Product Data Sheet

Product Name: Erlotinib mesylate
Cat. No.: GC36003

Chemical Properties

Cas. No.	248594-19-6		
SMILES	CS(=O)(O)=O.COCCOC1=CC2=NC=NC(NC3=CC=CC(C#C)=C3)=C2C=C1OCCOC		
Formula	$C_{23}H_{27}N_3O_7S$	M.Wt	489.54
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	Erlotinib mesylate		

Protocol

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Kinase experiment:	The kinase reaction is performed in 50 µL of 50 mM HEPES (pH 7.3), containing 125 mM NaCl, 24 mM MgCl2, 0.1 mM Na3VO4, 20 µM ATP, 1.6 µg/mL EGF, and 15 ng of EGFR, affinity purified from A431 cell membranes. The compound in DMSO is added to give a final DMSO concentration of 2.5%. Phosphorylation is initiated by addition of ATP and proceeded for 8 mm at room temperature, with constant shaking. The kinase reaction is terminated by aspiration of the reaction mixture and is washed 4 times with wash buffer. Phosphorylated PGT is measured by 25 mim of incubation with 50 µL per well HRP-conjugated PY54 antiphosphotyrosine antibody, diluted to 0.2 µg/mL in blocking buffer (3% BSA and 0.05% Tween 20 in PBS). Antibody is removed by aspiration, and the plate is washed 4 times with wash buffer. The colonmetric signal is developed by addition of TMB Microwell Peroxidase Substrate, 50 µL per well, and stopped by the addition of 0.09 M sulfuric acid, 50µL per well. Phosphotyrosine is estimated by measurement of absorbance at 450 nm. The signal for controls is typically 0.6-1.2 absorbance units, with essentially no back ground in wells without AlP, EGFR, or POT and is proportional to the time of incubation for 10 mm[1].

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Cell experiment:

To test the viability of cells treated with B-DIM, Erlotinib, or the combination, BxPC-3 and MIAPaCa cells are plated (3,000-5,000 per well) in a 96-well plate and incubated overnight at 37°C. A range of concentrations for both B-DIM (10-50 μM) and Erlotinib (1-5 μM) is initially tested. Based on the initial results, the concentration of B-DIM (20 μM) and Erlotinib (2 μM) are chosen for all assays. The effects of B-DIM (20 μM), Erlotinib (2 μM), and the combination on BxPC-3 and MIAPaCa cells are determined by the standard MTT assay after 72 h and is repeated three times. The color intensity is measured by a Tecan microplate fluorometer at 595 nm. DMSO-treated cells are considered to be the untreated control and assigned a value of 100%. In addition to the above assay, we have also done clonogenic assay for assessing the effects of treatment[2].

Animal experiment:

Mice[3] Bcrp1/Mdr1a/1b-/- and WT mice are treated p.o. or i.p. with 5 mg/kg Erlotinib. The i.p. administration is chosen assuming good drug absorption and complete bioavailability. Sampling is done from the tip of the lateral tail vein in three series. During the first series, whole blood samples are collected at 15 min and 0.5, 1.5, 5, and 10 h after injection. Based on the results of this initial group, the sampling times of the two subsequent series are adapted to 5 and 15 min and 0.5, 1.5, 4, and 8 h after injection. After collection, the blood samples are immediately centrifuged and plasma is stored at -20°C until high-performance liquid chromatographic analysis took place. Rats[4] Seven-week-old male Crl:CD (SD) rats (244-297 g) are used. The animals are treated with Erlotinib (10 mg/kg and 20 mg/kg) orally by gavage.

References:

[1]. Moyer JD, et al. Induction of apoptosis and

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cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res. 1997 Nov 1;57(21):4838-48.
[2]. Ali S, et al. Apoptosis-inducing effect of erlotinib is potentiated by 3,3'-diindolylmethane in vitro and in vivo using an orthotopic model of pancreatic cancer. Mol Cancer Ther, 2008, 7(6), 1708-1719.
[3]. Marchetti S, et al. Effect of the ATP-binding cassette drug transporters ABCB1, ABCG2, and ABCC2 on erlotinib hydrochloride (Tarceva)

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[4]. Adachi K, et al. Effects of erlotinib on lung injury induced by intratracheal administration of bleomycin (BLM) in rats. J Toxicol Sci. 2010 Aug;35(4):503-14.

Background

Erlotinib is a tyrosine kinase inhibitor which acts on the epidermal growth factor receptor (EGFR), inhibiting EGFR-associated kinase activity (IC_{50} = 2.5 ?M).[1,2] This inhibits tumor growth in human head and neck carcinoma HN5 tumor xenografts in mice with an ED_{50} value of 9 mg/kg.[1] Erlotinib also suppresses cyclin-dependent kinase 2 (Cdk2) activity in breast cancer cells (IC_{50} = 4.6 ?M) and JAK2 mutant $JAK2^{V617F}$ positive hematopoietic progenitor cells (IC_{50} = 5 ?M), which is associated with polycythemia vera, idiopathic myelofibrosis, and essential thrombocythemia.[3,4] Formulations containing erlotinib have been used to treat certain forms of cancer, including non-small cell lung cancer.[5,6]

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