Structure-Based Drug Design to Discover Potential Hit Molecules for the Trim71 protein to prevent Congenital Hydrocephalus

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ABSTRACT

Congenital Hydrocephalus (CH) is a condition characterized by excessive accumulation of cerebrospinal fluid (CSF) in the brain's ventricles, often linked to mutations in the *Trim71* gene. These mutations frequently result in a gain-of-function in the encoded LIN-41 protein, disrupting stem cell differentiation and neural development. Currently, no specific small-molecule inhibitors exist for TRIM71. This study utilizes structure-based drug design to identify potential hit molecules targeting the NHL domain of the TRIM71 protein. Using the Accelera PlayMolecule platform and molecular docking simulations, we screened libraries for compounds with favorable binding energies and drug-likeness. We identified Compound E as a promising candidate, exhibiting strong binding interactions (IC50 potential) and favorable blood-brain barrier permeability. These findings offer a potential therapeutic pathway for preventing the progression of CH.

INTRODUCTION

Congenital Hydrocephalus (CH) is a condition caused by brain malformations or birth defects leading to excessive accumulation of cerebrospinal fluid (CSF) in the brain's ventricles. CSF is crucial for delivering nutrients to the brain and spinal cord and is typically regulated and absorbed by the body. However, any disruption to the normal flow of CSF leads to it accumulating in the brain causing elevated intracranial pressure within the brain,[1] as in the case of patients with CH. CH affects around 1 in every 1000 infants and significantly hinders brain development due to the pressure exerted on the eyes, skull, and brain.[2]

Trim71 is a gene abundantly expressed in humans and plays a vital role in early embryogenesis and neural differentiation by binding to target mRNAs, triggering translational repression or mRNA degradation.[3] Qiuying Liu et al., and researchers explored CH-associated mutations in mice using crosslinking immunoprecipitation and sequencing (CLIP-seq) techniques. This study is important as the protein exhibits similar responses to humans.[4] The study revealed that

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mutated Trim71 proteins bind to different sets of target mRNAs, indicating a "gain of function." Specifically, R595H-Trim71 in mice binds to mRNA in the *Ctnnb1* gene, which encodes the β-catenin protein, essential for stem cell differentiation.[5] Repressing its translation blocks the production of essential proteins for neural development. Conversely, R783H-Trim71 binds to *Lsd1* mRNA, repressing its translation and causing defects in stem cell differentiation.[5]

In humans, patients with CH have gain-of-function mutations in their *TRIM71* gene, leading to the TRIM71 protein, LIN-41, binding to mRNAs and preventing normal protein production.[6] For instance, the R796H-Trim71 mutant binds to *lsd1* mRNA, repressing its translation, and inhibiting this mutant which alleviates defects in stem cell differentiation.[7]

Trim71 encodes the LIN-41 protein, an RNA-binding protein involved in a regulatory loop with *Let-7* miRNA. LIN-41 binds to *Let-7* miRNA, lowering its levels where there is a high concentration, while *Let-7* miRNA represses the *Trim71* gene when LIN-41 levels are too high.[8] In mutant *Trim71*, *Let-7* is unable to effectively repress the gene, leading to the uninhibited encoding of R783H-Trim71.[9] This combination of *Lsd1* repression and lack of *Let-7* inhibition causes defects in stem cell differentiation and neural lineage commitment, contributing to the development of CH.[10]

Tripartite motif (TRIM) proteins, a large subfamily of RING-type E3 ubiquitin ligases, play significant roles in homeostasis and immune response.[11] TRIM proteins typically have an N-terminal RING finger, a B-box, a coiled-coil domain, and diverse protein interaction modules at the C-termini. All CH-associated mutations in *Trim71* lie within its C-terminal RNA-binding domain (NHL domain).[12]

Molecular interactions between *Let-7* and the TRIM71 protein are part of a tightly regulated feedback loop. *Let-7* binds to the 3’ untranslated region of *TRIM71* mRNA, influencing its processes.[8,13] TRIM71 enhances pre-*Let-7* degradation through interactions with LIN28 and TUT4, inhibiting *Let-7* maturation and stabilizing *Let-7* targets, and represses mature *Let-7* activity through interaction with Ago2, a RISC effector protein.[14]

