上海源叶生物科技有限公司
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产品名称：SAN50900；FLT3-IN-3

CAS 号： 2229050-90-0

产品货号：S89217

生物活性：

Description	FLT3-IN-3 是一种有效的 FLT3 抑制剂，抑制 FLT3 WT 和 FLT3 D835Y，IC50 分别为 13 和 8 nM。
IC50 & Target	IC50: 13 nM (FLT3 WT), 8 nM (FLT3 D835Y)[1]

Cellular Effect

Cell Line	Type	Value	Description
BJ	GI_{50}	12.343 μM	Cytotoxicity against human BJ cells assessed as growth inhibition after 72 hrs by resazurin dye-based fluorescence analysis
BJ	GI_{50}	12.663 μM	Growth inhibition of human BJ cells after 72 hrs by calcein AM dye-based fluorescence assay
BaF3	GI_{50}	1.136 μM	Growth inhibition of mouse BA/F3 cells after 72 hrs by calcein AM dye-based fluorescence assay
CCRF-CEM	GI_{50}	0.82 μM	Cytotoxicity against human CEM cells assessed as growth inhibition after 72 hrs by resazurin dye-based fluorescence analysis
EOL1	GI_{50}	0.007 μM	Cytotoxicity against FIP1L1-PDGFRA positive human EOL-1 cells assessed as growth inhibition after 72 hrs by resazurin dye-based fluorescence analysis
HCC827	GI_{50}	0.327 μM	Growth inhibition of human HCC827 cells after 72 hrs by calcein AM dye-based fluorescence assay
HCC827	GI_{50}	1.011 μM	Cytotoxicity against human HCC827 cells assessed as growth inhibition after 72 hrs by resazurin dye-based fluorescence analysis
HUVEC	GI_{50}	5.866 μM	Growth inhibition of HUVEC after 72 hrs by calcein AM dye-based fluorescence assay
K562	GI_{50}	0.38 μM	Growth inhibition of human K562 cells after 72 hrs by calcein AM dye-based fluorescence assay
K562	GI_{50}	1.121 μM	Cytotoxicity against human K562 cells assessed as growth inhibition after 72 hrs by resazurin dye-based fluorescence analysis
Kasumi 1	GI_{50}	0.513 μM	Growth inhibition of human Kasumi-1 cells after 72 hrs by calcein AM dye-based fluorescence assay
MCF7	GI_{50}	0.197 μM	Growth inhibition of human MCF7 cells after 72 hrs by calcein AM dye-based fluorescence assay
MOLM-13	GI_{50}	0.001 μM	Growth inhibition of human MOLM13 cells after 72 hrs by calcein AM dye-based fluorescence assay
MRC5	GI_{50}	20.565 μM	Growth inhibition of human MRC5 cells after 72 hrs by calcein AM dye-based fluorescence assay
MV4-11	GI_{50}	0.002 μM	Growth inhibition of human MV4-11 cells after 72 hrs by calcein AM dye-based fluorescence assay

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	Sf9	IC_{50}	0.027 μM	Inhibition of recombinant human 3C cleavage site/N-terminal GST-His6 fused PDGFR alpha C-terminal fragment (Q551 to L1089 residues) harboring V561D mutant expressed in baculovirus infected Sf9 insect cells using AGLT peptide as substrate in presence of [gamma-33P]-ATP
	THP-1	GI_{50}	0.713 μM	Growth inhibition of human THP1 cells after 72 hrs by calcein AM dye-based fluorescence assay
	U-937	GI_{50}	0.664 μM	Growth inhibition of human U937 cells after 72 hrs by calcein AM dye-based fluorescence assay

In Vitro

FLT3-IN-3 (Compound 7d) inhibits the proliferation of FLT3-ITD positive MV4-11 and MOLM-13 cell lines very effectively at low nanomolar concentrations (GI50 values 2 and 1 nM, respectively)[1].

FLT3-IN-3 (1 nM, 10nM, 100 nM, 1 μM and 10 μM; 72 hours) inhibits the Ba/F3 FLT3-ITD cells with the GI50 of 0.034±0.015 μM, and inhibits the parental Ba/F3 cells with the GI50 value of 1.136±0.389 μM[1].

Concentrations as low as 1 nM are sufficient to block the autophosphorylation of the FLT3 receptor tyrosine kinase at three different tyrosine residues (589, 591, and 842). Moreover, this inhibition suppresses phosphorylation of several downstream targets of FLT3. Notably, FLT3-IN-3 (0.01, 0.1, 1, 10 and 100 nM; 1 hours) abolishes phosphorylation of STAT5 at Y694, which is a direct substrate of the oncogenic FLT3-ITD variant. The second pathway affected is the MAPK cascade: Two key components of this signaling pathway, ERK1/2 (T202/Y204) and MEK1/2 (S217/221), exhibit reduced phosphorylation upon treatment with FLT3-IN-3. FLT3-IN-3 also interfers with PI3K/AKT pathway which is confirmed by reduced phosphorylation of AKT at S473[1].

Cell Proliferation Assay[1]

Cell Line:	Murine Ba/F3 FLT3-ITD and parental Ba/F3 cells
Concentration:	1 nM, 10nM, 100 nM, 1 μM and 10 μM
Incubation Time:	72 hours
Result:	The GI50s for Ba/F3 FLT3-ITD cells and parental Ba/F3 cells are 0.034±0.015 μM and 1.136±0.389 μM, respectively.

