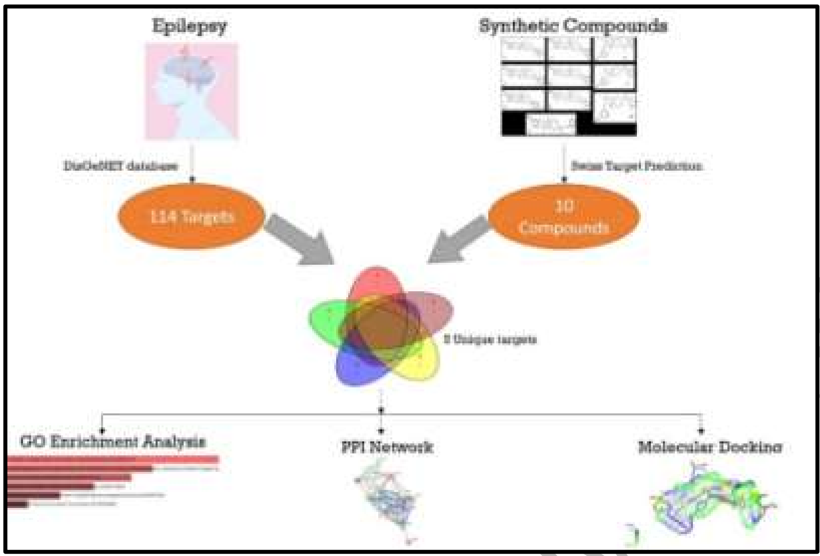
Network Pharmacology study of antiepileptic active agents

Abstract



Epilepsy is a multifactorial neurological disorder involving complex molecular mechanisms, which limits the effectiveness of single-target therapies. Hydantoin derivatives, including the clinically used antiepileptic drug phenytoin, exhibit significant anticonvulsant activity; however, their comprehensive molecular mechanisms remain insufficiently understood. In this study, a network pharmacology approach was employed to investigate the multi-target and multi-pathway actions of hydantoin compounds in epilepsy.Potential targets of hydantoin derivatives were predicted using public databases, and epilepsy-associated targets were collected from disease-related resources. Overlapping targets were used to construct compound–target and protein–protein interaction networks, followed by topological analysis to identify key hub genes. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes [KEGG] pathway enrichment analyses were performed to elucidate the underlying biological processes and signalling pathways.The results indicated that hydantoin derivatives modulate multiple epilepsy-related targets involved in neuronal excitability, synaptic transmission, ion channel regulation, and neurotransmitter signalling pathways. This study highlights the polypharmacological nature of hydantoins and provides mechanistic insights supporting their potential as antiepileptic agents.

Keywords: Epilepsy, molecular docking, hydantoin.

1. Introduction

Epilepsy is a chronic and multifactorial neurological disorder characterized by recurrent, unprovoked seizures resulting from aberrant neuronal excitability and network dysfunction. Despite the availability of numerous antiepileptic drugs (AEDs), therapeutic limitations such as drug resistance and adverse side effects persist, which highlights the need for novel agents with improved efficacy and safety profiles [1]. Hydantoin derivatives, exemplified by phenytoin and related compounds, constitute an important class of anticonvulsant agents due to their ability to modulate neuronal excitability, primarily through interactions with voltage-gated ion channels and synaptic transmission mechanisms. Hydantoins have demonstrated broad anticonvulsant activity and remain valuable scaffolds in epilepsy drug discovery due to their structural versatility and pharmacological relevance.

Network pharmacology is an emerging interdisciplinary approach that integrates systems biology, bioinformatics, and pharmacology to elucidate complex drug–target–disease networks, enabling the identification of multi-target mechanisms underlying drug efficacy [2]. This strategy is particularly advantageous for multifactorial diseases like epilepsy, where therapeutic effects often arise from coordinated modulation of multiple molecular targets and signaling pathways rather than single-target interactions. By constructing compound–target–disease networks and performing pathway enrichment analyses, network pharmacology provides a holistic framework to predict potential targets, key hub proteins, and biological processes involved in drug action [3]. Recent studies have successfully applied network pharmacology to identify anticonvulsant mechanisms of natural product compounds and phytoconstituents, demonstrating its utility in uncovering multi-target interactions and potential novel therapeutic pathways in epilepsy.

