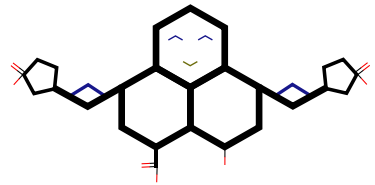


ALS-SynAegis: A Molecular Dynamics Study on TDP-43 Aggregation to Prevent Amyotrophic Lateral Sclerosis Onset

Aryaman Rtunjay

Tesla STEM High School

Project ID: BCHEM-017

Sammamish, Washington

United States

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a progressive onset neurodegenerative disease caused by the mislocalization and aggregation of a DNA-Binding protein, TDP-43.

- Occurs in at least 200,000 people globally, millions of unreported cases
- Mortality rate of 80% in 5 years, 90% in 10 years, and 95% in 20 years

What is unknown?

1. No research done in identifying specific aggregation tendencies of TDP-43
   - Specific residue interactions
   - Common aggregate structures and conformations

2. No work been done on designing inhibitor to target aggregates and disassemble them
   - Riluzole is only approved drug and causes minimal increase in survival time
   - Created over 30 years ago, non-optimized for TDP-43 aggregates as specific interactions were undeterminable

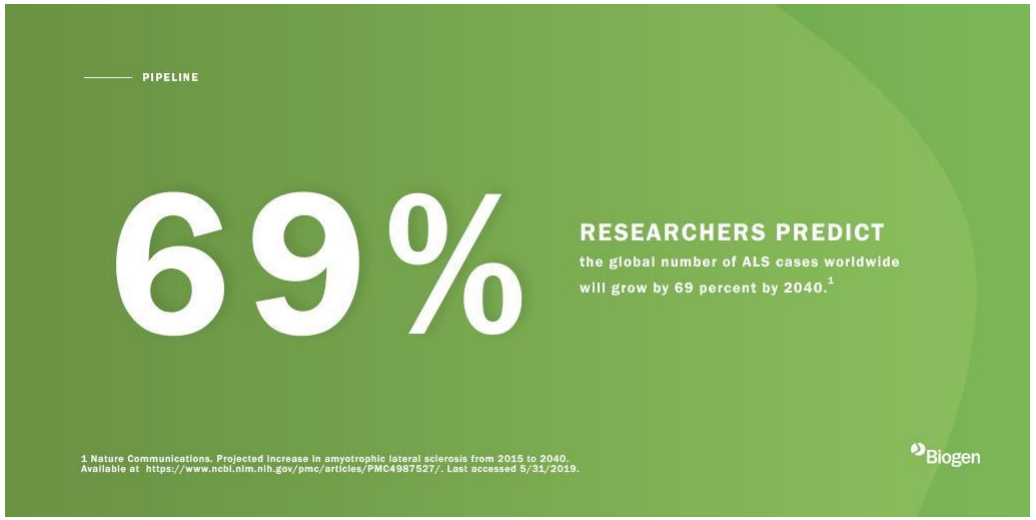


Figure 1: Prediction of ALS case growth by 2040 (Biogen 2019)

To prevent the onset of ALS, it is crucial to craft an improved inhibitor targeting TDP-43 aggregates, tailored to their specific aggregation patterns identified through a computational study

Research Objectives

Objective 1

Unravel specific TDP-43 **aggregation tendencies** and the **structural evolution** of aggregates focusing on the following traits

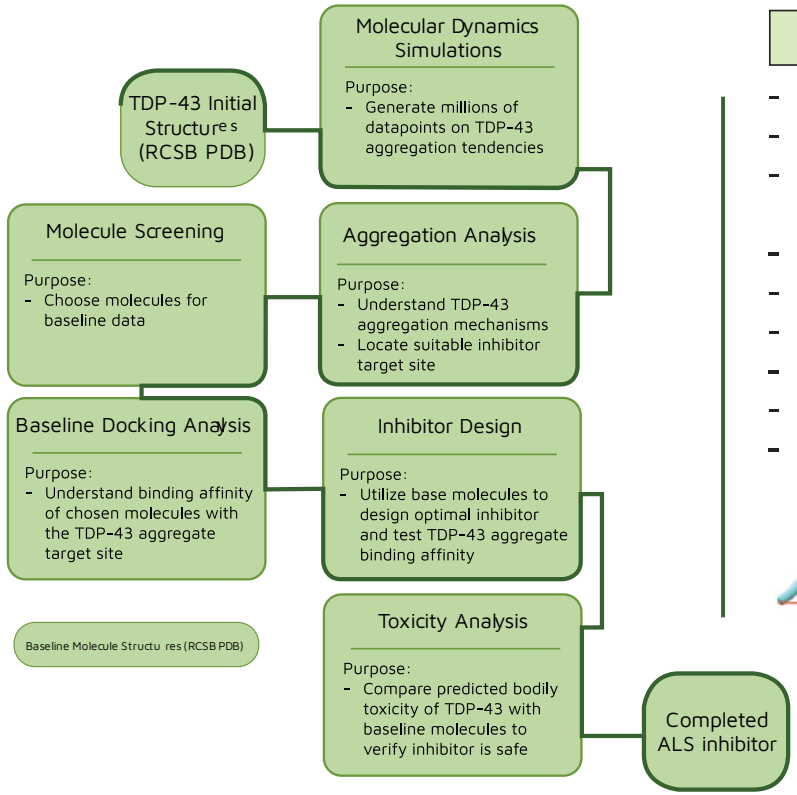
- Residue Contact Analysis
- Residue Hydrogen Bonding Analysis
- Formation of β-pleated sheets
- Most prominent aggregate structures

