High throughput quantitative pharmacology: cross platform comparison of ion channel activating compounds for pain

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Introduction

The two-pore domain potassium (**K2P**) family of ion channels represent an attractive opportunity for the development of **novel pain therapeutics**. Channel dysfunction has been shown to promote pain phenotypes and human genetic data has linked K2Ps to disease.

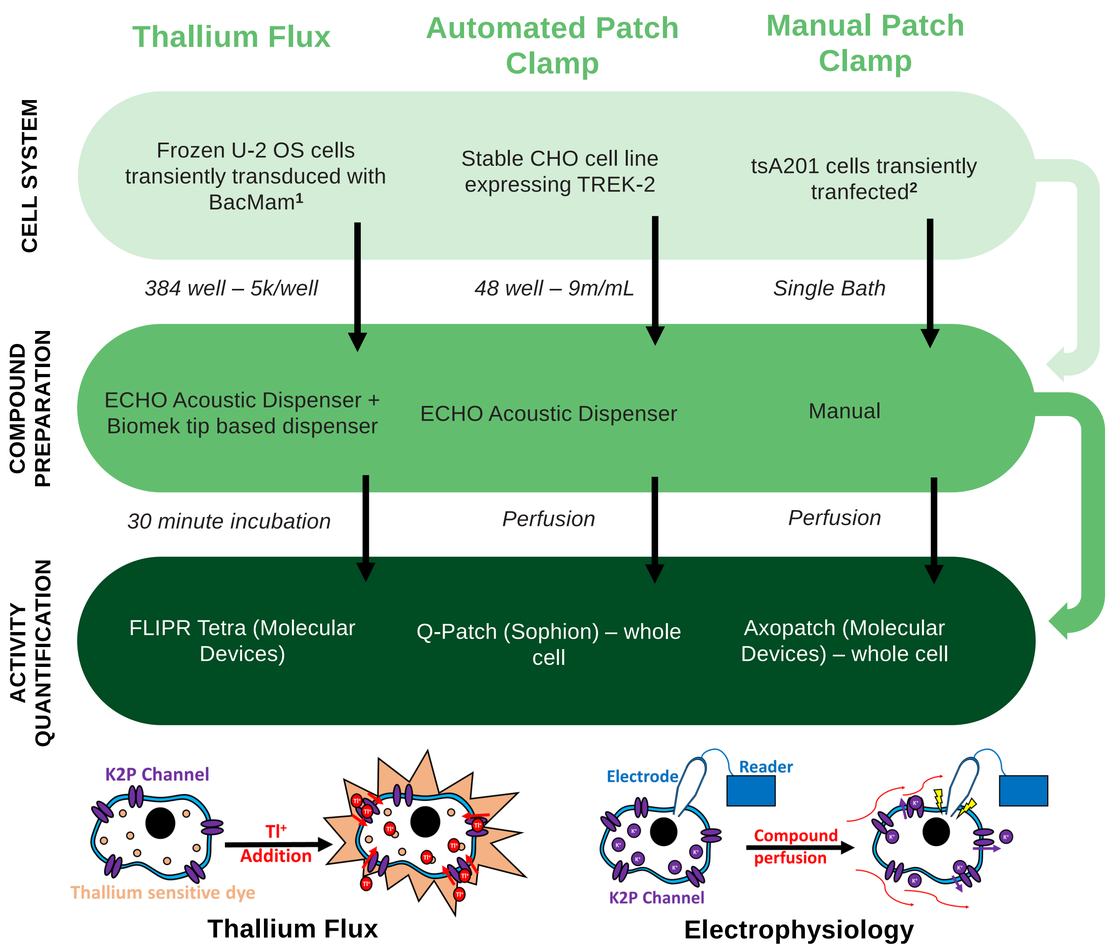
Given current therapies exhibit incomplete efficacy, addictive properties and sedation, there is an urgent need for new treatments. **K2P activators** show early promise but have not yet been clinically validated.

Here we define pharmacology of a subset of published **tool compounds** across multiple systems against **TREK-2** and **TREK-1** of the K2P family.

Aims

- Define pharmacology of tool compound activators against TREK-2 and TREK-1, investigating potency and efficacy.
- Validate activator tool compounds across multiple assay platforms, identifying most potent and robust molecules in each system.

