Product Datasheet

Technical Data

Formula	$C_{20}H_{21}FN_6O_5$					
Molecular Weight	444.42	**CAS No.**	518048-05-0			
Solubility (25°C)*	In vitro	DMSO	89 mg/mL (200.26 mM)			
		Water	Insoluble			
		Ethanol	Insoluble			
	In vivo (Add solvents to the product individually and in order)	Oral Administration	Homogeneous suspension	CMC-NA	≥5mg/ml	Taking the 1 mL working solution as an example, add 5 mg of this product to 1 ml of CMC-Na solution, mix evenly to obtain a homogeneous suspension with a final concentration of 5 mg/ml.

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Capsid Bio tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.
* Room temperature shipping (Stability testing shows this product can be shipped at 2-8°C for storage purposes.)

Preparing Stock Solutions

Concentration \ Volume / Mass	1 mg	5 mg	10 mg
1 mM	2.2501 mL	11.2506 mL	22.5012 mL
5 mM	0.4500 mL	2.2501 mL	4.5002 mL
10 mM	0.2250 mL	1.1251 mL	2.2501 mL
50 mM	0.0450 mL	0.2250 mL	0.4500 mL

Biological Activity

Description	Raltegravir is a potent **integrase (IN)** inhibitor for WT and S217Q PFV IN with IC50 of 90 nM and 40 nM in cell-free assays, respectively. It shows greater than 1000-fold selectivity for HIV-1 IN over several related Mg2+-dependent enzyme such as HCV polymerase, HIV reverse transcriptase, HIV RNaseH and human α-, β-, γ-polymerases.
Targets	Integrase (S217Q PFV) [1] (Cell-free assay): 40 nM Integrase (WT PFV) [1] (Cell-free assay): 90 nM
In vitro	PFV IN carrying the S217H substitution is 10-fold less susceptible to Raltegravir with IC50 of 900 nM. PFV IN displays 10% of WT activity and is inhibited by this compound with an IC50 of 200 nM, indicating a ~twofold decrease in susceptibility to the IN strand transfer inhibitor (INSTI) compared with WT IN. S217Q PFV IN is as sensitive to this compound as the WT enzyme. [1] It is metabolized by glucuronidation, not hepatically. This compound has potent in vitro activity against HIV-1, with a 95% inhibitory concentration of 31?0 nM, in human T lymphoid cell cultures. It is also active against HIV-2 when tested in CEMx174 cells, with an IC95 of 6 nM. Its metabolism occurs primarily through glucuronidation. Drugs that are strong inducers of the glucuronidation enzyme, UGT1A1, significantly reduce its concentrations and should not be used. It exhibits weak inhibitory effects on hepatic cytochrome P450 activity. It does not induce CYP3A4 RNA expression or CYP3A4-dependent testosterone 6-β-hydroxylase activity. [2] Its cellular permeativity is reduced in the presence of magnesium and calcium. [3] This compound and related HIV-1 integrase (IN) strand transfer inhibitors (INSTIs efficiently block viral replication. [4] In acutely infected human lymphoid CD4+ T-cell lines MT-4 and CEMx174, SIVmac251 replication is efficiently inhibited by this compound, which shows an EC90 in the low nanomolar range. [5]
In vivo	Raltegravir induces viro-immunological improvement of nonhuman primates with progressing SIVmac251 infection. One non-human primate shows an undetectable viral load following this compound monotherapy. [5]
Features	The 1st approved human immunodeficiency virus type 1 (HIV-1) integrase inhibitor.

