Electronic Supplementary Material (ESI) for Chemical Communications

Enhanced Stability and Activity of Temozolomide in Primary Glioblastoma Multiforme Cells with Cucurbit[n]uril

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S.1 Instrumentation and Materials

^{1}H NMR (400 MHz) spectra was recorded using a Bruker Avance QNP 400. Chemical shifts are recorded in ppm (δ) in D_2O with the internal reference set to δ 4.79 ppm. Titration experiments were performed by adding varying amounts of a solution of CB[7] in D_2O (1 mM) to a solution of TMZ in D_2O (1 mM) to give a final concentration of 0.5 mM TMZ and 0, 0.25, 0.50, 0.75 and 1.00 equivalents of CB[7].

ATR FT-IR spectroscopy was performed using a Perkin-Elmer Spectrum 100 series FT-IR spectrometer equipped with a universal ATR sampling accessory. UV-VIS studies were performed on a Varian Cary 4000 UV-Vis spectrophotometer.

ITC titration experiments were carried out on a VP-ITC from Microcal Inc. at 25 ^{o}C in 10 mM sodium phosphate buffer (pH=7). In a typical experiment, the host was in the sample cell at a concentration of 0.1 mM, and the guest was in the syringe at a 10 fold higher concentration. In the case of functional polymers, the concentration used is determined from the concentration of functional monomer units in solution and not the concentration of polymer. A titration consisted of 29 consecutive injections of 2-10 μL with at least 300 s intervals between injections. The first data point was removed from the data set prior to curve fitting. Heats of dilution were checked by titration well beyond saturation or by titration of the guest into a buffer solution and subtracted from the normalized enthalpies, but relatively small in all cases. The data were analyzed with Origin 7.0 software, using the one set of sites model.

All materials were purchased from Aldrich and used as received.

S.2 General Protocols

S.2.1 Culture Media

Cells were cultured under serum-free (SF) conditions.[1] The SF media consisted of phenol-free Neurobasal-A (NBA) (Invitrogen, UK) with 20 mM L-glutamine, 1% v/v penicillin, streptomycin and fungicid (PSF) solution, 20 ng ml^{-1} human epidermal growth factor (hEGF, Sigma, UK), 20 ng ml^{-1} human fibroblast growth factor (hFGF, RD systems, UK), 2% v/v B27 (Invitrogen, UK) and 1% N2 v/v (Invitrogen, UK). All cell cultures were grown in appropriate incubators at 37°C in 5% CO_2.

S.2.2 Cell Culture

Each GBM specimen was anonymised, weighed, chopped into small pieces and dissociated enzymatically using a mixture of Accutase (Invitrogen, UK) in HBSS Ca^{2+} / Mg^{2+} free. The suspension was filtered through a 40 μm strainer (Falcon, UK) to isolate single cells. For removal of red blood cell, the single cell suspension was washed with filtered red cell lysis buffer consisting of NH_4Cl (8.3 g), $KHCO_3$ (1.0 g) and 5% of EDTA (1.8 ml) in double distilled (dd) water (1 L). Cells were rewashed in HBSS Ca^{2+} / Mg^{2+} free before quantification of live cells by trypan blue exclusion. Cells were seeded at standard density of 15,000 cells per cm^2 in defined SF medium in 37 °C, 5% CO_2 incubator. All primary cultures monitored daily for primary neurosphere formation. Primary neurosphere cultures were rewashed and fed weekly for 2-6 weeks depending on the sample. After a variable priming period in which primary spheres size increased, spheres were harvested, washed once in HDSS Ca^{2+} / Mg^{2+} free before being seeded without dissociation on ECM-coated flasks (Nunc, Thermo Scientific) to grow as primary monolayer cultures. As the primary monolayer approached confluence cells were dissociated by incubation with Accutase as room temperature and washed with HBSS Ca^{2+} / Mg^{2+} free. The cell viability was assessed by trypan blue exclusion and cells reseeded onto ECM-coated flasks at a density of 15,000 cells per cm^2 to generate the subsequent monolayers. Cells were grown in 96 well plates, steriliszed coverslips in 24 well plates or in T25 and T75 flasks. Extracellular matrix gel (ECM) from Engelbreth Holm-Swarm mouse sarcoma (Sigma) was used as adhesive substrate after being dilute 1:10 in phenol-free NBA (Gibco) before coating the flasks and coverslips.

