# ZLN024 hydrochloride

**Cat. No.:** HY-16708A

**CAS No.:** 1883548-91-1

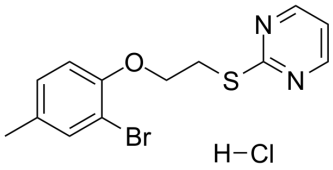
**Molecular Formula:** $C_{13}H_{14}BrClN_2OS$

**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)
$H_2O$ : < 0.1 mg/mL (insoluble)
* "≥" means soluble, but saturation unknown.

**Preparing Stock Solutions**

| Solvent / Mass / Concentration | 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 | 2.7648 mL |

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 (6.91 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (6.91 mM); Clear solution
3. 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 | AMPK α1β1γ1 | AMPK α2β1γ1 |
|---|---|---|
| 0.13 μM (EC50) | 0.42 μM (EC50) | 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)

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|---|---|
| | but not α1 (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 PP2Cα. 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 α1β1γ1 heterotrimer is performed and a new AMPK activator, ZLN024 is found. ZLN024 directly activates recombinant AMPK α1β1γ1 and its homologue α2β1γ1 in a concentration-dependent manner. ZLN024 increases the activity of α1β1γ1 by 1.5-fold and has an $EC_{50}$ of 0.42 μM, and it increases the activity of α2β1γ1 by 1.7-fold with an $EC_{50}$ of 0.95 μM. ZLN024 also directly activates recombinant AMPK α1β2γ1, by 1.7-fold with an $EC_{50}$ of 1.1 μM; and AMPK α2β2γ1, by 1.6-fold with an $EC_{50}$ of 0.13 μM[1].<br>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].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

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|---|---|
| **Kinase Assay** [1] | Before the scintillation proximity assay (SPA) assay, 200 nM recombinant AMPK protein (α1β1γ1, α2β1γ1, α1β2γ1, α2β2γ1, α1(1-394), α1(1-335), α1(1-312)) is constructed, expressed, purified and fully phosphorylated. The SPA reactions are performed in 96-well plates in a final volume of 50 μL containing 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 2 μM biotin-SAMS, 2 μM ATP and $7.4\times10^3$ Bq/well [γ-$^{33}$P]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 μL of stop solution containing 80 μg 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 μL 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].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration** [1] | Mice[1]<br>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.<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## 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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