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ARTICLE TYPE

Maleimide-functionalised platinum(IV) complexes as synthetic platform for targeted drug delivery

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Maleimide-functionalised Pt(IV) complexes with highly selective binding properties to thiol groups were synthesised as precursors for binding of thiol-containing tumour targeting molecules like human serum albumin.

The use of metal-based drugs in cancer therapy is well established due to the worldwide success of the platinum(II) drugs cisplatin, carboplatin and oxaliplatin.[1,2] Nevertheless, therapy with these compounds is characterised by severe adverse effects as a result of the unselective damage to healthy tissues and acquired or intrinsic resistance.[3] One approach to overcome the drawbacks of platinum(II) chemotherapeutics is the development of platinum(IV) complexes. This class of compounds is kinetically more inert and it is widely accepted that they act as prodrugs. Thus, platinum(IV) complexes open up the possibility to develop platinum drugs with reduced toxicity and enable potential oral application. The most prominent platinum(IV) drug is satraplatin which is currently investigated in advanced phase III clinical trials. [2,4] The additional axial ligands of platinum(IV) complexes allow the coupling of bioactive molecules like estrogen,[5] ethacrynic acid,[6] folic acid,[7] carbon nanotubes[8] or gold nanoparticles.[9]

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† Electronic Supplementary Information (ESI) available: [Materials and Methods, Synthetic procedures, Preinvestigations for HSA binding, X-ray diffraction data, RP-HPLC studies with cysteine, SEC-ICP-MS data]. See DOI: 10.1039/b000000x/

‡ This authors contributed equally to this work.

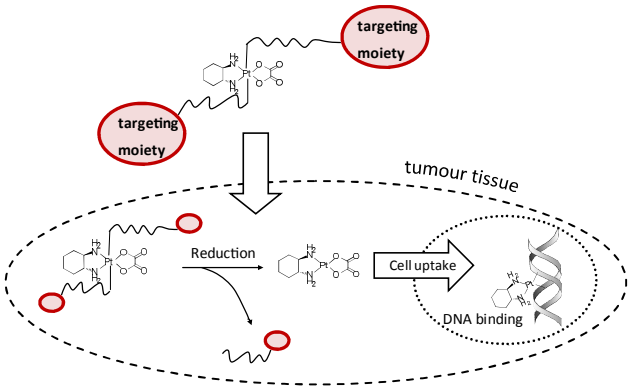


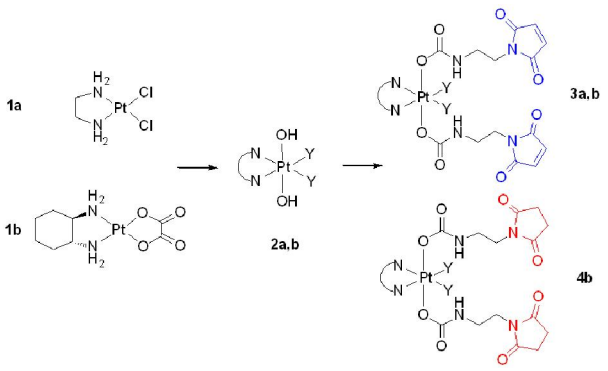
Fig 1. The concept of maleimide-functionalised platinum(IV) prodrugs.

These moieties are then released during the so called "activation by reduction" process accompanied by simultaneous generation of the corresponding reactive platinum(II) drugs.

However, there is increasing evidence that also platinum(IV) compounds are not specifically activated only in the tumour tissue but also in healthy tissues, especially in the presence of hemeproteins.[10-12] Thus, it is of interest to further enhance the tumour-targeting of platinum(IV) drugs.

A well-known targeting molecule is albumin, the most abundant protein in human blood serum which accumulates in the tumour tissues, due to the EPR ("enhanced permeability and retention") effect.[13] In addition, human serum albumin (HSA) possesses a single free thiol group (cysteine-34), which enables the selective and defined preparation of drug-HSA conjugates using maleimide as the coupling moiety. The favourable properties of HSA-coupled anticancer drugs were already clinically proven for doxorubicin (INNO206; aldoxorubicin), currently in phase II clinical studies,[14] and a HSA-based nanoparticle encapsulating paclitaxel (Abraxane®) approved for the treatment of breast cancer and non-small cell lung cancer (NSCLC).[15]

Herein, we report on the synthesis of the first platinum(IV) complexes containing a maleimide moiety in axial position, which enables a simple and fast coupling of thiol-containing



Scheme 1. Synthesis of novel platinum(IV) complexes (**a**: N∩N = 1,2-ethylenediamine, Y = Cl; **b**: N∩N = 1R,2R-diaminocyclohexane, Y_2 = oxalate)

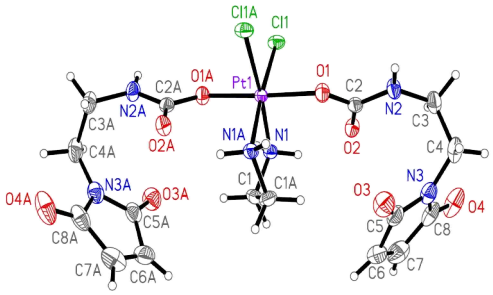


Fig 2. ORTEP plot of **3a** with atom labelling scheme, the thermal ellipsoids have been drawn at 50% probability level.

targeting molecules. This was confirmed by investigations with cysteine and HSA.

