

Product Data Sheet

Product Name: LRRK2-IN-1

Cat. No.: GC10809

Chemical Properties

Cas. No. 1234480-84-2

Chemical Name 2-[2-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidine-1-carbonyl]anilino]-5,11-dimethylpyrimido[4,5-b][1,4]benzodiazepin-6-one

SMILES CN1CCN(CC1)C2CCN(CC2)C(=O)C3=CC(=C(C=C3)NC4=NC=C5C(=N4)N(C6=CC=C(C=C6C(=O)N5C)C)OC

Formula $C_{31}H_{38}N_8O_3$ M.Wt 570.7

Solubility $\geq 28.55\text{mg/mL}$ in DMSO, $\geq 57.2\text{mg/mL}$ in EtOH Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

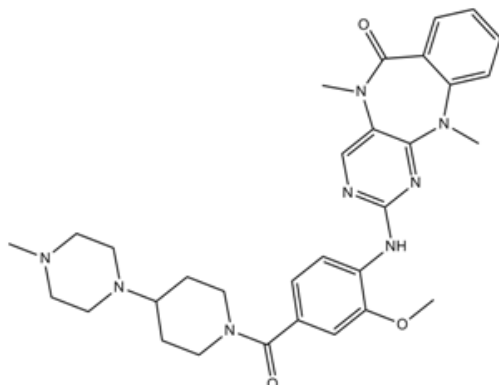
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Kinase experiment:

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 μ L containing 0.5 μ g 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 MgCl₂, 20 μ M Nictide, 0.1 μ M [γ -32P]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 μ L of the reaction mix onto P81 phosphocellulose paper and immersion in 50 mM phosphoric acid. Samples are washed extensively and the incorporation of [γ -32P]ATP into Nictide is quantified by Cerenkov counting. IC₅₀ values are calculated with GraphPad Prism using non-linear regression analysis.

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Cell experiment: Cells (104 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 μ M. 48 h post treatment, 10 μ L 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 μ L of 266 mM NH₄OH 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.

Animal experiment: 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 extinguished by cervical dislocation and kidney and brain tissue rapidly dissected and snap-frozen in liquid nitrogen.

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.

Background

LRRK2-IN-1 is a potent and selective inhibitor of LRRK2 with IC₅₀ value of 13 nM. [1]

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LRRK2 (Leucine-rich repeat kinase 2) is also known as dardarin. LRRK2 belongs to the leucine-rich repeat kinase family. LRRK2 is mostly located in the cytoplasm, however, it also associates with the outer membrane of the mitochondrion. LRRK2 interacts with parkin at the C-terminal R2 RING finger domain. Parkin interacts with LRRK2 at the COR domain. LRRK2 mutation will induce apoptotic cell death of neuroblastoma cells. Expression of LRRK2 mutants has a close relationship with autosomal dominant Parkinson's disease. The LRRK2 Gly2019Ser mutation is a common cause of familial Parkinson's disease. The Gly2019Ser mutation has been proved to cause Parkinson's disease, even though it is a small number of LRRK2 mutations. LRRK2 also has a relationship with Crohn's disease by genomewide association studies.

LRRK2-IN-1 dose-dependently inhibits the activity of wild-type and G2019S LRRK2 with IC₅₀ of 0.17 and 0.04 μ M respectively in HEK293 cells stably expressing GFP-tagged wild-type or mutated LRRK2.[2] LRRK2-IN-1 inhibits the activity of both wild-type and G2019S mutant LRRK2 with IC₅₀ values of 13 nM and 6 nM respectively in vitro enzyme assay with 0.1 mM ATP. LRRK2-IN-1 competes with ATP. LRRK2-IN-1 has a selective profile compared with other 442 diverse kinases and it has no inhibition effect with LRRK1. LRRK2-IN-1 induced endogenous LRRK2 phosphorylation in lymphoblastoid cells. LRRK2-IN-1 also induced Ser910/Ser935 dephosphorylation of LRRK2 in mice kidney at 100 mg/kg.[1]

References:

- [1]. Deng X, Dzamko N, Prescott A, Davies P, Liu Q, Yang Q, Lee JD, Patricelli MP, Nomanbhoy TK, Alessi DR et al: Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. *Nat Chem Biol*, 7(4):203-205.
- [2]. Hermanson SB, Carlson CB, Riddle SM, Zhao J, Vogel KW, Nichols RJ, Bi K: Screening for novel LRRK2 inhibitors using a high-throughput TR-FRET cellular assay for LRRK2 Ser935 phosphorylation. *PLoS One*, 7(8):e43580.

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