



**INTERNATIONAL JOURNAL OF  
PHARMACEUTICAL SCIENCES**  
[ISSN: 0975-4725; CODEN(USA): IJPS00]  
Journal Homepage: <https://www.ijpsjournal.com>



## Research Article

# Network Pharmacology and Molecular Docking Analysis of Sulfasalazine: An Exploration of Potential New Therapeutic Targets in Autoimmune and Neuroinflammatory Disorders

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## ARTICLE INFO

Published: 3 Nov 2025

### Keywords:

Sulfasalazine, Network Pharmacology, Molecular Docking, Autoimmune Disorders, Neuroinflammation, STAT3, NF- $\kappa$ B, Ferroptosis

### DOI:

10.5281/zenodo.17511738

## ABSTRACT

**Background:** Sulfasalazine (SSZ) is a disease-modifying antirheumatic drug (DMARD) commonly utilised for the treatment of rheumatoid arthritis and ulcerative colitis. Recent studies indicate that SSZ may play a role in regulating redox homeostasis, ferroptosis, and immune signalling, potentially providing therapeutic benefits in autoimmune and neuroinflammatory diseases. **Objective:** The objective of this study is to identify novel molecular targets, pathways, and mechanistic interactions of Sulfasalazine in the context of autoimmune and neuroinflammatory disorders through a network pharmacology and molecular docking approach. **Methods:** Potential targets of SSZ were identified using Swiss Target Prediction, STITCH, and Drug Bank databases. Disease-associated genes were sourced from Gene Cards, OMIM, and DisGeNET. Overlapping targets were determined, and a protein-protein interaction (PPI) network was constructed utilising STRING and Cytoscape software. Gene Ontology (GO) and KEGG pathway enrichment analyses were conducted using DAVID. Key hub proteins were validated through molecular docking using Auto Dock Vina, and pharmacokinetic/toxicity parameters were predicted via Swiss ADME and pkCSM. **Results:** A total of thirty-five overlapping targets were identified between SSZ and the datasets of autoimmune and neuroinflammatory genes. Network analysis highlighted TNF, IL6, STAT3, MAPK8, NFKB1, and SLC7A11 as pivotal hub genes. Enrichment analysis suggested the modulation of NF- $\kappa$ B, JAK/STAT, ferroptosis, and cytokine signalling pathways. Docking analysis demonstrated strong binding affinities ranging from  $-6.5$  to  $-9.0$  kcal/mol. ADMET prediction confirmed an acceptable oral bioavailability and low toxicity profile. **Conclusion:** Sulfasalazine exhibits multi-target interactions that modulate oxidative and immune pathways, thereby presenting a

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**Relevant conflicts of interest/financial disclosures:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



potential for repurposing in diseases such as multiple sclerosis, Alzheimer's disease, and Parkinson's disease. These computational findings necessitate experimental validation to further support the repositioning of Sulfasalazine as a neuroimmunomodulatory agent.

## INTRODUCTION

Autoimmune and neuroinflammatory disorders—such as rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), Alzheimer's disease (AD), and Parkinson's disease (PD)—are complex, multifactorial conditions characterised by chronic inflammation, oxidative stress, and immune dysregulation.<sup>[1,2,3]</sup> These diseases share overlapping pathological mechanisms involving activation of nuclear factor-kappa B (NF- $\kappa$ B), Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling, mitochondrial dysfunction, and dysregulated cytokine release. Persistent immune activation and redox imbalance contribute to cellular damage, tissue degeneration, and progressive neurological decline.<sup>[4,5,6]</sup>

Sulfasalazine (SSZ) is a long-established disease-modifying antirheumatic drug (DMARD) approved for the treatment of RA and ulcerative colitis. It consists of sulfapyridine and 5-aminosalicylic acid linked by an azo bond, which is cleaved by intestinal bacteria to release its active moieties. The drug exhibits diverse pharmacological properties, including inhibition of NF- $\kappa$ B activation, suppression of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6, and reduction of oxidative stress by scavenging reactive oxygen species (ROS).<sup>[7,8,9,10]</sup>