The starting compounds **1a** and **1b** were synthesised by standard literature procedures, followed by oxidation with H_2O_2 (**2a** and **2b,** Scheme 1).[16] The maleimide-containing linker was generated by conversion of maleimidopropanoic acid to the isocyanate, which was subsequently coupled to the platinum centre by reaction with the hydroxido groups of the corresponding platinum(IV) precursor in DMF yielding **3a** (67%) and **3b** (44%). As a reference complex, which is unreactive towards thiols, the succinimide functionalised compound **4b** was synthesised by an analogous procedure in good yields (53%). All complexes were characterised by one- and two-dimensional NMR spectroscopy, ESI-mass spectrometry and elemental analysis (see SI).

The results of an X-ray crystallographic study of § **3a** are shown in Fig. 2. The complex crystallised in the monoclinic space group C2/c, with octahedral coordination geometry. In the equatorial plane the bidentate ethane-1,2-diamine and two chlorido ligands are coordinated to the platinum(IV) core, whereas in the axial position the two 2-maleimideethylcarbamoyl moieties are bound. The bond lengths are in good accordance with comparable compounds in literature,[16,17] with Pt–Cl at around 2.3 Å and both Pt–O and Pt–N close to 2.0 Å (for bond lengths and angles see Tables S1, S2, SI).

The stability of the novel complexes in aqueous solution was measured with RP-HPLC over a period of 24 h. For both maleimide derivatives **3a** and **3b** slow hydrolysis could be

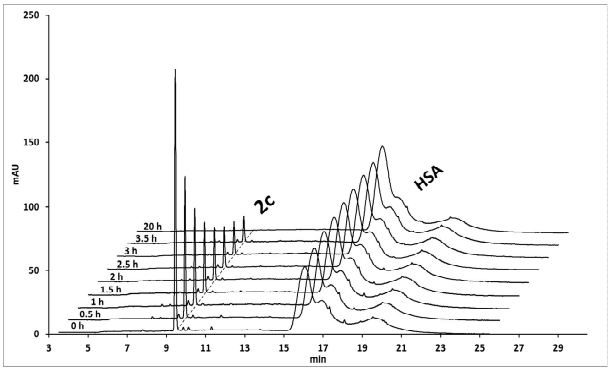


Fig 3. Binding studies of **3b** with albumin using RP-HPLC.

observed (<1%/h, Fig. S1, SI). In comparison, the recorded chromatograms of the succinimide derivative **4b** showed no change within 24 h suggesting that the changes in the case of **3a** and **3b** originate from slow hydrolysis of the maleimide moiety. Subsequently, the complexes were incubated with an excess of cysteine to analyse the reactivity of the thiol group with the maleimide moiety (Fig. S2, SI). The results showed an immediate and complete reaction of cysteine with complexes **3a** and **3b** (<2 min). In contrast, the succinimide derivative **4b** showed no reaction during the same time (1.5 h, Fig. S3, SI), which proves the selective binding of cysteine to the maleimide moiety without any reaction with the platinum centre. The binding rate of **3a** and **3b** to HSA was measured in aqueous solution in 4:1 albumin-to-Pt ratio. The analyses indicated a rapid albumin linkage with a half-time of ~1 h for both maleimide complexes (Fig. 3). To further investigate the albumin binding in detail, **3a**, **3b,** and **4b** where incubated with HSA for 4 h at various concentrations in phosphate buffer, followed by addition of an excess 2,2'-dithiodipyridine which reacts with the remaining free thiol groups of cysteine-34 and results in the generation of 2-thiopyridone, which can be quantified by UV-vis spectroscopy (see SI). In the case of **4b,** the thiol groups of HSA remain unreacted at all tested concentrations, whereas an exponential decrease of free SH was found for the maleimide-containing complexes **3a** and **3b** (Fig. 4).

This data reveals that around 80% of the maleimide-containing platinum(IV) compound is bound to HSA after 4 h incubation at a concentration of >30 µM. The original concentration of free SH groups from HSA was ~25 µM (see SI) and a binding-saturation

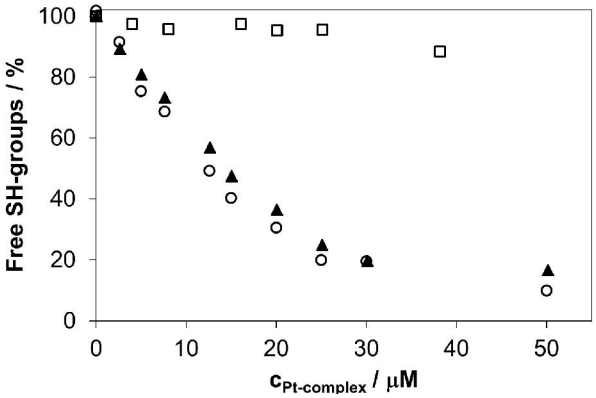


Fig 4. Free SH-content of HSA at various platinum complex/HSA ratios after 4 h incubation, **3a** (○), **3b** (▲), **4b** (□) [c_{HSA} = 99.2 µM; pH = 7.40, 20 mM phosphate puffer + 0.10 M NaCl, 2% (m/m) DMSO].

was reached at ca. 25–30 µM of the Pt-complex, which suggests a 1:1 Pt-HSA adduct possibly due to steric hindrance of the second maleimide group.

