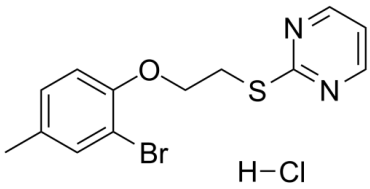
Data Sheet

Product Name:	ZLN024 (hydrochloride)
Cat. No.:	CS-3463
Molecular Formula:	C13H14BrClN2OS
Molecular Weight:	361.69
Target:	AMPK
Pathway:	Epigenetics; PI3K/Akt/mTOR
Solubility:	H2O : < 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 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. IC50 & Target: EC50: 0.42 μM, 0.95 μM, 1.1 μM and 0.13 μM (AMPK α1β1γ1, α2β1γ1, α1β2γ1 and α2β2γ1 heterotrimer)[1] **In Vitro:** ZLN024 allosterically stimulates active AMPK heterotrimers and the inactive α1 subunit truncations α1 (1-394) and α1 (1-335) 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]. **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 (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 7.4×10^3 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].

Animal Administration: ZLN024 is prepares 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

SMILES:

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

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