

Product Data Sheet

Product Name: CCT244747

Cat. No.: GC14772

Chemical Properties

Cas. No. 1404095-34-6

Chemical Name (R)-3-((1-(dimethylamino)propan-2-yl)oxy)-5-((4-methoxy-5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)amino)pyrazine-2-carbonitrile

SMILES C[C@](OC1=NC(NC(C=C2OC)=NC=C2C(C=N3)=CN3C)=CN=C1C#N)([H])CN(C)C

Formula $C_{20}H_{24}N_8O_2$

M.Wt 408.46

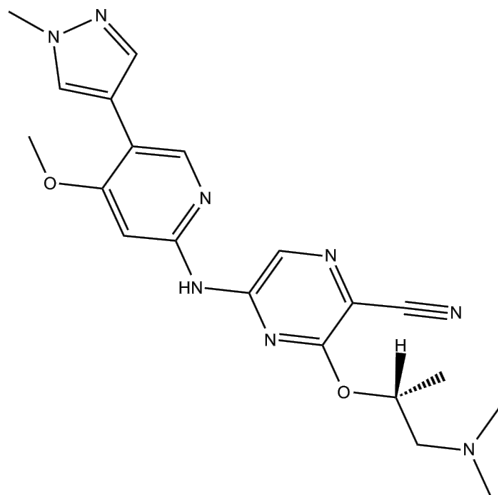
Solubility Soluble in DMSO

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.

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**Kinase
experiment:**

CHK1 kinase activity is measured in a microfluidic assay that monitored the separation of a phosphorylated product from its substrate. The assay is run on an EZ Reader II using separation buffer containing CR-8 (500 nM). An ECHO® 550 acoustic dispenser is used to generate duplicate 8 pt dilution curves directly into 384 polypropylene assay plates. For each compound a 50 µM stock concentration in 100% DMSO is used. The total amount of DMSO dispensed per well is 250 nL to give a final assay concentration of 2.5% DMSO and compound concentrations in the range 0.5-1000 nM. To this assay plate, 6 PL CHK1 (2 nM final concentration, in-house protein preparation), 2 PL peptide 10 (5-FAM-KKKVSRSGLYRSPSPENLNRPR-COOH, 1.5 PM final concentration) and 2 PL ATP (90 PM final concentration) all diluted in kinase buffer (HEPES 50 mM, NaN₃ 0.02%, BSA 0.01%, sodium orthovanadate 0.1 mM, DTT 1 mM, MgCl₂ 2 mM, Tween20 0.1%) are added. The plate is sealed and centrifuged (1 min, 1000 rpm) before incubation for 1 h at room temperature. The reaction is stopped by the addition of separation buffer (90 PL). The plate is read on an EZ Reader II, using a 12-sipper chip with instrument settings of -1.5 psi and 1750 ΔV. The percentage conversion of product from substrate is generated automatically and the percentage inhibition is calculated relative to blank wells (containing no enzyme and 2.5% DMSO) and total wells (containing all reagents and 2.5% DMSO). IC₅₀ values are calculated in GraphPad Prism5 using a non linear regression fit of the log (inhibitor) vs response with variable slope equation[1].

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Cell experiment: Compound cytotoxicity and the ability of CHK1 inhibitors to enhance SN38 (the active metabolite of the topoisomerase I inhibitor irinotecan) and gemcitabine (an antimetabolite) cytotoxicity is assessed using a 96 h sulforhodamine B assay (SRB). HT29 or SW620 cells are seeded at 1.6 to 3.2×10^3 cells per well in 96-well plates in a volume of $160 \mu\text{L}$ medium and allowed to attach for 36 h prior to treatment. For cytotoxicity assays, CHK1 inhibitors (10 mM stock in DMSO) are serially diluted in medium from a starting concentration of 250 PM and then 40 PL is added to appropriate wells in quadruplicate to give a final concentration range of 50-0.1 PM (10 concentrations). Genotoxic agents (SN38; 10 mM stock in DMSO) are serially diluted in medium from a starting concentration of 2 PM and 40 PL is added to each well in quadruplicate to give final concentrations from 200-0.39 nM (10 concentrations). Cells are incubated for 96 h (four doublings) at 37°C in a humidified 5% CO_2 environment and then fixed and stained with SRB. Appropriate controls are included and results are expressed as the concentration of test compound required to inhibit cell growth by 50% relative to untreated controls (SRB IC₅₀). Potentiation assays involved adding a fixed SRB IC₅₀ concentration of either gemcitabine or SN38 in a volume of $20 \mu\text{L}$ of medium (10 $\times$ final concentration), to each well in quadruplicate and mixing for 1 min. CHK1 inhibitor (10 mM stock) is serially diluted from a starting concentration of 50 PM in medium and 20 PL is added per well in quadruplicate to give a final concentration range of 5-0.039 PM (8 concentrations). After mixing for 1 min the cells are incubated at 37°C in a humidified atmosphere for 96 h (four doublings) prior to fixing and SRB staining. Untreated and genotoxic alone treated controls are included and results are expressed as the concentration of CHK1 inhibitor required to inhibit cell growth by 50% (potentiation IC₅₀). The potentiation index (PI) is used as a measure of the ability of the CHK1 inhibitor to enhance SN38 or gemcitabine cytotoxicity and is the ratio of the SRB IC₅₀ versus potentiation IC₅₀ ($\text{PI} = \text{SRB IC}_{50} / \text{Potentiation IC}_{50}$)[1].