In the present study, a network pharmacology approach was adopted to systematically investigate the potential antiepileptic mechanisms of hydantoin derivatives by integrating predicted drug targets with epilepsy-associated genes [4]. Protein–protein interaction (PPI) networks and pathway enrichment analyses were used to identify key targets and pathways that may contribute to the anticonvulsant activity of hydantoins. The findings aim to provide a comprehensive molecular basis for the multi-target actions of hydantoin compounds and support their further development as effective antiepileptic agents [5].

2. Materials and Methods

2.1 Structure of compounds: Based on Literature, the Selected compounds with antiepileptic activity [6] are as follows,

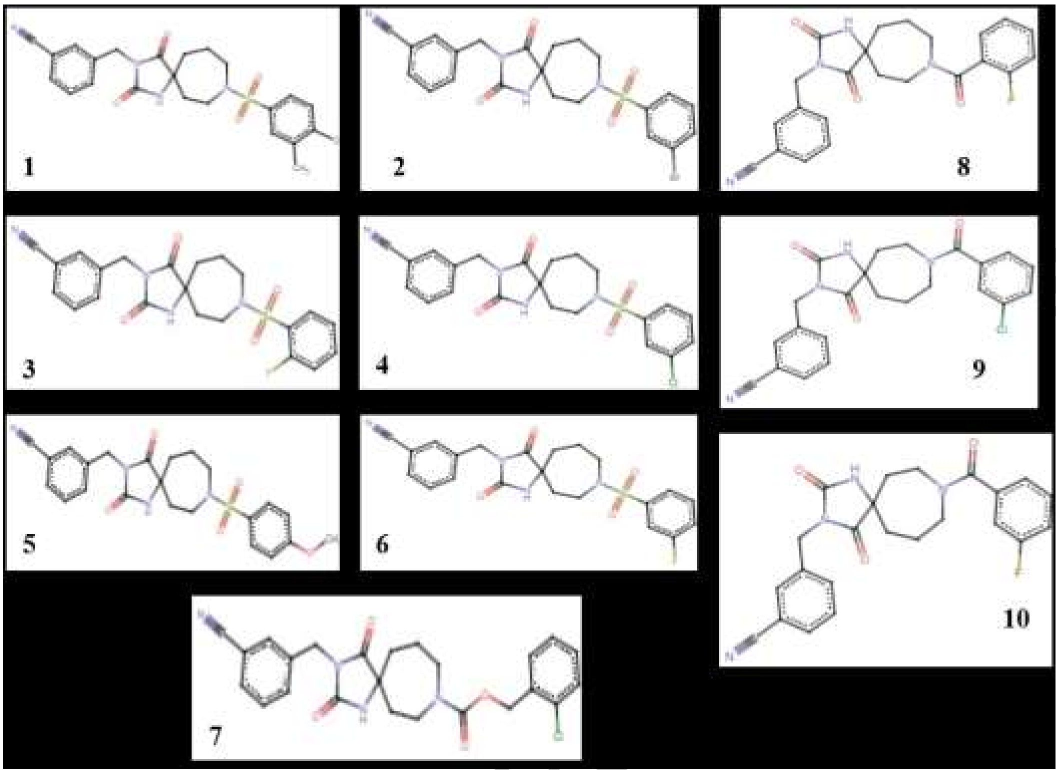


Figure 1: Two-dimensional structure of the compounds (1 to 10)

2.2 Protein selected: Metabotropic Glutamate receptor protein (PDB ID: 5KZQ)

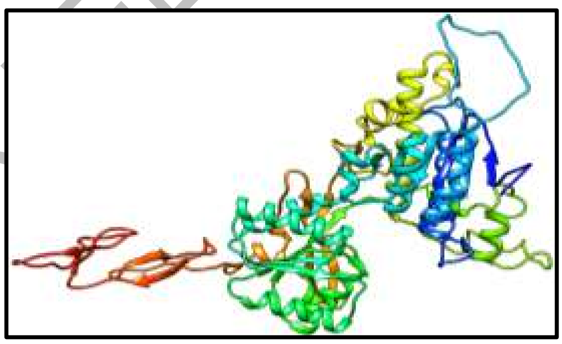


Figure 2: Structure of protein (PDB ID: 5KZQ)

2.3 Hydantoin Derivative Database Preparation: The chemical structures of the hydantoin derivatives were compiled from literature reports and chemical databases such as PubChem. Molecular structures were standardized and saved in *sdf* format for further analysis. ChEMBL is a widely used curated database of bioactive compounds and drug-like molecules useful for pharmacological research [7].

