SLC13A5 Inhibitor | BI01383298

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Summary

BI01383298 is a potent and selective inhibitor of human SLC13A5 for *in vitro use* with no structural homology to the substrate.

Chemical Structure

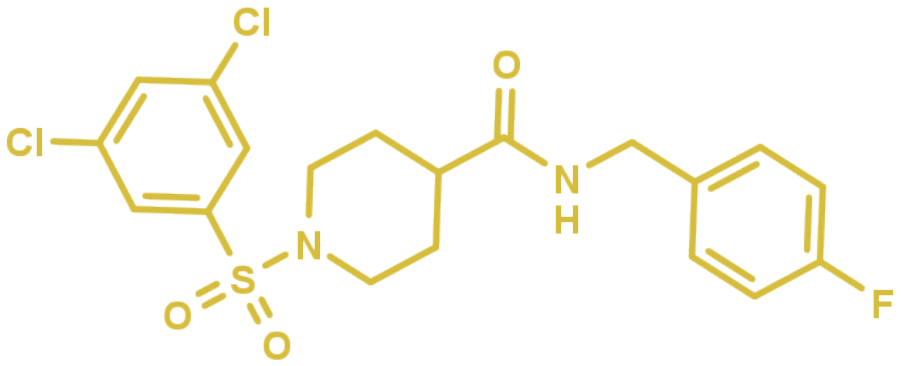

Figure 1: 2-D structure of BI01383298, a potent inhibitor of SLC13A5.

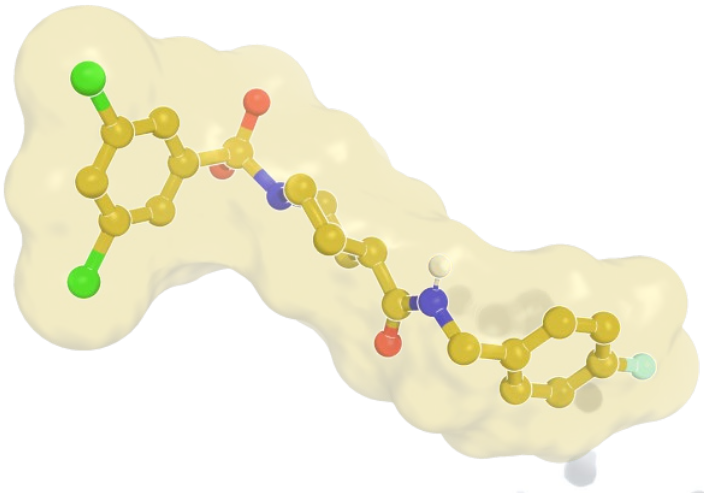

Figure 2: BI01383298, 3D conformation

Highlights

BI01383298 is a potent inhibitor of the human solute carrier SLC13A5, also known as sodium-coupled citrate transporter (hNaCT) with an IC_{50} of 56 nM and for *in vitro* use. As the compound is not a structural homolog of the substrate, citric acid, excellent selectivity for SLC13A5 over the closely related family members hSLC13A2 and hSLC13A3 and even murine SLC13A5 could be demonstrated (IC_{50} >100 µM each). The potency and selectivity of BI01383298 have been significantly improved over prior tool compounds[1-3]. In addition, BI01383298 is selective against hGlyT2, an unrelated transporter mediating glycine uptake. Interestingly, BI01372674, bearing only a single substituent exchange, is completely inactive.

Target information

SLC13A5, also known as the sodium-coupled citrate transporter (NaCT), is part of the SLC13 family of sodium/sulphate carboxylate co-transporters that comprises of five members. SLC13A5, along with SLC13A2 and SLC13A3, transports di- and tricarboxylates using sodium as a co-ion. SLC13A1 and SLC13A4 are not thought to transport di- and tricarboxylates. SLC13A5 is predominantly expressed in the plasma membrane of hepatocytes where it transports citrate from the circulatory system into hepatocytes. Lower levels are also observed in brain and testes.[4] SLC13A5 was first identified in *Drosophila melanogaster* where the name *I'm Not Dead Yet* or INDY was coined. Inactivation mutants of SLC13A5 in *D. melanogaster* and *Caenorhabditus elegans* led to increased lifespan.[5] Data from mouse models have implicated that the protein may have a role in various disease settings such as obesity and diabetes. This was supported by data that demonstrated that knockout mice were protected from adiposity.[6] Inhibition of the transporter also led to reduced lipid concentrations in a siRNA study,[7] and a substrate analogue was used to lower blood glucose levels.[1] More recently mutations in SLC13A5 have been linked to early-infantile epileptic encephalopathy,[8] while silencing of the SLC13A5 gene inhibits proliferation of human hepatocarcinoma cells.[9] Together, this data indicates that the development of pharmacological regulators of SLC13A5 may open new opportunities in the development of treatments for obesity, metabolic disorders and cancer.[10,11]

