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SIRT1 Emerges as a Convergent Target of Primidone in Traumatic Brain Injury: A Network Pharmacology and Molecular Docking Study

Sachin Gulia[1], Satyender Kumar[1]*, KM. Minakshi[1], Megha Thakur[1], Amit Lather[2], Tanuj Hooda[3]

[1]School of Pharmacy, Plot No. 32, 34, Knowledge Park III, Greater Noida, Uttar Pradesh 201310, India
[2]Hindu College of Pharmacy, Sonipat-131001, Haryana, India
[3]Department of Pharmaceutical Chemistry, Delhi Pharmaceutical Sciences and Research University, New Delhi, Delhi-110017
Corresponding author: Dr. Satyender Kumar, School of Pharmacy, Plot No. 32, 34, Knowledge Park III, Greater Noida, Uttar Pradesh 201310, India.
Kumar.raisingh@sharda.ac.in

ABSTRACT:

Purpose: Traumatic brain injury (TBI) is a leading cause of death and disability worldwide, and no disease-modifying pharmacological therapy has yet received regulatory approval. Drug repurposing offers a rapid route to bridge this gap. Primidone, a clinically established barbiturate anticonvulsant, remains largely unexplored in TBI despite evidence that it may exert neuroprotective actions beyond seizure suppression. This study used an integrative in silico framework to evaluate primidone's therapeutic relevance in TBI.

Materials and Methods: Primidone-associated targets were identified using SwissTargetPrediction and GeneCards, and TBI-associated genes were retrieved from the Online Mendelian Inheritance in Man (OMIM) database and GeneCards. Intersecting these datasets yielded 138 overlapping targets, which were mapped onto a protein–protein interaction (PPI) network in STRING and analyzed in Cytoscape. Hub genes were prioritized using the CytoHubba maximum clique centrality (MCC) algorithm, and Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses defined the biological processes and pathways engaged by these targets. Molecular docking using AutoDock Vina then evaluated primidone's binding affinity toward the prioritized hub proteins to validate the network-predicted interactions structurally.

Results: Network analysis identified ALB, JUN, PTGS2, CASP3, MMP9, CXCL8, HMOX1, APP, CYP2E1, and SIRT1 as the top ten hub genes, linking primidone to neuroinflammation, oxidative stress, apoptosis, and extracellular matrix remodeling. KEGG enrichment mapped these targets to apoptosis, NF-κB, PI3K-Akt, MAPK, and oxidative stress-response pathways. Molecular docking showed the strongest predicted affinity toward SIRT1 (−9.0 kcal/mol), followed by JUN (−6.8 kcal/mol) and MMP9 (−5.9 kcal/mol), with weaker affinities across the remaining hub proteins (−5.5 to −2.4 kcal/mol; Table 3). This ranking indicates that primidone's predicted interactions

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converge most strongly on SIRT1-mediated oxidative-stress regulation, with secondary, lower-affinity engagement spanning inflammatory, apoptotic, and extracellular-matrix pathways.

Conclusion: This systems-level analysis identifies primidone as a promising repurposing candidate for TBI, converging on a molecular network centered on SIRT1-mediated oxidative-stress regulation, alongside apoptotic and neuroinflammatory signaling. Given its established clinical safety profile, primidone holds translational potential as a repurposed neuroprotective intervention in TBI, warranting further preclinical and clinical evaluation.

INTRODUCTION

Traumatic brain injury (TBI) is widely regarded as a silent epidemic and ranks among the foremost causes of death and long-term disability worldwide.[1] Global Burden of Disease estimates indicate that TBI affects tens of millions of individuals annually, and although age-standardized incidence rates have declined modestly in high-income settings, the absolute number of cases and the associated disability burden continue to rise, with a disproportionate impact in low- and middle-income regions.[1] Despite this scale, no pharmacological agent has yet received regulatory approval for altering the disease course of TBI, and clinical management remains largely supportive.[2]

Beyond the primary mechanical insult, TBI initiates a protracted secondary injury cascade encompassing oxidative stress, neuroinflammation, apoptosis, and extracellular matrix disruption, each of which compounds neuronal loss and functional impairment over the days to weeks following injury.[3,4] Excessive generation of reactive oxygen species destabilizes redox homeostasis and damages macromolecules, thereby amplifying neuroinflammatory signaling,[3] while pro-inflammatory mediators such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α compromise blood–brain barrier integrity and accelerate neurodegenerative processes.[4,5] Because these cascades are mechanistically interconnected rather than sequential, therapeutic agents directed at a single node have thus far failed to translate into meaningful clinical benefit, underscoring the need for interventions capable of engaging multiple convergent pathways simultaneously.

Drug repurposing offers a pragmatic route to such multi-target neuroprotection by exploiting the established pharmacokinetic and safety profiles of existing agents to shorten the path from bench to bedside.[6,7] Primidone (PRM), a barbiturate anticonvulsant used clinically for epilepsy and essential tremor, is one such candidate. Following administration, PRM is metabolized to phenobarbital and phenylethylmalonamide,[8] retaining independent pharmacological activity through direct effects on voltage-gated sodium channels [8] and TRPM3 channels,[9] while its active metabolite phenobarbital contributes GABA-A receptor potentiation.[8] Despite this pharmacological breadth, PRM remains largely unexamined as a candidate for TBI, and its relationship to the molecular network underlying secondary injury has not been systematically characterized.

Network pharmacology provides a framework for addressing this gap by mapping a compound's predicted protein targets onto disease-relevant interactomes, thereby revealing hub proteins and pathways likely to mediate its pharmacological effect.[10] Molecular docking complements this systems-level analysis by testing, at atomic resolution, whether a compound can plausibly engage the specific proteins nominated by network analysis, providing structural support for network-derived hypotheses.[11,12] Combining these two approaches allows the polypharmacology of a repurposing candidate to be prioritized computationally before committing to costly experimental validation.

In this study, we applied this integrative in silico strategy to evaluate the neuroprotective potential of PRM in TBI. Predicted PRM targets, obtained from SwissTargetPrediction and GeneCards, were intersected with TBI-associated genes curated from OMIM and GeneCards to yield 138 shared targets. These were mapped onto a STRING-derived protein–protein interaction network and analyzed in Cytoscape, with hub genes prioritized using the CytoHubba MCC algorithm. Gene Ontology and KEGG enrichment analyses were then performed to define the biological processes and signaling pathways engaged by this target set. Finally, molecular docking with AutoDock Vina was used to evaluate PRM's binding affinity toward the prioritized hub proteins, providing structural validation of the network-predicted interactions. Together, this workflow was designed to determine whether PRM converges on the molecular machinery — centered on SIRT1-mediated oxidative stress regulation,

CASP3-driven apoptosis, PTGS2/CXCL8-linked neuroinflammation, and MMP9-mediated extracellular matrix remodeling — that governs secondary injury in TBI, and to establish a mechanistic rationale for its evaluation as a repurposed neuroprotective agent.

MATERIALS AND METHODS

Pharmacokinetic Properties and Toxicity Prediction of PRM

The chemical structure of PRM (PubChem CID: 4909; https://pubchem.ncbi.nlm.nih.gov/compound/Primidone) was retrieved from the PubChem database [13] and converted into a suitable three-dimensional format for computational analysis.

