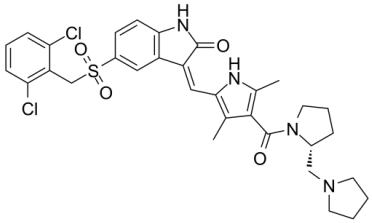
Data Sheet

Product Name:	PHA-665752
Cat. No.:	CS-0137
CAS No.:	477575-56-7
Molecular Formula:	C32H34Cl2N4O4S
Molecular Weight:	641.61
Target:	Apoptosis; Autophagy; c-Met/HGFR
Pathway:	Apoptosis; Autophagy; Protein Tyrosine Kinase/RTK
Solubility:	DMSO : 50 mg/mL (77.93 mM; Need ultrasonic); H2O : < 0.1 mg/mL (insoluble)



BIOLOGICAL ACTIVITY:

PHA-665752 is a selective, ATP-competitive, and active-site inhibitor of the catalytic activity of **c-Met** kinase (K_i=4 nM; IC_{50}=9 nM). PHA-665752 exhibits >50-fold selectivity for c-Met compared with a panel of diverse tyrosine and serine-threonine kinases. PHA-665752 induces **apoptosis** and cell cycle arrest, and exhibits cytoreductive antitumor activity[1][2]. IC50 & Target: Ki: 4 nM[1]
IC50: 9 nM (c-Met)[1] **In Vitro:** PHA-665752 is a potent and ATP-competitive inhibitor of c-Met kinase activity with a K_i of 4 nM and an IC_{50} of 9 nM[1].
PHA-665752 exhibits >50-fold selectivity for c-Met enzyme compared with the majority of kinases evaluated[1].
PHA-665752 shows potent inhibition of c-Met RTK autophosphorylation in NIH3T3 cells engineered to express high levels of c-Met and hepatocyte growth factor (HGF)[1].
PHA-665752 inhibits HGF-stimulated or constitutive phosphorylation of mediators of downstream of c-Met such as Gab-1, ERK, Akt, STAT3, PLC-γ, and FAK in multiple tumor cell lines[1].
PHA-665752 (0-1.25 μM; 18 hours) potently inhibits HGF and c-Met-driven phenotypes such as cell growth (proliferation and survival), cell motility, invasion, and/or morphology of a variety of tumor cells[1].
PHA-665752 (0-1.25 μM; 72 hours) induces apoptosis in both the presence and absence of HGF at concentrations that inhibited tyrosine phosphorylation of c-Met in GTL-16 cells[1].
PHA-665752 (0.0125-0.2 μM; 4 hours) potent inhibits HGF-induced c-Met phosphorylation in A549 cells[1].
In Vivo: PHA-665752 (7.5-30 mg/kg/day; i.v. ; for 9 days) exhibits statistically significant dose-dependent tumor growth inhibition of 68%, 39%, and 20% of vehicle control at the 30 mg/kg/day, 15 mg/kg/day, and 7.5 mg/kg/day doses, respectively[1].
PHA-665752 shows a potent cytoreductive activity in a gastric carcinoma xenograft model[1].

PROTOCOL (Extracted from published papers and Only for reference)

Cell assay [1] S114, GTL-16, NCI-H441, or BxPC-3 cells were seeded in 96-well plates at 9000 cells/well in medium with 10% FBS. After incubation for 48 h in low serum (0.5% FBS, S114; 0.1% FBS, GTL-16, NCI-H441, and BxPC-3), cells were treated with different concentrations of PHA-665752 for 18 h at 37°C. HGF (50 ng/ml) was added for 18 h before BrdUrd for studies involving GTL-16, H441, and BxPC-3. After incubation with BrdUrd labeling reagent for 1 h (Sigma Biochemicals, St. Louis, MO), cells were fixed and BrdUrd incorporation into newly synthesized DNA was assessed using anti-BrdUrd peroxidase-conjugated antibody followed by colorimetric determination at 630 nm. Animal administration [1] In vivo target modulation studies were performed in mice bearing S114 or GTL-16 tumor xenografts to determine the effect of PHA-665752 on c-Met phosphorylation in both single-dose and repeat-dose studies. For all of the target modulation studies, mice bearing established tumors (200–1200 mm3) were administered PHA-665752 or vehicle [L-lactate (pH 4.8) and 10% polyethylene glycol] via bolus i.v. tail vein injection at the desired dose in a volume of 150 μl. At the indicated times after administration, a blood sample was isolated from cardiac left ventricle using a syringe primed with heparin sulfate, mice

were euthanized, and their tumors were resected. Resected tumors were immediately frozen and pulverized using a liquid nitrogen-cooled cryomortar and pestle. Tumor powders were processed into lysates by homogenization in cold lysis buffer with protease and phosphatase inhibitors (35) . The amount of phosphorylated and total c-Met in each sample was determined by immunoblot analysis of protein lysates as described above. Enzyme assay [1] The IC50 values of PHA-665752 for the inhibition of c-Met and various other kinases were determined as described previously (34) . Briefly, c-Met kinase domain GST-fusion protein was used for the c-Met assay, whereas recombinant human full-length or GST-kinase domain fusion proteins were used in other enzyme assays. IC50 measurements of compound versus kinases were based on phosphorylation of kinase peptide substrates or poly-glu-tyr in the presence of ATP and divalent cation (MgCl2 MnCl2 10–20 mM). The linear range (i.e., the time period over which the rate remained equivalent to the initial rate) was determined for each kinase, and all of the kinetic measurements and IC50 determinations were performed within this range. Km values were calculated using the Eadie-Hofstee and Lineweaver-Burke methods with the final ATP concentrations within two to three times the Km value.

References:

[1]. Christensen JG, et al. A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive antitumor activity in vivo. Cancer Res. 2003 Nov 1;63(21):7345-55.

[2]. Ma PC. et al. A selective small molecule c-MET Inhibitor, PHA665752, cooperates with rapamycin. Clin Cancer Res. 2005 Mar 15;11(6):2312-9.

CAIndexNames:

2H-Indol-2-one, 5-[[(2,6-dichlorophenyl)methyl]sulfonyl]-3-[[3,5-dimethyl-4-[[(2R)-2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]carbonyl]-1H-pyrrol-2-yl]methylene]-1,3-dihydro-, (3Z)-

SMILES:

O=C(C1=C(NC(/C=C2C(NC3=C\2C=C(C=C3)S(=O)(CC4=C(C=CC=C4Cl)Cl)=O)=O)=C1C)C)N5[C@H](CCC5)CN6CCCC6

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA