

Product Data Sheet

Product Name: Tenofovir alafenamide hemifumarate

Cat. No.: GC37759

Chemical Properties

Cas. No. 1392275-56-7

SMILES O=C(O)/C=C/C(O)=O.NC1=NC=NC2=C1N=CN2C[C@@H](C)OC[P@](OC3=CC=CC=C3)(N[C@@H](C)C(OC(C)C)=O)=O.[0.5]

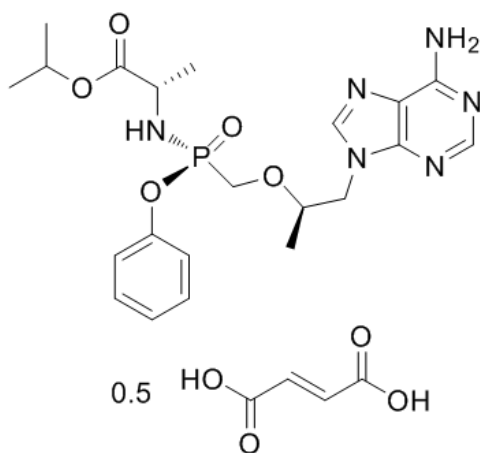
Formula $C_{21}H_{29}N_6O_5P \cdot \frac{1}{2} C_4H_4O_4$ M.Wt 534.5

Solubility DMSO: 50 mg/mL (93.55 mM) Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

Cell lines MT-2 cells

Caution: Product has not been fully validated for medical applications. For research use only.

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MT-2 cells (2.4 million) were incubated with virus for 3h at 37°C in 1mL screw-cap tubes. Virus was normalized to yield a signal-to-noise ratio (uninfected cells to infected cells) between 4 to 7, which was equivalent to a multiplicity of infection (MOI) ~0.003 for wild-type HIV-1 IIIB. Five-fold Tenofovir alafenamide hemifumarate gradient dilutions (0.01nM-1µM) were transferred in triplicate to the inside wells of 96-well plates (50µL per well). After incubation, infected MT-2 cells were seeded at 8500 per well in 100µL of media. After incubation for 5 days at 37°C, 100µL of CellTiterGlo reagent was added to each well of the assay plates and luminescence was measured using an Envision plate reader.

Reaction Conditions 0.01nM-1µM; 5 days

Applications Treatment of MT-2 cells with Tenofovir alafenamide hemifumarate showed potent antiviral activity in vitro with EC₅₀ values of 5nM.

Animal experiment [2]:

Animal models Naive Male Sprague-Dawley rats

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Naive male Sprague-Dawley rats were singly housed in polycarbonate caging with free access to food and water for the duration of the study, unless otherwise noted. At the time of dose initiation, animals were 11 to 14 weeks old and weighed between 372 and 549g. After an acclimation period of at least 7 days, rats were surgically implanted with a s.c. cannula while under isoflurane anesthesia. Cannulas were routed dorsally through the s.c. tissue extending from the lumbar region to an exteriorization site at the base of the neck. The cannulas were tethered through a swivel system outside the cage, and jackets were placed on the rats to hold the tethered system in place. Saline infusions

Preparation Method were maintained at a rate of 0.04ml/h until study initiation, which occurred after a recovery period of at least 10 days. Prior to study initiation, animals were assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. The study consisted of five treatment groups, with each group receiving Tenofovir alafenamide hemifumarate at a dose of 0, 30, 100, 300, or 1,000mg/kg/day. Within each treatment group, five animals were assigned to the main study group, and six animals were assigned to the recovery group. Each treatment group also contained two separate satellite groups: one group of six animals for Histopathology analysis and one group of eighteen animals for PBMC sample collection.

Dosage form 0-1000mg/kg/day; 28 days; subcutaneous infusion

Applications Tenofovir alafenamide hemifumarate resulted in increased incidence and severity of fibrosis and mononuclear cell inflammation at the infusion site in male rats.

