Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Electronic Supplementary Information

Anti-colorectal Cancer Activity of an Organometallic Osmium Arene Azopyridine Complex

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Materials

The A2780 human ovarian carcinoma and A549 human lung cancer cell lines were purchased from European Collection of Animal Cell Cultures (Salisbury, UK), RPMI-1640 media and trypsin from Invitrogen, bovine serum from Biosera, penicillin, streptomycin, L-buthionine sulfoximine (L-BSO), trichloroacetic acid (TCA) and sulforhodamine B (SRB) from Sigma-Aldrich, and tris[hydroxymethyl]aminomethane from Formedium. Complex **1** (FY026) was synthesised and characterised as previously described (ref 8).

Experimental Procedures

ICP-MS. ICP-MS analyses were carried out on an Agilent Technologies 7500 Series ICP-MS instrument. The water used for ICP-MS analysis was double deionised (DDW) using a USF Elga UHQ water deionizer. The osmium Specpure plasma standard (Alfa Aesar, 1000 ppm in 5% HCl) was diluted with DDW to 20 ppm. The standards for calibration were freshly prepared by diluting this stock solution with 3% HNO_3 in DDW. The concentrations used were 100, 60, 20, 10, 5, 4, 2, 1, 0.4 and 0.1 ppb.

ROS Detection. DCFH-DA was handled under an N_2 atmosphere, dissolved in DMSO to give a 10 mM stock solution. A2780 cells were seeded at 5000 cells/well into black 96-well plate and incubated for 24 h at 310 K, 5% CO_2, high humidity. Cells were incubated for 20 min with DCFH-DA (10 µM). To remove extracellular probe, the cells were washed twice with PBS. The cells were then kept in PBS solution and either the osmium complex **1**, or **1** plus L-BSO (50 µM), or hydrogen peroxide (50 µM, as the positive control) were added. The fluorescence was recorded over a period of 4 h at 310 K by excitation at 480 nm and emission at 530 nm using a TECAN plate reader.

Combination treatment with 1 and L-BSO. To determine the effect of decreased GSH levels on the cytotoxicity of **1,** A2780 human ovarian and A549 human lung cancer cells were co-incubated with 50 µM L-BSO and various concentrations of **1** for 24 h. Cell viability was then determined using the sulforhodamine (SRB) assay.

Redox potentials. The electrochemical study was carried out using the same conditions as for the ruthenium analog reported previously.[1]

Animals. Six to ten week old female BALB/cOlaHsd-Foxn1^{nu} mice (Harlan, Blackthorn, UK), maintained with food (Teklad 2018 diet, Harlan) and water *ad libitum*, were used according to UKCCCR guidelines for the welfare of animals [2]. Regulated procedures were carried out under a UK Home Office Project License.

Tumour System. HCT-116 (human colon adenocarcinoma line) tumours were excised from a donor animal, placed in sterile physiological saline containing antibiotics and cut into small fragments of approximately 2 mm^3. Under brief general inhalation anaesthesia, fragments were implanted in the left flank of each mouse using a trocar. Once the tumours could accurately be measured by calipers (mean tumour volume of 32 mm^3), the mice were allocated into groups by restricted randomisation to keep group mean tumour size variation to a minimum.

Plasma and Tissue Distribution Studies. Tumour-bearing mice (n = 3 per time point) were sacrificed at 5 min, 60 min and 240 min following intravenous administration of a single 10 mg kg^{-1} dose of FY026. Tumour, liver, lung, kidneys and plasma were collected, weighed and stored at 193 K until analyzed. Osmium concentrations in different samples were measured by ICP-MS using the methodology described above. Tumour and tissue samples were homogenized in phosphate buffered saline, and

digested in HNO_3 at 353 K overnight to give clear solutions. These were then diluted as appropriate for evaluation by ICP-MS.

Chemotherapy Studies. The compounds were administered by single injections, with the day of therapy designated day 0. The maximum soluble dose of FY026 when administered as a single intravenous injection was established as 40 $mgkg^{-1}$. The efficacy of FY026 was compared with the standard anticancer agent cisplatin which was administered as a single dose intraperitoneally at its maximum tolerated dose of 8 $mg\ kg^{-1}$. The effects of therapy were assessed as previously described.[3] Briefly, daily 2-dimensional caliper measurements of the tumours were taken, with volumes calculated using the formula (a^2 x b) / 2, where a is the smaller and b the larger diameter of the tumour. Tumour volume was then normalised to the respective volume on day 0, and semi-log plots of relative tumour volume (RTV) versus time were made. Mann-Whitney U tests were performed to determine the statistical significance of any differences in growth rate (based on tumour volume doubling time) between control and treated groups, and between the 2 compounds.