The whole-exome sequencing on CH patients revealed genetic mutations in over 20% of sporadic CH cases.[5] In Qiuxing Liu et al.'s study, multiple CH-associated mutations were found within the RNA-binding domain of Lin-41, a stem cell-specific RNA-binding protein encoded by the *Trim71* gene. Using CRISPR technology and mouse stem cells, the study explored how *Trim71* mutations (R595H-Trim71 and R783H-Trim71) affected neural differentiation, confirming the importance of *Trim71* in brain development.[4]

Current treatment for CH involves neurological shunting, a procedure where a small plastic tube directs excess CSF to another body part to be reabsorbed. However, this treatment carries risks of infection, hemorrhages, and growth complications, underscoring the need for drug discovery.[15,16] Hence, we think that a small molecule that could bind Trim71 protein and prevent CSF accumulation in the brain could eventually stop the progression of CH.

No inhibitors for the TRIM71 protein are available till date, making it an attractive target for structure-based drug design. The RING domain in TRIM71, common across many E3 ubiquitin ligases, is essential for ubiquitination activity, transferring ubiquitin to substrate proteins. Hence, it is beneficial to study molecules that target various E3 ubiquitin ligases when designing a novel inhibitor for TRIM71. Chaikuad et al.'s study has identified molecules binding to RING domains of E3 ubiquitin ligases, and testing these against TRIM71 is essential.[17]

The molecule by Astex Pharmaceuticals targeting E3 Ubiquitin-protein ligase XIAP, exhibited the lowest IC50 value (IC50 = 0.0170 nM). This molecule interacts with Trp323, Glu314, and His343 residues of the TRIM71 protein. We aim to identify new molecules that could be potential TRIM71 inhibitors, which can bind similarly to the E3 Ubiquitin ligase inhibitors to maintain the interactions.

Our study aims to identify new compounds to bind to TRIM71 with low IC50 values, good blood-brain barrier (BBB) permeability, and compliance with drug-likeness rules.

MATERIALS AND METHODS

Accelera's PlayMolecule platform (version 1.0) was utilized to perform molecular modeling studies.[18] This platform employs advanced machine learning algorithms and provides a range of tools, including ligand preparation, protein preparation, docking, and predictions for viable binding pockets.

The Protein Prepare tool was used to preprocess the target protein before docking by adding missing side chains, assigning correct protonation states to amino acids, and removing any steric clashes if present. The crystal structure of the NHL domain of TRIM71 protein is known and was downloaded from the RCSB Protein Database (PDB ID: 7QRX).[17] The Ace Prep tool was utilized to prepare various molecules as ligands. Ligands were input using SMILES strings or the draw molecule feature, generating numerous conformations. Protonation states were predicted under physiological conditions (pH 7.4), and CHARMM36 force field was used for energy minimization, with all other default settings.

The Deep Site tool predicted potential binding sites on the protein (PDB ID: 7QRX) with a pocket score threshold of 0.8, ensuring only high-confidence sites were considered. This algorithm analyzes the protein's surface topology, physicochemical properties, and spatial arrangement of atoms. Docking simulations were conducted using the Ace Dock tool, targeting the identified binding pockets of TRIM71. The ligand conformations prepared in Ace Prep were screened to identify the best fit, and compared with the binding energy scores for each ligand conformation.

The DockThor program was employed to assess the binding site(s) in the NHL domain of TRIM71.[19] Using advanced algorithms and 3D screening, DockThor simulated multiple poses and interactions to determine optimal ligand binding conformations and affinities. The inhibitor

generated from DockThor was used as a template for Ace Dock docking simulations, enabling the overlay of hundreds of potential ligand conformations to identify the best fits.

Schrödinger's molecular modeling suite, Maestro, was used for RMSD analysis and protein-ligand interaction diagrams. The Ligand Interaction Diagram (LID) tool generated 2D interaction diagrams showing Hydrogen Bonds, Pi-stacking interactions, Hydrophobic contacts, and more.[20]

Finally, the substructure search was conducted using the emolecules database search to identify compounds containing specific partial structures.

