DISCOVERY AND CHARACTERISATION OF A NOVEL TRPML1 AGONIST

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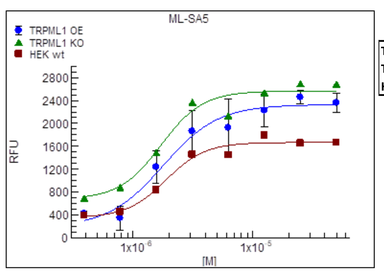
Introduction

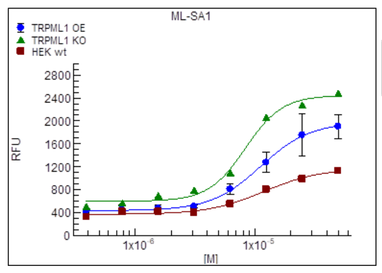
- **The transient receptor potential channel mucolipin 1 (TRPML1) plays an important role in neurological development and function and is the most characterised member of the mucolipin subfamily of transient receptor potential cation channels (TRPML) localised to lysosomes.**
- **To identify novel TRPML1 agonists, assays suitable for high throughput screening were developed and then used to screen the BioAscent Diversity library of 125K compounds.**
- **Computer-aided drug design (CADD) instrumental to design conformationally locked macrocyclic analogues with improved potency and retained selectivity.**

TRPML1 Assay Development

Assay development was carried out at BioAscent on two cell lines (produced by Thermo Scientific), HEK293_TRMPL1 containing overexpressed TRPML1 (OE) and a HEK293_TRPML1 knock-out (KO). Assays were developed using a fluorescent Ca^{2+} dye (Calcium 6, Molecular Devices) on the FLIPR Penta (Molecular Devices) for the OE, KO and HEK293 parental cell lines which were validated using the commercially available TRMPL1 activators, ML-SA5 and ML-SA1 (n=4). Surprisingly, the two activators produced increases in intracellular Ca^{2+} with similar EC_{50}s in all three cell lines (Figure 1). Suggesting non-TRMPL1 mediated effects upon Ca^{2+} handling



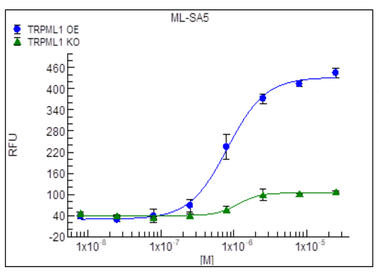
	ML-SA5 EC_{50} [M]
TRPML1 OE	1.70E-06
TRPML1 KO	1.71E-06
HEK wt	1.88E-06



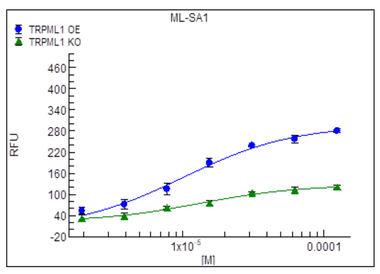
	ML-SA1 EC_{50} [M]
TRPML1 OE	1.12E-05
TRPML1 KO	8.36E-06
HEK wt	1.20E-05

Figure 1: The TRPML1 activators ML-SA5 and ML-SA1 cause a dose-dependent increase in cytosolic Ca^{2+} in the TRMPL1 OE, TRMPL1 KO and parental HEK293 cell lines (n = 4).

To isolate the intracellular, TRPML1-specific Ca^{2+} release the experimental protocol was adapted to remove extracellular Ca^{2+} by replacing the cell growth media with a Ca^{2+} free buffer (HBSS (-/-), 20mM HEPES, 0.05% Pluronic Acid pH7.0) containing 1mM EGTA (Figure 2).



ML-SA5	Max RFU	EC50 (M)
OE	444.66	9.13E-07
KO	107.00	1.14E-06
Fold	4.16	



ML-SA1	Min-Max	EC50 (M)
OE	280.33	1.04E-05
KO	121.00	1.34E-05
Fold	2.32	

Figure 2: The TRPML1 activators ML-SA5 and ML-SA1 cause a dose-dependent increase in cytosolic Ca^{2+} in TRMPL1 OE cells with minimal effect upon the TRMPL1 KO cell line after removal of extracellular Ca^{2+}.

