ENaC Inhibitor | BI-8668

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Summary

BI-8668 is a potent and well-characterized *in vitro* and *in vivo* tool compound, structurally distinct from amiloride derived ENaC inhibitors. A structurally analogous negative control BI-0377 is also available.

Chemical Structure

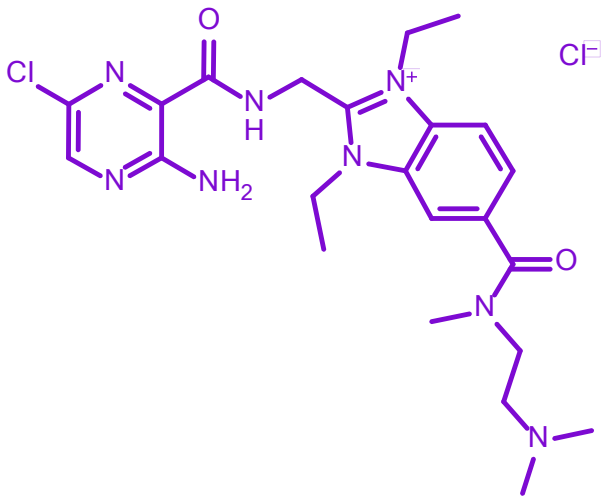

Figure 1: 2-D structure of BI-8668, a well-characterized *in vitro* and *in vivo* ENaC inhibitor

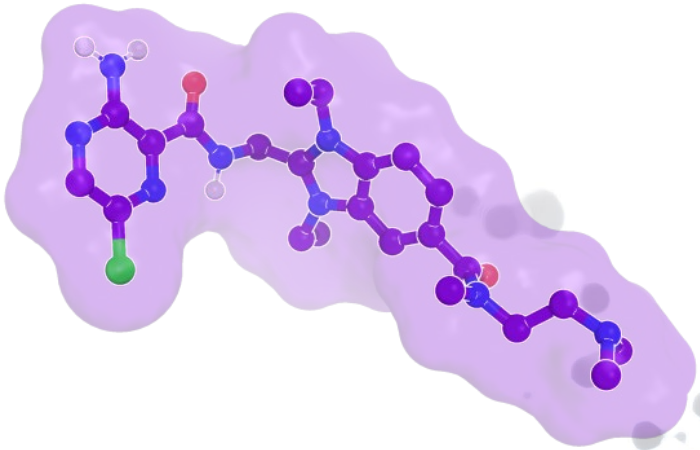

Figure 2: BI-8668, 3D structure

Highlights

BI-8668 is a highly potent and well characterized epithelial sodium channel (ENaC) inhibitor, being structurally distinct from amiloride derived compounds. It possesses a high aqueous solubility, a high microsomal and hepatocyte stability, and a moderate Caco permeability. It is suitable for *in vitro* and *in vivo* use. The structurally analogous BI-0377 can be used as a negative control.

Target information

The epithelial Na^+ channel ENaC is expressed at the apical surface of epithelia of the lung, colon, kidney, salivary gland ducts, and sweat glands.[1] ENaC mediates the diffusion of luminal sodium and water across the apical membrane of epithelial cells. It plays an essential role in electrolyte and blood pressure homeostasis, as well as in airway surface fluid homeostasis, which is important for proper clearance of mucus. Further it controls the reabsorption of sodium in the kidney, colon, lungs, and eccrine sweat glands.[2]

The currently solved cryo-EM structure shows that ENaC is a heterotrimeric transmembrane protein composed of α, β, and γ-subunits that form a pore with high selectivity for Na^+ and Li^+ over K^+. Each subunit is encoded by a different gene, namely SCNN1A, SCNN1B, and SCNN1G.[3]

In cystic fibrosis airways, the lack of cystic fibrosis transmembrane conductance regulator CFTR and increased ENaC activity lead to mucus dehydration that causes mucus obstruction, neutrophilic infiltration, and chronic bacterial infection.