Methods



Results

Automated Patch clamp electrophysiology

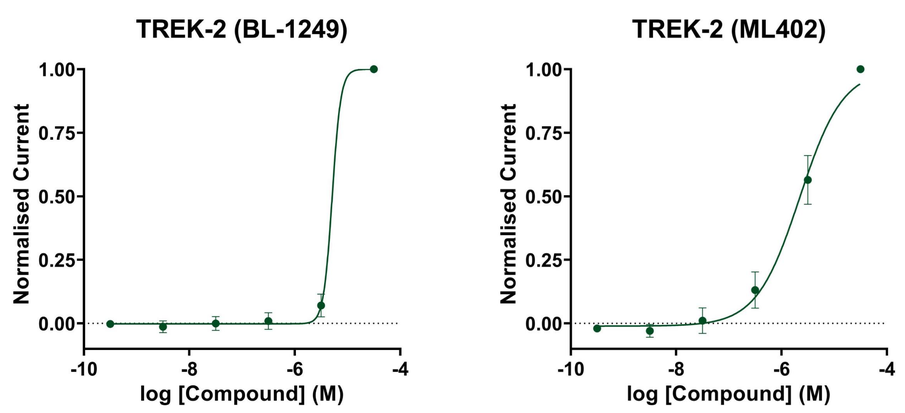


Figure 1 – Representative 6 point dose response curves of tool compounds showing significant activity in Q-patch recordings. Outward current normalized to pre-addition baseline (0) and maximal compound response (1). All other compounds tested showed no activation at top concentrations, apart from ML67-33 displaying partial activity. Results ± S.E.M, n=5 from two independent experiments. Data analysed using Analyzer 6.6.44 (Sophion).

References

1. Wright PD, Veale EL, McCoull D, Tickle DC, Large JM, Ococks E, Gothard G, Kettleborough C, Mathie A, Jerman J. Terbinafine is a novel and selective activator of the two-pore domain potassium channel TASK3. Biochemical and biophysical research communications. 2017 Nov 4;493(1):444-50.
2. Wright PD, McCoull D, Walsh Y, Large JM, Hadrys BW, Gaurilcikaite E, Byrom L, Veale EL, Jerman J, Mathie A. Pranlukast is a novel small molecule activator of the two-pore domain potassium channel TREK2. Biochemical and biophysical research communications. 2019 Nov 26;520(1):35-40.

Results

Thallium flux

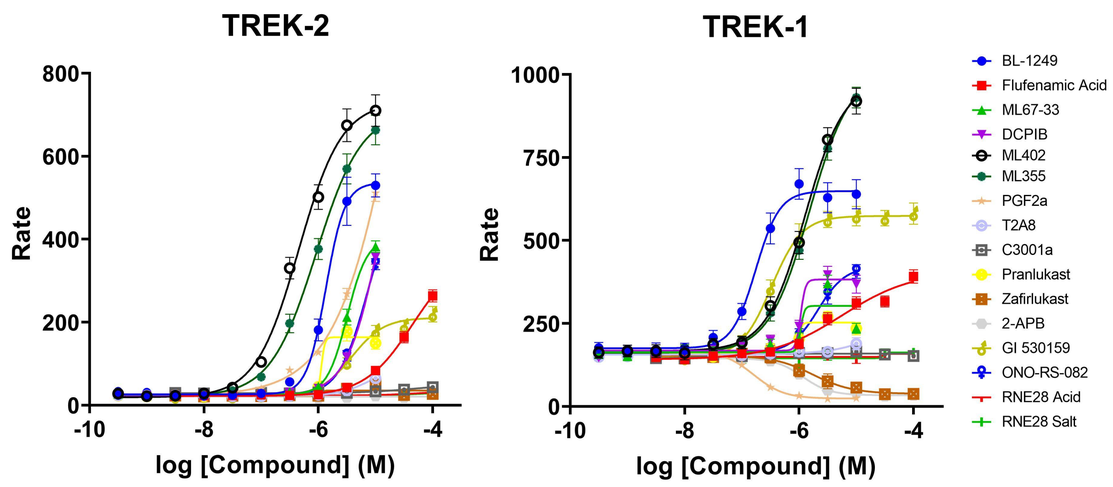


Figure 2 – Activity of tool molecules in thallium flux assay. Representative 10 point dose response curves of tool compounds. Compounds with published pEC50s > 5 were run at top concentrations of 100µM, all other compounds top concentration of 10µM. Rate refers to fluorescence increase between 13 and 19s post thallium addition in U-2 OS cells transduced with 4%/2% TREK-2/TREK-1 respectively. Results ± S.E.M from three independent experiments (n=2 per experiment). Data analysed using Graphpad prism.

