**Product Data Sheet**

**PF-04447943**

**Cat. No.:** HY-15441  
**CAS No.:** 1082744-20-4  
**Molecular Formula:** C₂₀H₂₅N₇O₂  
**Molecular Weight:** 395.46

**Target:** Phosphodiesterase (PDE)  
**Pathway:** Metabolic Enzyme/Protease

**Storage:**  
- Powder: -20°C for 3 years, 4°C for 2 years  
- In solvent: -80°C for 6 months, -20°C for 1 month

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**SOLVENT & SOLUBILITY**

**In Vitro**  
DMSO: ≥ 54.6 mg/mL (138.07 mM)  
*“≥” means soluble, but saturation unknown.*

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>Mass 1 mg</th>
<th>Mass 5 mg</th>
<th>Mass 10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td></td>
<td>2.5287 mL</td>
<td>12.6435 mL</td>
<td>25.2870 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>0.5057 mL</td>
<td>2.5287 mL</td>
<td>5.0574 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>0.2529 mL</td>
<td>1.2644 mL</td>
<td>2.5287 mL</td>
</tr>
</tbody>
</table>

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

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**BIOLOGICAL ACTIVITY**

**Description**  
PF-04447943 is a potent inhibitor of human recombinant PDE9A (IC₅₀=12 nM) with >78-fold selectivity, respectively, over other PDE family members (IC₅₀>1000 nM).

**IC₅₀ & Target**  
IC₅₀: 12 nM (PDE9A)[¹]

**In Vitro**  
Using recombinant human, rhesus, and rat PDE9A2 in a cell free assay PF-0447943 is shown to have a Kᵢ of 2.8±0.26, 4.5±0.13, and 18.1±1.9 nM (n=4, 11 and 9 respectively). PF-0447943 is found to be highly selective over other PDE enzymes (PDE1, Kᵢ=8600±2121 nM, n = 5; PDE2A3, Kᵢ>99,000 nM; PDE3A, Kᵢ>50,000 nM; PDE4A, Kᵢ>29,000 nM; PDE5A, Kᵢ=14,980±5025 nM, n=5; PDE6C, Kᵢ=5324±2612 nM, n=4; PDE7A2, Kᵢ>75,000 nM; PDE8A, Kᵢ>50,000 nM; PDE10, Kᵢ>51,250±20,056 nM, n=4; PDE11, Kᵢ>80,000 nM) and no other significant activity at ~60 other receptors/enzymes. In HEK whole cells expressing rhesus PDE9A2, PF-04447943 inhibits ANP (0.3 μM) stimulated cGMP with an IC₅₀ of 375±36.9 nM (n=16)[²].

[¹] MedChemExpress.com  
[²] MedChemExpress.com
### In Vivo
Based on i.v. and p.o. dosing, pharmacokinetic studies with PF-04447943 in the rat indicates a $T_{\text{max}}$ of 0.3 h, $T_{1/2}$ of 4.9 h, Cl of 21.7 mL/min/kg and an oral bioavailability of 47%. Thirty minutes following oral administration in rats (1-30 mg/kg), PF-04447943 concentrations dose-dependently increase in blood, brain and cerebrospinal fluid (CSF). The 

