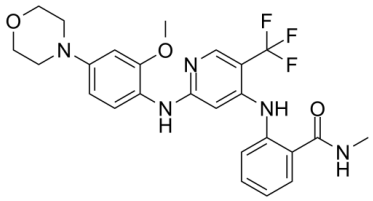
Data Sheet

Product Name:	PND-1186
Cat. No.:	CS-1584
CAS No.:	1061353-68-1
Molecular Formula:	C25H26F3N5O3
Molecular Weight:	501.50
Target:	Apoptosis; FAK
Pathway:	Apoptosis; Protein Tyrosine Kinase/RTK
Solubility:	DMSO : ≥ 34 mg/mL (67.80 mM)



BIOLOGICAL ACTIVITY:

PND-1186 (VS-4718; SR-2516) is a potent and reversible inhibitor of **FAK** with an **IC_{50}** of 1.5 nM in cell assay. IC50 & Target: IC50: 1.5 nM (FAK)[1] **In Vitro:** Using the recombinant FAK kinase domain as a glutathione-S-transferase (GST) fusion protein in an in vitro kinase assay, PND-1186 inhibits FAK activity with IC_{50} of 1.5 nM. PND-1186 has an IC_{50} of ~100 nM in breast carcinoma cells as determined by anti-phospho-specific immunoblotting to FAK Tyr-397. Whereas 1.0 μM PND-1186 (>5-fold above IC_{50}) has limited effects on cell proliferation, under non-adherent conditions or when grown as spheroids or colonies in soft agar, 0.1 μM PND-1186 blocks FAK and p130Cas tyrosine phosphorylation, promotes caspase-3 activation, and triggers cell apoptosis. PND-1186 inhibits 4T1 breast carcinoma subcutaneous tumor growth correlated with elevated tumor cell apoptosis and caspase 3 activation[1]. **In Vivo:** 100 mg/kg PND-1186 treatment significantly reduces final 4T1 tumor weight 2-fold (n=8, $p<0.05$) whereas 30 mg/kg PND-1186 slightly reduces final tumor weight but is not significantly different compare to control (n=8, $p>0.05$). Both 30 and 100 mg/kg administration of PND-1186 significantly increases tumor TUNEL staining compare to vehicle-treated controls. As elevated cleaved caspase-3 staining is also found in the tumors of PND-1186-treated mice[1]. PND-1186 displays a multi-exponential decay with a terminal half life ($t_{1/2}$) of 1.72 hours after i.v. injection. Following i.p. and p.o. dosing, PND-1186 is rapidly absorbed with terminal half lives ($t_{1/2}$) of 2.15 to 2.65 h, and bioavailability (%F) from 14.8 to 42.2%. PND-1186 bioavailability is greater upon intraperitoneal versus oral dosing[2].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: PND-1186 is dissolved in DMSO and stored, and then diluted with appropriate media (DMSO 0.1%-0.2%) before use[1].[1] For cell growth analyses, adherent or suspended cells are treated with PND-1186 for the indicated times, collected as a single cell suspension by limited trypsin treatment, fixed with 70% ethanol, collected by centrifugation and washed with PBS. Cell pellets are resuspended in 300 μL of PBS containing propidium iodide (PI) (10 μg/mL), DNAse-free RNAse (100 μg/mL), and then incubated at 37°C with agitation for 1 h. Samples are analyzed by flow cytometry and cell cycle analyses are performed by ModFit LT3.2 software. Hypodiploid DNA content as a measure of cell apoptosis is detected by PI staining. For cell apoptosis analyses, adherent or suspended cells are treated with PND-1186 and collected as above, stained for phycoerythrin (PE)-conjugated annexin V binding and 7-amino-actinomycin (7-AAD) reactivity, and analyzed within 1 h by flow cytometry. Quadrant gates are positioned based on cell autofluorescence (negative) staurosporine-treated (positive) controls. Apoptosis is calculated to be the percent of annexin V-positive cells. In the soft agar assays, apoptosis is quantified by visual inspection of at least 200 cells and is defined as the appearance of membrane blebbing or cell shrinkage. Apoptosis is also detected by appearance of cleaved caspase-3 antibody reactivity in protein lysates by immunoblotting[1]. **Animal Administration:** PND-1186 is solubilized in polyethylene glycol 400 (PEG400) in PBS (1:1) (Mice)[1].[1]Mice[1]

Six to eight week old female C57BL6 and BALB/c mice are used. For subcutaneous tumor growth, 1×10^6 mCherry-labeled 4T1 cells in 100 μL PBS are injected into the hindflank of Balb/C mice. After 8 days, mice with equal volume tumors (as measured using vernier calipers and determined by length×width^2/2) are grouped (n=8 per group) and PND-1186 solubilized in polyethylene glycol 400

(PEG400) in PBS (1:1) is injected (100 µL) subcutaneously in the neck region at 30 mg/kg or 100 mg/kg every 12 hours. Control animals receive PEG400:PBS injections and at 13 days, tumors are imaged in situ using an Olympus OV100 Intravital Fluorescence Molecular Imaging System, tumors are excised and weighed, half is frozen in OCT, and half is solubilized in protein lysis buffer for FAK phosphorylation analyses.

References:

[1]. Tanjoni I, et al. PND-1186 FAK inhibitor selectively promotes tumor cell apoptosis in three-dimensional environments. Cancer Biol Ther. 2010 May 15;9(10):764-77.

[2]. Walsh C, et al. Oral delivery of PND-1186 FAK inhibitor decreases tumor growth and spontaneous breast to lung metastasis in pre-clinical models. Cancer Biol Ther. 2010 May 15;9(10):778-90.

CAIndexNames:

Benzamide, 2-[[2-[[2-methoxy-4-(4-morpholinyl)phenyl]amino]-5-(trifluoromethyl)-4-pyridinyl]amino]-N-methyl-

SMILES:

O=C(NC)C1=CC=CC=C1NC2=CC(NC3=CC=C(N4CCOCC4)C=C3OC)=NC=C2C(F)(F)F

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