Western Blot Analysis[1]

Cell Line:	MV4-11 cells
Concentration:	0.01, 0.1, 1, 10 and 100 nM
Incubation Time:	1 hours
Result:	Concentrations as low as 1 nM were sufficient to block the autophosphorylation of the FLT3 receptor tyrosine kinase at three different tyrosine residues (589, 591, and 842).

In Vivo

A single dose of FLT3-IN-3 (Compound 7d; 10 mg/kg; i.p.) in mice with subcutaneous MV4-11 xenografts causes sustained inhibition of FLT3 and STAT5 phosphorylation over 48 hours[1].

Animal Model:	Female athymic nu/nu mice with subcutaneously implanted MV4-11 xenografts[1]
Dosage:	10 mg/kg
Administration:	Intraperitoneal (i.p.) injection; 48 hours

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<table>
<tr><td></td><td>Result:</td><td>Effectively inhibited FLT3-ITD autophosphorylation in MV4-11 xenografts.</td></tr>
<tr><td>Solvent&Solubility</td><td colspan="2">
细胞实验:
DMSO 中的溶解度 ：250 mg/mL (509.54 mM; 超声助溶; 吸湿的 DMSO 对产品的溶解度有显著影响，请使用新开封的 DMSO）
<table>
<tr><td rowspan="4">Preparing Stock Solutions</td><td>Solvent / Mass / Concentration</td><td>1 mg</td><td>5 mg</td><td>10 mg</td></tr>
<tr><td>1 mM</td><td>2.0382 mL</td><td>10.1908 mL</td><td>20.3815 mL</td></tr>
<tr><td>5 mM</td><td>0.4076 mL</td><td>2.0382 mL</td><td>4.0763 mL</td></tr>
<tr><td>10 mM</td><td>0.2038 mL</td><td>1.0191 mL</td><td>2.0382 mL</td></tr>
</table>
*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。
动物实验：
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
方案 一
请依序添加每种溶剂： 10% DMSO → 40% PEG300 → 5% Tween-80 → 45% Saline
Solubility: ≥ 2.08 mg/mL (4.24 mM); 澄清溶液
此方案可获得 ≥ 2.08 mg/mL（饱和度未知）的澄清溶液。
以 1 mL 工作液为例，取 100 μL 20.8 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；再向上述体系中加入 50 μL Tween-80，混合均匀；然后再继续加入 450 μL 生理盐水 定容至 1 mL。
生理盐水的配制：将 0.9 g 氯化钠，溶解于 ddH_2O 并定容至 100 mL，可以得到澄清透明的生理盐水溶液。
方案 二
请依序添加每种溶剂： 10% DMSO → 90% (20% SBE-β-CD in Saline)
Solubility: ≥ 2.08 mg/mL (4.24 mM); 澄清溶液
此方案可获得 ≥ 2.08 mg/mL（饱和度未知）的澄清溶液。
以 1 mL 工作液为例，取 100 μL 20.8 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。
2 g SBE-β-CD（磺丁基醚 β-环糊精）粉末定容于 10 mL 的生理盐水中，完全溶解至澄清透明。
方案 三
请依序添加每种溶剂： 10% DMSO → 90% Corn Oil
Solubility: ≥ 2.08 mg/mL (4.24 mM); 澄清溶液
此方案可获得 ≥ 2.08 mg/mL（饱和度未知）的澄清溶液，此方案实验周期在半个月以上的动物实验酌情使用。
以 1 mL 工作液为例，取 100 μL 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
</td></tr>
</table>

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参考文献	[1]. Gucký T, et al. Discovery of N2-(4-Amino-cyclohexyl)-9-cyclopentyl-N6-(4-morpholin-4-ylmethyl-phenyl)- 9H-purine-2,6-diamine as a Potent FLT3 Kinase Inhibitor for Acute Myeloid Leukemia with FLT3 Mutations. J Med Chem. 2018 May 10;61(9):3855-3869.

完整储备液配制表：

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。

可选溶剂	质量 / 溶剂体积 / 浓度	1 mg	5 mg	10 mg	25 mg
DMSO	1 mM	2.0382 mL	10.1908 mL	20.3815 mL	50.9539 mL
	5 mM	0.4076 mL	2.0382 mL	4.0763 mL	10.1908 mL
	10 mM	0.2038 mL	1.0191 mL	2.0382 mL	5.0954 mL
	15 mM	0.1359 mL	0.6794 mL	1.3588 mL	3.3969 mL
	20 mM	0.1019 mL	0.5095 mL	1.0191 mL	2.5477 mL
	25 mM	0.0815 mL	0.4076 mL	0.8153 mL	2.0382 mL
	30 mM	0.0679 mL	0.3397 mL	0.6794 mL	1.6985 mL
	40 mM	0.0510 mL	0.2548 mL	0.5095 mL	1.2738 mL
	50 mM	0.0408 mL	0.2038 mL	0.4076 mL	1.0191 mL
	60 mM	0.0340 mL	0.1698 mL	0.3397 mL	0.8492 mL
	80 mM	0.0255 mL	0.1274 mL	0.2548 mL	0.6369 mL
	100 mM	0.0204 mL	0.1019 mL	0.2038 mL	0.5095 mL