A cell based assay to identify small molecules that stabilize mutant Clarin-1

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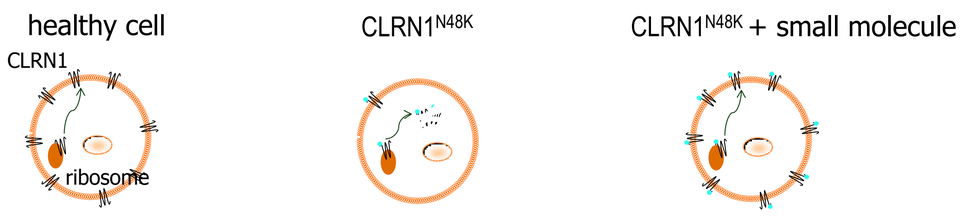
discovery from charles river

1 Introduction

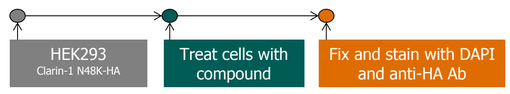
Usher syndrome III (USHIII) is characterised by progressive loss of vision and hearing.
One of the prevalent forms of the disorder is caused by a single point mutation in the clarin-1 gene, which encodes for the four transmembrane protein CLRN1. The replacement of the asparagine at position 48 with lysine prevents glycosylation, which affects folding and trafficking of the CLRN1^{N48K} leading to degradation in the proteasome.
Subsequent lack of expression at the cell surface has been shown to lead to degeneration of the mechanosensory hair cells of the inner ear.
No treatment is currently available to stop or slow the progression of USHIII.
Thus, a generally applicable strategy is needed to develop therapeutic agents for treating USHIII.

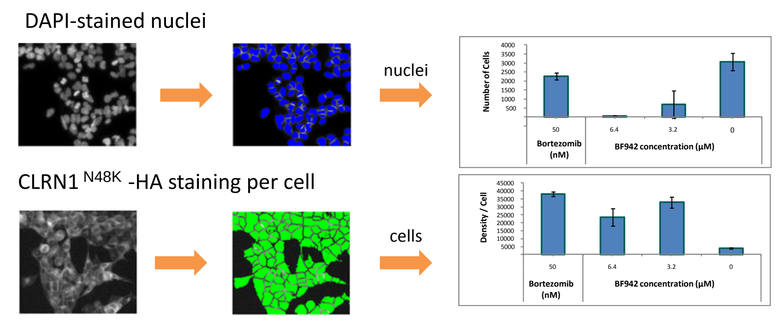


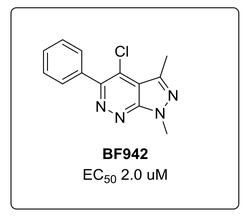
It was postulated that a small molecule that inhibits degradation of the mutant CLRN1^{N48K} and restores trafficking to the cell surface would prevent disease progression and provide an avenue of intervention for USHIII.
Although generic proteasome inhibitors would prevent degradation of CLRN1^{N48K} this would not constitute a valuable treatment for USHIII due to the potential side effects of non-selective proteasome inhibitors.

2 Functional assay

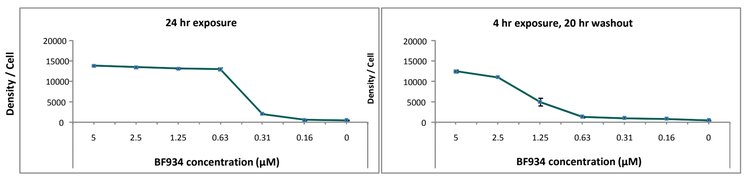
A high content cell based assay was developed that detects CLRN1 at the cell surface of HEK293 cells expressing CLRN1^{N48K}





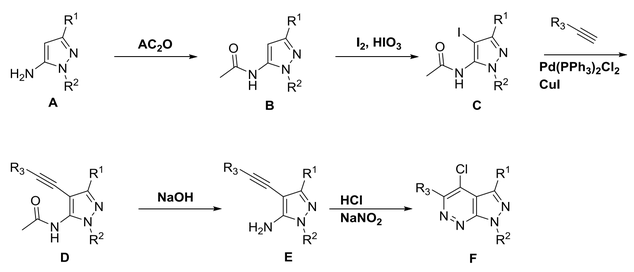


A screen of 50,000 compounds led to the discovery of **BF942.**



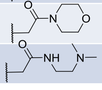
Washout experiments with a more potent compound (**BF934**) show sustained efficacy postulated to be due to covalent binding to the target.

