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：HLM006474
产品别名：HLM006474

生物活性：

Description	HLM006474 is a pan E2F inhibitor, which inhibits E2F4 DNA-binding with an IC50 of 29.8 μM in A375 cells.
IC_{50} & Target	IC50: 29.8 μM (E2F4 DNA-binding)[1]
In Vitro	HLM006474 shows little activities against E2F4 DNA-binding in A375 cells at 10 and 20 μM, apparently inhibits E2F4 DNA-binding at 40 μM, and increasingly suppressses the effect at 60 and 80 μM concentrations. HLM006474 (40 μM) inhibits E2F4 activity via suppression of its DNA-binding activity and down regulation of its expression. HLM006474 (40 μM) also significantly induces apoptosis in A375 and 231 cell lines for 24 hours. HLM006474 dramaticly reduces cyclin D3 protein expression, and is a potent inducer of PARP cleavage[1]. HLM006474 reduces the viability of both SCLC and NSCLC lines with IC50 ranging from 15 to 75 μM. HLM006474 (60 μM) increases the expression of several known E2F-regulated genes after short treatments in H292 and H1299 cell lines. HLM006474 (20 μM) weakly synergizes with paclitaxel, but there is antagonism between HLM006474 and cisplatin and gemcitabine in H1299 cells[2]. HLM006474 leads to a decrease in the mRNA levels of MAD2. Furthmore, HLM006474 apparently suppresses the increase of Mad2 protein and pRb-S780 signal but not the level of Skp2 protein in human lung cancer A549 cellss[3].
Solvent&Solubility	***In Vitro:*** **DMSO : 25 mg/mL (62.58 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5033 mL	12.5163 mL	25.0325 mL
	5 mM	0.5007 mL	2.5033 mL	5.0065 mL
	10 mM	0.2503 mL	1.2516 mL	2.5033 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 (6.26 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.26 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 (6.26 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (6.26 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 (6.26 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.26 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]. Ma Y, et al. A small-molecule E2F inhibitor blocks growth in a melanoma culture model. Cancer Res. 2008 Aug 1;68(15):6292-9. [2]. Kurtyka CA, et al. E2F inhibition synergizes with paclitaxel in lung cancer cell lines. PLoS One. 2014 May 15;9(5):e96357.

实验参考：

Cell Assay	The antiproliferative activity of compounds and their combinations is evaluated using a high-throughput CellTiter-Blue cell viability assay. For the assays, 1000 cells in 24 μL are plated in 384-well plates and incubated overnight at 37°C, 5% CO_2. The next day, the drugs are diluted in media and 6 μL of these dilutions added to appropriate wells using an automated pipetting station. Four replicate wells are used for each drug concentration. The cells are incubated with the drug for 120 hours, at which time, 5 μL CellTiter-Blue reagent is added. Cell viability is assessed by the ability of the remaining treated cells to bioreduce resazurin to resorufin (579 nm Ex/584 nm Em). Fluorescence is read with a Synergy HT microplate reader. IC_{50}s are determined using a sigmoidal equilibrium model regression using XLfit version 4.3.2 and is defined as the concentration of drug required for a 50% reduction in growth/viability. For 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell viability assays, CellTiter 96 AQueous One Solution is added according to vendor instructions to cells for 2 hours following treatment with drug at the noted dose for 72 hours. All experiments are performed in triplicate and repeated at least three times[2].
References	[1]. Ma Y, et al. A small-molecule E2F inhibitor blocks growth in a melanoma culture model. Cancer Res. 2008 Aug 1;68(15):6292-9. [2]. Kurtyka CA, et al. E2F inhibition synergizes with paclitaxel in lung cancer cell lines. PLoS One. 2014 May 15;9(5):e96357.