Product Data Sheet

Product Name: BFH772
Cat. No.: GC15340

Chemical Properties

Cas. No.	890128-81-1		
Chemical Name	6-((6-(hydroxymethyl)pyrimidin-4-yl)oxy)-N-(3-(trifluoromethyl)phenyl)-1-naphthamide		
SMILES	O=C(C1=C2C=CC(OC3=NC=NC(CO)=C3)=CC2=CC=C1)NC4=CC=CC(C(F)(F)F)=C4		
Formula	$C_{23}H_{16}F_3N_3O_3$	M.Wt	439.39
Solubility	DMF: 30 mg/ml,DMSO: 30 mg/ml,DMSO:PBS (pH 7.2) (1:2): 0.33 mg/ml	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	BFH772		

Protocol

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Kinase experiment: In vitro kinase assay is based on a filter binding assay, using the recombinant GST-fused kinase domains expressed in baculovirus and purified over glutathione-sepharose, γ-[33P]ATP as the phosphate donor, and poly(Glu:Tyr 4:1) peptide as the acceptor. Each GST-fused kinase is incubated under optimized buffer conditions [20 mM Tris-HCl buffer (pH 7.5), 1-3 mM $MnCl_2$, 3-10 mM $MgCl_2$, 3-8 μg/mL poly(Glu:Tyr 4:1), 0.25 mg/mL polyethylene glycol 20000, 8 μM ATP, 10 μM sodium vanadate, 1 mM DTT] and 0.2 μCi γ-33P ATP in a total volume of 30 μL in the presence or absence of a test substance for 10 min at ambient temperature. The reaction is stopped by adding 10 mL of 250 mM EDTA. Using a 384-well filter system, half the volume is transferred onto an Immobilon-polyvinylidene difluoride membrane. The membrane is then washed extensively and dried, and scintillation counting is performed. IC50s for compounds are calculated by linear regression analysis of the percentage inhibition[1].

Cell experiment: Different Ba/F3 cell lines rendered IL-3 independent by transduction with various constitutively active tyrosine kinases are grown in RPMI 1640 medium containing 10% fetal calf serum. For maintenance of parental Ba/F3 cells, the medium is additionally supplemented with 10 ng/mL interleukin-3 (IL-3). For proliferation assays, Ba/F3 cells are seeded on 96-well plates in triplicates at 10000 cells per well and incubated with various concentrations of compounds for 72 h followed by quantification of viable cells using a resazurin sodium salt dye reduction readout (commercially known as Alamar Blue assay). IC50s are determined with the XLFit Excel Add-In using a four-parameter dose response model[1].

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Animal experiment:

Mice[1] Female FVB mice weighing between 18 and 20 g are housed in groups of six. Porous chambers containing VEGF (2 μg/mL) in 0.5 mL of 0.8% w/v agar (containing heparin, 20 U/mL) are implanted subcutaneously in the flank of the mice (n=6 per group). VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and TIE-2 levels of the tissue. Mice are treated either orally once daily with compounds or vehicle (PEG200 100%, 5 mL/kg) starting 4-6 h before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 h after the last dose. Tissue weight is taken and then a lysate prepared for TIE-2 ELISA analysis .Rats[1] Catheters are implanted into the femoral artery and vein of naïve female rats strain OFA for BFH772, and BAW2881, or in the jugular vein and femoral artery in female Sprague-Dawley rats for compounds 4, 9, and 10. Animals are allowed to recover for 96 h and are housed in single cages with free access to food and water throughout the experiment. Female OFA rats received 2.5 mg/kg of BAW2881 dissolved in ethanol/dimethylisosorbide/polyethylene glycol400/D5W (10/15/35/40 v/v) or 1 mg/kg of BFH772 dissolved in N-methyl pyrrolidone/polyethylene glycol200 (30:70, v/v) via injection into the femoral vein. D5W is glucose 5%/water (v/v). Oral administration: BAW2881 and BFH772 are formulated as a micronized suspension (dissolved/suspended in 0.5% carboxymethyl cellulose in distilled water) and administered by gavage to female OFA rats to deliver a dose of 25 mg/kg for BAW2881 or 3 mg/kg BFH772 (n=4 rats per group). For compounds 4, 9, and 10, female Sprague-Dawley rats at 8 weeks of age received an intravenous dose of 3 mg/kg 4, 9, and 10, formulated in ethanol/NMP/polyethylene glycol400/D5W (10/10/50/30) (n=2 rats per group), or a suspension in 0.5% carboxymethyl cellulose in distilled water dosed at 50 mg/kg (n=3 rats per group). At the allotted times, blood samples are collected into heparinized tubes, and the amount of compound in plasma determined by HPLC/MS-MS.

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References:

[1]. Bold G, et al. A Novel Potent Oral Series of VEGFR2 Inhibitors Abrogate Tumor Growth by Inhibiting Angiogenesis. J Med Chem. 2016 Jan 14;59(1):132-46.

Background

IC50: 3 nM

BFH772 is a VEGFR2 inhibitor.

The VEGF family including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF signals through VEGFR1, VEGFR2, and VEGFR3 cell surface tyrosine kinase receptors that are located on the host vascular endothelium, lymphatic, and hematopoietic systems.

In vitro: BFH772 was highly effective at targeting VEGFR2 kinase, however, lost 500-fold potency on FLK-1, FLT-1, and FLT-4. BFH772 also targeted B-RAF, RET, and TIE-2, albeit with at least 40-fold lower potency. BFH772 inhibited the ligand induced autophosphorylation of RET, PDGFR, and KIT kinases. BFH772 was selective against the kinases of EGFR, ERBB2, INS-R, and IGF-1R and against the cytoplasmic BCR-ABL kinase [1].

In vivo: The dose response curves of BFH772 at 0.3, 1, and 3 mg/kg showed that even at

the lowest concentrations, this naphthalene-1-carboxamide inhibited VEGF induced tissue weight and TIE-2 levels but only reached statistical significance at 1 mg/kg and above. Moreover, BFH772 at 3 mg/kg orally dosed once per day could potently inhibit melanoma growth (54-90% for primary tumor and 71-96% for metastasis tumor) when compared with control ratios [1].

Clinical trial: A proof of concept study to evaluate the safety, tolerability, and efficacy of 12 week administration of BFH772 ointment in rosacea patients has completed, but no result data are released [2].

References:
[1] Bold G, et al. A Novel Potent Oral Series of VEGFR2 Inhibitors Abrogate Tumor Growth by Inhibiting Angiogenesis. J Med Chem. 2016 Jan 14;59(1):132-46.
[2] http://adisinsight. springer.com/trials/700244037