Product Data Sheet

Product Name: CEP-32496 hydrochloride
Cat. No.: GC17860

Chemical Properties

Cas. No.	1227678-26-3		
Chemical Name	1-[3-(6,7-dimethoxyquinazolin-4-yl)oxyphenyl]-3-[5-(1,1,1-trifluoro-2-methylpropan-2-yl)-1,2-oxazol-3-yl]urea;hydrochloride		
SMILES	CC(C)(C1=CC(=NO1)NC(=O)NC2=CC(=CC=C2)OC3=NC=NC4=CC(=C(C=C43)OC)OC)C(F)(F)F.Cl		
Formula	$C_{24}H_{23}ClF_3N_5O_5$	M.Wt	553.92
Solubility	Soluble in DMSO	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	CEP-32496 hydrochloride		

Protocol

Cell experiment:

A375 cells are seeded at 10,000 cells per well in DMEM with 10% fetal calf serum and allowed to attach. The cells are washed with PBS and switched to DMEM with 0.5% of serum and incubated overnight. The test compounds (e.g., Agerafenib (CEP-32496); 10 μM) are then added at various concentrations with a final DMSO concentration of 0.5% and incubated for 72 h. At the end of incubation, a Cell Titer Blue is added per instructions, and incubation is continued for 3 h. Remaining viable cells are quantified by measuring the strength of the fluorescence signal using SoftMax Pro (excitation at 560 nm and emission at 590 nm). IC50 values are derived using a 9-point curve and are presented as mean values from experiments performed in duplicate[1].

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Animal experiment:

Mice[1]Six to eight week old athymic nu/nu nude mice (20-25 g) are inoculated subcutaneously with Colo-205 tumor cells (1×106/mouse) in the right flank. Upon reaching an average tumor volume of 150-200 mm3 (10-12 days post implantation), animals are randomized into treatment groups (n=10 mice/group). Each group is dosed orally for 14 days with either vehicle only (22% HPβCD) or with Agerafenib (CEP-32496) at 10, 30, or 100 mg/kg twice daily (BID), and each dose of drug is given in a volume of 0.1 mL per 20 g of body weight, adjusted for the body weight of the animal. Tumor volumes are measured three times weekly using vernier calipers, and volumes are calculated[1].

References:

[1]. Rowbottom MW, et al. Identification of 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropan-2-yl)isoxazol-3-yl)urea hydrochloride (CEP-32496), a highly potent and orally efficacious inhibitor of V-RAF murine sarcoma viral oncogene homologue B1 (BRAF) V600E.

Background

CEP-32496 is a selective inhibitor of BRAFV600E with IC50 value of 669 nM [1].
BRAF is a member of Raf kinase family and plays an important role in sending signals (directing cell growth) inside cells. BRAF involves in regulating MAP kinase/ERKs signaling pathway and mutant BRAFV600E activates MAPK pathway, it has been shown that BRAF mutant BRAFV600E is correlated with several human cancers [2].
CEP-32496 is a potent BRAFV600E inhibitor and has a different selectivity with the reported BRAFV600E inhibitor RG7204. Using Ambit Kinomescan Assay, it was shown that CEP-32496

potently binding to RAF family with Kd value ranged from 14 to 39 nmol/L. When tested with a panel of BRAF mutant (A375, SK-MEL-28, Colo-679, HT-144, and Colon-205) cell lines, CEP-32496 showed a selective cytotoxicity profile against tumor cell lines with V600E mutant while had no effect on wild type cell lines (HCT116, Hs578T, LNCaP, DU145, PC-3) [1]. CEP-32496 treatment exhibited high cytotoxicity against human tumor cell lines with expression of BRAFV600E while had little effect on wild type BRAF expressed cell lines [2].
In nude mice model with Colo-205 tumor cells subcutaneous xenograft, oral administration of CEP-32496 significantly inhibited pMEK normalization, induced tumor stasis (40%) while control group did not have this phenomenon in a dose of 30 mg/kg [1].
CEP-32496 also inhibits MEK phosphorylation with IC 50 value of 60 nM [1].

References:

[1].James, J., et al., CEP-32496: a novel orally active BRAF(V600E) inhibitor with selective cellular and in vivo antitumor activity. Mol Cancer Ther, 2012. 11(4): p. 930-41.

[2].Rowbottom, M.W., et al., Identification of 1-(3-(6,7-dimethoxyquinazolin-4-yloxy)phenyl)-3-(5-(1,1,1-trifluoro-2-methylpropa n-2-yl)isoxazol-3-yl)urea hydrochloride (CEP-32496), a highly potent and orally efficacious inhibitor of V-RAF murine sarcoma viral oncogene homologue B1 (BRAF) V600E. J Med Chem, 2012. 55(3): p. 1082-105.