上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：E7046
产品别名：E7046

生物活性：

Description	E7046 is an orally bioavailable and specific EP4 antagonist, with IC_{50} of 13.5 nM and K_i of 23.14 nM, exhibiting anti-tumor activities.	
IC_{50} & Target	EP4	EP4
	13.5 nM (IC_{50})	23.14 nM (Ki)
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 CD8C 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].	

Solvent&Solubility

In Vitro:

DMSO : ≥ 100 mg/mL (206.87 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0687 mL	10.3436 mL	20.6872 mL
	5 mM	0.4137 mL	2.0687 mL	4.1374 mL
	10 mM	0.2069 mL	1.0344 mL	2.0687 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.17 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.17 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (5.17 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.17 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.17 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.17 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. X. Bao, et al. Combination of a Novel EP4 Antagonist E7046 and Radiation Therapy Promotes Anti-tumor Immune Response and Tumor Rejection in Preclinical Tumor Models. International Journal of Radiation Oncology Biology Physics [2]. Diana I. Albu, et al. EP4 Antagonism by E7046 diminishes Myeloid immunosuppression and synergizes with Treg-reducing IL-2-Diphtheria toxin fusion protein in restoring anti-tumor immunity. OncoImmunology.

实验参考：

Cell Assay	Bone marrow (BM) cells are flushed from femurs of BALB/c mice using sterile CM. Freshly harvested (BM) cells (0.5×10^6) 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 Dynabeadsstimulated and CFSE (1 mM)-stained T cells. T cell proliferation is assessed by CFSE dilution using flow cytometry. [2]
Animal Administration	For the tumor isograft efficacy studies, 6-week old female BALB/c mice are implanted with cancer cells: 1×10^5 CT26 or 4T1 cells or 8×10^5 H22 cells per mouse s.c., or 1×10^5 EMT6 cells in the mammary fat pad. C57BL/6 mice are implanted s.c. with 1×10^6 Pan02 cells per mouse, and A/J mice are implanted s.c. with 2-3 mm^3 SAI/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 1×10^5 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 CD4CT and CD8CT 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). [2]
References	[1]. X. Bao, et al. Combination of a Novel EP4 Antagonist E7046 and Radiation Therapy Promotes Anti-tumor Immune Response and Tumor Rejection in Preclinical Tumor Models. International Journal of Radiation Oncology Biology Physics [2]. Diana I. Albu, et al. EP4 Antagonism by E7046 diminishes Myeloid immunosuppression and synergizes with Treg-reducing IL-2-Diphtheria toxin fusion protein in restoring anti-tumor immunity. OncoImmunology.