_3)(Py)Cl_2$ and MPEG-PyPt conjugate upon UVA irradiation were monitored by MALDI-TOF-MS, and the data showed peaks at m/z equal to 984.066 and 970.0146, which can be assigned to the bis-adducts of t,t-$Pt(NH_3)$(pyridine)$(5'\text{-GMP})_2$ and t,t-Pt(pyridine)$(5'\text{-GMP})_2$(loss of one NH_3), respectively (FigureS6-S7), indicating the robust biding with guanosine, the major target in cellular DNA. PyPt-NP showed no UVA absorbance change at 286 nm in the dark but intermittent UVA irradiation resulted in a synchronous drop in UVA absorbance (Figure 2f), denoting the great dark stability and robust UVA light response of PyPt-NP. Taken together, the possible mechanism of drug cleavage from the polymer chain could be ester hydrolysis at acidic conditions but direct breakdown Pt(IV) to Pt(II) would be envisaged as shown in Scheme S3.

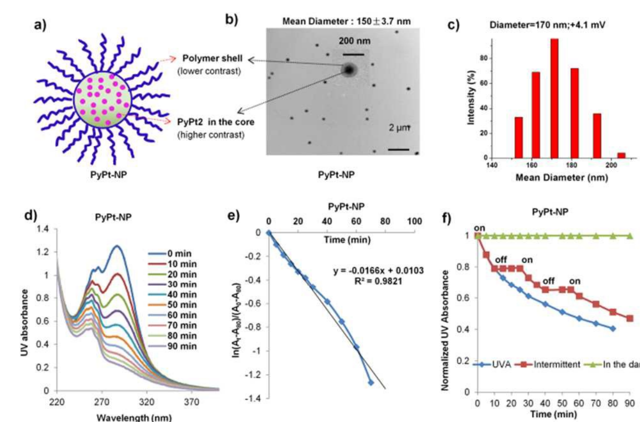

Figure 2. Characterization of PyPt-NP. (a) Schematic illustration of PyPt-NP; (b) TEM and (c) DLS images of PyPt-NP. To show light response, PyPt-NP was irradiated continuously. UV absorbance was monitored over time and a first order kinetics was

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found in the first 80 minutes (d and e). Absorbance at 286 nm of PyPt-NP in the dark and upon intermittent UVA irradiation was collected (f).

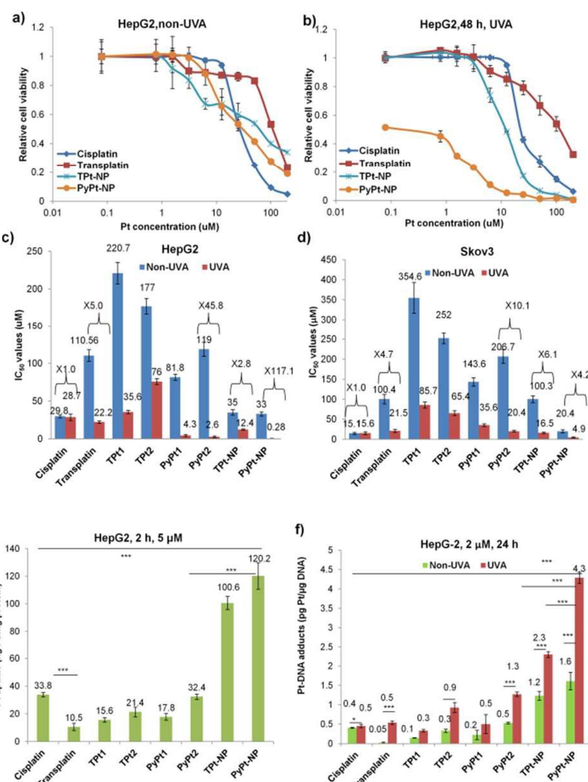

Figure 3. *In vitro* evaluation of PyPt-NP. Representative dose dependent cell viability curve of PyPt-NP on HepG-2 cells in the dark (a, non-UVA) and upon UV irradiation at 1.8 mW/cm^2 for 1 h(b, UVA). Transplatin and TPt-NP were used as controls. IC_{50} values were collected for all these drugs including cisplatin, transplatin, TPt1, TPt2, PyPt1, PyPt2, TPt-NP and PyPt-NP in the dark and upon UVA irradiation on HepG2 (c) and SKOV3 (d) cells. Uptake (e) and intracellular Pt-DNA adducts (f) were measured after 2 h and 24 h treatment of drugs respectively.