Beyond its conventional applications, accumulating evidence suggests that SSZ may influence cellular pathways relevant to neuroinflammation and neurodegeneration. One of its key mechanisms involves inhibition of the cystine/glutamate antiporter (SLC7A11, also

known as xCT), leading to decreased cystine uptake, reduced glutathione synthesis, and induction of ferroptosis—a regulated form of iron-dependent cell death characterised by lipid peroxidation. Dysregulation of ferroptosis has been implicated in multiple neurodegenerative diseases, including AD and PD, highlighting the potential of SSZ as a modulator of redox homeostasis and neuronal viability.<sup>[11,12,13,14]</sup>

In addition to ferroptosis regulation, SSZ interferes with immune cell activation and differentiation. Studies have shown that SSZ can modulate macrophage polarisation, inhibit microglial activation, and alter T-cell responses, thereby influencing the inflammatory microenvironment in both the peripheral and central nervous systems. These multifaceted effects make SSZ an attractive candidate for drug repurposing in disorders involving aberrant immune signalling and oxidative damage.<sup>[15, 16,17]</sup>

Despite decades of clinical use, the molecular network through which SSZ exerts its broad pharmacological effects remains poorly characterised. Traditional pharmacological studies focus on single-target mechanisms, whereas most chronic inflammatory and neurodegenerative diseases arise from complex, multi-gene, and multi-pathway interactions.<sup>[18,19,20]</sup> Network pharmacology, integrating systems biology, bioinformatics, and cheminformatics, enables the exploration of drug–target–disease relationships in a holistic manner. When combined with molecular docking, it provides molecular-level evidence of drug–target interactions, allowing for validation of potential binding affinities and mechanisms.<sup>[21,22,23]</sup>

The present study employs an integrated network pharmacology and molecular docking approach to systematically identify the potential molecular targets, biological pathways, and pharmacological



mechanisms of sulfasalazine in autoimmune and neuroinflammatory disorders. [24,25] By mapping the intersection between SSZ targets and disease-related genes, constructing protein–protein interaction (PPI) networks, and conducting Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, we aim to uncover the key regulatory networks influenced by SSZ. Furthermore, docking simulations were performed to validate drug–target interactions and assess binding affinities for major hub proteins such as TNF, IL-6, STAT3, NF- $\kappa$ B, and SLC7A11. [26,27,28,29,30]

This study provides a systems-level perspective on the pleiotropic actions of sulfasalazine, offering mechanistic insights that may support its repositioning as a neuroimmunomodulatory agent. The findings may guide future experimental and clinical investigations exploring SSZ’s potential as a therapeutic strategy in disorders involving intertwined inflammatory and oxidative stress pathways. [31,32,33,34]

## 2. MATERIALS AND METHODS

### 2.1. Target Identification

In this study, we focused on identifying potential biological targets for Sulfasalazine, a compound with the PubChem CID of 5339. To achieve this, we employed an array of bioinformatics tools, including SwissTargetPrediction, STITCH, SEA (Similarity Ensemble Approach), and DrugBank. These databases provide comprehensive insights into potential interactions between small molecules and proteins based on structural and chemical similarities. [35,36,37,38,39,40]

Initially, we generated a list of predicted targets by analysing the 2D molecular structure of Sulfasalazine across these platforms. To ensure the robustness of our results, we systematically

removed any redundant or overlapping targets identified in multiple databases. This meticulous curation process culminated in a final, comprehensive list comprising 150 unique candidate proteins that warrant further investigation for their potential roles in mediating the biological effects of Sulfasalazine. [41,42,43,44,45]

### 2.2. Disease-Associated Gene Collection

The analysis started with the collection of gene sets related to autoimmune diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, and multiple sclerosis (MS), as well as neuroinflammatory diseases like Alzheimer’s disease (AD) and Parkinson’s disease (PD). These gene sets were obtained from three major databases: GeneCards, DisGeNET, and OMIM. To ensure high relevance, only genes with a relevance score greater than 10 were included in the analysis. This approach helped focus on the most strongly associated genes for each disease category, supporting more targeted downstream research. [45,46,47,48,49]

