Afatinib dimaleate

Cat. No.: HY-10261A

CAS No.: 850140-73-7

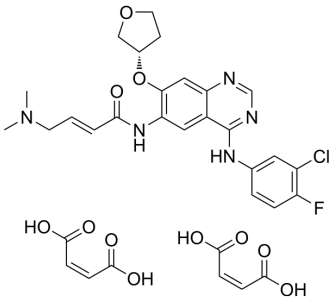
Molecular Formula: $C_{32}H_{33}ClFN_5O_{11}$

Molecular Weight: 718.08

Target: EGFR; Autophagy; Apoptosis; c-Met/HGFR; Akt; p38 MAPK

Pathway: JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Autophagy; Apoptosis; PI3K/Akt/mTOR; MAPK/ERK Pathway

Storage: 4°C, sealed storage, away from moisture
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro

H_2O : 50 mg/mL (69.63 mM; Need ultrasonic)
DMSO : ≥ 35 mg/mL (48.74 mM)
* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.3926 mL	6.9630 mL	13.9260 mL
5 mM	0.2785 mL	1.3926 mL	2.7852 mL
10 mM	0.1393 mL	0.6963 mL	1.3926 mL

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

In Vivo

1. Add each solvent one by one: PBS
Solubility: 100 mg/mL (139.26 mM); Clear solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

Afatinib (BIBW 2992) dimaleate is an orally active, potent and irreversible dual specificity inhibitor of ErbB family (EGFR and HER2), with IC_{50} values of 0.5 nM, 0.4 nM, 10 nM and 14 nM for $EGFR^{wt}$, $EGFR^{L858R}$, $EGFR^{L858R/T790M}$ and HER2, respectively. Afatinib dimaleate can be used for the research of esophageal squamous cell carcinoma (ESCC), non-small cell lung cancer (NSCLC) and gastric cancer[1][2][3][4].

IC_{50} & Target

$EGFR^{L858R}$ 0.4 nM (IC_{50})	EGFR 0.5 nM (IC_{50})	$EGFR^{L858R/T790M}$ 10 nM (IC_{50})	HER2 14 nM (IC_{50})
HER3			

In Vitro

Afatinib dimaleate (100 nM) sufficiently prevents heregulin-stimulated HER3 phosphorylation[1].

Afatinib dimaleate (0-10000 nM) effectively inhibits anchorage-independent proliferation of NIH-3T3 cells ectopically expressing EGFR mutants, and inhibits cell proliferation of H1666, H3255, and NCI 1975 cells[1].
Afatinib dimaleate (48-72 h)shows growth inhibition in HKESC-1, HKESC-2, SLMT-1 and EC-1 cells[2].
Afatinib dimaleate (0-1 μM, 24-48 h) inhibits AKT and MAPK pathways, and inhibits EGFR and AKT phosphorylation in ESCC cell lines[2].
Afatinib dimaleate (0-1 μM, 16-48 h) induces G0/G1 cell cycle arrest in HKESC-2 and EC-1[2].
Afatinib dimaleate (0-1 μM, 24-48 h) effectively induces apoptotic cell death in HKESC-2 and EC-1[2].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Proliferation Assay[1]

Cell Line:	NIH-3T3 cells, H1666, H3255, and NCI 1975 cells
Concentration:	0, 1, 10, 100, 1000, 10000 nM
Incubation Time:	
Result:	Effectively inhibited anchorage-independent proliferation of NIH-3T3 cells ectopically expressing EGFR mutants. Showed inhibition of anchorage independent cell proliferation of various lung cancer cell lines (H1666, H3255, and NCI 1975 cells), with IC_{50} values of 60 nM, 0.7 nM and 99 nM, respectively.

Cell Viability Assay[2]

Cell Line:	HKESC-1, HKESC-2, SLMT-1 and EC-1 cell lines
Concentration:	
Incubation Time:	48 and 72 hours
Result:	Observed over 95% of growth inhibition. The respective IC_{50} concentrations at 48 hours (HKESC-1=0.078 μM, HKESC-2=0.115 μM, KYSE510=3.182 μM, SLMT-1=4.625 μM and EC-1=1.489 μM) and 72 hours (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) were all in lower micro-molar range.

