Electronic Supplementary Information for Dalton Transactions

Issues surrounding standard cytotoxicity testing for assesing activity of non-covalent DNA-binding metallo-drugs.

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Supplementary Information

Experimental

Cell culture

The MDA-MB-231 (human breast carcinoma) cell line was maintained in continuous logarithmic culture in Dulbecco's Modified Eagle's Medium (DMEM) (+ 4.5 g/l glucose, Gibco BRL™, Invitrogen Corporation, GB) supplemented with 10% FBS (Invitrogen), 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 10 mM Hepes buffer (Sigma) and antibiotics (Antibiotics Antimycotic 100× diluted to 1×, Sigma). Cells from confluent monolayers were removed from flasks by 1% trypsin (Trypsin-EDTA 10× diluted to 1× using PBS, Sigma). Cell viability was determined by the trypan blue dye exclusion test.

Extended incubation cellular toxicity

10,000 cells were seeded in 100 μL complete medium per well of 96-multiwell flatbottom microtiter plates (Corning Costar) and incubated for 24 hrs at 37 °C, 5 % CO_2 for cells to adhere. Cells were then treated with 100 μL compound of concentrations between 6.5 and 200 μM followed by further incubation of 1-6 days. At the end of each incubation period cell viability was established by the MTT assay and IC_{50} value calculated.

Volume cellular toxicity

10,000 cells were seeded in 100 μl of complete medium in each well of 96-multiwell flatbottom microtiter plates (Corning Costar) and incubated at 37 °C, 5 % CO_2 for 24 hours

prior to drug testing to allow cell adhesion. Following this incubation medium was removed, cells were washed with 50 μl warm PBS which was then removed. Cells were then treated with 75 μL cylinder/cisplatin in medium with concentrations of between 100 and 3.125 μM. Remaining wells were incubated with 75 μL medium as a control. This method was repeated with volumes of 100, 150 and 200 μL. Plates were incubated for 72 hrs before cell viability was assessed via the MTT assay.

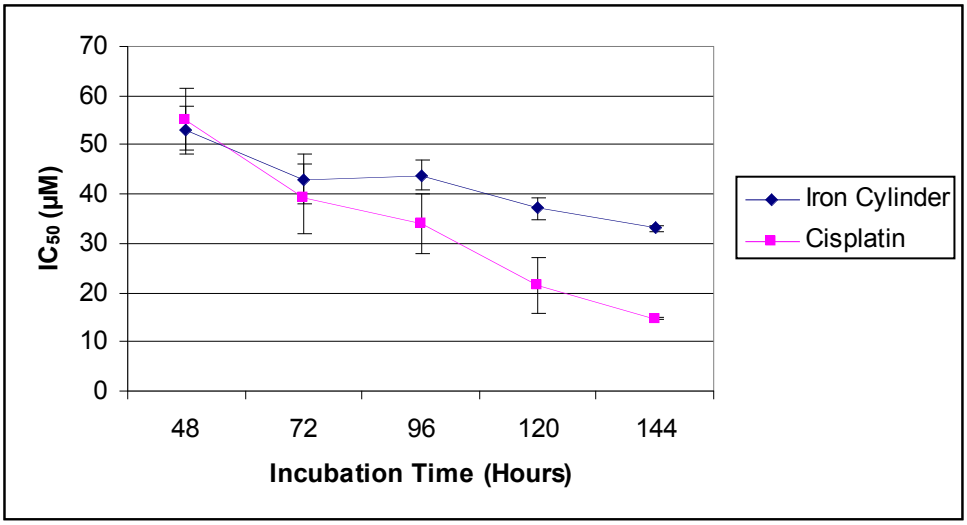
MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay is commonly used to determine mitochondrial reductive function and hence is a good indicator of cell death or inhibition of growth.

