RESOLUTE — Research Empowerment on Solute Carriers

SLC12A2 assay development

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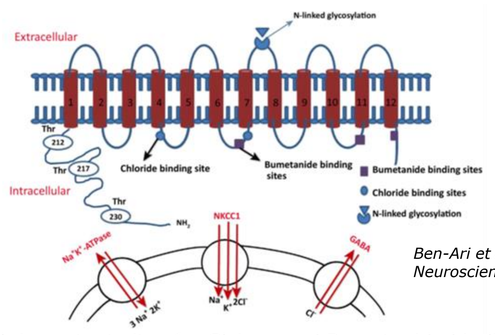
Introduction

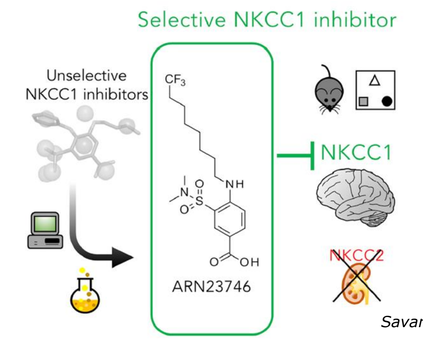
- **SLC12A2** (coding for the electroneutral transporter **NKCC1**) mediates uptake of **Na^+**, **K^+**, and **Cl^-** in a ratio of **1:1:2** and is inhibited by "loop diuretics" such as **furosemide** and **bumetanide**
- It is localized on the **plasma membrane** (PM); it plays a role in **neuronal Cl^- homeostasis** and represents a **target for brain pathologies**
- It has also been shown to participate in the **maintenance and regulation of cell volume** (transport of ions is also associated to water transport)
- **Activation** of the transporter has been demonstrated by osmotic stress and is **regulated by phosphorilation**



Ben-Ari et al., Trends in Neurosciences; 2017

Figure 1 *– Structure of NKCC1 with important sites for Cl^- ions and Bumetanide binding, as well as phosphorylation sites.*

- Treatment with **Bumetanide** has shown positive outcomes in clinical case studies of patients with **neurological/psychiatric conditions**. However, Bumetanide has a strong diuretic effect due to its inhibition of the kidney Cl^- transporter NKCC2 (SLC12A1) → critical issues with drug compliance and health concerns
- **Inhibitor compounds** that present **selectivity for NKCC1 versus NKCC2** may have a strong and consistent therapeutic value



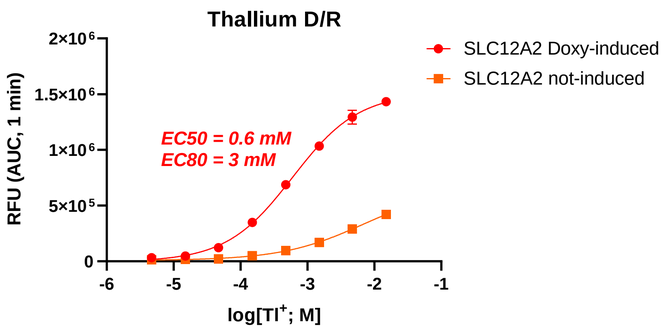
Savardi et al., Chem; 2020

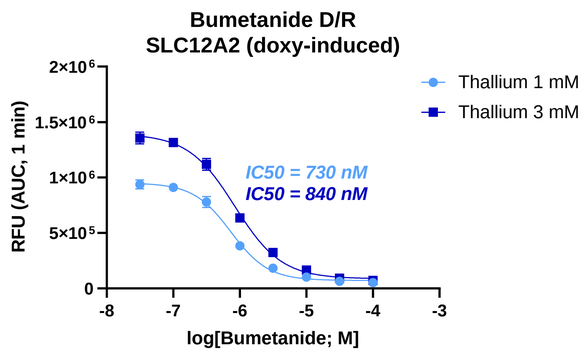
Figure 2 *– A novel NKCC1 inhibitor devoid of diuretic effects (ARN23746), may be clinically relevant for treatment of several neurological conditions characterized by impaired Cl homeostasis.*

Assay development and HTS optimization was successfully carried out in 384-well plate format:

- **Cell line: HEK-293 Jump In T-REx/SLC12A2** (OE cell line from CeMM, N-terminally tagged SLC; IF expression confirmed, SLC localized on PM)
- **Read-out: Thallium (Tl^+) influx measurements** using a Tl^+-sensitive fluorescent dye (**Potassium assay kit**)