RESULTS AND DISCUSSION

Trim71 belongs to the E3 Ubiquitin Ligase family and displays similar binding site residues and interactions with E3 Ubiquitin ligase inhibitors. Trim71's RING domain, common across all E3 ubiquitin ligases, recognizes specific classes of substrates (such as RNA-binding proteins, transcription factors, cell cycle regulators, and signaling pathway components).[21]

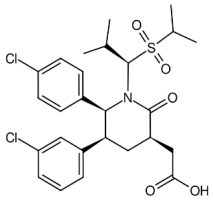
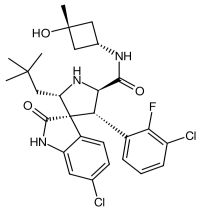
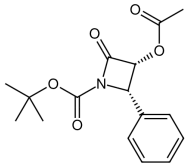
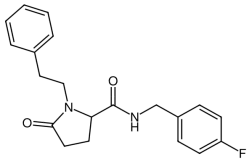
This domain contains conserved cysteine and histidine residues coordinating zinc ions, which maintain the structural integrity of E3 ubiquitin proteins. Key amino acids include Cys449, Cys452, Cys455, Cys458, Cys465, Cys468, and His461. These features facilitate interactions with E2 enzymes, crucial for protein degradation.[22]

In addition to its essential residues, the RING domain has hydrophobic residues that interact with pockets on the surface of E3 enzymes. These interactions often involve leucine, isoleucine, and phenylalanine to stabilize binding.[23] Key residues in the binding pocket of TRIM71 are Arg751, Arg625, and Phe766.

A few known inhibitors of E3 Ubiquitin Ligase were screened against TRIM71 protein (PDB 7QRX) using AceDock. The IC50 values along with their docking scores are provided in Table 1.

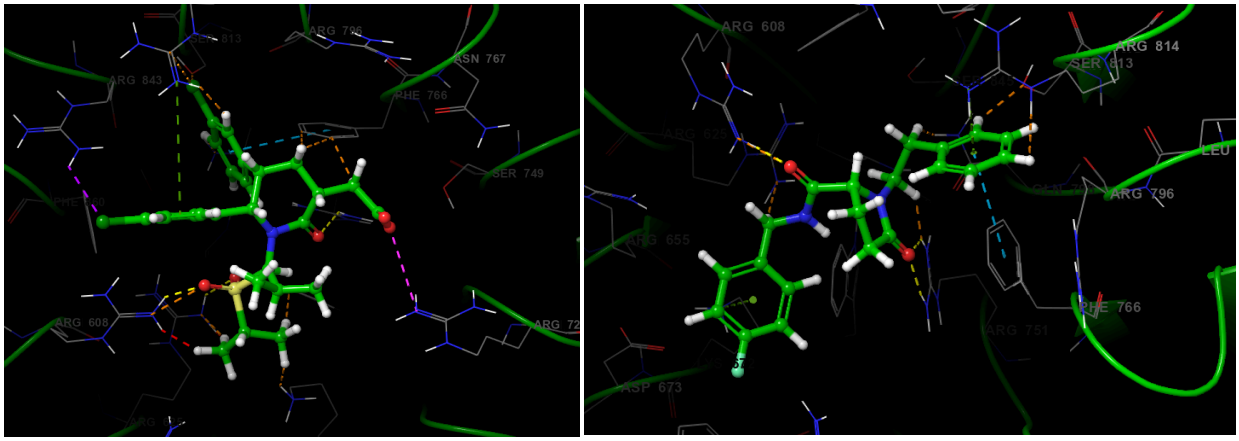
Figure 1. Crystal structure of TRIM71 (PDB: 7QRX)

Table 1. Known inhibitors of E3 Ubiquitin Ligase screened for TRIM71

Structure	IC50	Docking Score (kcal/mol)	Interactions
Compound A (CHEMBL4463050) 	Ki = 0.0450 nM	-18.529	Arg751, Arg625, Arg608, Phe766, Arg814
Compound B (CHEMBL4537250) 	Ki = 0.440 nM	-21.11	Arg751, Arg720
Compound C 	IC50 > 1.00E+4 nM	-17.521	Arg751, Phe860, Lys672
Compound D 	IC50 > 5.00E+4 nM	-23.351	Arg751, Phe766, Arg614

Compound A has interactions with residues Arg751, Arg625, Arg608, Phe766, and Arg814 of TRIM71, compound B has interactions with residues Arg751, Arg720, compound C has interactions with residues Arg751, Phe860, Lys672, and compound 4 has interactions with residues Arg751, Phe766, Arg614. Figure 2 shows the ligand interactions of compound A (left) and compound B within the binding site of TRIM71 protein.

