

Product Data Sheet

Product Name: OTSSP167 hydrochloride

Cat. No.: GC12155

Chemical Properties

Cas. No. 1431698-10-0

Chemical Name 4-[7-acetyl-8-[[4-[(dimethylamino)methyl]cyclohexyl]amino]-1H-1,5-naphthyridin-2-ylidene]-2,6-dichlorocyclohexa-2,5-dien-1-one;hydrochloride

SMILES CC(=O)C1=CN=C2C=CC(=C3C=C(C(=O)C(=C3)Cl)Cl)NC2=C1NC4CCC(CC4)CN(C)C.Cl

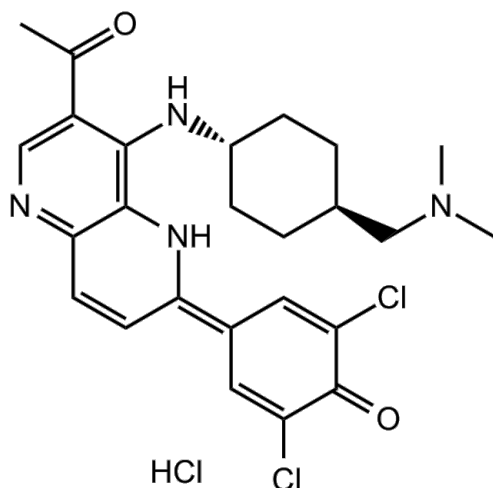
Formula C₂₅H₂₉Cl₃N₄O₂ M.Wt 523.88

Solubility DMF: 10 mg/ml, DMSO: 30 mg/ml, DMSO:PBS (pH 7.2)(1:9): 0.1 mg/ml, Ethanol: 1 mg/ml
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.

Structure



Protocol

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

**Kinase
experiment:**

For in vitro kinase assay, MELK recombinant protein (0.4 µg) is mixed with 5 µg of each substrate in 20 µL of kinase buffer containing 30 mM Tris-HCl (pH), 10 mM DTT, 40 mM NaF, 10 mM MgCl₂, 0.1 mM EGTA with 50 µM cold-ATP and 10 Ci of [γ-³²P]ATP for 30 min at 30°C. The reaction is terminated by addition of SDS sample buffer and boiled for 5 min prior to SDS-PAGE. The gel is dried and autoradiographed with intensifying screens at room temperature. OTSSP167 (final concentration of 10 nM) is dissolved in DMSO and added to kinase buffer before the incubation.

Cell experiment:

In vitro cell viability is measured by the colorimetric assay using Cell Counting Kit-8. Cells are plated in 100 µL in 96-well plates at a density that generates continual linear growth (A549, 1×10³ cells; T47D, 3×10³ cells; DU4475, 4×10³ cells; 22Rv1, 6×10³ cells; and HT1197, 2×10³ cells, in 100 µL per well). The cells are allowed to adhere overnight before exposure to OTSSP167 for 72 hours at 37°C. Plates are read using a spectrophotometer at a wavelength of 450 nm. All assays are carried out in triplicate.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Animal experiment: MDA-MB-231 cells are injected into the mammary fat pads of NOD.CB17-Prkdcscid/J mice. A549, MIAPaCa-2 and PC-14 cells (1×10^5 cells) are injected subcutaneously in the left flank of female BALB/cSLC-nu/nu mice. DU145 cells are injected subcutaneously in the left flank of male BALB/cSLC-nu/nu mice. When MDA-MB-231, A549, DU145, MIAPaCa-2, and PC-14 xenografts has reached an average volume of 100, 210, 110, 250, and 250 mm³, respectively, animals are randomized into groups of 6 mice (except for PC-14, for which groups of 3 mice are used). For oral administration, OTSSP167 and other compounds are prepared in a vehicle of 0.5% methylcellulose and given by oral gavage at the indicated dose and schedule. For intravenous administration, compounds are formulated in 5% glucose and injected into the tail vein. An administration volume of 10 mL per kg of body weight is used for both administration routes. Tumor volumes are determined every other day using a caliper.

References:

- [1]. Chung S, Suzuki H, Miyamoto T, et al. Development of an orally-administrative MELK-targeting inhibitor that suppresses the growth of various types of human cancer. *Oncotarget*. 2012 Dec 21.
- [2]. Ji W, et al. OTSSP167 Abrogates Mitotic Checkpoint through Inhibiting Multiple Mitotic Kinases. *PLoS One*. 2016 Apr 15;11(4):e0153518.
- [3]. Cho YS, et al. The crystal structure of MPK38 in complex with OTSSP167, an orally administrative MELK selective inhibitor. *Biochem Biophys Res Commun*. 2014 Apr 25;447(1):7-11.
- [4]. Jurmeister S, et al. Identification of potential therapeutic targets in prostate cancer through a cross-species approach. *EMBO Mol Med*. 2018 Feb 5. pii: e8274.
- [5]. Meel MH, et al. MELK inhibition in Diffuse Intrinsic Pontine Glioma. *Clin Cancer Res*. 2018 Jul 30. pii: clincanres.0924.2018.

Background

OTSSP167 is an inhibitor of maternal embryonic leucine zipper kinase(MELK) with IC₅₀ value of 0.41nM [1].

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

MELK belongs to the AMPK serine/threonine kinase family and involves in the mammalian embryonic development. It overexpresses in various types of human cancer. OTSSP167 is synthesized from a compound which is a hit of a high-throughput screening. In vitro anti-proliferative assay shows that OTSSP167 suppresses cell growth of various cancer cell lines, such as A549(IC₅₀=6.7nM), T47D(IC₅₀=4.3nM), DU4475(IC₅₀=2.3nM) and 22Rv1(IC₅₀= 6.0nM). In vivo assay also shows OTSSP167 can significantly suppress tumor growth in the xenograft model using various cancer cell lines. The effects are observed by both intravenous administration and oral administration. When investigating the substrates of MELK, OTSSP167 is found to inhibit the phosphorylation of DBNL and PSMA1 in vitro. Since PSMA1 is essential for survival of cancer cells, the reduction of phosphorylated PSMA1 caused by OTSSP167 can subsequently suppress mammosphere formation of cancer stem cells [1].

References:

[1] Suyoun Chung, Hanae Suzuki, Takashi Miyamoto et al. Development of an orally-administrative MELK-targeting inhibitor that suppresses the growth of various types of human cancer. *Oncotarget*. 2012, 3: 1629-1640.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA