Targeting CSF-1 with Small Molecules to Inhibit Interaction with CSF-1R in Order to Find Novel Therapies for Alzheimer's Disease

Kelly Feng

Received December 28, 2024
Accepted June 22, 2025
Electronic access September 15, 2025

Alzheimer's disease is a neurodegenerative disease caused by many factors, the pathology focused on in this paper is the activation of inflammatory proteins. There has been minimal progress made on treatment for this disease, with only 2 main symptom-repressing medications: cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) antagonists. However, there is still a 98% clinical treatment failure for Alzheimer's, indicating the unmet need to find new therapies. Recent studies have found that colony-stimulating factor 1 receptor (CSF-1R), one of the main expressions in microglia and a glial cell found in the brain, can be inhibited to slow neuroinflammation. As part of a drug discovery effort, this study aims to identify small molecules that bind to CSF-1 with the future goal of evaluating their ability to inhibit downstream signaling. These effects can later be compared to direct CSF-1R inhibition to assess their potential in reducing neuroinflammation and treating Alzheimer's disease. Computational tools and virtual screening were used to find potential binding spots for CSF-1. First, Pocketquery and ZINCPharmer found the 20 compatible compounds that bind CSF-1. From these 20, Swissdock predicted the molecular interactions between the target protein and a small molecule and analyzed the Gibbs free energy values. The following results' druggability, found by Lipinski's Rules, and predicted toxicity were analyzed in SwissADME and Protox respectively. They all had good results. This suggests that the resulting top 2 compounds, ZINC39990688 and ZINC57675840, are good potential candidates for binding CSF-1 based on their overall results. Future research will focus on subjecting the top 2 compounds to biophysical and cellular screening, followed by animal model testing.

Keywords: Alzheimer's disease, Drug Discovery, Small molecules, Virtual screening, Computational tools, Neuroinflammation, CSF-1R

1 Introduction

Alzheimer's is a dangerous and widespread neurodegenerative disease that causes memory loss, behavioral changes, and inhibits language processing abilities. It is the most common form of dementia, the loss of cognitive function. It has an annual global cost of $\sim$ \$1 \$ trillion[1,2]. Currently, around 50 million people worldwide have Alzheimer's, a number expected to double every 5 years and increase to 152 million by 2050[3].

Alzheimer's has numerous pathologies that play a part in cognitive decline[4,5]. The one this study focuses on is neuroinflammation. Alzheimer's disease is characterized by multiple pathological mechanisms that contribute to cognitive decline, with neuroinflammation being the key factor focused on in this paper. In response to aggregated amyloid-beta (Aβ) peptides, glial cells such as microglia and astrocytes become activated and release inflammatory mediators, including cytokines and chemokines. This chronic inflammation disrupts normal brain function, impairs Aβ clearance, and causes neuronal damage[6–8].

Beyond neuroinflammation, several other mechanisms contribute to Alzheimer's pathology. The amyloid-beta hypothesis suggests that misfolded Aβ peptides aggregate into plaques, disrupting synaptic function and triggering oxidative stress, which leads to cellular damage[5,9]. The cholinergic hypothesis says that cognitive decline in Alzheimer's is driven by the loss of cholinergic neurons and reduced acetylcholine levels[10]. Tau pathology is another major component that accelerates this progression. Tau proteins, which normally stabilize microtubules in neurons, get hyperphosphorylated. This leads to neurofibrillary tangles that destabilize microtubules and impair neuronal transport, ultimately destabilizing the cytoskeleton, disrupting neuron transport, and contributing to synaptic dysfunction. The accumulation of these disruptions can lead to specialized transporter proteins in the blood-brain barrier (BBB) to keep essential nutrients, like glucose, from reaching the brain and prevent the clearing of toxic beta-amyloid and tau proteins[11**?**]. Over time, these interconnected pathological changes accumulate, ultimately leading to widespread neuronal death and cognitive decline[8].

Currently, there is nothing able to completely cure the underlying causes of Alzheimer's, only medications that help manage symptoms, such as cholinesterase inhibitors and N-methyl-D-

