

Supplementary information for

PBT2 acts through a different anti-cancer mechanism of action than other 8-hydroxyquinolines: An X-ray fluorescence imaging study

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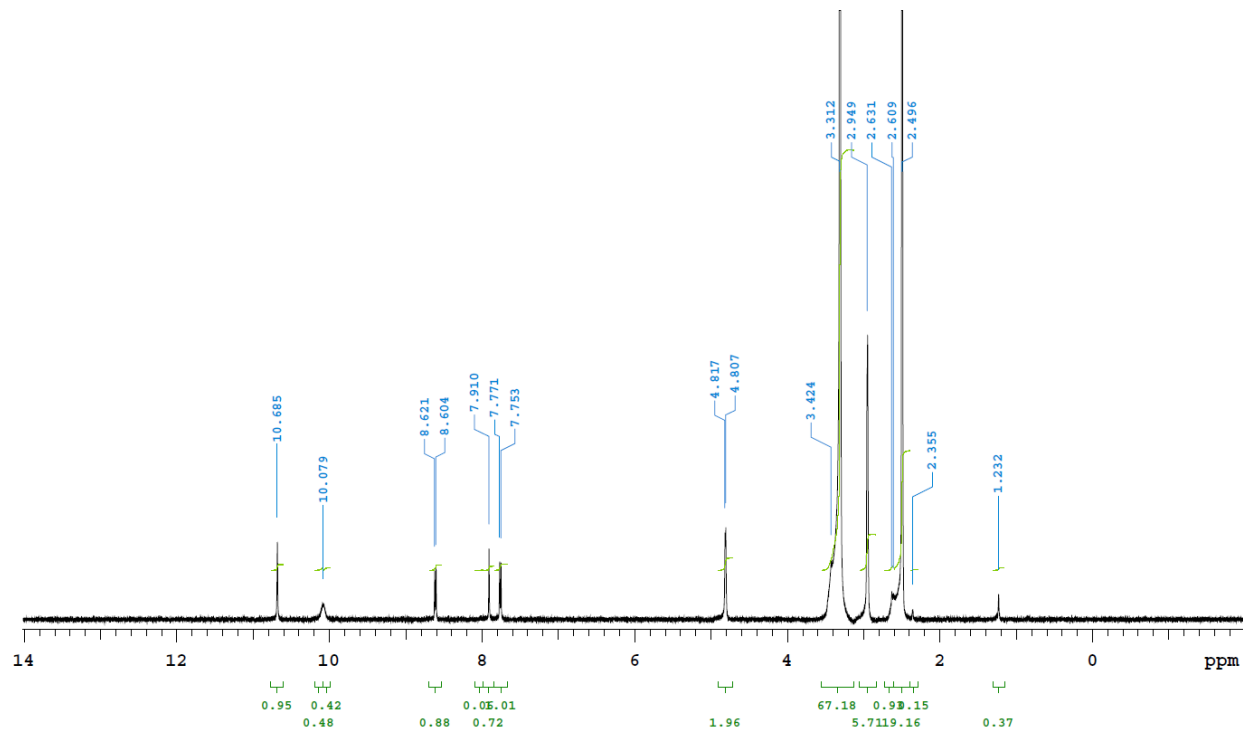


Figure S.1. $^1\text{H-NMR}$ of PBT2 in d_6 DMSO. No unknown impurities were found. Spectrum is consistent with NMR resonances reported by Barnham *et al.*¹

Table S.1. Number and area of individual cells imaged using X-ray fluorescence imaging over all experiments.^a

Treatment	Number of Cells Imaged (<i>n</i>)	Mean Area of Cells Imaged (μm^2)
Untreated	4	259.5
Vehicle control (DMSO)	3	373.5
Vehicle control (50% DMSO, 50% H ₂ O)	7	311.9
5 μM CuCl ₂ (DMSO)	8	317.8
15 μM CuCl ₂ (DMSO)	9	364.9
5 μM CuCl ₂ (50% DMSO, 50% H ₂ O)	7	365.3
15 μM CuCl ₂ (50% DMSO, 50% H ₂ O)	4	267.5
5 μM PBT2 ^b	5	321.8
5 μM PBT2, 5 μM CuCl ₂ ^b	9	370.2
5 μM PBT2, 15 μM CuCl ₂ ^b	8	275.0
5 μM CQ	15	327.5
5 μM CQ, 5 μM CuCl ₂	10	271.4
5 μM CQ, 15 μM CuCl ₂	8	381.8
30 μM CQ	3	436.1
5 μM B2Q	9	356.0
5 μM B2Q, 5 μM CuCl ₂	6	248.6
5 μM B2Q, 15 μM CuCl ₂	9	349.0
30 μM B2Q	3	139.7

^a Number of cells imaged (*n*) in each treatment varies due to the size of cells including projections and the orientation of cells in a raster-scanned area. ^b PBT2 treatments were dissolved in 50% DMSO, 50% diH₂O

