Product Data Sheet

Erlotinib

Cat. No.: HY-50896 CAS No.: 183321-74-6 Molecular Formula: $C_{22}H_{23}N_3O_4$ Molecular Weight: 393.44

Target: EGFR; Autophagy

Pathway: JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Autophagy

Storage: Powder -20°C 3 years

In solvent

4°C 2 years -80°C 1 year

-20°C 6 months

SOLVENT & SOLUBILITY

In Vitro

DMSO: 3.94 mg/mL (10.01 mM; ultrasonic and warming and heat to 40°C)

Preparing Stock Solutions	Solvent Mass Concentration	1 mg	5 mg	10 mg
	1 mM	2.5417 mL	12.7084 mL	25.4168 mL
	5 mM	0.5083 mL	2.5417 mL	5.0834 mL
	10 mM	0.2542 mL	1.2708 mL	2.5417 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- 1. Add each solvent one by one: 0.5% CMC-Na/saline water Solubility: 10 mg/mL (25.42 mM); Suspended solution; Need ultrasonic
- 2. Add each solvent one by one: 50% PEG300 >> 50% saline Solubility: 10 mg/mL (25.42 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description	Erlotinib (CP-358774) is a directly acting EGFR tyrosine kinase inhibitor, with an IC $_{50}$ of 2 nM for human EGFR. Erlotinib reduces EGFR autophosphorylation in intact tumor cells with an IC $_{50}$ of 20 nM. Erlotinib is used for the treatment of non-small cell lung cancer ^[1] . Erlotinib is a click chemistry reagent, it contains an Alkyne group and can undergo copper-catalyzed azide-alkyne cycloaddition (CuAAc) with molecules containing Azide groups.	
IC ₅₀ & Target	EGFR 2 nM (IC ₅₀ , Cell Free Assay)	
In Vitro	Erlotinib (CP-358774) is also a potent inhibitor of the recombinant intracellular (kinase) domain of the EGFR, with an IC ₅₀ of 1 nM. The proliferation of DiFi cells is strongly inhibited by Erlotinib with an IC ₅₀ of 100 nM for an 8-day proliferation assay ^[1] .	

The combination of B-DIM and Erlotinib (2 μ M) results in a significant inhibition of colony formation in BxPC-3 cells when compared with either agent alone. The combination of B-DIM and Erlotinib (2 μ M) results in a significant induction of apoptosis only in BxPC-3 cells when compare with the apoptotic effect of either agent alone^[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

Under the experimental conditions, the combination of B-DIM and Erlotinib (50 mg/kg, i.p.) treatment shows significant decrease (P <0.01) in tumor weight compared with untreated control [2]. Erlotinib (20 mg/kg, p.o.) significantly attenuates Cisplatin (CP)-induced body weight (BW) loss when compared to the CP+vehicle (V) rats (P<0.05). Erlotinib treatment significantly improves renal function in CP-N(normal control group, NC) rats. The CP+Erlotinib (E) rats show significant reduction of the levels of Serum creatinine (s-Cr) (P<0.05), blood urea nitrogen (BUN) (P<0.05), urinary N-acetyl- β -D-glucosaminidase (NAG) index (P<0.05), and significant increase of urine volume (UV) (P<0.05) and Cr clearance (Ccr) (P<0.05) compare to the CP+V rats [3]MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]

The kinase reaction is performed in 50 μ L of 50 mM HEPES (pH 7.3), containing 125 mM NaCl, 24 mM MgCl₂, 0.1 mM Na₃VO₄, 20 μ M ATP, 1.6 μ g/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 μ L per well HRP-conjugated PY54 antiphosphotyrosine antibody, diluted to 0.2 μ g/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 μ L per well, and stopped by the addition of 0.09 M sulfuric acid, 50 μ L 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 Assay [2]

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 μ M) and Erlotinib (1-5 μ M) is initially tested. Based on the initial results, the concentration of B-DIM (20 μ M) and Erlotinib (2 μ M) are chosen for all assays. The effects of B-DIM (20 μ M), Erlotinib (2 μ M), 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration [2][3]

Mice^[2]

Female ICR-SCID (6-7 weeks old) mice are randomized into the following treatment groups (n=7): (a) untreated control; (b) only B-DIM (50 mg/kg body weight), intragastric once every day; (c) Erlotinib (50 mg/kg body weight), everyday i.p. for 15 days; and (d) B-DIM and Erlotinib, following schedule as for individual treatments. All mice are killed on day 3 following last dose of treatment, and their body weight is determined. One part of the tissue is rapidly frozen in liquid nitrogen and stored at -70°C for future use and the other part is fixed in formalin and processed for paraffin block. H&E staining of fixed tissue section is used to confirm the presence of tumor(s) in each pancreas.

Six-week-old male Sprague-Dawley (SD) rats weighing 180 to 210 g are used. Cisplatin (CP) is freshly prepared in saline at a concentration of 1 mg/mL and then injected intraperitoneally in SD rats (n=28) at a dose of 7 mg/kg on day 0. To investigate the effect of Erlotinib, 28 CP-N rats are divided into two groups. Separate groups (n=14) each of animals are administered with either Erlotinib (20 mg/kg) (CP+E, n=14) or vehicle (CP+V, n=14) daily by oral gavage from day -1 (24 hours prior to the CP injection) to day 3. Vehicle-treated groups receive an equivalent volume of saline. Five male SD rats at the age of 6 weeks are used as a normal control group (NC, n=5). The NC rats are given an equivalent volume of saline daily by oral gavage from day -1 to day 3. At day 4 (96 hours after CP injection), each rat is anesthetized and sacrificed by exsanguination after the

cardiac puncture; blood is collected by cardiac puncture and kidneys are collected. Renal tissue is divided; separate portions are snap-frozen in liquid nitrogen or fixed in 2% paraformaldehyde/phosphate-buffered saline (PBS) for later use. All surgery is performed under diethyl ether gas anesthesia, and all efforts are made to minimize suffering.

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CUSTOMER VALIDATION

- Cell Res. 2020 Oct;30(10):833-853.
- Nat Immunol. 2022 Feb;23(2):251-261.
- Bioact Mater. 2021 Nov 4;13:312-323.
- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- Nat Commun. 2023 Apr 24;14(1):2342.

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REFERENCES

[1]. Moyer JD, et al. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res. 1997, 57(21), 4838-4848.

[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]. Wada Y, et al. Epidermal growth factor receptor inhibition with erlotinib partially prevents cisplatin-induced nephrotoxicity in rats. PLoS One. 2014 Nov 12;9(11):e111728.

Caution: Product has not been fully validated for medical applications. For research use only.

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