aspartate (NMDA) antagonists [12–16]. One critical new treatment on Alzheimer's being studied is the expression of colony stimulating factor 1-receptor (CSF-1R) in microglia, prominent immune cells in the central nervous system that control brain homeostasis and cleanse cellular waste [17]. CSF-1R, one of the main expressions on microglia, is critical for helping the cells proliferate, regulate neuron function, and develop nervous tissue [18]. In response to threats, CSF-1R expression in microglia increases and produces inflammatory proteins that lead to chronic neuroinflammation. Because of this, both are strongly connected to many neurodegenerative diseases [19]. New studies have found that CSF-1R inhibitors reduce the phosphorylation of CSF-1R and its downstream signaling pathways, such as PI3K-AKT, ERK1/2 and JAK/STAT, which promote cellular proliferation, survival, and differentiation; this overall leads to reduced neuroinflammation and potentially improved cognition [20–22]. A mouse model study conducted by Jinming Han et al. saw CSF-1R inhibitors decreasing microglia by 30%-100% [22]. This study and one by Adrian Olmos-Alonso et al. done on CSF-1R inhibitors also saw a reduction in hippocampal-dependent memory deficits, neural loss, inflammatory transcripts and cytokines, plaque burden, inflammatory gene expression, and synaptic degeneration [23]. So far, the inhibition of CSF-1R has produced promising results in preventing cognitive decline in clinical studies, indicating its value in the field of possible medications for neurodegenerative diseases like Alzheimers.

This study aims to identify small molecules that bind CSF-1 with the future goal of assessing their impact on downstream signaling inhibition. In this experiment, protein-protein interactions were studied rather than drugs or marketed CSF-1R antagonists to identify the investigated compounds. Thus, comparing compound binding sites to CSF-1R's CSF-1 interaction is not needed and validation with marketed CSF-1 antagonists is not relevant. This study targets the ligand CSF-1, instead of the receptor because it can serve as an indirect strategy to suppress receptor activation and signaling without completely disrupting the receptor's normal physiological functions. This assesses its functional role, helping to validate the receptor as a therapeutic target. There is currently also not much research into this yet, further indicating studies like these are necessary. Studying CSF-1 inhibition alongside CSF-1R targeting may help enhance the effectiveness of current CSF-1R inhibitors, such as PLX3397, which inhibits pathways like C-KIT, PDGFR and FLT3 but have been associated with adverse effects like hepatotoxicity, by potentially reducing toxicity and improving therapeutic outcomes. Additionally, the mouse model study by Jinming Han et al. showed region-specific microglial loss following CSF-1 functional blockade or genetic targeting. Their findings also suggest that CSF-1 may contribute to microglial expansion through autocrine signaling mechanisms [22].

Compounds targeting the CSF-1 binding site were identified using pharmacophore modeling, and then further evaluated for their molecular interactions, druggability, and toxicity to assess their safety and suitability for drug study and use.

2 Results and Discussion

2.1 Results and Discussion for Pocketquery 0.896664, 0.794025, 0.787968, and 0.702748 and Corresponding ZINCPharmer Compounds for CSF-1

Pocketquery was used to find spots for protein-protein interactions between CSF-1 and small molecules. It calculates a score from zero to one by analyzing the properties of overlapping residues with small-molecule binding sites in ligand-bound structures to estimate the likelihood that chemical mimicry of the cluster would result in a small-molecule inhibitor. Higher scores indicate consistency and compatibility between the properties and the overlapping clusters [24]. A variety of Pocketquery scores were chosen for diversity and thoroughness: one with a score of 0.896664, one with 0.794025, another with 0.787968, and the last with a score of 0.702748, as shown in figure 1. These were then exported to ZINCPharamer, which finds compounds in the ZINC database using pharmacophore search technology. A pharmacophore is the arrangement of the necessary features of an interaction. ZINCPharamer includes the feature types hydrophobic, hydrogen bond donor/acceptor, and aromatic. Lower ZINCPharmer RMSD scores indicated less pharmacophore map deviation. The mass affects how well a compound fits the shape and pharmacophore space, and the number of rotatable bonds (RBnds) influences binding strength, as higher flexibility can lead to entropy penalties that reduce binding affinity. They both affect how well a molecule fits and interacts with the binding site and the resulting scores [25,26].

The 20 ZINCPharmer compounds were found by clicking the RMSD twice to sort the compounds by highest Gibbs value to lowest; to ensure diverse results, some of the lowest RMSD score compounds (e.g., 0.001) were used and some bigger ones were used (e.g., 0.101, 0.152). Table 1 includes the Pocketquery scores, corresponding ZINCPharmer IDs, RMSD scores, RBnds, and mass. The top Pocketquery result with the score of 0.896664 had HydrogenDonor, "HydrogenAccepter, and Aromatic. 5 ZINCPharmer results were used from this group; one of the more promising ones had a RMSD score of 0.001, 9 RBnds, and a mass of 451. The pharmacophore maps for all Pocketquery results are shown in figure 2. Using the results from the experiment, evaluation of the 20 ZINCPhamer compounds can be done to gain more knowledge on the binding specifications of CSF-1.

