opnMe by Boehringer Ingelheim

Slowpoke 1 (SLO-1) channel agonist

BI-3972

Boehringer Ingelheim

Table of contents

Summary

BI-3972 is a potent and selective agonist of the homotetrameric calcium-activated potassium channel Slowpoke 1 (SLO-1). SLO-1 is ubiquitously expressed in metazoa and has been implied in a wide range of physiological roles. Functionally, BI-3972 has exhibited strong nematocidal activity against *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Dirofilaria immitis*, as well as large and small *Strongylus* species in mammals. It is supplied together with the negative control BI-4083.

Chemical Structure

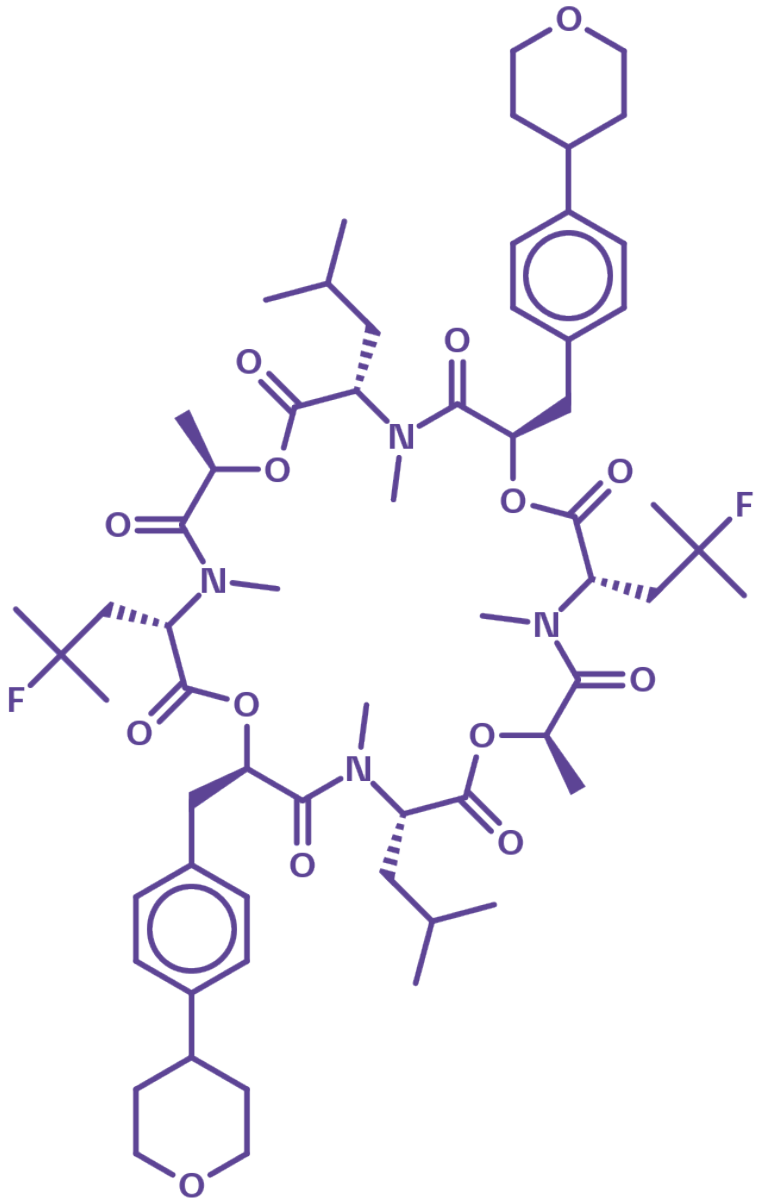

Figure 1: Structure of BI-3972, a SLO-1 agonist

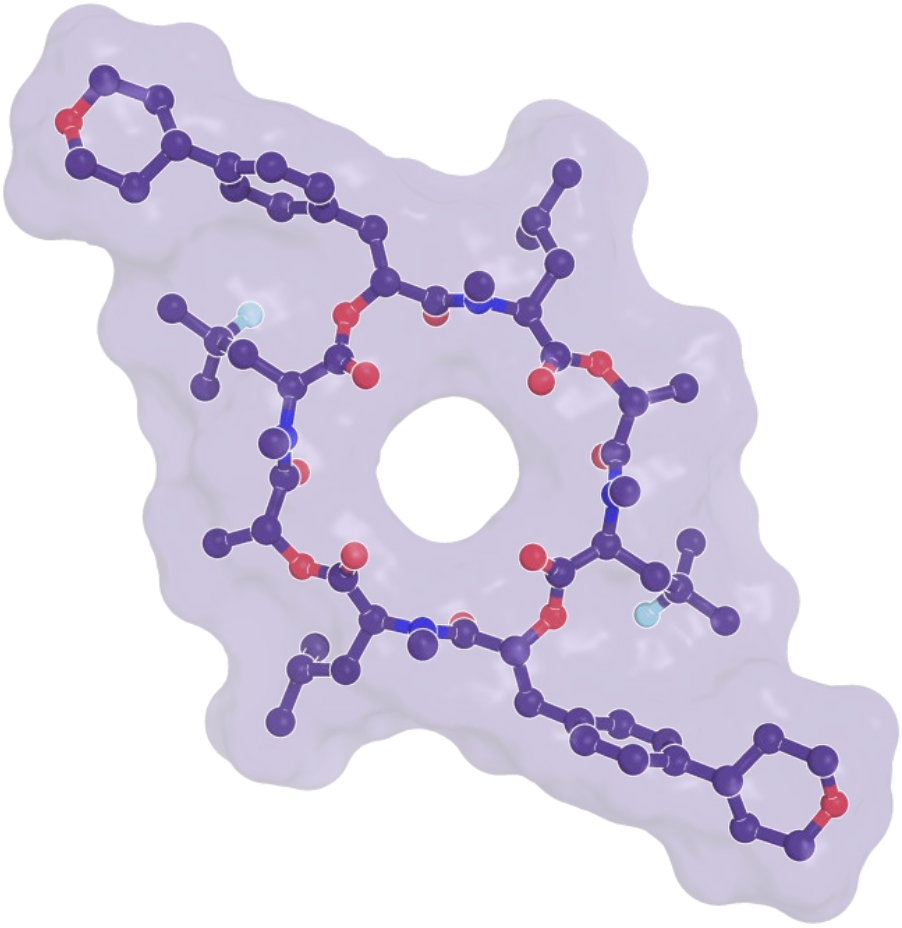

Figure 2: Low energy 3D conformation of SLO-1 agonist BI-3972

Highlights

BI-3972 is an agonist of the homotetrameric potassium channel Slowpoke 1 (SLO-1). SLO-1 is ubiquitously expressed in metazoa and has been implied in a wide range of physiological roles. Functionally, BI-3972 has been shown to have nematocidal activity against *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Dirofilaria immitis*, and *Strongylus* species.

Target information

SLO-1, a large-conductance calcium- and voltage-activated homotetrameric potassium channel, belongs to the evolutionarily conserved K^+ channel family. Activated by cellular depolarization and cytosolic calcium, it plays a critical role in regulating excitatory neurotransmitter release.

The channel was first identified in the *Drosophila melanogaster* slowpoke mutant, which exhibited abnormal locomotion and reduced flight ability. Due to its unusually high conductance, SLO-1 was classified as a "big K^+ conductance" channel. It is highly conserved across animal phyla, where it regulates cell excitability in a phylogenetically consistent manner.

In nematodes, SLO-1 modulates both activatory and inhibitory receptors in the nervous system. In *C. elegans*, it controls excitatory neurotransmitter release and is expressed in the nerve ring and body wall muscles. Mutations in the *slo-1* gene result in a distinct locomotor phenotype, characterized by an increased frequency of reversals during movement.

SLO-1 is also the molecular target of emodepside, a broad-spectrum anthelmintic. Emodepside activates the SLO-1 K^+ channel, leading to inhibition of nematode motility, pharyngeal pumping, and egg-laying[1-7].

