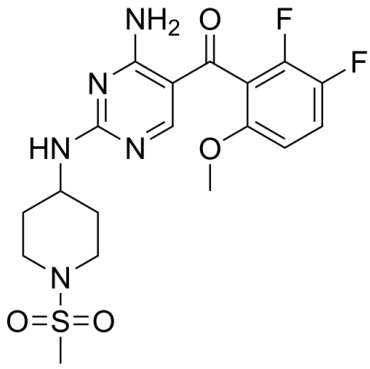
Data Sheet

Product Name:	R547
Cat. No.:	CS-0022
CAS No.:	741713-40-6
Molecular Formula:	$C_{18}H_{21}F_2N_5O_4S$
Molecular Weight:	441.45
Target:	Apoptosis; CDK; GSK-3
Pathway:	Apoptosis; Cell Cycle/DNA Damage; PI3K/Akt/mTOR; Stem Cell/Wnt
Solubility:	10 mM in DMSO



BIOLOGICAL ACTIVITY:

R547 is a potent, selective and orally active ATP-competitive **CDK** inhibitor, with **K_i**s of 2 nM, 3 nM and 1 nM for CDK1/cyclin B, CDK2/cyclin E and CDK4/cyclin D1, respectively[1][2][3][4][5]. ***In Vitro:*** R547 effectively inhibits CDK1/cyclin B, CDK2/cyclin E, and CDK4/cyclin D1 (Ki = 1–3 nmol/L) and is inactive (Ki > 5,000 nmol/L) against a panel of >120 unrelated kinases in cell-free assays[4].
R547 effectively inhibits the proliferation of tumor cell lines independent of multidrug resistant status, histologic type, retinoblastoma protein, or p53 status, with IC_{50}s <0.60 μM[4].
R547 reduces phosphorylation of the cellular retinoblastoma protein at specific CDK phosphorylation sites at the same concentrations that induced cell cycle arrest[4].
R547 has anti-proliferative activity in tumor cells independent of p53, retinoblastoma, or MDR status[4].
R547 blocks tumor cells in G1 plus G2 and induces apoptosis[4].
R547 induces apoptosis as measured by DNA fragmentation[4].
R547 inhibits phosphorylation of retinoblastoma protein in humantumor cells[4].
In Vivo: R547 has significant in vivo efficacy with daily oral and once weekly i.v. dosing[4].
R547 inhibits phosphorylation of retinoblastoma protein in tumors[4].

PROTOCOL (Extracted from published papers and Only for reference)

Cell assay [1] The effect of the compound on cell cycle progression was determined using dual-variable flow cytometry. Cells were labeled with propidium iodide and bromodeoxyuridine (BrdUrd) to measure both the DNA and BrdUrd contents of the cells. A modification of the method as described by Bussink et al. (35) was followed for determination of percentage cells in S phase. Cells were plated in 10-cm dishes at 5 × 104/mL in 10 mL of growth medium and incubated at 37°C with 5% CO2. After 24 hours, 30 μL of a stock solution of the test compound in 100% DMSO were added to individual dishes to obtain a final concentration equivalent to the IC50, IC90, and 3 × IC90 of the compound as determined in antiproliferative assays. To the control dish, 30 μL of 100% DMSO were added (final concentration of DMSO in all plates is 0.3%). Cells were pulsed with 20 μmol/L BrdUrd for 1 hour before harvesting, and supernatant and cells were collected at the indicated time points. Cells were resuspended in 0.5 mL ice-cold PBS and fixed by slow addition of 5 mL ice-cold 70% ethanol while vortexing. Cells were washed twice with PBS and resuspended in 1 mL of a 2 N HCl/0.5% Triton X-100 solution for 30 minutes at room temperature. After centrifugation, 1 mL of 0.1 mol/L Na2B4O7 was added to the pellet and cell nuclei were washed with PBS, resuspended in 100 μL of 0.5% Tween 20/1% BSA/PBS containing 20 μL of anti-BrdUrd FITC (Becton Dickinson, San Jose, CA), and incubated in the dark for 30 minutes. Samples were washed with the Tween 20/BSA/PBS buffer, resuspended in 500 μL PBS containing 50 μg of propidium iodide (Sigma), and analyzed by flow cytometry. Enzyme assay [1] Enzyme reactions were initiated by adding recombinant histidine-tagged enzyme and retinoblastoma substrate to