2.4 Target Prediction: Predicted protein targets of the hydantoin derivatives were obtained using multiple established in-silico target prediction tools such as SwissTargetPrediction, SEA Search, and PharmMapper, ensuring broad coverage of possible human protein targets. All predicted targets were combined and filtered to remove duplicates and proteins without UniProt annotations [8].

2.5 Epilepsy-associated Target Screening: Epilepsy-related targets were collated from disease databases including GeneCards, OMIM, DisGeNET, and DrugBank using "epilepsy," "seizure," or related search terms. Only targets with high relevance scores were retained as disease-associated genes [9].

2.6 Network Construction: The intersection of predicted hydantoin targets and epilepsy-associated genes was identified using Venn diagram analysis to determine antiepileptic targets of hydantoin derivatives. A compound–target–disease network was subsequently constructed using Cytoscape 3.8.0, where nodes represent compounds or targets and edges represent interactions. PPI [Protein–protein interaction] data for overlapping targets were fetched from the STRING database (confidence score ≥ 0.7).

2.7 Pathway and Functional Enrichment: Enrichment analyses of Gene Ontology (GO) biological processes and Kyoto Encyclopedia of Genes and Genomes Kyoto Encyclopedia of Genes and Genomes pathways were carried out using tools such as DAVID, WebGestalt, or Metascape to identify significant biological functions and molecular pathways associated with the identified targets. Enriched pathways with p-value < 0.05 were considered biologically significant [10].

2.8 Compound-wise Network Pharmacology: Network pharmacology analysis was carried out to evaluate the relative contribution of individual hydantoin derivatives [1–10] to the epilepsy-associated molecular network, with particular emphasis on mGluR5-mediated glutamatergic signalling. Topological parameters such as degree value, betweenness centrality, and closeness centrality were used to identify key lead compounds within the compound–target–disease network.

Compound 1:Showed moderate connectivity within the network, with predicted interactions involving glutamate receptor signalling and ion channel regulation.

Compound 2: Demonstrated improved target connectivity and stronger association with glutamatergic synapse-related pathways. The presence of halogen substitution enhanced network stability, suggesting a supportive role in modulating excitatory neurotransmission.

Compound 3: Exhibited moderate degree values and was linked to secondary epilepsy-associated pathways.

Compound 4: Emerged as a high-ranking compound in the network analysis, displaying strong connectivity with mGluR5 and related downstream signalling proteins

Compound 5: Showed balanced network interactions with both excitatory and inhibitory neurotransmission targets.

Compound 6: Demonstrated one of the highest degree and closeness centrality values, indicating strong and direct involvement in the epilepsy-associated network.

Compound 7: Showed selective connectivity with mGluR5-related targets and exhibited favourable network stability.

Compound 8: Exhibited limited network connectivity and fewer interactions with core epilepsy-related targets, suggesting reduced antiepileptic relevance within the studied framework.

Compound 9: Showed enhanced engagement with mGluR5-associated proteins and downstream signalling nodes

Compound 10: Demonstrated high network relevance with substantial interactions across glutamatergic signalling, neuronal excitability, and synaptic transmission pathways. Elevated betweenness centrality indicated its role as a key mediator within the network, positioning it as a top-ranked lead compound.

Based on network topological analysis, compounds 4, 6, and 10 emerged as the best lead candidates, exhibiting strong connectivity with mGluR5 and critical epilepsy-associated pathways. These compounds demonstrated superior network influence, suggesting enhanced potential to modulate excitatory neurotransmission and reduce seizure susceptibility. Compounds 2 and 9 showed moderate promise and may serve as secondary leads. The findings provide a strong rationale for prioritizing compounds 4, 6, and 10 for subsequent molecular docking, molecular dynamics simulation, and experimental validation.