After incubation of cells with a range of concentrations of compound the MTT assay, in combination with cell viability of controls containing no compound can be used to obtain an IC_{50} value. This is the concentration of compound where 50 % of cells are viable. 50 μl MTT solution (5mg/ml in PBS, Sigma) was added to each well and incubated for 2 hours. Medium was subsequently removed from wells and resulting formazan crystals solubilised in 100 μl DMSO. Culture plates were rocked gently for 30 mins to solubilise before optical density was measured using a microplate reader (Bio Rad) at 590 nm.

Incubation Time (Hours)	IC_{50} Fe cylinder (μM)	IC_{50} Cisplatin (μM)
24	>100	>100
48	53 ± 6.3	55 ± 4.8
72	43 ± 7.1	39 ± 5.0
96	44 ± 6.0	34 ± 3.0
120	37 ± 5.6	21 ± 2.1
144	33 ± 0.1	15 ± 0.5

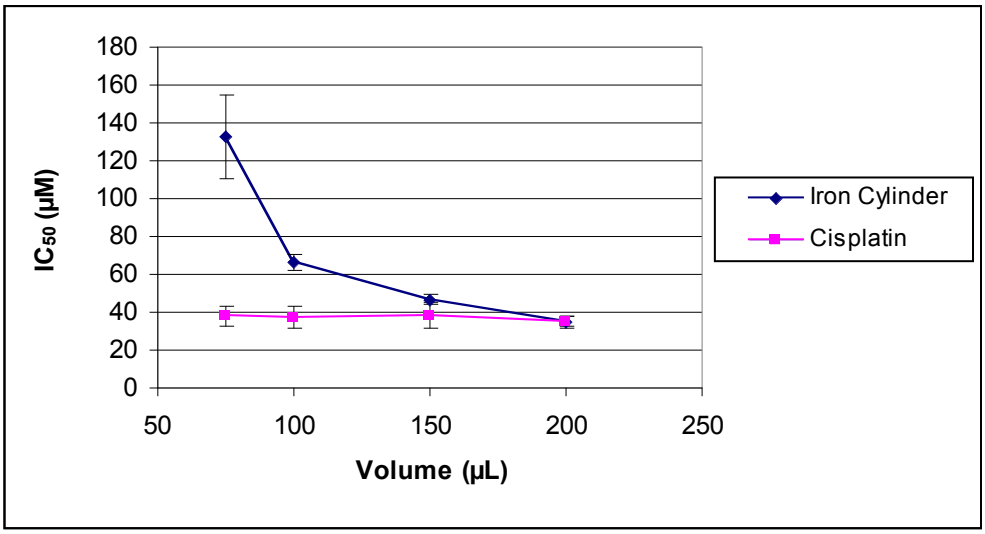
Activity against the breast cancer cell line MDA-MB-231 with iron cylinder and cisplatin following 48, 72, 96, 120 and 144 hour incubations, as assessed using the MTT assay. Data are reported as mean IC_{50} values ± SEM from 3 independent experiments.



Activity against the breast cancer cell line MDA-MB-231 with iron complex and cisplatin following 48, 72, 96, 120 and 144 hour incubations, as assessed using the MTT assay. Data are reported as IC_{50} versus time. Graph represents mean ± SEM from 3 independent experiments.

Volume (µL)	75	100	150	200
IC_{50} Fe Cylinder	> 100	66 ± 4.4	47 ± 2.3	35 ± 3.0
IC_{50} Cisplatin	38 ± 5.6	37 ± 6.1	38 ± 7.0	35 ± 3.0

Activity against the breast cancer cell line MDA-MB-231 with iron cylinder and cisplatin following 72 hour incubation with varying volumes of compound, as assessed using the MTT assay. Data are reported as mean IC_{50} values ± SEM from 3 independent experiments.



Activity against the breast cancer cell line MDA-MB-231 with iron complex and cisplatin following 72 hour incubation with varying volumes of compound, as assessed using the MTT assay. Data are reported as IC_{50} versus volume. Graph represents mean ± SEM from 3 independent experiments.