Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/11.1.20250107

Research article
2025 | Volume 11 | Issue 1 | Pages 1-7

ARTICLEINFO

Received
December 19, 2024
Revised
January 23, 2025
Accepted
February 26, 2025

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Keywords
TSPO
Schizophrenia
Computational drug design
ADMET profiling
Molecular docking

How to Cite
Mehmood F, Ghaffar MJ, Sohail M, Jameel M, Mujahid U, Maqbool M, Sajid M. *In silico* efforts to screen potential natural compounds against Schizophrenia by targeting TSPO. Biomedical Letters 2025; 11(1): 1-7.

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In silico efforts to screen potential natural compounds against Schizophrenia by targeting TSPO

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Abstract

Schizophrenia (SZ) is a chronic and complex mental disorder. About 1% of the population of the world is affected by SZ as a serious neuropsychiatric disorder. Early adulthood persons facing brain hormonal changes, viral infection, defects in genetic encoding as well as stressful environmental factors are more susceptible to developing symptoms of SZ. Translocator protein (TSPO) is located in the outer mitochondrial membrane and plays an important role in several cellular processes including transport of cholesterol and synthesis of steroid hormones, mitochondrial respiration and ATP production, cell proliferation and apoptosis, and immunomodulation. TSPO expression is increased in chronic psychiatric patients and has been implicated as a modulator of inflammation and apoptosis. making it a potential target for drug development. In current efforts, a computational approach of 3D structure prediction, molecular docking, and Absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis was applied to screen potential compounds against SZ by targeting the TSPO translocator protein. Various Structures of the target protein were predicted, and a reliable structure was picked for further analysis of molecular docking. Molecular docking was performed against the natural compound library and the top-ranked compounds were picked for further analysis. Current experiments revealed that all the compounds were binding at similar binding pockets, and the top-ranked compounds were reported in the studies and were further evaluated based on ADMET analysis. After performing ADMET analysis and evaluating the compounds based on their interactional analysis and safety profiling it was observed that the compound 029-886-365 can be used against SZ by targeting TSPO.

Introduction

Schizophrenia (SZ) is a chronic and complex mental disorder. About 1% population of the world is affected by SZ as a serious neuropsychiatric disorder [1, 2]. Early adulthood persons facing brain hormonal changes, viral infection, defects in genetic encoding as well as stressful environmental factors are more susceptible to developing symptoms of SZ [3]. The main symptoms of SZ are organized in three sections: positive (delusions, hallucinations, and thought disorders), negative (social withdrawal, anhedonia, and flattened affect), and cognitive (poverty of speech, deficits in working memory, attention difficulties) [4, 5]. Current antipsychotic medications show promising efficacy but the advancement in SZ treatment remains limited by unacceptable negative side effects that prevent their use to address cognitive dysfunction in patients [6, 7]. Therefore, the discovery of target-specific drugs with higher clinical effectiveness is still necessary [8].

In this regard, translocator protein (TSPO) has emerged as a promising therapeutic target for SZ. TSPO is an 18 kDa protein located in the outer mitochondrial membrane and plays an important role in several cellular processes including the transport of cholesterol and synthesis of steroid hormones, mitochondrial respiration and ATP production, cell proliferation and apoptosis, and immunomodulation [9, 10]. TSPO expression is increased in chronic psychiatric patients and has been implicated as a modulator of inflammation and apoptosis making it a potential target for drug development [11, 12]. The rise in TSPO levels is believed to indicate ongoing neuroinflammatory processes and glial activation which are considered main factors in the development of SZ. TSPO levels correlate with microglial activation which plays a role in neuroinflammation and can trigger the release of pro-inflammatory cytokines and oxidative stress, ultimately linked to the disease pathophysiology.

For drug discovery and finding drug-like compounds computational approaches such as molecular docking, 3D structure prediction, and ADMET analysis play crucial roles [13, 14]. Molecular docking analysis helps in the understanding and analysis of bindings of drug-like compounds and targets. 3D structure prediction in drug discovery enables accurate modeling of target proteins, facilitating drug design [15, 16]. ADMET analysis evaluates the pharmacokinetic and toxicological properties of screened compounds, ensuring their drug-likeness and safety profile [17, 18]. Computational drug design has helped identify various biological compounds for neurological disorders including SZ [2, 13, 19-22] and various computational compounds have been reported against cancer [23, 24]. Moreover, numerous epitope-based vaccines have also been reported by utilizing *in silico* approaches [25-28]. In current efforts, various computational approaches and tools are applied to find the inhibitors of the TSPO for the treatment of SZ. These approaches are essential in finding potential TSPO inhibitors based on their binding energies, molecular interactions, and safety profiles. Current studies aim to identify novel therapeutic compounds by using computational methods that can influence TSPO activity offering a promising approach to control the progression and symptoms of SZ.