Objective 2

Design an inhibitor molecule to **destabilize TDP-43** aggregates that satisfy the drug criteria specified below:

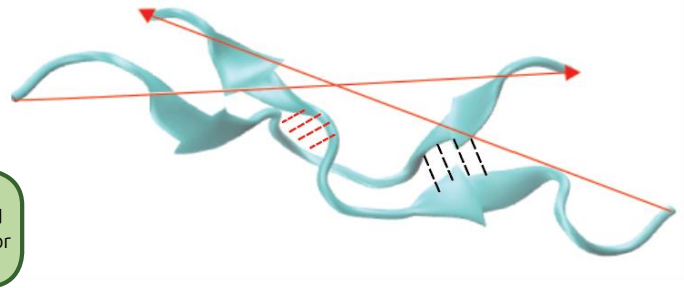
1. Binding Affinity better than Riluzole
2. Molecular mass between 400 and 600 Da (Banks 2009)
3. < 12 Hydrogen Bond Donors (Lipinski 1997)
4. < 5 Hydrogen Bond Acceptors (Lipinski 1997)
5. logP value < 5 (Lipinski 1997)
6. No Molecular Toxicophores present in structure

Methodology



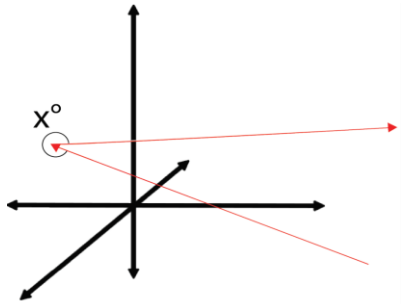
TDP-43 Aggregation Tendencies

- Simulated **Hexameric and Dimeric** TDP-43
- Simulated **A315E and A315T** mutant variants
- Leads to investigation of both **Familial-ALS** and **Sporadic-ALS**
- **Replica Exchange** Techniques for max accuracy
- Utilized **Purdue ANVIL** Computing cluster
- Total of **35 million GPU Cores** utilized
- **41.6 billion frames** across **8 total simulations**
- Simulation lengths of **800-1100 ns**
- **~3,120 computing hours** of simulations



Analysis Methodology

- Residue Contact Prominence
- Prevalent Hydrogen Bonding Positions
- Determined via analyzing all amino acid residue interactions
- β-sheet Direction
  - Done via end-to-end vector cosine angle calculations
- Root Mean Square Deviation
  - Used to visualize the most common clusters throughout simulation



1. System Setup

- CHARMM36m force field
- 22 sodium ions and 28 chloride ions for charge neutralization

$$V = \sum_{bonds} k_b(b-b_0)^2 + \sum_{angles} k_\theta(\theta-\theta_0)^2 + \sum_{dihedrals} k_\phi[1+\cos(n\phi-\delta)] + \sum_{impropers} k_\omega(\omega-\omega_0)^2 + \sum_{Urey-Bradley} k_u(u-u_0)^2 + \sum_{nonbonded} \left\{ \epsilon_{ij} \left[\left(\frac{R_{min_{ij}}}{r_{ij}} \right)^{12} - 2\left(\frac{R_{min_{ij}}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{\epsilon_r r_{ij}} \right\}$$

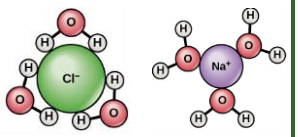
Equation modelling CHARMM36m forcefield

2. Box Setup

- Dodecahedron shaped box used for MD Simulations.
- ~70% volume of cubic boxes reduces computational intensity
- Few solvent molecules used

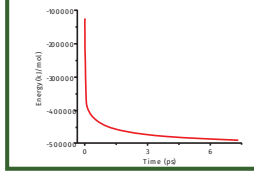
3. Solvation & Ions

- Water molecules as solvent; all atoms simulated
- Neutralized charge with sodium cations & chloride anions



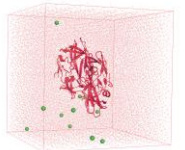
4. Energy Minimization

- Solvated system energy goes through minimization
- Alleviates steric clashes
- Utilized steepest descent
- Minimization ran for 6 ps