S.2.3 MTS Determined TMZ and TMZ@CB[7] Response Assay

Cells were seeded, at a density of 3,000 cells per well, into 3 96-well plates pre-coated with ECM and incubated for 2 days in 200 μl of defined SF media in 37 °C and 5% CO_2. There were 12 replicas in each repeat per cell line. Following incubation, either TMZ, TMZ/CB[7] complex or CB[7] was added to concentrations of 10 mM, 5 mM, 1 mM and 500 μM, 100 μM, 50 μM and 10 μM. The cells were then incubated for a further 24 hours prior to the addition of 5 μl of MTS solution into each well. Following a further incubation period of 4 hours, in which the tetrazolium was reduced by the mitochodria in the living cells to formazan, optical absorbance values at 490 nm from each well was measured using a plate reader (ELX 800, Bio-Tek Instruments, Inc.). Absorbance values measured from wells containing no cells were used as controls. Percentage cell viability was then assessed by adjusted absorbance values whereby the average absorbance values of the control wells was subtracted from the experimental well values and the corresponding value then analysed as a percentage of the absorbance from the 0 μM wells.

References

[1] Al-Mayhani, T. M. F.; Ball, S. L. R.; Zhao, J.-W.; Fawcett, J.; Ichimura, K.; Collins, P. V.; Watts, C. *J. Neurosci. Methods* **2009**, *176*, 192–199.

S.2.4 Characterisation of CB[7] Binding with Temozolomide

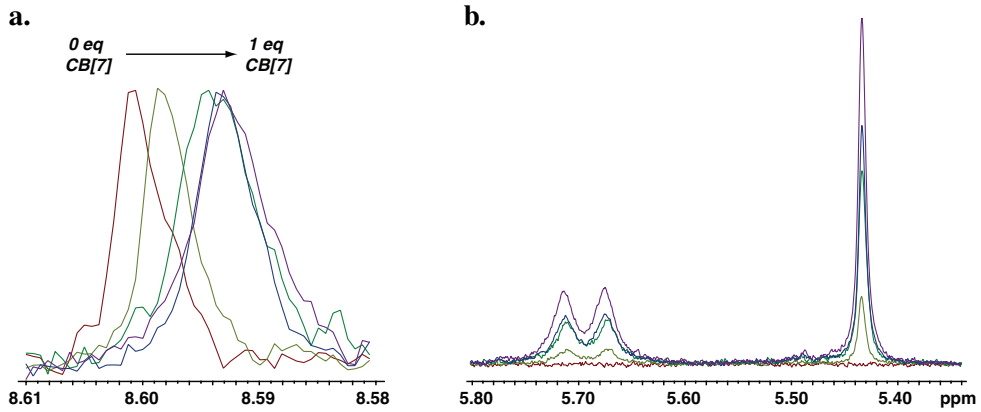

Figure S1: a, ^{1}H-NMR stack of a titration of CB[7] (0, 0.25, 0.5, 0.75, 1 eq) into a solution of TMZ (30 mM) in water at pH 7.0 demonstrating a clear shift in the aromatic protons of TMZ upon binding. **b,** The peaks associated with the CB[7] proton clearly increase with addition of CB[7] whilst the intensity of the TMZ peaks remains constant.

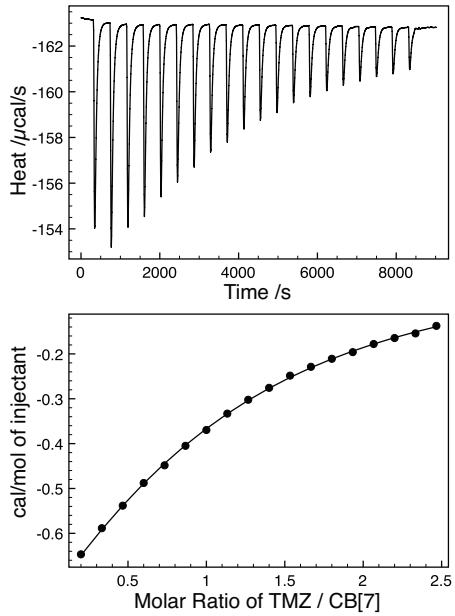

Figure S2: Isothermal titration calorimetry data for the binding of TMZ to CB[7] at 25 °C in water. TMZ was titrated at 5 mM into a 0.5 mM solution of CB[7]. The top plot displays the power applied as a function of time. The bottom plot displays integrated enthalpy values as a function of the molar ratio of TMZ titrated into CB[7] solution.