ISSN 1998 – 409X

ORIGINAL ARTICLE

Molecular Docking of Polyphenols to Triggering Receptor Expressed on Myeloid Cells 2 Identifies Rosmarinic Acid as a Promising Microglial Receptor Ligand

Venkata Durga Jyothi Undavalli[1], Vijaya Lakshmi Bodiga[2], Hasinee Puppala[1], Gopika Mathi[1], Bhaskar Venkata Saketh Kesiraju[1], Chanikya Uppala[1], Sri Lakshmi Kamala JagruthiMudavatu[1], Praveen Kumar Vemuri[1]

[1]Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India, [2]Department of Biochemistry and Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, Telangana, India

Abstract

Introduction: Triggering receptor expressed on myeloid cells 2 (TREM2) is a microglial immunoreceptor whose dysfunction increases Alzheimer's disease risk, making it an attractive target for small-molecule modulation. Salvianolic acid B (SalB) and rosmarinic acid (RA) are brain-relevant polyphenols with documented anti-inflammatory and neuroprotective effects, including modulation of microglial polarization and TREM2/DAP12-related pathways; however, direct structural evidence for their engagement of TREM2 remains limited. **Objectives:** To evaluate the potential direct binding of SalB and RA to the apical complementarity-determining region (CDR)-like loops of the human TREM2 ectodomain using structure-based molecular docking. **Materials and Methods:** We employed structure-based molecular docking to assess whether SalB and RA can bind the apical CDR-like loops of the human TREM2 ectodomain. The TREM2 extracellular domain (PDB ID: 6YYE, chain A) was prepared and docking cavity was defined around the basic apical loops spanning residues 42–52 and 110–120. Ligand structures (PubChem CIDs 6451084 and 5281792) were docked using Molegro Virtual Docker with PLP/MolDock scoring, followed by pose refinement and interaction analysis. **Results:** RA exhibited a more favorable MolDock Score and interaction energy than SalB (−155.997 vs. −102.437), supported by an extensive network of hydrogen bonds, electrostatic, and π interactions with key residues, including ARG52, ARG62, VAL63, and ASP104. In contrast, SalB formed fewer interactions and showed mild internal strain. **Conclusion:** These findings suggest that RA is a higher-ranked candidate for direct TREM2 engagement than SalB under the tested conditions, aligning with experimental evidence that both compounds predominantly modulate TREM2-related microglial responses indirectly. Overall, the predicted binding poses and interaction motifs provide testable hypotheses for future molecular dynamics simulations and biophysical and cellular assays to confirm or refute direct TREM2 targeting by SalB and RA.

Key words: Microglia, molecular docking, neuroinflammation, rosmarinic acid, salvianolic acid B, triggering receptor expressed on myeloid cells 2

INTRODUCTION

Triggering receptor expressed on myeloid cells 2 (TREM2) is a lipid-sensing immunoreceptor predominantly expressed on microglia and other tissue macrophages, where it regulates survival, phagocytosis, and the switch from homeostatic to disease-associated states in neurodegenerative disease.[1,2] Loss-of-function TREM2 variants increase the risk of Alzheimer's disease (AD) and other dementias, underscoring the therapeutic interest in small molecules that can modulate TREM2 signaling and restore protective microglial functions.[3,4] Recent computational efforts have structurally defined druggable pockets within the TREM2 ectodomain and have identified small-molecule

Address for correspondence:
Praveen Kumar Vemuri, Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India.
E-mail: vemuripraveen@gmail.com

Received: 18-08-2026
Revised: 20-09-2026
Accepted: 26-09-2026

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agonists binding to TREM2 with sub-micromolar affinity and enhancing microglial phagocytosis and receptor-proximal signaling, highlighting molecular docking as a key first step in rational ligand discovery for this receptor.[5,6]