Materials and Methods

In current computational analyses, 3D structure prediction, molecular docking, and ADMET analysis were performed to elucidate potential therapeutic compounds against the target protein (**Fig. 1**). The sequence of the selected protein having 169 residues in FASTA format was retrieved from UniProt knowledge-based database [29]. For the 3D structure prediction of the target protein, the protein sequence was subjected to BLASTp [30] in order to find a suitable template for homology modeling. Sequence with higher similarity and a percent identity of 81% was analyzed and selected as a suitable template for 3D structure prediction and the protein sequence was submitted to I-tasser [31] and Robetta [32]. Both the servers predicted different 3D models of the target protein that were further evaluated by calculating the Z score, Q mean and analyzing the Ramachandran plot of the predicted structures [33, 34]. Energy minimization of the 3D protein structure was performed for 1000 steps, the conjugate-gradient method was applied by utilizing the Amber ff98 forcefield. Molecular docking analyses were performed in order to screen potential compounds having maximum interactions with the target protein. The molecular docking analysis was performed against the natural compound library having 113,000 small natural compounds. AutoDock Vina [35]was utilized for molecular docking analyses and the exhaustiveness was set as 8. For molecular docking analyses, the 3D structure of the target protein was prepared, and the required hydrogen atoms were added to the predicted structure at appropriate positions. 44.53, 53.35, 48.86, Å in *x*-, *y*, and *z*-axis

respectively were set as the grid size for molecular docking studies. Molecular docking analyses were performed and the whole receptor protein structure was covered having grid spacing of 64.71, 64.19, and 64.37Å. PyMol [36], Discovery Studio [37], Ligplot [38], and UCSF Chimera 1.9 [39] were utilized for the interactional analyses and the interacting residues of all the docked complexes were analyzed and visualized.

ChemDraw Ultra 12.0 [40] was used to retrieve the canonical SMILES of the screened compounds. Absorption Distribution Metabolism Excretion and Toxicity (ADMET) properties were calculated for all the screened compounds through ADMETsar [41] and Protox-3.0 [42]. Furthermore, the number of rotatable bonds, H-bond donors, and acceptors were also calculated. The risks of reproductive behavior, tumorigenicity, and mutagenicity including the drug-like properties of all the selected compounds were calculated to assess the toxic behavior of the compounds. The properties of ADMET play a significant key to studying the distribution and absorption behavior of the compound in an organism.

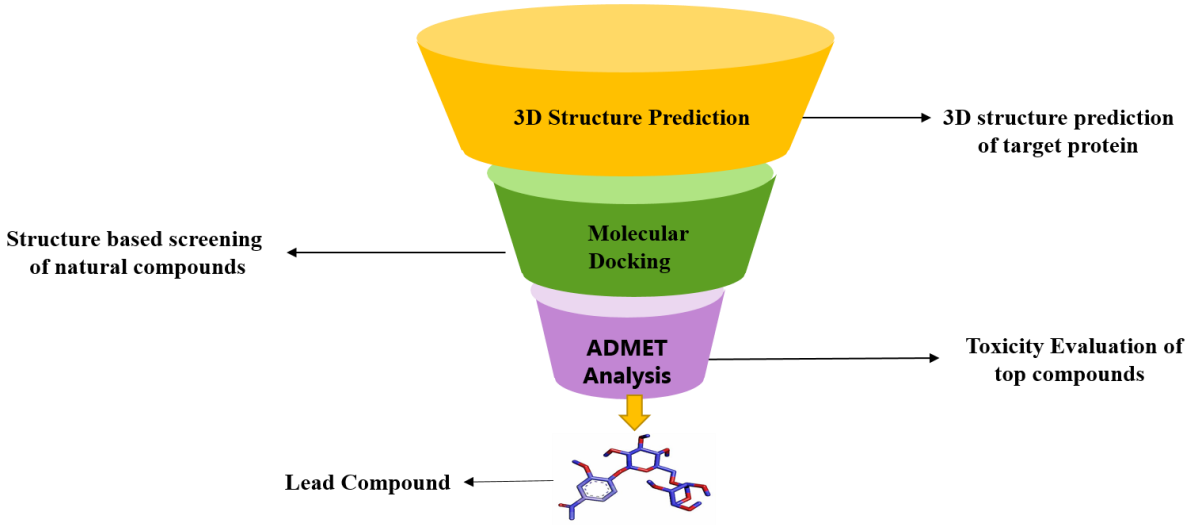

Fig. 1: Applied methodology to screen natural compounds against the target protein

