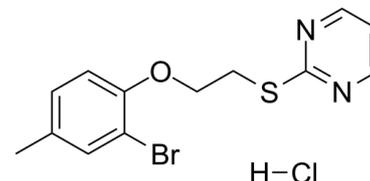


Data Sheet

Product Name:	ZLN024 (hydrochloride)
Cat. No.:	CS-3463
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
Solubility:	H ₂ O : < 0.1 mg/mL (insoluble); DMSO : ≥ 46 mg/mL (127.18 mM)



BIOLOGICAL ACTIVITY:

ZLN024 hydrochloride is an **AMPK** allosteric activator. ZLN024 directly activates recombinant AMPK $\alpha 1\beta 1\gamma 1$, AMPK $\alpha 2\beta 1\gamma 1$, AMPK $\alpha 1\beta 2\gamma 1$ and AMPK $\alpha 2\beta 2\gamma 1$ heterotrimer with **EC₅₀s** of 0.42 μ M, 0.95 μ M, 1.1 μ M and 0.13 μ M, respectively. IC₅₀ & Target: EC₅₀: 0.42 μ M, 0.95 μ M, 1.1 μ M and 0.13 μ M (AMPK $\alpha 1\beta 1\gamma 1$, $\alpha 2\beta 1\gamma 1$, $\alpha 1\beta 2\gamma 1$ and $\alpha 2\beta 2\gamma 1$ heterotrimer)^[1] **In Vitro**: ZLN024 allosterically stimulates active AMPK heterotrimers and the inactive $\alpha 1$ subunit truncations $\alpha 1$ (1-394) and $\alpha 1$ (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]. **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].

PROTOCOL (Extracted from published papers and Only for reference)

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 $\times 10^3$ 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].

Animal Administration: ZLN024 is prepared in vehicle (0.5% methylcellulose) (Mice)^[1].^[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.

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.

CAIndexNames:

Pyrimidine, 2-[[2-(2-bromo-4-methylphenoxy)ethyl]thio]-, hydrochloride (1:1)

SMILES:

CC1=CC=C(OCCSC2=NC=CC=N2)C(Br)=C1.[H]Cl

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

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