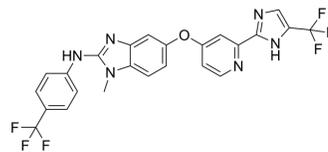


## RAF265

<b>Cat. No.:</b>	HY-10248		
<b>CAS No.:</b>	927880-90-8		
<b>Molecular Formula:</b>	C <sub>24</sub> H <sub>16</sub> F <sub>6</sub> N <sub>6</sub> O		
<b>Molecular Weight:</b>	518.41		
<b>Target:</b>	Raf; VEGFR; Autophagy; Apoptosis		
<b>Pathway:</b>	MAPK/ERK Pathway; Protein Tyrosine Kinase/RTK; Autophagy; Apoptosis		
<b>Storage:</b>	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : ≥ 26 mg/mL (50.15 mM)  
 Ethanol : 10 mg/mL (19.29 mM; Need ultrasonic)  
 \* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	1.9290 mL	9.6449 mL	19.2898 mL
	5 mM	0.3858 mL	1.9290 mL	3.8580 mL
	10 mM	0.1929 mL	0.9645 mL	1.9290 mL

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

#### In Vivo

- Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 1 mg/mL (1.93 mM); Clear solution
- Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline)  
Solubility: 1 mg/mL (1.93 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% EtOH >> 90% corn oil  
Solubility: 1 mg/mL (1.93 mM); Clear solution; Need warming

### BIOLOGICAL ACTIVITY

#### Description

RAF265 is a potent and orally active RAF/VEGFR2 inhibitor.

#### IC<sub>50</sub> & Target

VEGFR2

RAF

#### In Vitro

The MTT assay reveals that in HT29 and MDAMB231 cells, RAF265 alone shows significant activity with IC<sub>20</sub> values of 1 to 3 μ

M and IC<sub>50</sub> values of 5 to 10 μM. In A549 and HCT116 cells, IC<sub>20</sub> values are 1 μM for both, but RAF265 concentrations up to 10 μM do not reach IC<sub>50</sub> values. However, in the presence of 1 nM RAD001, the IC<sub>50</sub> for RAF265 is 5 μM in A549 cells and 10 μM in HCT116 cells<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### In Vivo

In single-compound efficacy studies, optimal dosing of RAD001 and RAF265 is 5 to 12 mg/kg daily and 30 mg/kg every two days, respectively. However, combination tolerability studies in nontumor-bearing mice define dose-limiting toxicity as a 10% weight loss with the combination of RAD001 at a dose of 12 mg/kg daily and RAF265 at a dose of 20 mg/kg every two days. Therefore, the combination of RAF265 at a dose of 12 mg/kg qd and RAD001 at a dose of 12 mg/kg qd seems to be the maximal tolerated dose. RAD001 and RAF265 are both given at a dose of 12 mg/kg qd, alone or concurrently, over 6 days. After a 2-day stop, the compounds are given for another 6 days, and the treatment is then stopped. To confirm the potential of the combination of RAF265 and RAD001, the antitumor effect of the combination is tested in HCT116 xenografts (KRAS mut, PIK3CA mut). In HCT116 xenografts, RAD001 or RAF265 given alone shows 60% to 65% and 71% to 72% TVI%, respectively<sup>[1]</sup>.

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## PROTOCOL

#### Cell Assay <sup>[1]</sup>

The MTT assay and Bliss additivity model are used to assess the effect of the combination on cell viability. Human A549 and H460 lung, HT29 and HCT 116 colon, and MDAMB231 breast cancer cell lines are used. In each well of a 96-well plate,  $1 \times 10^4$  cells are grown in 200 μL of medium. After 24 h, RAD001, RAF265, or the combination is added to achieve a final concentration of 0.1 to 10 nM and 0.1 to 10 μM, respectively. After 48 h of treatment, 20 μL of 5 mg/mL MTT solution in PBS is added to each well. After 4 h, supernatant is removed and formazan crystals are discarded in 200 μL of DMSO. Absorbance is then measured at 595 nm using an absorbance plate reader. Data are expressed as the percentage of viable cells in treated relative to nontreated conditions<sup>[1]</sup>.

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#### Animal Administration <sup>[1]</sup>

Mice<sup>[1]</sup>

The efficacy of the combination is also tested in vivo. A total of  $3 \times 10^6$  A549, H460, HCT116, or MDAMB231 cells are injected s.c. into the flank region of 6-wk-old female athymic mice. When tumors reach 50 mm<sup>3</sup>, the mice are randomized into four groups (n=7/group) for the following treatment: vehicle, RAF265 (12 mg/kg daily), RAD001 (12 mg/kg daily), or both. All drug are administered over 14 d (6 d on, 2 d off, 6 d on), and the drug combination is administered concurrently. Control mice receive the respective vehicles of both drugs. Animal weight and tumor volumes are taken twice weekly and expressed relative to initial tumor volume. Tumors are measured until achieving a relative volume of 10 times the initial volume, and the time to this end point is noted. Drug efficacy is assessed based on the tumor growth curve, growth delay, and tumor volume inhibition percentage. The tumor growth curve is designed to depict the evolution of the relative tumor size over time. The tumor volume inhibition percentage (TVI%) is calculated<sup>[1]</sup>.

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):eaaq1093.
- J Med Virol. 2022 Oct 17.
- Clin Sci (Lond). 2019 Apr 16;133(8):919-932.
- Viruses. 2022 Oct 15;14(10):2261.
- BMC Pulm Med. 2023 May 11;23(1):162.

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## REFERENCES

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[1]. Mordant P, et al. Dependence on phosphoinositide 3-kinase and RAS-RAF pathways drive the activity of RAF265, a novel RAF/VEGFR2 inhibitor, and RAD001 (Everolimus) in combination. Mol Cancer Ther. 2010 Feb;9(2):358-68.

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

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