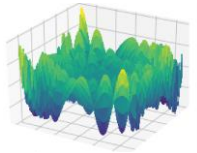
5. Equilibration

- First stage: Protein Restriction
- Second Stage: NVT Ensemble
- Third Stage: NPT Ensemble
- Fourth Stage: Removed restraint on protein and equilibrated



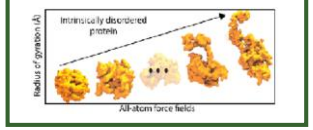
6. Production Run

- Equilibrated system used for production simulation
- 64 replicas, periodic exchanges
- Captures diverse states and rare events in structure



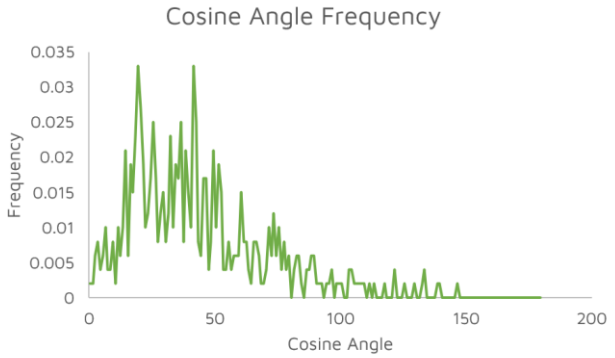
7. Analysis

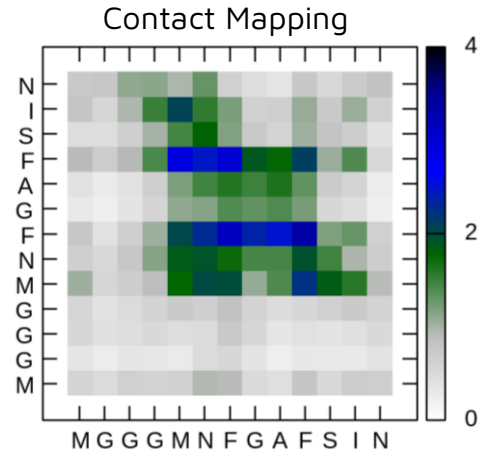
- Properties Investigated
  - Structural Stability
- Tools Used
  - RMSD
  - Gyration
  - Contacts/Hbonds
  - End-to-End Vectors

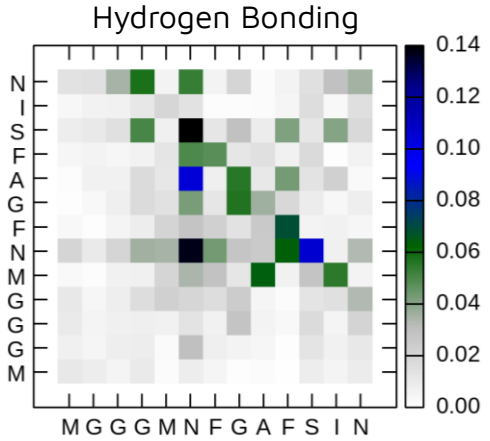


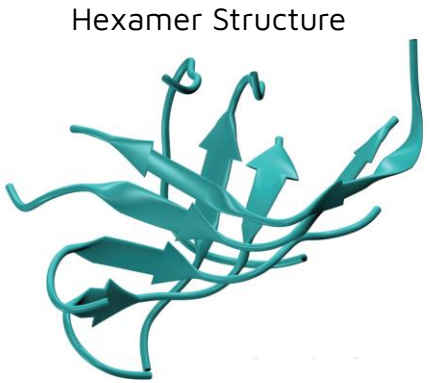
Aggregation Tendencies

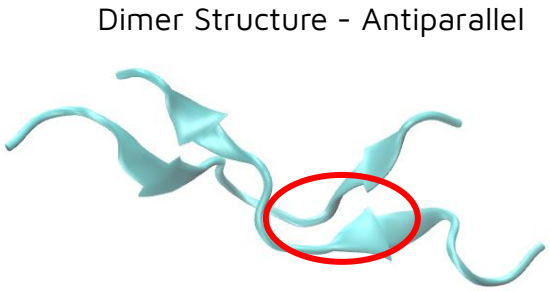
1. Residue Contact Mapping
   - Primary contacts between 311-MNFGAF-316
   - Found high contacts on PHE-313,316
   - No significant difference in parallel vs antiparallel contact maps
2. Hydrogen Bond Mapping
   - Primary contacts between 312-NFGAFS-317
   - Found most hydrogen bonds on ASP-312 and on SER-317
   - Precise target site can now be determined
3. RMSD Clustering Analysis
   - Hexamer showed dimeric interactions coming together to form one large aggregate
   - Dimer clusters: ~1-2 beta sheets → High structural stability
4. Cosine Angle
   - Most probable states at 0 to 75 degrees
   - Signifies most beta sheets formed are parallel

