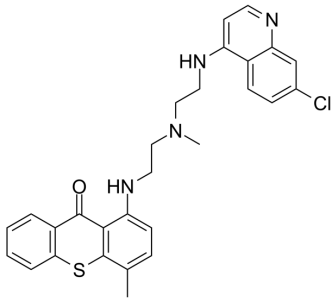
ROC-325

Cat. No.:	HY-103706
CAS No.:	1859141-26-6
Molecular Formula:	$C_{28}H_{27}ClN_4OS$
Molecular Weight:	503.06
Target:	Autophagy; Apoptosis
Pathway:	Autophagy; Apoptosis
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 4.55 mg/mL (9.04 mM; Need ultrasonic and warming)

H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.9878 mL	9.9392 mL	19.8783 mL
5 mM	0.3976 mL	1.9878 mL	3.9757 mL
10 mM	---	---	---

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

BIOLOGICAL ACTIVITY

Description

ROC-325 is a potent and orally active autophagy inhibitor with a strong anticancer activity. ROC-325 induces the deacidification of lysosomes, accumulation of autophagosomes, and disrupted autophagic flux. ROC-325 also induces renal cell carcinoma apoptosis[1].

IC_{50} & Target

Autophagy[1]

In Vitro

ROC-325 antagonizes renal cell carcinoma (RCC) growth and survival in an ATG5/7-dependent manner, induces apoptosis, and exhibits favorable selectivity. ROC-325 inhibits cells growth with IC_{50} values of 4.9 μM, 11 μM, 4.6 μM, 5.4 μM, 7.4 μM, 11 μM, 8.2 μM, 5.8 μM, 5.0 μM, 11 μM, 8.4 μM and 6.0 μM for A498, A549, CFPAC-1, COLO-205, DLD-1, IGROV-1, MCF-7, MiaPaCa-2, NCI-H69, PC-3, RL and UACC-62 cells, respectively. ROC-325 induces hallmark features of autophagy inhibition and antagonizes autophagic flux[1].

ROC-325 triggers a highly significant increase in cathepsin D (CTSD) levels. Treatment with 5 μM ROC-325 for 24 hours leads to the formation of LC3B punctae and a robust increase in LC3B levels in both A498 and 786-0 RCC cells. Immunoblotting analysis conducted in both A498 and 786-0 cells demonstrates that ROC-325 promotes a dose-dependent increase in LC3B

	expression in a manner that correlated with a corresponding increase in the levels of p62 and cathepsin D[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Oral administration of ROC-325 (25 mg/kg, 40 mg/kg, 50 mg/kg,) to mice bearing 786-0 RCC xenografts is well tolerated, significantly more effective at inhibiting tumor progression than Hydroxychloroquine, and inhibits autophagy in vivo[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Renal cancer cells are incubated with ROC-325 for 24 hours. Cells are harvested and then lysed. Approximately 50 μg of total cellular protein from each sample are subjected to SDS-PAGE, proteins are transferred to nitrocellulose membranes, and the membranes are blocked with 5% nonfat milk in a Tris-buffered saline solution containing 0.1% Tween-20 for 1 hour. The blots are then probed overnight at 4°C with primary antibodies, washed, and probed with species-specific secondary antibodies coupled to horseradish peroxidase. Immunoreactive material is detected by enhanced chemiluminescence[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Cell viability is estimated by the MTT assay. Cells are seeded into 96-well microcultureplates at 10,000 cells per well and allowed to attach for 24 hours. Cells are then treated with ROC-325 for 72 hours. Following ROC-325 treatment, MTT is added and formazan absorbance is quantified using a microplate reader. The estimated cell viability under each experimental condition is calculated by normalizing the respective formazan optical density to the density of control cells. Proapoptotic effects following in vitro ROC-325 exposure are quantified by propidium iodide (PI) staining and fluorescence-activated cell sorting (FACS) analysis of sub-G_0/G_1 DNA content and by measurement of active caspase-3 by flow cytometry using a commercial kit[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	786-0 renal cancer cells (5×10^6) are suspended in a mixture of HBSS and Matrigel and subcutaneously implanted into female nude mice. Tumor-bearing animals from each cell line xenograft are randomized into treatment groups. Mice are treated with vehicle (water), ROC-325 (25, 40, and 50 mg/kg PO) QD×5 for 6 weeks. Mice are monitored daily and tumor volumes are measured twice weekly. At study completion, tumors from representative animals are excised from each group, formalin-fixed, and paraffin-embedded for immunohistochemical analysis[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Carew JS, et al. Disruption of Autophagic Degradation with ROC-325 Antagonizes Renal Cell Carcinoma Pathogenesis. Clin Cancer Res. 2017 Jun 1;23(11):2869-2879.

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