Drug-likeness, physicochemical properties, and pharmacokinetic behavior — absorption, distribution, metabolism, and excretion (ADME) — were evaluated using SwissADME,[14] a widely used web-based tool for predicting the pharmacokinetic and medicinal chemistry properties of small molecules. Key physicochemical descriptors, including molecular weight, topological polar surface area (TPSA), hydrogen bond donor and acceptor counts, number of rotatable bonds, and lipophilicity (logP), were determined to estimate oral bioavailability, membrane permeability, and the theoretical potential to cross the blood–brain barrier. Lipinski's rule of five was applied to screen for drug-likeness and the likelihood of favorable oral pharmacokinetic behavior in humans.

In silico toxicity prediction was performed using ProTox-II [15] to assess the potential safety profile of PRM. Predicted endpoints included median lethal dose (LD50), hepatotoxicity, nephrotoxicity, cardiotoxicity, neurotoxicity, mutagenicity, and immunotoxicity, providing a comprehensive estimate of organ-specific and systemic toxicological risk. Additional parameters, including bioavailability score, gastrointestinal absorption, P-glycoprotein substrate/inhibitor status, and cytochrome P450 isoform interactions, were also recorded to characterize the compound's suitability for central nervous system (CNS) delivery and its likely route of metabolic clearance.

Screening of PRM Targets

The three-dimensional structure of PRM (PubChem CID: 4909) was converted into canonical SMILES notation for use across in silico target-prediction platforms. SwissTargetPrediction (https://www.swisstargetprediction.ch/) [16] was used to predict candidate protein targets on the basis of two- and three-dimensional chemical similarity to known ligands with experimentally validated targets. Only targets with a predicted probability score greater than 0.1 were retained, to prioritize high-confidence interactions while minimizing spurious associations.

To supplement and cross-validate this target set, the GeneCards database (https://www.genecards.org/) [17] was queried. GeneCards integrates genomic, transcriptomic, proteomic, and clinical data to support comprehensive identification of genes associated with a given compound or disease. Gene symbols were standardized and cross-referenced against the UniProt Knowledgebase (https://www.uniprot.org/) [18] to ensure nomenclature accuracy, restrict the dataset to human proteins, and remove redundant entries. This curation yielded a high-confidence PRM target dataset relevant to CNS function, neurotransmission, and neuroprotection.

Identification of TBI-Associated Genes

Genes associated with TBI were retrieved from GeneCards [17] and the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/) [19] using "TBI" as the search keyword. Retrieved genes were pooled into a single list, and duplicate entries were removed to generate a non-redundant, high-confidence set of TBI-associated genes for downstream network and pathway analysis.

Identification of Intersection Targets

Intersection targets — genes common to both the PRM and TBI datasets — were identified using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).[20] These overlapping genes represent candidate mediators of PRM's potential therapeutic effects in TBI. The intersection set was further annotated using UniProt [18] and GeneCards [17] to characterize gene ontology terms, biological pathways, and molecular functions, enabling identification of targets implicated in neuroprotection, neurotransmitter modulation, anti-inflammatory responses, and oxidative stress regulation.

Additional curation excluded non-human proteins, pseudogenes, and poorly characterized entries, ensuring a high-quality

dataset for downstream network pharmacology analyses. The final intersection gene set was used for protein–protein interaction (PPI) network mapping, molecular docking validation, and pathway enrichment analysis to elucidate the potential mechanisms underlying PRM's therapeutic effects in TBI.

Construction and analysis of the protein–protein interaction network

To explore the molecular interplay underlying PRM's proposed neuroprotective effect in TBI, the shared PRM–TBI targets were mapped onto a protein–protein interaction (PPI) network built in STRING (https://string-db.org/).[21] Analysis was restricted to Homo sapiens, and a minimum interaction score of 0.4 was applied — STRING's medium-confidence setting — to avoid excluding true interactions while filtering out low-confidence noise.

Network topology was visualized and analyzed in Cytoscape (version 3.10.2; https://cytoscape.org/),[22] into which the STRING output was imported directly. Node degree, network density, and clustering coefficient were calculated to characterize the overall architecture of the network.

Hub genes were identified using the CytoHubba plug-in,[23] applying the maximum clique centrality (MCC) algorithm — a method noted for its strong performance in pinpointing biologically important nodes within interaction networks. The 10 genes with the highest MCC scores were selected as candidate hub genes, consistent with a potential regulatory role in PRM-mediated neuroprotection, and were taken forward into functional enrichment analysis.

Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis

To characterize the biological roles of the overlapping targets, Gene Ontology (GO) and KEGG[26] pathway enrichment analyses were performed using the DAVID bioinformatics platform (https://davidbioinformatics.nih.gov/) ,[24] with statistical significance defined as $P < 0.05$ after false discovery rate correction for multiple testing. Enrichment results, restricted to Homo sapiens, were rendered as bubble plots using SRplot (https://www.bioinformatics.com.cn/) .[25] Within the GO framework, enriched terms were grouped into the three standard ontology categories: molecular function (MF), cellular component (CC), and biological process (BP).

KEGG pathway enrichment analysis highlighted signaling pathways relevant to PRM's targets in TBI, spanning oxidative stress response, apoptosis regulation, neuroinflammation, neuronal survival, neurotransmission, neuroprotection, and post-injury functional recovery. Bubble size in the resulting plots indicated gene count per term, while color intensity reflected statistical significance.

Construction of Compound–Target–Pathway (C–T–P) Network

A compound–target–pathway (C–T–P) network was constructed to provide a systems-level view of PRM's potential therapeutic effects in TBI. The network integrated PRM, its predicted high-confidence protein targets, and the top enriched TBI-related pathways identified from KEGG analysis. Visualization and network construction were performed using Cytoscape 3.10.2 (https://cytoscape.org/).[22]

In the network, node types and colors were used to distinguish compounds, protein targets, and signaling pathways. Edges represented interactions or associations between nodes, including PRM–protein interactions and protein–pathway associations. Node size was proportional to degree centrality, highlighting highly connected proteins that may serve as key mediators of PRM's neuroprotective effects.

Topological parameters, including node degree, betweenness centrality, and closeness centrality, were calculated to identify hub targets and key pathways within the network. These hubs likely play pivotal roles in the modulation of oxidative stress, apoptosis, neuroinflammation, and neurotransmission following TBI.

This C–T–P network provides a comprehensive, systems-level framework to visualize PRM's multi-target and multi-pathway therapeutic potential, supporting further functional validation and mechanistic studies. By integrating computational target prediction with pathway enrichment, this approach helps elucidate the complex pharmacological mechanisms of PRM in the context of TBI.

Molecular Docking

Molecular docking was performed to investigate possible interactions between PRM and its ten prioritized TBI-associated

hub-protein targets — SIRT1 (PDB: 4I5I), JUN (PDB: 5T01), MMP9 (PDB: 4H3X), ALB (PDB: 2BXG), PTGS2 (PDB: 5IKR), CYP2E1 (PDB: 3E6I), HMOX1 (PDB: 3HOK), CXCL8 (PDB: 5D14), CASP3 (PDB: 2J30), and APP (PDB: 3KTM) — identified from the protein–protein interaction network analysis described above. The crystal structures of these ten proteins in three dimensions were accessed at the RCSB Protein Data Bank (https://www.rcsb.org/) [27] and thoroughly prepared using AutoDockTools 1.5.7.[12] Protein preparation entailed removal of water molecules, co-crystallized ligands, and heteroatoms, addition of polar hydrogen atoms, assignment of Gasteiger charges, and modeling of missing side chains or residues where required, to preserve structural integrity. Realistic enzymatic conditions were maintained in solution by retaining active site cofactors and metal ions.