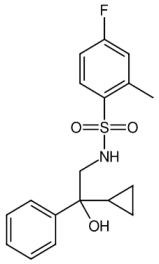
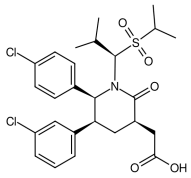
Figure 2. Docked structures of Compound A (left) and Compound B (right) to PDB 7QRX (Binding score: -18.529 and -21.11kcal/mol)



Using inhibitors for one E3 ubiquitin ligase protein as a scaffold can aid in designing drugs for other proteins within the family. Selectivity is also an important factor to consider while designing TRIM71 inhibitors. After analyzing inhibitors with the lowest IC50 values and discerning their binding energy scores to Trim71's binding pocket, a comprehensive substructure search identified additional compounds that could serve as TRIM71-specific inhibitors.

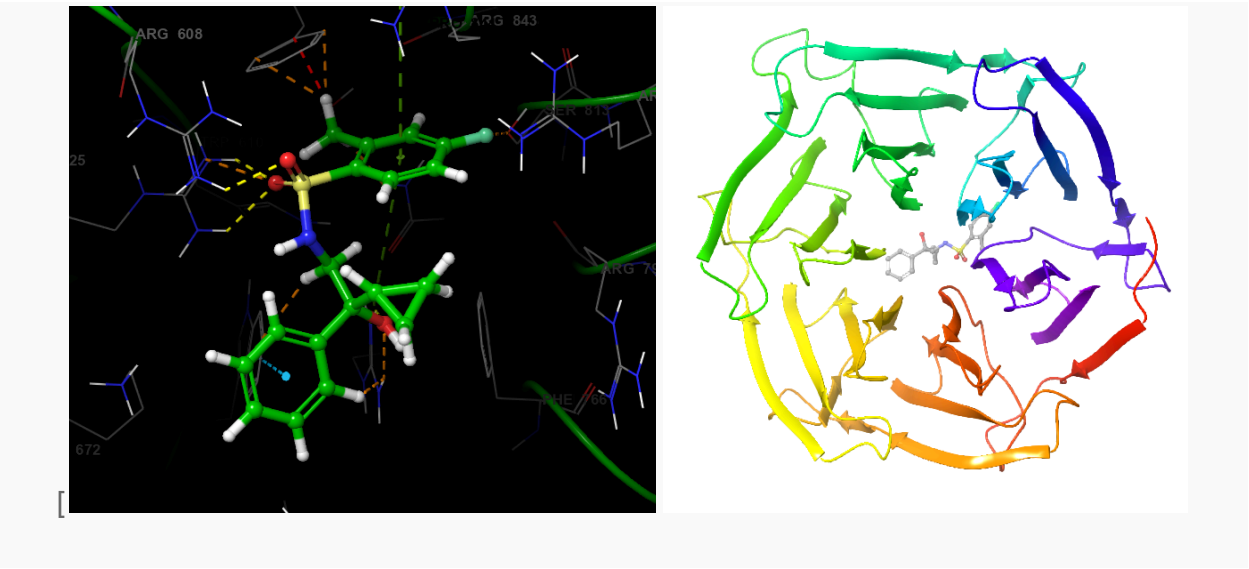
The substructure search retained key pharmacophores on known E3 ubiquitin ligase inhibitors, ensuring that analyzed molecules exhibit essential features and bind exclusively to Trim71. After screening multiple molecules, two promising candidates for TRIM71 inhibition were identified (shown in Table 2). These molecules either had a bio-isostere for the key features in the known E3 Ubiquitin Ligase inhibitors and/or demonstrated better binding capabilities due to complementary groups.

