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产品名称：
N-[4-[(4-Ethyl-1-piperazinyl)methyl]-3-(trifluoromethyl)phenyl]-N'-[4-[[6-(methylamino)-4-pyrimidinyl]oxy]phenyl]urea
产品别名：AST 487；NVP-AST 487

生物活性：

Description	AST 487 is a RET kinase inhibitor with IC_{50} of 880 nM, inhibits RET autophosphorylation and activation of downstream effectors, also inhibits Flt-3 with IC_{50} of 520 nM.
IC_{50} & Target	IC50: 880 nM (RET), 170 nM (KDR), 790 nM (Flt-4), 500 nM (c-Kit), 520 nM (Flt-3), 20 nM (Abl)[1]
In Vitro	A number of other kinases are also similarly inhibited by AST 487 (NVP-AST487) in the in vitro kinase assays, including KDR (IC_{50}=170 nM), Flt-4 (IC_{50}=790 nM), Flt-3 (IC_{50}=520 nM), c-Kit (IC_{50}=500 nM), and c-Abl (IC_{50}=20 nM). AST 487 potently inhibits the growth of human thyroid cancer cell lines with activating mutations of *RET* but not of lines without *RET* mutations. Both GDNF/GFRα1 and persephin-induced calcitonin mRNA are markedly inhibited by coincubation with 100 nM of AST 487 in MTC-M cells[1]. AST 487 is a novel, mutant FLT3 inhibitor. AST 487 is tested in biochemical assays for inhibition of Flt-3 kinase activity. The K_i is determined to be 0.12 μM. Besides Flt-3, NVP-AST487 inhibits RET, KDR, c-Kit, and c-Abl kinase with IC_{50} values below 1 μM. Treatment of FLT3-ITD-Ba/F3 cells and D835Y-Ba/F3 cells with AST 487 potently inhibits cellular proliferation (IC_{50}<5 nM). AST 487 treatment of FLT3-ITD-Ba/F3 cells with 0.01 μM AST 487 results in complete cell killing compare with approximately 50% killing of AML patient samples at the same concentration[2].
In Vivo	After a single oral administration of 15 mg/kg of AST 487 to OF1 mice, a mean peak plasma level (C_{max}) of 0.505±0.078 μM SE is achieved after 0.5 h. Similar levels of AST 487 are found in the plasma of mice up to 6 h after oral administration, with a C_{last} of 21±4 nM at 24 h. The oral bioavailability is calculated to be 9.7% with a $t_{1/2}$terminal elimination of 1.5 h[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 100 mg/mL (188.84 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.8884 mL	9.4418 mL	18.8836 mL
5 mM	0.3777 mL	1.8884 mL	3.7767 mL
10 mM	0.1888 mL	0.9442 mL	1.8884 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 (4.72 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.72 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 (4.72 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.72 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 (4.72 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.72 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]. Akeno-Stuart N, et al. The RET Kinase Inhibitor NVP-AST487 Blocks Growth and Calcitonin Gene Expression through Distinct Mechanisms in Medullary Thyroid Cancer Cells. Cancer Res. 2007 Jul 15;67(14):6956-64. [2]. Weisberg E, et al. Antileukemic effects of the novel, mutant FLT3 inhibitor NVP-AST487: effects on PKC412-sensitive and -resistant FLT3-expressing cells. Blood. 2008 Dec 15;112(13):5161-70.

实验参考：

Cell Assay	The trypan blue exclusion assay is used to determine proliferation of cells cultured in the presence and absence of NVP-AST 487. Cell viability is reported as percentage of control (untreated) cells, and data are presented as the average of 2 independent experiments, except where indicated. Error bars represent the standard error of the mean for each data point. Apoptosis of drug-treated cells is measured using the Annexin-V-Fluos Staining Kit. Cell-cycle analysis is performed[2].
Animal Administration	Mice[1] Female athymic nude mice are kept under optimized hygienic conditions (maximum of 10 mice per Makrolon type III cage) with free access to food and water. Tumors are established by s.c. injection of 1×10^6 and 5×10^6 of NIH3T3-RETC634W and TT cells, respectively, in 100 μL of HBSS per mouse. Treatable tumors, i.e., mean tumor volume of 100 mm^3, developed within 10 days of NIH3T3-RETC634W cell injection, and within 20 days of TT cell injection. NVP-AST487 is given p.o., once daily by gavage. The compound is formulated by dissolving the appropriate amount of powder in N-methylpyrrolidone/PEG300 (1:10 v/v). The mice are randomized into four treatment groups of eight mice each. The first three groups received daily oral administrations of NVP-AST487 at 50, 30, and 10 mg/kg, respectively, for 3 weeks. The fourth group received treatment with vehicle. Tumor growth and body weights are monitored twice weekly. Tumor volumes are determined

	according to the formula: length×diameter^2×π/6. Tumors are collected and frozen in liquid nitrogen at the end of the efficacy study, 6 h after the last administration.
Kinase Assay	Glutathione S-transferase (GST)-fused kinase domains are expressed in baculovirus and purified over glutathione-sepharose. Kinase activity is tested by measuring the phosphorylation of a synthetic substrate [poly(Glu, Tyr)], by purified GST-fusion kinase domains of the respective protein kinase in the presence of radiolabeled ATP; the ATP concentrations used are optimized within the K_m range for the individual kinases. Briefly, each kinase is incubated under optimized buffer conditions in 20 mM of Tris-HCl (pH 7.5), 1 to 3 mM of $MnCl_2$, 3 to 10 mM of $MgCl_2$, 10 μM of Na_3VO_4, 1 mM of DTT, 0.2 μCi [^{33}P]ATP, 1 to 8 μM of ATP, 3 to 8 μg/mL of poly(Glu/Tyr, 4:1), and 1% DMSO in a total volume of 30 μL in the presence or absence of NVP-AST487 for 10 min at ambient temperature. Reactions are terminated by adding 10 μL of 250 mM EDTA, and the reaction mixture is transferred onto an Immobilon polyvinylidene difluoride membrane. Filters are washed (0.5% H_3PO_4), soaked in ethanol, dried and counted in a liquid scintillation counter. IC_{50}s for AST 487 are calculated by linear regression analysis of the percentage inhibition[1].
References	[1]. Akeno-Stuart N, et al. The RET Kinase Inhibitor NVP-AST487 Blocks Growth and Calcitonin Gene Expression through Distinct Mechanisms in Medullary Thyroid Cancer Cells. Cancer Res. 2007 Jul 15;67(14):6956-64. [2]. Weisberg E, et al. Antileukemic effects of the novel, mutant FLT3 inhibitor NVP-AST487: effects on PKC412-sensitive and -resistant FLT3-expressing cells. Blood. 2008 Dec 15;112(13):5161-70.