TRPML1 HTS

The BioAscent diversity library of ~125,000 compounds was screened at a single concentration (10 µM, N=1) against the TRPML1 OE cell line using the assay described above (Figure 3). All compound transfers were carried out on an ECHO 550 acoustic liquid handler (Beckman Coulter).

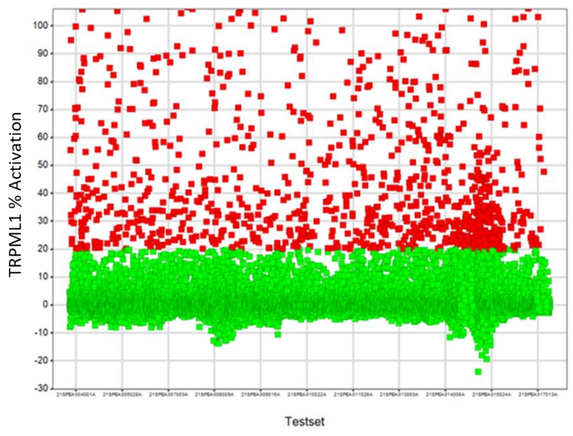


Figure 3: TRPML1 OE primary screen. ~1700 compound eliciting a response of ≥15% activation relative to 25 µM ML-SA5 control are highlighted in red and were progressed to active confirmation/deselection.

TRPML1 Active Confirmation and De-selection (TRMPL1 KO)

To identify specific activators of TRPML1 ~1700 primary screen actives were retested (10 µM, N=2) in the TRPML1 OE cell line and the TRPML1 KO cell line (Figure 4).

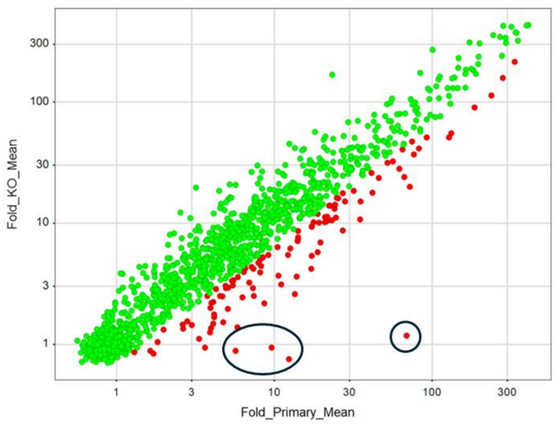


Figure 4: Correlation of mean fold response over baseline (n=2) for hit compounds tested the OE (x-axis) and KO (y-axis) cell lines.

109 compounds were selected (highlighted in red) for further potency testing on the basis that they elicited a fold response >1.5 times in the TRPML1 OE cells.
Three compounds (circled) were of particular interest as they showed no apparent activity in the KO cell line but a 5-10 fold response over baseline in the OE cell line.
One compound (circled) showed a good response in one replicate only.

TRPML1 Potency Testing

109 compounds were tested for potency as 7-point 1:2 dilution series' with a top concentration of 30 µM (0.75% DMSO final assay concentration) in both TRPML1 OE and KO cells. Data were analysed as fold response over baseline for both assays.

9 compounds showed little or no activity in the KO cells and some degree of dose-dependent activity in the OE cells and were retested as 11-point 1:2 dilution series' with a top concentration of 75 µM (0.75% DMSO final assay concentration).

2 compounds (BCC0092140 and BCC0044046) demonstrated little/no activity in KO cells and high nanomolar/low micromolar potency in OE assay (Figure 5).

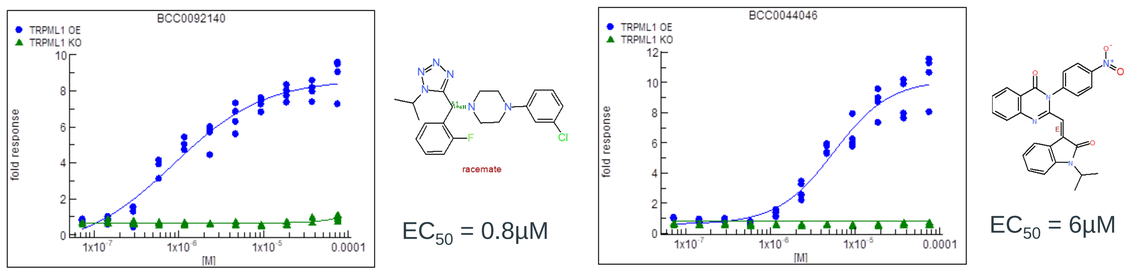


Figure 5: Dose-response data for the two most promising compounds.