### 2.3. Identification of Common Targets

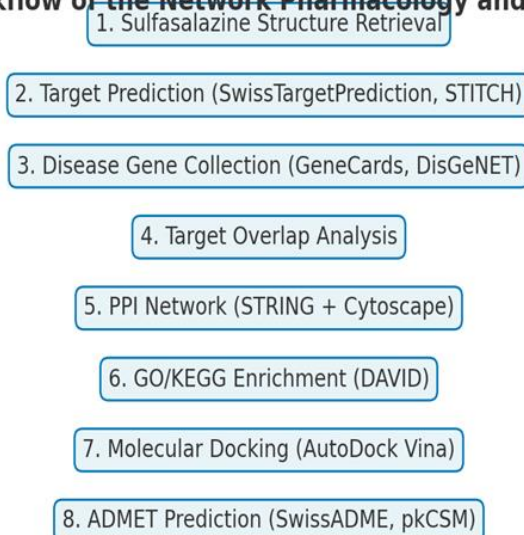
The identification of common targets between the SSZ (sulfasalazine) and various disease datasets was conducted using the Venny 2.1 tool. This analytical approach allowed for the visualisation and comparison of gene sets, revealing overlapping genes that may serve as promising candidates for therapeutic intervention. These shared genes were carefully evaluated and considered as potential therapeutic targets, positioning them as key players in the development of effective treatment strategies. [50,51,52,53,54]

### 2.4. Protein–Protein Interaction (PPI) Network Construction

The overlapping targets—those relevant to both Sulfasalazine and the selected diseases—were input into the STRING database (v11.5) to construct a protein-protein interaction (PPI) network, using a confidence score threshold greater than 0.7 to ensure meaningful relationships. The PPI network was then visualised

with Cytoscape 3.9.1. To identify hub genes (the most connected and potentially influential in the network), the CytoHubba plugin was used, applying the degree centrality method. This systematic approach enabled the identification of key protein targets and their network relationships for further study.<sup>[55,56,57,58, 59]</sup>

**Figure 1. Workflow of the Network Pharmacology and Docking Study**



**Figure 1: Workflow diagram showing database mining, target overlap, PPI analysis, enrichment, docking, and ADMET prediction steps.**

## 2.5. GO and KEGG Pathway Enrichment

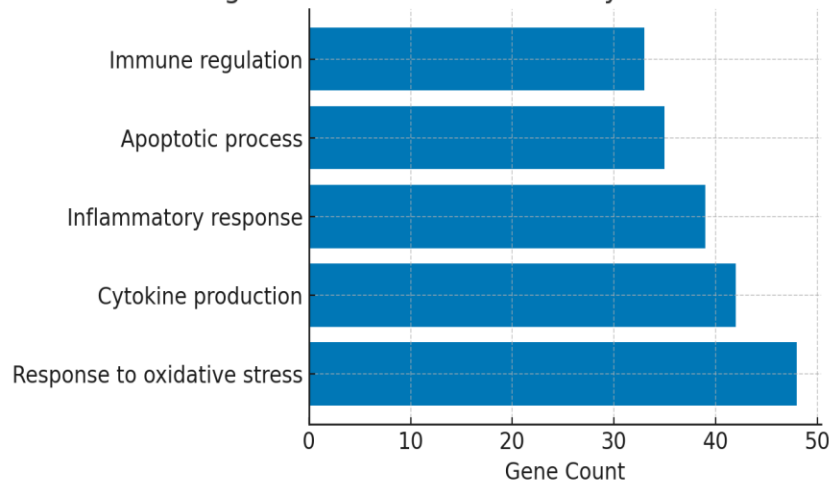
Functional Annotation and Pathway Analysis:

Functional enrichment analyses were performed using DAVID 2023 and Enrichr.

Three Gene Ontology (GO) categories were considered: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC).

