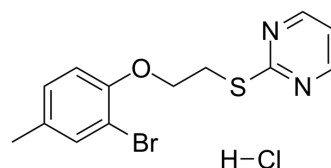


ZLN024 hydrochloride

Cat. No.:	HY-16708A
CAS No.:	1883548-91-1
Molecular Formula:	C ₁₃ H ₁₄ BrClN ₂ OS
Molecular Weight:	361.69
Target:	AMPK
Pathway:	Epigenetics; PI3K/Akt/mTOR
Storage:	4°C, sealed storage, away from moisture * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro	DMSO : ≥ 46 mg/mL (127.18 mM) H ₂ O : < 0.1 mg/mL (insoluble) * "≥" means soluble, but saturation unknown.																						
	<table border="1"> <thead> <tr> <th rowspan="2">Preparing Stock Solutions</th> <th rowspan="2">Solvent Concentration</th> <th colspan="3">Mass</th> </tr> <tr> <th>1 mg</th> <th>5 mg</th> <th>10 mg</th> </tr> </thead> <tbody> <tr> <td></td> <td>1 mM</td> <td>2.7648 mL</td> <td>13.8240 mL</td> <td>27.6480 mL</td> </tr> <tr> <td></td> <td>5 mM</td> <td>0.5530 mL</td> <td>2.7648 mL</td> <td>5.5296 mL</td> </tr> <tr> <td></td> <td>10 mM</td> <td>0.2765 mL</td> <td>1.3824 mL</td> <td>2.7648 mL</td> </tr> </tbody> </table> <p>Please refer to the solubility information to select the appropriate solvent.</p>	Preparing Stock Solutions	Solvent Concentration	Mass			1 mg	5 mg	10 mg		1 mM	2.7648 mL	13.8240 mL	27.6480 mL		5 mM	0.5530 mL	2.7648 mL	5.5296 mL		10 mM	0.2765 mL	1.3824 mL
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In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (6.91 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (6.91 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (6.91 mM); Clear solution 																						

BIOLOGICAL ACTIVITY

Description	ZLN024 hydrochloride is an AMPK allosteric activator. ZLN024 directly activates recombinant AMPK α1β1γ1, AMPK α2β1γ1, AMPK α1β2γ1 and AMPK α2β2γ1 heterotrimer with EC ₅₀ s of 0.42 μM, 0.95 μM, 1.1 μM and 0.13 μM, respectively.		
IC ₅₀ & Target	AMPK α2β2γ1 0.13 μM (EC50)	AMPK α1β1γ1 0.42 μM (EC50)	AMPK α2β1γ1 0.95 μM (EC50)
In Vitro	ZLN024 allosterically stimulates active AMPK heterotrimers and the inactive α1 subunit truncations α1 (1-394) and α1 (1-335)		

but not $\alpha 1$ (1-312). AMPK activation by ZLN024 requires the pre-phosphorylation of Thr-172 by at least one upstream kinase and protects AMPK Thr-172 against dephosphorylation by PP2C α . ZLN024 activates AMPK in L6 myotubes and stimulates glucose uptake and fatty acid oxidation without increasing the ADP/ATP ratio. Using the established scintillation proximity assay (SPA) assay, random screening against the AMPK $\alpha 1\beta 1\gamma 1$ heterotrimer is performed and a new AMPK activator, ZLN024 is found. ZLN024 directly activates recombinant AMPK $\alpha 1\beta 1\gamma 1$ and its homologue $\alpha 2\beta 1\gamma 1$ in a concentration-dependent manner. ZLN024 increases the activity of $\alpha 1\beta 1\gamma 1$ by 1.5-fold and has an EC₅₀ of 0.42 μ M, and it increases the activity of $\alpha 2\beta 1\gamma 1$ by 1.7-fold with an EC₅₀ of 0.95 μ M. ZLN024 also directly activates recombinant AMPK $\alpha 1\beta 2\gamma 1$, by 1.7-fold with an EC₅₀ of 1.1 μ M; and AMPK $\alpha 2\beta 2\gamma 1$, by 1.6-fold with an EC₅₀ of 0.13 μ M^[1].

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

In Vivo

C57BKS db/db mice are administered a 15 mg/kg/day dose of ZLN024 by daily gavage for 5 weeks; 250 mg/kg/day Metformin (Met) is used as a positive control. During the treatment period, there is no significant alteration in food intake and body weight compared with the vehicle group. After 4 weeks of treatment, ZLN024 improves glucose tolerance. ZLN024 reduces the fasting blood glucose by 15%. Liver tissue weight, triacylglycerol and the total cholesterol content are decreased^[1].

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PROTOCOL

Kinase Assay ^[1]

Before the scintillation proximity assay (SPA) assay, 200 nM recombinant AMPK protein ($\alpha 1\beta 1\gamma 1$, $\alpha 2\beta 1\gamma 1$, $\alpha 1\beta 2\gamma 1$, $\alpha 2\beta 2\gamma 1$, $\alpha 1$ (1-394), $\alpha 1$ (1-335), $\alpha 1$ (1-312)) is constructed, expressed, purified and fully phosphorylated. The SPA reactions are performed in 96-well plates in a final volume of 50 μ L containing 20 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 1 mM DTT, 2 μ M biotin-SAMS, 2 μ M ATP and 7.4 \times 10³ Bq/well [γ -³³P]ATP. The reactions are initiated by the addition of 50 nM recombinant AMPK protein to the reaction solutions, followed by incubation at 30°C for 2 hr. The reactions are then terminated by the addition of 40 μ L of stop solution containing 80 μ g Streptavidin-coated SPA beads per well, 50 mM EDTA and 0.1% Triton X-100 in PBS, pH 7.5, followed by incubation for 1 hr. Finally, 160 μ L of suspension solution containing 2.4 M CsCl, 50 mM EDTA and 0.1% Triton X-100 in PBS, pH 7.5, is added to the reaction solution to suspend the SPA beads completely. The SPA signals are measured in a Wallac Microbeta plate counter 30 min later^[1].

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Animal Administration ^[1]

Mice^[1]

C57BKS *db/db* mice are maintained under a 12 hr light-dark cycle with free access to water and food. At 8 weeks of age, male *db/db* mice are randomly assigned to the various treatment groups by body weight and glucose levels (n=6-8). The treatment groups for the 5-week chronic study are as follows: vehicle (0.5% methylcellulose), ZLN024 (15 mg/kg) and Metformin (250 mg/kg). The treatments are orally administered once daily. The body weights and food intake are measured daily. After 5 weeks of treatment, the mice are killed after a final dose, and the tissues are collected for further analysis.

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CUSTOMER VALIDATION

- Cell Death Differ. 2022 Jan 29.

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REFERENCES

[1]. Zhang LN, et al. Novel small-molecule AMP-activated protein kinase allosteric activator with beneficial effects in *db/db* mice. PLoS One. 2013 Aug 20;8(8):e72092.

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

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