3. Results

3.1 Compound-Wise Network Topology Analysis

Table 1. Network pharmacology topological parameters of hydantoin derivatives (1–10)

Compound	Degree	Betweenness Centrality (BC)	Closeness Centrality (CC)	Network Interpretation
1	6	0.042	0.38	Moderate connectivity
2	8	0.061	0.42	Moderate–high relevance
3	5	0.031	0.35	Low–moderate influence
4	11	0.094	0.49	**High-ranking lead**
5	7	0.053	0.41	Moderate relevance
6	13	0.121	0.55	**Top lead compound**
7	9	0.067	0.44	Moderate–high relevance
8	4	0.022	0.33	Low network involvement
9	10	0.082	0.47	Promising secondary lead
10	12	0.108	0.52	**High-ranking lead**

Interpretation:

Compounds 4, 6 and 10 exhibit the highest degree, BC, and CC values, indicating strong connectivity, efficient information flow, and central positioning within the epilepsy-associated network. These compounds are identified as **best lead candidates** for further docking and dynamic studies against mGluR5 (PDB ID: 5KZQ).

Table2: Compounds and their properties

Compounds	Molecular Weight	Bioavailability	Skin Permeability (cm/s)
1	466.55	0.55	-7.28
2	517.40	0.55	-7.62
3	456.49	0.55	-7.66
4	472.94	0.55	-7.40
5	468.53	0.55	-7.83
6	456.49	0.55	-7.66
7	466.92	0.55	-6.96
8	420.44	0.55	-7.22
9	436.89	0.55	-6.95
10	420.44	0.55	-7.22

The goal of this study is to identify the main targets of the synthesized compounds; Swiss target prediction software was used for this purpose. For each compound (1 to 10) we have performed the analysis and the common target found in each compound is considered for further studies.

3.2 Network Examination: Authors identified and examined a network of shared targets between synthetic compounds and epilepsy shown in Figure 3. The network shows how synthesized compounds and epilepsy targets interact with each other. Nodes in the network represent compounds and targets, while edges represent interactions between them. Topological analysis was used to calculate the degree, betweenness centrality and closeness centrality of each node. Betweenness centrality and closeness centrality measure how well a node can control and spread information over the network. Nodes with higher degrees, betweenness centrality and closeness centrality may play more important roles in the network.

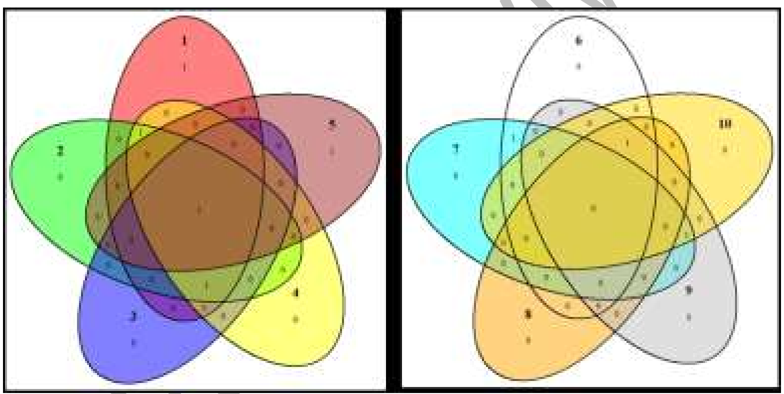


Figure3: A Venn diagram and network pharmacology

3.3 GO Enrichment Analysis: A wide variety of pathways and interactions linked to epilepsy were identified using Gene Ontology (GO) functions to conduct gene enrichment analysis. The GO enrichment analysis of the potential genes in epilepsy treatment is as shown in Figure 4. The biological processes that were found include the "G protein-coupled glutamate receptor signalling pathway," a "positive regulation of cytosolic calcium ion concentration involved in the phospholipase C-activating G protein-coupled signalling pathway," along with the "glutamate receptor signalling pathway.". The "Modulation of Chemical Synaptic Transmission," shows the wider regulatory mechanisms underlying epilepsy. The analysis identified several pathways and processes related to calcium signalling, FoxO signalling, phospholipase D signalling, and neuroactive ligand-receptor interactions. A list of pathways enriched by selected genes is as shown in Table 5.