Salvianolic acid B (SalB; PubChem CID: 6451084) is a water-soluble polyphenolic constituent of *Salvia miltiorrhiza* (Danshen) with potent antioxidant and anti-inflammatory properties and an increasingly well-documented neuroprotective profile.[7-10] In clinical models, SalB attenuates neuroinflammation by suppressing activation of the NLRP3 inflammasome, shifting microglial polarization from a pro-inflammatory M1 phenotype toward an M2 phenotype, and reducing production of caspase-1 and interleukin-1β in Aβ-stimulated microglia.[10,11] SalB was shown to also improve cognitive impairment in mouse models by modulating microglial pathways (including TREM2/DAP12 signaling) and downregulating amyloidogenic processing, suggesting its upstream modulator of receptors or co-receptors that integrate metabolic and inflammatory cues in the brain.[8,12] Beyond TREM2-related pathways, Salvianolic acids have been docked and functionally validated at other targets, such as BACE1, SIRT1, and ischemia-related signaling cascades.[9,13] Importantly, however, no biophysical data currently confirm direct binding of SalB to TREM2.

Rosmarinic acid (RA; PubChem CID: 5281792) is another plant-derived polyphenol, abundant in Lamiaceae species, with well-characterized antioxidative and anti-inflammatory actions in the central nervous system.[14] RA suppresses LPS-induced M1 microglial activation and promotes polarization toward the anti-inflammatory M2 phenotype through the PDPK1/Akt/HIF axis, thereby dampening cytokine release and ameliorating neuroinflammation *in vivo*.[14] In d-galactose or Aβbased AD models, RA – often in combination with tacrine – has been reported to increase hippocampal TREM2 expression, reduce Aβ plaque burden, and support a shift from Iba-1-positive M1 microglia to Arg-1-positive M2 microglia, suggesting that RA may indirectly enhance TREM2-mediated microglial responses.[15] At the same time, docking and spectroscopic studies have mapped RA binding to other proteins, such as TRPC1 and human transferrin, with measurable affinities and induced conformational changes, while no direct RA-TREM2 docking or biophysical data have been reported.[16,17]

TREM2's natural ligands include anionic and zwitterionic lipids, apolipoprotein E, and heparan sulfate, which interact with basic and hydrophobic patches on the V-type immunoglobulin domain, particularly around arginine-rich apical and lateral surfaces.[2,18] Biophysical mapping has identified residues, such as ARG62 and neighboring hydrophobic residues as key contributors to these basic sites, providing a structural context for exploring small-molecule binding.[18] Together, SalB and RA exemplify bioactive, brain-relevant polyphenols with convergent microglia-modulating and neuroprotective properties, yet whether they can directly occupy a TREM2 binding pocket remains unknown.[7,14]

In this study, we used molecular docking to evaluate whether SalB and RA can engage the apical, complementarity-determining region (CDR)-like loops of human TREM2, a region that overlaps with mapped basic ligand-binding surfaces.[5,6,18] Our objective was not to establish direct agonism but to generate structural and energetic hypotheses that can guide subsequent molecular dynamics simulations and experimental validation of putative SalB-TREM2 and RA-TREM2 interactions.

MATERIALS AND METHODS

Protein preparation

The crystal structure of the human TREM2 extracellular domain in complex with single-chain variable fragment (scFv-2) (PDB ID: 6YYE), co-crystallized with NAG-2-acetamido-2-deoxy-beta-D-glucopyranose at a resolution of 3.36 Å, was retrieved from the RCSB-PDB database (https://www.rcsb.org/structure/6YYE). The asymmetric unit of 6YYE contains four chains: Two copies of the human TREM2 V-type Ig domain (chains A and B, residues 19–131) and two engineered scFv-2 chains (chains C and D) used solely for crystallization stabilization. Protein preparation, modification, and refinement were performed using Accelrys Discovery Studio Client v21.10, following standard protocols for structure-based docking.[5]

