opnMe by Boehringer Ingelheim

Voltage-gated Na^+channel 1.1 ($Na_v1.1$) activator

BI-7150

Boehringer Ingelheim

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Summary

BI-7150 is a potent and selective activator of the voltage-gated sodium channel $Na_v1.1$. BI-7283 is available as negative control.

Chemical Structure

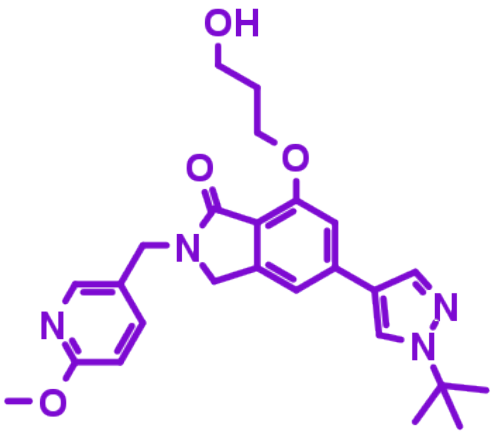

Figure 1: 2D structure of BI-7150, a $Na_v1.1$ activator

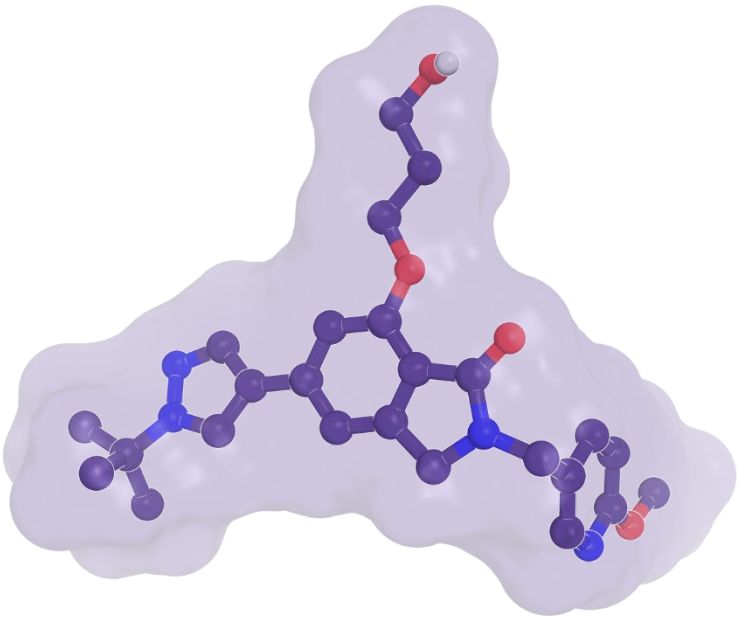

Figure 2: BI-7150, 3D conformation

Highlights

BI-7150 is a potent small-molecule activator of voltage-gated sodium channel $Na_v1.1$ that can be used for *in vitro* studies. The compound is representative of a structural class which is different from other known $Na_v1.1$ activators[1]. BI-7150 displays good aqueous solubility, as well as reduced protein binding.

Target information

The voltage gated sodium channel $Na_v1.1$ comprises a single pore-forming α-subunit and two β-subunits. $Na_v1.1$ is primarily expressed in the central nervous system, where it is involved in neuronal membrane depolarization and thereby in action potential firing. Within the CNS, $Na_v1.1$ is predominantly expressed in the GABAergic parvalbumin positive fast spiking interneurons. This sub-population of interneurons regulates the excitatory state of the glutamatergic pyramidal neurons and thereby is involved in controlling the excitatory / inhibitory balance (E/I balance)[1-8]. Inappropriate function of fast-spiking interneurons can result in disinhibition of pyramidal cells and network desynchronization, without detectable changes in excitatory pyramidal neurons[2-3]. The resulting imbalance between excitation and inhibition is thought to contribute to hyperexcitability and seizures.

$Na_v1.1$. is encoded by the *SCN1A* gene, for which up to 900 missence, nonsense and truncation mutations have been identified. Such mutations can lead to loss of function or gain of function phenotypes[4-5]. While the pathogenic mechanism of such mutations is not yet understood, a clear genetic link has been established between *SCN1A* mutations and Dravet syndrome, also known as the severe myoclonic epilepsy of infancy[3,6].