ENaC inhibitors are active in cellular water resorption assays due to their mode of action. *In vivo* effects (rat lung water resorption models or mucociliary clearance model (MCC) in sheep) however, are only seen with suitably optimized compounds.[4]

Suitable PK properties are crucial to achieve long duration of action and to avoid renal side effects, also upon inhalative administration. ENaC inhibition is generally seen to be a potential treatment of cystic fibrosis.[5,6]

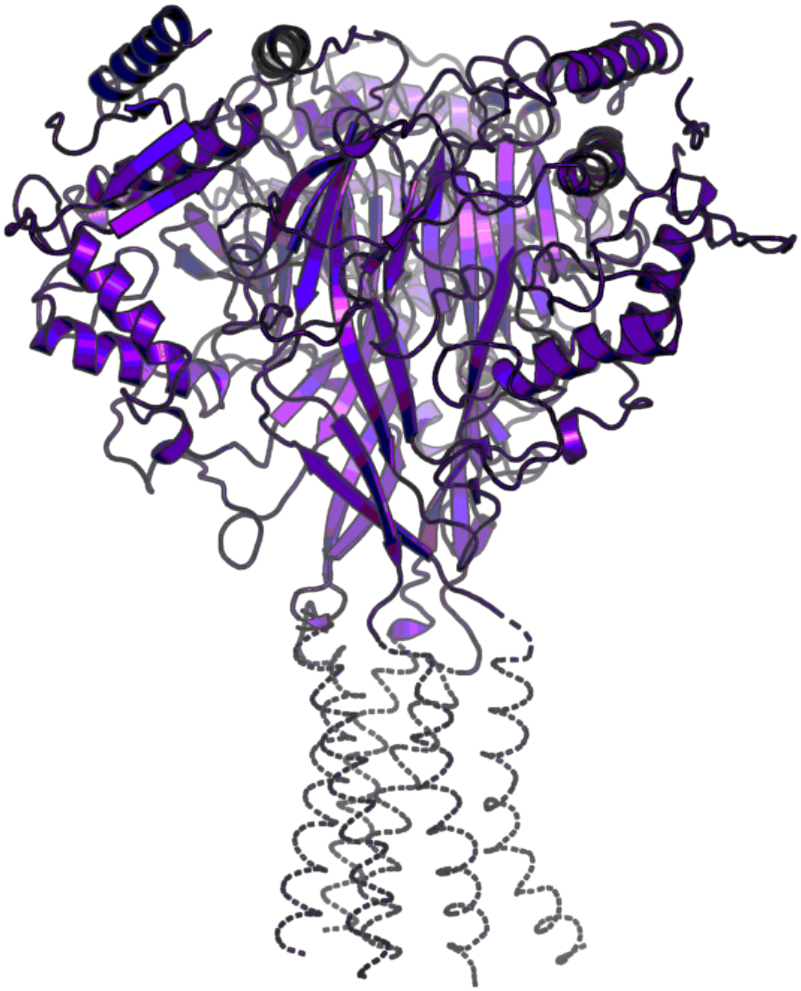

Figure 3: 3D structure of ENaC as revealed by cryo-electron microscopy (PDB code: 6BQN; transmembrane helices shown as dashed lines as these were not fully resolved in the structure determination.[3]

In vitro activity

BI-8668 effectively inhibits the Na+ current on human airway epithelium in an Ussing Chamber setup with an IC_{50} of 17 nM. Further, BI-8668 displays 81% inhibition in an M-1 water resorption assay at 3 µM.

PROBE NAME	BI-8668	BI-0337
MW [Da]	559.9	539.5
Inhibition of M-1 water resorption (%Inhib) @3 µM [%][a]	81	n.d.
Ussing chamber (IC_{50}) [nM][b]	17	>10.000

[a] Measurement of ENaC-mediated water permeability through cell monolayers of the cell line M-1 (M-1 cells: mouse kidney tubules cells) and the blocking capacity of ENaC-inhibitors. The transported volume will be determined with tritiated water. A potent ENaC-inhibitor results in reduced water transport leading to more remaining liquid in the apical (upper) compartment of the cell layer.

[b] Inhibition of Na+ current by Ussing Chamber - MucilAir™ P0: IC_{50} were derived form a 5-steps concentration curve. (1 nM -> 10,000 nM; n=6)

In vitro DMPK and CMC parameters

In vivo DMPK and CMC parameters for BI-8668 and the negative control BI-0337 are tabulated below. BI-8668 possesses a high aqueous solubility, a high microsomal and hepatocyte stability and a moderate Caco permeability. It is devoid of cytochrome P450 inhibition and shows low plasma protein binding.