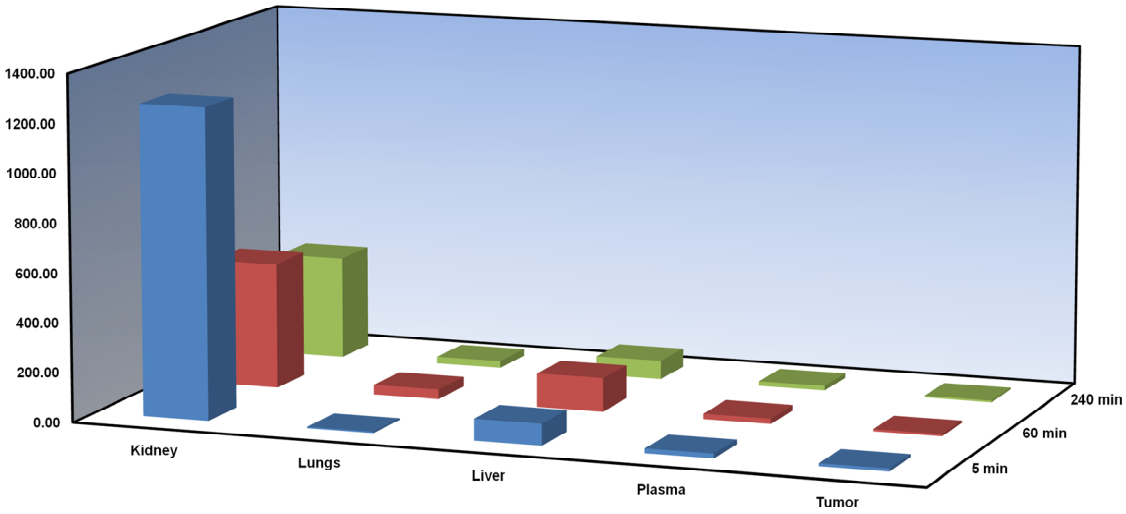

Fig. S1 Concentration-versus-time point profiles of complex 1 in kidney, lung, liver, plasma and tumour. Points represent the mean concentration for three mice. Data are in Table 1.

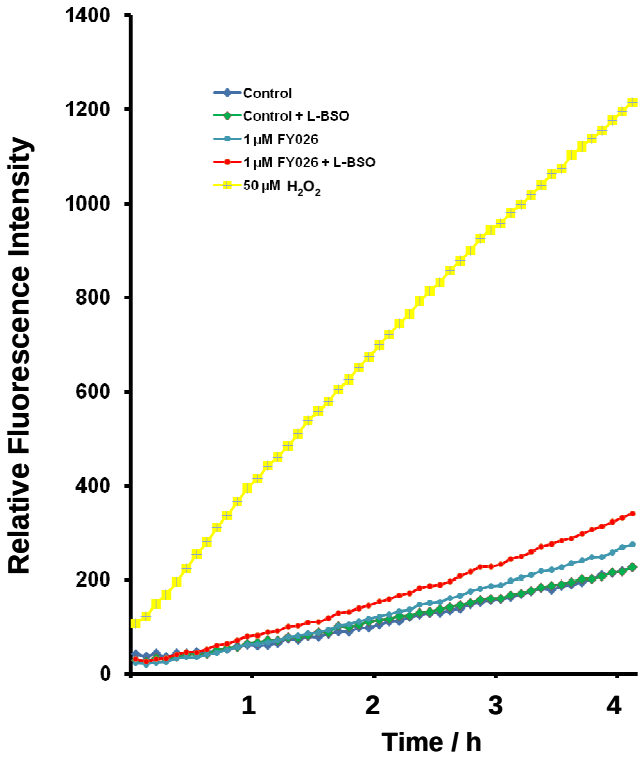

Fig. S2 Relative changes in DCF fluorescence detected over time after exposure to 1 µM complex **1** (FY026), 1 µM **1** with 50 µM L-BSO, and 50 µM H_2O_2. For each experiment, the fluorescence was averaged over 8 wells.

References

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