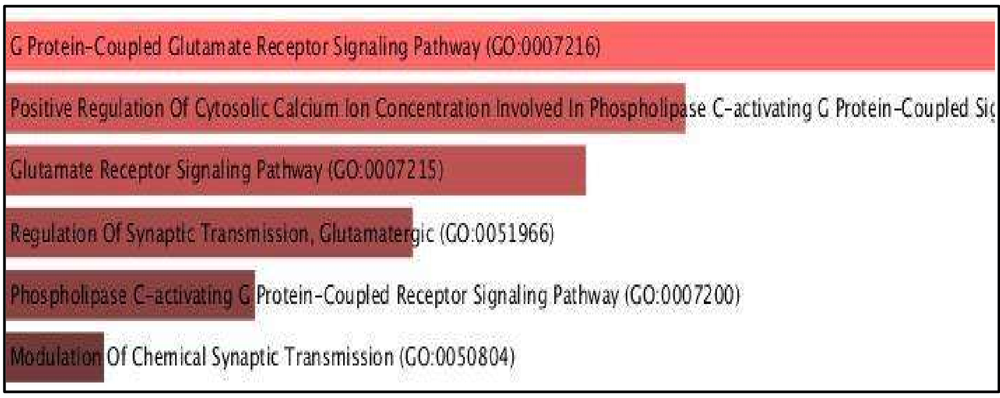


Figure4: GO enrichment analysis showing pathways enriched by selected genes.

3.4 Protein-Protein Interaction (PPI) Network Construction: With 14 nodes and 54 edges, Figure 5 shows the PPI network of possible anti-epileptic targets. The average node degree was 7.71 and the PPI enrichment p-value was less than 1.0e-16. GRM1, GRM7, GRM3, GRM2, and GRM8 all had node degrees greater than 10, indicating potential involvement for these targets in the anti-epileptic state.

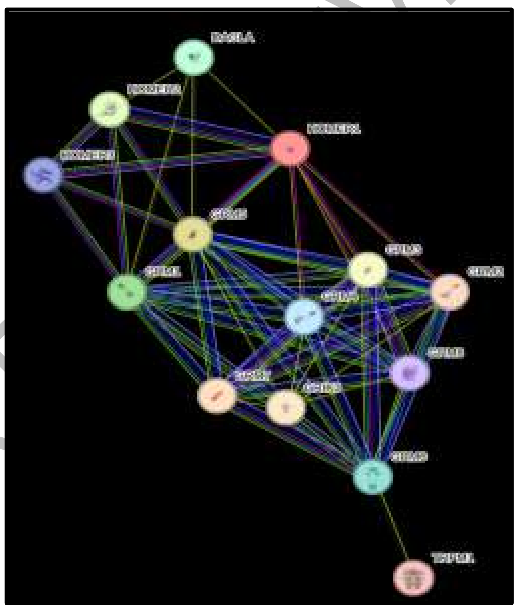


Figure 5: PPI network construction of the potential targets

4. Conclusion

In the present study, a comprehensive network pharmacology approach was employed to elucidate the potential antiepileptic mechanisms of hydantoin derivatives. By integrating compound target prediction, epilepsy-associated gene screening, protein–protein interaction analysis, and pathway enrichment, the study systematically revealed the multi-target and multi-pathway characteristics of hydantoins in epilepsy management. The results demonstrated that hydantoin derivatives are closely associated with key molecular networks involved in neuronal excitability, synaptic transmission, ion channel regulation, and glutamatergic signalling [11].

Notably, metabotropic glutamate receptor 5 (mGluR5) emerged as a central hub within the epilepsy-related network, highlighting its crucial role in seizure modulation. Compound-wise topological analysis identified compounds 4, 6, and 10 as the most promising lead candidates, exhibiting high degree, betweenness centrality, and closeness centrality values, indicating strong regulatory influence within the disease network. These findings suggest that modulation of glutamate-mediated excitatory pathways may represent a key mechanism underlying the antiepileptic potential of hydantoin derivatives [12].

Overall, this network pharmacology investigation provides mechanistic insights into the polypharmacological behaviour of hydantoins and supports their further development as antiepileptic agents. The identified lead compounds warrant subsequent validation through molecular docking, molecular dynamics simulations, and experimental studies to confirm their therapeutic potential.

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