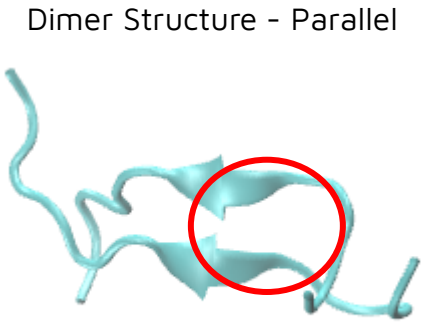






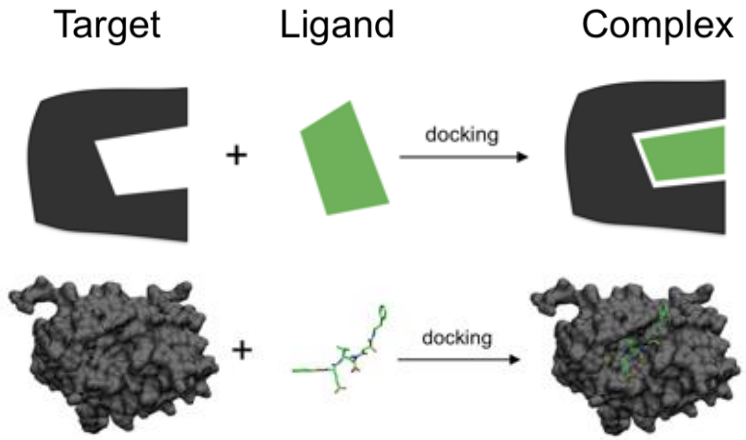


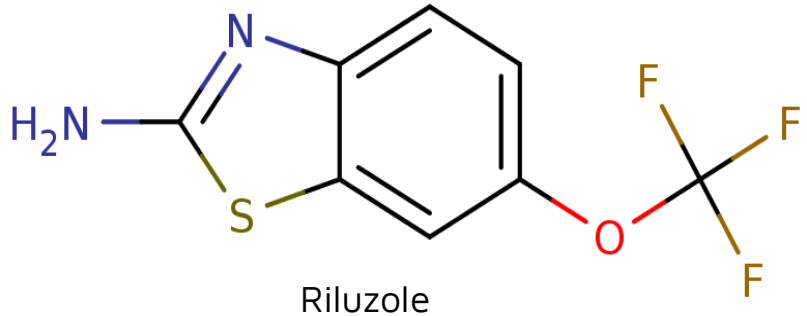




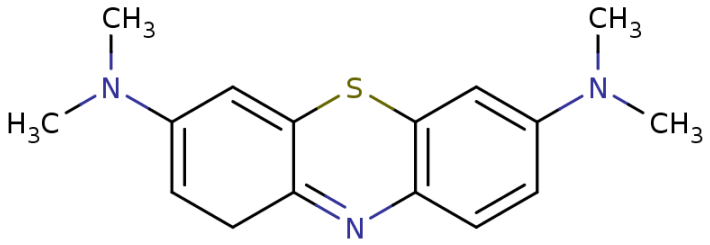
Baseline Docking Analysis

- Previous studies describe 100 molecules able to reduce TDP-43 toxicity
  - Analyzed molecules based on reported ALS subsystem and effectiveness in destabilizing TDP-43 aggregates
  - 5 molecules selected for docking analysis in AutoDock Vina
- Tested chosen molecules on most common aggregate from the 40 billion simulated structures