384-well plates containing diluted test compounds. Final reaction conditions were such that the ATP concentration was 3× the respective enzyme Km for ATP in the presence of 25 mmol/L HEPES (pH 7.0), 6.25 mmol/L MgCl2, 1.5 mmol/L DTT, 0.002% Tween 20, and 0.2 mg/mL bovine serum albumin (BSA). After a 25-minute incubation at 37°C, reactions were terminated by addition of anti-phosphorylated retinoblastoma (Ser780) antibody (Cell Signaling Technology, Beverly, MA). The phosphorylated retinoblastoma was analyzed by adding lance europium anti-rabbit IgG and anti-His-allophycocyanin, resulting in fluorescence resonance energy transfer between europium anti-rabbit and allophycocyanin, and quantified by fluorescence intensity ratio 665 nm/615 nm (excited at 340 nm). IC50s were calculated from net readings at 665 nm, normalized for europium readings at 615 nm. The kinase insert domain-containing receptor, fibroblast growth factor receptor, platelet-derived growth factor receptor, and epidermal growth factor receptor kinase assays are also homogeneous time-resolved fluorescence assays. Protein kinase A, protein kinase B, protein kinase Cα, protein kinase Cβ, FYN, extracellular signal-regulated kinase 2, p38, mitogen-activated protein kinase 2, serum/glucocorticoid-regulated kinase, and EPHB3 assays were conducted using an assay based on IMAP Technology (Molecular Devices Corp., Sunnyvale, CA) that enables quantitation of kinase activity via preferential binding of phosphorylated fluorescent peptide substrates to immobilized metal beads. These reactions were carried out at ATP concentrations of 3× the Km for the respective enzyme. SRC, focal adhesion kinase, AURORA, glycogen synthase kinase (GSK) 3β, and insulin-like growth factor receptor kinase assays are fluorescence resonance energy transfer assays run at the Km for ATP. R547 was also evaluated for activity against 123 kinases at a single 10 μmol/L concentration in the Upstate kinase selectivity screen (Kinase Profiler, Kinase Profiler-FP, and PI Profiler, Upstate Biotechnology, Lake Placid, NY), and the IC50s determined in the Upstate IC50 Profiler Express for kinases identified as hits in the initial screen.

References:

[1]. Chu XJ, DePinto W, Bartkovitz D, So SS, Vu BT, Packman K, Lukacs C, Ding Q, Jiang N, Wang K, Goelzer P, Yin X, Smith MA, Higgins BX, Chen Y, Xiang Q, Moliterni J, Kaplan G, Graves B, Lovey A, Fotouhi N.Discovery of [4-Amino-2-(1-methanesulfonylpiperidin-4-ylamino)pyrimidin-5-yl](2,3-difluoro-6-methoxyphenyl)methanone (R547), a potent and selective cyclin-dependent kinase inhibitor with significant in vivo antitumor activity.J Med Chem. 2006 Nov 2;49(22):6549-60.

[2]. Bayés M, Rabasseda X, Prous JR.Gateways to clinical trials. Methods Find Exp Clin Pharmacol. 2007 Jul-Aug;29(6):427-37.

[3]. Martin F, Thomson TM, Sewer A, Drubin DA, Mathis C, Weisensee D, Pratt D, Hoeng J, Peitsch MC.Assessment of network perturbation amplitudes by applying high-throughput data to causal biological networks.BMC Syst Biol. 2012 May 31;6:54.

[4]. DePinto, Wanda et al In vitro and in vivo activity of R547: a potent and selective cyclin-dependent kinase inhibitor currently in phase I clinical trials Molecular Cancer Therapeutics (2006), 5(11), 2644-2658

[5]. Jones, Clifford D.; Andrews, David M. Imidazole pyrimidine amides as potent, orally bioavailable cyclin-dependent kinase inhibitors Bioorganic & Medicinal Chemistry Letters (2008), 18(24), 6486-6489

CAIndexNames:

Methanone, [4-amino-2-[[1-(methylsulfonyl)-4-piperidinyl]amino]-5-pyrimidinyl](2,3-difluoro-6-methoxyphenyl)-

SMILES:

O=S(N1CCC(NC2=NC(N)=C(C(C3=C(C(F)=CC=C3OC)F)=O)C=N2)CC1)(C)=O

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