产品名称：EAI045
产品别名：EAI045

生物活性：

Description

EAI045 is an allosteric and the fourth-generation inhibitor of mutant **EGFR** with **IC_{50}**s of 1.9, 0.019, 0.19 and 0.002 μM for EGFR, $EGFR^{L858R}$, $EGFR^{T790M}$ and $EGFR^{L858R/T790M}$ at 10 μM ATP, respectively.

IC_{50} & Target

EGFR	$EGFR^{L858R}$	$EGFR^{T790M}$	$EGFR^{L858R/T790M}$
1.9 μM (IC_{50})	0.019 μM (IC_{50})	0.19 μM (IC_{50})	0.002 μM (IC_{50})

In Vitro

EAI045 potently inhibits EGFR Y1173 phosphorylation in H1975 cells (EC50=2 nM), but not in HaCaT cells. EAI045 is an inhibitor of the L858R/T790M mutant with 1000-fold selectivity versus wild type EGFR at 1 mM ATP. Profiling of EAI045 against a panel of 250 protein kinases reveals exquisite selectivity; no other kinases are inhibited by more than 20% at 1 μM EAI045[1]. EAI045 has high potency and selectivity for L858R/T790M mutation. In L858R/T790M-mutant NSCLC cell line H1975 cells, EAI045 decreases but does not completely abolish the EGFR autophosphorylation. In stably transfected NIH-3T3 cells harboring the L858R/T790M EGFR mutant, EAI045 shows the same activity. In L858R-mutant H3255 cells, EAI045 exhibits moderate activity. In the HaCaT cells, a keratinocyte cell line with wild-type EGFR, EAI045 does not show any activity of inhibiting EGFR phosphorylation. It confirms the selectivity of EAI045 for mutant EGFR[2].

In Vivo

In a genetically engineered mouse model of L858R/T790Mmutant-driven lung cancer , remarkable tumor regression is observed in L858R/T790M-mutant mice treated with the combination of EAI045 and cetuximab. No response is seen in those mice treated with EAI045 alone. The same effect is seen in both L858R/T790M/C797S- engineered Ba/F3 cells and in mice carrying the L858R/T790M/C797S tumor xenografts. These assays clearly show that EAI045 can overcome resistance from acquired T790M and C797S mutations[2].

Solvent&Solubility

In Vitro:

DMSO : 100 mg/mL (260.82 mM; Need ultrasonic)

H2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.6082 mL	13.0412 mL	26.0824 mL
	5 mM	0.5216 mL	2.6082 mL	5.2165 mL
	10 mM	0.2608 mL	1.3041 mL	2.6082 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 (6.52 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.52 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 (6.52 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (6.52 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 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 (6.52 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.52 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]. Jia Y, et al. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature. 2016 Jun 2;534(7605):129-32. [2]. Wang S, et al. EAI045: The fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. Cancer Lett. 2017 Jan 28;385:51-54.

实验参考：

Cell Assay	For the experiment studying the effect of EGF pre-treatment on EAI045 target modulation, H1975 cells are harvested and plated in 0.5% FBS/RPMI Pen/Strep. On the following day, cells are pre-treated with 0.5% FBS/RPMI media with or without 10 ng EGF/mL for 5 minutes. Compound is added and assay is carried out[1].
Animal Administration	Mice: Cetuximab is administrated at 1 mg/mouse every other day by intraperitoneal injection. The TL, TD and TLCS mice are monitored by MRI to quantify lung tumor burden before being assigned to various study treatment cohorts, which are non-blinded and not formally randomized. All treated mice had an equal initial tumor burden. MRI evaluation is repeated every 2 weeks during treatment. The animals are imaged with a rapid acquisition with relaxation enhancement sequence in the coronal and axial planes with a 1-mm slice thickness gating with respiratory rates. The tumor burden volumes are quantified[1].
References	[1]. Jia Y, et al. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature. 2016 Jun 2;534(7605):129-32. [2]. Wang S, et al. EAI045: The fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. Cancer Lett. 2017 Jan 28;385:51-54.