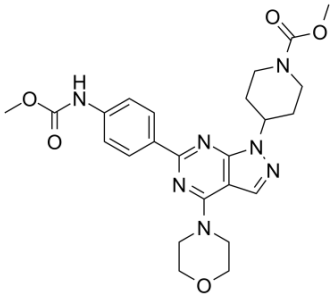
WYE-354

Cat. No.:	HY-12034
CAS No.:	1062169-56-5
Molecular Formula:	$C_{24}H_{29}N_7O_5$
Molecular Weight:	495.53
Target:	mTOR; Autophagy; Apoptosis
Pathway:	PI3K/Akt/mTOR; Autophagy; Apoptosis
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 : 6.67 mg/mL (13.46 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0180 mL	10.0902 mL	20.1804 mL
	5 mM	0.4036 mL	2.0180 mL	4.0361 mL
	10 mM	0.2018 mL	1.0090 mL	2.0180 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: ≥ 0.67 mg/mL (1.35 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 0.67 mg/mL (1.35 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 0.67 mg/mL (1.35 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

WYE-354 is an ATP-competitive mTOR inhibitor with an IC_{50} of 5 nM. WYE-354 also inhibits PI3Kα and PI3Kγ with IC_{50}s of 1.89 μM and 7.37 μM, respectively. WYE-354 inhibits both mTORC1 and mTORC2. WYE-354 induces autophagy activation in vitro[3].

IC_{50} & Target

mTOR 5 nM (IC_{50})	mTORC1	mTORC2	PI3K alpha 1.89 μM (IC_{50})
PI3K gamma	Autophagy		

	7.37 μM (IC_{50})	
In Vitro	In the DELFIA measuring His6-S6K1 T389 phosphorylation, WYE-354 inhibits recombinant mTOR enzyme with an IC_{50} of 5 nM [1]. Cell viability is analyzed by MTS assay. G-415 and TGBC-2TKB cell lines are treated with increasing concentrations of WYE-354 (0.1, 1, 5 and 10 μM) for 24, 48, and 72 hours. WYE-354 significantly reduces cell viability starting at a 1 μM concentration after a 24 hours exposure, in both studied cell lines (P<0.001). A decrease in cell viability is not observed at a dose of 100 nM, except for the TGBC-2TKB cell line after 72 hours of treatment[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.	
In Vivo	The effect of Rapamycin and WYE-354 on tumor growth is evaluated in xenograft GBC tumor models. 2×10^6 or 5×10^6 cells of G-415 or TGBC2TKB, respectively, are xenotransplanted into NOD-SCID mice subcutaneously. When tumors reach an average volume of 100 mm^3, the mice are treated either with Rapamycin or WYE354. Rapamycin is administered i.p. at a concentration of 10 mg/kg, daily for 5 days per week for 3 weeks, while WYE-354 is administrated at a daily i.p. dose of 50 mg/kg for 5 days. Mice are sacrificed 30 days after the initiation of the treatments and an autopsy is performed that include removal of the entire tumor area. Mice treated with WYE-354 exhibit 68.6% and 52.4% reduction in average tumor size (P<0.01; P<0.01), as well as 82.9% and 45.5% (P<0.01; ns) reduction in tumor weight, respectively[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.	

PROTOCOL

Kinase Assay [1]	The routine inhibitor assays are performed in 96-well plates for 2 h at room temperature in 25 μL containing 6 nM Flag-TOR(3.5) (estimated 5-10% purity), 1 μM His6-S6K and 100 μM ATP. The assays are performed and detected by DELFIA employing the Euphospho-p70S6K T389 antibody. Some assays employ a commercially purchased batch of mTOR. For inhibitor versus ATP matrix competition, mTOR kinase reactions are carried out with varying concentrations of ATP (0, 25, 50 100, 200, 400 and 800 μM) in combination with varying concentrations of inhibitor. The assays contain 12 nM Flag-TOR(3.5), 1 μM His-S6K and are incubated for 30 min. The assay results are similarly detected by DELFIA and processed for generation of double-reciprocal plots[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [2]	G-415 and TGBC-2TKB cell lines are plated onto 96 well plates at a density of 2×10^3 cells per well. After an overnight attachment period cells are treated with WYE-354. The number of viable cells is determined at certain intervals using CellTiter 96 Aqueous One Solution Cell Proliferation assay. 20 μL CellTiter 96 solution is added to each well and the plates are incubated for 2 hour after which the absorbance of each well is read at a wavelength of 490 nm using a multiwell plate reader. All assays are performed in quintuplicate, and each assay is repeated three times[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2]	Mice[2] 8 to 12-week- old NOD-SCID mice are subcutaneously injected in one flank with either 2×10^6 or 5×10^6 cells of G-415 or TGBC2TKB, respectively, and re-suspended in 200 μL of PBS with 30% of Matrigel. When the average tumor reach 100 mm^3, mice are randomly separated into four groups and treated with Rapamycin or WYE-354 and its respective vehicles. Rapamycin is administered at a daily intraperitoneal (i.p) dose of 10 mg/kg for 5 days per week for 3 weeks, while WYE-354 is administrated at a daily i.p dose of 50 mg/kg for 5 days. Tumor volumes are estimated twice a week. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Int J Mol Sci. 2020 Feb 19;21(4):1387.
- ACS Chem Biol. 2012 Jun 15;7(6):982-7.

- Biochim Biophys Acta. 2018 Feb 18;1865(5):709-720.
- Front Pharmacol. 2020, 11:580407.

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REFERENCES

[1]. Yu K et al. Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. Cancer Res. 2009 Aug 1;69(15):6232-40.

[2]. Weber H, et al. Rapamycin and WYE-354 suppress human gallbladder cancer xenografts in mice. Oncotarget. 2015 Oct 13;6(31):31877-88.

[3]. Lijun Wang, et al. Autophagy inhibition sensitizes WYE-354-induced anti-colon cancer activity in vitro and in vivo. Tumour Biol. 2016 Sep;37(9):11743-11752.

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