Figure 1 visualizes small molecules in CSF-1 protein binding pockets. Figure 2 section 1 shows the 5 pharmacophore maps found for 0.896664, section 2 shows 5 pharmacophore maps found for scores 0.794025, section 3 shows the 6 pharmacophore maps found for 0.787968, section 4 shows the 4 pharmacophore

Table 1 20 ZINCPharmer Compounds That Bind CSF-1 with RMSD Scores (PDB ID: 4WRM)

	ZINC ID	Root Mean Square Deviation (RMSD)	Rotatable bonds (RBnds)	Mass
Pocketquery 0.896664 Size 1 (TYR 6) Dist 0	ZINC33271702	0.001	9	451
Pocketquery 0.896664 Size 1 (TYR 6) Dist 0	ZINC06550301	0.001	7	385
Pocketquery 0.896664 Size 1 (TYR 6) Dist 0	ZINC92594700	0.001	11	498
Pocketquery 0.896664 Size 1 (TYR 6) Dist 0	ZINC11866333	0.001	10	400
Pocketquery 0.896664 Size 1 (TYR 6) Dist 0	ZINC43761295	0.001	7	317
Pocketquery 0.794025 Size 1 (LEU 85) Dist 0	ZINC39990688	0.01	4	374
Pocketquery 0.794025 Size 1 (LEU 85) Dist 0	ZINC92532202	0.005	5	306
Pocketquery 0.794025 Size 1 (LEU 85) Dist 0	ZINC92514950	0.009	5	306
Pocketquery 0.794025 Size 1 (LEU 85) Dist 0	ZINC86165953	0.01	12	202
Pocketquery 0.794025 Size 1 (LEU 85) Dist 0	ZINC36612309	0.01	7	268
Pocketquery 0.787968 Size 2 (TYR 6, HIS 9) Dist 7.2702	ZINC92532202	0.007	6	385
Pocketquery 0.787968 Size 2 (TYR 6, HIS 9) Dist 7.2702	ZINC43553817	0.101	14	491
Pocketquery 0.787968 Size 2 (TYR 6, HIS 9) Dist 7.2702	ZINC15079784	0.069	7	358
Pocketquery 0.787968 Size 2 (TYR 6, HIS 9) Dist 7.2702	ZINC71415573	0.038	6	347
Pocketquery 0.787968 Size 2 (TYR 6, HIS 9) Dist 7.2702	ZINC41007105	0.176	12	342
Pocketquery 0.787968 Size 2 (TYR 6, HIS 9) Dist 7.2702	ZINC64540858	0.152	10	457
Pocketquery 0.702748 Size 2 (LEU 85, ARG 86) Dist 5.6775	ZINC74103384	0.008	7	378
Pocketquery 0.702748 Size 2 (LEU 85, ARG 86) Dist 5.6775	ZINC89572453	0.008	4	320
Pocketquery 0.702748 Size 2 (LEU 85, ARG 86) Dist 5.6775	ZINC57675840	0.009	9	363
Pocketquery 0.702748 Size 2 (LEU 85, ARG 86) Dist 5.6775	ZINC91768992	0.016	4	320

maps found for 0.702748. The green pharmacophore is a hydrophobic molecular feature, violet is aromatic, the yellow is a hydrogen bond acceptor, and the white is a hydrogen bond donor. These figures are important because they show critical interactions needed for activity[26].

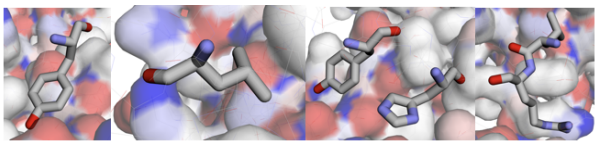

Fig. 1 Pocketquery CSF-1 (PDB ID: 4WRM) Chain B Scores 0.896664, 0.794025, 0.787968, 0.702748 (left to right)

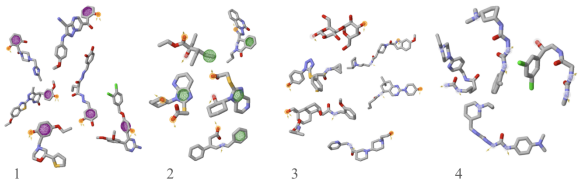

Fig. 2 Pocketquery Pharmacophore Maps (1): ZINC33271702, ZINC06550301, ZINC92594700, ZINC11866333, and ZINC43761295; (2) ZINC39990688, ZINC92532202, ZINC92514950, ZINC86165953, and ZINC36612309 with CSF-1; (3) ZINC92532202, ZINC43553817, ZINC15079784, ZINC71415573, ZINC41007105, and ZINC64540858; and (4) ZINC74103384, ZINC89572453, ZINC57675840, and ZINC91768992 with CSF-1 (PDB ID: 4WRM)

2.2 Swissdock Results and Discussion for Top 20 Compounds

SwissDock was used to predict molecular interactions between CSF-1 and the 20 ZINCPharmer compounds. For each compound, SwissDock generated all found binding site clusters and binding poses (elements) with corresponding Gibbs free energy values (an indicator of thermodynamic favorability). SwissDock estimates Gibbs free energy values by evaluating multiple energy components including electrostatics, van der Waals interactions, desolvation effects, and internal ligand strain. The cluster with the lowest Gibbs free energy value was recorded as an indicator of binding strength because lower scores indicate stronger binding affinity with less deviation[27].

