**Epothilone B**

**Cat. No.:** HY-17029  
**CAS No.:** 152044-54-7  
**Molecular Formula:** C₂₇H₄₁NO₆S  
**Molecular Weight:** 507.68  
**Target:** Microtubule/Tubulin; Fungal; Apoptosis; Antibiotic  
**Pathway:** Cell Cycle/DNA Damage; Cytoskeleton; Anti-infection; Apoptosis  
**Storage:** Powder -20°C 3 years  
In solvent -80°C 6 months  
-20°C 1 month

### SOLVENT & SOLUBILITY

#### In Vitro

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>1 mM</td>
<td>1.9697 mL</td>
<td>9.8487 mL</td>
<td>19.6974 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.3939 mL</td>
<td>1.9697 mL</td>
<td>3.9395 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.1970 mL</td>
<td>0.9849 mL</td>
<td>1.9697 mL</td>
</tr>
</tbody>
</table>

* "≥" means soluble, but saturation unknown.

#### In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
   Solubility: ≥ 2.08 mg/mL (4.10 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
   Solubility: ≥ 2.08 mg/mL (4.10 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil  
   Solubility: 2.08 mg/mL (4.10 mM); Clear solution; Need warming

### BIOLOGICAL ACTIVITY

**Description**  
Epothilone B is a microtubule stabilizer with a $K_i$ of 0.71 μM. It acts by binding to the αβ-tubulin heterodimer subunit which causes decreasing of αβ-tubulin dissociation.

**IC₅₀ & Target**  
EC0.01: 1.8 μM (Microtubule/Tubulin)[1]

**In Vitro**  
Epothilone B inhibits HCT116 cells with IC₅₀ of 0.8 nM in HCT-116 cell line cytotoxicity assay[1]. Epothilone B (Patupilone) is a microtubule (MT) targeting agent. As shown by MTT cell proliferation assay, after 72 h of treatment Epothilone B efficiently...
inhibits cell growth with an IC\textsubscript{50} of 6 nM, while concentrations ≤1 nM are not cytotoxic. Epothilone B significantly inhibits transwell cell migration at the non-cytotoxic concentration of 1 nM, and the effect is more evident at 10 nM\cite{2}. Epothilone B (Patupilone) is a novel, non-taxane-related and nonneurotoxic microtubule-stabilizing agent in human medulloblastoma cell lines. Epothilone B reduces the proliferative activity in the D341 cell line, with an IC\textsubscript{50} of 0.53 nM; in the D425Med cell line, with an IC\textsubscript{50} of 0.37 nM; and in the DAOY cell line, with an IC\textsubscript{50} of 0.19 nM. In the D341Med cell line, the effect of Epothilone B on clonogenic survival is at dose range of Epothilone B similar to the level of proliferative activity and viability (IC\textsubscript{50}, 0.50-0.75 nM). However, the clonogenicity of D425Med and DAOY cells is already strongly reduced at a 10-fold lower concentration of Epothilone B (IC\textsubscript{50}, 30 pM). These results overall demonstrate that Epothilone B is highly potent against different medulloblastoma cell lines\cite{3}.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**In Vivo**

Treatment with Epothilone B (Patupilone) or ionizing radiation alone results in a partial tumor growth suppression over 10 days, whereas combined treatment exerts a strong supra-additive tumor growth control, with complete tumor regression in the follow-up period (P<0.005, for ionizing radiation or Epothilone B alone vs combined treatment)\cite{3}.

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**PROTOCOL**

**Kinase Assay**\cite{3}

Asp-Glu-Val-Asp (DEVD)ase activity is determined in cytosolic cell extracts. Cells are treated with increasing concentrations of Epothilone B (Patupilone) for 6, 12, 24, and 48 h. Cells are harvested thereafter by trypsin/EDTA, centrifuged, and washed with precooled PBS. The cell pellet is suspended in 5 volumes of precooled buffer A (20 mM HEPES-KOH [pH 7.5], 10 mM KCl, 1.5 mM MgCl\textsubscript{2}, 1 mM sodium EDTA, 1 mM sodium EGTA, 1 mM dithiothreitol [DTT], 250 mM sucrose, and 0.1 mM phenylmethylsulfonyl fluoride [PMSF]) supplemented with protease inhibitors (5 mg/mL pepstatin A, 10 mg/mL leupeptin, 2 mg/mL aprotinin, 2 mg/mL DTT, and 1 mM of PMSF). After incubation on ice for 15 min, the cells are disrupted by freezing and thawing. Cell lysates are centrifuged at 1000g for 10 min at 4°C, and the supernatant is further centrifuged at 100 00g for 30 min. The resulting supernatant (S-100 fraction) is stored at −80°C. To determine caspase 3-like activity, 75 μg of protein from the S-100 fraction is incubated at 37°C with the colorimetric caspase 3 substrate N-acetyl-Asp-Glu-Val-Asp p-nitroanilide (100 mM; Ac-DEVD-pNA) and 1 mM dATP in a final volume of 120 μL. Cleavage of the caspase substrate is monitored at 405 nm using a GenTec spectrophotometer\cite{3}.

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**Cell Assay**\cite{2}

Human glioblastoma cells (U87MG, ATCC) are routinely maintained at 37°C and 5% CO\textsubscript{2} in EMEM medium, with NEAA, containing 10% fetal bovine serum, 2 mM of glutamine, 1% penicillin and streptomycin. U87MG cells are used for no more than 15 passages. Cells are seeded in 96-well plates (5000 cells/well). After 24 h cells are treated with Epothilone B. Growth inhibition of U87MG cells is measured after 72 h of drug treatment by using the MTT cell proliferation assay\cite{2}.

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**Animal Administration**\cite{3}

D425Med cells (6×10\textsuperscript{6}) are injected subcutaneously on the backs of 4-6-week-old athymic nude mice. Tumor volumes are determined from caliper measurements of tumor length (L) and width (l) according to the formula (L×l\textsuperscript{2})/2. Tumors are allowed to expand to a volume of 200 mm\textsuperscript{3} (±10%) before treatment start. With the use of a customized shielding device, mice are given strictly loco regional radiotherapy of 3×3 Gy on 3 consecutive days using a Gulmay 200 kV X-ray unit at 100 cGy/min at room temperature. Epothilone B (2 mg/kg; dissolved in 30% PEG-300/70% saline) is applied intravenously 24 h before the first treatment with ionizing radiation at day 0 of the treatment; n=5 per group. Tumor growth is monitored daily.

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REFERENCES

