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


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Abstract

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 tyrosine prevents glycosylation, which affects folding and trafficking of the CLRN1 protein, leading to degradation in the proteasome.

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 postulated to be due to covalent binding interaction between the target and the electrophilic chlorine.

Introduction

USH III is caused by a single point mutation in the CLRN1 gene, which encodes for the four transmembrane protein CLRN1. The replacement of the asparagine at position 48 with tyrosine prevents glycosylation, which affects folding and trafficking of the CLRN1 protein, leading to degradation in the proteasome.

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<sup>N48K</sup> 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<sup>N48K</sup> this would not constitute a valuable treatment for USHIII due to the potential side effects of non-selective proteasome inhibitors.

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 aryl group gave an order of magnitude improvement in potency.

Screening.

A high content cell based assay was developed that detects CLRN1 at the cell surface of HEK293 cells expressing CLRN1<sup>N48K</sup>

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

A model using mice expressing CLRN1 under control of the Atoh1 gene enhancer on a CLRN1<sup>−/−</sup> background was produced (Tg(Atoh1)). This model closely mimicked the delayed-onset progressive hearing loss profile manifested by Usher III patients.

Mice were treated by ip administration of BF944 in a dose escalating regimen from 10 log/mg on day 10 to 30 log/mg on day 45. Hearing loss was measured at different frequencies using auditory brainstem response (ABR) recording on day 46 and day 55.

Efficacy study

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Conclusion

We have demonstrated a robust screening approach, which led to the discovery and optimisation of a series of compounds. BF944 showed statistically significant efficacy in a mouse model of hearing loss for CLRN1<sup>N48K</sup> USHIII.

Mice treated with BF944 showed 1000-10000 times more sensitive hearing than untreated mice.

BF944 showed greater exposure than other compounds.

Single dose PK carried out on juvenile mice.

BF944 showed greater exposure than other compounds.

Treated (Tg;KI/KI) vs untreated (Tg;KI/KI) experiments.

BF944 showed significantly better hearing than untreated mice.