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产品名称：2,3,4,5-四(4-吡啶基)噻吩
产品别名：GANT 58；NSC 75503

生物活性：

Description	GANT 58 is a potent Gli antagonist that inhibits GLI1-induced transcription with IC50 of 5 μM.
IC_{50} & Target	IC50: 5 μM (Gli)[1]
In Vitro	GANT58 is a downstream inhibitor of Hh signaling. GANT58 is an indeed inhibitor of Hh signaling downstream of Smo and Sufu. GANT58 mainly acts at the nuclear level because transcription induced by GLI1 with a mutated nuclear export signal is still blocked. GANT58 efficiently inhibits in vitro tumor cell proliferation in a GLI-dependent manner and successfully blocks cell growth using human prostate cancer cells harboring downstream activation of the Hh pathway[1]. GANT58 (NSC75503) has been shown to inhibit transcriptional activation by GLI1 (as well as by the other GLI species). GANT58 has been shown to inhibit GLI transactivation[2].
In Vivo	Nude mice are injected s.c. with GLI1-positive 22Rv1 prostate cancer cells, and tumors are established (median size ≈250 mm^3). Nude mice are treated with daily s.c. injections at a concentration of 50 mg/kg of cyclopamine, GANT61, GANT58, or solvent only (n=4-5 for each group). However, after 3 days, cyclopamine-treated animals presented with severe ulcerations at the injection sites. Therefore, changing the treatment regimen to injections only every second day. To be able to compare all compounds, this protocol is also introduced for the GANTs, although mice treated with these compounds showed no such signs of toxicity. All injections are done 2-3 cm away from the tumors. During an 18-day treatment period, suppression of tumor cell growth is observed for all compounds. Treatment with cyclopamine or GANT58 results in the inhibition of additional xenograft growth and limited the increase in tumor size[1].
Solvent&Solubility	***In Vitro:*** **DMSO : 9.09 mg/mL (23.16 mM;** Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.5479 mL	12.7395 mL	25.4790 mL
5 mM	0.5096 mL	2.5479 mL	5.0958 mL
10 mM	0.2548 mL	1.2740 mL	2.5479 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: ≥ 0.91 mg/mL (2.32 mM); Clear solution 此方案可获得 ≥ 0.91 mg/mL (2.32 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 9.1 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: ≥ 0.91 mg/mL (2.32 mM); Clear solution 此方案可获得 ≥ 0.91 mg/mL (2.32 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 9.1 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: ≥ 0.91 mg/mL (2.32 mM); Clear solution 此方案可获得 ≥ 0.91 mg/mL (2.32 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 9.1 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Lauth M, et al. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc Natl Acad Sci U S A. 2007 May 15;104(20):8455-60. [2]. Joo J, et al. GLI1 is a central mediator of EWS/FLI1 signaling in Ewing tumors. PLoS One. 2009 Oct 27;4(10):e7608.

实验参考：

Cell Assay	HEK293 cells are transfected with GLI1 expression plasmid, together with the reporter plasmids 12× GliBS-Luc and R-Luc on 10 cm plates (day 0). Twenty-four hours later, cells are seeded in white 96 well plates with clear bottom at a density of 15,000 cells per well. Cells are allowed to attach, and compounds are added at a final concentration of 10 μM in DMSO (0.5% final DMSO concentration) (day 1.5). Cells are grown for another 24 h, subsequently lysed, and then analyzed by using the Dual Luciferase kit. Plates are read on a Berthold Technologies microplate luminometer. Subconfluent cells are grown in reduced FBS (2.5%) for 48 h in the presence of 5 μM test compound (or DMSO) on white 96 well plates with clear bottom. Subsequently, cells are labeled for 2 h with BrdU, fixed, and analyzed. Samples are read on a Molecular Devices SpectraMax Gemini EM[1].
Animal Administration	Mice[1] 5×10^6 22Rv1 cells are suspended in a total volume of 100 μL of a 1:1 mixture of RPMI medium 1640:Matrigel (E1270). The cell suspension is injected s.c. at the posterior flank of female BALB/c nude mice (nu/nu). Tumors are grown until they reached a median size of ≈250 mm^3 (5-6 days). Animals are randomly divided into four groups (n=4-5) and treated with solvent only (corn oil:ethanol, 4:1) or compounds in solvent (50 mg/kg) for 16 days s.c. injections of compounds are performed several centimeters away from the tumor. Tumor volumes are calculated by the formula length×width×0.5×(length+width). At the end of the treatment period, animals are given a BrdU pulse (50 mg/kg) for 30 min, and tumors are removed. All animal experiments are approved by local ethics authorities.

References	[1]. Lauth M, et al. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc Natl Acad Sci U S A. 2007 May 15;104(20):8455-60. [2]. Joo J, et al. GLI1 is a central mediator of EWS/FLI1 signaling in Ewing tumors. PLoS One. 2009 Oct 27;4(10):e7608.