E7046

Cat. No.: HY-103088
CAS No.: 1369489-71-3
Molecular Formula: C₂₂H₁₈F₅N₃O₄
Molecular Weight: 483.39
Target: Prostaglandin Receptor
Pathway: GPCR/G Protein
Storage: Powder -20°C 3 years
  4°C  2 years
  In solvent -80°C 6 months
  -20°C 1 month

SOLVENT & SOLUBILITY

In Vitro DMSO : ≥ 100 mg/mL (206.87 mM)
* “≥” means soluble, but saturation unknown.

Preparing Stock Solutions

<table>
<thead>
<tr>
<th>Solvent Concentration</th>
<th>Preparing Stock Solutions</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td></td>
<td>2.0687 mL</td>
<td>10.3436 mL</td>
<td>20.6872 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>0.4137 mL</td>
<td>2.0687 mL</td>
<td>4.1374 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>0.2069 mL</td>
<td>1.0344 mL</td>
<td>2.0687 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 (5.17 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
Solubility: ≥ 2.5 mg/mL (5.17 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.5 mg/mL (5.17 mM); Clear solution

BIOLOGICAL ACTIVITY

Description E7046 is an orally bioavailable and specific EP4 antagonist, with IC₅₀ of 13.5 nM and Kᵢ of 23.14 nM, exhibiting anti-tumor activities.

IC₅₀ & Target

<table>
<thead>
<tr>
<th>IC₅₀</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5 nM (IC₅₀)</td>
<td>EP4</td>
</tr>
<tr>
<td>23.14 nM (Ki)</td>
<td>EP4</td>
</tr>
</tbody>
</table>
**In Vitro**

E7046 reverses the immunosuppressive effects of PGE2 on activation and differentiation of human myeloid cells through selective EP4 antagonism\(^2\).

**In Vivo**

In the CT-26 tumor model, the E7046/RT combination causes the anti-tumor memory response of 9 animals. In the 4T1 model, the combination of E7046 and RT also produces significant better tumor growth inhibition activity compared with each treatment alone. The combination significantly improves survival by inhibiting the subsequent spontaneous lung metastasis of 4T1 tumors\(^1\). E7046 (150 mg/kg) inhibits the growth of multiple syngeneic tumor models. Blockade of EP4 signaling promotes anti-tumor DC differentiation and slows tumor growth in mice. E7046 treatment reduces the growth or even rejected established tumors in vivo in a manner dependent on both myeloid and CD8\(^+\) T cells. Furthermore, co-administration of E7046 and E7777, an IL-2-diphtheria toxin fusion protein that preferentially kills Tregs, synergistically disrupts the myeloid and Treg immunosuppressive networks, resulting in effective and durable anti-tumor immune responses in mouse tumor models\(^2\).

**PROTOCOL**

### Cell Assay \(^2\)

Bone marrow (BM) cells are flushed from femurs of BALB/c mice using sterile CM. Freshly harvested (BM) cells \((0.5\times10^6)\) are differentiated in the presence of 20 ng/mL recombinant mouse GM-CSF, ±PGE2 (10 nM), at 37°C, for 8 d. CM C fresh GM-CSF ± PGE2 is changed on days 3 and 6. After in vitro differentiation, cells are analyzed by flow cytometry. For certain experiments, CT26, 4T1 cell supernatants, and/or EP1 (SC-57089), EP2 (ER-880696), EP3 (L-798106), or EP4 (E7046) antagonists at 1 mM, are added to the BM cells. To assess the effect of differentiated BM cells on T cell proliferation, mouse BM cells differentiated are co-cultured for 72 hours with anti-CD3/CD28 Dynabeads stimulated and CFSE (1 mM)-stained T cells. T cell proliferation is assessed by CFSE dilution using flow cytometry.

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

### Animal Administration \(^2\)

For the tumor isograft efficacy studies, 6-week old female BALB/c mice are implanted with cancer cells: \(1\times10^5\) CT26 or 4T1 cells or \(8\times10^5\) H22 cells per mouse s.c., or \(1\times10^5\) EMT6 cells in the mammary fat pad. C57BL/6 mice are implanted s.c. with \(1\times10^6\) Pan02 cells per mouse, and A/J mice are implanted s.c. with 2-3 mm\(^3\) SA1/N tumor fragments. To investigate the role of T cells in the anti-tumor response, 6 week old female nude mice (which lack T cells) are injected s.c. with \(1\times10^5\) CT26 cells. When tumors reach approximately 50-100 mm\(^3\), tumor-bearing mice are randomly assigned to vehicle or treatment groups, and treatment regimens begin. E7046 is administered per oral (p.o.) as a 100 or 150 mg/kg suspension in 0.5% MC, daily for 21 d (QDx21). For the combination studies, E7777 is administered intravenously (i.v.) at 2.5 mg/mouse in saline, as 2 to 3 doses injected one week apart (Q7Dx2-3). Tumor volumes and body weights are recorded 2-3 times a week. For comparison with current immunotherapies, in addition to vehicle control and E7046 C E7777 groups, mice are assigned to anti-PD1 antibodies or anti-mouse PD-1 C antimouse CTLA4 antibodies treatment groups. Anti-PD-1 and anti-CTLA-4 antibodies (1 mg/mL), are administered i.p. in 100 mL, 3 times 4 d apart (Q4Dx3) for a total of 300 mg each. Isotype controls are administered i.p. at 1 mg/mL to the control group. For the CD4\(^+\)CT and CD8\(^+\)CT lymphocyte depletion, antimouse CD4 or anti-mouse CD8 antibodies or their isotype controls are administered in 100 mL, i.p. at 2.5 mg/mL every 4 days, for a total of 4 injections per mouse (1 mg). To deplete macrophages, clodronate-containing or control liposomes are administered i.p. at 1 mg/mouse, every other day for a total of 7 injections. For the live H22 cell challenge, mice whose initial tumors have completely and stably disappeared are paired with age-matched naive controls and injected s.c. with \(8\times10^5\) H22 cells, on the opposite side from the original implantation site. Tumor volumes and body weights are measured 2 times weekly throughout all experiments.

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

**REFERENCES**


---

www.MedChemExpress.com

---

2