Electronic Supplementary Information

Enhancement of selectivity of an organometallic anticancer agent by redox modulation

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In all cases, independent two-sample t-tests with unequal variances, Welch's tests, were carried out to establish statistical significance of the variations ($p < 0.001$ for ***, $p < 0.01$ for **, and $p < 0.05$ for *).

Table 1. Selectivity factors.

	IC_{50} (nM ± SD)		Selectivity factor
	A2780 ovarian cancer cells	MRC5 foetal lung fibroblasts	$IC_{50\text{-MRC5}}/IC_{50\text{-A2780}}$
FY26	160 ± 10	4550 ± 60	28.4 ***
FY26 + 5 μM LBSO	69 ± 5	4380 ± 25	63.5 ***

Table 2. GSH determination in A2780 cells – values normalised to the negative controls. All values compared to the untreated controls for statistical significance calculations.

	L-BSO (μM)			
	0	1	5	50
GSH	1.00 ± 0.01	0.95 ± 0.02	0.56 ± 0.03***	0.37 ± 0.02***

	FY26 IC_{50}		FY26 $2xIC_{50}$	
		+ L-BSO		+ L-BSO
GSH	0.92 ± 0.01**	0.54 ± 0.03***	0.87 ± 0.02**	0.47 ± 0.03***

Table 3. Induction of ROS and superoxide determined by flow cytometry experiments in MRC5 fibroblasts. All values compared to the untreated controls for statistical significance calculations.

	% populations			
	FL-1-/FL-2-	FL-1+/FL-2-	FL-1-/FL-2+	FL-1+/FL-2+
	Lower left	Lower right	Upper left	Upper right
FY26 IC_{50}	17.2 ± 0.9 ***	59 ± 2 ***	5.6 ± 0.8 *	18.3 ± 0.7 ***
Positive control	0.8 ± 0.3 ***	1.5 ± 0.6	2.1 ± 0.4	96 ± 2 ***
Negative control	96 ± 3	1.2 ± 0.4	2.8 ± 0.7	0.1 ± 0.1

Table 4. Induction of ROS and superoxide in A2780 cells determined by flow cytometry experiments. All values compared to the untreated controls for statistical significance calculations.

	% populations			
	FL-1-/FL-2-	FL-1+/FL-2-	FL-1-/FL-2+	FL-1+/FL-2+
	Lower left	Lower right	Upper left	Upper right
FY26 IC_{50}	3.4 ± 0.8 ***	0.5 ± 0.3	80 ± 3 ***	15 ± 2 ***
FY26 IC_{50} + 5 μM L-BSO	3.5 ± 0.9 ***	0.8 ± 0.3	76 ± 4 ***	19 ± 1 ***
5 μM L-BSO	98 ± 2	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.2
Positive control	1.2 ± 0.4 ***	3.6 ± 0.9 *	0.4 ± 0.2	96 ± 4 ***
Negative control	99 ± 2	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.2

Table 5. Induction of apoptosis in A2780 cells using flow cytometry experiments. All values compared to the untreated controls for statistical significance calculations.

	% populations			
	FL-1-/FL-2-	FL-1+/FL-2-	FL-1-/FL-2+	FL-1+/FL-2+
	Lower left Viable cells	Lower right Early apoptosis	Upper left Non-viable	Upper right Late apoptosis
FY26 IC_{50}	73 ± 3 ***	1.2 ± 0.8	16 ± 2 ***	8.6 ± 0.9 ***
FY26 IC_{50} + 5 μM L-BSO	75 ± 4 ***	0.8 ± 0.2	14 ± 3 ***	10 ± 1 ***
5 μM L-BSO	96 ± 3	1.8 ± 0.8	0.6 ± 0.2	0.2 ± 0.1
Positive control	13 ± 2 ***	4.5 ± 0.6	76 ± 4 ***	6.2 ± 0.4 ***
Negative control	97 ± 2	2.4 ± 0.6	0.8 ± 0.4	0.2 ± 0.1

Table 6. Variations in mitochondrial membrane potential in A2780 cells determined by JC-1 dye using flow cytometry. All values are compared to the untreated controls for statistical significance calculations.