The 20 ZINCPharmer results were entered into Swissdock, and the following are the 5 results with the best Gibbs free energy scores: ZINC92532202 had 34 total clusters and the cluster with the lowest Gibbs result was -9.31 kcal/mol with 5 elements, ZINC39990688 had 33 total clusters and the lowest Gibbs result was -11.78 kcal/mol with 2 elements, ZINC91768992 had 52 total clusters and the lowest Gibbs result was -9.45 kcal/mol with 5 elements, ZINC57675840 had 35 total clusters and the lowest Gibbs result was -9.37 kcal/mol with 3 elements, and ZINC64540858 had 36 total clusters and the lowest Gibbs result was -10.81 kcal/mol with 2 elements. All results are on table 2.

All Gibbs values found were between 6.67 kcal/mol (ZINC86165953) and 11.78 kcal/mol (ZINC39990688), and aside from the outlier with a Gibbs value of 6.67 kcal/mol, the rest were around 8 or above, indicating very good binding potential.

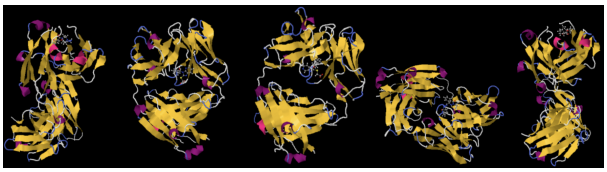

Fig. 3 Swissdock Results for the Interaction of (A) ZINC33271702, (B) ZINC06550301, (C) ZINC92594700, (D) ZINC11866333, and (E) ZINC43761295 with CSF-1 (PDB ID: 4WRM)

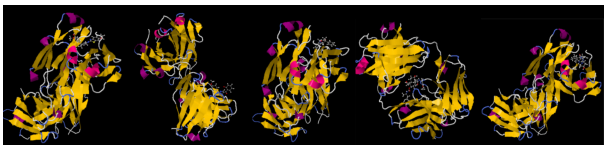

Fig. 4 Swissdock Results for the Interaction of (A) ZINC39990688, (B) ZINC92532202, (C) ZINC92514950, (D) ZINC86165953, and (E) ZINC36612309 with CSF-1 (PDB ID: 4WRM)

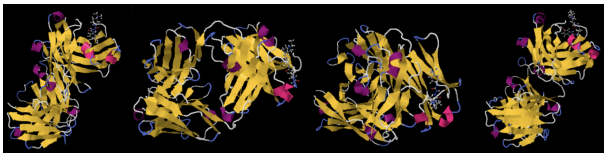

Fig. 5 Swissdock Results for the Interaction of (A) ZINC74103384, (B) ZINC89572453, (C) ZINC57675840, and (D) ZINC91768992 with CSF-1 (PDB ID: 4WRM)

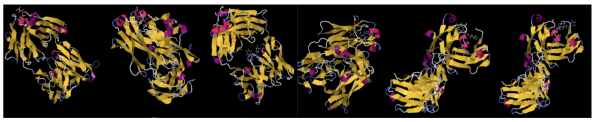

Fig. 6 Swissdock Results for the Interaction of (A) ZINC92532202, (B) ZINC43553817, (C) ZINC15079784, (D) ZINC71415573, (E) ZINC41007105, and (F) ZINC64540858 with CSF-1 (PDB ID: 4WRM)

2.3 SwissADME Results and Discussion for Top 5 Compounds

The 5 top results from the previous experiment were sent to SwissADME to check for good druggability properties[28]. To test for good absorption and permeation, Lipinksi's Rules were

Table 2 Swissdock Predicted Binding Modes for the Top 20 Compounds Binding CSF-1 (PDB ID: 4WRM)