To find whether the design of sterically hindered PyPt2 for nanoparticle delivery can be translated into better anticancer potency, PyPt-NP was evaluated in the dark and upon UVA irradiation on HepG2 and SKOV3 cancer cells. TPt-NP loaded with TPt2 without steric hindrance was set as a control. UVA light irradiation and polymer was found non-toxic on cells [16] (Figure S8 and Figure S9). Compared with drugs in the dark, upon UV irradiation, representative dose dependent curves changed little for cisplatin but shifted down significantly for transplatin, TPt-NP and PyPt-NP. Notably, PyPt-NP displayed the highest toxicity unpon UVA irradiation (Figure 3a and 3b). As shown in Figure 3c, cisplatin, transplatin, TPt2, PyPt2, TPt-NP and PyPt-NP on HepG2 cells showed IC_{50UVA} of 28.7, 22.2,76, 2.6, 12.4 and 0.28 μM, while these were 29.8, 110.6, 177, 119, 35 and 33 μM respectively in the dark(IC_{50dark}). The photosensitivity and enhanced efficacy of transplatin upon UVA irradiation were in accordance with previous report [10]. Compared to TPt-NP (IC_{50UVA}=12.4 μM), PyPt-NP showed an IC_{50UVA} of 0.28 μM, almost a 44-fold increase was observed, which proves the steric hindrance to some extent greatly augmented anti-cancer potency. More interestingly, PyPt-NP (IC_{50UVA}=0.28 μM) was >100 times more toxic than the gold standard Pt agent cisplatin (IC_{50UVA}=28.7 μM), of which this kind of enhancement was rarely reported for the Pt(IV) prodrug drug delivery system. The greater potency of PyPt-NP was also found on SKOV-3 cells (Figure 3d).

Nanoparticle delivery was supposed to increase the uptake of drugs. As shown in Figure 3e, all the free drugs ranging from cisplatin to PyPt2 demonstrated similar uptake at a level of 10 to 40 ng Pt/mg protein. However, both TPt-NP and PyPt-NP showed much higher uptake of drugs, up to > 100 ng Pt/mg protein, which equals to almost 2.5 to 10 times more drugs internalization. To visualize the nanoparticle, confocal laser scanning microscopy showed Rhodamine B labelled PyPt-NPs were distributed in the whole A2780DDP cells (Figure S10). Taken together, this result demonstrated the ability of maximizing the drug internalization by nanoparticles compared to small molecules. The greater uptake of sterically hindered Pt drugs into the cells could partially explain the higher efficacy of PyPt-NP.

The ultimate target of Pt drugs was supposed to be nuclear DNA[1]. It is believed Pt(IV) drugs should be first reduced to Pt(II), they can bind to DNA then [17]. Therefore, photo-activation of PyPt-NP to Pt(II) could result in robust DNA binding, resulting in a higher amount of Pt-DNA adducts after photo-irradiation. As shown in Figure 3f, cisplatin showed no photo-irradiation dependent Pt-DNA adducts formation ability (<0.5 pg Pt/ug DNA). Transplatin as an

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exception as reported before, it did show more Pt-DNA adducts after irradiation which could explain its greater potency upon UVA light irradiation (0.05 pg Pt/ug DNA *v.s.* 0.5 pg Pt/ug DNA). All other drugs had photosensitivity thus photoactivation did increase the Pt-DNA adducts. Notably, PyPt-NP produced Pt-DNA adducts at a weight ratio of 4.3 pg Pt/ug DNA, the highest among all groups.

Taken together, we have reported here for the first time that introducing a sterically hindered ligand to photosensitive platinum (IV) prodrugs and delivery of these types of drugs could result in great enhance in anticancer efficacy. Specifically, PyPt-NP showed >100 times more effective than cisplatin. This simple strategy could open the door for the designing new platinum(IV) agents for combating drug resistance and might bring benefits in the future clinic practice.

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