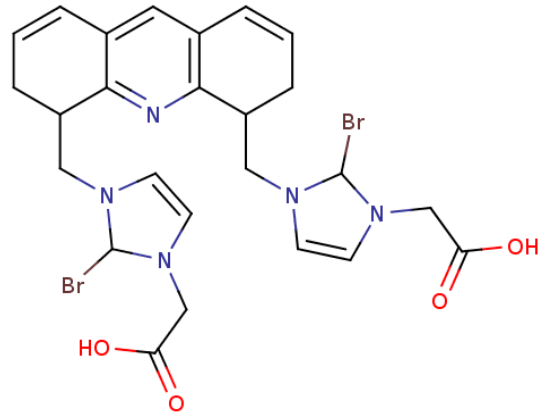




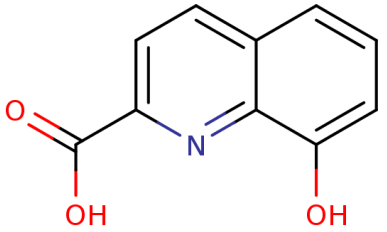
Riluzole



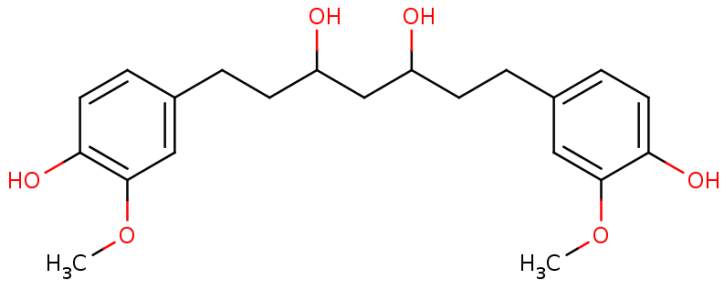
Methylene Blue



AIM4



Hydroxyquinoline

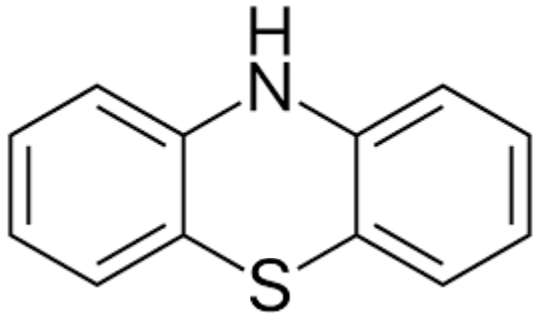


Curcumin

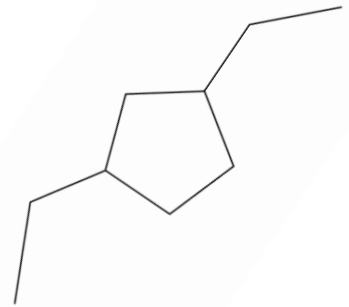
Inhibitor Design

- Best molecules from baseline ranked
- Specific groups chosen from baseline for initial inhibitor design
- 12 iterations to create final inhibitor
- Candidate groups for Inhibitor:
  1. Phenothiazine
  2. Cyclopentane Ethyl Propane
  3. Carboxyl Group
  4. Methyl/Ethyl Group
  5. Nitrile Group
  6. Hydroxyl Group

