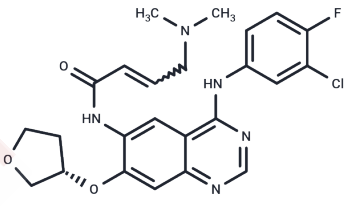
Data Sheet (Cat.No.T2303)

TargetMol

(S)-Afatinib

Chemical Properties



CAS No. : 439081-18-2

Formula: C24H25ClFN5O3

Molecular Weight: 485.94

Appearance: no data available

Storage: keep away from moisture,keep away from direct sunlight
Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	(S)-Afatinib (BIBW2992) is an irreversible EGFR family inhibitor with IC50s of 0.5/0.4/10/14/1 nM for EGFRwt, EGFR (L858R), EGFR (L858R/T790M), HER2, and HER4, respectively.
Targets(IC50)	EGFR,HER
In vitro	(S)-Afatinib shows potent activity against wild-type and mutant forms of EGFR and HER2 (IC50s: 0.5, 0.4, 10, 14 nM for EGFRwt, EGFR L858R, EGFR L858R/T790M, and HER2, respectively). In the human breast cancer cell line, treatment with 100 nM (S)-Afatinib was sufficient to prevent heregulin-stimulated HER3 phosphorylation [1]. Esophageal squamous cell carcinoma (ESCC) cell lines were sensitive to afatinib with IC50 concentrations at lower micromolar range (at 72 hour incubation: HKESC-1 = 0.002 μM, HKESC-2 = 0.002 μM, KYSE510 = 1.090 μM, SLMT-1 = 1.161 μM and EC-1 = 0.109 μM). The phosphorylation of ErbB family downstream effectors such as pAKT, pS6 and pMAPK were significantly inhibited in HKESC-2 and EC-1. Apoptosis was observed in both cell lines at 24 hours after exposure to afatinib [2].
In vivo	Daily oral treatment with BIBW2992 at 20 mg/kg for 25 days resulted in dramatic tumor regression and downregulation of EGFR and AKT phosphorylation. Xenograft tumor formation by the NCIH1975 cell line, expressing EGFR L858R/T790M, was effectively controlled by BIBW2992, with a T/C value of 12% for doses of 20 mg/kg [1]. Afatinib could effectively inhibit HKESC-2 tumor growth in mice without obvious toxicity. Afatinib alone has shown excellent growth inhibitory effect on ESCC in in vivo models [2].
Kinase Assay	The wild type tyrosine kinase domain of the human EGFR, as well as the EGFR L858R/T790M double mutant, were fused to Glutathione-S-transferase (GST) and extracted as described in Supplementary methods. The L858R mutant was purchased from Upstate. Enzyme activity was then assayed in the presence or absence of serial inhibitor dilutions performed in 50% Me2SO. A random polymer pEY (4:1) from Sigma was used as substrate. Biotinylated pEY was added as a tracer substrate. The kinase domain of HER2 was cloned using baculovirus system and extracted similarly to that of EGFR kinase domain. Detailed procedures for EGFR, HER2, SRC, BIRK and VEGFR2 kinase activity assays are included in Supplementary information [1].
Cell Research	Cells (1×10^4) were transferred into each well of a 96-well plate and cultured over night in serum-free media for EGFR phosphorylation assay. After addition of test compounds on the next day, the plates were then incubated at 37°C for 1 hour. EGF-stimulation was

	done at 100 ng/ml for 10 min at room temperature. Cells were washed with ice cold PBS before extraction with 120 µl per well HEPEX buffer and shaken for 1 h at room temperature. In all 2×10^4 cells per well was used for HER2 phosphorylation assay. Streptavidin precoated plates were coated with anti-EGFR-biotin at 1:100 dilution with blocking buffer and c-erb2/HER2 oncoprotein Ab-5(Clone N24)-Biotin. Extracts from above steps were then transferred to the antibody-coated wells and incubated for 1 h at room temperature. Assessment of color development is described in Supplementary information. Extinction was measured at 450 nm. The data generated were analysed by the program PRISM. Normalized values were used to calculate the IC50 by a nonlinear regression curve fit (variable slope) [1].
Animal Research	Six weeks old female athymic nude mice (nu/nu) weighing about 16-20 gram were housed by Laboratory Animal Services Centre of The Chinese University of Hong Kong. The experiment was conducted by researchers under license from the Hong Kong Government Department of Health and according to approval given by Animal Experimentation Ethics Committee of the Chinese University of Hong Kong. ESCC xenografts were established by inoculating HKESC-2 (0.6 × 10^5 cells re-suspended in 50 µl of HBSS-buffer) subcutaneously into both flanks of the nude mice. When tumor size reached to 4-6 mm diameter, they were randomized in either treatment (15 mg/kg) or vehicle control group. Afatinib for treatment was prepared by dissolving in 0.5% methylcellulose before administration. Either drug or vehicle was administered to mouse by oral gavage in a schedule of 5 days on plus 2 days off for two weeks. Drug efficacy was evaluated by monitoring the change in tumor size with caliper. Tumor volume was calculated with the formula Tumor Volume = (width2 × length)/2 [2].

Solubility Information

Solubility	H2O: < 1 mg/mL (insoluble or slightly soluble), DMSO: 90 mg/mL (185.21 mM),Sonication is recommended. Ethanol: 12 mg/mL (24.69 mM),Sonication is recommended. 10% DMSO+40% PEG300+5% Tween 80+45% Saline: 9 mg/mL (18.52 mM),Suspension. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.0579 mL	10.2893 mL	20.5787 mL
5 mM	0.4116 mL	2.0579 mL	4.1157 mL
10 mM	0.2058 mL	1.0289 mL	2.0579 mL
50 mM	0.0412 mL	0.2058 mL	0.4116 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.

Reference

Li D, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene. 2008 Aug 7;27(34):4702-11.
Luo P, Yan H, Du J, et al. PLK1 (polo like kinase 1)-dependent autophagy facilitates gefitinib-induced hepatotoxicity by degrading COX6A1 (cytochrome c oxidase subunit 6A1). Autophagy. 2021 Oct;17(10):3221-3237.
Lu H H, Lin S Y, Roc Weng R, et al. Fucosyltransferase 4 shapes oncogenic glycoproteome to drive metastasis of lung adenocarcinoma. EBioMedicine. 2020, 57: 102846
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Shang J, Ning S, Chen Y, et al. MDL-800, an allosteric activator of SIRT6, suppresses proliferation and enhances EGFR-TKIs therapy in non-small cell lung cancer. Acta Pharmacologica Sinica. 2021, 42(1): 120-131
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Lu H H, Lin S Y, Weng R R, et al. Fucosyltransferase 4 shapes oncogenic glycoproteome to drive metastasis of lung adenocarcinoma[J]. EBioMedicine. 2020, 57: 102846
Lu H H, Lin S Y, Roc Weng R, et al. Fucosyltransferase 4 shapes oncogenic glycoproteome to drive metastasis of lung adenocarcinoma[J]. EBioMedicine. 2019
Luo P, Yan H, Du J, et al. PLK1 (polo like kinase 1)-dependent autophagy facilitates gefitinib-induced hepatotoxicity by degrading COX6A1 (cytochrome c oxidase subunit 6A1)[J]. Autophagy. 2020: 1-17.