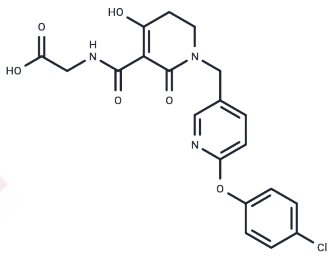
Data Sheet (Cat.No.T5392)

TargetMol

TP0463518

Chemical Properties



CAS No. : 1558021-37-6

Formula: C20H18ClN3O6

Molecular Weight: 431.83

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	TP-0463518 is a highly potent HIF prolyl hydroxylase (PHD) inhibitor (IC50s: 13 nM and 18 nM for human and rat PHD2, respectively).
Targets(IC50)	HIF/HIF Prolyl-Hydroxylase
In vitro	TP0463518 competitively inhibited human PHD2 with a Ki value of 5.3?nM. TP0463518 also inhibited human PHD1/3 with IC50 values of 18 and 63?nM as well as monkey PHD2 with an IC50 value of 22?nM.
In vivo	In normal mice and rats, TP0463518 significantly increased the serum EPO levels at doses of 5 and 20?mg/kg, respectively. TP0463518 also increased the serum EPO level in 5/6 nephrectomized chronic kidney disease model rats at a dose of 10?mg/kg, with a correlation factor for serum EPO and the serum TP0463518 levels of 0.82. TP0463518 was promptly removed with a half-life of 5.2?h and increased the area under the curve (AUC) of EPO at a dose of 5?mg/kg.
Kinase Assay	The PHDs inhibition studies were performed using fluorescence polarization.FITC-HIF and 2-oxoglutarate were mixed with enzyme solution in a reaction buffer (20 mM Tris-HCl [pH 7.5],5 mM KCl,1.5 mM MgCl2,10 μM FeSO4,2 mM ascorbic acid,1 mM DTT) with or without various concentrations of TP0463518.The concentrations of FITC-HIF and 2-oxoglutarate were twice the Km values of each enzyme.The reaction temperature was 30?C,and the reaction time was optimized to each PHD enzyme to obtain the initial velocity (9 to 20 min).At the end of the reaction,a stop solution containing 20 mM of EDTA and anti-hydroxylated HIF antibody was added to the reaction buffer.Then,the fluorescence (ex: 480 nm,em: 535 nm) was measured using EnVision to calculate the millipolarization (mP) value.The mP values and the corresponding hydroxylated HIF concentration were proportional,so we used the mP values as the activities.The IC50 values were calculated using SAS version 9.2 using a nonlinear least-squares method.To determine the mode of inhibition,the activity of PHD2 was measured with various concentrations of 2-oxoglutarate (0.025 to 8 μM) and TP0463518 (0 to 40 μM).Then the apparent Vmax and Km corresponding to each TP0463518 concentration were compared.The mode of inhibition was confirmed using a double reciprocal plot.
Animal Research	Nine-week-old Balb/c mice were randomly assigned to a vehicle or a 5 to 40 mg/kg dose of TP0463518 group. The mice were orally treated with 0.5% methylcellulose or a TP0463518 dosing suspension. Blood was collected at 6 h after administration from the orbital plexus under deep anesthesia, and euthanasia was performed without

Animal Research	awakening. An aliquot of blood was mixed with EDTA, and the remaining blood sample was left to stand at room temperature for 15 min. The samples were then centrifuged (2130 x g for 10 min at 4°C) to prepare the plasma and serum.

Solubility Information

Solubility	DMSO: 125 mg/mL (289.47 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+90% Saline: < 10 mg/mL (23.16 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. 10% DMSO+40% PEG300+5% Tween 80+45% Saline: 10 mg/mL (23.16 mM),Solution. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.3157 mL	11.5786 mL	23.1573 mL
5 mM	0.4631 mL	2.3157 mL	4.6315 mL
10 mM	0.2316 mL	1.1579 mL	2.3157 mL
50 mM	0.0463 mL	0.2316 mL	0.4631 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Kato S, et al. TP0463518, a novel inhibitor for hypoxia-inducible factor prolyl hydroxylases, increases erythropoietin in rodents and monkeys with a good pharmacokinetics-pharmacodynamics correlation. Eur J Pharmacol. 2018 Nov 5;838:138-144.