Structural Groups

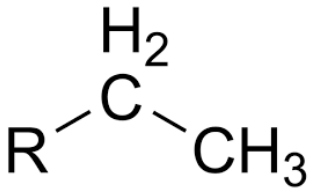


Phenothiazine



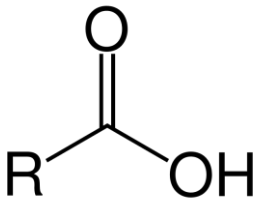
1,3-diethylcyclopentane

Functional Groups

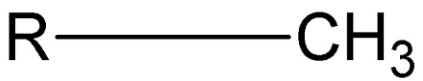


Ethyl

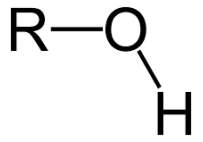
Nitrile



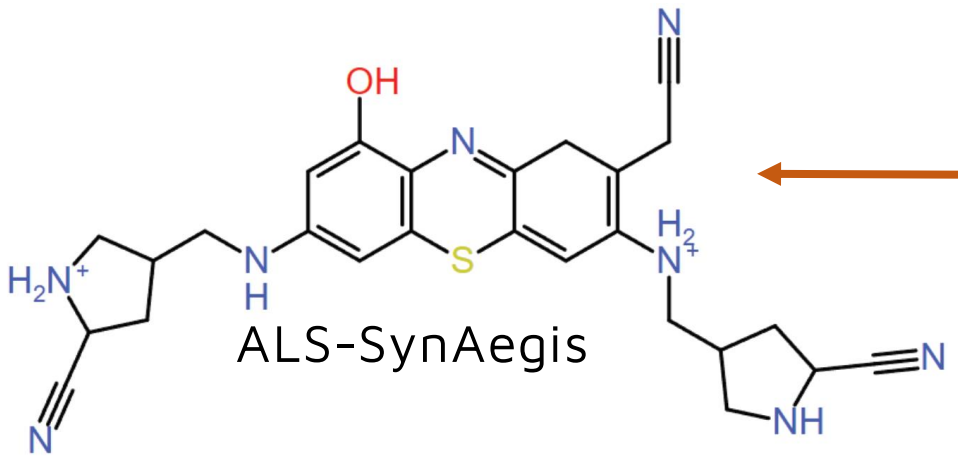
Carboxyl



Methyl



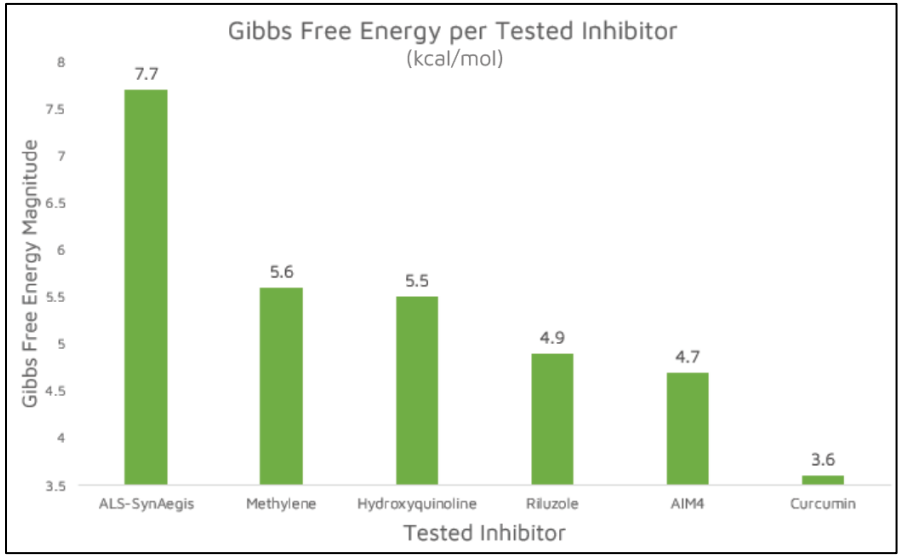
Hydroxyl

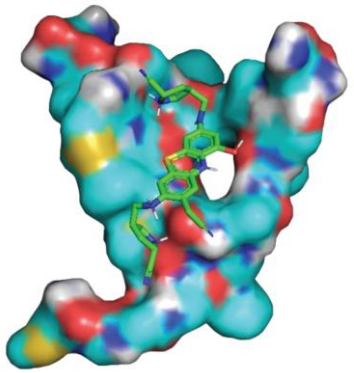


ALS-SynAegis

Results: Affinity Analysis

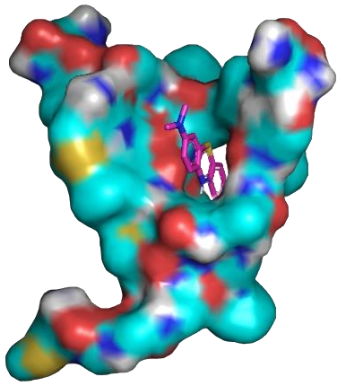
Molecule	Highlighted Trait	ALS-SynAegis Improvement
Methylene Blue	Clinical Trials	36%
Hydroxyquinoline	BBB Permeability	38%
Riluzole	FDA Approved	55%
AIM4	Computational	62%
Curcumin	Natural Inhibitor	111%