The PRM ligand was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/),[13] energy-minimized using the MMFF94 force field,[28] and converted to docking format PDBQT. States of protonation and tautomers were also confirmed under physiological conditions (pH 7.4).

Docking simulation of each protein was performed with AutoDock Vina,[11] and a grid box was centered on the active site. The size of the grid was determined to completely cover the binding pocket and catalytic residues, but provide adequate room to allow ligand flexibility. Several docking poses of each ligand–protein complex were computed, and conformations ranked by predicted binding affinity. To determine whether docking was accurate and reproducible, the root-mean-square deviation (RMSD) was noted.

Interactions between proteins and their ligand, with explicit measurements of hydrogen bonds, hydrophobic contacts, π-π contacts, and electrostatic interactions after docking, were analyzed in PyMOL (Schrödinger, LLC) [29] and Discovery Studio Visualizer.[30] These analyses made it possible to identify binding residues of importance and to give structural clues as to the possible mechanism.

To ensure reproducibility, docking procedures were performed in triplicate for each target protein, and the most consistent binding poses were retained for downstream computational analyses, such as network pharmacology integration and pathway interpretation.

RESULTS

Pharmacokinetic Properties and Toxicity Prediction of PRM

PubChem [13] was used to find the structural data of PRM (CID 4909), and predicted pharmacokinetics of this drug was calculated by SwissADME.[14] PRM exhibited good drug-likeness, oral bioavailability together with anticipated blood-brain barrier permeability, which conforms in every way to Lipinski's rule of five. These characteristics indicate effective oral delivery and access to central nervous system (CNS) locales that are vital to its therapeutic application in TBI and seizure control (Table 1).

In silico analysis of absorption, distribution, metabolism, and excretion (ADME) parameters indicated high gastrointestinal absorption, moderate lipophilicity (XLogP3-AA 1.8), and a low number of rotatable bonds (2), which collectively support good oral bioavailability and metabolic stability. The topological polar surface area (TPSA) of 61.1 Å² further corroborates PRM's potential to permeate cellular membranes and cross the blood-brain barrier, a critical requirement for CNS-acting drugs.

In silico toxicological predictions using ProTox-II [15] revealed low predicted toxicity in major organs, including no hepatotoxicity, nephrotoxicity, cardiotoxicity, neurotoxicity, or mutagenicity. The predicted LD50 (~500 mg/kg) suggests a moderate acute toxicity level, indicating a reasonable therapeutic safety margin for preclinical and clinical studies. Interestingly, a predicted risk of respiratory toxicity was observed, which may relate to central depressant effects reported in clinical use, underscoring the importance of monitoring respiratory parameters during administration (Table 2).

Additional toxicity endpoints, including carcinogenicity, immunotoxicity, and cytotoxicity, were predicted to be inactive, supporting the safety profile of PRM for long-term therapeutic use. The combination of favorable pharmacokinetic parameters and a relatively low toxicity risk suggests PRM is a suitable candidate for CNS-targeted interventions, particularly in neuroprotective contexts such as TBI. These computational findings provide a rationale for further in vitro and in vivo validation studies, which could include pharmacodynamic evaluation, metabolic stability assays, and CNS penetration studies in animal models.

Natural Resources for Human Health
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Table 1. Pharmacological and Molecular Properties of PRM

Property	Value
Molecular weight	218.25 g/mol
Chemical formula	C12H14N2O2
XLogP3-AA	1.8
Rotatable bonds	2
Hydrogen bond acceptors	3
Hydrogen bond donors	1
Topological polar surface area	61.1 Å²
Complies with Lipinski rule	Yes
Gastrointestinal absorption	High
Blood-brain barrier permeability	Yes

Table 2. Toxicity Profile of PRM (In Silico)

Parameter	Prediction	Interpretation
LD50 (mg/kg)	~500	Moderate acute toxicity
Hepatotoxicity	Inactive	Low risk of liver damage
Nephrotoxicity	Inactive	Low risk of kidney damage
Cardiotoxicity	Inactive	Low risk of heart-related issues
Neurotoxicity	Inactive	Low risk of neurological effects
Respiratory Toxicity	Active	Potential risk to respiratory system
Mutagenicity	Inactive	Low risk of genetic mutations
Carcinogenicity	Inactive	Low risk of cancer
Immunotoxicity	Inactive	Low risk of immune system effects
Cytotoxicity	Inactive	Low risk of cell toxicity

Screening targets of PRM against TBI

To identify the potential therapeutic targets of PRM, predicted protein targets were retrieved using SwissTargetPrediction (http://www.swisstargetprediction.ch/)[16] and GeneCards (https://www.genecards.org/).[17] These targets represent proteins that PRM is likely to interact with, particularly in the central nervous system, reflecting its known pharmacological activities such as modulation of voltage-gated sodium channels, TRPM3 channel inhibition, and — via its active metabolite phenobarbital — GABAergic signaling.

Concurrently, 6,078 TBI-associated genes were compiled from the OMIM [19] and GeneCards [17] databases, representing proteins involved in TBI pathophysiology, including neuroinflammation, oxidative stress, excitotoxicity, and apoptotic pathways.

By intersecting the PRM targets with TBI-related genes, a total of 138 overlapping targets were identified (Figure 1). These

shared targets represent the potential molecular mediators through which PRM may exert neuroprotective effects in TBI. The overlapping targets were visualized using a Venn diagram, providing a focused subset for downstream analyses, including protein–protein interaction (PPI) network construction, gene ontology (GO) and KEGG pathway enrichment, and molecular docking studies.

This integrative approach highlights the multi-target therapeutic potential of PRM, emphasizing its relevance for CNS-targeted interventions in TBI and supporting further experimental validation.

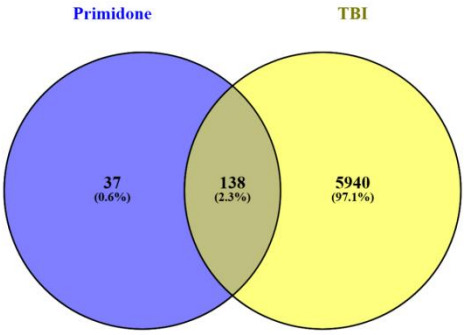


Figure 1. Venn diagram showing intersection of PRM and TBI targets

Construction, Analysis, and Pathway Enrichment of the PPI Network of PRM

To elucidate the therapeutic potential of PRM in TBI, protein–protein interaction (PPI) analysis was performed using the STRING database.[21] A total of 138 common target genes were identified by intersecting PRM-predicted targets with TBI-related genes. The PPI network was constructed with a medium confidence score of 0.400, integrating both experimental and curated interaction sources. The resulting network (Figure 2) consisted of 137 nodes (genes) and 1,095 edges (interactions), with an average node degree of 16.0, reflecting a high level of interconnectivity and functional cross-talk among targets.