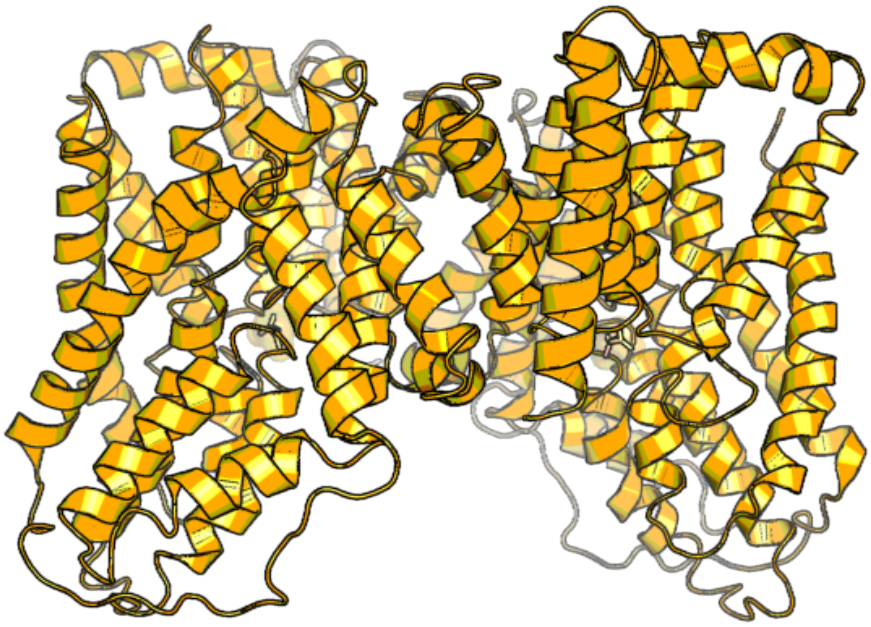

Figure 3: Structure of a SLC related to SLC13A5, as revealed by X-ray crystallography (PDB code 5UL7)

In vitro activity

BI01383298 displays an IC_{50} (hSLC13A5) = 56 nM and an IC_{50} (HepG2) = 24 nM, while being highly selective (IC_{50} > 100 µM for all other SLC13 family members, and for murine SLC13A5).

PROBE NAME / NEGATIVE CONTROL	BI01383298	BI01372674
MW [Da]	445.0	503.1
HEK293-hSLC13A5 (IC_{50}) [µM][a]	0.056	>100
HepG2 (IC_{50}) [µM][b]	0.024	>100
HEK293-hSLC13A2 (IC_{50}) [µM][a]	>100	n.d.

HEK293-hSLC13A3 (IC_{50}) [µM][a]	>100	n.d.
HEK293-mSLC13A5 (IC_{50}) [µM][a]	>100	>70
HEK293-GLYT2 (IC_{50}) [µM][c]	>100	n.d.

Citrate uptake inhibition was measured for all citrate transporters and glycine uptake measured to GLYT2. Potency was assessed for the probe candidate and the negative control on uptake of 14C-citrate into cells over-expressing SLC13A5, SLC13A2, SLC13A3, mouse SLC13A5 and in HEPG2 cells.

[a] The IC_{50} were measured in a recombinant human SLC13A5 citrate uptake assay, using human embryonic kidney 293Flp-In-cells overexpressing the human SLC13A5 transporter.

[b] The IC_{50}s were obtained in a HepG2 citrate uptake assay.

[c] The IC_{50}s were obtained in a human GlyT2 glycine uptake assay.

For all assays outlined for this molecule, detailed experimental conditions can be obtained via the "Contact us" form on opnMe.com.

In vitro DMPK and CMC parameters

BI01383298 is a permeable but poorly soluble tool compound with moderate microsomal stability. Efforts to improve the solubility led to the discovery of BI01372674, the negative control.

PROBE NAME / NEGATIVE CONTROL	BI01383298	BI01372674
logP	4.64	1.8
Solubility @ pH 6.8 [µg/ml]	<1	70
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	37	0.3
CACO efflux ratio	0.4	64
Microsomal stability (human/mouse/rat) [% Q_H]	48/50/45	<23/<23/n.d.

Hepatocyte stability (human/mouse/rat) [% Q_H]	28/95/84	10/47/52
Plasma protein binding (human/mouse/rat) [%]	99.2/-/-	86.1/97.7/89.0
hERG [inh. % @ 10 µM]	17	n.d.
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2D6 (IC_{50}) [µM]	>50	>50
CYP 1A2 (IC_{50}) [µM]	>50	>50
CYP 3A4 (IC_{50}) [µM]	>50	>50

In vitro pharmacology

BI01383298, along with an active probe from the same structural class with better solubility and reference compound PF-06649298,[1,3] and negative control BI01372674 in a thermostability assay, at various concentrations (Figure 4). It was shown that BI01383298 directly stabilizes hSLC13A5, and that both the probe and the negative control demonstrate target engagement *in vitro*. The thermostabilisation data was obtained with the Prometheus label-free system from Nanotemper.