Results and Discussion

The objective of the current *in silico* analysis was to elucidate potent natural compounds against schizophrenia by targeting the TPSO protein. Computational drug design analyses were performed to identify and investigate the potential of the natural compounds against schizophrenia by targeting the translocator protein. To analyze the structural features of the protein, 3D structure prediction of the target protein was performed using homology modeling, threading, and *ab initio* means. Analyzing BLASTp results, the most suitable template was retrieved and utilized for 3D structure prediction of the target protein, and the canonical sequence of the target protein was also submitted to different servers for 3D structure prediction. A total of 25 structures of the target protein were predicted for the target protein. All the 3D predicted structures were evaluated by different means including overall Quality factor, Z score and Ramachandran plot. Suitable structures were selected for further analysis after the evaluation of the 3D predicted structures. The overall quality factor and Z score were observed, and it was observed that the values for the overall quality factor and Z score were 93.3% and 87% respectively. The Ramachandran plot of the selected structure revealed that 81.2% of the residues were falling in the favorably allowed region, 17.3% of the residues were falling in the allowed region and only 1.5% of the residues were falling in the disallowed region revealing the efficacy of the predicted 3D structure (**Fig. 2**).

The predicted 3D structure of the target protein was analyzed and visualized to check the efficacy of the predicted structures (**Fig. 3**). Different regions of the selected structure were visualized to analyze the efficacy of the target protein. Energy minimization of the 3D predicted structure was performed in order to

fix the torsion angles and steric collisions to relax the utilized system.

The natural compound library was utilized for molecular docking analyses and to perform high-throughput virtual screening. Molecular docking analysis revealed that all the compounds from the natural compound library were binding at similar pockets. For further analysis, the top-ranked five compounds having the least binding energy were selected sharing the maximum number of residues (**Table 1**).

