Product Data Sheet

Product Name: WHI-P180 hydrochloride
Cat. No.: GC37933

Chemical Properties

Cas. No.	153437-55-9		
SMILES	COC1=CC2=NC=NC(NC3=CC=CC(O)=C3)=C2C=C1OC.[H]Cl		
Formula	$C_{16}H_{16}ClN_3O_3$	M.Wt	333.77
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	WHI-P180 hydrochloride		

Protocol

Kinase experiment:

Inhibitors (WHI-P180) are pre-incubated in the plate for 15 min with 5 µL kinase and assay buffer at the following concentrations; 13 pM RET and 150 pM KDR. The reaction is initiated by the addition of 5 µL ATP and substrate at 2×final reaction concentrations. For RET, this is 18 µM and 2 µM; for KDR, this is 16 µM and 1 µM, respectively. Reactions are performed at ATP Km for each target. The assay is allowed to proceed at room temperature for 20 min before terminating with the addition of 10 µL HTRF detection buffer containing EDTA supplemented with TK-antibody labelled with Eu3+-Cryptate (1:100 dilution) and streptavidin-XL665 (128 nM). Following incubation at room temperature for 1 h, FRET signal is measured[1].

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Cell experiment:

IL3-dependent BaF3 cells are modified to express an activated recombinant kinase. Following removal of IL3, the modified cells are dependent on the activity of the recombinant kinase for survival and proliferation. The BaF3 cell lines, expressing KIF5B-RET and KDR are maintained in RPMI-1640 media containing 10% FBS and appropriate antibiotics. Non-modified BaF3 cells (WT) are maintained in RPMI-1640 media containing 10% FBS and supplemented with 10 ng/mL recombinant mouse IL3. For assessment of compound IC50, cells are plated into 384-well plates at 1500 or 3000 cells per well in 30 µL culture medium and compounds dispensed using an acoustic liquid handling platform. Following incubation of the cells for 48 h at 37 °C in a humidified 5% CO2 atmosphere, viability is determined by addition of 10 µL CellTiter-Glo reagent and measurement of luminescence[1].

Animal experiment:

Mice: A high performance liquid chromatography (HPLC)-based quantitative detection method is used to measure plasma WHI-P180levels in mice. The plasma concentration-time data is fit to a single compartment pharmacokinetic model by using the WinNonlin program to calculate the pharmacokinetic parameters. A cutaneous anaphylaxis model is used to examine the pharmacodynamic effects of WHI-P180 on anaphylaxis-associated vascular hyperpermeability[3].

References:

[1]. Newton R, et al. The discovery of 2-substituted phenol quinazolines as potent RET kinase inhibitors

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with improved KDR selectivity. Eur J Med Chem. 2016 Apr 13;112:20-32.
[2]. Ghosh S, et al. 4-[3-Bromo-4-hydroxyphenyl)amino]-6,7-dimethoxyquinazolin-1-ium chloride methanol solvateand 4-[(3-hydroxyphenyl)amino]-6,7-dimethoxy-1-quinazolinium chloride. Acta Crystallogr C. 2001 Jan;57(Pt 1):76-8.
[3]. Chen CL, et al. Pharmacokinetics and biologic activity of the novel mast cell inhibitor, 4-(3-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline in mice. Pharm Res. 1999 Jan;16(1):117-22.

Background

WHI-P180 is a multi-kinase inhibitor with IC_{50} values of 4.5 and 66 nM for the human proto-oncogene RET and kinase insert domain receptor (KDR), respectively.[1] It also inhibits EGFR (IC_{50} = 4 μM) and binds to tau-tubulin kinase 1 (TTBK1; K_ds = 0.46 and 0.24 μM for phosphorylated and non-phosphorylated TTBK1, respectively).[2,3] WHI-P180 inhibits JAK3 and JAK3-driven graft *versus* host disease responses in mice receiving

allogenic bone marrow and splenocyte grafts.[2,4] WHI-P180 (25 mg/kg, i.p) inhibits IgE-induced vascular hyperpermeability in a mouse model of passive anaphylaxis.[5]

1.Newton, R., Bowler, K.A., Burns, E.M., et al.The discovery of 2-substituted phenol quinazolines as potent RET kinase inhibitors with improved KDR selectivityEur. J. Med. Chem.1320-32(2016) 2.Ghosh, S., Jennissen, J.D., Liu, X.P., et al.4-[3-Bromo-4-hydroxyphenyl)amino]-6,7-dimethoxyquinazolin-1-ium chloride methanol solvate and 4-[(3-hydroxyphenyl)amino]-6,7-dimethoxy-1-quinazolinium chlorideActa. Crystallogr. C.57(Pt 1)76-78(2001) 3.Xue, Y., Wan, P.T., Hillertz, P., et al.X-ray structural analysis of tau-tubulin kinase?1 and its interactions with small molecular inhibitorsChemMedChem8(11)1846-1854(2013) 4.Cetkovic-Cvrlje, M., Roers, B.A., Schonhoff, D., et al.Treatment of post-bone marrow transplant acute graft-versus-host disease with a rationally designed JAK3 inhibitorLeuk. Lymphoma.43(7)1447-1453(2002) 5.Chen, C.-L., Malaviya, R., Navara, C., et al.Pharmacokinetics and biologic activity of the novel mast cell inhibitor, 4-(3-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline in micePharm. Res.16(1)117-122(1999)