Product Data Sheet

Product Name: INCB3344 R-isomer
Cat. No.: GC36307

Chemical Properties

Cas. No.			
SMILES	CCO[C@H]1CN([C@]2([H])CC[C@](C3=CC(OCO4)=C4C=C3)(O)CC2)C[C@@H]1NC(CNC(C5=CC(C(F)(F)F)=CC=C5)=O)=O		
Formula	$C_{29}H_{34}F_3N_3O_6$	M.Wt	577.59
Solubility	DMSO: ≥ 106.5 mg/mL (184.39 mM)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	INCB3344 R-isomer		

Protocol

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Kinase experiment:

Cells are seeded in 96-well plates in media supplemented with 10% fetal bovine serum (FBS) and transferred to serum-free media [with 0.04% bovine serum albumin (BSA)] after 24 h. In experiments investigating ligand-dependent RTK phosphorylation, corresponding growth factors are added for up to 20 min. After incubation of cells with PF-2341066 for 1 h and/or appropriate ligands for the designated times, cells are washed once with HBSS supplemented with 1 mM Na3VO4, and protein lysates are generated from cells. Subsequently, phosphorylation of selected protein kinases is assessed by a sandwich ELISA method using specific capture antibodies used to coat 96-well plates and a detection antibody specific for phosphorylated tyrosine residues. Antibody-coated plates are (a) incubated in the presence of protein lysates at 4°C overnight; (b) washed seven times in 1% Tween 20 in PBS; (c) incubated in a horseradish peroxidase-conjugated anti-total-phosphotyrosine (PY-20) antibody (1:500) for 30 min; (d) washed seven times again; (e) incubated in 3,3′,5,5′-tetramethyl benzidine peroxidase substrate to initiate a colorimetric reaction that is stopped by adding 0.09 N H2SO4; and (f) measured for absorbance in 450 nm using a spectrophotometer.

Cell experiment:

Tumor cells are seeded in 96-well plates at low density in media supplemented with 10% FBS (growth media) and transferred to serum-free media (0% FBS and 0.04% BSA) after 24 h. Appropriate controls or designated concentrations of PF-2341066 are added to each well, and cells are incubated for 24 to 72 h. Human umbilical vascular endothelial cells (HUVEC) are seeded in 96-well plates in EGM2 media for 5 to 6 h at > 20,000 cells per well and transferred to serum-free media overnight. The following day, appropriate controls or designated concentrations of PF-2341066 are added to each well, and after 1 h incubation, HGF is added to designated wells at 100 ng/mL. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay is done to determine the relative tumor cell or HUVEC numbers.

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Animal experiment:

Athymic mice bearing xenografts (300-800 mm3) are given PF-2341066 in water by oral gavage at designated dose levels. At designated times following PF-2341066 administration, mice are humanely euthanized, and tumors are resected. Tumors are snap frozen and pulverized using a liquid nitrogen-cooled cryomortar and pestle, protein lysates are generated, and protein concentrations are determined using a BSA assay. The level of total and phosphorylated protein is determined using a capture ELISA or immunoprecipitation-immunoblotting method.

References:

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Background

INCB3344 R-isomer is the R-isomer of INCB3344. INCB3344 is a potent CCR2 antagonist.