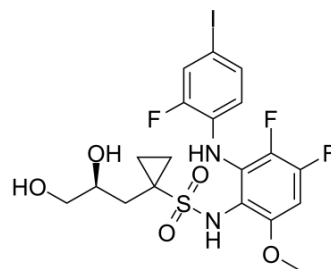


Refametinib

Cat. No.:	HY-14691		
CAS No.:	923032-37-5		
Molecular Formula:	C ₁₉ H ₂₀ F ₃ IN ₂ O ₅ S		
Molecular Weight:	572.34		
Target:	MEK		
Pathway:	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 : ≥ 31 mg/mL (54.16 mM)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent		1 mg	5 mg	10 mg
	Concentration	Mass			
	1 mM		1.7472 mL	8.7361 mL	17.4721 mL
	5 mM		0.3494 mL	1.7472 mL	3.4944 mL
	10 mM		0.1747 mL	0.8736 mL	1.7472 mL

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

In Vivo

- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
 Solubility: ≥ 2.5 mg/mL (4.37 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
 Solubility: ≥ 2.5 mg/mL (4.37 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Refametinib (BAY 869766; RDEA119) is an orally available, potent, non-ATP-competitive, selective, allosteric MEK1/MEK2 inhibitor with IC₅₀s of 19 nM and 47 nM, respectively.

IC₅₀ & Target

MEK1 19 nM (IC ₅₀)	MEK2 47 nM (IC ₅₀)
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In Vitro

Refametinib (BAY 869766; RDEA119) selectively binds directly to an allosteric pocket in the MEK1/2 enzymes. Refametinib potently inhibits MEK activity in enzyme inhibition assays in a non-ATP-competitive manner (MEK1 IC₅₀=19 nM, MEK2 IC₅₀=47 nM) determined through incorporation of radioactive phosphate from ATP into ERK as substrate. Refametinib potently

inhibits MEK activity as measured by phosphorylation of ERK1/2 across several human cancer cell lines of different tissue origins and BRAF mutational status with EC₅₀ values ranging from 2.5 to 15.8 nM. Refametinib inhibits anchorage-dependent growth of human cancer cell lines harboring the gain-of-function V600E BRAF mutant with GI₅₀ values ranging from 67 to 89 nM. In contrast, Refametinib has significantly less growth-inhibitory potency against cell lines with wild-type BRAF (A431 cells) or MDA-MB-231 cells harboring a BRAF mutation G464V that shows minimal (<2-fold increase) enhancement of inherent kinase activity. Under anchorage-independent conditions, GI₅₀ values for all cell lines tested are similar (40-84 nM). MDA-MB-231 and A431 cells are significantly more sensitive to Refametinib under anchorage-independent conditions^[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

Refametinib (BAY 869766; RDEA119) is an orally available, potent, non-ATP-competitive, highly selective inhibitor of MEK1/2, which is active in human tumor xenograft models and is well tolerated within the therapeutic exposure range in animals. The human melanoma A375 tumor xenograft is found to be sensitive to Refametinib treatment with 54% and 68% tumor growth inhibition (TGI) seen with 25 and 50 mg/kg/d administered orally on a once daily ×14 schedule. Significant tumor growth delay (TGD) and regressions are also observed in A375 tumors on this once-daily schedule. For example, five to eight complete or partial responses (CR/PR) and up to six tumor-free survivors (TFS) are observed. Administering Refametinib every other day at 100 mg/kg is less effective than daily dosing with either 25 or 50 mg/kg. When Refametinib is dosed on a twice-daily schedule, it is more effective than once-daily schedules^[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]

MEK1 kinase activity is determined using mERK2 K52A T183A as the substrate. Recombinant MEK1 enzyme (5 nM) is first activated by 0.02 unit or 1.5 nM of RAF1 in the presence of 25 mM HEPES (pH 7.8), 1 mM MgCl₂, 50 mM NaCl, 0.2 mM EDTA, and 50 μM ATP for 30 min at 25°C. The reactions are initiated by adding 2 μM of mERK2 K52A T183A and 2.5 μCi [³³P]ATP in a total volume of 20 μL. The MEK2 kinase activity is determined similarly except that activation by RAF1 is not needed and 11 nM of MEK2 enzyme (active) are used in the assays. Kinase profiling is performed. The Z'-LYTE biochemical assay is used. Refametinib is assayed in quadruplicate at 10 μM against 205 kinases^[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[1]

For anchorage-dependent growth inhibition experiments, A375, SK-Mel-28, A431, Colo205, HT-29, MDA-MB-231, and BxPC3 cell lines are plated in white 384-well plates at 1,000/20 μL/well or white 96-well microplates at 4,000/100 μL/well. After 24-h incubation at 37°C, 5% CO₂, and 100% humidity, Refametinib is incubated for 48 h at 37°C and assayed using CellTiter-Glo. For the 96-well anchorage-independent growth assay, wells of an "ultralow binding" plate are filled with 60 μL of a 0.15% agarose solution in complete RPMI 1640. Then, 60 μL of complete RPMI 1640 containing 9,000 cells in 0.15% agarose are added per well. After 24 h, 60 μL of a 3× drug solution in agarose-free complete RPMI 1640 are added. After 7 d, 36 μL of 6× MTS reagent are added per well. After 2 h at 37°C, absorbance at 490 nm is determined on the M5 plate reader. Nonlinear curve fitting is performed using GraphPad Prism 4^[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration ^[1]

Mice^[1]
Female athymic nude mice are used for all the efficacy studies except for the Colo205 study 2, which used male mice. Mice are injected s.c. in the flank with 1×10⁶ tumor cells (Colo205 and A431) or ~1 mm³ tumor fragments (A375 and HT-29). Tumor volumes are monitored by caliper measurements. For efficacy analysis, treatment is initiated when tumors are 80 to 185 mm³. Refametinib (25 and 50 mg/kg/d) is administered by oral gavage once daily or twice daily for 14 d or once every 2 d for 14 doses^[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

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- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
 - Neuro Oncol. 2019 Mar 18;21(4):486-497.
 - Comput Struct Biotechnol J. 2019 Feb 8;17:352-361.
 - ACS Comb Sci. 2019 Dec 9;21(12):805-816.
 - Oncotarget. 2020 Nov 3;11(44):3921-3932.

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REFERENCES

[1]. Iverson C, et al. RDEA119/BAY 869766: a potent, selective, allosteric inhibitor of MEK1/2 for the treatment of cancer. Cancer Res. 2009 Sep 1;69(17):6839-47.

Caution: Product has not been fully validated for medical applications. For research use only.

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