The network was subsequently imported into Cytoscape[22] for advanced visualization and topological analysis (Figure 4), where nodes represented protein targets and edges denoted their interactions. Using CytoHubba,[23] the Maximum Clique Centrality (MCC) algorithm was applied to identify the most influential nodes. This analysis revealed the top 10 hub genes: ALB, JUN, PTGS2, CASP3, MMP9, CXCL8, HMOX1, APP, CYP2E1, and SIRT1 (Figure 3). Notably, these hub genes are implicated in critical processes such as neuroinflammation (CXCL8, PTGS2, JUN), apoptosis (CASP3, APP), oxidative stress response (HMOX1, SIRT1, CYP2E1), and extracellular matrix remodeling (MMP9), all of which are central to secondary injury cascades in TBI.

To further explore the functional relevance of these targets, Gene Ontology (GO) and KEGG pathway enrichment analyses were performed (Figure 5). The enriched GO biological processes included response to oxidative stress, regulation of apoptotic signaling pathway, inflammatory response, neurogenesis, and synaptic plasticity. In terms of molecular function, enriched categories included cytokine activity, MAP kinase activity, and transcription factor binding.

KEGG pathway analysis highlighted several key TBI-associated signaling cascades:

- NF-κB signaling pathway (involving JUN, CXCL8, PTGS2) – regulating neuroinflammation and cytokine release.
- Apoptosis pathway (CASP3, APP, MMP9) – mediating neuronal death and axonal injury.
- Oxidative stress-related pathways (HMOX1, CYP2E1, SIRT1) – linked to mitochondrial dysfunction and ROS detoxification.
- MAPK signaling (JUN, PTGS2, CXCL8) – controlling stress responses and neuronal survival.
- Neurodegeneration-associated pathways (APP, SIRT1) – highlighting shared mechanisms between TBI and chronic neurodegenerative diseases.

Collectively, these enrichment results indicate that PRM may exert neuroprotective effects through multi-target modulation of apoptosis, neuroinflammation, and oxidative stress, thereby potentially attenuating the secondary injury cascade in TBI. The integration of PPI topology with GO/KEGG enrichment strengthens the systems-level evidence for PRM's therapeutic role as a repurposed neuroprotective agent in TBI.

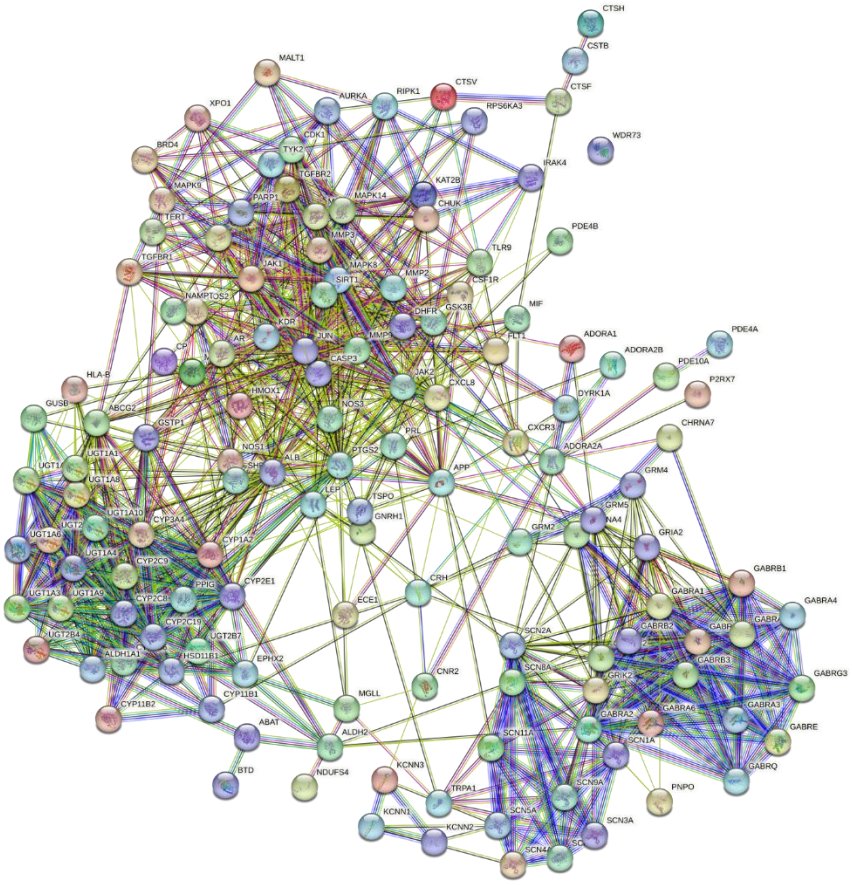


Figure 2. The PPI Network illustrates protein interactions derived from STRING: Nodes symbolize proteins with edges depict the interactions between them.

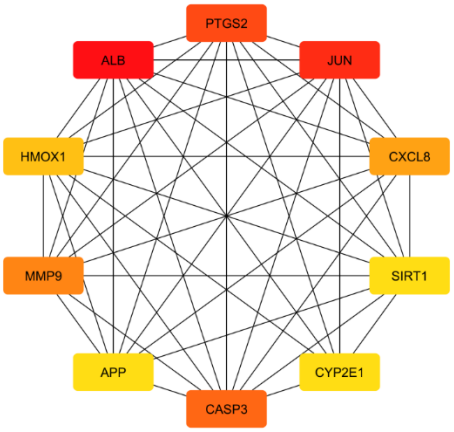


Figure 3. The core PPI network, created using the MCC method from CytoHubba: Nodes representing the top 10 targets based on the shortest path, with edges indicating their interactions

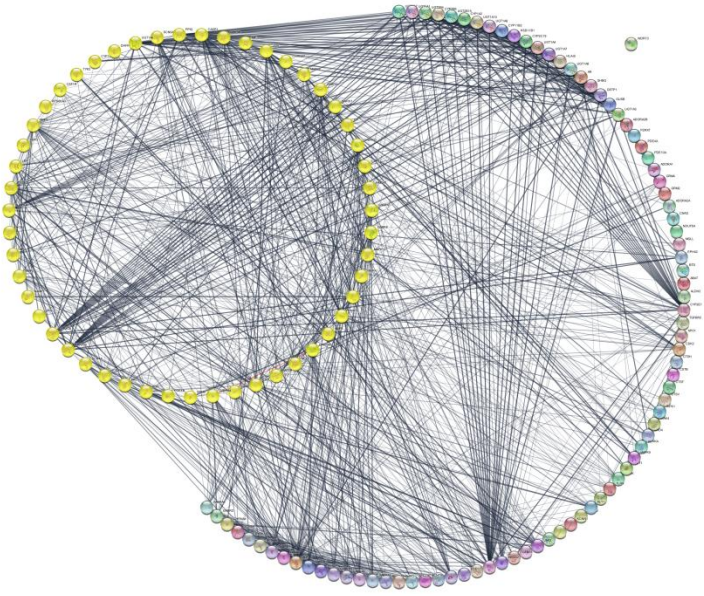


Figure 4. The PPI Network generated using Cytoscape software: Nodes symbolize proteins with edges depict the interactions between them.