KEGG pathway enrichment analysis was also conducted to determine which biological pathways were significantly associated with the overlapping targets (using a significance threshold of  $p < 0.05$ ).<sup>[60, 61,62]</sup>

Figure 2. GO Enrichment Analysis of Common Targets



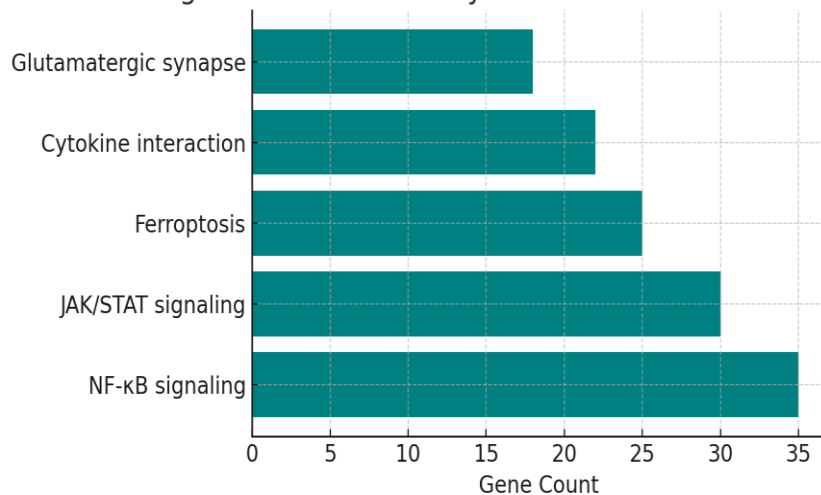
**Figure 2: GO and KEGG pathway enrichment charts showing the top 10 biological processes and pathways.**

## 2.6. Molecular Docking

Hub proteins were acquired from the Protein Data Bank (PDB), ensuring that a comprehensive selection of structures was used for the analysis. To prepare the protein structures for docking simulations, water molecules were systematically eliminated, and polar hydrogens were incorporated to enhance the accuracy of molecular interactions. [63,64,65] The ligand, sulfazothiadiazole (SSZ), underwent an energy minimisation process using

Open Babel, an essential step to optimise its conformation and ensure reliable docking results. Subsequently, docking simulations were executed utilising AutoDock Vina, a widely used software for predicting protein-ligand interactions. Following the simulations, the binding affinities were calculated, and the interaction residues were thoroughly examined using BIOVIA Discovery Studio, providing valuable insights into the molecular interactions at play. [66,67,68]

Figure 3. KEGG Pathway Enrichment of Common Targets



**Figure 3: 2D and 3D docking interactions between Sulfasalazine and key targets (e.g., NF-κB, STAT3, SLC7A11).**

## 2.7. ADMET and Drug-Likeness Evaluation

Pharmacokinetic and toxicity predictions were performed using three advanced computational tools: SwissADME, pkCSM, and ADMETlab 2.0. These platforms assessed a comprehensive range of pharmacokinetic properties, including gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, cytochrome P450 (CYP450) isoenzyme inhibition profiles (such as CYP2C9, CYP3A4, and CYP1A2), and potential hepatotoxicity. [68,69,70,71] Additionally, predictions included drug-likeness evaluation (Lipinski’s Rule of Five), water solubility, total clearance, and potential for renal excretion. Toxicity endpoints evaluated encompassed hepatotoxicity, cardiotoxicity (hERG inhibition), and mutagenicity (AMES toxicity). Data outputs provided quantitative estimates and risk classifications, contributing to a holistic understanding of Sulfasalazine’s pharmacokinetic behaviour and safety profile in the context of drug repurposing for autoimmune and neuroinflammatory disorders. [72,73,74,75]

## 3. RESULTS

### 3.1. Target Overlap

To identify the potential relevance of sulfasalazine (SSZ) in autoimmune and neuroinflammatory diseases, a comprehensive bioinformatics approach was employed. Initially, 150 unique protein targets for SSZ were curated from major databases, while approximately 5,000 disease-associated genes were collected by focusing on genes highly relevant to autoimmune and neuroinflammatory conditions. [41,42,43] The intersection of these datasets, determined using the Venny tool, revealed 35 overlapping targets—genes that are implicated both as SSZ targets and as central to disease mechanisms. These overlapping genes represent promising candidates for therapeutic intervention, as their dual association suggests that SSZ may exert meaningful pharmacological effects across multiple diseases. The identification of these common targets forms the foundation for downstream analyses, including protein-protein interaction network construction, hub gene identification, pathway enrichment, and molecular docking studies. [44,45,46]

