Supplementary Information
Derivatisation of buforin IIb, a cationic henicosapeptide, to afford its complexation to platinum(II) resulting in a novel platinum(II)-buforin IIb conjugate with anti-cancer activity

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Synthesis of malBuforin IIb
Attachment of the protected malonate-linker to the Rink Amide MBHA resin-bound buforin IIb was performed manually in a 5 ml or 25 ml syringe (0.1 mmol or 0.2 mmol scale, respectively) fitted with a Teflon frit and stopcock. The resin was agitated in DCM for 30 min to facilitate swelling after which the DCM was drained. Quantities of the reagents were calculated relative to the original substitution of the resin. To the pre-swollen resin was added a solution of 5-(tert-butoxy)-4-(tert-butoxycarbonyl)-5-oxopentanoic acid (4.5 eq) and a solution of HATU (4.0 eq) in DMF, quickly followed by the addition of DIEA (9 eq). The vessel was subsequently agitated for three hours after which the medium was drained. The resin was washed with DMF (2x) and DCM (1x) and dried under vacuum. Successful coupling was monitored using the qualitative Kaiser test; an additional coupling procedure was performed for positive tests. The peptide was then cleaved from the resin and concomitantly deprotected. The cleavage cocktail (6 ml for 0.1-0.125 mmol of resin) consisted of trifluoroacetic acid (80 %) and scavengers triisopropylsilane (TIPS, 5 %), water (5 %), thioanisole (TA, 5 %) and 1,2-ethanedithiol (EDT, 5 %). The mixture was gently stirred for 4.5 hours. The resin was filtered and the peptide precipitated by addition of cold diethyl ether (-20 °C, ~10 ml), isolated by centrifugation and washed twice with 5 ml of diethyl ether. The dry pellet was dissolved in H₂O and the solution lyophilized. The peptide was purified using reverse-phase semi-preparative HPLC on a C18 column (250 x 10 mm) using a linear gradient of 5-65 % acetonitrile water, both containing 0.1 % TFA. The purity of the peptide was checked by analytical reverse-phase HPLC on a C18 column, Fig. 3 (ESI). ESI-MS: m/z: 906 [M+H]⁺³, 679 [M+H]⁺⁴, 543 [M+H]⁺⁵. MALDI-TOF MS: 2672.45 [M-CO₂]. Expected: 2716.67, Fig. 3 (ESI).

Synthesis of cis-[Pt(NH₃)₄(malBuf₂H₂)]
To generate cis-[Pt(NH₃)₄(malBuf₂H₂)], iodoplatin (0.192 g, 0.39 mmol) and AgNO₃ (0.13 g, 0.76 mmol) in deionised water (5 ml) were first stirred for 3 hrs at 55 °C in the dark. The insoluble AgI was filtered off to afford a 0.038 M solution of cis-[Pt(NH₃)₂(OH)₂]²⁺ (1.64 ml, 0.062 mmol) was added to malBuf (34 mg, 0.12 mmol) and the pH of the resulting solution adjusted to 6.4-6.8 with additions of 0.1 M NaOH. The solution was then stirred for 24 hrs in the dark at room temperature. Removal of excess cis-[Pt(NH₃)₂(OH)₂]²⁺ was achieved using gel filtration on a Sephadex-G10 column. An iodine test was performed to identify peptide in the collected fractions which were combined, frozen and lyophilized to afford cis-[Pt(NH₃)₂(malBuf₂H₂)], as a white solid. ESI-MS: m/z: 982 [M+H]⁺³, 737 [M+H]⁺⁴, 589 [M+H]⁺⁵, Figure 4 (ESI). ¹⁹⁵Pt NMR: -2011 ppm.
Fig. 1 (ESI) HPLC trace of buforin IIb

Retention Time 28.60
Purity 99.31 %
Fig. 2 (ESI) HPLC trace malBuforin IIb

Retention Time 29.51
Purity 96.01%
Fig. 3 (ESI) Mass spectra of Buforin IIb and its malonate derivative, Malbuf

MALDI-TOF of Buforin IIb

MALDI-TOF of malBuf

ESI-MS of malBuf
Fig. 4 (ESI) Mass spectrum of $\text{Cis-}[\text{Pt(NH}_3\text{)}_2(\text{malBuf}_2\text{H})]$