Pathway Enrichment Analysis of PRM Targets

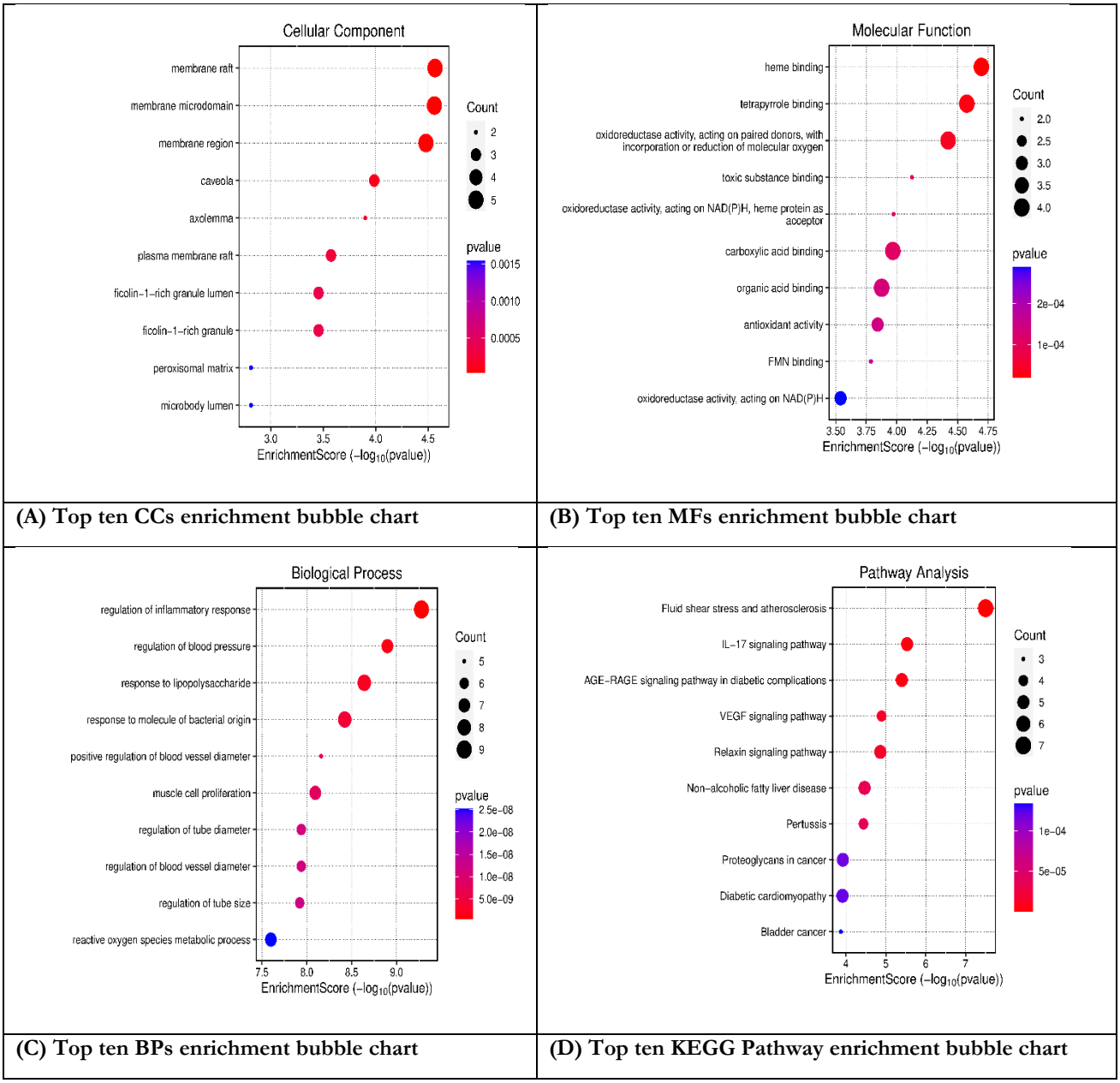
To gain deeper insights into the biological significance of the identified PRM–TBI targets, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) [26] enrichment analyses were performed. A total of 138 common genes were subjected to enrichment analysis, and the results revealed significant involvement in key biological processes, cellular components, and molecular functions relevant to neuroprotection and TBI pathology.

GO enrichment analysis highlighted several enriched terms across three major domains. For cellular components (CCs), the targets were primarily localized to the postsynaptic density, synaptic membrane, mitochondrial outer membrane, and cytosolic signaling complexes, indicating their role in synaptic transmission and intracellular stress regulation (Figure 5A). In terms of molecular functions (MFs), enriched terms included enzyme binding, cytokine receptor activity, oxidoreductase activity, and transcription factor binding, reflecting the involvement of PRM in modulating oxidative stress, inflammatory signaling, and apoptotic cascades (Figure 5B). For biological processes (BPs), the most significant enrichments included regulation of apoptosis, reactive oxygen species (ROS) metabolic process, inflammatory response, regulation of NF-κB signaling, and neuronal death (Figure 5C).

KEGG pathway enrichment analysis identified 187 pathways, with the top ten ($p < 0.05$) being: apoptosis, NF-κB signaling pathway, PI3K-Akt signaling, MAPK signaling, TNF signaling, oxidative stress response, Alzheimer's disease, Parkinson's disease, calcium signaling pathway, and neuroactive ligand-receptor interaction (Figure 5D). Notably, several hub genes such as CASP3, MMP9, CXCL8, PTGS2, and HMOX1 were central to apoptotic and inflammatory cascades, while APP, JUN, and SIRT1 were linked to neurodegenerative disease pathways and synaptic plasticity.

Collectively, these results suggest that PRM exerts neuroprotective potential in TBI by modulating multiple interconnected biological processes, particularly through regulation of oxidative stress, neuroinflammation, apoptotic pathways, and synaptic signaling networks. Such findings reinforce the hypothesis that PRM operates as a multi-target agent, influencing diverse but converging mechanisms that underlie TBI pathology.

Natural Resources for Human Health
eISSN: 2583-1194



(A) Top ten CCs enrichment bubble chart

(B) Top ten MFs enrichment bubble chart

(C) Top ten BPs enrichment bubble chart

(D) Top ten KEGG Pathway enrichment bubble chart

Figure 5. (A) Dot-plot diagram for CCs enrichment (The pathway shorted by Fold enrichment, dots show the number of genes counts x-axis shows -logP and colour show the pvalue);(B) Dot-plot diagram for MFs enrichment (The pathway shorted by Fold enrichment, dots show the number of gene count x-axis shows -logP and colour show the pvalue);(C) Dot-plot diagram for BPs enrichment (The pathway shorted by Fold enrichment, dots show the number gene count, the x-axis shows -logP and colour show the pvalue);(D) Dot plot diagram for KEGG Pathway enrichment analysis (The pathway shorted by Fold enrichment, dots show the number of gene count, the x-axis shows –logP and colour show the p-value).

Construction of the Compound–Target–Pathway (C–T–P) Network

To visualize the multi-target, multi-pathway architecture underlying PRM's predicted neuroprotective activity in TBI, a C–T–P network was constructed in Cytoscape 3.10.2, [22] integrating PRM (compound node), its ten prioritized hub targets (JUN, MMP9, CASP3, PTGS2, APP, ALB, CXCL8, SIRT1, HMOX1, CYP2E1), and sixteen KEGG-enriched pathways associated with TBI pathophysiology (Figure 6). The resulting network comprised 27 nodes, with PRM directly connected to all ten hub proteins, confirming its predicted engagement of the complete hub-gene set identified by the PPI analysis. Each target, in turn, was linked to a distinct subset of downstream signaling or disease pathways, giving the network a modular, multi-cluster

architecture.