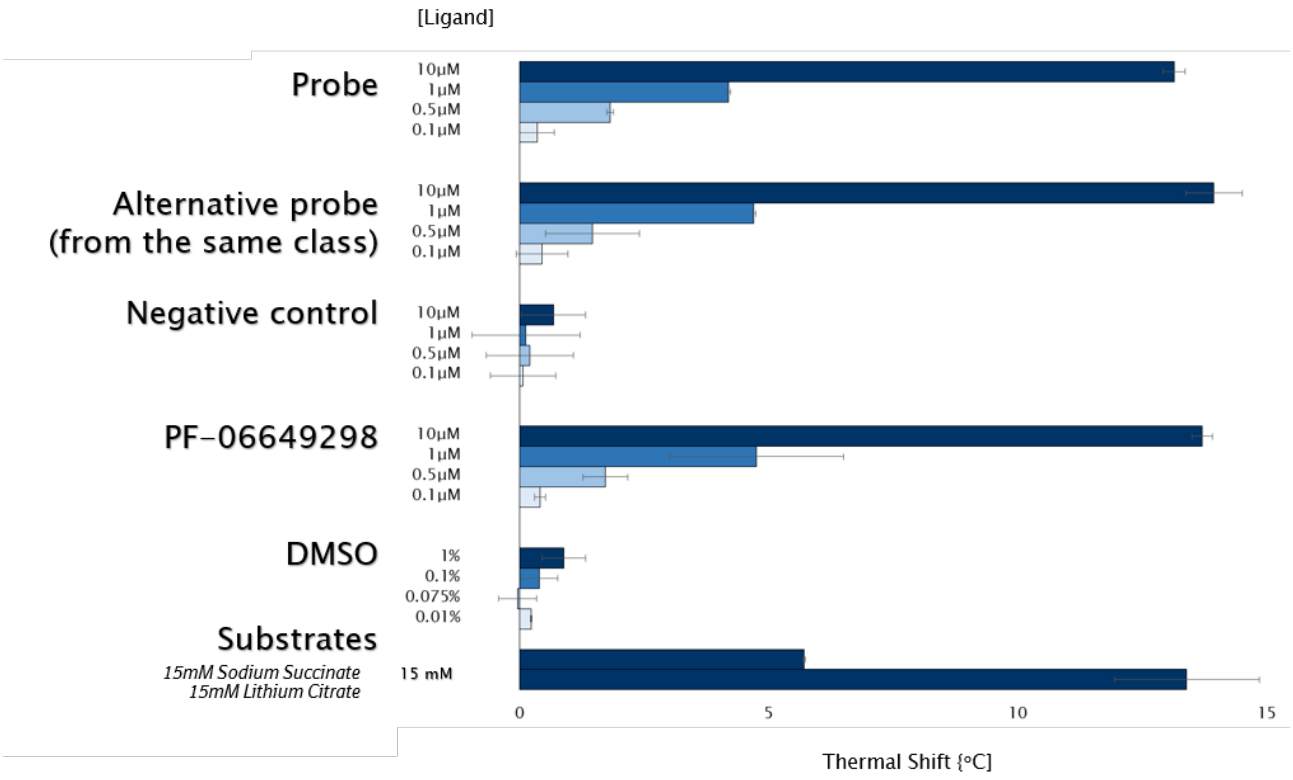

Figure 4: Thermostabilisation of human SLC13A5 by BI0138298 at concentrations ranging from 0.1 mM to 10 mM (three biological samples, each measured in 4 or 8 replicates).

Negative control

BI01372674 is provided as the negative control. It bears only a single substituent exchange by comparison with the active probe, and is completely inactive on both hSLC13A5 and HepG2, IC_{50} >100 µM for both.

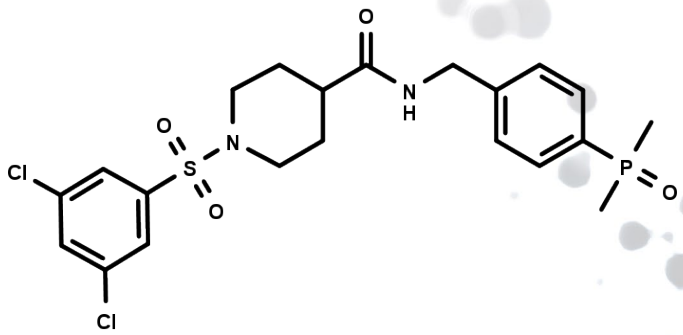

Figure 5: BI01372674, which serves as a negative control

Selectivity

BI01383298 was subjected to a selectivity panel profiling where it showed >100-fold selectivity for 42 out of 44 targets and still >10-fold selectivity for the other two ones.

SELECTIVITY DATA AVAILABLE	BI01383298	BI01372674
SafetyScreen44™ with kind of support of eurofins	Yes	data in progress - will be added soon
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

PF-06649298 (see Reference 1)

Summary

BI01383298 is a potent inhibitor of SLC13A5 with no structural homology to the substrate for *in vitro* use. It is selective over other family members (hSLC13A2, hSLC13A3, mSLC13A5) and other transporters. It is offered together with its negative control BI01372674.

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

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