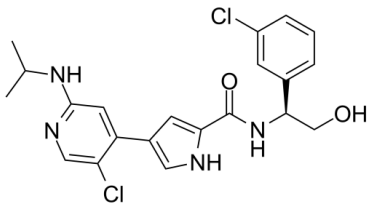
Data Sheet

Product Name:	Ulixertinib
Cat. No.:	CS-2115
CAS No.:	869886-67-9
Molecular Formula:	$C_{21}H_{22}Cl_2N_4O_2$
Molecular Weight:	433.33
Target:	ERK
Pathway:	MAPK/ERK Pathway; Stem Cell/Wnt
Solubility:	DMSO : 100 mg/mL (230.77 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

Ulixertinib (BVD-523; VRT752271) is a potent, orally active, highly selective, ATP-competitive and reversible covalent inhibitor of **ERK1/2** kinases, with an **IC_{50}** of <0.3 nM against ERK2. Ulixertinib (BVD-523; VRT752271) inhibits the phosphorylated ERK2 (pERK) and downstream kinase RSK (pRSK) in an A375 melanoma cell line[1][2]. IC50 & Target: IC50: <0.3 nM (ERK2)[2] **In Vitro:** Combined Ulixertinib (BVD-523; 10, 20, 30 μM; 48 hours) and VS-5584 treatment causes significant induction of cell death in human pancreatic cancer (HPAC) cells in PDAC cell lines BxPC-3, MIAPaCa-2, and CFPAC-1[3]. **In Vivo:** In the pharmacokinetic study, the sensitivity and specificity of the assay are found to be sufficient for accurately characterizing the plasma pharmacokinetics of Ulixertinib (VRT752271) in Balb/C mice[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [2]The MEK autophosphorylation assay is performed using the ADP Glo Kinase Assay kit. Activated MEK protein is expressed and purified in-house. Enzyme and substrate solutions are prepared in assay buffer consisting of 50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 0.1 mM EGTA, 10 mM DTT and 0.01 % Tween 20. 6 nM MEK protein is prepared in assay buffer and 2 μL is dispensed into each well of a 384-well white small volume medium bind plate containing test and reference control compounds. Compound plates are dosed with a 12 point dose response curve from 10 μM down to 0.0625 nM in order to calculate compound IC_{50}s, with a total DMSO concentration in the assay of 1%. Following a 15 minute pre-incubation of enzyme and compound at room temperature, 2 μL of a 20 μM Ultra Pure ATP solution in assay buffer is added to the wells, and the reaction is allowed to progress for 90 minutes at room temperature before the addition of 4 μL ADP Glo reagent R1 to quench the reaction. The plate is incubated at room temperature for 45 minutes before the addition of 8 μL Kinase Detection Reagent, and then the luminescence signal is allowed to equilibrate for 60 minutes before the plates are read on a Pherastar plate reader. **Cell Assay:** [2]A375 cells are cultured in cell media composed of DMEM, 10% (v/v) Foetal Calf Serum and 1% (v/v) L-Glutamine. After harvesting, cells are dispensed into black, 384-well Costar plates to give 200 cells per well in a total volume of 40 μL cell media, and are incubated overnight at 37°C, 90% relative humidity and 5% CO_2 in a rotating incubator. Test compounds and reference controls are dosed directly into the cell plates, into the inner 308 wells. The cells are dosed over a 12 point range from 30 μM down to 0.03 nM in order to calculate compound IC_{50}s, with a total DMSO concentration in the assay of 0.3%. The cell plates are then incubated for 72 hours at 37°C. Cells are fixed and stained by the addition of 20 μL 12% formaldehyde in PBS/A (4% final concentration) and 1:2000 dilution of Hoechst 33342, with a 30 minute room temperature incubation, and then washed with PBS/A. A cell count is performed on the stained cell plates using a Cellomics ArrayScanTM VTI imaging platform. A Day 0 cell plate is also fixed, stained and read to generate a cell count baseline for determining compound cytotoxic effects as well as anti-proliferative effects.

References:

[1]. Ward RA, et al. Structure-Guided Design of Highly Selective and Potent Covalent Inhibitors of ERK1/2. J Med Chem. 2015 Jun 11;58(11):4790-801.

[2]. Kumar R, et al. Determination of ulixertinib in mice plasma by LC-MS/MS and its application to a pharmacokinetic study in mice. J Pharm Biomed Anal. 2016 Jun 5;125:140-4.

[3]. Changwen Ning, et al. Targeting ERK Enhances the Cytotoxic Effect of the Novel PI3K and mTOR Dual Inhibitor VS-5584 in Preclinical Models of Pancreatic Cancer. Oncotarget. 2017 Jul 4;8(27):44295-44311.

CAIndexNames:

1H-Pyrrole-2-carboxamide, 4-[5-chloro-2-[(1-methylethyl)amino]-4-pyridinyl]-N-[(1S)-1-(3-chlorophenyl)-2-hydroxyethyl]-

SMILES:

O=C(C1=CC(C2=CC(NC(C)C)=NC=C2Cl)=CN1)N[C@@H](C3=CC=CC(Cl)=C3)CO

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