A recent publication featured four cryo-EM structures of the prototype insect SLO channel from *Drosophila melanogaster* in the Ca^{2+}-bound and Ca^{2+}-free conformations and in complex with the ligands verruculogen and emodepside. This represented a first step towards the understanding on the structural and mechanistic details outlining how SLO-1 could be modulated by small molecule inhibitors and activators[8].

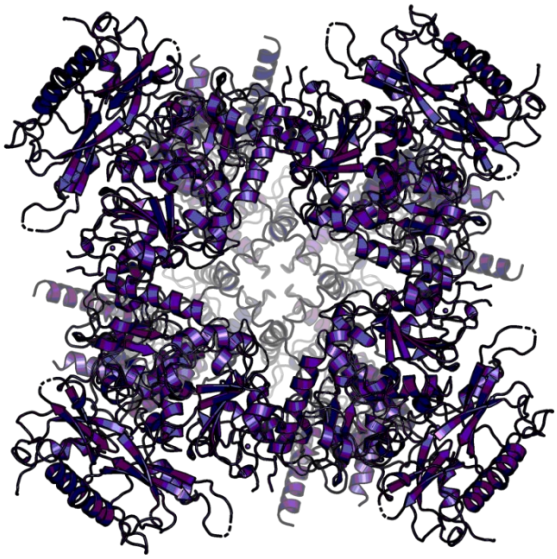

Figure 3: 3D structure of metazoan SLO-1 bound to agonist BI-3972, view from the extracellular side (Cryo-EM structure solved at Boehringer Ingelheim)

In vitro activity

BI-3972 underwent a series of phenotypic assessment. In larval development assays, BI-3972 effectively inhibits the metamorphosis of *Haemonchus contortus* with an EC_{50} value of 621 nM. Similarly, it prevents the larval development of *Cooperia oncophora* with a MIC_{90} of 10 nM. Against the dog heartworm *Dirofilaria immitis*, BI-3972 very potently inhibits the motility of microfilarial (EC_{50} = 28 nM) and L4 stages (EC_{50} = 0.21 nM).

In contrast, the negative control compound, BI-4083, is inefficacious in the *Haemonchus contortus* larval development assay, with an EC_{50} value exceeding 10,000 nM. Additionally, BI-4083 exhibits significantly lower activity in the *Dirofilaria* assays, with EC_{50} values of 2088 nM against the microfilarial and 53 nM against the L4 stages. Overall, the negative control is approximately 100-fold less active than the SlO-1 agonist BI-3972.

Probe name / Negative control	BI-3972	BI-4083
MW [Da][a]	1153.4	1189.4
H. contortus larval development (EC_{50}) [nM][b]	621 ± 882 (n=12)	>10,000 (n=4)
C. oncophora larval development (MIC_{90}) [nM][b]	10 ± 3.6 (n=4)	n.d.
D. immitis microfilaria (EC_{50}) [nM][c]	28 ± 16 (n=5)	2088 ± 691 (n=4)
D. immitis L4 motility (EC_{50}) [nM][d]	0.21 ± 0.35 (n=6)	53 ± 29 (n=3)

[a] *For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs*

[b] L1 stage *Haemonchus contortus* or *Cooperia oncophora* are delivered to wells of a microtiter plate containing nutrient medium. Compounds dissolved in DMSO (1% final conc.) are added. Plates are incubated for four days at 27 °C and 85% relative humidity. The resulting worms (L3s) are imaged, and quantitative motility descriptors are calculated.

[c] *Dirofilaria immitis* microfilariae are suspended in RPMI media supplemented with antibiotics/antimycotic and delivered to wells of a microtiter plate. Compounds dissolved in DMSO (1% final conc.) are added. Plates are incubated for 72 hours days at 37 °C and 5% CO_2. Worms are imaged, and quantitative motility descriptors are calculated.

[d] *Dirofilaria immitis* L4 -stage worms delivered to microplate wells containing a 1:1 mixture of iMDM:NCTC-109 media supplemented with antibiotic/antimycotic. Compounds dissolved in DMSO (1% final conc.) are added. Plates are incubated for 72 hours days at 37 °C and 5% CO_2. Worms are imaged, and quantitative motility descriptors are calculated.