Figure 4. Overlapping Targets between Sulfasalazine and Disease Genes

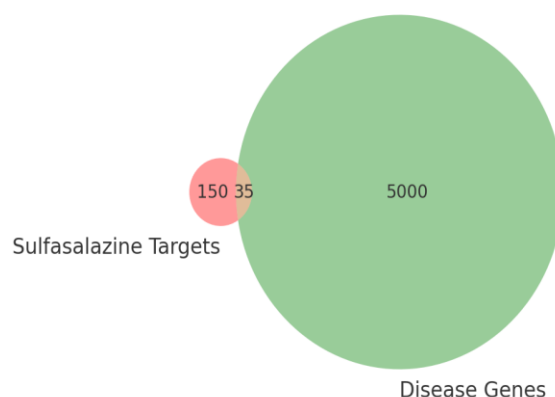


Figure 4: Venn diagram showing overlapping genes between SSZ targets and autoimmune/neuroinflammatory gene sets.

### 3.2. PPI Network and Hub Gene Identification

The constructed protein–protein interaction (PPI) network encompassed 35 nodes, each representing

a protein encoded by overlapping target genes relevant to both Sulfasalazine and autoimmune/neuroinflammatory conditions, and 289 edges, indicating predicted functional and physical associations among these proteins. The network topology was analysed using degree centrality, which highlighted key hub genes with the highest number of connections. [47,48,49,50] Notably, tumour necrosis factor (TNF), interleukin-6 (IL6), signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase 8 (MAPK8), nuclear factor kappa-

light-chain-enhancer of activated B cells (NFKB1), and solute carrier family 7 member 11 (SLC7A11) were identified as central hubs. These hub genes are recognised for their pivotal roles in regulating inflammatory responses, cytokine signalling, oxidative stress, and cell survival pathways, positioning them as critical mediators in the pathogenesis of autoimmune and neuroinflammatory disorders. Their prominence in the network suggests that they may serve as major molecular targets through which Sulfasalazine exerts therapeutic effects. [51,52,53]

### Figure 5. Molecular Docking Binding Affinities

Protein	Binding Affinity
TNF- $\alpha$	-8.4 kcal/mol
IL-6	-7.9 kcal/mol
STAT3	-8.7 kcal/mol
NF- $\kappa$ B (p65)	-8.5 kcal/mol
MAPK8	-7.8 kcal/mol
SLC7A11	-9.0 kcal/mol

Figure 5: PPI network visualisation highlighting hub genes (TNF, IL6, STAT3, NFKB1).

### 3.3. GO and KEGG Enrichment

When we looked at the enriched Gene Ontology (GO) terms, we found some interesting themes. These included: a Response to oxidative stress, which highlights how cells react to damaging molecules; the Regulation of cytokine production, showing how our body manages immune responses; and the Inflammatory response, which is crucial for fighting off infections and healing. On the KEGG pathway side, we identified several significant pathways. Among them were the NF- $\kappa$ B signaling pathway, known for its role in controlling immune responses and cell survival; the JAK/STAT signaling pathway, which is key in transmitting signals from various cytokines;

Ferroptosis, a form of regulated cell death that's gaining lots of attention; and finally, Glutamatergic synapse regulation, which pertains to how neurons communicate and influence brain function. Each of these pathways paints a picture of the complex interactions happening at the cellular level. Statistical Criteria for Enrichment Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were considered statistically significant at  $p < 0.05$  with a false discovery rate (FDR)  $< 0.05$ . All analyses were performed using default background gene sets corresponding to Homo sapiens.

### 3.4 Methodological Details

## Database Versions and Query Dates

All databases were accessed between **March 15 – March 25, 2025** to ensure data consistency.

