Supplementary Information

Platinum complexes act as shielding agents against virus infection

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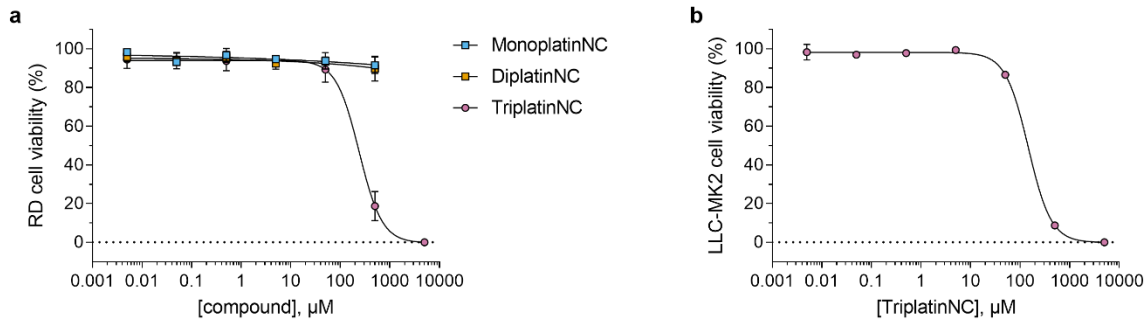
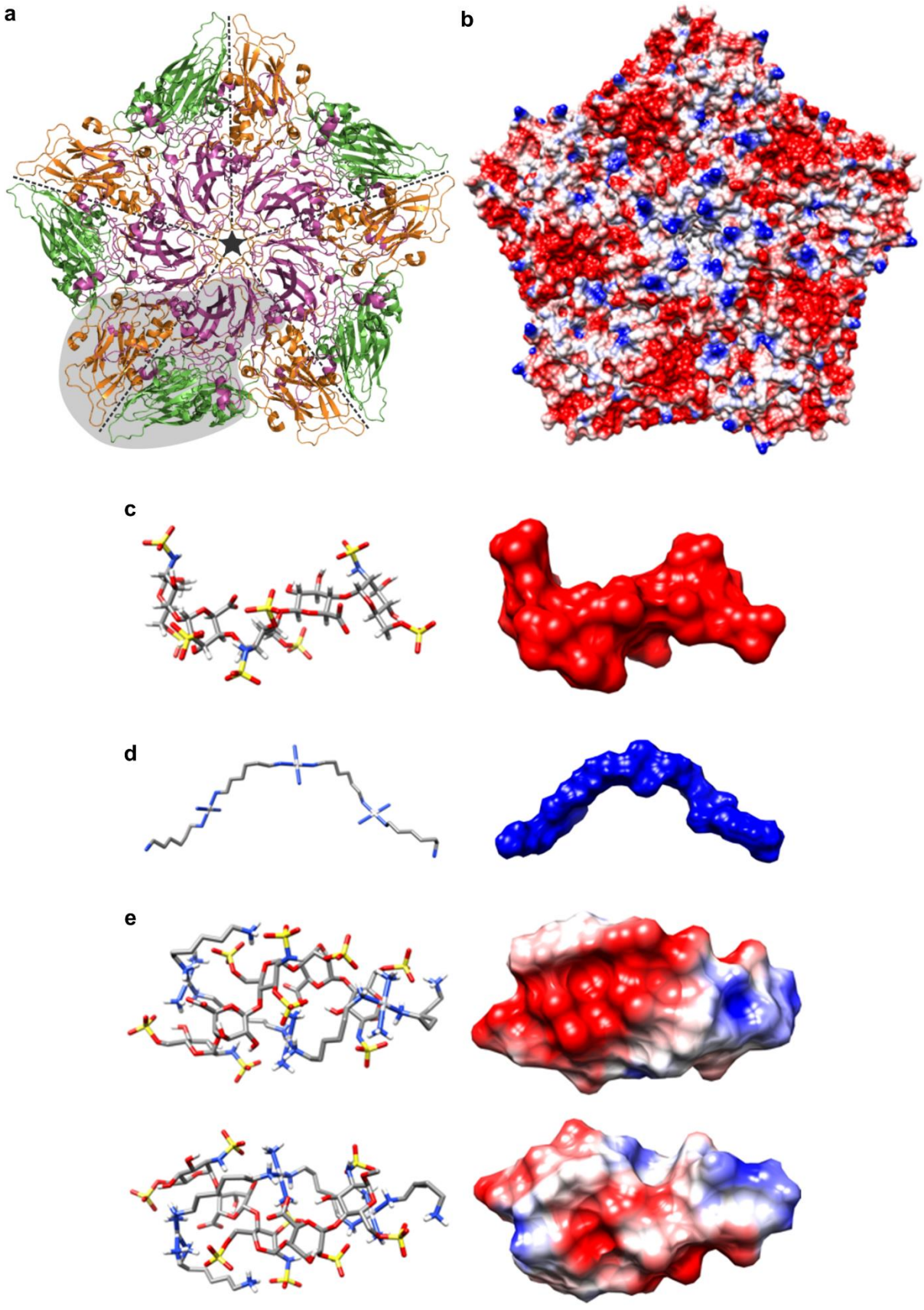
Supplementary Figure 1