### PROTOCOL

#### Kinase Assay

PDE enzyme assays are carried out. PDE1A-C, PDE2A, PDE3A/B, PDE4A-D, PDE7A/B, PDE8A/B, PDE9A, PDE10A, and PDE11 are generated from full-length recombinant clones. PDE5 is isolated from human platelets, and PDE6 is isolated from bovine retina. PDE activity is measured by using a scintillation proximity assay (SPA). The effects of PDE inhibitors are investigated by assaying a fixed amount of enzyme and varying inhibitor concentrations in the presence of substrate concentrations of 1/3 $K_m$ values for each enzyme, so that the IC$_{50}$ value approximates the $K_i$ value. PF-4447943 is dissolved in 100% DMSO and diluted to the required concentrations in 15% DMSO water. The enzyme stocks are all thawed slowly and diluted in assay buffer containing 50 mM Tris-HCl (pH 7.5 at room temperature) and 1.3 mM MgCl$_2$. In addition, the PDE1 assay buffers contain 2.8 mM CaCl$_2$. The PDE1C assay also requires the addition of the activator calmodulin at a final assay concentration of 100 units/mL. Incubations are initiated by the addition of diluted enzyme to 384-well plates containing test drugs and radioligand (50 nM $[^3]$H$cGMP$ for PDE1, PDE2, PDE5, PDE6, PDE9, PDE10, and PDE11 and 20 nM $[^3]$H$cAMP$ for PDE3, PDE4, PDE7, and PDE8). The assays are incubated for 30 min at room temperature (60 min for PDE5 and PDE6). The reactions are stopped by the addition of phosphodiesterase SPA beads at a final assay concentration of 0.2 mg/well. PDE9 requires the extra addition of a high concentration (10 μM) of a potent PDE9 inhibitor before beads to stop the reaction completely. Activities of test compounds are assessed by measuring the amount of $[^3]$H$cGMP$ or $[^3]$H$cAMP$ produced from $[^3]$H$cGMP$ or $[^3]$H$cAMP$ radioligand, respectively. Levels of $[^3]$H$cGMP$ or $[^3]$H$cAMP$ binds to SPA beads are determined by paralux counting of the assay plates in a Microbeta Trilux Counter 10 h after bead addition. Nonspecific binding is determined by radioligand binding in the presence of a saturating concentration of a potent PDE inhibitor. The IC$_{50}$ value of each test compound (concentration at which 50% inhibition of specific binding occurs) is calculated by nonlinear regression (curve fitting) of the concentration response

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

#### Cell Assay

The rhesus PDE9A2 construct is subcloned into a pcDNA3.3 TOPO vector and HEK 293 cells, stably transfected to constitutively express rhesus PDE9A2 and hNPR1, are incubated with PF-04447943 (30 μM to 1.5 nM) in assay media at a density of 10,000 cells/well, for 30 min at 37°C. Cyclic GMP formation is stimulated by incubation with 0.3 μM ANP (Atrial Natriuretic Peptide) for another 30 min at 37°C. Following incubation, cells are lysed with Antibody/Lysis buffer and ED Reagent for 1 h at room temperature. After a subsequent incubation with EA Reagent for 30 min at room temperature, followed by incubation with Substrate Reagent for 1 h at room temperature, cGMP concentrations are determined by measuring luminescence on the Envision Microplate Luminometer. The maximal inhibition (100% activity) in the cell based assay is determined using 30 μM PF-04447943 and 0% activity is defined by the DMSO control. PF-04447943 is titrated in quadruplicate, in a 10 point titration. Percentage inhibition is calculated using the maximal inhibition value and IC$_{50}$ values are calculated from concentration response curves using Prism software. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### Animal

Mice and Rats

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Administration [2]

For the mouse studies, male C57Bl/6J mice are administered PF-04447943 (3, 10, 30 mg/kg p.o.). For the rat studies rats (strain, weight range and supplier as described in the novel object recognition study below) are administered PF-04447943 10 mg/kg i.v. and p.o. At various times after administration the animals are anesthetized with isoflurane; blood samples are withdrawn via cardiac puncture and placed in EDTA tubes on ice. Plasma is separated and frozen at −70°C until assayed for drug concentration. The animals are decapitated, the brain is removed, then homogenized in 3 mL of water per gram of tissue and centrifuged for 15 min at 13,500 g. Sample analysis is conducted using an Acquity UPLC system coupled with a SCIEX API4000 Q-Trap mass spectrometer. Two μL of the sample extract is analyzed using an Acquity UPLC® BEH C18 column (1.7 μm particle size, 50×2.1 mm I.D.) operated at 60°C. The flow rate is 0.7 mL/min. A gradient mobile phase consisting of solvent A (20/80 Methanol/Water, 10 mM Ammonium Acetate) and solvent B (10 mM Ammonium Acetate in Methanol with 0.6 mL/L 10% acetic Acid) is used. The total run time for each sample is 1.2 min. PF-04447943 and the internal standard eluted at 0.61 and 0.67 min, respectively. PF-04447943 and the internal standard are monitored in the positive ion mode at the transition from m/z 396.2 to 203.1 and m/z 477.3 to 266.2, respectively. Quantification is performed using Analyst 1.4 based on duplicate standard curves.

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

CUSTOMER VALIDATION


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REFERENCES


[2]. Hutson, P. H, et al. The selective phosphodiesterase 9 (PDE9) inhibitor PF-04447943 (6-((3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) enhances synaptic plasticity and