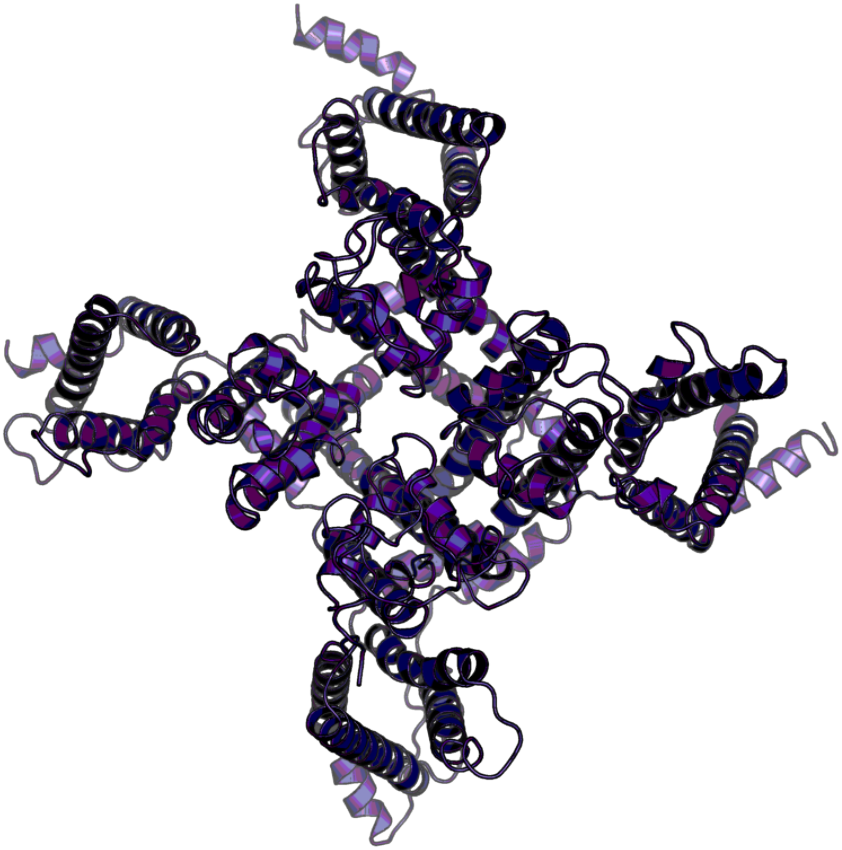

Figure 3: Model of the voltage-gated sodium channel $Na_v1.1$ (PDB code: 7DTD)[2].

In vitro activity

Potentiation of $Na_v1.1$ was measured via an automated sychropatch electrophysiology assay and based on sodium currents caused by the opening (activation) of the voltage-gated sodium channel recombinantly expressed in HEK 293 cells. Channel activation was evoked by the depolarization of the cell, which changes the channel into its open state. The transfer of sodium ions in the open state can be recorded using the patch clamp technique.

Compounds were tested in an 11-point concentration curve in quadruplicate starting at 30µM and using a 1:3 dilution factor with a constant DMSO concentration of 0.3%. Once cells were plated and compounds had been added, a holding potential of −100 mV was applied during the first 50 ms to measure baseline recordings. This was followed by a depolarization to 0 mV for 50 ms. Over the course of these 50 ms, peak current, TAU (time constant of inactivation), AUC (area under the curve), and residual current were recorded and analyzed. Subsequent to this depolarization, the holding potential of −100 mV was reapplied for 50 ms prior to the next of a total of 46 sweeps.

Probe name / Negative control	BI-7150	BI-7283
MW [Da, free base][a]	450.5	475.6
Nav 1.1 TAU (EC_{150}) [nM][b]	119	14,500
Nav 1.1 AUC (EC_{150}) [nM][c]	45	4,440

[a] The molecule is supplied in salt form. For the molecular weight of the salt form, please refer to the vial label
[b] EC_{150} is defined as the compound concentration necessary to increase the $Na_v1.1$ ion throughput over 50 ms by 50% relative to stimulation in the presence of vehicle. The biological activity of compounds is determined by the following methods:

1) Syncropatch 384PE platform and software

The activity of voltage-dependent sodium (Na_v) channels and cpd-dependent modulation of Na_v channel activity were recorded with the automated patch clamp platform Syncropatch 384PE utilizing NPC-384 resistance chips (384-well format). Data acquisition was controlled with PatchControl384 software, and export and analysis were performed with DataControl384 software (all Nanion Technologies, Munich, Germany) and customized analysis software.

2) Buffers used in the experiments

The following extracellular solutions were utilized: *Cell catch buffer* containing (in mM) 140 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Glucose, 10 HEPES (pH adjusted to 7.4 with NaOH). *Seal enhancer buffer* containing (in mM) 80 NaCl, 3 KCl, 35 $CaCl_2$, 10 $MgCl_2$,10 HEPES (pH adjusted to 7.4 with NaOH; cell catch buffer). *Washing/run buffer* containing (in mM) 140 NaCl, 4 KCl, 5 $CaCl_2$, 1 $MgCl_2$, 10 Glucose, 10 HEPES (pH adjusted to 7.4 with NaOH; cell catch buffer).

The intracellular solution was prepared by mixing to two buffers (90 vol% of buffer I with 10% of buffer II). Buffer I contained (in mM) 140 CsF, 10 NaCl, 1 EGTA, 10 HEPES and Buffer II contained (in mM) 20 EDTA, 20 $MgCl_2$. After addition of 1 mM Na_2ATP, the pH of the intracellular buffer was adjusted to 7.2 (with CsOH).