The structure was pre-processed by assigning bond orders, charges, and hybridizations, and explicit hydrogen atoms were added wherever missing. Disulfide bonds were added between sulfur atoms within 3.2 Å of each other, and corresponding cysteine residues were renamed to CYX to reflect covalent linkage. All water molecules and hetero atoms were inspected, and bond orders (for the hetero groups) were checked for correctness. The ionization and tautomeric states of the protein were set for a physiological pH of 7.0, and the hydrogen-bond network was optimized by reorienting thiol and hydroxyl groups, the imidazole ring of histidine, and the amide groups of asparagine and glutamine to maximize favorable interactions. The resulting structure was refined by energy minimization until convergence at a backbone root mean square deviation (RMSD) of approximately 0.30 Å, ensuring removal of local strain without distorting the experimental fold.[5]

Structural characterization of the target

To prioritize biological relevance, chains C and D (scFv-2) were excluded from further consideration because they are non-native antibody fragments that do not occur in physiological TREM2 complexes.[18] Although TREM2 is

present as two nearly identical copies in the asymmetric unit (chains A and B), with a backbone RMSD < 0.5 Å, chain A was selected as the rigid receptor for docking.[6] This choice is consistent with prior *in silico* and biophysical work using monomeric TREM2 ectodomains and reduces computational noise while preserving all native secondary structures and loop orientations relevant for ligand recognition.[5,6]

The docking site was defined around the apical CDR-like loops of the Ig domain, which have been identified as hotspots for TREM2-ligand interactions and overlap with basic patches implicated in binding heparan sulfate and lipid ligands.[18] In 6YYE, the scFv binds a lateral surface of TREM2, leaving the apical loops (approximately residues 42–52 and 110–120) solvent-exposed and in a "competent" conformation for ligand engagement [Figure 1].[5] A spherical docking region encompassing these apical loops and their adjacent basic/hydrophobic residues was generated in Molegro Virtual Docker (MVD) and used as the binding cavity in subsequent docking runs.[5]

Ligand preparation

Two ligands were selected for docking: (SalB; PubChem CID: 6451084) and (RA; PubChem CID: 5281792), retrieved from the PubChem compound database in 2D format and converted to 3D structures.[19] Ligand preparation was conducted using accelrys discovery studio client v21.10.[5] Implicit hydrogens were added to each ligand, and chemically reasonable protonation states were assigned at physiological pH, with charged groups neutralized where appropriate before enumerating alternative ionization states.[5,19] A short conformational search was performed to relieve strain in rotatable bonds and ensure correct stereochemistry for ligands with multiple chiral centers, followed by energy minimization to obtain low-energy starting conformers for docking.[19]

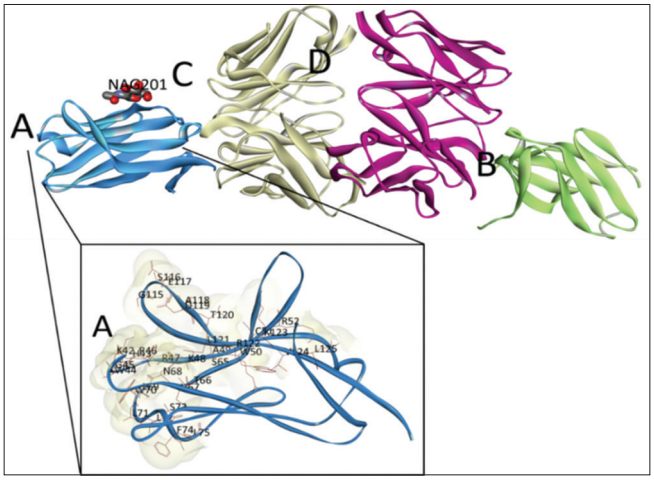


Figure 1: Structure of thuman TREM2, PDB ID: 6YYE. The structure ishoswn with 4 chains (A and B) Chains (A and B) (TREM2 receptor) with chains (C and D) (single-chain variable fragment helper). (Inset) Chain A was selected as the rigid receptor for docking showing complementarity determining region-like, shown with surface of the immunoglobulin domain which are established hotspots for TREM2 ligand interaction. TREM2: Triggering receptor expressed on myeloid cells