Manual Patch clamp electrophysiology

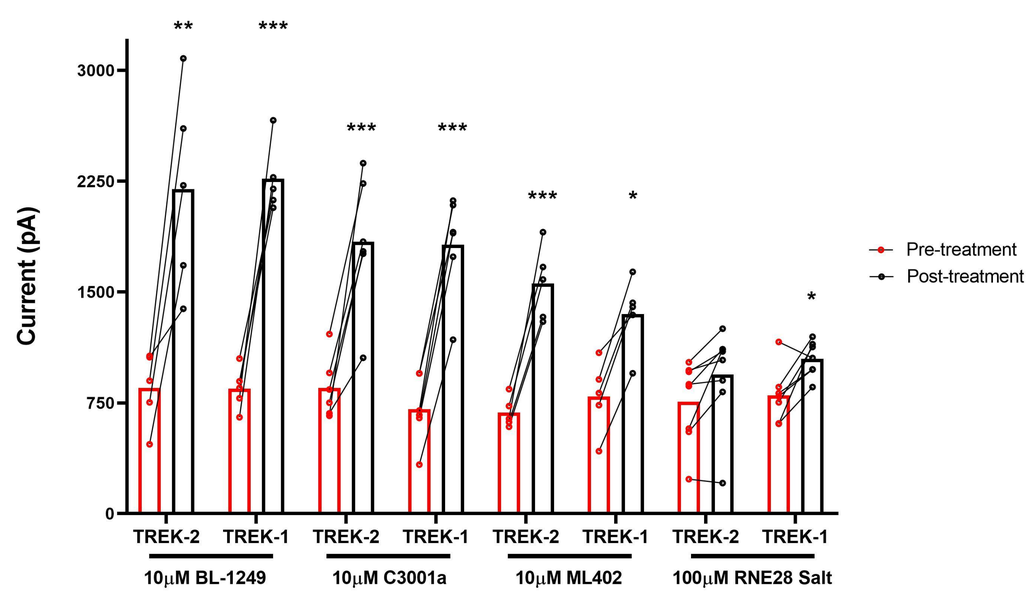


Figure 3 – Outward current in tsA201 cells overexpressing TREK-2 or TREK-1 pre and post compound treatment; cells were subject to continuous compound perfusion during treatment. Compounds tested at top concentrations used previously in thallium flux. Results from one experiment (n=5); * = p<0.05, ** = p<0.005, *** = p<0.0005 as determined using a paired T-test. Compounds were chosen that showed less activity than expected, or no activity, in thallium flux (C3001a and RNE28) and compared to robust activators (BL-1249 and ML402)

	TREK-2		TREK-1	
	Thallium flux	**Electrophysiology**	**Thallium flux**	**Electrophysiology**
BL-1249	Activator (pEC50 = 5.9 ± 0.1)	Activator	Activator (pEC50 = 6.8 ± 0.1)	Activator**
Flufenamic Acid	Activator (pEC50 = 4.4 ± 0.2)	ND	Activator (pEC50 = 5.2 ± 0.2)	ND
ML67-33	Activator (pEC50 = 5.5 ± 0.1)	Partial activator*	Activator (pEC50 = 5.6 ± 0.2)	ND
DCPIB	Activator (pEC50 = 4.9 ± 0.4)	ND	Activator (pEC50 = 5.9 ± 0.1)	ND
ML402	Activator (pEC50 = 6.4 ± 0.1)	Activator	Activator (pEC50 = 5.9 ± 0.1)	Activator**
ML355	Activator (pEC50 = 6.0 ± 0.1)	ND	Activator (pEC50 = 5.8 ± 0.1)	ND
PGF2a	Activator (pEC50 = 5.5 ± 0.7)	ND	Inhibitor (pIC50 = 6.8 ± 0.1)	ND
T2A8	Inactive	ND	Inactive	ND
C3001a	Inactive	Activator (manual patch only)	Inactive	Activator**
Pranlukast	Partial activator	ND	Partial activator	ND
Zafirlukast	Inactive	ND	Inhibitor (pIC50 = 5.8 ± 0.2)	ND
2-APB	Inactive	ND	Inhibitor (pIC50 = 5.9 ± 0.1)	ND
GI 530159	Inactive	Inactive*	Activator (pEC50 = 6.5 ± 0.1)	ND
ONO-RS-082	Activator (pEC50 = 5.2 ± 0.2)	Inactive*	Activator (pEC50 = 5.7 ± 0.1)	ND
RNE28 Acid	Inactive	Inactive*	Inactive	ND
RNE28 Salt	Inactive	Inactive	Inactive	Inactive**

Table 1 – Comparative pharmacology of tool compounds; activity of compounds defined relative to maximum activity of BL-1249 (Activator = >40%; Partial activator = 20 – 40%; Inactive = <20%); * = only run in automated patch clamp format; ** = only run in manual patch clamp format; ND = no data

Conclusions

- Potency and efficacy of compound panel defined against TREK-2 and TREK-1 in multiple assay systems
- BL-1249 and ML 402 demonstrate robust activation across numerous platforms
- Several compounds present novel activity profiles (notably 2-APB inhibition at TREK-1, and ONO-RS-082 activation at TREK-2)
- Future work - validation of compounds in alternate assays (e.g. membrane potential dye) and understand functional consequences in physiologically relevant systems (e.g. DRG cultures)