Use of the SEE-Tx® Drug Discovery Platform to Identify Pharmacological Chaperones for Glutaric Acidemia Type 1

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Background

Glutaric acidemia type 1 (GA1) is a rare inherited metabolic disorder caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH), affecting 1 in 125,000 to 1 in 250,000 newborns. Current treatment challenges include strict dietary management, emergency interventions during metabolic crises, and limited efficacy in preventing neurological complications in about 15% of patients.

Pharmacological chaperones are small molecules that can bind to and stabilize misfolded proteins, potentially rescuing their function. In GA1, these chaperones could stabilize mutant GCDH enzymes, increasing their activity and reducing toxic metabolite accumulation. This approach offers a targeted therapy that may complement existing treatments and improve long-term outcomes for GA1 patients.

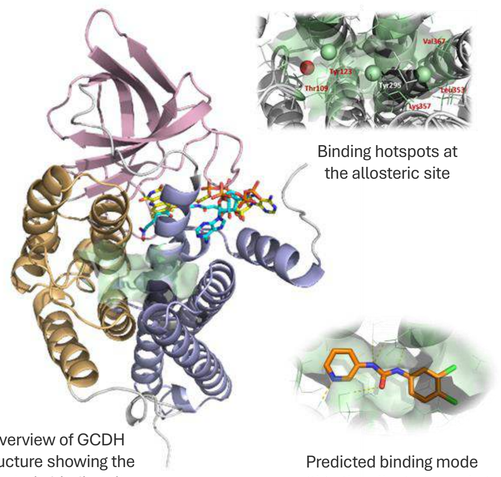
AIM: Identify allosteric regulators for the enzyme glutaryl-CoA dehydrogenase (GCDH) using SEE-Tx technology.

SEE-Tx® Drug Discovery Platform

1. Druggability Assessment
Allosteric site discovery with mixed solvent simulations

2. Virtual Screening
Millions of compounds filtered and screened guided by hotspots

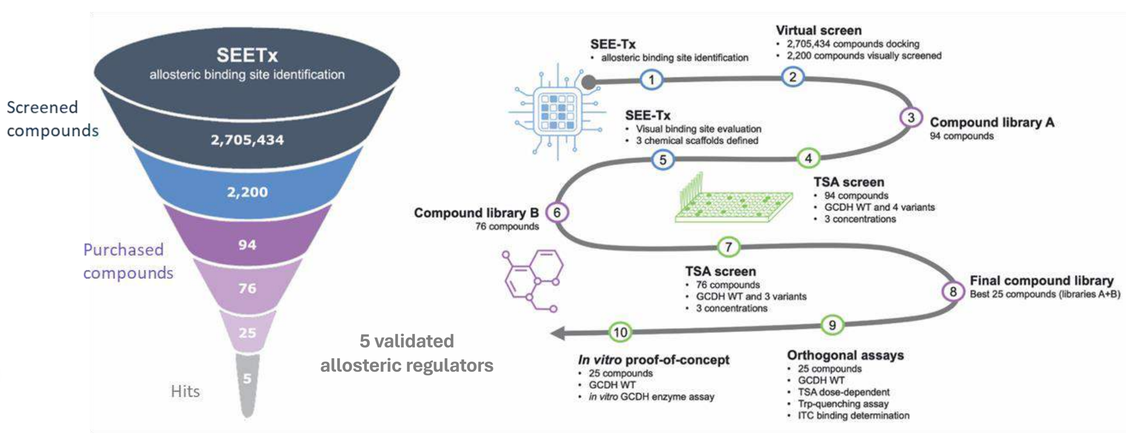
3. Experimental Validation
MoA confirmation using biophysical and biochemical assays



Overview of GCDH structure showing the allosteric binding site

Binding hotspots at the allosteric site

Predicted binding mode within the allosteric pocket



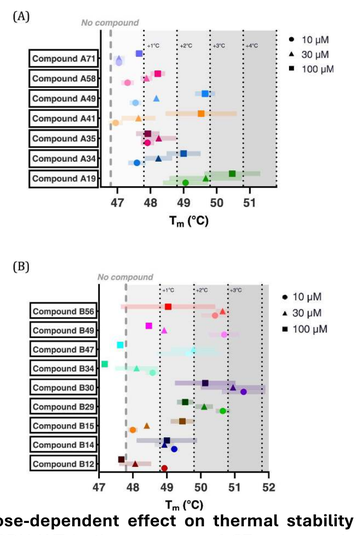
SEE-Tx's physics-based technology utilized the published 3D structure of GCDH to identify an allosteric binding site and predict its druggability.

This guided a high-throughput virtual screening, using a unique automated methodology to explore commercial compound libraries. Through this computational approach, we identified and purchased structurally diverse small molecules as virtual hits.

This was followed by a step-by-step experimental validation approach using thermal shift assays and additional orthogonal biophysical and biochemical assays, which allowed the identification of 5 validated allosteric regulators of GCDH.