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References:

- [1] Callebaut C, Stepan G, Tian Y, Miller MD. In Vitro Virology Profile of Tenofovir Alafenamide, a Novel Oral Prodrug of Tenofovir with Improved Antiviral Activity Compared to That of Tenofovir Disoproxil Fumarate. *Antimicrob Agents Chemother*. 2015;59(10):5909-5916.
- [2] Zane D, Roller S, Shelton J, et al. A 28-Day Toxicity Study of Tenofovir Alafenamide Hemifumarate by Subcutaneous Infusion in Rats and Dogs. *Microbiol Spectr*. 2021;9(1):e0033921.

Background

Tenofovir alafenamide hemifumarate (GS-7340 hemifumarate) is a prodrug of the HIV-1 nucleotide reverse transcriptase (RT)^[1]. Tenofovir alafenamide hemifumarate is converted into the active metabolite tenofovir-diphosphate (TFV-DP) in vivo by carboxylate esterase-1 (CES1) in hepatocytes and subsequent phosphorylation^[2]. TFV-DP competitively inhibits viral RT and acts as a chain terminator by incorporating into nascent viral DNA, thereby blocking HIV-1 replication^[3]. Tenofovir alafenamide hemifumarate is commonly used in antiviral research against HIV-1 and HBV^[4].

In vitro, treatment of MT-2 cells with Tenofovir alafenamide hemifumarate (0.01 nM–1 μM; 5 days) showed potent antiviral activity in vitro with EC₅₀ values of 5 nM^[5].

In vivo, Tenofovir alafenamide hemifumarate (10 μg/kg/day; subcutaneous implant; 12 weeks) induced severe local inflammation and necrosis in the peri-implant tissue of rhesus macaques^[6]. Tenofovir alafenamide hemifumarate (0–1000 mg/kg/day; 28 days; subcutaneous infusion) resulted in increased incidence and severity of fibrosis and mononuclear cell inflammation at the infusion site in male rats^[7].

References:

- [1] Gibson AK, Shah BM, Nambiar PH, Schafer JJ. Tenofovir Alafenamide. *Ann Pharmacother*. 2016;50(11):942-952.
- [2] Birkus G, Bam RA, Willkom M, et al. Intracellular Activation of Tenofovir Alafenamide and the Effect of Viral and Host Protease Inhibitors. *Antimicrob Agents Chemother*. 2015;60(1):316-322.
- [3] Uglietti A, Zanaboni D, Gnarin M, Maserati R. Emtricitabine/tenofovir in the treatment of HIV infection: current PK/PD evaluation. *Expert Opin Drug Metab Toxicol*. 2012;8(10):1305-1314.

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- [4] Hong X, Cai Z, Zhou F, et al. Improved pharmacokinetics of tenofovir ester prodrugs strengthened the inhibition of HBV replication and the rebalance of hepatocellular metabolism in preclinical models. *Front Pharmacol.* 2022;13:932934.
- [5] Callebaut C, Stepan G, Tian Y, Miller MD. In Vitro Virology Profile of Tenofovir Alafenamide, a Novel Oral Prodrug of Tenofovir with Improved Antiviral Activity Compared to That of Tenofovir Disoproxil Fumarate. *Antimicrob Agents Chemother.* 2015;59(10):5909-5916.
- [6] Su JT, Simpson SM, Sung S, et al. A Subcutaneous Implant of Tenofovir Alafenamide Fumarate Causes Local Inflammation and Tissue Necrosis in Rabbits and Macaques. *Antimicrob Agents Chemother.* 2020;64(3):e01893-19.
- [7] Zane D, Roller S, Shelton J, et al. A 28-Day Toxicity Study of Tenofovir Alafenamide Hemifumarate by Subcutaneous Infusion in Rats and Dogs. *Microbiol Spectr.* 2021;9(1):e0033921.

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