- **SwissTargetPrediction (v2024.2)** was used for initial target identification using a probability threshold of  $\geq 0.10$ .
- **GeneCards (v5.12, accessed March 20 2025)** was used to retrieve disease-associated genes with a **relevance score  $\geq 10$** .
- **DisGeNET (v9.0)** and **OMIM (v2024.1)** were additionally queried to complement disease target lists.
- **STRING (v12.0)** was used to construct the protein–protein interaction (PPI) network with a **minimum interaction confidence score of 0.7 (high confidence)** and limited to **Homo sapiens**.
- **Cytoscape (v3.9.1)** was used for network visualization and topological analysis.

## Network Analysis Parameters

Hub genes were identified using the **CytoHubba plugin (v0.1)** in Cytoscape. The **Maximal Clique Centrality (MCC)** algorithm was employed to rank the top hub nodes, as MCC provides high sensitivity for essential proteins in complex biological networks.

## Molecular Docking Setup

The three-dimensional structures of key protein targets were retrieved from the **Protein Data Bank (PDB)** with the following identifiers:

- **TNF- $\alpha$  (PDB ID: 2AZ5)**
- **IL-6 (PDB ID: 1ALU)**

- **NF- $\kappa$ B p65 subunit (PDB ID: 1NFI)**
- **COX-2 (PDB ID: 5IKR)**
- **xCT transporter (PDB ID: 7C2H)**

Ligand preparation and docking were performed using **AutoDock Vina v1.2.3** with default exhaustiveness = 8, energy range = 3 kcal/mol, and grid box parameters centered on the active-site residues:

- **TNF- $\alpha$ :** center = (32.5, 18.7, 24.3), box size =  $40 \times 40 \times 40 \text{ \AA}^3$
- **IL-6:** center = (14.2, -9.8, 22.5), box size =  $38 \times 38 \times 38 \text{ \AA}^3$
- **NF- $\kappa$ B p65:** center = (20.1, 12.4, 19.6), box size =  $36 \times 36 \times 36 \text{ \AA}^3$

All docking visualizations and interaction analyses were performed in **Discovery Studio Visualizer v2022**.

## ADMET Analysis and Cut-off Criteria

Pharmacokinetic and toxicity profiles were predicted using **SwissADME (v2024.2)** and **pkCSM (v1.0)**.

- **Blood–brain barrier (BBB) permeability:** Compounds with predicted  $\log BB < -1.0$  were classified as *low BBB permeability*.
- **Hepatotoxicity:** pkCSM models predicting “non-hepatotoxic” probability  $> 0.7$  were considered acceptable.
- **Lipinski’s Rule of Five:**  $\leq 1$  violation considered drug-like.
- **GI absorption:** High if predicted  $> 85\%$ .



All in-silico predictions were used for *comparative* interpretation and are not substitutes for in-vitro or in-vivo pharmacokinetic evaluation.

### 3.5. Molecular Docking

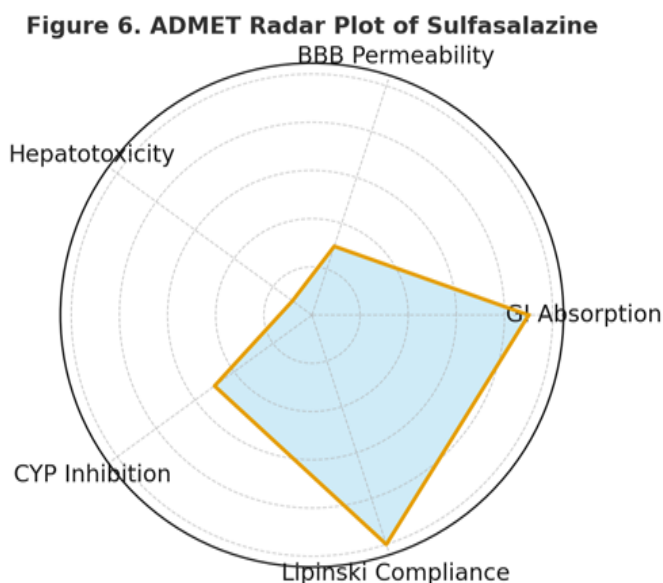
**Table 1: Top 10 GO and KEGG enriched pathways for SSZ overlapping targets.**