JUN, CASP3, MMP9, and APP formed a densely interconnected cluster linked to MAPK signaling, TNF signaling, IL-17 signaling, apoptosis, and neurodegeneration-associated pathways shared across multiple diseases, consistent with their shared involvement in neuroinflammatory and neurodegenerative cascades. PTGS2 bridged this cluster to Toll-like receptor and NF-κB signaling, while CXCL8 formed a second, chemokine-centered node connected to NF-κB signaling, chemokine signaling, and cytokine–cytokine receptor interaction, reflecting its role in leukocyte recruitment and inflammatory amplification following injury. A third, metabolically oriented cluster comprising SIRT1, HMOX1, and CYP2E1 mapped to FoxO signaling, ferroptosis, HIF-1 signaling, arachidonic acid metabolism, and cytochrome P450-mediated xenobiotic metabolism, pathways central to redox homeostasis and iron-dependent cell death regulation (Figure 6). ALB connected exclusively to thyroid hormone synthesis, consistent with its established function as a plasma carrier protein rather than a direct signaling mediator.

This clustering indicates that PRM's predicted network effects converge on three broadly distinct but interconnected mechanistic axes — neuroinflammatory/apoptotic signaling (JUN–CASP3–MMP9–APP–PTGS2), chemokine-mediated inflammation (CXCL8), and oxidative/redox metabolism (SIRT1–HMOX1–CYP2E1) — supporting a multi-target rather than single-pathway mode of action in TBI.

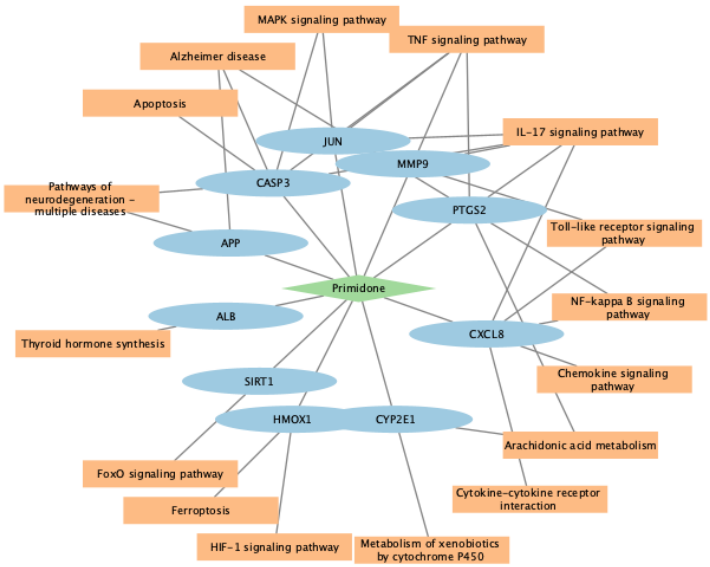


Figure 6. Compound–target–pathway (C–T–P) network of PRM integrating its ten prioritized hub-gene targets and sixteen KEGG-enriched pathways associated with TBI. Node size is proportional to degree centrality; PRM is directly connected to all ten hub proteins.

Molecular Docking Analysis of PRM with Core TBI Targets

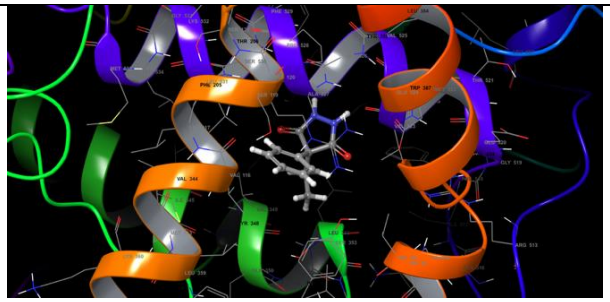
To provide structural support for the network-pharmacology findings, PRM was docked against ten prioritized TBI-associated hub proteins: SIRT1, JUN, MMP9, ALB, PTGS2, CYP2E1, HMOX1, CXCL8, CASP3, and APP (Figure 7). Docking was performed using AutoDock Vina,[11] with predicted binding affinities expressed in kcal/mol; more negative values indicate more favorable predicted interactions within the applied scoring function.

SIRT1 showed the most favorable predicted interaction (−9.004 kcal/mol; PDB: 4I5I), followed by JUN (−6.756 kcal/mol; PDB: 5T01) and MMP9 (−5.910 kcal/mol; PDB: 4H3X). SIRT1 is a stress-responsive deacetylase implicated in oxidative-stress regulation and neuronal survival, whereas JUN/c-Jun participates in AP-1-mediated cellular stress and inflammatory responses. Notably, PDB 5T01 represents the c-Jun DNA-binding domain rather than full-length JUN; therefore, the predicted interaction should be interpreted as a structural hypothesis for the crystallized domain. MMP9 is associated with extracellular-matrix remodeling, neuroinflammation, and blood–brain barrier disruption, making its predicted interaction relevant to secondary TBI processes.

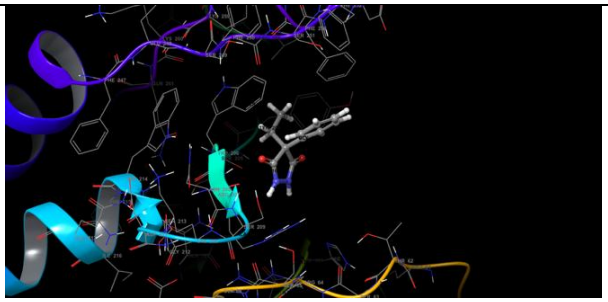
Intermediate docking scores were observed for ALB (−5.511 kcal/mol; PDB: 2BXG), PTGS2 (−5.456 kcal/mol; PDB: 5IKR), CYP2E1 (−5.262 kcal/mol; PDB: 3E6I), and HMOX1 (−4.525 kcal/mol; PDB: 3HOK). Among these, PTGS2, CYP2E1, and HMOX1 are particularly relevant to inflammatory and oxidative-stress responses. ALB, as a circulating transport protein, may primarily reflect protein-binding potential rather than a direct neuroprotective mechanism.

CXCL8 (−4.228 kcal/mol; PDB: 5D14), CASP3 (−3.974 kcal/mol; PDB: 2J30), and APP (−2.427 kcal/mol; PDB: 3KTM) showed comparatively less favorable predicted interactions. CXCL8 is involved in inflammatory chemokine signaling, CASP3 in apoptotic execution, and APP in neuronal and neurodegenerative processes. Thus, these interactions provide additional computational evidence of potential target engagement within the broader TBI-associated network, although their lower docking scores indicate weaker predicted interactions relative to the highest-ranked targets under the present protocol.

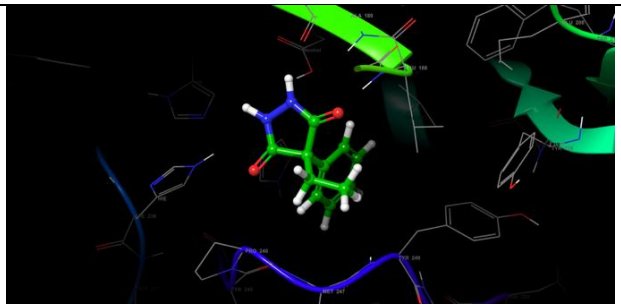
Overall, the docking profile demonstrated target-selective variation, with SIRT1 emerging as the highest-ranked computational interaction, followed by JUN and MMP9. Integration with the network-pharmacology results suggests that PRM may potentially interact with multiple molecular nodes involved in oxidative stress, inflammation, apoptosis, and extracellular-matrix remodeling. These findings are hypothesis-generating and do not establish direct binding, target modulation, or therapeutic efficacy; experimental validation is required to determine the biological significance of the predicted interactions.