Thallium Dose-Response (D/R) and Bumetanide D/R

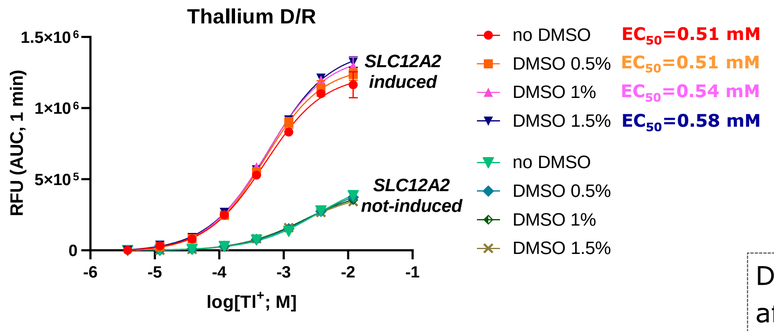




- Cells induction: **24h** (Doxycycline 1 µg/mL)
- Fluorescent Dye loading: **1h at RT** (in Chloride-free buffer)
- Double injection protocol (DMSO 0.5%, **FLIPR^{TETRA}**). Tl^+ is injected in a buffer containing 25 mM Cl^-

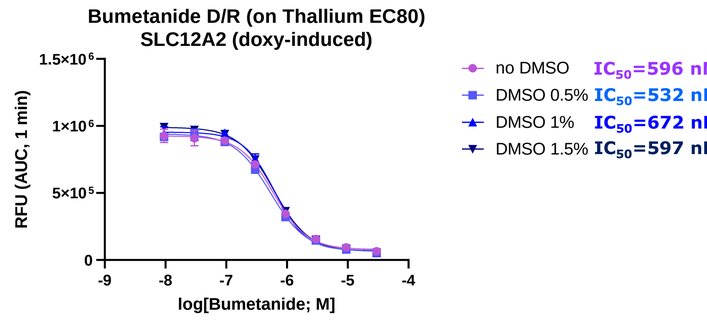
- Tl^+ D/R: significant difference between **induced and not-induced** condition
- Fluorescent signal is **fully abolished** by Bumetanide

DMSO sensitivity



DMSO up to 1.5% is not affecting:

- **EC_{50}/IC_{50}** values
- **Assay window**

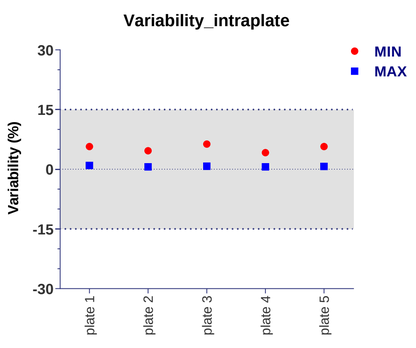


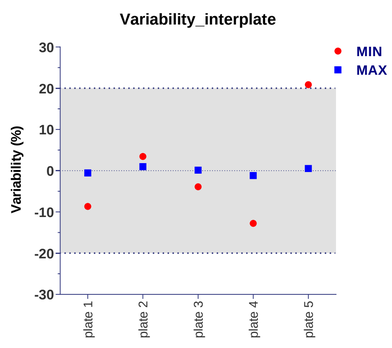
Multiplate test

A multiplate test was carried out on **5 plates** to assess the robustness of the assay and its eligibility for HTS purposes

- **MAX**: Bumetanide IC100 (30 µM, DMSO 0.5%)
- **MIN**: DMSO 0.5%
- rZ' factor **≥ 0.5**
- Variability intraplate: **≤ 15%**
- Variability interplate : **≤ 20%**

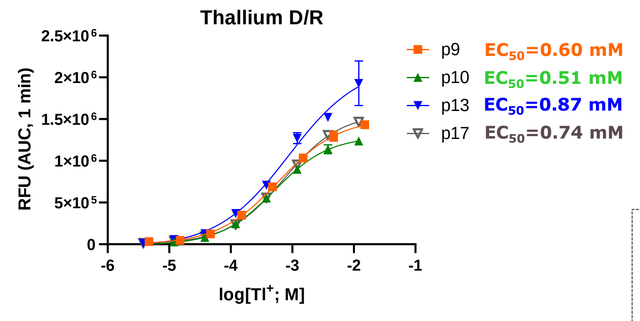
	RZ'
Plate 1	0.80
Plate 2	0.84
Plate 3	0.79
Plate 4	0.86
Plate 5	0.81





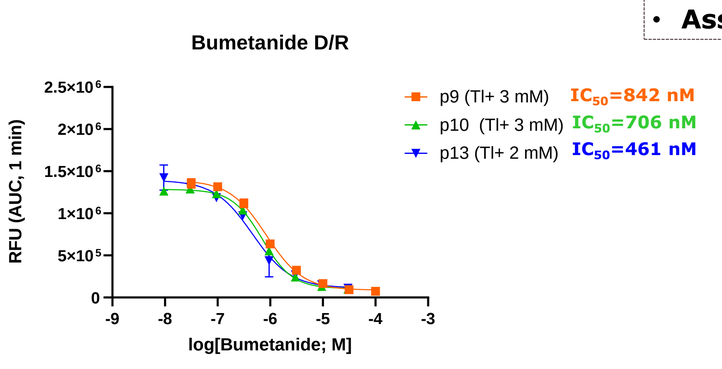
Assay quality criteria are fulfilled

Cell line stability



Cell line is stable up to p17:

- **EC_{50}** and **IC_{50}** values are conserved
- **Assay window** is reproducible



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