In vitro DMPK and CMC parameters

The depsipeptide BI-3972 is larger, more lipophilic, and less water-soluble compared to Rule of 5 (Ro5)-like compounds. Despite these characteristics, BI-3972 demonstrates standard drug-like permeability and metabolic stability, while showing no significant inhibition in cytochrome P_{450} assays.

Probe name / Negative control	BI-3972	BI-4083
logD @ pH 2 / 7.4 / 11	>6 / 5.5 / >6	>6 / n.a. / >6
Solubility @ pH 7 [µg/ml]	<1	Not detectable
Caco-2 permeability AB @ pH 7.4 [$*10^{-6}$ cm/s]	36	1.2
Caco-2 efflux ratio	2.5	21.7
Microsomal stability (human/mouse/rat) [% Q_H]	<23 / <23 / <22	<23 / <23 / <22
Hepatocyte stability (human/mouse/rat) [% Q_H]	n.d. / 64 / 29	n.d. / <12 / 33
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2C8 (IC_{50}) [µM]	>50	>50
CYP 2C9 (IC_{50}) [µM]	>50	>50
CYP 2C19 (IC_{50}) [µM]	>50	>50
CYP 2D6 (IC_{50}) [µM]	>50	>50

In vivo DMPK parameters

BI-3972 is characterized by good bioavailability, a high volume of distribution, and a long half-life. However, its uptake and clearance rates are species dependent. Tissue distribution studies conducted in dogs revealed that the compound preferentially distributes to tissues in the following order, based on tissue-to-plasma (C_{last}) ratios:

Subcutaneous tissue (C_{last} = 1793), Skin (C_{last} = 1138), perirenal fat (C_{last} = 1061), hind limb muscle (C_{last} = 140). This data highlights the compound's propensity for accumulation in subcutaneous tissue, skin, and fat, with significantly lower distribution to muscle.

BI-3972 was originally investigated by the Animal Health division of Boehringer Ingelheim. This explains the species selection below.

BI-3972	Mouse	Dog	Horse
Clearance [L/kg/d][a]	5.8	40	1.4
$T_{1/2}$ [h][a]	23	43	233
T_{max} [h][b]	4	1	3.4
C_{max} [nM][b]	1968	95	2.9
F [%][b]	52	52	17
V_{ss} [L/kg][a]	7.9	57	14

[a] i.v. dose: mouse = 2 mg/kg; dog = 0.25 mg/kg; horse = 0.02 mg/kg
[b] p.o. dose: mouse = 10 mg/kg; dog = 1 mg/kg; horse = 0.05 mg/kg

In vivo pharmacology/efficacy

The cyclooctadepsipeptide compound, BI-3972, demonstrated full efficacy against both macrocyclic lactone-resistant and susceptible strains of heartworm in dogs following oral administration (PO)[8,9].

Negative control

The structural close analogue BI-4083 can be used as negative control.

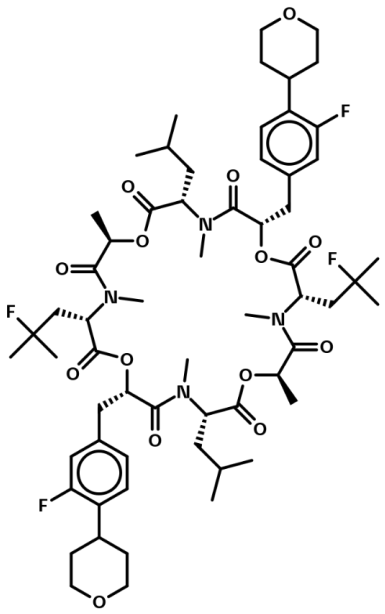

Figure 4: The negative control BI-4083

Selectivity

In the SafetyScreen44™, BI-3972 showed >50% inhibition at a 10 µM concentration for Na^+/SITE2/R and COX-2@CE, while exhibiting no significant activity against other targets tested (44 in total). The negative control BI-4083 inhibited COX-2@CE, LCK_Kinase, and COX-1@CE (>50% inhibition at 10 µM) but showed no activity against the remaining 41 targets of the panel.