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**Animal
experiment:**

Female BALB/c mice (6 weeks old) are kept in a controlled environment with food and sterilized water available ad libitum. Animals weighed 20 (± 2) g at the time of experiment. Dosing solutions are prepared by dissolving the compounds in 10% DMSO and 5% Tween20 in 85% saline. The compounds are administered i.v. and p.o., individually. Animals are warmed before receiving a single i.v. bolus injection into a lateral tail vein. Oral administration is by oral gavage. Blood is collected at selected time points (1 h and 6 h after dosing) by cardiac puncture under anesthesia into heparinized syringes, transferred to micro centrifuge tubes, and centrifuged at $4500 \times g$ for 2 min to obtain plasma. Quantitative analysis is performed by high performance liquid chromatography tandem mass spectrometry on a triple quadrupole instrument using multiple reaction monitoring of selected transitions with olomoucine used as internal standard. Quantitation is performed against a standard curve ranging from concentrations of 2-1000 nM in the matrix measured. Quality controls are included at the level of 25, 250 and 750 nM. If required, samples are diluted in the matrix of interest[1].

References:

- [1]. Lainchbury M, et al. Discovery of 3-alkoxyamino-5-(pyridin-2-ylamino)pyrazine-2-carbonitriles as selective, orally bioavailable CHK1 inhibitors. *J Med Chem.* 2012 Nov 26;55(22):10229-40.
- [2]. Walton MI, et al. CCT244747 is a novel potent and selective CHK1 inhibitor with oral efficacy alone and in combination with genotoxic anticancer drugs. *Clin Cancer Res.* 2012 Oct 15;18(20):5650-61.
- [3]. Patel R, et al. An orally bioavailable Chk1 inhibitor, CCT244747, sensitizes bladder and head and neck cancer cell lines to radiation. *Radiother Oncol.* 2017 Mar;122(3):470-475.

Background**Description:**

IC50: 29-170 nM

CHK1 is a serine/threonine kinase that is activated in response to single strand breaks (SSBs) in

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DNA caused by either direct DNA damage or replication stress. Activation of CHK1 initiates a signaling cascade culminating in cell cycle arrest leading to DNA repair, senescence or death. Inhibition of CHK1 abrogates cell cycle arrest, inhibits DNA repair and induces tumor cell death following DNA damage by a range of chemotherapeutic agents. CCT244747 is a potent and selective CHK1 inhibitor.

In vitro: CCT244747 inhibited cellular CHK1 activity, significantly enhanced the cytotoxicity of a few anticancer drugs and abrogated drug-induced S and G2 arrest in multiple tumor cell lines. Biomarkers of CHK1 activity and cell cycle inactivity were induced by genotoxics and inhibited by CCT244747, producing enhanced DNA damage and apoptosis [1].

In vivo: Active tumor concentrations of CCT244747 were obtained following oral administration. The antitumor activities of both gemcitabine and irinotecan were significantly enhanced by CCT244747 in human tumor xenografts, giving concomitant biomarker modulation indicative of CHK1 inhibition [1].

Clinical trial: Up to now, CCT244747 is still in the preclinical development stage.

Reference:

[1] Walton MI, Eve PD, Hayes A, Valenti MR, De Haven Brandon AK, Box G, Hallsworth A, Smith EL, Boxall KJ, Lainchbury M, Matthews TP, Jamin Y, Robinson SP, Aherne GW, Reader JC, Chesler L, Raynaud FI, Eccles SA, Collins I, Garrett MD. CCT244747 is a novel potent and selective CHK1 inhibitor with oral efficacy alone and in combination with genotoxic anticancer drugs. Clin Cancer Res. 2012 Oct 15;18(20):5650-61.

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