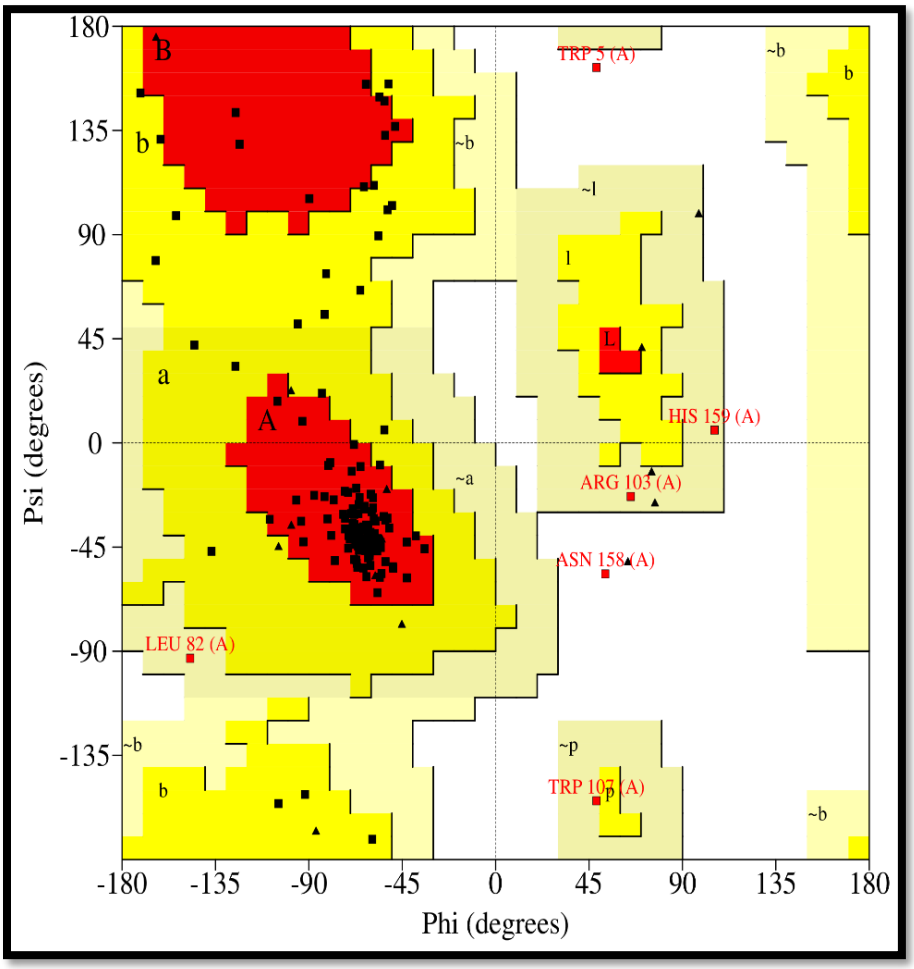

Figure 2: Ramachandran plot of the 3D predicted structure revealing different regions of the residue

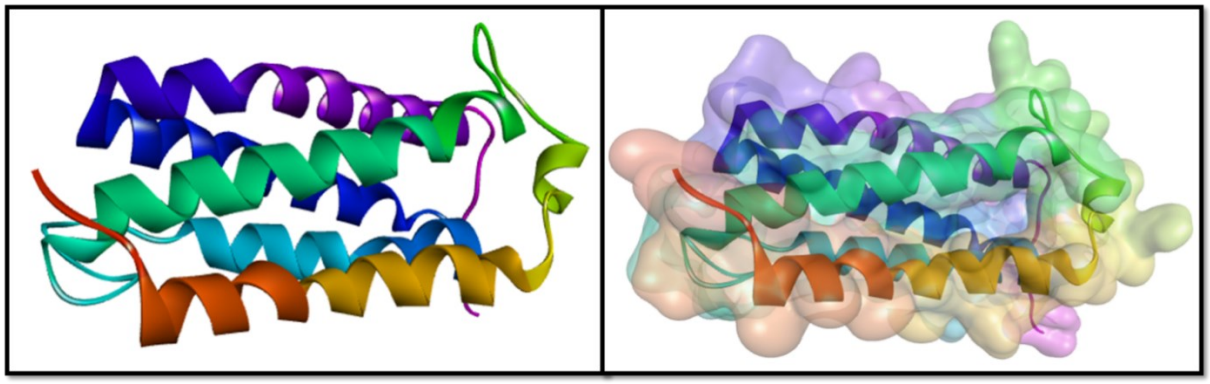

Figure 3: 3D predicted protein structure of the target protein

Table 1: Binding energy and binding site residues of the top 5 compounds.

Molprot ID's	Binding Energy (Kcal/mol)	Binding site Residues
029-885-912	-8.7	Gly-50, Gly-22, Cys-19, Trp-53, Tyr-57, Pro-15, Ser-16, Thr-12, Leu-13, Ser-58, Gly-54, Thr-55, Pro-51
029-885-996	-8.3	Cys-19, Gly-54, Trp-53, Tyr-57, Trp-95, Pro-96, Phe-100, Gly-22, Gly-50, His-46, Ser-23, His-27, Trp-47, Val-26, Pro-51
029-886-352	-8.7	Gly-54, Thr-12, Pro-15, Leu-13, Ser-16, Cys-19, Asn-92, Trp-95, Pro-96, Trp53, Gly-18, Tyr-57, Gly-22, His-46, Val-26, Gly-50, Ser-23, Pro-51
029-886-365	-8.2	Phe-100, Trp-95, His-27, Ser-23, Val-26, Pro-51, His-46, Gly-54, Cys-19, Tyr-57, Gly-18, Gly-50, Gly-22, Trp-53
029-886-398	-8.3	Trp-95, Pro-96, Tyr-57, Gly-50, Gly-18, Ser-16, Pro-15, Cys-19, Gly-54, Pro-51, Ser-23, His-46, Val-36, Gly-22, Trp-53

The top-ranked 5 compounds had reliable interactions with the target protein and shared a high number of residues including Gly-22, Cys-19, Trp-53, Tur-57, Gly-54, and Pro-51. According to the molecular studies and interactional analysis, the top-ranked five compounds showed reliable results and were selected for further analysis. ADMET analysis was performed and the toxicity of the top-ranked selected compounds along with different properties that play crucial roles in the drug discovery process were calculated and critically analyzed in order to screen a compound that can be utilized as a drug candidate to treat schizophrenia (**Table 2**).

According to ADMET analysis, all the compounds showed reliable results although the compound having the best interactions and ADMET properties were further analyzed, and logical conclusions were deduced from the screened bioactive compound (**Fig. 4**).