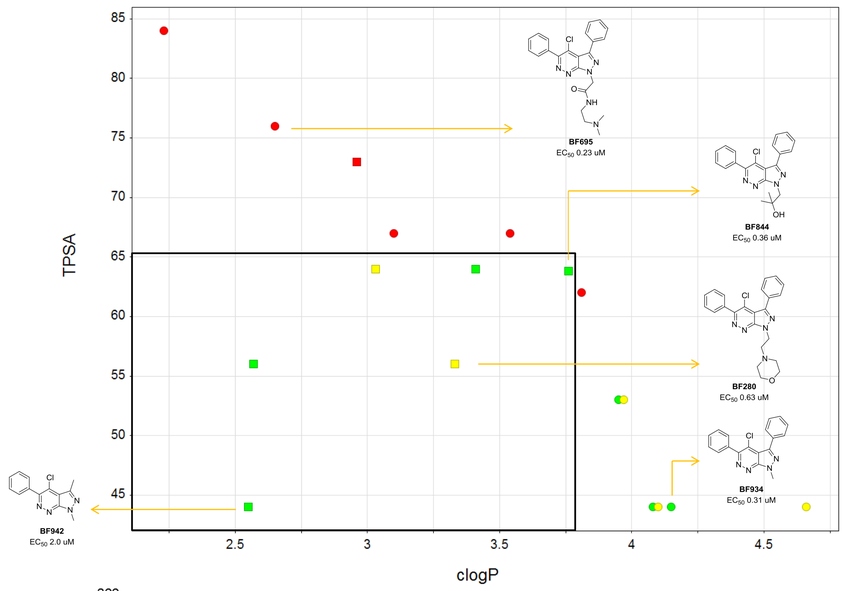
3 Chemistry development

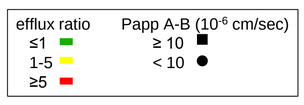


Further compounds to explore the SAR were prepared in 5 steps from commercially available materials. The key final step involving a Richter cyclisation.

Replacement of the chlorine in position 4 with any moiety other than a halogen led to complete loss in activity, as did replacement of either of the nitrogen atoms in the pyridazine ring, this provided further evidence that the molecules were operating via a covalent binding interaction between the target and the electrophilic chlorine.
Replacement of the methyl group at R1 with an aryl group gave an order of magnitude improvement in potency (**BF934**).
Similarly, an aryl group at R3 was required. However, we discovered that there was scope for changes at R2 to modulate physicohemical properties to improve solubility and ADME properties whilst maintaining potency.

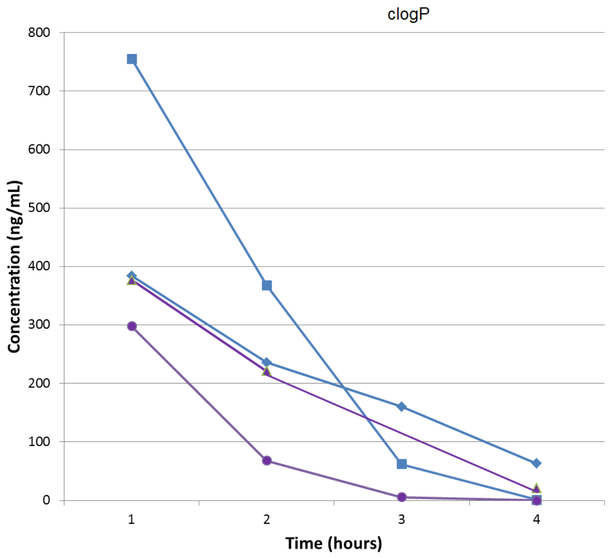
Compound	R1	R2	R3	EC_{50}µM	Solubility $_{(Aq)}$ µM
BF942	Me	Me	Ph	2.0	105
BF934	Ph	Me	Ph	0.31	<5
BF136	Ph	Me	Cy	>25	ND
BF353	Ph		Ph	0.45	19
BF280	Ph		Ph	0.63	19
BF981	Ph		Ph	0.31	67
BF844	Ph		Ph	0.36	32
BF378	Ph		Ph	0.40	36
BF695	Ph		Ph	0.23	>200

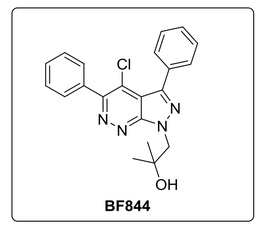




Compounds with TPSA<65Å^2 and cLogP<4 exhibit good permeability and low efflux, which translated into good retina and brain exposure.

Compound	Brain: plasma	Retina: plasma
BF353	0.4	0.2
BF981	1.8	0.47
BF695	0.07	ND

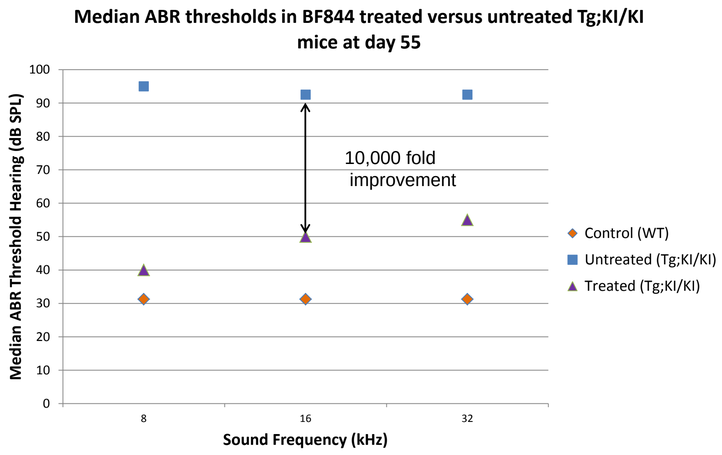




Single dose PK carried out on juvenile mice. **BF844** showed greater exposure than other compounds.

4 Efficacy study

A model using mice expressing CLRN1 under control of the *Atoh1* gene enhancer on a CLRN1^{N48K} background was produced (Tg:KI/KI).
This model closely mimicked the delayed-onset progressive hearing loss profile manifested by Usher III patients.
Mice were treated by ip administration of **BF844** in a dose escalating regimen from 10mg/kg on day 10 to 30mg/kg on day 45.
Hearing loss was measured at different frequencies using auditory brainstem response (ABR) recording on day 46 and day 55.



5 Conclusion

We have demonstrated a robust screening approach, which led to the discovery and optimisation of a series of compounds.
BF844 showed statistically significant efficacy in a mouse model of hearing loss for CLRN1^{N48K} USHIII.
Mice treated with **BF844** showed 1000-10000 times more sensitive hearing than untreated mice.

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USHER III INITIATIVE

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