产品名称：**LRRK2-IN-1**
产品别名：**LRRK2-IN-1**

生物活性：

Description	LRRK2-IN-1 is a potent and selective LRRK2 inhibitor with IC50 of 6 nM and 13 nM for LRRK2 (G2019S) and LRRK2 (WT), respectively.
IC_{50} & Target	IC50: 13 nM (WT), 6 nM (G2019S)
In Vitro	Wild-type and G2019S transduction results in 2.5 fold higher TR-FRET signal which can be inhibited by LRRK2-IN-1 in a dose-dependent manner with IC50 values of 0.08 μM and 0.03 μM, respectively[1]. LRRK2-IN-1 possessed an IC50 of 45 nM for inhibition of DCLK2 and exhibits an IC50 of greater than 1 μM when evaluated in biochemical assays for AURKB, CHEK2, MKNK2, MYLK, NUAK1, and PLK1. LRRK2-IN-1 is confirmed to inhibit MAPK7 with an EC50 of 160 nM. LRRK2-IN-1 induces a dose dependent inhibition of Ser910 and Ser935 phosphorylation accompanied by loss of 14-3-3 binding to both wild type LRRK2 and LRRK2[G2019S] stably transfected into HEK293 cells[2]. LRRK2-IN-1 is moderately cytotoxic with IC50 of 49.3 μM in HepG2 cells. LRRK2-IN-1 exhibits genotoxicity in the presence and absence of S9 at 15.6 and 3.9 μM, respectively[3]. LRRK2-IN-1 inhibits proliferation, migration, and induces cell death with hallmarks of apoptosis of HCT116 and AsPC-1 cells[4].
In Vivo	LRRK2-IN-1 (100 mg/kg, i.p.) induces dephosphorylation of LRRK2 in the kidney of the mice[2]. Peritumoral injection of LRRK2-IN-1 (100 mg/kg) results in a significant decrease in tumor volume and weight of AsPC-1 tumor xenografts[4].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 50 mg/mL (87.61 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7523 mL	8.7613 mL	17.5226 mL
	5 mM	0.3505 mL	1.7523 mL	3.5045 mL
	10 mM	0.1752 mL	0.8761 mL	1.7523 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 (4.38 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.38 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 (4.38 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.38 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 (4.38 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.38 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]. Hermanson SB, et al. Screening for Novel LRRK2 Inhibitors Using a High-Throughput TR-FRET Cellular Assay for LRRK2 Ser935 Phosphorylation.PLoS One. 2012;7(8):e43580. Epub 2012 Aug 28. [2]. Deng, Xianming., et al. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nature Chemical Biology (2011), 7(4), 203-205. [3]. Koshibu K, et al. Alternative to LRRK2-IN-1 for Pharmacological Studies of Parkinson's Disease. Pharmacology. 2015;96(5-6):240-7. [4]. Weygant N, et al. Small molecule kinase inhibitor LRRK2-IN-1 demonstrates potent activity against colorectal and pancreatic cancer through inhibition of doublecortin-like kinase 1. Mol Cancer. 2014 May 6;13:103.

实验参考：

Cell Assay	Cells (10^4 cells per well) are seeded into a 96-well tissue culture plate in triplicate. The cells are cultured in the presence of LRRK2-IN-1 with DMSO as a vehicle at 0, 0.31, 0.63, 1, 2, and 5, 10, and 20 μM. 48 h post treatment, 10 μL of TACS MTT Reagent is added to each well and the cells are incubated at 37°C until dark crystalline precipitate become visible in the cells. 100 μL of 266 mM NH_4OH in DMSO is then added to the wells and placed on a plate shaker at low speed for 1 minute. After shaking, the plate is allowed to incubate for 10 minutes protected from light and the OD550 for each well is read using a microplate reader. The results are averaged and calculated as a percentage of the DMSO (vehicle) control +/- the standard error of the mean. [4]
Animal Administration	LRRK2-IN-1 is dissolved in Captisol and administered by intraperitoneal injection into wild type male C57BL/6 mice at a dose of 100 mg/kg. Control mice are injected with an equal volume of Captisol. At 1 and 2 h time points, mice are extinguwashed by cervical dislocation and kidney and brain tissue rapidly dissected and snap-frozen in liquid nitrogen. [2]
Kinase Assay	Active GST-LRRK2 (1326-2527), GST-LRRK2 [G2019S] (1326-2527), GST-LRRK2 [A2016T] (1326-2527) and GST-LRRK2 [A2016T+G2019S] (1326-2527) enzyme is purified with glutathione sepharose from HEK293 cell lysate 36 h following transient transfection of the appropriate cDNA constructs. Peptide kinase assays, performed in duplicate, are set up in a total volume of 40 μL containing 0.5 μg LRRK2 kinase (which at approximately 10% purity gives a final concentration of 8 nM) in 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 10 mM $MgCl_2$, 20 μM Nictide, 0.1 μM [γ-^{32}P]ATP (500 cpm/pmol) and the indicated concentrations of inhibitor dissolved in DMSO. After incubation for 15 min at 30°C, reactions are terminated by spotting 35 μL of the reaction mix onto P81 phosphocellulose paper and immersion in 50 mM phosphoric acid. Samples are washed extensively

	and the incorporation of [γ-^{32}P]ATP into Nictide is quantified by Cerenkov counting. IC_{50} values are calculated with GraphPad Prism using non-linear regression analysis. [2]
References	[1]. Hermanson SB, et al. Screening for Novel LRRK2 Inhibitors Using a High-Throughput TR-FRET Cellular Assay for LRRK2 Ser935 Phosphorylation.PLoS One. 2012;7(8):e43580. Epub 2012 Aug 28. [2]. Deng, Xianming., et al. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nature Chemical Biology (2011), 7(4), 203-205. [3]. Koshibu K, et al. Alternative to LRRK2-IN-1 for Pharmacological Studies of Parkinson's Disease. Pharmacology. 2015;96(5-6):240-7. [4]. Weygant N, et al. Small molecule kinase inhibitor LRRK2-IN-1 demonstrates potent activity against colorectal and pancreatic cancer through inhibition of doublecortin-like kinase 1. Mol Cancer. 2014 May 6;13:103.