Legend to Supplementary Figure 1

Supplementary Figure 1. Cytotoxicity of PPCs towards RD and LLC-MK2 cells. Compound cytotoxicity assays were conducted in the conditions of dose-response experiments and measured by alamarBlue. (**a**) Toxicity of MonoplatinNC, DiplatinNC and TriplatinNC over a 24 h incubation with RD cells. (**b**) Toxicity of TriplatinNC over a 72 h incubation with LLC-MK2 cells. Data points represent the average of 2 independent experiments, ± SD (*n* = 2).

Supplementary Figure 2



Legend to Supplementary Figure 2

Supplementary Figure 1. Structure and electrostatic charge distribution of an EV71 capsid pentamer, FPX, TriplatinNC and the FPX:TriplatinNC adduct. (**a**) Structure of an EV71 capsid pentamer (PDB 3ZFE). VP1, VP2 and VP3 are represented in magenta, green and orange, respectively. VP4, which is internal, was omitted for clarity. An asymmetric unit composed of VP1-3 is highlighted in light grey and the 5-fold axis of symmetry of the pentamer represented with a black star. (**b-e**) Electrostatic Potential (ESP) mapped onto van der Waals surfaces and coloured from red (-5 kcal/mol.e, negatively charged) to blue (+5 kcal/mol.e, positively charged) for (**b**) the EV71 capsid pentamer, (**c**) FPX, (**d**) TriplatinNC and **e**) the FPX:TriplatinNC adduct (alternate views shown). The structures of FPX, TriplatinNC and the FPX:TriplatinNC adduct were optimized at M06l/6-311+G(2d,2p) level of density functional theory.[1] ESP surfaces were mapped using the "Coulombic surface colouring" function in UCSF Chimera v.1.13.1, from amino acid residue electrostatic charges (**b**) or quantum mechanical calculations (**c-e**).

Detailed Materials and Methods

Cells, viruses and compounds. Human enterovirus A71 (EV71) H-strain (ATCC Ref.: VR-1432) was propagated in rhabdomyosarcoma (RD) cells (ATCC Ref.: CCL-136), at 35 °C in a humidified atmosphere of 5% CO_2, using DMEM supplemented with 2% FBS and 1% penicillin/streptomycin. Human metapneumovirus (hMPV, Viratree Ref.: MPV-GFP1) was propagated in rhesus monkey kidney LLC-MK2 cells (ATCC Ref.: CCL-7), at 33 °C in a humidified atmosphere of 5% CO_2, using optiMEM supplemented with 5 µg/mL trypsin, 1% penicillin/streptomycin and 100 µg/mL $CaCl_2$. Fondaparinux sodium and suramin sodium were purchased from Sigma Aldrich.

***In vitro* dose-dependent inhibition assays.** Assays were conducted in 96-well plates. Confluent RD or LLC-MK2 cells were infected with EV71 or hMPV for 24 h or 72 h, respectively. Infection was measured by *in situ* ELISA. For treatment during all stages of infection, compounds were serially diluted in virus (660 or 500 focus-forming units (ffu) per well of EV71 or hMPV, respectively), and applied to cells for 1 h at 4 °C. Infected cells were subsequently transferred to 35 °C for the rest of the infection. For preincubation with cells treatment, compound dilutions were applied to cells for 1 h at 4 °C after which compound was removed and cells washed twice with media. Cells were then infected with 1200 pfu (EV71) or 1000 ffu (hMPV) per well for 1 h at 35 °C, the inocula discarded and the cells washed twice with media. Fresh media was applied to cells and plates were left to incubate at 35 °C for the rest of the infection. The 50% inhibitory concentration of compounds was determined from non-linear regression of normalised infection values using the software GraphPad Prism v.8.