ZINCPharmer Result	# of Clusters	Estimated ΔG (kcal/mol)	Elements
ZINC39990688	33	-11.78	2
ZINC64540858	36	-10.81	2
ZINC91768992	52	-9.45	5
ZINC57675840	35	-9.37	3
ZINC92532202	34	-9.31	5
ZINC74103384	55	-9.12	7
ZINC92514950	36	-8.87	5
ZINC11866333	33	-8.59	2
ZINC71415573	45	-8.53	2
ZINC43553817	36	-8.52	1
ZINC43761295	33	-8.3	3
ZINC06550301	38	-8.15	1
ZINC89572453	40	-8.13	3
ZINC92122254	43	-8.08	3
ZINC33271702	36	-8.07	1

used: no more than 5 H-bond donors, logarithm of the partition coefficient (LogP) no more than 5, molecular mass less than 500 daltons (g/mol), and no more than 10 H-bond acceptors. More than 5 H-Bond donors and 10 H-Bond Acceptors can increase polarity and thus affect absorption and permeability. LogP measures how well a compound can dissolve in nonpolar solvents compared to water; a score below 5 indicates a good balance between the water solubility and the fat solubility. Lastly, the molecular mass must be below 500 daltons as greater weights result in poorer absorption. All results showed no violation of the rules above.

The compounds with the best results were ZINC39990688 (ΔG -11.78 kcal/mol) with 6 H-Bond acceptors, 1 H-bond donor, LogP of 2.84, a molecular mass of 373.39, high GI absorption, and was moderately soluble as shown in figure 7-1; and ZINC57675840 (ΔG -9.45 kcal/mol) with 3 H-Bond acceptors, 4 H-bond donors, LogP of 2.39, molecular mass of 363.48, high GI absorption, and was very soluble as shown in figure 7-3. All data is shown in table 3. The results also all had good solubility and high gastrointestinal (GI) absorption except ZINC64540858. Even so, all the results are still excellent drug candidates because they follow Lipinski's Rules.

2.4 Protox Results and Discussion for Toxicity Predictions of ZINC39990688 and ZINC57675840

ZINC39990688 and ZINC57675840, the most promising compounds found thus far, were sent to Protox to analyze toxicity predictions. The LD50, the amount of something that is lethal to half of the experimental subjects, was measured. An LD50 score greater than 100 mg/kg is recommended as higher values indicate lower toxicity and require larger doses to be lethal.

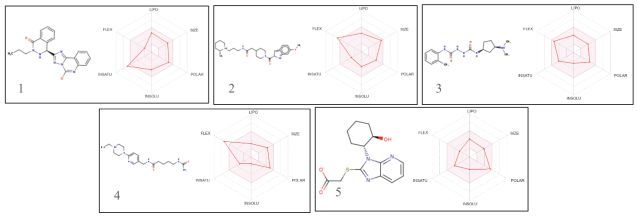

Fig. 7 (1) ZINC39990688 (ΔG -11.78 kcal/mol), (2) ZINC64540858 (ΔG -10.81 kcal/mol), (3) ZINC57675840 (ΔG -10.81 kcal/mol), (4) ZINC57675840 (ΔG -9.37 kcal/mol), (5) ZINC92532202 (ΔG -9.31 kcal/mol) SwissADME Results

Additionally, the predicted toxicity class (1-5) is related to the LD50 score, with higher classes meaning higher LD50 scores. Above class 3 was required in this case. Areas of potential toxic activity were also observed and measured in terms of how toxic they are compared to the average for active molecules[29].

ZINC39990688 had a predicted LD50 of 300 mg/kg, level 3 toxicity class, and 7 predicted active toxicity targets: neurotoxicity, nephrotoxicity, respiratory toxicity, carcinogenicity, mutagenicity, BBB-barrier, and GABA receptor (GABAR). The compound exceeded the average respiratory toxicity amount by a couple percent while the rest remained below the average for active molecules, meaning they are predicted to be non-toxic in that category. This data is shown in table 4 and figure 8. ZINC64540858 had a predicted LD50 of 1000 mg/kg, level 4 toxicity class, and 4 predicted active toxicity targets: neurotoxicity, respiratory toxicity, immunotoxicity, and BBB-barrier. All toxicity percentages for the active targets remained below the average for active molecules. This is shown in table 4 and figures 8 and 9.

Table 3 SwissADME Lipinski's Rules Druggability Results for Top 5 Compounds

	H-Bond Acceptors	H-Bond Donors	LogP	Molecular Mass (g/mol)	Water Solubility	GI Absorption	BBB Permeant	Druglikeness (Lipinski)
ZINC39990688 (ΔG -11.78 kcal/mol)	6	1	2.84	373.39	Moderately Soluble	High	No	Yes 0 Violation
ZINC64540858 (ΔG -10.81 kcal/mol)	3	3	4.52	456.62	Soluble	Low	No	Yes 0 Violation
ZINC57675840 (ΔG -9.37 kcal/mol)	3	4	2.39	363.48	Very Soluble	High	No	Yes 0 Violation
ZINC92532202 (ΔG -9.31 kcal/mol)	5	1	1.98	306.36	Soluble	High	No	Yes 0 Violation
ZINC91768992 (ΔG -9.45 kcal/mol)	2	5	2.12	320.41	Soluble	High	No	Yes 0 Violation

