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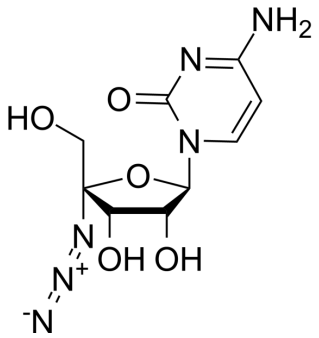
R-1479

Cat. No.:	HY-10444
CAS No.:	478182-28-4
Molecular Formula:	$C_9H_{12}N_6O_5$
Molecular Weight:	284.23
Target:	HCV; DNA/RNA Synthesis
Pathway:	Anti-infection; Cell Cycle/DNA Damage
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 : 100 mg/mL (351.83 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.5183 mL	17.5914 mL	35.1828 mL
	5 mM	0.7037 mL	3.5183 mL	7.0366 mL
	10 mM	0.3518 mL	1.7591 mL	3.5183 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 (8.80 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (8.80 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (8.80 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

R-1479 (4'-Azidocytidine), a nucleoside analogue, is a specific inhibitor of RNA-dependent RNA polymerase (RdRp) of HCV. R-1479 inhibits HCV replication in the HCV subgenomic replicon system (IC_{50}=1.28 μM)[1][2][3]. R-1479 is a click chemistry reagent, it contains an Azide group and can undergo copper-catalyzed azide-alkyne cycloaddition reaction (CuAAc) with molecules containing Alkyne groups. Strain-promoted alkyne-azide cycloaddition (SPAAC) can also occur with molecules containing DBCO or BCN groups.

IC_{50} & Target

IC50: 1.28 μM (HCV replication)[1]

In Vitro	R-1479 (R1479) inhibits HCV RNA replication with a mean IC_{50} value of 1.28 μM when measured as dose-dependent reduction of Renillaluciferase activity after a 72 h incubation of proliferating replicon cells. R-1479 shows no effect on cell viability or proliferation of HCV replicon or Huh-7 cells at concentrations up to 2 mM[1]. The most potent and non-cytotoxic derivative is R-1479 with an IC_{50} of 1.28 μM in the HCV replicon system. The triphosphate of R-1479 is prepared and shown to be an inhibitor of RNA synthesis mediated by NS5B (IC_{50}=320 nM), the RNA polymerase encoded by HCV. R-1479 displays good activity in the replicon assay with no measurable cytotoxic or cytostatic effect[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	The membrane-associated, native HCV replicase complex is isolated from 2209-23 HCV replicon cells and a derived cell line carrying HCV replicon RNA with a S282T mutation in the NS5B coding sequence. The in vitro replicase assay contain 10 μL of cytoplasmic membrane fraction, 50 mM HEPES (pH 7.5), 10 mM KCl, 10 mM dithiothreitol, 5 mM $MgCl_2$, 20 μg/mL actinomycin D, 1 mM ATP, 1 mM GTP, 1 mM UTP, 30 μCi of [α-^{33}P]CTP (3000 Ci/mmol, 10 mCi/mL), 1 unit/μL SUPERase•In, 10 mM creatine phosphate, and 200 μg/mL creatine phosphokinase in a final volume of 25 μL. Inhibition by nucleotide analogs is determined[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	The effect of R-1479 on the incorporation of tritiated thymidine into cellular DNA is measured using the [^{3}H]thymidine incorporation scintillation proximity assay system. MTT and WST-1 assay systems are used to measure cell viability. The ATP bioluminescence assay kit HSII is used to measure intracellular ATP levels[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Infect Dis. 2016 Sep 1;214(5):707-11.
- Antivir Res. 2020 Jun;178:104786.
- Antiviral Res. 2019 Oct;170:104570.

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REFERENCES

[1]. Klumpp K, et al. The novel nucleoside analog R1479 (4'-azidocytidine) is a potent inhibitor of NS5B-dependent RNA synthesis and hepatitis C virus replication in cell culture. J Biol Chem. 2006 Feb 17;281(7):3793-9.

[2]. Smith DB, et al. Design, synthesis, and antiviral properties of 4'-substituted ribonucleosides as inhibitors of hepatitis C virus replication: the discovery of R1479. Bioorg Med Chem Lett. 2007 May 1;17(9):2570-6.

[3]. Nguyen NM, et al. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. J Infect Dis. 2013 May 1;207(9):1442-1450.

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