3) Cell types

Experiments were performed utilizing HEK293 cells stably expressing SCN1A (NM_006920 (var 2); pD3.2 vector) and SCN1B (NM_001037.4; pD6.2 vector) encoding human Na_v channel α subunit and β1 subunit, respectively (termed HEK-$Na_v1.1$ cells; from SB DrugDiscovery).

4) Compound plate preparation:

Compound plates were prepared by Compound Logistic in Axygen deep well plates 384 (Thermo Fisher Scientific, Waltham, US), compound wells contained a volume of 300 nL. Each compound titration was prepared in quadruplicates in 100% DMSO. Starting from the highest concentration of 10 mM, compounds were in diluted 1:3 on the plate to create a 11-point concentration-response curve. Compounds were then diluted by adding 50µl/well of run buffer containing a 0.1% Pluronic F-127 solution. An extra 1:2 dilution was carried out by the Syncropatch during the experiments, reaching a final top concentration of 30µM and 0.3% DMSO. Controls were manually prepared freshly on the day of the experiment. 0.3% DMSO was used as negative control and ATX II (30 nM in 0.3% DMSO) served as positive control.

5) Voltage protocol, data sampling, analysis, and quality control

From a holding potential of -100 mV, Na_v channels were activated by depolarizing voltage steps to 0 mV for 50 ms every 20 s. A total of 24 baseline sweeps and 17 sweeps after compound application were recorded, leak subtraction was applied in between sweeps. The following parameters were analyzed: baseline (leak current), peak inward current, AUC (area under the curve), residual current after channel inactivation, TAU, R^2, seal resistance, cell capacitance, R_s (series resistance). Recordings were only analyzed, if current amplitude before application of cpd was >-200 pA, and if R_s was 30 MΩ throughout recordings. Recordings were discarded, if the following quality control criteria were not met: Residual current > 50 pA, baseline current <-100 pA.

[c] EC150 is defined as the compound concentration necessary to increase the Nav1.1 ion throughput over 50 ms by 50% relative to stimulation in the presence of vehicle.

In vitro DMPK and CMC parameters

BI-7150 shows good aqueous solubility and permeability as well as acceptable metabolic stability across human, rat, and mouse hepatocytes. The compound's protein binding in human and mouse plasma appears to be high, but still with reasonable fraction unbound, in line with a measured logD of 2.9.

Probe name / negative Control	BI-7150	BI-7283
logD @pH 2 / pH 7.4 / pH 11	2.2 / 2.9 / n.a.	1.5 / n.a. / 3.4
Solubility @ pH 7 [µg/ml]	222.2	73
MDCK permeability P_{AB} @ 1µM [10^{-6} cm/s]	19	n.a.
MDCK efflux ratio	5.3	n.a.
Microsomal stability (human/mouse/rat) [% Q_H]	<33 / n.a. / 25	70 / 65 / 51
Hepatocyte stability (human/mouse/rat) [% Q_H]	40 / 73 / 36	n.a.
Plasma protein binding (human/mouse) [%]	90.7 / 94.4	n.a.
CYP 3A4 (IC_{50}) [µM]	>50	n.a.
CYP 2C8 (IC_{50}) [µM]	24.1	n.a.
CYP 2C9 (IC_{50}) [µM]	>50	n.a.
CYP 2C19 (IC_{50}) [µM]	>50	n.a.
CYP 2D6 (IC_{50}) [µM]	>50	n.a.

In vivo pharmacology

Despite its good aqueous solubility, brain-blood barrier penetration and bioavailability, use of BI-7150 *in vivo* is not recommended due to adverse effects observed in murine models.

Negative control

BI-7283 was designed as a structurally close analogue of BI-7150 and can be used as a negative control.

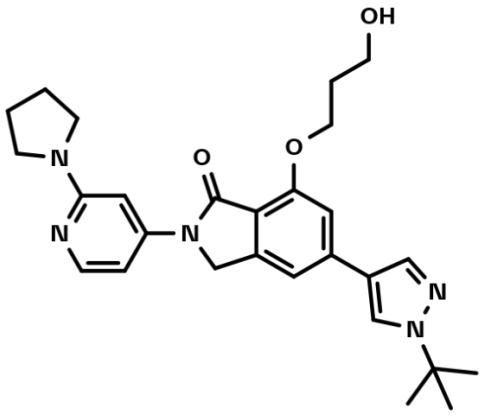

Figure 4: BI-7283 which serves as a negative control

Selectivity

BI-7150 was tested on 44 targets in a selectivity panel and showed ≥1,000fold selectivity for 42 targets (≤ 50%inhibition @ 10 µM). In two assays (5HT2H/H and LCK_CE) the compound showed inhibition between 55% and 98% @ 10µM.

The negative control BI-7283 showed in 9 out of 44 targets inhibition with more than 50% @ 10 µM (D2SH_AGON, NEUP/H, M3/H, MU/H, ACE/HU, M2/H, M1/H, Na+/SITE2, HERG_DOFETILIDE).

Selectivity data available	BI-7150	BI-7283
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

There is no X-ray crystal structure available.

Reference molecule(s)

$Na_v1.1$ activator 1, HY-126429[1]

AA43279[2]

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

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