Table 4 Protox Toxicity Predictions for LD50, Toxicity Class, and Active Toxicity Targets for Compounds ZINC39990688 and ZINC64540858

	Predicted LD50 (mg/kg)	Predicted Toxicity Class	# of Predicted Activities with Active Toxicity	Predicted Active Toxicity Targets
ZINC39990688 (ΔG -11.78 kcal/mol)	300	3	7	Neurotoxicity, Nephrotoxicity, Respiratory toxicity, Carcinogenicity, Mutagenicity, BBB-barrier, GABA receptor (GABAR)
ZINC57675840 (ΔG -10.81 kcal/mol)	1000	4	4	Neurotoxicity, Respiratory toxicity, Immunotoxicity, BBB-barrier

ZINC39990688, otherwise the most promising compound with a high Gibbs score and druggability, seems to be more toxic than ZINC64540858, but overall, both results remain promising. This toxicity information can be used to determine the potential limitations to the druggability of both compounds. Current known CSF-1R inhibitors like PLX3397 show hepatotoxicity, while these 2 molecules don't[22]. Potential structural modifications to reduce toxicity include reducing electronic density, modifying specific chemical groups, and improving binding selectivity[30].

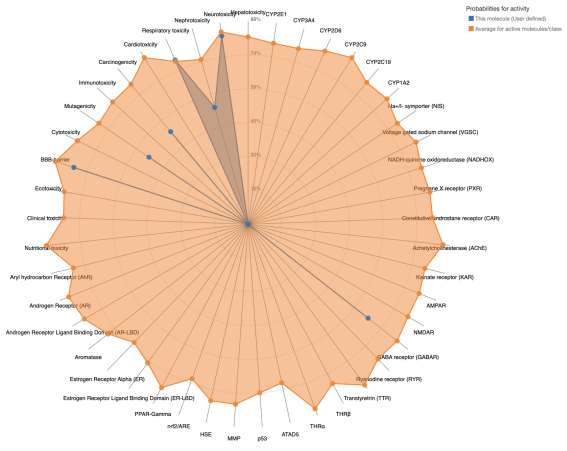

Fig. 8 ZINC39990688 ProTox Toxicity Radar Chart with 7 Active Toxic Predictions and Respiratory Toxicity Exceeding Average

3 Discussion

Alzheimer's is a neurodegenerative disease that is caused partially due to inflammation in the brain. Much of this inflammation is driven by abnormally active microglia. Recent studies have shown that one of their key surface receptors, CSF-1R, can be inhibited to help delay or reduce neuroinflammation. The goal of the experiment was to find small molecules with good

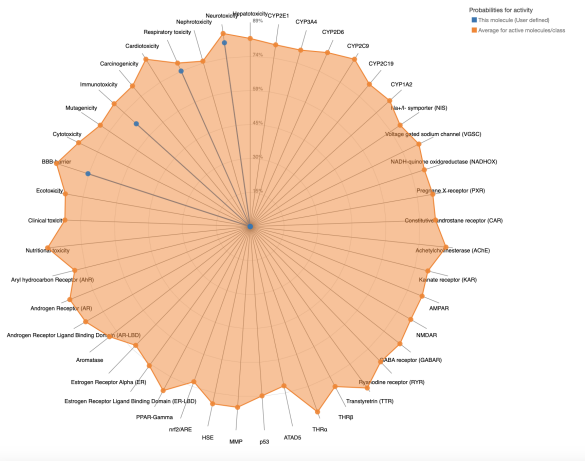

Fig. 9 ZINC39990688 ProTox Toxicity Radar Chart with 7 Active Toxic Predictions and Respiratory Toxicity Exceeding Average

molecular interactions and druggability to CSF-1 in order to mimic inhibitors for CSF-1R with the future goal of comparing their effects to direct CSF-1R inhibition to help evaluate their potential to reduce or slow neuroinflammation and contribute treatments for Alzheimer's disease. Two suitable compounds were indeed found, showing that this study helps further research into CSF-1 and CSF-1R and how they can potentially help slow or even stop Alzheimer's.

First, Pocketquery found areas of protein-protein interaction for CSF-1; 5 of the top Pocketquery results were used and had scores of 0.896664, 0.794025, 0.787968, and 0.702748. From these 5 results, millions of ZINCPharmer pharmacophore maps and compounds were found, and only 20 were used. Swissdock, which predicts molecular interactions between compounds and small molecules, narrowed the 20 compounds to 5 compounds. The top 5 Swissdock results with the best Gibbs value were ZINC39990688 (ΔG -11.78 kcal/mol), ZINC64540858 (ΔG -10.81 kcal/mol), ZINC91768992 (ΔG -9.45 kcal/mol), ZINC92532202 (ΔG -9.31 kcal/mol), and ZINC57675840 (ΔG -9.37 kcal/mol). All five had good druggability when tested with SwissADME and followed Lipinski's Rules. The two best compounds from SwissADME also showed good toxicity predictions via ProTox. The research objective was met as potential compounds that bind CSF-1 were found and had good druggability results.