TRPML1 Follow up

BCC0092140 was re-synthesised and the racemic mixture was subjected to chiral SFC separation and retested. Although both where active, one enantiomer was more so with an EC_{50} of 0.481µM in the TRPML1 OE cell line, and an EC_{50} > 48.8µM in the TRPML1 KO. Based on the docking studies (Fig. 6, left panel) into the cryo-EM structure of TRPML1, we were able to assign the absolute configuration to the two BCC0092140 enantiomers. In addition, the most active BCC0092140 enantiomer was better positioned in a binding pocket occupied by the agonist ML-SA1, rather than at the orthosteric binding site occupied by the endogenous agonist phosphatidylinositol 3,5-bisphosphate. We speculated that locking into a macrocycle the fluorophenyl and the isopropyl rings of BCC0092140 could reduce the unfavourable entropic contributions to the binding energy, possibly resulting in increased potency. Structure-based design was used to prioritise a few macrocyclic concepts and three macrocycles (see table below) were able to keep the potency compared to the parent compound in the TRPML1 OE cell line. One of them with an unsaturated linker even showed an increased potency in the TRPML1 OE cell line (EC_{50} 0.270 ± 0.188 µM (n=4)). Finally, all three macrocyclic compounds displayed the same lack of activity in the TRPML1 KO cell line (EC_{50} > 48.8 µM).

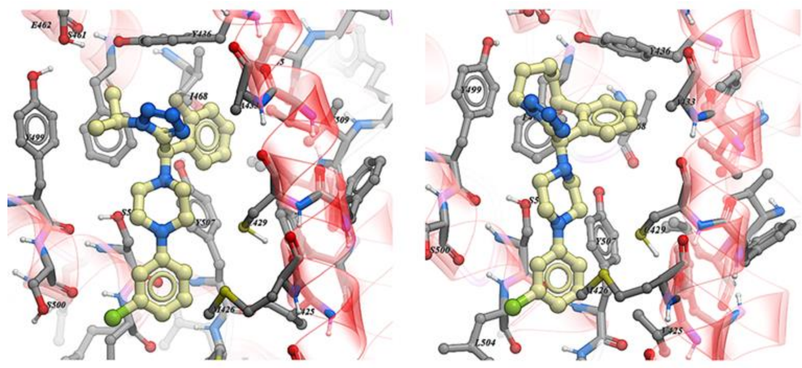


Figure 6: Docking pose of the most active BCC0092140 enantiomer (left panel) and (R)-Compound 19 (right panel) into the TRPML1 Cryo-EM structure.

	17	18	19	ML-SA5
TRPML1 OE EC_{50} (µM)	1.04	0.270	1.41	0.885
TRPML1 KO EC_{50} (µM)	> 48.8	> 48.8	> 48.8	1.00

Summary

After developing suitable Ca2+ fluorescent assays and screening a compound library of ~125,000 compounds, a compound (BCC0092140) was identified, and its activity confirmed. The racemic compound was prepared, and the enantiomers separated. Both antipodes were tested and evaluated. The more active enantiomer showed better activity and much better selectivity over ML-SA5 as a TRPML1 agonist in vitro. BCC0092140 should serve as a better tool compound for future studies on the activation of the TRPML1 channel. We were able to improve the potency by a structure-based design driven approach yielding several promising macrocyclic leads. Further optimization of this scaffold is currently underway.

Conclusions

- Accelerated the discovery process compared to Arkuda in-house capabilities alone.
- Cost-effective approach using our well-established HTS platforms.
- Access to specialized computational skills and cutting-edge technologies.
- Identified promising novel TRPML1 agonists with potential for therapeutic development.

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