Supporting Information

The MT-Stabilizer, Dictyostatin, Exhibits Prolonged Brain Retention and Activity: Potential Therapeutic Implications

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Compounds

Discodermolide, dictyostatin and EpoD were prepared as previously described.[1-4] In all cases, sample purity was >95% as determined by NMR and LC/MS analyses.

Compound Dosing in Mice

Discodermolide, dictyostatin and EpoD solutions were prepared at 4 mM in 100% DMSO, and 2-month old CD1 mice received i.p. injections at a dose of 5 mg/kg (volumes of 55-70 μl based on body weight). Groups (n = 3) of mice were sacrificed at various times after dosing, with blood and brains collected using protocols approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Determination of Plasma and Brain Compound Concentrations

Mouse brains (one hemisphere) were homogenized in 10 mM ammonium acetate, pH 5.7 (1:2; w/v) using a handheld sonic homogenizer. Mouse plasma was obtained from blood that was collected into 1.5 ml tubes containing 0.5M EDTA solution and subsequently centrifuged at 4500g at 4^0C for 10 min. Aliquots (50 μl) of brain homogenates or plasma were mixed with 0.2 ml of acetonitrile, centrifuged at 15,000g, and the resulting supernatant was used for LC-MS/MS analysis employing an Aquity UPLC and a TQ MS that was controlled using MassLynx software (Waters Corporation, Milford, MA, USA). Compounds were analyzed using multiple reaction monitoring (MRM) of their specific collision-induced ion transitions (see Table below for details). Samples were separated on an Aquity BEH C18 column (1.7 μm, 2.1 x 50 mm) at 35˚C. Operation was in positive electrospray ionization mode, with mobile phase A of 0.1% (v/v) formic acid, and B of acetonitrile with 0.1% (v/v) formic acid. Injections of 5 μl were separated at a flow rate of 0.6 mL/min using a gradient from 5% to 95% B over

two minutes, followed by wash and re-equilibration steps. The MS was operated with a desolvation temperature of 450˚C and a source temperature of 150˚C. Desolvation and source nitrogen gas flows were 900 L/hr and 50 L/hr, respectively. Source and MS/MS voltages were optimized for each compound using the MassLynx auto tune utility (see Table below). To account for possible matrix effects on analytes, standard curves were generated for each compound from brain homogenate and plasma samples that had compound added at concentrations ranging from 0.01 to 10 μM. The standard curve samples were extracted and analyzed in an identical fashion as the corresponding tissue-derived samples, and peak areas were plotted against concentration and a linear regression curve was used to obtain estimated concentrations of the tissue-derived samples (r^2 values of >0.99). In all cases, the tissue-derived sample peak areas fell within the linear portion of standard curves that were prepared and analyzed concurrently with the samples.

MS/MS Conditions

Compound	**Ion Transition**	**Cone (V)**	**Collision (V)**
Dictyostatin	533.4>515.3	14	10
Discodermolide	594.5>334.3	20	12
Epothilone D	492.2>304.2	45	24

Approximation of the unbound fraction of dictyostatin in plasma and brain was as previously described.[12]

AcTub Assay

HEK293 cells were maintained in DMEM containing 10% FCS, 1% penicillin/streptomycin solution and 1% glutamine in 5% CO2 at 37˚C. Cells were plated into 6-well plates at a density of 400,000 cells/ml in 2 ml of DMEM/well the day before an experiment. On the day of an experiment, 1 ml of media was removed from each well and 1 ml of DMEM containing either a test compound or medium alone was added. Compounds were diluted from a DMSO stock solution to prepare a 2x solution. Compounds were incubated at 37˚C for 4 hours and the plates were then placed on ice. The media in each well is removed and the well was washed with ice cold PBS, followed by the addition of 0.2 ml of ice cold RIPA (0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40, 5 mM EDTA, pH8.0) buffer containing a commercial protease inhibitor mix (1:1000) and 1 μM of the deacetylase inhibitor, trichostatin A. Each well was scraped and the RIPA lysate was added to Beckman Coulter micro centrifuge tubes, which were spun at 45,000 rpm for 30 min at 4˚C. The supernatant was subsequently removed and protein concentration was determined for each sample using the Pierce BCA kit. The supernatant samples were then analyzed in an AcTub ELISA, as previously

described.[5] For AcTub determinations in mouse brain samples, mice (n=3/treatment) were administered vehicle (DMSO) or dictyostatin (5 mg/kg i.p.). Mouse brains were homogenized as described above, and aliquots were analyzed using an AcTub ELISA as previously described.[12]

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