Molecular docking

Molecular docking was performed in MVD, which implements a piecewise linear potential (PLP) and the MolDock scoring functions that have been validated in multiple docking benchmarks.[5] The binding cavity was defined around the apical CDR-like loops of TREM2 chain A, as described above.[5] For each ligand, multiple independent docking runs were carried out (e.g., 20 runs per ligand), and for each run MVD generated an ensemble of poses within the pre-defined cavity.[5]

Docking parameters were set as follows: Grid resolution 0.20 Å, maximum iterations 1,500, and population size 50 for the evolutionary search algorithm.[5] Simplex evolution was configured with a maximum of 300 steps and a neighborhood distance factor of 1.0 to refine poses within the local energy landscape.[5] After docking, energy minimization and hydrogenbond optimization were applied to each ligand-receptor complex.[5] Binding affinity and pose quality were evaluated based on the MolDock Score and associated interaction terms (Interaction, Internal, Electro, ElectroLong, HBond, and torsional contributions), with more negative scores indicating more favorable predicted binding.[5]

For each ligand, the topscoring poses were visually inspected to remove solutions with severe steric clashes, deeply buried unsatisfied polar atoms, or unrealistic orientations relative to the protein surface.[5] Among the remaining plausible poses, the best-ranked complex was selected according to the MolDock, re-rank Score – a consensus between several refined scoring components – and used for detailed interaction analysis.[5] Post-dock energies were further minimized using Nelder–Mead simplex minimization with a non-grid force field implementing explicit hydrogen-bond directionality.[5] Docking was performed on the static crystallographic structure and did not explicitly account for membrane embedding or full glycosylation patterns beyond the NAG moieties present in 6YYE; these aspects are considered as limitations in the Discussion.[18]

RESULTS

Molecular docking

Molecular docking simulations were performed using MVD to evaluate the binding affinity of SalB (PubChem CID: 6451084) and RA (PubChem CID: 5281792) to the TREM2.

The MolDock Score, a PLP-based scoring function in MVD that approximates binding free energy through

steric, electrostatic, and hydrogen bonding terms, indicated superior binding for RA at −155.997 compared to SalB at −102.437, suggesting stronger overall interactions for the former. This difference arises primarily from the Interaction energy component (−117.803 for RA vs. −103.178 for SalB), reflecting favorable protein-ligand contacts, while the positive Internal strain (0.740 for SalB vs. −38.195 for RA, indicating conformational relaxation) further favors rosmarinic acid.

Further, re-rank Scores, derived from a more rigorous consensus of four advanced scoring functions emphasizing steric clashes, hydrogen bonds, and electrostatics; provide a refined estimate of binding pose reliability, with lower values denoting stability. RA exhibited a slightly superior Rerank Score of −66.168 over SalB's −68.343, though both fall within a comparable range indicative of viable binding poses. This proximity underscores that while initial docking favors rosmarinic acid, refined evaluation highlights subtle energetic equivalence, potentially influenced by the compounds' polyphenolic scaffolds forming similar hydrogen-bonding networks with TREM2's ligand-binding Ig domain.

Key interaction terms reveal distinct binding profiles: RA's higher Torsions (14 vs. 7) and Heavy Atoms (52 vs. 26) suggest greater conformational flexibility and contact surface, contributing to its lower Electro (−3.708 vs. −1.170) and ElectroLong (−4.823 vs. −4.156) penalties, alongside comparable H-bond contributions (−2.781 vs. −4.967). SalB, despite fewer contacts, benefits from optimized HBond energy, aligning with its known inhibition of TREM2/DAP12 pathways in microglial models. No cofactor or soft constraints were involved, emphasizing direct ligand-protein engagements [Table 1].