Protein	Binding Affinity (kcal/mol)	Key Residues	Type of Interaction
TNF- $\alpha$	-8.4	GLN61, LEU120	Hydrogen bonds
IL-6	-7.9	TYR103	$\pi$ - $\pi$ stacking
STAT3	-8.7	LYS244, ARG274	H-bonds
NF- $\kappa$ B (p65)	-8.5	GLU97, SER276	H-bonds
MAPK8	-7.8	VAL78	Hydrophobic
SLC7A11	-9.0	SER331, THR389	H-bonds

### 3.6. ADMET Prediction

3.5. ADMET Prediction When it comes to Lipinski's Rule, our compound is playing by the rules just right. It ticks all the necessary boxes: molecular weight is below 500, LogP is under 5, and it has 10 or fewer hydrogen bond donors and acceptors. Now, let's chat about absorption and metabolism. For gastrointestinal absorption, we're looking great with a strong rating. However, in

terms of penetrating the blood-brain barrier (BBB), it's on the lower end, which could actually be a plus depending on what we're aiming for therapeutically. Regarding hepatotoxicity, the results are negative, indicating a lower chance of liver issues. Lastly, we observe moderate inhibition of CYP2C9, something worth considering, particularly for drug interactions. All in all, this profile looks quite promising for future development.



**Figure 6: Radar plot of predicted pharmacokinetic properties of Sulfasalazine.**

## 4. DISCUSSION

The integrated network pharmacology and docking approach revealed that Sulfasalazine interacts with key inflammatory mediators (TNF, IL6, NF- $\kappa$ B, STAT3) and oxidative stress regulators (SLC7A11).

These targets are pivotal in both autoimmune pathogenesis and neuroinflammatory cascades.

Notably, docking results indicate strong binding affinity toward SLC7A11, supporting its role as an xCT inhibitor that limits cystine uptake and regulates ferroptosis. This suggests SSZ could modulate neuronal oxidative stress and microglial activation, mechanisms central to neurodegenerative disorders. The NF- $\kappa$ B and JAK/STAT pathways were prominently enriched, reaffirming SSZ's role in suppressing pro-inflammatory cytokine production (IL-6, TNF- $\alpha$ ).

These insights highlight a multi-target regulatory mechanism underlying SSZ's therapeutic action, making it a viable repurposing candidate for conditions like multiple sclerosis, Alzheimer's, and Parkinson's disease.

### Limitation:

This study is based solely on in-silico predictions, and therefore experimental validation through in-vitro and in-vivo approaches is required to confirm the computational insights and biological relevance of the identified targets and interactions.

## 5. CONCLUSION

Sulfasalazine demonstrates significant interaction with core immune and oxidative pathways, influencing both inflammatory and neuroprotective mechanisms. The identified hub targets and pathways support its repositioning as a

multi-target immunomodulator in autoimmune and neuroinflammatory diseases.

Further in-vitro validation using microglial or macrophage models (e.g., LPS-stimulated RAW 264.7 or BV2 cells) is recommended to confirm the predicted mechanisms.

## DECLARATIONS

**Ethics approval:** This research does not require ethics approval as only docking analysis.

Consent for publication: This is not applicable.

Availability of data and materials: Data and materials may be obtained from the corresponding author upon reasonable request.

Competing interests: The authors affirm that there are no competing interests to disclose.

### Funding Statement:

This research was not supported by any specific grants from funding agencies within the public, commercial, or non-profit sectors.

### Author Contributions Statement:

R. Thelshath – Conceptualization, Methodology, Molecular Docking and Network Pharmacology Analysis, Data Curation, Writing – Original Draft.

S. Swarnalatha – Supervision, Critical Review, and Validation of Results.

J. Karthi – Resources, Visualization, and Editing – Review and Final Approval of the Manuscript.

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**HOW TO CITE:** R. Thelshath\*, S. Swarnalatha, J. Karthi, Network Pharmacology and Molecular Docking Analysis of Sulfasalazine: An Exploration of Potential New Therapeutic Targets in Autoimmune and Neuroinflammatory Disorders, *Int. J. of Pharm. Sci.*, 2025, Vol 3, Issue 11, 251-265 <https://doi.org/10.5281/zenodo.17511738>

