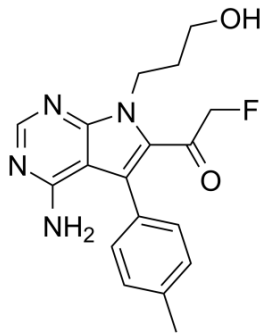
FMK

Cat. No.:	HY-52101A
CAS No.:	821794-92-7
Molecular Formula:	$C_{18}H_{19}FN_4O_2$
Molecular Weight:	342.37
Target:	Ribosomal S6 Kinase (RSK)
Pathway:	MAPK/ERK Pathway
Storage:	4°C, stored under nitrogen * In solvent : -80°C, 6 months; -20°C, 1 month (stored under nitrogen)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (292.08 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.9208 mL	14.6041 mL	29.2082 mL
	5 mM	0.5842 mL	2.9208 mL	5.8416 mL
	10 mM	0.2921 mL	1.4604 mL	2.9208 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: ≥ 2.5 mg/mL (7.30 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.30 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

FMK is a an irreversible RSK2 kinase inhibitor, that covalently modifies the C-terminal kinase domain of RSK.

In Vitro

Pretreatment of ARVMs with 3 μM fmk attenuates the increase in Ser386 phosphorylation, but it has no inhibitory effect on the increase in Thr577 phosphorylation[1]. FMK inhibits relatively few protein kinases in the panel, although it does inhibit protein tyrosine kinases, such as Src, Lck, Yes and Eph-A2, as well as S6K1. FMK will not inhibit RSK if the N-terminal kinase domain are activated by a mechanism that is independent of the C-terminal domain[2]. Fmk potently inactivates the CTD auto-kinase activity of RSK1 and RSK2 with high specificity in mammalian cells. Targeting RSK2 by a specific small molecule RSK inhibitor fmk attenuates FGFR3-induced cytokine-independent growth in Ba/F3 cells. FMK inhibits cytokine-independent proliferation of Ba/F3 cells conferred by FGFR3[3].

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

PROTOCOL

Kinase Assay [3]	The S6 peptide kinase assay is carried out according to the manufacturer's protocol using RSK2 immunoprecipitates. To determine the ability of FGFR3 to phosphorylate RSK2, 500 ng of purified recombinant RSK2 variants are incubated with 500 ng of recombinant active FGFR3 in 10 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.01% Trixton-X-100, 10 mM $MnCl_2$, and 200 μM ATP for 30 min at 30°C. Phosphorylation of Y529 RSK2 is detected by specific phospho-antibody. To determine kinase activity of RSK2 CTD variants, purified recombinant RSK2 CTD proteins (500 nM) are incubated with 500 nM of active ERK in 20 mM HEPES [pH 8.0], 10 mM $MgCl_2$, 2 mM tris-(2-carboxyethyl)-phosphine (TCEP), and 200 μM ATP for 1 hr at 30°C. Kinase reactions are initiated by the addition of 5 μCi of [γ-^{32}P] ATP and 100 μM peptide substrate (CTD-tide), followed by incubation for 20 min at room temperature. Kinase activity is determined using the standard disk phospho-cellulose assay. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [3]	RSK2 expressing Ba/F3 cell lines are generated by retroviral transduction as described by using Ba/F3 cells stably expressing FGFR3 TDII with pMSCV-puro plasmids encoding myc-tagged RSK2 variants, followed by antibiotic selection. For cell viability assays, 1×10^5 Ba/F3 cells stably expressing FGFR3 are cultured in 24-well plates with media containing increasing concentrations of FMK, acidic FGF (10 nM), and heparin (30 μg/mL) in the absence of IL-3. The relative cell viability at each experimental time point is determined by using the Celltiter96AQ$_{ueous}$ One solution proliferation kit. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Mol Cell. 2020 Oct 15;80(2):296-310.e6.
- Cancers. 2019 Nov 20;11(12):1827.

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REFERENCES

[1]. Cuello F, et al. Evidence for direct regulation of myocardial Na+/H+ exchanger isoform 1 phosphorylation and activity by 90-kDa ribosomal S6 kinase (RSK): effects of the novel and specific RSK inhibitor fmk on responses to alpha1-adrenergic stimulation.

[2]. Bain J, et al. The selectivity of protein kinase inhibitors: a further update. Biochem J. 2007 Dec 15;408(3):297-315.

[3]. Kang S, et al. FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. Cancer Cell. 2007 Sep;12(3):187-9.

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