b-AP15

Cat. No.: HY-13989  
CAS No.: 1009817-63-3  
Molecular Formula: C₂₂H₁₇N₃O₆  
Molecular Weight: 419.39  
Target: Deubiquitinase; Apoptosis  
Pathway: Cell Cycle/DNA Damage; Apoptosis  
Storage: Powder  
-20°C  3 years  
4°C  2 years  

* The compound is unstable in solutions, freshly prepared is recommended.

**SOLVENT & SOLUBILITY**

**In Vitro**  
DMSO : ≥ 44 mg/mL (104.91 mM)  
* "≥" means soluble, but saturation unknown.

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td></td>
<td>2.3844 mL</td>
<td>11.9221 mL</td>
<td>23.8442 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td></td>
<td>0.4769 mL</td>
<td>2.3844 mL</td>
<td>4.7688 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td></td>
<td>0.2384 mL</td>
<td>1.1922 mL</td>
<td>2.3844 mL</td>
</tr>
</tbody>
</table>

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**  
1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: 2.5 mg/mL (5.96 mM); Suspended solution; Need ultrasonic

**BIOLOGICAL ACTIVITY**

**Description**  
b-AP15 is a specific inhibitor of the deubiquitinating enzymes UCHL5 and Usp14.

**IC₅₀ & Target**  
UCHL5/Usp14[1]

**In Vitro**  
Purified 19S proteasomes (5 nM) are treated with indicated concentrations of b-AP15 and DUB activity is determined by detection of Ub-AMC cleavage. The IC₅₀ value (2.1±0.411 μM) is determined from log concentration curves in Graph Pad Prism using non linear regression analysis. b-AP15 as a previously unidentified class of proteasome inhibitor that abrogates the deubiquitinating activity of the 19S regulatory particle. b-AP15 inhibited the activity of two 19S regulatory-particle-associated deubiquitinases, ubiquitin C-terminal hydrolase 5 (UCHL5) and ubiquitin-specific peptidase 14 (USP14), resulting in accumulation of polyubiquitin. b-AP15 induced tumor cell apoptosis that is insensitive to TP53 status and overexpression of the apoptosis inhibitor BCL2[1]. The ability of b-AP15 is determined...
to inhibit proteasome deubiquitinase activity using Ub-AMC as the substrate. An IC$_{50}$ of 16.8±2.8 μM is observed[2].

b-AP15 is a specific USP14 and UCHL5 inhibitor, which blocks growth and induces apoptosis in MM cells[3].

**In Vivo**

b-AP15 (2.5 mg/kg) inhibits tumor growth in syngenic mice models with less frequent administration schedules. We administered b-AP15 to C57BL/6J mice with Lewis lung carcinomas (LLCs) using a 2-d-on, 2-d-off schedule and to BALB/c mice with orthotopic breast carcinoma (4T1) using a 1-d-on, 3-d-off schedule. b-AP15 significantly inhibited tumor growth in both models, with T/C=0.16 (P≤0.01) for the C57BL/6J mice and T/C=0.25 (P≤0.001) for the BALB/c mice. A reduction in the number of pulmonary metastases also is observed in the group of mice with 4T1 breast carcinomas treated with b-AP15[1].

**PROTOCOL**

**Kinase Assay**[1]

For deubiquitinase inhibition assays, 19S regulatory particle (5 nM), 26S (5 nM) UCH-L1 (5 nM), UCH-L3 (0.3 nM), USP2CD (5 nM) USP7CD (5 nM) or BAP1 (5 nM) is incubated with DMSO or b-AP15 and monitored the cleavage of ubiquitin-AMC (1,000 nM) using a Wallac VICTOR Multilabel counter or a Tecan Infinite M1000 equipped with 380 nm excitation and 460 nm emission filters[1].

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

**Cell Assay**[2]

Cell viability is monitored by either the fluorometric microculture cytotoxicity assay or the MTT assay. For the MTT assay, cells are seeded into 96-well flat-bottomed plates overnight and exposed to drugs, using DMSO as the control. At the end of incubations, 10 μl of a stock solution of 5 mg/mL MTT is added into each well, and the plates are incubated 4 hours at 37°C. Formazan crystals are dissolved with 100 μL 10% SDS/10 mM HCl solution overnight at 37°C. Absorbance is measured using an enzyme-linked immunosorbent assay (ELISA) plate reader at 590 nm[2].

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**Animal Administration**[1]

Mice[1]

For the squamous carcinoma model, 1×10$^6$ FaDu cells are subcutaneously injected into the right rear flank of female SCID mice. Tumor growth is measured by the formula length×width$^2$×0.44. When tumors have grown to a size of approximately 200 mm$^3$ (defined as day 0), mice are randomized to receive either vehicle (n=10) or b-AP15 (n=15) at 5 mg per kg of body weight by daily subcutaneous injection. For the colon carcinoma model, we subcutaneously injected 2.5×10$^6$ HCT-116 colon carcinoma cells overexpressing Bcl2 into the right flank of female nude mice. We treated mice with 5 mg of b-AP15 per kg of body weight by intraperitoneal injection. For the lung carcinoma model, we subcutaneously injected 2×10$^5$ LLC cells into the right rear flank of female C57/B6 mice. When tumors had grown to a size of approximately 50 mm$^3$ (defined as day 0), we randomized mice to receive either vehicle (n=4) or b-AP15 (n=4) at 5 mg per kg of body weight intraperitoneally, with a treatment cycle consisting of 2 d of treatment followed by 2 d of rest (2 d on, 2 d off) for 2 weeks.

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

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