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产品名称：
N-(3-Chloro-4-methylphenyl)-2-[2,6-dinitro-4-(trifluoromethyl)phenyl]-hydrazinecarbothioamide
产品别名：Kobe0065

生物活性：

Description	Kobe0065 is a novel and effective inhibitor of Ras-Raf interaction, competitively inhibiting the binding of H-Ras·GTP to c-Raf-1 RBD with a Ki value of 46±13 μM.
IC_{50} & Target	H-Ras
In Vitro	Kobe0065-family compounds bind to Ras•GTP and exhibit antiproliferative activity toward cancer cells carrying the activated ras oncogenes. The compounds efficiently inhibit the interaction of Ras•GTP with their multiple effectors including Raf, PI3K, and RalGDS and a regulator/effector Sos and show rather broad binding specificity toward various Ras family small GTPases, which may account for their higher potency at the cellular level compared with that of the in vitro binding inhibition[1]. The phosphorylation of downstream kinases MEK and ERK is effectively attenuated by 20 μM Kobe0065 and Kobe2602 in NIH3T3 cells transiently expressing H-RasG12V[2].
In Vivo	Kobe0065 and Kobe2602 exhibit antitumor activity on a xenograft of human colon carcinoma SW480 cells carrying the K-ras(G12V) gene by oral administration[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 42.5 mg/mL (94.49 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.2233 mL	11.1163 mL	22.2326 mL
	5 mM	0.4447 mL	2.2233 mL	4.4465 mL
	10 mM	0.2223 mL	1.1116 mL	2.2233 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 (5.56 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.56 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。

References	[1]. Shima, Fumi, et al. In silico discovery of small-molecule Ras inhibitors that display antitumor activity by blocking the Ras-effector interaction. Proceedings of the National Academy of Sciences of the United States of America (2013), 110(20), 8182-8187, [2]. Shima F, et al. Discovery of small-molecule Ras inhibitors that display antitumor activity by interfering with Ras·GTP-effector interaction. Enzymes. 2013;34 Pt. B:1-23

实验参考：

Cell Assay	Cells (2×10^3) are seeded in a 96-well plate and cultured in DMEM containing 2% (vol/vol) FBS in the presence of one of the compounds. Viable cell numbers are measured by formazan formation using a Cell Counting Kit 8. Apoptotic cells are detected by a standard TUNEL assay using an In Situ Cell Detection kit. [1]
Animal Administration	Cells (5×10^6) are implanted into the right flanks of female athymic nude mice (6-8 wk old). After tumor sizes reached appr 50 mm^3 on average, compounds suspended in Cremophor:ethanol:water (1:1:6) are administered orally for five consecutive days per week for 17 d. Tumor volumes (V) are calculated. Dissected tumors after 17-d administration of the 80 mg/kg compounds are fixed in 4% (wt/vol) paraformaldehyde and embedded in paraffin. Their sections are subjected to immunohistochemistry with an anti-ERK1/2 antibody or an anti-CD31 antibody using a HISTMOUSE-PLUS kit. Apoptotic cells are detected by a TUNEL assay. Statistical significance for groups of three or more is determined by one-way ANOVA with Tukey's test for post hoc analysis. [1]
References	[1]. Shima, Fumi, et al. In silico discovery of small-molecule Ras inhibitors that display antitumor activity by blocking the Ras-effector interaction. Proceedings of the National Academy of Sciences of the United States of America (2013), 110(20), 8182-8187, [2]. Shima F, et al. Discovery of small-molecule Ras inhibitors that display antitumor activity by interfering with Ras·GTP-effector interaction. Enzymes. 2013;34 Pt. B:1-23