CNX-1351

Cat. No.: HY-16596

CAS No.: 1276105-89-5

Molecular Formula: $C_{30}H_{35}N_7O_3S$

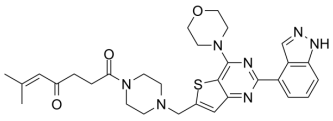
Molecular Weight: 573.71

Target: PI3K

Pathway: PI3K/Akt/mTOR

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (174.30 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7430 mL	8.7152 mL	17.4304 mL
	5 mM	0.3486 mL	1.7430 mL	3.4861 mL
	10 mM	0.1743 mL	0.8715 mL	1.7430 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (4.36 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.36 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

CNX-1351 is a potent and isoform-selective targeted covalent PI3Kα inhibitor with IC_{50} of 6.8 nM.

IC_{50} & Target

PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ
6.8 nM (IC_{50})	166 nM (IC_{50})	240.3 nM (IC_{50})	3020 nM (IC_{50})

In Vitro

CNX-1351 is able to potently (EC_{50}<100 nM) and specifically inhibit signaling in PI3Kα-dependent cancer cell lines, and this leads to a potent antiproliferative effect (GI_{50}<100 nM). CNX-1351 inhibits PI3K signaling in SKOV3 cells, with potency (EC_{50} of 10-100 nM) similar to that of the pan-PI3K inhibitor. To investigate the functional consequence of inhibiting PI3Kα in cells, two cell lines with different PIK3CA activating mutations, SKOV3 ovarian cancer cells (H1047R) and MCF-7 breast cancer cells (E545K), are treated with CNX-1351 and growth is monitored. Both PIK3CA-driven cell lines are growth inhibited by exposure

	to CNX-1351 for 96 h (GI_{50} of 78 and 55 nM, respectively)[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	CNX-1351 inhibits p-Akt^{Ser473} in mouse spleens and bonds to PI3Kα in vivo. CNX-1351 is delivered into the intraperitoneal cavity of nude mice at 100 mg/kg once a day for 5 consecutive days (n=3 mice per group). Spleens are harvested from the mice at the indicated times after the last dose (1-24 h) and interrogated by immunoblot for P-Akt^{Ser473} or for PI3Kα occupancy. Inhibition of PI3K signaling is detected as a decrease in P-Akt^{Ser473} at 1 and 4 h after last dose[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	CNX-1351 is tested in a panel of 10 lipid kinases. CNX-1351s tested in a 10-concentration IC_{50} curve with 3-fold serial dilution starting at 1 μM. Reactions are carried out at 10 μM ATP. An HTRF assay format is used for PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ; ADP-GLO assay format is used for other kinases. The following substrates are used: for HTRF, phosphatidylinositol 4,5-bisphosphate; for SPHK1 and SPHK2, sphingosine; for other ADP-GLO enzymes, phosphatidylinositol. For general kinase selectivity, CNX-1351 is run in a kinase selectivity panel at using HotSpot technology and radioisotope-based P81 filtration. CNX-1351 is dissolved in pure DMSO to the final 1 μM test concentration. Substrates for the various kinases tested against CNX-1351 are prepared fresh daily in reaction buffer. Any required cofactors are then added to the substrate solution followed by kinase addition and preincubated for 30 min at room temperature. ^{33}P-ATP (10 μM) is delivered into the reaction mixture to initiate the reaction, and reaction continued for 2 h at room temperature. The reaction is terminated, and any unreacted phosphate is washed away using 0.1% phosphoric acid prior to detection utilizing a proprietary technology. The study is performed in duplicate, and 10 μM staurosporine, a nonselective, ATP-competitive kinase inhibitor, is used as the positive control[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	SKOV3 cells or MCF-7 cells are plated in SKOV3 proliferation assay medium (DMEM supplemented with 5-10% FBS and pen/strep) at a density of 5000 cells in 180 μL volume per well in Costar no. 3610 white 96-well clear flat-bottom plates and incubated overnight in a humidified 37°C incubator. A standard curve ranging from 10 000 to 50 000 cells is set up in a separate plate and allowed to adhere to the plate for 4-6 h, at which time the plate is developed using Cell Titer-Glo. The next morning, 3-fold compound dilutions ranging from 10 000 to 40 nM are prepared in proliferation medium containing 1% DMSO. Then 20 μL of each dilution is added to the SKOV3 or MCF-7 cells plated the previous day, resulting in a dose-response curve from 1000 to 4 nM. The cells are incubated for 96 h and then developed with Cell Titer Glo.The cell numbers at the end of the assay are determined using the standard curve generated at the start of the assay. Growth inhibition is calculated using the following formulas, and GI50 values are determined[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice[1] Female nu/nu (n=3/group) are administered compound (GDC-0941 or CNX-1351) delivered ip at 100 mg/kg once daily for 5 consecutive days. After delivery of the last dose, spleens from treated animals are harvested at 1, 4, and 24 h time points. Spleens are immediately frozen in liquid nitrogen. Samples are stored at -80°C until processing for homogenates. Homogenates are made by adding approximately 100 μL of spleen sample to a Precellys homogenizing tube containing 300 μL of cell extraction buffer plus Complete protease inhibitor and PhosStop phosphatase inhibitor and kept on ice. The sample is homogenized in a Precellys 24 homogenizer for 15 s followed by centrifugation at 16000g for 20 min at 4°C. The supernatant is moved to a new tube, and the protein concentration is determined by BCA Assay. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Nacht M, et al. Discovery of a potent and isoform-selective targeted covalent inhibitor of the lipid kinase PI3Kα. J Med Chem. 2013 Feb 14;56(3):712-21.

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

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