Trifunctional High-Throughput Screen Identifies Promising Scaffold To Inhibit Grp94 and Treat Myocilin-Associated Glaucoma

Huard DJE1, Crowley VM2, Du Y3, Cordova RA4, Sun Z4, Tomlin MO1, Dickey CA4, Koren J 3rd4, Blair L4, Fu H3, Blagg BSJ5, Lieberman RL1

1 School of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, United States.
2 Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas 66045, United States.
3 Emory Chemical Biology Discovery Center, Department of Pharmacology, Emory University, Atlanta, Georgia 30332, United States.
4 Byrd Alzheimer Institute, Department of Molecular Medicine, University of South Florida, Tampa, Florida 33612, United States.
5 Department of Chemistry and Biochemistry, The University of Notre Dame, Notre Dame, Indiana 46556, United States.

Gain-of-function mutations within the olfactomedin (OLF) domain of myocilin result in its toxic intracellular accumulation and hasten the onset of open-angle glaucoma. The absence of myocilin does not cause disease; therefore, strategies aimed at eliminating myocilin could lead to a successful glaucoma treatment. The endoplasmic reticulum Hsp90 paralog Grp94 accelerates OLF aggregation. Knockdown or pharmacological inhibition of Grp94 in cells facilitates clearance of mutant myocilin via a non-proteasomal pathway.

Here, we expanded our support for targeting Grp94 over cytosolic paralogs Hsp90α and Hsp90β. We then developed a high-throughput screening assay to identify new chemical matter capable of disrupting the Grp94/OLF interaction. When applied to a blind, focused library of 17 Hsp90 inhibitors, our miniaturized single-read in vitro thioflavin T-based kinetics aggregation assay exclusively identified compounds that target the chaperone N-terminal nucleotide binding site. In follow up studies, one compound (2) decreased the extent of co-aggregation of Grp94 with OLF in a dose-dependent manner in vitro, and enabled clearance of the aggregation-prone full-length myocilin variant I477N in cells without inducing the heat shock response or causing cytotoxicity.

Comparison of the co-crystal structure of compound 2 and another non-selective hit in complex with the N-terminal domain of Grp94 reveals a docking mode tailored to Grp94 and explains its selectivity. A new lead compound has been identified, supporting a targeted chemical biology assay approach to develop a protein degradation-based therapy for myocilin-associated glaucoma by selectively inhibiting Grp94.

PMID: 29402077