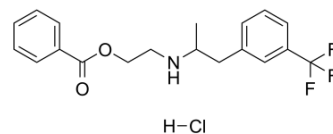


## Benfluorex hydrochloride

<b>Cat. No.:</b>	HY-B1058		
<b>CAS No.:</b>	23642-66-2		
<b>Molecular Formula:</b>	C <sub>19</sub> H <sub>21</sub> ClF <sub>3</sub> NO <sub>2</sub>		
<b>Molecular Weight:</b>	387.82		
<b>Target:</b>	Others		
<b>Pathway:</b>	Others		
<b>Storage:</b>	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : ≥ 100 mg/mL (257.85 mM)  
 H<sub>2</sub>O : 3.33 mg/mL (8.59 mM; Need ultrasonic)  
 \* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	2.5785 mL	12.8926 mL	25.7852 mL
	5 mM	0.5157 mL	2.5785 mL	5.1570 mL
	10 mM	0.2579 mL	1.2893 mL	2.5785 mL

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

#### In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.5 mg/mL (6.45 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
Solubility: ≥ 2.5 mg/mL (6.45 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil  
Solubility: ≥ 2.5 mg/mL (6.45 mM); Clear solution

### BIOLOGICAL ACTIVITY

<b>Description</b>	Benfluorex hydrochloride (JP-992 hydrochloride) is a hepatic nuclear factor 4 alpha (HNF4α) activator.
<b>IC<sub>50</sub> &amp; Target</b>	HNF4α <sup>[1]</sup>
<b>In Vitro</b>	Benfluorex hydrochloride consistently activates insulin promoter activity as measured by an increased number of GFP-

positive cells. Benfluorex hydrochloride increases the number of GFP-positive cells in a dose-responsive manner and increases the level of endogenous insulin mRNA. Consistent with being HNF4 $\alpha$  activator, Benfluorex hydrochloride stimulates HNF4 $\alpha$  expression. Benfluorex hydrochloride alters HNF4 $\alpha$  protease sensitivity, while the inactive control compound does not<sup>[1]</sup>. Benfluorex hydrochloride decreases, in a concentration-dependent manner, the synthesis of acid-soluble products and ketone bodies from oleate, whereas the production of <sup>14</sup>CO<sub>2</sub> into citric acid cycle is markedly increased by Benfluorex hydrochloride. Benfluorex hydrochloride inhibits in a dose-dependent manner the rates of gluconeogenesis from lactate/pyruvate (10/1 nM)<sup>[2]</sup>.

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

## PROTOCOL

### Kinase Assay <sup>[1]</sup>

HepG2 cells are treated with DMSO or Benfluorex hydrochloride at a concentration of 20  $\mu$ M or 40  $\mu$ M for 16 hr. Total cell protein is extracted, measured by BCA protein assay. Each sample is split into two aliquots for proteolysis without (-) or with (+) Subtilisin. Twenty  $\mu$ g of cell lysate is incubated with or without protease (20 ng/mL subtilisin) for 35 minutes at room temperature. Western blot is then performed with primary anti-HNF4 $\alpha$  polyclonal antibody (1:1000 dilution) and secondary HRP conjugated anti-goat IgG (1:2000 dilution), detected with chemiluminescence ECL kit<sup>[1]</sup>.

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### Cell Assay <sup>[2]</sup>

Hepatocytes are isolated by in situ perfusion of the liver with 0.025% collagenase. Hepatocytes (1 to 2 $\times$ 10<sup>6</sup> cells/mL) are incubated at 37°C in 2 mL of oxygenated (O<sub>2</sub>:CO<sub>2</sub>; 95:5) Krebs-Henseleit bicarbonate buffer (pH 7.4) for 1 h in a gyratory shaking water bath. Benfluorex hydrochloride is dissolved in DMSO and added (10  $\mu$ L) to the incubation medium at a final concentration of 0.1 or 1 nM<sup>[2]</sup>.

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

## REFERENCES

[1]. Lee SH, et al. Identification of alverine and benfluorex as HNF4 $\alpha$  activators. ACS Chem Biol. 2013 Aug 16;8(8):1730-6.

[2]. Kohl C, et al. Effects of benfluorex on fatty acid and glucose metabolism in isolated rat hepatocytes: from metabolic fluxes to gene expression. Diabetes. 2002 Aug;51(8):2363-8.

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

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