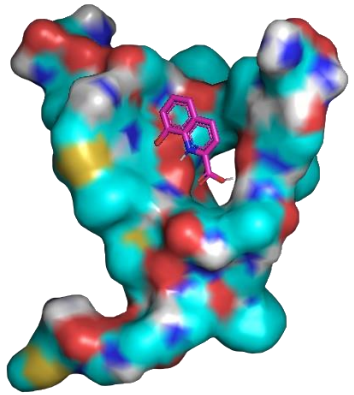




ALS-SynAegis

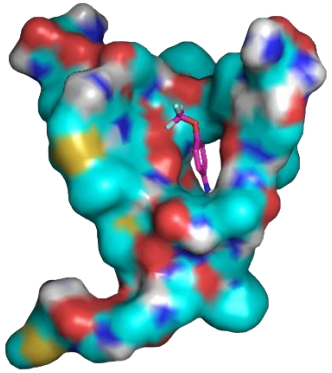
Methylene Blue

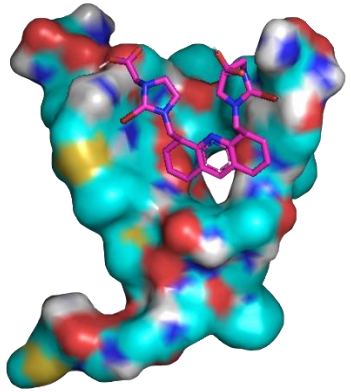




Hydroxyquinoline

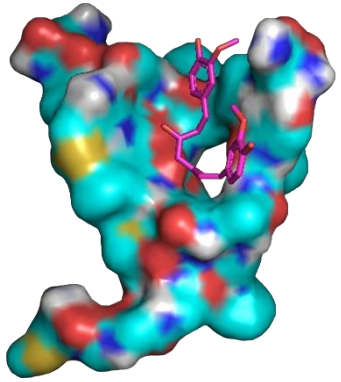
Riluzole





AIM4

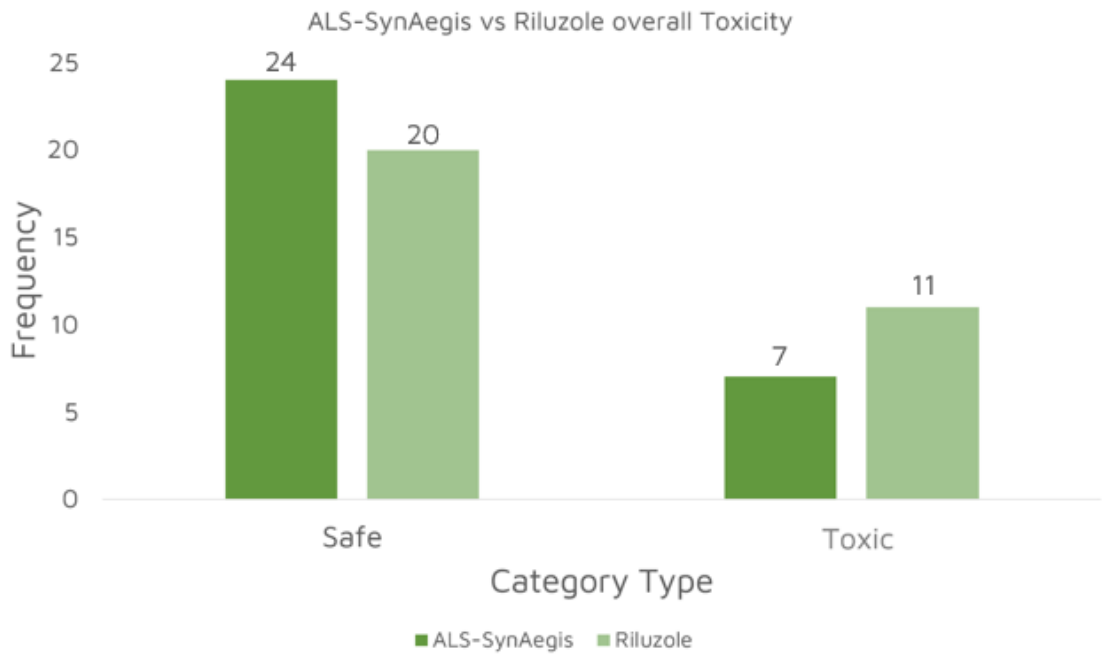
Curcumin



Results: Toxicity Analysis

- Toxicity Results (Compared to Riluzole)
  - 35% reduction in overall toxicity
  - 20% increase in categories showing safety
  - 36% decrease in categories showing toxicity
- Potential Side Affects
  - 19% reduction in Interstitial Lung Disease
  - 42% reduction in Liver Injury

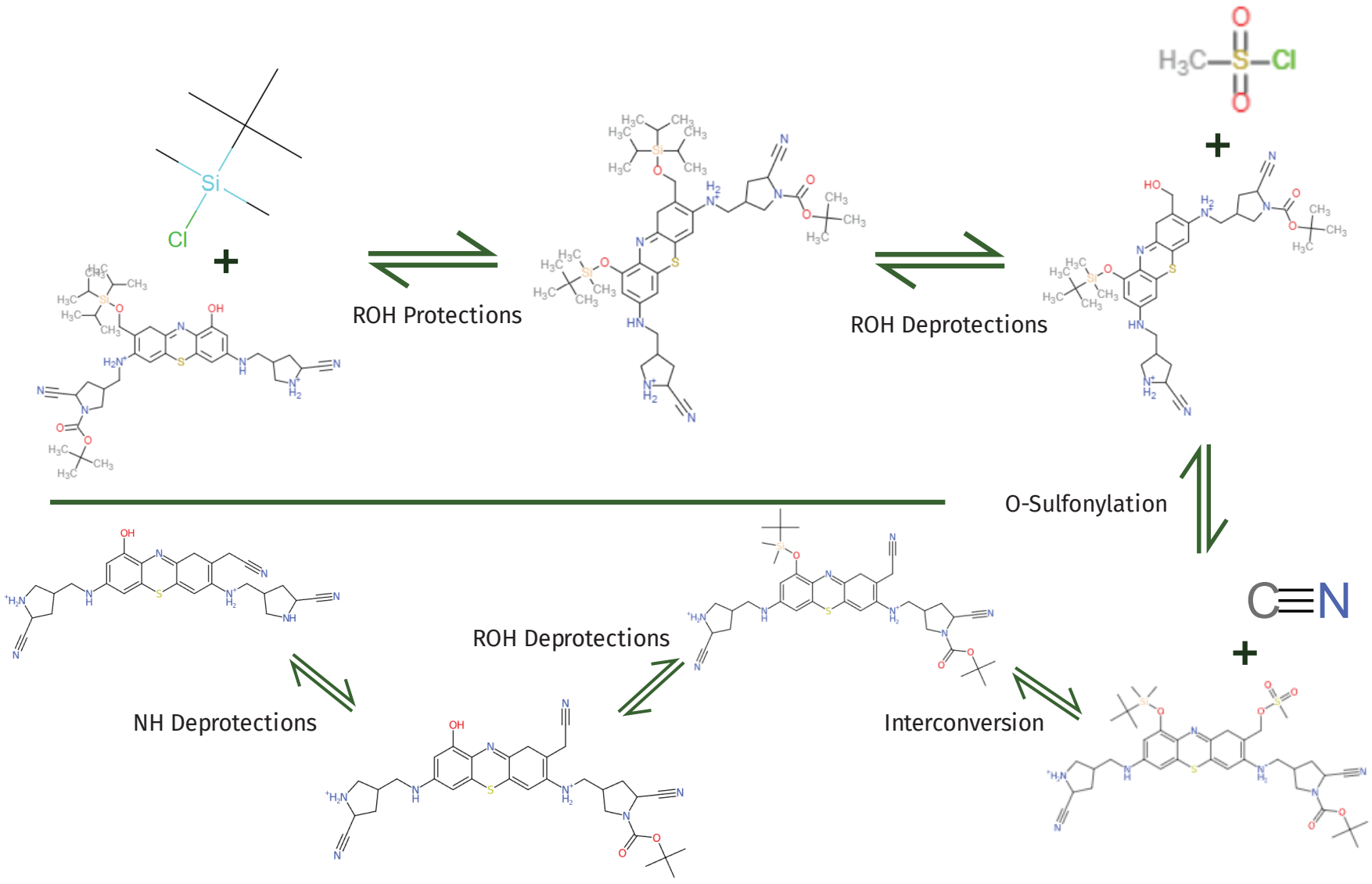
Molecule	Toxicity Score
ALS-SynAegis	0.156
Riluzole	0.239
Methylene Blue	0.348
Hydroxyquinoline	0.259
Curcumin	0.191
AIM4	0.141