Selectivity data available	BI-3972	BI-4083
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No

Reference molecule(s)

Other available tool compounds: Emodepside is a similar cyclo-octadepsipeptide which is commercially available[9].

Supplementary data

2D structure files can be downloaded free of charge from opnMe.

References

1. Guest M., Bull K., Walker R. J., Amliwala K., O'Connor V., Harder A., Holden-Dye L., Hopper N. A. The calcium-activated potassium channel, SLO-1, is required for the action of the novel cyclo-octadepsipeptide anthelmintic, emodepside, in Caenorhabditis elegans *Int J Parasitol* **2007**, 37(14), 1577–1588 DOI: 10.1016/j.ijpara.2007.05.006. PubMed: 17583712.
2. Welz C., Krüger N., Schniederjans M., Miltsch S. M., Krücken J., Guest M., Holden-Dye L., Harder A., Samson-Himmelstjerna G. von Slo-1-channels of parasitic nematodes reconstitute locomotor behaviour and emodepside sensitivity in Caenorhabditis elegans slo-1 loss of function mutants *PLoS Pathog* **2011**, 7(4), e1001330 DOI: 10.1371/journal.ppat.1001330, PubMed: 21490955.
3. Lucas S. J., Bortolotto Z. A., Collingridge G. L., Lodge D. Selective activation of either mGlu2 or mGlu3 receptors can induce LTD in the amygdala *Neuropharmacology* **2013**, 66, 196–201 DOI: 10.1016/j.neuropharm.2012.04.006, PubMed: 22531751.
4. Klokouzas A., Wu C.-P., van Veen H. W., Barrand M. A., Hladky S. B. Cgmp and glutathione-conjugate transport in human erythrocytes *Eur J Biochem* **2003**, 270(18), 3696–3708 DOI: 10.1046/j.1432-1033.2003.03753.x, PubMed: 12950253.

5. Holden-Dye L., O'Connor V., Hopper N. A., Walker R. J., Harder A., Bull K., Guest M. Slo, SLO, quick, quick, slow: Calcium-activated potassium channels as regulators of Caenorhabditis elegans behaviour and targets for anthelmintics *Invert Neurosci* **2007**, 7(4), 199–208 DOI: 10.1007/s10158-007-0057-z, PubMed: 17962986.
6. Martin R. J., Buxton S. K., Neveu C., Charvet C. L., Robertson A. P. Emodepside and SL0-1 potassium channels: A review *Exp Parasitol* **2012**, 132(1), 40–46 DOI: 10.1016/j.exppara.2011.08.012, PubMed: 21910990.
7. Kulke D., Samson-Himmelstjerna G. von, Miltsch S. M., Wolstenholme A. J., Jex A. R., Gasser R. B., Ballesteros C., Geary T. G., Keiser J., Townson S., Harder A., Krücken J. Characterization of the Ca2+-gated and voltage-dependent K+-channel Slo-1 of nematodes and its interaction with emodepside *PLoS Negl Trop Dis* **2014**, 8(12), e3401 DOI: 10.1371/journal.pntd.0003401, PubMed: 25521608.
8. Raisch T., Brockmann A., Ebbinghaus-Kintscher U., Freigang, J., Gutbrod O., Kubicek J., Maertens B., Hofnagel O., Raunser S. Small molecule modulation of the Drosophila Slo channel elucidated by cryo-EM Nat Commun 2021, 12 (7164), DOI: https://doi.org/10.1038/s41467-021-27435-w, PubMed: 34887422.
9. Risch F., Kazakov A., Specht S., Pfarr K., Fischer P. U., Hoerauf A., Hübner M. P. The long and winding road towards new treatments against lymphatic filariasis and onchocerciasis *Trends Parasitol* **2024**, 40(9), 829–845 DOI: 10.1016/j.pt.2024.07.005, PubMed: 39122645.