# Afatinib

**Cat. No.:** HY-10261

**CAS No.:** 850140-72-6

**Molecular Formula:** $C_{24}H_{25}ClFN_5O_3$

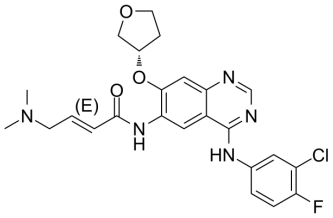
**Molecular Weight:** 485.94

**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:**

| | | |
|---|---|---|
| Powder | -20°C | 3 years |
| | 4°C | 2 years |
| In solvent | -80°C | 6 months |
| | -20°C | 1 month |



## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : 100 mg/mL (205.79 mM; Need ultrasonic)

| Preparing Stock Solutions | Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 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 |

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

**In Vivo**

1. Add each solvent one by one: 0.5% Methylcellulose/saline water
   Solubility: 5 mg/mL (10.29 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.14 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.14 mM); Clear solution
4. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 2.5 mg/mL (5.14 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

Afatinib (BIBW 2992) 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 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 | HER2 | $EGFR^{L858R}$ | $EGFR^{L858R/T790M}$ |
|---|---|---|---|---|
| | 0.5 nM ($IC_{50}$) | 14 nM ($IC_{50}$) | 0.4 nM ($IC_{50}$) | 10 nM ($IC_{50}$) |

**In Vitro**

Afatinib (100 nM) sufficiently prevents heregulin-stimulated HER3 phosphorylation[1].
Afatinib (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 (48-72 h)shows growth inhibition in HKESC-1, HKESC-2, SLMT-1 and EC-1 cells[2].
Afatinib (0-1 μM, 24-48 h) inhibits AKT and MAPK pathways, and inhibits EGFR and AKT phosphorylation in ESCC cell lines[2].
Afatinib (0-1 μM, 16-48 h) induces G0/G1 cell cycle arrest in HKESC-2 and EC-1[2].
Afatinib (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]

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|---|---|
| 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 (0-20 mg/kg, Orally, daily for 25 days) shows dramatic tumor regression and downregulation of EGFR, HER2, HER3 and AKT phosphorylation[1].

Afatinib (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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## 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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