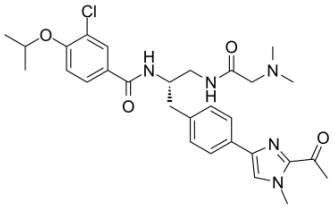
PF-2771

Cat. No.:	HY-19530
CAS No.:	2070009-55-9
Molecular Formula:	$C_{29}H_{36}ClN_5O_4$
Molecular Weight:	554.08
Target:	Kinesin
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 32 mg/mL (57.75 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Mass / Solvent / Concentration	1 mg	5 mg	10 mg
1 mM	1.8048 mL	9.0240 mL	18.0479 mL
5 mM	0.3610 mL	1.8048 mL	3.6096 mL
10 mM	0.1805 mL	0.9024 mL	1.8048 mL

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

BIOLOGICAL ACTIVITY

Description

PF-2771 is a potent and selective centromere protein E (CENP-E) inhibitor, inhibiting CENP-E motor activity with an IC_{50} of 16.1 nM; PF-2771 is used as an anticancer agent.

IC_{50} & Target

CENP-E

16.1 nM (IC_{50})

In Vitro

PF-2771 is a potent and selective CENP-E inhibitor, inhibiting CENP-E motor activity with an IC_{50} of 16.1 nM. PF-2771 shows no inhibitory effect on the ATPase activities of highly related kinesins (0% inhibition of Eg5/KSP, chromokinesin, and MCAK at both 1 or 10 μM PF-2771). PF-2771 exhibits inactive activity against 74 protein kinases (all < 23% inhibition with 1 μM PF-2771, < 40% with 10 μM PF-2771). PF-2771 is cytotoxic to the basal-like breast cancer tumor cell survival, with EC_{50}s of < 0.1 μM, and with no cytotoxicity on the normal and premalignant cell lines (EC_{50} > 5 μM). PF-2771 (100 nM) reusults in a chromosomal congression defect in MDA-MB-468 cells[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo	PF-2771 (100 mg/kg, every day i.p.) potently inhibits CENP-E motor function, and causes tumor regression in SCID mice bearing AA1077 mammary tumors[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Microtubule-activated CENP-E kinesin ATPase activity is measured spectrophotometrically by coupling the hydrolysis of ATP to ADP to NADH oxidation (decrease in 340 nm absorbance) through the activities of pyruvate kinase (PK) and lactate dehydrogenase (LDH). Reactions contained 2 mM phosphoenolpyruvate, 0.28 mM NADH, 5 mM $MgCl_2$, 1 mM DTT, 15 μM taxol, 0.7 μM MT (preformed porcine microtubules), 50 μM ATP, 10 U/mL PK, and 10 U/mL LDH in 15 mM PIPES buffer (pH 7.0). Reactions are initiated with a 20 nM CENP-E addition (Human: 1-342; WT) at 30°C. The IC_{50} values are determined by a nonlinear, least squares fit of the data to the four-parameter dose-response curve equation. PF-2771 kinesin biochemical selectivity toward other kinesins is tested in triplicate at both 1 and 10 μM PF-2771 in a variant of the CENP-E enzymatic assays that have the following enzyme concentrations: 200 nM chromokinesin motor domain, 200 nM Eg5 motor domain, 226 nM MCAK motor domain. PF-2771 biochemical mechanism is determined by measuring CENP-E enzymatic activity as a function of ATP and PF-2771 concentrations (0, 2.5, 5, 10, 20, 30, 45 nM PF-2771; 1,000, 500, 250, 125, 62.5, 31.2, 15.6, 7.81, 3.90, 1.95, 0.977 μM ATP)[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	PF-2771 is added to cells seeded in 96-well plates. The number of cells seeded (1,000-3,000) depended on growth characteristics of each cell type and normalized proliferation rates. Ten different concentrations of compound (PF-2771) used are based on a half-log increment between 1 nM and either 1 or 25 μM. Cells are incubated at 37°C for 7 days before assessing viability with the CellTiter-Glo reagent. Untreated control cells are 80% to 90% confluent after 7 days of culture. Data are fitted into a sigmoidal curve-fitting program to calculate IC_{50} values[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	HCC1806 tumor cells (3×10^6) are implanted in the mammary fat pad of CB17/Icr.Cg-PrkdcscidLystbg female mice. PF-2771 is administered intraperitoneally (i.p.) to groups of 12 mice at 3, 10, and 30 mg/kg every day for 14 days and at 100 mg/kg every day for 4 days followed by 3 days off and then another 4-day cycle. Tumor volumes are recorded twice weekly by calipers with the final measurement taken 3 days after the last dose. Tumor growth inhibition (TGI) is calculated using the formula $100 \times (1 - \Delta T/\Delta C)$, where ΔT (treated) and ΔC (control) are the mean tumor volume changes between 1 day after the last dose and the first-day treatment. Time-to-progression endpoint and associated tumor growth delay determinations are calculated using median days to reach to two doublings of initial tumor size. Statistical comparisons are made using one-way ANOVA with Dunnett posttests. Other tumor models had similar methodology except where noted. PDX-AA1077 is a patient-derived xenograft model developed from tumor tissue from a triple-negative patient engrafted to the mammary glands of NSG female mice to create passage 1 tumor-bearing mice. Surgically resected tumor tissue is cut into 2- to 4-mm^3 fragments and subcutaneously implanted into the flank of SCID-bg mice. Mice with palpable tumors are randomized before dosing. An HCC1599 tumor cell line xenograft model is established from tumor fragments of established tumors and reimplanted into flank of female SCID mice. When average tumor size reached 250 mm^3, animals are randomized into five groups of 10 mice. Animals are treated with vehicle (daily dosing), 10 mg/kg docetaxel (once per week dosing), 25 mg/kg docetaxel (once per week dosing), 20 mg/kg paclitaxel (twice per week dosing), or 100 mg/kg of PF-2771 (every day, i.p.)[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Kung PP, et al. Chemogenetic evaluation of the mitotic kinesin CENP-E reveals a critical role in triple-negative breast cancer. Mol Cancer Ther. 2014 Aug;13(8):2104-15.

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

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