Protocol (from reference)

Kinase Assay:[1]

- **PFV integration assay**

  For quantitative strand transfer assays, donor DNA substrate is formed by annealing HPLC grade oligonucleotides 5′-GACTCACTATAGGGCACGCGTCAAAATTCCATGACA and 5′-ATTGTCATG GAATTTTGACGCGTGCCCTATAGTGAGTC. Reactions (40 μL) contains 0.75 μM PFV IN, 0.75 μM donor DNA, 4 nM (300 ng) supercoiled pGEM9-Zf(−) target DNA, 125 mM NaCl, 5 mM $MgSO_4$, 4 μM $ZnCl_2$, 10 mM DTT, 0.8% (vol/vol) DMSO, and 25 mM BisTris propane–HCl, pH 7.45. Raltegravir is added at indicated concentrations. Reactions are initiated by addition of 2 μL PFV IN diluted in 150 mM NaCl, 2 mM DTT, and 10 mM Tris-HCl, pH 7.4, and stopped after 1 hour at 37 °C by addition of 25 mM EDTA and 0.5% (wt/vol) SDS. Reaction products, deproteinized by digestion with 20 μg proteinase K for 30 minutes at 37 °C followed by ethanol precipitation, are separated in 1.5% agarose gels and visualized by staining with ethidium bromide. Integration products are quantified by quantitative real-time PCR, using Platinum SYBR Green qPCR SuperMix and three primers: 5′-CTACTTACTCTAGCTTCCCGGCAAC, 5′-TTCGCCAGTTAATAGTTTGCGCAAC, and 5′-GACTCACTATAGGGCACGCGT. PCR reactions (20 μL) contained 0.5 μM of each primer and 1 μL diluted integration reaction product. Following a 5-min denaturation step at 95 °C, 35 cycles are carried out in a CFX96 PCR instrument, using 10 seconds denaturation at 95 °C, 30 seconds annealing at 56 °C and 1 minutes extension at 68 °C. Standard curves are generated using serial dilutions of WT PFV IN reaction in the absence of this compound.

Cell Assay:[5]

- **Cell lines**

  Human MT-4 cells
- **Concentrations**

  0.0001-1 μM
- **Incubation Time**

5 days

- **Method**

  Human MT-4 cells are infected for 2 hours with the SIVmac251, HIV-1 (IIIB) and HIV-2 (CDC 77618) stocks at a multiplicity of infection of, approximately, 0.1. Cells are then washed three times in phosphate buffered saline, and suspended at 5 × 10^5/mL in fresh culture medium (to primary cells 50 units/mL of IL-2 are added) in 96-well plates, in the presence or absence of a range of triplicate raltegravir concentrations (0.0001 μM -1 μM). Untreated infected and mock-infected controls are prepared too, in order to allow comparison of the data derived from the different treatments. Viral cytopathogeniciy in MT-4 cells is quantitated by the methyl tetrazolium (MTT) method (MT-4/MTT assay) when extensive cell death in control virus-infected cell cultures is detectable microscopically as lack of capacity to re-cluster. The capability of MT-4 cells to form clusters after infection. Briefly, clusters are disrupted by pipetting; and, after 2 hours of incubation at 37 °C, the formation of new clusters is assessed by light microscopy (100 × magnification). Cell culture supernatants are collected for HIV-1 p24 and HIV-2/SIVmac251 p27 core antigen measurement by ELISA. In CEMx174-infected cell cultures, which show a propensity to form syncytia induced by the virus envelope glycoproteins, syncytia are counted, in blinded fashion, by light microscopy for each well at 5 days following infection.

Animal Study:[5]

- **Animal Models**

  Indian Rhesus macaques

- **Dosages**

  50 mg/kg or 100 mg/kg

- **Administration**

  Oral administration

References

https://pubmed.ncbi.nlm.nih.gov/21030679/ https://pubmed.ncbi.nlm.nih.gov/19231980/ https://pubmed.ncbi.nlm.nih.gov/22450971/

+ Expand

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SHIPPING & STORAGE

Capsid Bio products are transported at room temperature. If you receive the product at room temperature, please rest assured, the Capsid Bio Quality Inspection Department has conducted experiments to verify that the normal temperature placement of one month will not affect the biological activity of powder products. After collecting, please store the product according to the requirements described in the datasheet. Most Capsid Bio products are stable under the recommended conditions.

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