However, the study had many limitations. The Pocketquery scores found for CSF-1 were not the most ideal; only one scored above 0.8 while the rest were between 0.7-0.795. The corresponding ZINCPharmer compounds found were also in the millions and some good results could have been missed. Furthermore, when the 5 final results' druggability were tested, all except ZINC64540858 had high gastrointestinal (GI) absorption. This means ZINC64540858 is incompatible with oral administration. In addition, all final results showed low toxicity to the BBB barrier and neurotoxicity, however, all values were still within the acceptable (orange) range, indicating it is predicted to be non-toxic. Moreover, both compounds were not BBB-permeant, so follow up in vitro BBB transport studies are necessary to test their therapeutic viability for Alzheimer's disease. SwissDock ΔG values are also derived from single-point calculations and do not account for receptor flexibility or thermal averaging. To address this limitation, complementary approaches such as molecular dynamics simulations and ensemble docking can be employed to capture a more accurate and dynamic picture of protein-ligand interactions. Lastly, all experimentation was done online through computational tools and virtual screening, therefore, most results are predictions based on scientific databases. These databases, however, are all used professionally and thus source credibility.

Some future follow up experiments include comparing the CSF-1 and small molecule binding to direct CSF-1R inhibition to help evaluate the potential to reduce neuroinflammation and further Alzheimer's disease therapies, and conducting a more in-depth review/analysis of the 20 Pocketquery and ZINCPharmer results to find better connections and similarities between compounds. Additionally, since Gibbs values are theoretical predictions, a follow up study on binding affinity for the compounds using molecular dynamics simulations could be employed to further assess the stability of ligand-protein interactions over time. Kinetic stability, enthalpic contributions, and water solvation effects can also be done for further studies In addition, in-person biophysical screening labs could also help validate the binding between the compounds and the target protein CSF-1 by purchasing samples of CSF-1, ZINC39990688, and ZINC57675840 and conducting Surface Plasmon Resonance (SPR), Microscale Thermophoresis (MST), Isothermal Titration Calorimetry (ITC), and cell based assay tests. The real time binding interactions can be seen between the compounds and CSF-1 during SPR, the structures of the compounds can be studied in detail with MST, the thermodynamics of the binding can be analyzed with ITC, and the compounds' biological effects on different cell lines can be studied with the cell based assay tests. These will provide a more in-depth study of the compounds' binding specifics. Chemical clustering can be another follow up experiment that helps group similar molecules together based on their chemical structure and compare them. For this, we can prioritize commercially available compounds. Additionally, RCSB PDB can later be used to analyze common structural motifs between the two found molecules and other ligands to look for similar interactions with CSF-1 binding sites. Decoys can also be generated using DUD-E to validate the virtual screening protocol and assess the ability of the computational pipeline to distinguish true binders from non-binders, as well as to estimate false positive rates. Future cross-docking or consensus scoring sites like Autodock can also be used to verify binding poses. In

terms of toxicity, another follow up experiment can compare toxicity profiles with known CSF-1R inhibitors. Later, a follow up mouse model study could be done with ZINC39990688 and ZINC57675840. The experiment could also be redone with a focus on CSF-1R.

By finding compounds that bind CSF-1 to later compare the downstream effects to CSF-1R inhibition's effects, potential treatment dealing with the inhibition of CSF-1R expression on microglia for Alzheimer's disease can be studied and improved.

4 Methods

The research was an in silico virtual screening study using computational tools. These methods and tools were chosen because of their professionalism, thoroughness, and extensive/large databases. Pocketquery, ZINCPharmer, Swissdock, SwissAME, and Protox were used in this research project. The final compounds were measured by their Pocketquery scores, Gibbs values, RMSD-scores, compliance of Lipinski's Rules, and toxicity predictions. They were measured based on how well they bind CSF-1 and how safe they are on humans. Both statistical tests and qualitative analysis were used to predict relationships between compounds, their effects, and chemical and biological observations of the data.

First, Pocketquery was used to find residues for CSF-1 and the results were exported to ZINCPharmer to find 20 total Pharmacophore maps for specific compounds. After, Swissdock was used to predict the molecular interactions and Gibbs values for the 20 compounds. Afterwards, using the ZINC IDs of the top 5 compounds found from Swissdock, druggability results were found via SwissADME. Finally, these 5 compounds were narrowed down to 2 before Protox toxicity predictions.

