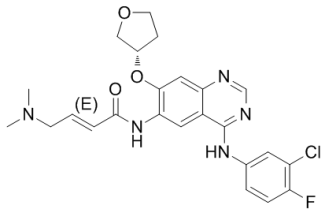
Data Sheet

Product Name:	Afatinib
Cat. No.:	CS-0043
CAS No.:	850140-72-6
Molecular Formula:	$C_{24}H_{25}ClFN_5O_3$
Molecular Weight:	485.94
Target:	Autophagy; EGFR
Pathway:	Autophagy; JAK/STAT Signaling; Protein Tyrosine Kinase/RTK
Solubility:	10 mM in DMSO



BIOLOGICAL ACTIVITY:

Afatinib is an irreversible, dual **EGFR/HER2** inhibitor, shows potent activity against wild-type and mutant forms of EGFR and HER2, with **IC50** of 0.5 nM, 0.4 nM, 10 nM and 14 nM for $EGFR^{wt}$, $EGFR^{L858R}$, $EGFR^{L858R/T790M}$ and HER2, respectively.
IC50 & Target: IC50: 0.5 nM ($EGFR^{wt}$), 0.4 nM ($EGFR^{L858R}$), 10 nM ($EGFR^{L858R/T790M}$), 14 nM (HER2)[1]
In Vitro: In cell-free in vitro kinase assays, Afatinib (BIBW2992) dimaleate shows potent activity against wild-type and mutant forms of EGFR and HER2, similar to Gefitinib in potency for L858R EGFR, but about 100-fold more active against the Gefitinib-resistant L858R-T790M EGFR double mutant, with an IC_{50} of 10 nM. BIBW2992 is furthermore comparable to Lapatinib and Canertinib for in vitro potency against HER2, with an IC_{50} of 14 nM. The most sensitive kinase in this evaluation is lyn with an IC_{50} of 736 nM[1]. Afatinib is an irreversible inhibitor of these ErbB family receptors. Esophageal squamous cell carcinoma (ESCC) cell lines are sensitive to Afatinib with IC_{50} concentrations at lower micro-molar range (at 48 hour incubation: HKESC-1=78 nM, HKESC-2=115 nM, KYSE510=3.182 μM, SLMT-1=4.625 μM and EC-1=1.489 μM; and at 72 hour incubation: HKESC-1=2 nM, HKESC-2=2 nM, KYSE510=1.090 μM, SLMT-1=1.161 μM and EC-1=109 nM) with a maximum growth inhibition over 95%. Afatinib can strongly induce G_0/G_1 cell cycle arrest in HKESC-2 and EC-1 in a dose- and time-dependent manner[2].
In Vivo: Afatinib (15 mg/kg) strongly inhibits the growth of HKESC-2 tumor once the treatment began. Average tumor sizes of vehicle and treatment at end point are 348±24 mm^3 and 108±36 mm^3 respectively, showing significantly difference between them. And apparently tumor size does not bounce back in a short period of time after the end of Afatinib administration. Without rapid change of body weight throughout the treatment shows that the toxicity of Afatinib is minimal and this drug is well tolerated[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]The EGFR kinase domain-GST fusion proteins are extracted from Sf9 biomasses, 72 hours post infection, with HEPEX (20 mM HEPES pH 7.4, 100 mM NaCl, 10 mM β-glycerophosphate, 10 mM para-nitro-phenylphosphate, 30 mM NaF, 5 mM EDTA, 5% glycerol, 1% Triton X-100, 1 mM Na_3VO_4, 0.1% SDS, 0.5 μg/mL pepstatin A, aprotinin 20 KIU/mL, Leupeptin 2 μg/mL, Benzamidine 1 mM, 2.5 μg/mL 3,4-dichloroisocoumarin, 2.5 μg/mL trans-epoxysuccinyl-L-leucyl-L-amido butane and 0.002% PMSF) and used for the determination of the IC_{50} values. Each 100 μL enzyme reaction contains 10 μL of Afatinib (BIBW2992) in 50 % Me_2SO, 20 μL of substrate solution (200 mM HEPES pH 7.4, 50 mM Mg-acetate, 2.5 mg/mL poly (EY), 5 μg/mL bio-pEY) and 20 μL enzyme preparation. The enzymatic reaction is started by addition of 50 μL of a 100 μM ATP solution made in 10 mM $MgCl_2$. Assays are carried out at room temperature for 30 minutes and terminated by the addition of 50 μL of stop solution (250 mM EDTA in 20 mM HEPES pH 7.4). 100 μL are transferred to a streptavidin coated microtiterplate, after an incubation time of 60 min at room temperature the plate is washed with 200 μL of wash solution (50 mM Tris, 0.05% Tween20). A 100 μL aliquot of a HRPO- labeled anti-PY antibody (PY20H Anti-Ptyr:HRP) 250 ng/mL are added to the wells. After 60 min of incubation, the plate is washed three times with a 200 μL wash solution.

The samples are then developed with a 100 µL TMB Peroxidase Solution (A:B=1:1). The reaction is stopped after 10 min. The plate is transferred to an ELISA reader and extinction is measured at OD_{450nm}. All data points are performed in triplicates[1]. **Cell Assay:** Afatinib is dissolved in DMSO (100 mM) and stored (-80°C), and then diluted in corresponding medium just before addition to cell cultures[2].[2]Human ESCC cell lines, EC-1, HKESC1 and HKESC2, SLMT1, and KYSE510 are cultured in RPMI with 10% fetal bovine serum (FBS). Cytotoxicity is assessed by a colorimetric assay using MTT. Tumour cells are cultured in 48-well plates (3000-8000 cells per well) in respective culture medium. Afatinib in complete medium is added at 24 hr after cell plating and incubated at 37°C with 5% CO_2 for 48 and 72 hr. Cell growth inhibition is expressed as the percentage of absorbance of control cultures measured at 570 nm with a microplate reader and 50% of the maximum growth inhibition (IC_{50}) is calculated by GraphPad PRISM. In each experiment, triplicate wells are performed for each drug concentration (n=3), and assay is repeated in three independent experiments[2]. **Animal Administration:** Afatinib is dissolved in 0.5% methylcellulose (Mice)[2].[2]Mice[2]
Six weeks old female athymic nude mice (nu/nu) weighing about 16-20 gram are used. ESCC xenografts are established by inoculating HKESC-2 (6×10^4 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 are randomized in either treatment (15 mg/kg) or vehicle control group. Afatinib for treatment is prepared by dissolving in 0.5% methylcellulose before administration. Either drug or vehicle is administered to mouse by oral gavage in a schedule of 5 days on plus 2 days off for two weeks. Drug efficacy is evaluated by monitoring the change in tumor size with caliper. Tumor volume is calculated with the formula Tumor Volume=$(width^2\times length)/2$.

References:

[1]. 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.

[2]. Wong CH, et al. Preclinical evaluation of afatinib (BIBW2992) in esophageal squamous cell carcinoma (ESCC). Am J Cancer Res. 2015 Nov 15; 5(12):3588-99.

[3]. Wang XK, et al. Afatinib circumvents multidrug resistance via dually inhibiting ATP binding cassette subfamily G member 2 in vitro and in vivo. Oncotarget. 2014 Dec 15;5(23):11971-85.

[4]. Yoshioka T, et al. Antitumor activity of pan-HER inhibitors in HER2-positive gastric cancer. Cancer Sci. 2018 Apr;109(4):1166-1176.

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