PROBE NAME	BI-8668	BI-0337
logD pH 2 / pH 7.4 / pH 11	-0.5/ 0.2/ 0.58	-0.8/ n.d./ n.d.
Solubility in PBS pH 7.4 [µg/ml]	110	81
CACO permeability (PEAB) @ pH 7.4 [$*10^{-6}$ cm/s]	<0.3	0.1

CACO efflux ratio	1.2	0.9
Microsomal stability (human/mouse/rat) [% Q_H]	<23/<23/<22	<23/<23/<22
Hepatocyte stability (human/mouse/rat) [% Q_H]	23.5/<12/19.5	<4/<12/4
Plasma protein binding (human/mouse/rat) [%]	38/43/29	n.d./24/20
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	n.d.

In vivo DMPK parameters

In vivo DMPK parameters for BI-8668 are tabulated below.

PROBE NAME	BI-8668	BI-0337
Actual dose [umol/kg]	0.5	n.d.
Clearance (mouse,rat) [ml/(min*kg)]	46 / 59	n.d.
MRT (disp, mouse,rat) [h]	0.39 / 0.17	n.d.
V_{ss} (mouse,rat) [l/kg]	1.10 / 0.58	n.d.
t_{max} (mouse,rat) [h]	n.d. / 0.1	n.d.
C_{max} (mouse,rat; dose 0.05 µmol/kg) [nM]	n.d./ 34	n.d.

In vivo activity/pharmacology

BI-8668 at 3 µg/kg displays up to 33% inhibition of fluid absorption compared to control animals in an airway fluid absorption assay in wistar rats.

PROBE NAME	BI-8668
Inhibition of airway fluid absorption (%Inhib) @ 0.3 µg/kg [%][a]	27
Inhibition of airway fluid absorption (%Inhib) @ 3.0 µg/kg [%][a]	33

[a] Ringer Lactat solution (control) or test solution (0.1mg/kg cpd) was instilled into the trachea of male wistar rats. After 3 hours, the absorption of fluid from the lungs was determined by measuring difference between control and compound treated lungs (weight of both lungs lobes). Concomitantly the serum levels of aldosterone were determined as a measure of systemic ENaC inhibition. The compound effect was expressed in percent inhibition of fluid absorption compared to control animals.

In vivo aldosterone stimulation in rat airway fluid absorption assay (0.3 µg/kg and 3 µg/kg dosing) after 3 h is below 50%, indicating that there is no relevant ENaC inhibition in kidney.

Negative control

The molecule BI-0337 is structurally very close to BI-8668. Nonetheless, it offers a >500-fold lower potency in the Ussing chamber assay (MucilAir™) on human airway epithelium and therefore can be used as an *in vitro* negative control.

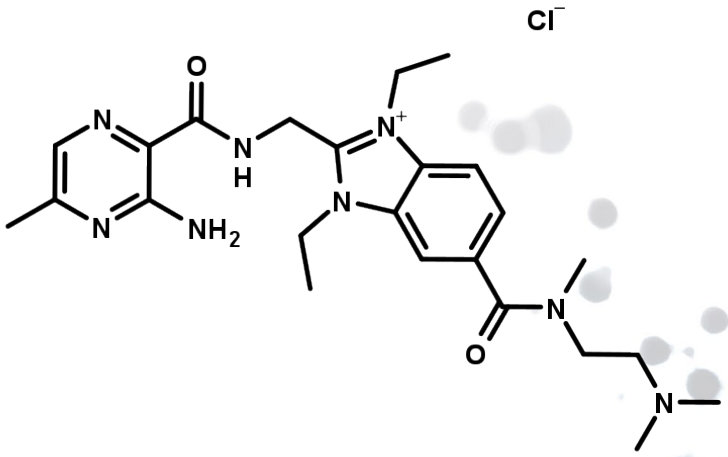

Figure 4: BI-0337 which serves as a negative control

Selectivity

BI-8668 was tested on 50 targets in a selectivity panel and showed ≥1,000-fold selectivity for 47/50 targets (≤ 50% inhibition @ 10 µM). For three panel targets (M3, M2, α1), percent inhibition data indicate at least 50-fold selectivity. The negative control BI-0337 showed more than 50% inhibition @ 10 µM in 4 out of 44 targets.

SELECTIVITY DATA AVAILABLE	BI-8668	BI-0337
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	no
DiscoverX®	No	no
Dundee	No	no

Reference molecule

P-552-02 is an ENaC inhibitor based on the amiloride pharmacophore.[7]

Supplementary data

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

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