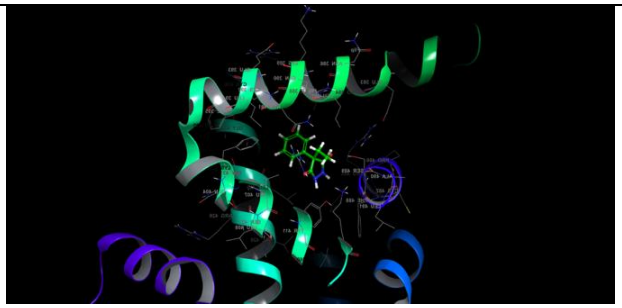
(A) PTGS2 / COX-2



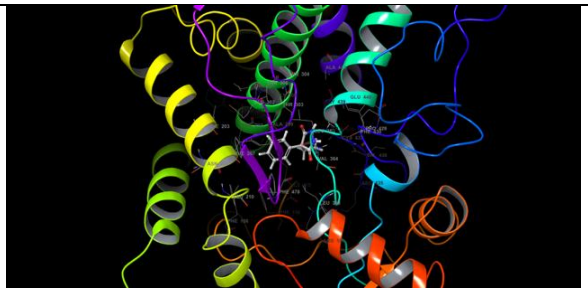
(B) CASP3 / Caspase-3



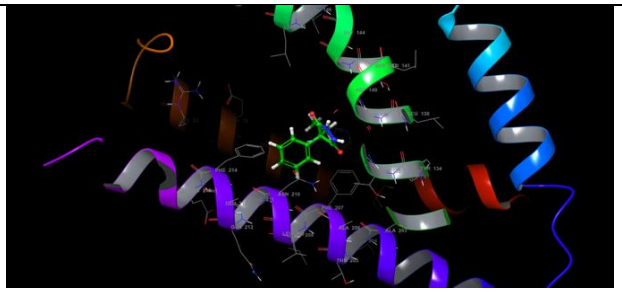
(C) MMP9 / MMP-9



(D) SIRT1



(E) CYP2E1



(F) HMOX1 / HO-1

Natural Resources for Human Health
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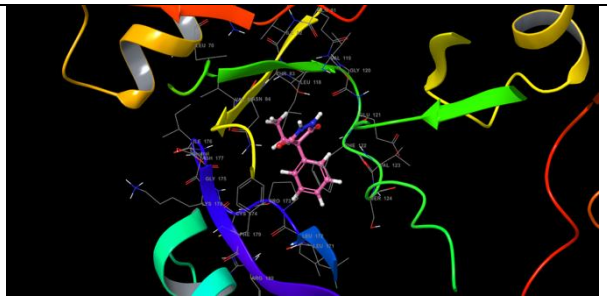
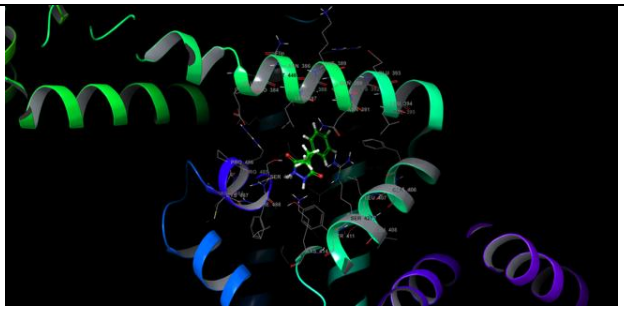
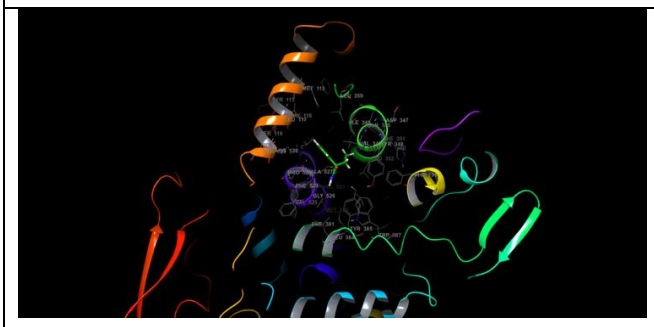


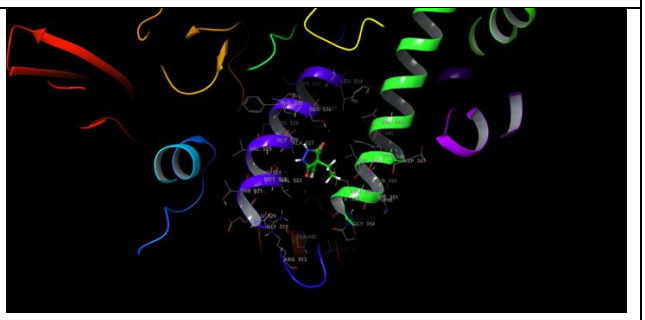
(G) APP



(H) ALB / HSA



(I) JUN



(J) CXCL8

Figure 7. Docking configurations of PRM with the core TBI target proteins: (A) PTGS2 / COX-2, (B) CASP3 / Caspase-3, (C) MMP9 / MMP-9, (D) SIRT1, (E) CYP2E1, (F) HMOX1 / HO-1, (G) APP, (H) ALB / HAS, (I) JUN, (J) CXCL8.

Table 3. Molecular docking of PRM with TBI-associated target proteins

Molecule	Target / Gene	PDB ID	Binding Affinity (kcal/mol)
PRM	Sirtuin-1 (SIRT1)	4I5I	-9.004
	c-Jun (JUN)	5T01	-6.756
	Matrix Metalloproteinase-9 (MMP9)	4H3X	-5.910
	Human Serum Albumin (ALB/HSA)	2BXG	-5.511
	Prostaglandin-Endoperoxide Synthase 2 (PTGS2/COX-2)	5IKR	-5.456
	Cytochrome P450 2E1 (CYP2E1)	3E6I	-5.262
	Heme Oxygenase-1 (HMOX1/HO-1)	3HOK	-4.525
	CXCL8 / Interleukin-8 (CXCL8/IL-8)	5D14	-4.228
	Caspase-3 (CASP3)	2J30	-3.974
	Amyloid Precursor Protein (APP)	3KTM	-2.427

DISCUSSION

TBI is defined less by the initial mechanical insult than by the protracted secondary injury cascade it triggers — a self-amplifying interplay of excitotoxicity, oxidative stress, neuroinflammation, and neurotransmitter dysregulation that unfolds over hours to weeks and determines much of the eventual functional outcome.[3,2] Antiepileptic drugs remain the mainstay of pharmacological management in this setting, but they were developed to raise seizure threshold, not to interrupt secondary

injury, and single-target agents have consistently underperformed against a pathology this mechanistically diffuse. Multi-target drug repurposing offers a more tractable route to this problem: rather than designing a new molecule to hit several nodes at once, it asks whether an existing, clinically characterized drug already does so.[10]