	% populations	
	Low fluorescence FL-2	High fluorescence FL-2
FY26 IC_{50}	9.8 ± 0.6 ***	90.1 ± 0.9 ***
FY26 IC_{50} + 5 μM L-BSO	9.3 ± 0.3***	90.6 ± 0.8 ***
5 μM L-BSO	10 ± 1 ***	92 ± 3 ***
Positive control	0.2 ± 0.1 ***	99.7 ± 0.1 ***
Negative control	96.9 ± 0.5	3.0 ± 0.5

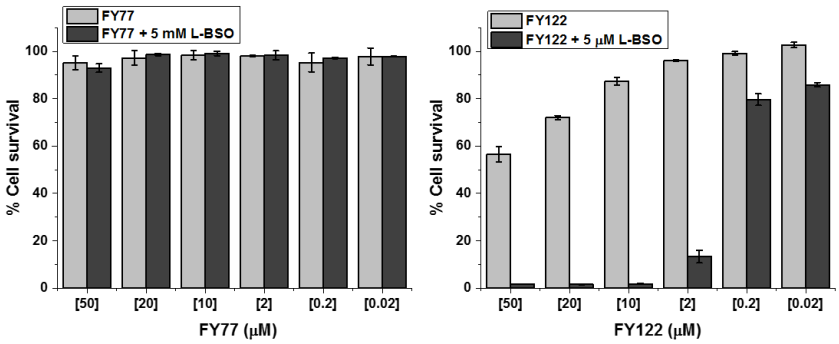

Figure 1. Percentages of survival of A2780 ovarian cancer cells exposed to various concentrations of structurally related osmium complexes FY77 ([Os(η^6-bip)(Cl-Azpy)Cl]PF_6 IC_{50}>100 μM) and FY122 ([Os(η^6-*p*-cym)(OH-Impy)I]PF_6 IC_{50}= 30 ± 2 μM). The data are compared to A2780 cells exposed under similar conditions to the osmium complexes and co-administered with 5 μM L-BSO. The drug exposure and recovery times were 24 h and 72 h, respectively.

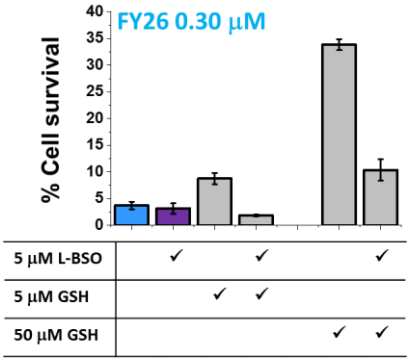

Figure 2. Effect of co-administration of 0.30 μM FY26 with 5 μM L-BSO in the presence/absence of GSH (5 and 50 μM) on the percentage of cell survival of A2780 ovarian cancer cells.

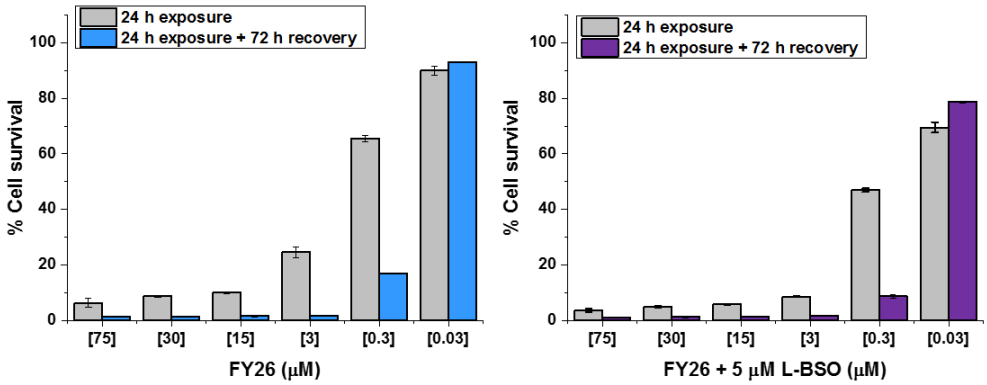

Figure 3. Percentages of survival of A2780 ovarian cancer cells exposed to different concentrations of FY26 +/- 5 μM L-BSO. Results in color represent those experiments that included 72 h recovery time in drug-free medium, while results in grey did not. In both cases the drug exposure time was 24 h.