These results position RA as a promising TREM2 binder, potentially enhancing microglial homeostasis akin to other polyphenols that upregulate TREM2 expression and suppress pro-inflammatory MAPK signaling. SalB demonstrates moderate affinity, consistent with its neuroprotective effects through TREM2 pathway modulation. Future validation through molecular dynamics simulations and experimental assays, such as surface plasmon resonance, is recommended to confirm pose stability and functional agonism at TREM2's hydrophobic or basic ligand sites.

Table 1: Comparative docking scores for TREM2 ligands

Ligand	MolDock score	Rerank score	Interaction	Protein	Internal	Torsions	Electro	ElectroLong	HBond	LE1	LE3
Rosmarinic acid (ID: 6451084)	−155.997	−66.168	−117.803	−117.803	−38.195	14.000	−3.708	−4.823	−2.781	−3.000	−1.272
Salvianolic acid B (ID: 5281792)	−102.437	−68.343	−103.178	−103.178	0.740	7.000	−1.170	−4.156	−4.967	−3.940	−2.629

Interaction profiling of RA and SalB in the CDR-like loops of TREM-2

The molecular docking interaction profiles for RA (PubChem CID: 5281792) and SalB (PubChem CID: 6451084) with TREM2 provide mechanistic insights into their respective MolDock Scores (−155.997 and −102.437), where more negative values indicate stronger predicted binding affinity [Figures 2 and 3].

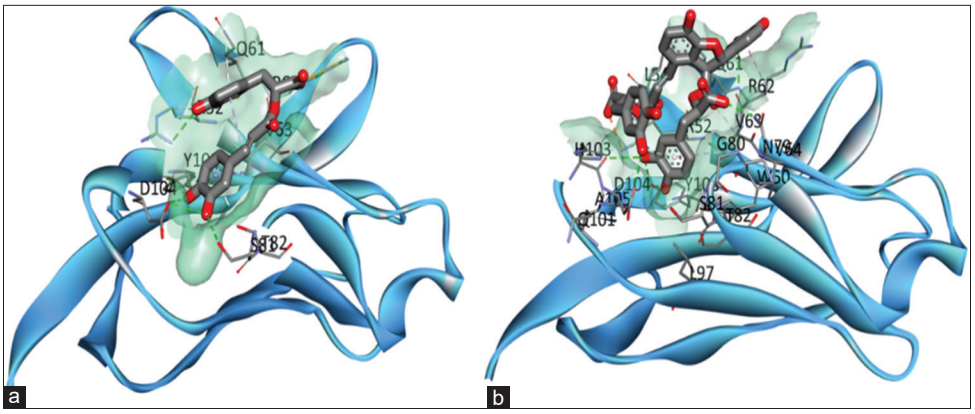


Figure 2: Interaction of ligands with TREM2 chain A. The interaction of (a) rosmarinic acid and (b) Salvianolic acid B in the complementarity determining region-like loops in the TREM2. TREM2: Triggering receptor expressed on myeloid cells