To investigate the drug protein binding properties of **3b** in fetal calf serum (FCS), SEC-ICP-MS measurements of sulphur and platinum were performed (Fig 5). The majority (>70%) of platinum was detected in the frame of 8–9 min, which corresponds to the retention time of albumin (see SI). Only small amounts of **3b** were found in the low molecular weight fraction, while **4b** was exclusively detected in this fraction.

To test the impact of albumin binding on the anticancer activity of the new compounds *in vivo* the murine CT-26 colon cancer model was used. The use of this syngeneic murine tumour model was necessary due to the recently reported importance of the immune system for the anticancer activity of oxaliplatin.[10] Both platinum complexes are well tolerated with no significant loss of body weight (data not shown). As depicted in Fig. 6, both drugs had potent anticancer potential resulting in significantly reduced tumour growth in case of **4b** and even disease stabilisation in case of the maleimide-containing **3b.** Thus, **3b** had a significantly higher anticancer activity (p <0.01 at day 15) against CT-26 tumours than **4b** resulting in a distinctly lower mean tumour burden (of 0.38 g vs. 0.18 g for **4b** and **3b**, respectively at day 15). Together, this suggests that the albumin binding of **3b** leads either to prolonged plasma half-life of the drug and/or a more selective accumulation in the malignant tissue due to the EPR effect.

Based on our data it seems feasible that the combination of maleimide-mediated platinum(IV) binding to serum albumin together with the "activation by reduction" principle can be used to enhance the tumour-targeting of platinum drugs.

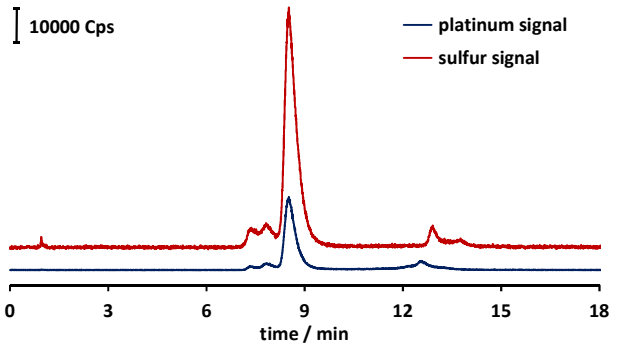


Fig 5. Size-exclusion chromatography (SEC-)ICP-MS determination of **3b** (50 µM) in fetal calf serum after 2h of incubation.

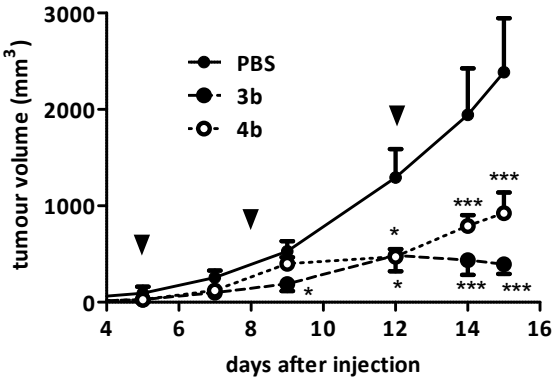


Fig 6. **In vivo anticancer activity.** CT-26 cells were injected subcutaneously in the right flank of BALB/c mice. Mice were treated on day 5, 8, and 12 (indicated by ▼) i.v. with 18 mg/kg **3b** and **4b**. Tumour volumes were calculated as described in the Supporting Information. Each experimental group contained four animals. Data are means ± SEM Statistical analysis was performed by two-way ANOVA with Bonferroni post-test (*, p <0.05; **, p <0.01; ***, p <0.001 compared to control mice).

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Notes and references

§ *Crystallographic details:* ***3a****:* $C_{16}H_{22}Cl_2N_6O_8Pt$, *Mr = 692.39,* 0.12 x 0.10 x 0.02 mm*, monoclinic, C2/c, a = 26-9636(16) Å, b = 7.7770(4) Å, c = 11.3395(6) Å, β = 106.782(2)°, V = 2276.6(2) Å*^{3}*, Z = 4,* ρ_{calcd} = 2020 g cm^{-3}, *T* = 150(2) K, λ = 0.71073 Å, µ = 6454 mm^{-1}, R_1 = 0.0181, wR_2 = 0.0438, GOF = 1.007; description of data collection and refinement see supporting information; CCDC XXX contains the supplementary crystallographif data for this paper (The Cambridge Crystallographic Data Centre, www.ccdc.cam.ac.uk/data_request/cif)

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