The present docking results support this rationale for PRM. Across the ten PPI-derived hub proteins, SIRT1 returned the most favorable predicted affinity (−9.004 kcal/mol; Table 3), with JUN and MMP9 following as the next-most-favorable interactions. This ordering is biologically coherent. SIRT1 is an NAD^+-dependent deacetylase that governs mitochondrial biogenesis and redox homeostasis via targets such as PGC-1α, and in a rodent lateral fluid-percussion TBI model, pharmacological inhibition of SIRT1 significantly exacerbated post-traumatic mitochondrial damage, apoptotic markers, and p38 MAPK activation, while SIRT1 expression itself was neuroprotective against secondary injury.[31] A recent review specifically nominates sirtuins, and SIRT1 in particular, as an underexploited therapeutic target class for TBI.[32] The present docking result is broadly consistent with this body of evidence, though it does not itself establish that PRM engages SIRT1 in a cell. JUN/c-Jun, the second-ranked hit, is the central component of the stress-activated AP-1 transcription factor complex that couples JNK signaling to neuronal apoptosis; MMP9, third-ranked, is a matrix metalloproteinase implicated in blood–brain barrier breakdown during the acute post-injury window. The remaining seven targets returned progressively weaker affinities, with PTGS2, CYP2E1, and HMOX1 — three proteins central to oxidative and inflammatory signaling — clustering in an intermediate range, and CXCL8, CASP3, and APP at the lower end of the panel (Table 3).

One caveat merits explicit statement. Vina's scoring function was developed to rank many candidate ligands against a single binding pocket; its comparability across ten structurally unrelated pockets of differing size and chemistry is limited, and a substantial affinity gap between SIRT1 and, say, APP may partly reflect pocket geometry rather than a true biological preference. This does not invalidate the ranking as a prioritization tool, but the SIRT1 result should be read as the strongest candidate for follow-up rather than as quantitatively superior evidence of engagement.

These structural observations converge with the network-level enrichment findings. GO annotation of the 138 shared PRM–TBI targets localized enrichment to the mitochondrial outer membrane, synaptic membrane, and postsynaptic density (cellular component), and to oxidoreductase activity, cytokine receptor activity, and transcription factor binding (molecular function) — a combination that points toward redox regulation and inflammatory signaling at the synapse and mitochondrion rather than toward classical neurotransmitter-receptor pharmacology. KEGG enrichment reinforced this picture: apoptosis, NF-κB signaling, PI3K-Akt signaling, MAPK signaling, TNF signaling, and oxidative stress response pathways dominated the top-ranked terms, alongside Alzheimer's disease and Parkinson's disease pathways that share substantial molecular overlap with TBI-induced neurodegeneration, consistent with SIRT1's established role at the intersection of these two disease processes.[32] Taken together, the enrichment profile and the docking hierarchy tell the same story from two independent computational angles: PRM is predicted to act primarily through oxidative-stress and apoptotic-signaling nodes rather than through direct ion-channel or neurotransmitter-receptor engagement — a mechanistically distinct axis from PRM's established anticonvulsant pharmacology.[8,9]

The repurposing case for PRM is strengthened by its age rather than weakened by it: as a barbiturate anticonvulsant in continuous clinical use since the 1950s, its pharmacokinetics, standard dosing ranges, and adverse-event profile are already characterized in humans, removing much of the Phase I burden facing a de novo candidate.[6,7] This mirrors related repurposing efforts by our group in TBI, including ranolazine acting via ESR1/NMDA-linked mechanisms[33] and oleanolic acid attenuating secondary injury cascades,[34] both validated through combined in silico and in vivo approaches. The SwissADME-predicted blood–brain barrier permeability reported here (Table 1) is consistent with PRM's established CNS activity as an antiepileptic and antitremor agent. One practical nuance specific to the TBI setting is worth noting: the blood–brain barrier itself is transiently more permeable in the acute post-injury window as a direct consequence of the injury cascade this study models, which affects both the achievable CNS concentration of a repurposed candidate and the optimal timing of its administration — a variable that standard steady-state pharmacokinetic parameters, derived from non-injured populations, do not directly capture and that would need to be addressed in an injury-specific pharmacokinetic study.

Several limitations temper these conclusions. First, every stage of this workflow is predictive rather than confirmatory: target prediction, disease-gene association, and docking are all in silico estimates, each carrying its own false-positive and false-negative risk, and none of the reported interactions has been demonstrated biochemically or in a cell. Second, as noted above, cross-target comparison of Vina scores is only weakly calibrated; the ranking is best treated as a prioritization heuristic rather

than a quantitative measure of relative affinity. Third, several docking targets represent isolated domains or specific crystallized conformations rather than full-length, physiologically dynamic proteins — most notably JUN, where the crystal structure captures only the DNA-binding domain — and docking used rigid or semi-flexible receptor structures that cannot capture the conformational plasticity or induced-fit binding a real protein–ligand interaction may involve. Fourth, the disease-gene dataset depends on the specific search parameters and database versions used at the time of retrieval; different search criteria would yield a different starting gene set and, potentially, a different hub-gene ranking. These limitations are characteristic of the network-pharmacology/docking approach generally and do not undermine the value of the analysis as a hypothesis-generating step, but they define the boundary of what can be claimed from it.

Within these boundaries, the convergence of independent lines of computational evidence — hub-gene centrality, pathway enrichment, and docking affinity all pointing toward SIRT1-centered oxidative-stress regulation — constitutes a coherent, testable hypothesis rather than a settled mechanism. What this analysis offers is not proof that PRM will be neuroprotective in TBI, but a prioritized, mechanistically motivated shortlist — SIRT1 foremost, JUN and MMP9 secondarily — for the experimental work that would actually establish that.

CONCLUSION AND FUTURE PERSPECTIVES

This study applied an integrated network-pharmacology and molecular-docking workflow to ask whether PRM, a barbiturate anticonvulsant with no prior literature in TBI, engages the molecular machinery of secondary injury. Intersecting predicted PRM targets with TBI-associated genes yielded 138 candidates, from which ten hub proteins were prioritized by network centrality; docking against all ten converged on SIRT1 as the strongest predicted interaction, with JUN and MMP9 as secondary hits, spanning oxidative-stress regulation, AP-1-mediated stress signaling, and extracellular-matrix/blood–brain barrier biology, respectively.

The immediate next step is direct biochemical confirmation rather than a further round of computation. A SIRT1 deacetylase activity assay, using PRM as a test compound against a validated substrate such as an acetylated p53 or PGC-1α peptide, would establish whether the predicted interaction translates into measurable enzymatic modulation — a tractable, low-cost experiment that could be completed before committing to an animal model. If activity is confirmed, a rodent TBI model with existing precedent for SIRT1 pharmacology, such as the lateral fluid-percussion model used in prior SIRT1–TBI work,[31] would allow PRM to be tested against a SIRT1 inhibitor (e.g., sirtinol) or activator control, with mitochondrial membrane potential, cleaved caspase-3, and functional neurobehavioral outcomes as readouts. Because PRM's human dosing range is already established for epilepsy and essential tremor, a pilot in vivo study could reasonably anchor its dose selection to existing clinical pharmacokinetic data rather than requiring a de novo dose-ranging study, meaningfully shortening the path from this computational finding to a first efficacy readout.

More broadly, this analysis adds PRM to a small but growing list of repurposing candidates nominated for TBI through network pharmacology and, independent of whether PRM specifically advances, reinforces SIRT1 as a recurring, mechanistically plausible node for future screening efforts in this indication.[32]

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