Time of compound addition assay. Confluent RD cells in 96-well plates were infected at a high multiplicity of infection of 1 for 1 h at 4 °C (t_{-1}) to allow for virus binding and a synchronous infection between wells. Inocula were discarded, cells washed twice with media and transferred at 35 °C (t_0) in fresh media for 8 h at 35 °C, which corresponds to a single cycle of EV71 replication. Infected cells were treated at different time points with 200 µM of suramin or 100 µM of TriplatinNC, leading to different overall times of exposure of the compounds to cells: t_{-1} (virus binding at 4 °C, 9 h total exposure), t_0 (virus entry at 35 °C, 8 h total exposure), t_1, t_2, t_4, t_6 and t_8 after virus entry (7, 6, 4, 2, 0 h total exposure, respectively. Infection was stopped after 8 h of infection and measured by *in situ* ELISA.

***In situ* ELISA.** Assays were conducted as previously described.[2] Briefly, cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. Cells were then permeabilised and endogenous peroxidases inhibited with 1% IGEPAL and 0.35% H_2O_2 in PBS for 20 min at 37 °C. Infected cells were immuno-stained using a primary anti- EV71 VP1 (mouse, clone MAB979, Merck Millipore) or hMPV N (mouse, clone NBP1-21631, Novus Biologicals) antibody diluted 1:2000 in 0.02% Tween-20, 5% skim-milk/PBS, and a secondary goat anti-mouse IgG(H+L)-HRP-conjugated antibody (BioRad) diluted 1:6000 in 0.02% Tween-20, 5% skim-milk/PBS. The secondary antibody was detected using the OptEIA TMB substrate (BD Biosciences), and reactions stopped with 0.6 M H_2SO_4. Absorbances at 450 nm were read using an X-Mark Microplate Absorbance Spectrophotometer (BioRad) and converted to % of infection by normalising to negative control (cells only, no virus or compound) and positive control (cells and virus, no compound) wells.

Cytotoxicity assays. Compound cytotoxicity was determined in the conditions of dose-response experiments, in the absence of virus. Compound dilutions were applied to RD cells for 24 h, at 35 °C, after which a 10^{th} of the well volume of alamarBlue (Thermo Fisher Scientific) was added to each well for 3 h at 35 °C. Absorbances at 570 nm and 600 nm were measured in each well using an X-Mark Microplate Absorbance Spectrophotometer (BioRad) and were converted to % cell viability following the manufacturer's instructions. The 50% cytotoxic concentration (CC_{50}) of compounds was determined from non-linear regression using the software GraphPad Prism v.8.

Virus purification. Clarified infection supernatants were PEG-precipited with 8% PEG-8000 overnight and spun 45 min at 5,000 *g* at 4 °C. The pellet was resuspended in PBS, dounce-homogenised and ultra-centrifuged through a 30% sucrose cushion in PBS for 3 h at 4 °C and 100,000 *g* in a SW32.1 Ti rotor (Beckman Coulter). The pellet was resuspended in PBS, dounce-homogenised and ultra-centrifuged through a non-linear 30-60% sucrose gradient in PBS for 3 h at 4 °C and 100,000 *g* in a SW32.1 Ti rotor. The 40% and 50% sucrose fractions, containing infectious virus, were pooled and the virus was pelleted twice in PBS for 3 h at 4 °C and 100,000 *g* in a SW32.1 Ti rotor to remove the sucrose. The resulting purified virus was resuspended in deuterated PBS and UV-inactivated for 15 min.

Saturation Transfer Difference NMR spectroscopy. Experiments were performed in deuterated PBS at pH 7.4 and 283 K. Compounds were dissolved in 180 µL of purified virus, at a final concentration of 2.2 mM. For competition experiments, spectra were acquired in the presence of the first compound and acquired again upon addition of an equimolar concentration of the second compound. Data was collected using a 600 MHz Bruker Avance NMR spectrometer equipped with a standard cryoprobe. On- and off-resonance frequencies were set to -1 ppm and 330 ppm, respectively. EV71 was saturated with 40 Gaussian-shaped pulses of 50 ms each, resulting in a saturation time of 2 s. Water signal was reduced by applying the 3-9-19 WATERGATE sequence. STD spectra were obtained by subtraction of on-resonance spectra from off-resonance spectra.

Supplementary References

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2 D. F. Earley, B. Bailly, A. Maggioni, A. R. Kundur, R. J. Thomson, C.-W. Chang and M. von Itzstein, *ACS Infect. Dis.*, 2019, **5**, 1708–1717.