Category	ALS-SynAegis	Riluzole
Liver Injury	0.58	1.0
Respiratory Disease	0.81	1.0
T. Pyriformis	0.73	0.99
Fathead Minnow	0.67	0.98
NR-AhR	0.02	0.95
NR-GR	0.34	0.76
NR-TR	0.26	0.68

Retrosynthesis Verification

- 14 possible synthesis pathways discovered
- Average pathway 6-7 steps long
- Average confidence of 72% across all possible pathways
- 34% average yield across all steps, 47% yield on final synthesis step
- Overall classification as high confidence of synthesis occurring
- Only 4 total initial products required for full formation of molecule



Conclusion

- Discovered novel features of TDP-43 aggregates such as:
  - Found most common contacting and hydrogen bonding residues in the aggregate
  - Unveiled that beta sheet direction does not cause a significant affect in aggregate formation
  - Discovered novel target site for inhibitor molecules
- Computationally developed a novel compound that:
  - Inhibits TDP-43 aggregation
  - Better drug-like properties and binding affinities than current inhibitors
  - Predicted to be less cytotoxic than FDA approved drug

Inhibitor Criteria	Passed?	Value
Binding Affinity (kcal/mol) better than Riluzole	✓	-7.7
Between 400-600 Da molecular mass	✓	502.2
< 12 Hydrogen Bond Donors	✓	5
< 10 Hydrogen Bond Acceptors	✓	8
logP < 5	✓	0.98
No Molecular Toxicophores Found	✓	0

Molecular Toxicophores

Show 18 entries Search:

Toxicophore SMARTS	Molecular Toxicophore Verification
O=N(~O)a	Absent
a[NH2]	Absent
a[N;X2]=O	Absent
CO[N;X2]=O	Absent
N[N;X2]=O	Absent
O1[c,C]-[c,C]1	Absent
C1NC1	Absent
N=[N+]=[N-]	Absent
C=[N+]=[N-]	Absent
N=N-N	Absent
c[N;X2]!@;=[N;X2]c	Absent
[OH,NH2][N,O]	Absent
[OH]Na	Absent
[Cl,Br,I]C	Absent
[Cl,Br,I]C=O	Absent
[N,S]!@[C;X4]!@[CH2][Cl,Br,I]	Absent
[cH]1[cH]ccc2c1c3c(cc2)cc[cH][cH]3	Absent
[cH]1cccc2c1[cH][cH]c3c2ccc[cH]3	Absent

Showing 1 to 18 of 36 entries Previous 1 2 Next

Molecular Toxicophores

Show 18 entries Search:

Toxicophore SMARTS	Molecular Toxicophore Verification
a[NH2]	Present
O=N(~O)a	Absent
a[N;X2]=O	Absent
CO[N;X2]=O	Absent
N[N;X2]=O	Absent
O1[c,C]-[c,C]1	Absent
C1NC1	Absent
N=[N+]=[N-]	Absent
C=[N+]=[N-]	Absent
N=N-N	Absent
c[N;X2]!@;=[N;X2]c	Absent
[OH,NH2][N,O]	Absent
[OH]Na	Absent
[Cl,Br,I]C	Absent
[Cl,Br,I]C=O	Absent
[N,S]!@[C;X4]!@[CH2][Cl,Br,I]	Absent
[cH]1[cH]ccc2c1c3c(cc2)cc[cH][cH]3	Absent
[cH]1cccc2c1[cH][cH]c3c2ccc[cH]3	Absent

Showing 1 to 18 of 36 entries Previous 1 2 Next

Key References

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