Neratinib

Cat. No.: HY-32721

CAS No.: 698387-09-6

Molecular Formula: $C_{30}H_{29}ClN_6O_3$

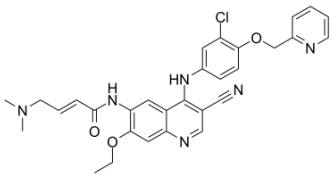
Molecular Weight: 557.04

Target: EGFR

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

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 : 8.33 mg/mL (14.95 mM; Need ultrasonic)
H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.7952 mL	8.9760 mL	17.9520 mL
5 mM	0.3590 mL	1.7952 mL	3.5904 mL
10 mM	0.1795 mL	0.8976 mL	1.7952 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 0.83 mg/mL (1.49 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2 mg/mL (3.59 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

Neratinib is an orally available, irreversible tyrosine kinase inhibitor with IC_{50}s of 59 nM and 92 nM for HER2 and EGFR, respectively.

IC_{50} & Target

EGFR	HER2
92 nM (IC_{50})	59 nM (IC_{50})

In Vitro

Neratinib has inhibition of tyrosine kinases KDR and Src with IC_{50} of 0.8 μM and 1.4 μM, respectively, being 14- and 24-fold less active compared with HER2. Neratinib displays no activity against other serine-threonine kinases such as Akt, cyclin D1/cdk4, cyclin E/cdk2, cyclin B1/cdk1, IKK-2, MK-2, PDK1, c-Raf, and Tpl-2, as well as the tyrosine kinase c-Met. Neratinib

	selectively inhibits the proliferation of 3T3 cells transfected with the HER2 (3T3/neu), as well as two other HER-2-overexpressing SK-Br-3 and BT474 cells with IC_{50} values of 2-3 nM, displaying > 230-fold potency compared with non-transfected 3T3 cells as well as MDA-MB-435 and SW620 which are EGFR- and HER2-negative. Neratinib also inhibits the proliferation of EGFR-dependent A431 cells with an IC_{50} of 81 nM. Neratinib reduces HER2 receptor autophosphorylation in BT474 cells with an IC_{50} of 5 nM, and EGF-dependent phosphorylation of EGFR in A431 cells with IC_{50} of 3 nM. Blocking of HER-2 by Neratinib results in inhibition of downstream MAPK and Akt pathways with IC_{50} of 2 nM. Neratinib inhibits the cyclin D1 expression and the phosphorylation of the Rb-susceptibility gene production in BT474 cells with IC_{50} of 9 nM, leading to G1-S arrest and ultimately decreased cell proliferation[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Orally treated neratinib significantly inhibits the growth of 3T3/neu xenografts, with inhibition of 34%, 53%, 98%, and 98% at dose of 10, 20, 40, and 80 mg/kg/day, respectively. Consistent with the inhibition of HER-2 phosphorylation by 84% within 1 hour of administration at 40 mg/kg/day, Neratinib inhibits the growth of BT474 xenografts by 70-82%, 67%, and 93% at dose of 5, 10, and 40 mg/kg/day, respectively. Neratinib is also effective against SK-OV-3 xenografts with inhibition of 31% and 85% at 5 and 60 mg/kg/day, respectively. Neratinib is less potent against EGFR-dependent A431 xenografts than HER-2-dependent tumors, with 32% and 44% inhibition at 5 and 20 mg/kg/day, respectively. Neratinib displays little activity against MCF-7 and MX-1 xenografts expressing low levels of HER-2 and EGFR, with only 28% inhibition at 80 mg/kg/day, suggesting that Neratinib has selective activity for cells expressing HER-2 or EGFR[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Neratinib is prepared as 10 mg/mL stocks in DMSO and diluted in 25 mM HEPES (pH 7.5; 0.002 ng/mL-20 μg/mL). Purified recombinant COOH-terminal fragments of HER2 (amino acids 676-1255) or epidermal growth factor receptor (EGFR) (amino acids 645-1186) [diluted in 100 mM HEPES (pH 7.5) and 50% glycerol] is incubated with increasing concentrations of Neratinib in 4 mM HEPES (pH 7.5), 0.4 mM $MnCl_2$, 20 μM sodium vanadate, and 0.2 mM DTT for 15 minutes at room temperature in 96-well ELISA plates. The kinase reaction is initiated by the addition of 40 μM ATP and 20 mM $MgCl_2$ and allowed to proceed for 1 hour at room temperature. Plates are washed, and phosphorylation is detected using Europium-labeled anti-phospho-tyrosine antibodies (15 ng/well). After washing and enhancement steps, signal is detected using a Victor2 fluorescence reader (excitation wavelength 340 nm, emission wavelength 615 nm). The concentration of Neratinib that inhibits receptor phosphorylation by 50% (IC_{50}) is calculated from inhibition curves. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Cells are exposed to various concentrations of Neratinib for 2, or 6 days. Cell proliferation is determined using sulforhodamine B, a protein binding dye. Briefly, cells are fixed with 10% trichloroacetic acid and washed extensively with water. Cells are then stained with 0.1% sulforhodamine B and washed in 5% acetic acid. Protein-associated dye is solubilized in 10 mM Tris, and absorbance is measured at 450 nM. The concentration of Neratinib that inhibits cell proliferation by 50% (IC_{50}) is determined from inhibition curves. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Tumor cells (maintained in tissue culture) or tumor fragments are implanted s.c. in the flanks of female athymic (nude) mice. For estrogen-dependent cell lines (BT474, MCF-7, and SK-OV-3), animals are implanted with hormone pellets 1 week before implantation of tumors. Additionally, SK-OV-3 cells are suspended in Matrigel basement membrane matrix for implantation. Treatment is initiated after tumors hads reached a size of 90-200 mg, following random assignment of the animals to different treatment groups (staging, day 0). For 3T3/neu xenografts, treatment is initiated the day after tumor implantation (day 0). HKI-272 is formulated in 0.5% methocellulose-0.4% polysorbate-80 (Tween 80) and administered daily, p.o., by gavage. Tumor mass [(length × $width^2$)/2] is determined every 7 days. Tumor outgrowth in all xenograft studies, except 3T3/neu, is expressed as relative tumor growth: the ratio of the mean tumor mass to the mean tumor mass on day 0. Inhibition of tumor growth is calculated relative to vehicle-treated controls. Statistical significance of inhibition is demonstrated using one-tailed Student's t test (equal variance) after log transformation of the data. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450). pii: eaaq1093.
- Sci Transl Med. 2018 Jun 20;10(446). pii: eaao2565.
- Ann Rheum Dis. 2020 Sep 7;annrheumdis-2020-217904.
- Cell Syst. 2019 Jul 24;9(1):35-48.e5.
- Mol Cancer Ther. 2018 Mar;17(3):603-613.

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REFERENCES

[1]. Rabindran SK, et al. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. Cancer Res, 2004, 64(11), 3958-3965.

[2]. 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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