Primary screening

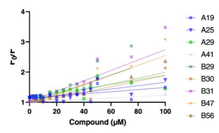
Thermal Stability Assay (TSA) – binding
170 virtual hits



Dose-dependent effect on thermal stability of GCDH WT in the presence of GT compounds at three different concentrations (10, 30 &100 µM).

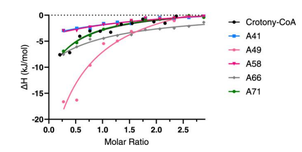
Secondary screening – Orthogonal assays

Tryptophan quenching assay – binding
25 stabilizers



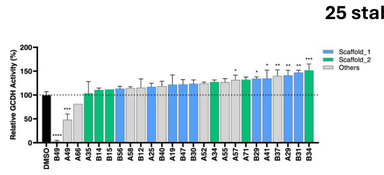
Intrinsic fluorescence of GCDH WT is quenched due to the **binding** of GT compounds **at increasing concentrations**.

Isothermal titration calorimetry - binding
25 stabilizers

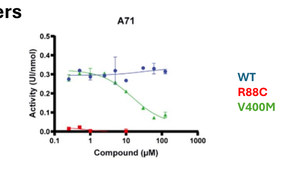


ITC analysis of the interaction of GCDH WT with GT compounds. Crotonyl-CoA was used as a positive binding control.

Enzyme activity – functional effects
25 stabilizers



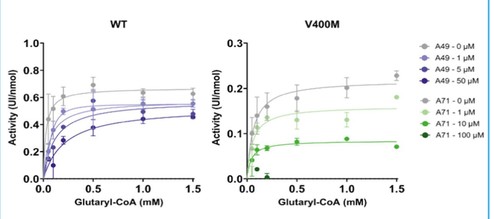
1. Effect of the final GT compound library on the catalytic activity of recombinant GCDH WT. **Most compounds increased GCDH specific activity at** a concentration of **10 µM**.



2. Effect of the leading GT compound on enzymatic activity of recombinant GCDH WT and variants R88C and V400M at increasing concentrations (0.25 to 125 µM). No residual activity could be detected for variant A433E, and it was almost absent for variant R88C.

Extra assays performed to verify compound binding at the allosteric site identified

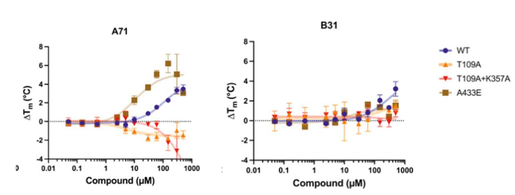
Enzyme kinetics competition assay – binding
5 binders



Competition assay for GCDH WT and GCDH V400M proteins with 2 GT inhibitors at 4 different concentrations (0-100 µM) and increasing substrate concentrations (0 to 1.5 mM).

- A49 compound is likely binding to the WT enzyme's active site, directly competing with the substrate. The observed effects suggest that this is a reversible inhibition, as is typical for **competitive inhibitor**.
- A71 compound showed a clear **non-competitive** interaction; Binding interaction confirmed by ITC.

TSA using allosteric site variants – binding
5 binders



Dose-dependent effect on thermal stability of GCDH WT and mutations mapping to the proposed allosteric binding site in the presence of GT leading compounds.

Specific pocket site variants: T109A, and a double mutant T109A+K357A to further disrupt potential hydrophobic interactions. GCDH WT and the pocket independent variant A433E were used as controls.

- A71 compound stabilized both the WT and the disease variant A433E. However, this stabilizing effect was not observed with the pocket site mutations (T109A and T109A+K375A), supporting the **allosteric binding site interaction with GCDH protein**.
- B31 compound does not show stabilizing effect in the double mutant (T109A+K375A), supporting the **existence of an interaction with the allosteric binding site when bound to GCDH**.

A two-step biophysical screening approach was employed, using a thermal shift assay (TSA) as the primary screen to monitor the thermal denaturation of GCDH WT and four variants in the absence and presence of 170 compounds. To further characterize the top 25 stabilizing hit compounds, a series of orthogonal assays, including tryptophan quenching, isothermal titration calorimetry (ITC), and enzyme activity assays, were conducted. These assays led to the identification of 5 novel binders for the GCDH protein. To confirm the effect of these five lead compounds and validate the allosteric pocket, two additional approaches were implemented: enzyme-substrate competition assays and compound stability assays after pocket mutation.

Conclusions

Bibliography: *J. Med. Chem.* 2024, 67, 19, 17087-17100

- SEE-Tx computational platform successfully identified a novel druggable allosteric site in GCDH, the enzyme deficient in GA1.
- Through virtual screening and experimental validation, five lead compounds were discovered that bind to and stabilize GCDH, demonstrating concurrent effects on enzyme activity, protein stabilization, and binding.
- These identified allosteric regulators show potential as pharmacological chaperones for GA1, offering a promising starting point for developing new therapies that address protein misfolding without competing with the natural substrate.