Table 2: ADMET properties of the top ranked 5 compounds.

ADMET properties	029-885-912	029-885-996	029-886-352	029-886-365	029-886-398
H-bond acceptors	8	8	9	10	8
H-bond donors	4	4	4	4	4
Neurotoxicity	0.83	0.73	0.78	0.86	0.88
Respiratory toxicity	0.67	0.76	0.66	0.64	0.82
Carcinogenicity	0.53	0.79	0.73	0.63	0.55
Cytotoxicity	0.76	0.73	0.71	0.79	0.84
Cytochrome CYP1A2	0.79	0.89	0.83	0.80	0.88

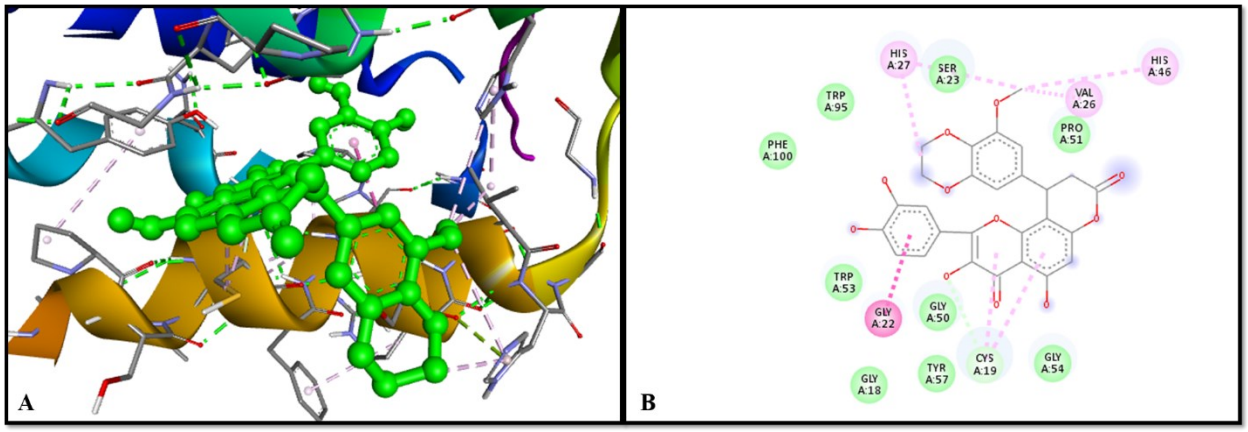

Figure 4: A) Binding pocket and interacting regions of target protein, **B)** 2D diagram of the protein-ligand complex

TPSO is considered a potential therapeutic target in order to treat SZ, in the current analysis computational approaches were utilized to screen potential compounds against schizophrenia by targeting TPSO. Computational analysis aids biological sciences by finding solutions to complex biological problems. Conventional drug design methods are costly and time-consuming; however, computational drug design approaches are cost-effective and consume less time to narrow down and make logical conclusions. In

current studies, 3D structure prediction of TPSO protein was performed to gain structural insights of the protein. The structure prediction of the target protein showed reliable results. After selecting the suitable structure, the binding site of the protein was observed and analyzed through molecular docking analysis. Molecular docking analysis was performed against the natural compound library and the binding site residues of the target protein were observed. The molecular docking analysis revealed that residue Gly-22, Cys-19, Trp-53, Tur-57, Gly-54, and Pro-51 played crucial roles in the interaction between protein and small compounds. All compounds from the natural compound library were binding at similar pockets sharing the maximum number of residues. After analyzing the interactions and binding energies top top-ranked five compounds were selected and ADMET analyses were performed to evaluate the compounds based on toxicity and other metabolic properties. Detailed computational analysis suggested that the compound 029-886-365 may be potent against schizophrenia by targeting TPSO.

Conclusion

After performing the current computational analysis, efforts were made to screen novel compounds against TSPO in order to treat SZ and according to the analysis, it is observed that the reported natural compound shows strong binding with the target protein and may prove potent against schizophrenia by targeting TSPO protein. However, the computational experiments showed reliable results to suggest that the reported natural compound may prove effective against SZ by targeting TSPO translocator protein, and considering the above-mentioned results during experimental studies may expect similar studies.

Acknowledgments

The authors acknowledge Mr. Hassaan Bin Waseem for continued guidance and support throughout this project.

Conflict of interest

The authors declare no conflict of interest.

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