**PF-04447943**

**Cat. No.:** HY-15441  
**CAS No.:** 1082744-20-4  
**Molecular Formula:** C_{20}H_{25}N_{7}O_{2}  
**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>Concentration</th>
<th>Mass (1 mg)</th>
<th>Mass (5 mg)</th>
<th>Mass (10 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td>2.5287 mL</td>
<td>12.6435 mL</td>
<td>25.2870 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.5057 mL</td>
<td>2.5287 mL</td>
<td>5.0574 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</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.

**In Vivo**  
1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
   Solubility: ≥ 2.5 mg/mL (6.32 mM); Clear solution  
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
   Solubility: ≥ 2.5 mg/mL (6.32 mM); Clear solution  
3. Add each solvent one by one: 10% DMSO >> 90% corn oil  
   Solubility: ≥ 2.5 mg/mL (6.32 mM); Clear solution

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**BIOLOGICAL ACTIVITY**

**Description**  
PF-04447943 is a potent inhibitor of human recombinant PDE9A (IC_{50}=12 nM) with >78-fold selectivity, respectively, over other PDE family members (IC_{50}>1000 nM).

**IC_{50} & Target**  
IC50: 12 nM (PDE9A)\(^{[1]}\)  

**In Vitro**  
Using recombinant human, rhesus, and rat PDE9A2 in a cell free assay PF-04447943 is shown to have a K_{i} of 2.8±0.26.
4.5±0.13, and 18.1±1.9 nM (n=4, 11 and 9 respectively). PF-04447943 is found to be highly selective over other PDE enzymes (PDE1, $K_i=8600±2121$ nM, n = 5; PDE2A3, $K_i>99,000$ nM; PDE3A, $K_i=50,000$ nM; PDE4A, $K_i>29,000$ nM; PDE5A, $K_i=14,980±5025$ nM, n=5; PDE6C, $K_i=5324±2612$ nM, n=4; PDE7A2, $K_i>75,000$ nM; PDE8A, $K_i=50,000$ nM; PDE10, $K_i>51,250±20,056$ nM, n=4; PDE11, $K_i>80,000$ nM) and no other significant activity at ~60 other receptors/enzymes. In HEK whole cells expressing rhesus PDE9A2, PF-04447943 inhibits ANP (0.3 $\mu$M) stimulated cGMP with an $IC_{50}$ of 375±36.9 nM (n=16). MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**In Vivo**

Based on i.v. and p.o. dosing, pharmacokinetic studies with PF-04447943 in the rat indicates a $T_{max}$ 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 brain:plasma exposure ratios 30 min after dosing range from 0.13 at the 1 mg/kg dose to 0.33 at the 30 mg/kg dose. CSF levels are approximately 50% of brain levels. In mice, PF-04447943 (3, 10, 30 mg/kg p.o.) dose-dependently increases plasma and brain concentrations of PF-04447943 while the brain to plasma ratio ranged from 0.26 to 0.7 although this is not entirely dose dependent. CSF cGMP levels increase in a dose-dependent manner from a basal level of 3 pmol/mL to 13.3 pmol/mL (3.5-fold) at the 30 mg/kg dose. CSF cGMP levels also increase in a dose-dependent manner from a basal level of 3 pmol/mL in vehicle treated animals to 13.3 pmol/mL (3.5-fold) at the 30 mg/kg dose. CSF cGMP levels are elevated at all doses tested with a maximal effect of 3.5 fold increase above controls at 30 mg/kg. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**PROTOCOL**

**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 $\mu$M 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 $\mu$M 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 $\mu$M 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 Administration**

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. 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**

- Neurochem Int. 2020 Feb;133:104630.

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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

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