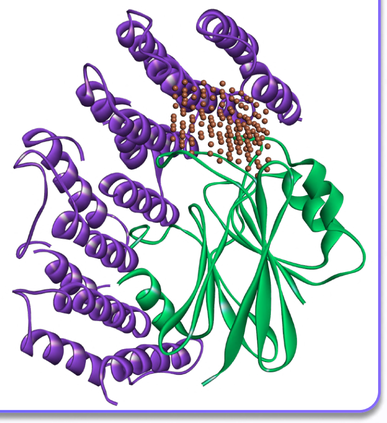
BACKGROUND:

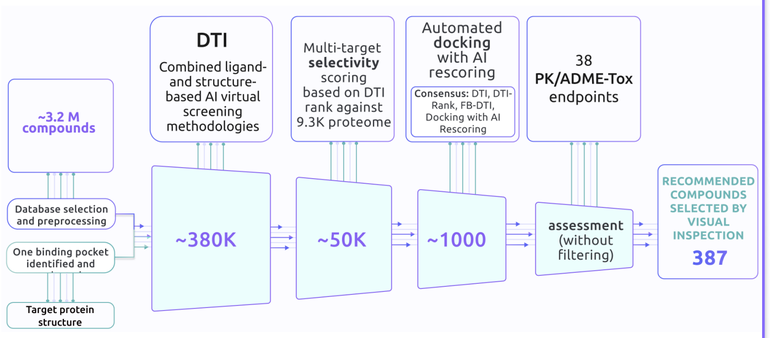
Challenging target: A complex of two proteins with unknown binding sites. A binding pocket was identified by Receptor.AI at the protein-protein interface using a proprietary AI model for the binding site identification. Two low-quality ligands are known with only mediocre binding affinity.

Goal: Design of "molecular glue" to stabilise the membrane complex of 2 proteins associated with autophagy and neurodegeneration.



METHODOLOGY:

Virtual screening: Enamine stock collection of 3.2M compounds was subject to virtual screening. 1000 top-ranked candidate compounds were selected. 387 compounds were used for experimental validation.



3.2 M	387	7	250%+
compounds screened	hit candidates selected	potent compounds identified	expression induced by lead compound

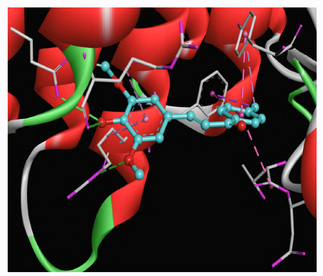
RESULTS:

Biological validation: The experimental validation was conducted on the HEK 293 cell line to assess modulation of target protein expression. Protein expression level was assessed by the Western blot. The hit criteria for the screening was set as a 120% increase in target protein concentration against the control, which resulted in the identification of seven hits. Following the initial screening, a dose-response analysis was carried out for these seven hits to to confirm their potency.

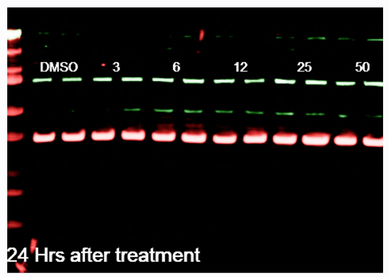
Hit compounds: The activity of all seven identified hit compounds, along with the absence of cytotoxicity, was substantiated through dose-response analysis.

Compound	Expression increase	
	Round 1	Round 2
#1	1.63	1.32
#2	2.62	2.71
#3	1.55	1.40
#4	1.97	1.57
#5	1.61	2.27
Competitor #1	1.53	1.69
Competitor #2	1.44	1.14

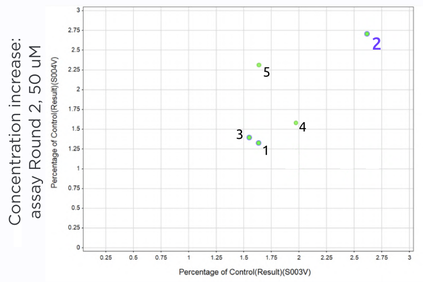
Four active scaffolds were chosen for future series expansion, specifically the scaffolds of compound 1, 3, 4 and the shared scaffolds of compounds 2, 5-7. The most promising hit compound 2 exhibited a nearly sub-micromolar EC_{50} in a cellular functional assay with a 400% higher potency compared to the best existing rivals.



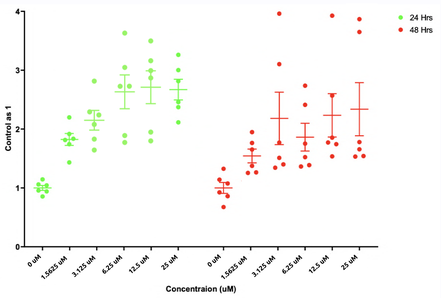
The binding pose for the compound 2 mentioned above



Western blot, Compound 2, HEK 293 cell line, 24h after treatment



Target protein concentration increase: assay Round 1, 50 uM



Dose-Response relationship for Compound 2. Treatment time 24 and 48 hours.