Western Blot Analysis[2]

Cell Line:	HKESC-2 cells and EC-1 cells
Concentration:	0, 0.01, and 0.1 μM (HKESC-2 cells), 0, 0.1 and 1 μM (EC-1 cells)
Incubation Time:	24 and 48 hours
Result:	Reduced the phosphorylation of EGFR and the endogenous expression level of HER2 receptors in ESCC cells. Suppressed AKT phosphorylation in a dose and time dependent manner. Significantly reduced the phosphorylation level of the downstream effectors of the AKT-mTOR axis especially in HKESC-2 cells. Inhibited the two major downstream pathways of the ErbB/HER axis, namely, AKT and MAPK pathways in ESCC cell lines.

Cell Cycle Analysis[2]

Cell Line:	HKESC-2 cells and EC-1 cells
Concentration:	0, 0.01, and 0.1 μM (HKESC-2 cells), 0, 0.1 and 1 μM (EC-1 cells)
Incubation Time:	16, 24, and 48 hours
Result:	Induced G0/G1 cell cycle arrest in both tested ESCC cell lines in a time and dose dependent manner. In HKESC-2 cells, the percentage of cells in G0/G1 phase was increased from

	38.2% to 68.1% at 0.01 μM of afatinib and to 74.7% at 0.1 μM of afatinib, from 24 hours (82.4% G0/G1 arrest at 0.01 μM and 86.2% at 0.1 μM) to 48 hours (from 74.7% to 88.2% for 0.01 μM and 91.0% for 0.1 μM). In EC-1 cells, the percentage of cells arrested in the G0/G1 phase was increased from 59.1% to 66.6% and 72.2% at 24 and 48 hours respectively.

Apoptosis Analysis[2]

Cell Line:	HKESC-2 cells and EC-1 cells
Concentration:	0, 0.01, and 0.1 μM (HKESC-2 cells), 0, 0.1 and 1 μM (EC-1 cells)
Incubation Time:	24 and 48 hours
Result:	Effectively induced cell death by triggering apoptotic mechanisms in ESCC cell lines. Showed a stronger expression level of cleaved Poly (ADP-ribose) polymerase (PARP) in these cell lines.

In Vivo

Afatinib dimaleate (0-20 mg/kg, Orally, daily for 25 days) shows dramatic tumor regression and downregulation of EGFR, HER2, HER3 and AKT phosphorylation[1].
Afatinib dimaleate (15 mg/kg, Orally, in a schedule of 5 days on plus 2 days off, for two weeks) strongly inhibits the growth of HKESC-2 tumor[2].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Model:	Athymic NMRI-nu/nu female mice (21–31 g, five to six-week-old, transgenic murine lung cancer model and xenograft models)[1]
Dosage:	15 mg/kg, 20 mg/kg
Administration:	Orally, daily for 25 days
Result:	Resulted in dramatic tumor regression with a cumulative treated/control tumor volume ratio (T/C ratio) of 2% in a standard xenograft model of the epidermoid carcinoma cell line A431, and downregulation of EGFR and AKT phosphorylation. Induced regression of large tumors in this HER2-driven model, effectively controlled xenograft tumor formation by the NCIH1975 cell line, expressing EGFR L858R/T790M, with a T/C value of 12% for doses of 20 mg/kg. Induced more than 50% percent tumor reduction after a 4-week treatment period. Downregulated EGFR, HER2 and HER3 phosphorylation.

Animal Model:	Six weeks old female athymic nude mice (nu/nu) (16-20 g)[2]
Dosage:	15 mg/kg
Administration:	Oral gavage in a schedule of 5 days on plus 2 days off, for two weeks
Result:	Strongly inhibited the growth of HKESC-2 tumor. Average tumor sizes of vehicle and treatment at end point are 348 ± 24 mm^3 and 108 ± 36 mm^3 respectively.

CUSTOMER VALIDATION

- Cancer Cell. 2022 Dec 7;S1535-6108(22)00562-1.
- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.

- Nat Commun. 2019 Apr 18;10(1):1812
- Cell Rep Med. 2023 Jan 10;100911.
- Biomaterials. 16 September 2022.

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[1]. 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.

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

[3]. 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.

[4]. 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

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