All references used were specifically sourced from credible cites such as PubMed, CDC, various hospital websites, and scientific journals. Older references were used to collect general information. More concept specific sources, especially those researching CSF-1R, were recent to ensure accurate and up to date data. The virtual screening and computational tool databases used were also professional websites.

5 Acknowledgments

I would like to thank Dr. Moustafa Gabr (Weill Cornell Medicine) for mentoring and supporting this project.

References

1 V. J. De-Paula *et al.*, *Alzheimer's disease*, 2012.

2 L. Gustafson, *What is dementia?*, 1996.

3 Centers for Disease Control and Prevention, *About Dementia*, 2024, https://www.cdc.gov/alzheimers-dementia/about/index.html, Accessed: 2025-08-27.

4 P. Scheltens, B. De Strooper, M. Kivipelto, H. Holstege, G. Chetelat, C. E. Teunissen, J. Cummings and W. M. van der Flier, *Alzheimer's disease*, 2021.

5 Mayo Clinic Staff, *Alzheimers disease – Symptoms and causes*, 2024, https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447, Accessed: 2025-08-27.

6 S. Bhatia *et al.*, *Mitochondrial Dysfunction in Alzheimer's Disease: Opportunities for Drug Development*, 2022.

7 L. Zhan *et al.*, *Autophagy therapeutics: preclinical basis and initial clinical studies*, 2018.

8 J. Xie, L. V. Hoeke and R. E. Vandenbrouke, *The impact of systemic inflammation on Alzheimers disease pathology*, 2021, https://www.frontiersin.org/articles/10.3389/fimmu.2021.796867/full.

9 G.-F. Chen *et al.*, *Amyloid beta: structure, biology and structure-based therapeutic development*, 2017.

10 A. V. T. Jr and J. J. Buccafusco, *The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development*, 2003.

11 Mayo Clinic, *Alzheimers disease*, 2024, https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447.

12 A. Sobue, O. Komine and K. Yamanaka, *Neuroinflammation in Alzheimers disease: microglial signature and their relevance to disease inflammation and regeneration*, 2023, https://biomedcentral.com/articles/10.1186/s41232-023-00277-3.

13 Z. Breijyeh and R. Karaman, *Comprehensive Review on Alzheimer's Disease: Causes and Treatment*, 2020.

14 F. R. Buccellato *et al.*, *Treatment of Alzheimer's Disease: Beyond Symptomatic Therapies*, 2023.

15 Clinical Leader, *Why do so many Alzheimers clinical trials fail?*, 2024, https://www.clinicalleader.com/doc/why-do-so-many-alzheimer-s-clinical-trials-fail-0001.

16 B. E. Jewett and B. Thapa, *Physiology, NMDA Receptor*, 2022.

17 R. Wang and P. H. Reddy, *Role of Glutamate and NMDA Receptors in Alzheimer's Disease*, 2017.

18 M. Augusto-Oliveira *et al.*, *What Do Microglia Really Do in Healthy Adult Brain?*, 2019.

19 B. Hu *et al.*, *Insights Into the Role of CSF1R in the Central Nervous System and Neurological Disorders*, 2022.

20 C.-Y. Wen *et al.*, *CSF-1R: A promising therapeutic target for various diseases*, 2024.

21 J. Miao, H. Ma, Y. Yang, Y. Liao, C. Lin, J. Zheng, M. Yu and J. Lan, *Microglia in Alzheimers disease: pathogenesis, mechanisms, and therapeutic potentials*, 2023, https://www.frontiersin.org/journals/aging-neuroscience/articles/10.3389/fnagi.2023.1201982/full.

22 J. Han *et al.*, *Inhibition of colony stimulating factor-1 receptor (CSF-1R) as a potential therapeutic strategy for neurodegenerative diseases: opportunities and challenges*, 2022.

23 A. Olmos-Alonso *et al.*, *Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology*, 2016.

24 University of Pittsburgh, *PocketQuery Help*, 2012, `http://pocketquery.csb.pitt.edu/help.html`.

25 D. R. Koes and C. J. Camacho, *ZINCPharmer: pharmacophore search of the ZINC database*, 2012.

26 ZINCPharmer, *Welcome to ZINCPharmer*, 2012, `http://zincpharmer.csb.pitt.edu/`.

27 SwissDock, *SwissDock*, `http://www.swissdock.ch/`.

28 SwissADME, *SwissADME*, 2017, `http://www.swissadme.ch/`.

29 ProTox-3.0, *Prediction of TOXicity of Chemicals*, `http://tox.charite.de/protox3/`.

30 C. Limban *et al.*, *The use of structural alerts to avoid the toxicity of pharmaceuticals*, 2018.