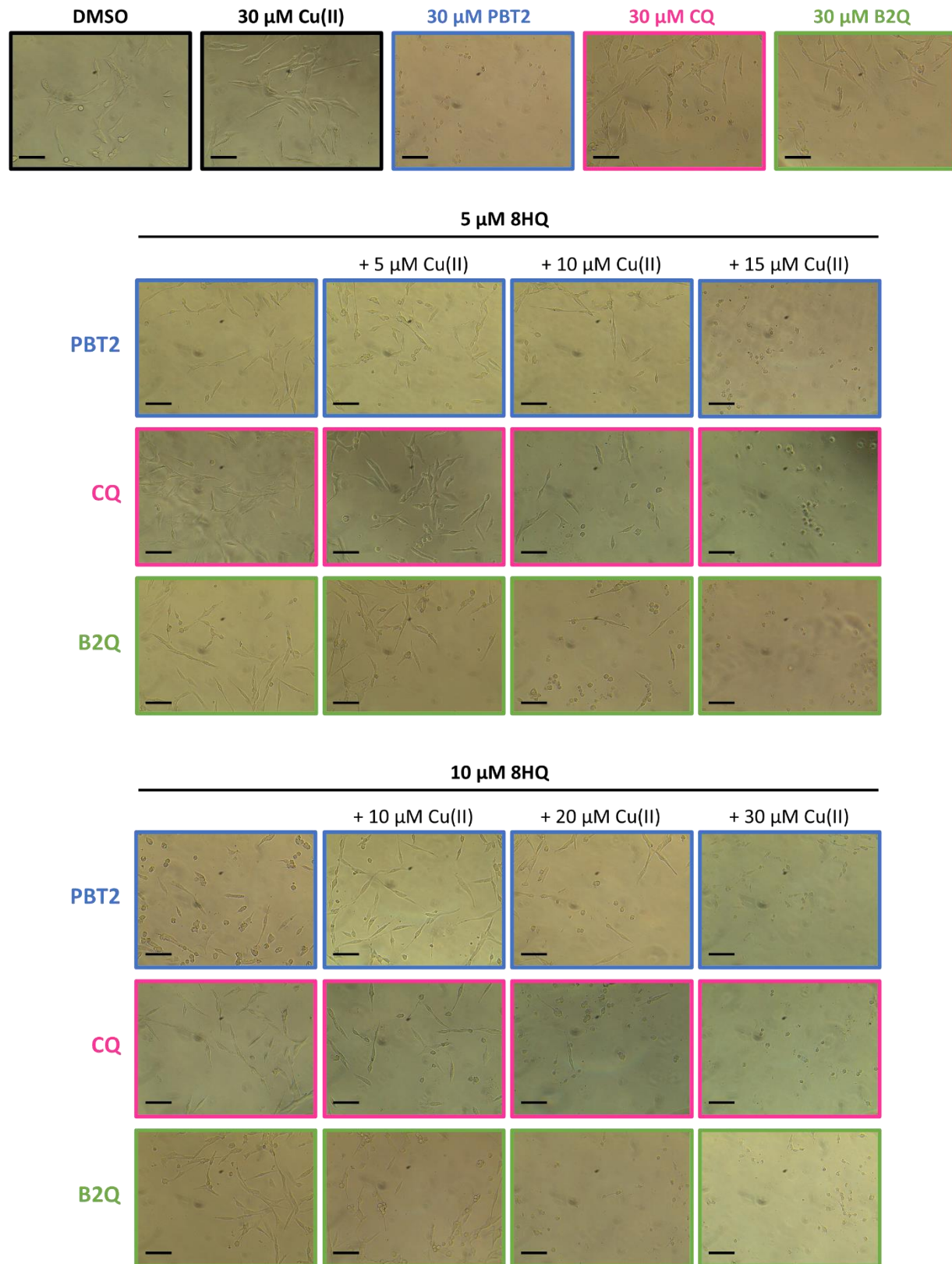


Figure S.2. Light microscope images of C6 glioma cells treated with 8HQs with or without Cu(II) 24 h post-treatment. Scale bars represent 100 μm .

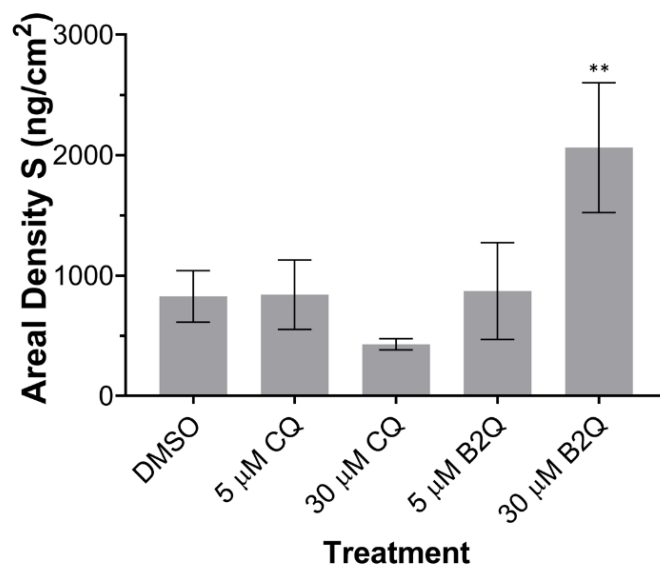


Figure S.3. Areal density of sulfur in X-ray fluorescence images of C6 glioma cells treated with either 5 or 30 μ M CQ or B2Q. Bars represent the mean of each group where $n > 3$ (see Section 2.4 and Table S.1 for details). Error bars indicate standard deviation. Statistical significance is denoted as ** ($p = 0.0016$).

Reference

1. Barnham, K. J., Gautier, E. C. L., Kok, G. B., and Krippner, G. (2008) Preparation of 8-hydroxyquinolines for treatment of neurological conditions, U.S. Pat. Appl. Publ., Patent version number 20080161353 A1, United States.