Table 2. Compounds identified for TRIM71 protein via substructure search

Structure	Docking Score (kcal/mol)	Interactions
Compound E 	-30.29 (HIT molecule)	Arg625, Phe766, Trp704, Arg751
Compound F 	-18.295	Arg625, Phe766, Trp704, Arg751

Compound E and Compound F exhibit pi-cation interactions with Arg751 and pi-pi interactions with Phe766, and Trp704. All the key interactions are maintained in compounds E and F suggesting their potential as TRIM71 binders. Virtual screening of these molecules for Trim71 revealed that compound E had the best binding energy score and closest fit.

Figure 3. Docked structure of Compound E to PDB 7QRX (left, binding score -30.29 kcal/mol), ligand interaction diagram (Right)



The compounds were also evaluated for their physiochemical properties to assess their blood-brain barrier penetration and bioavailability. Blood-brain barrier assessment is crucial for TRIM71 inhibitors as the compounds should be able to cross the BBB to reach their target sites in the brain. If the compounds cannot cross BBB, they will prove ineffective as TRIM71 inhibitors as TRIM71 activity will not be influenced by these compounds. The ADME predictor tool, SwissADME, was employed to predict pharmacokinetic data and drug-likeness in all the compounds shown in Tables 1 and 2. Out of the compounds that evolved from substructure search, Compound E, was found to be BBB penetrant apart from satisfying Lipinski's rule of five, signifying its potential as a TRIM71-specific inhibitor (Table 3).

Table 3. Predicted physiochemical properties of compounds using SWISSADME

Molecule	Formula	MW	#Heavy atoms	TPSA	XLOGP3	ESOL Log S	GI absorption	BBB permeant	Pgp substrate	log Kp (cm/s)	Lipinski #violations	Bioavailability Score	PAINS #alerts
Molecule 1	C26H31CI2NO5S	540.5	35	100.13	5.52	-6.39	Low	No	Yes	-5.68	1	0.56	0
Molecule 2	C28H32CI2FN3O3	548.48	37	90.46	4.73	-6.06	High	No	Yes	-6.29	1	0.55	0
Molecule 3	C16H19NO5	305.33	22	72.91	2.15	-2.89	High	Yes	No	-6.64	0	0.55	0
Molecule 4	C20H21FN2O2	340.39	25	49.41	2.72	-3.56	High	Yes	No	-6.45	0	0.55	0
Molecule 5	C18H20FNO3S	349.42	24	74.78	2.76	-3.72	High	Yes	Yes	-6.47	0	0.55	0
Molecule 6	C26H31CI2NO5S	540.5	35	100.13	5.52	-6.39	Low	No	Yes	-5.68	1	0.56	0

This study underscores the significance of the TRIM71 RING domain in facilitating interactions and identifies promising compounds for selective TRIM71 inhibition. Future work may involve further validation of these compounds in experimental settings.

CONCLUSION

TRIM71, an E3 ubiquitin ligase, is a key protein involved in various cellular processes, including regulating microRNA activity and maintaining stem cells. Its role in diseases such as congenital hydrocephalus and certain cancers has made it a subject of interest in biomedical research. Currently, there are no known small-molecule inhibitors specifically targeting TRIM71. Given the absence of direct inhibitors, current research efforts are focused on understanding TRIM71's molecular mechanisms and interactions to identify potential therapeutic strategies. Our in-silico modeling studies have identified promising hit compounds for the NHL domain of TRIM71 protein. In conclusion, our study on the NHL domain of TRIM71 protein has revealed Compound E as a promising candidate. This molecule has shown potential as an inhibitor for TRIM71 in our virtual screening studies including good predicted physiochemical properties and drug likeliness. The virtual hit molecules we propose in this study are readily available from commercial suppliers, facilitating testing using biochemical assays in a future study.

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Notes
The authors declare no competing financial interest.

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ABBREVIATIONS

CH, Congenital Hydrocephalus; CSF, Cerebrospinal Fluid; TRIM, Tripartite Motif; BBB, Blood-Brain Barrier; CLIP-seq, crosslinking immunoprecipitation and sequencing.

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