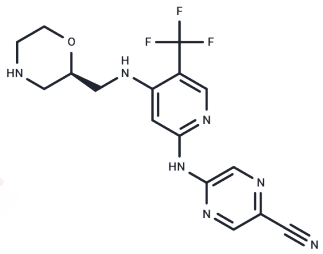
Data Sheet (Cat.No.T7080)

TargetMol

CCT245737

Chemical Properties



CAS No. : 1489389-18-5

Formula: C16H16F3N7O

Molecular Weight: 379.34

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	CCT245737 is an orally active, selective Chk1 inhibitor with an IC50 of 1.3 nM, and is >1,000-fold selective over CHK2 and CDK1.
Targets(IC50)	Chk
In vitro	**METHODS**: K562 cells were treated with CCT245737 (0.01, 0.05, 0.1, 0.5, 1, 2, 5, 24 hours), and the total expression of CHK1 protein was detected by Western Blot; K562 cells treated with VP16 (5 μM), CCT245737 (5 μM) or the combination of VP16 and CCT245737 (0.01, 0.05, 0.1, 0.5, 1, 2, 5, 24 hours) were analyzed by Western blot. **RESULTS** Increasing CCT245737 concentrations did not significantly reduce total CHK1 protein levels, and CHK1 was only inhibited at a high concentration of CCT245737 (5 μM); CCT245737 could effectively inhibit CHK1 activation by inhibiting VP16-induced CHK1 autophosphorylation at Ser296 and promoting the accumulation of DNA damage in VP16-treated K562 cells. [3]
In vivo	**METHODS**: CCT245737 (10 mg/kg, intravenous/oral) was administered to mice and the pharmacokinetics in vivo were tested. **RESULTS** The peak plasma concentration of CCT245737 after intravenous injection was 4 μmol/, the half-life was 2.86 h, the AUC was 9.96 μmol.h/L, the plasma clearance was 2.1 L/h/kg, and the distribution volume was large (0.19 L); the equivalent oral dose was almost the same as the AUC, indicating complete oral bioavailability (F = 105%). [1]
Kinase Assay	Commercial in vitro 33P radiometric kinase assays is carried out against 124 human kinases using 10 μM CCT245737 at ATP concentrations corresponding to the kinase Km, ATP [2].
Cell Research	Cytotoxicity is determined as the drug concentration that gives 50% inhibition of tumour cell proliferation (GI50) using a 96 h Sulforhodamine B (SRB) assay. Inhibition of intracellular CHK1 activity is measured using a cell-based ELISA for the abrogation of an etoposide-induced G2 checkpoint (mitosis induction assay, MIA). The IC50 for G2 checkpoint abrogation (MIA) is determined in the presence of nocodazole using UCN01 as a positive control. The activity index (AI) is used as a measure of the compounds ability to induce mitosis relative to its toxicity (i.e., ratio of MIA IC50: 96 h SRB GI50). Routine potentiation studies are carried out using a fixed concentration (GI50) of either gemcitabine or SN38 in combination with a range of CCT245737 concentrations to determine the combination GI50 of CCT245737. The ability of CCT245737 to enhance gemcitabine or SN38 cell killing is expressed as a potentiation index (PI) equal to the

Cell Research	ratio of the GI50 for CCT245737 alone versus the combination GI50 for CCT245737. PI values > 1 indicate the potentiation of the genotoxic activity. In addition, a series of experiments is carried out using fixed, non- or minimally toxic concentrations of CCT245737 (≤GI20) with a range of different concentrations of gemcitabine or SN38 to determine the extent to which CCT245737 enhances drug cytotoxicity compared with the genotoxic agent alone, i.e. conventional PI (ratio GI50 genotoxic alone: GI50 genotoxic combined with non-toxic CCT245737 concentration, Con PI)[2].
Animal Research	Human HT29 colorectal carcinoma cells are injected s.c into the flanks of female NCr athymic mice 6-8 weeks of age. Dosing commenced 5 days after transplantation when tumours reach a mean diameter of 5.5 mm. Gemcitabine (100 mg/kg i.v.) is dosed in saline on days 0, 7 and 14 and compounds 4 (CCT245737) and 41 (150 mg/kg p.o.) in 10% DMSO 20% PEG 400, 5% Tween 80, 65% water, 24 and 48 h after each dose of gemcitabine. Tumours are measured and body weights recorded three times weekly. Animals are culled on an individual basis when tumours reach a predetermined humane endpoint (mean diameter <15 mm)[1].

Solubility Information

Solubility	Ethanol: 5 mg/mL (13.18 mM),Sonication is recommended. H2O: Insoluble, DMSO: 48.33 mg/mL (127.41 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+ 45% Saline: 6 mg/mL (15.82 mM),Suspension. 10% DMSO+90% Saline: < 4.83 mg/mL (12.73 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. 10% DMSO+40% PEG300+5% Tween 80+45% Saline: 4.83 mg/mL (12.73 mM),Solution. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.6362 mL	13.1808 mL	26.3616 mL
5 mM	0.5272 mL	2.6362 mL	5.2723 mL
10 mM	0.2636 mL	1.3181 mL	2.6362 mL
50 mM	0.0527 mL	0.2636 mL	0.5272 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Walton MI,et al. The clinical development candidate CCT245737 is an orally active CHK1 inhibitor with preclinical activity in RAS mutant NSCLC and Eμ-MYC driven B-cell lymphoma. Oncotarget. 2016 Jan 19;7(3):2329-42.
Liang J, Niu Z, Zhang B, et al. p53-dependent elimination of aneuploid mitotic offspring by entosis. Cell Death & Differentiation. 2020: 1-15
Liang J, Niu Z, Zhang B, et al Liang J, Niu Z, Zhang B, et al. p53-dependent elimination of aneuploid mitotic offspring by entosis. Cell Death & Differentiation. 2020: 1-15.
Osborne JD,et al. Multiparameter Lead Optimization to Give an Oral Checkpoint Kinase 1 (CHK1) Inhibitor Clinical Candidate: (R)-5-((4-((Morpholin-2-ylmethyl)amino)-5-(trifluoromethyl)pyridin-2-yl)amino)pyrazine-2-carbonitrile (CCT245737). J Med Chem. 2016 Jun 9;59(11):5221-37.
Fan Z, et al. Checkpoint kinase-1 inhibition and etoposide exhibit a strong synergistic anticancer effect on chronic myeloid leukemia cell line K562 by impairing homologous recombination DNA damage repair. Oncol Rep. 2020 Nov;44(5):2152-2164.
Liang J, Niu Z, Zhang B, et al. Liang J, Niu Z, Zhang B, et al. p53-dependent elimination of aneuploid mitotic offspring by entosis[J]. Cell Death & Differentiation. 2020: 1-15.