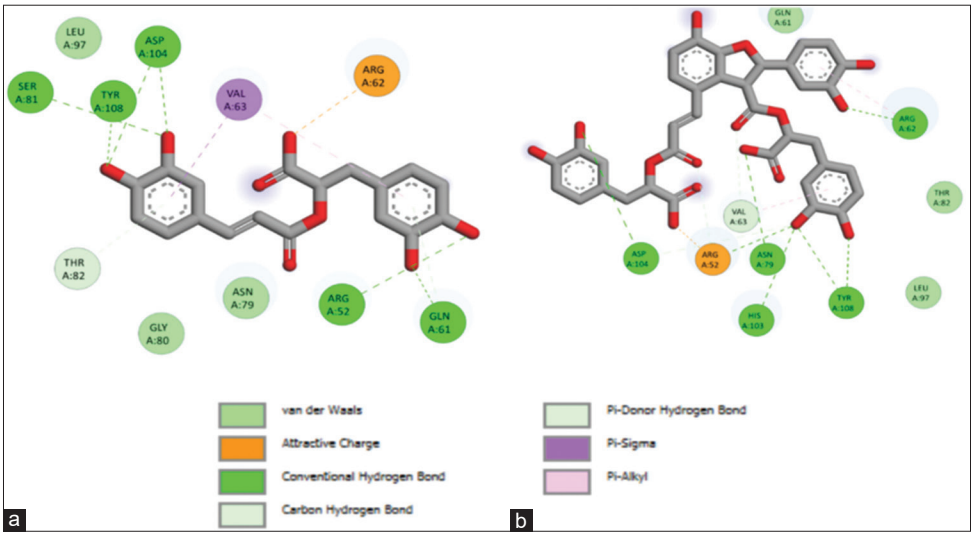


Figure 3: 2D interaction map of ligands with TREM2 chain A. The interaction of (a) rosmarinic acid and (b) Salvianolic acid B in the complementarity determining region-like loops in the TREM2. TREM2: Triggering receptor expressed on myeloid cells 2

Markedly lower MolDock Score of RA stems from an extensive network of stabilizing interactions, including one electrostatic attractive charge between ARG62:NH1 (positive) and ligand O2 (negative), alongside five conventional hydrogen bonds involving ARG52:NH1–O4, ARG62:NE–O2, SER81:OG–O7, and TYR108:OH–O8 from the protein, plus three ligand-to-protein hydrogen bonds (O3–GLN61:O, O7–ASP104:OD1, O8–ASP104:OD1). These are augmented by a carbon hydrogen bond (ARG52:CD–O4), two pi-donor hydrogen bonds (GLN61:NE2 and THR82:OG1 to ligand pi-orbitals), a pi-sigma interaction (VAL63:CG1 to pi-orbitals), and a pi-alkyl contact (VAL63 alkyl to pi-orbitals), yielding a highly favorable Interaction energy (−117.803) and negative Internal strain (−38.195) that reflects conformational relaxation without penalty. This multifaceted engagement, particularly with TREM2's basic site residues (e.g., ARG52, ARG62) critical for anionic ligand recognition, aligns with the compound's greater number of heavy atom contacts (52) and torsions (14), driving the overall energetic superiority.

In contrast, SalB exhibits a less favorable MolDock Score due to fewer and less diverse stabilizing contacts, despite a prominent salt bridge (ARG52:NH2–O13) and five conventional hydrogen bonds from protein residues (ARG52:NH1–O11, ARG62:N–O5, ASN79:ND2–O8, TYR108:OH–O11/O12) plus three ligand-to-protein bonds (O11–HIS103:O, O11–O7 intra-ligand, O15–ASP104:OD1). Three carbon hydrogen bonds (ARG52:CD–O10, VAL63:CA–O3, ASP104:CA–O11), one pi-donor hydrogen bond (O15 to intra-ligand pi-orbitals), and two pi-alkyl interactions (ARG62/VAL63 alkyl to pi-orbitals) contribute to its Interaction energy (−103.178), but positive Internal strain (0.740) signals minor conformational tension. The reduced contact diversity and reliance on TREM2 residues, such as ARG52 and TYR108, without equivalent electrostatic or pi-sigma terms, limits energetic gain relative to RA, consistent with fewer heavy atoms (26) and torsions (7).

The Interaction and Protein energies mirror these profiles (−117.803/−117.803 for RA vs. −103.178/−103.178 for

SalB), with RA's edge amplified by stronger Electro (−3.708 vs. −1.170) and comparable HBond (−2.781 vs. −4.967) contributions from its hydrogen-bond-rich pose [Table 2]. TREM2's ligand-binding domain favors such polyvalent networks, as seen in prior biophysical mappings of basic/hydrophobic sites involving ARG62 and VAL63, underscoring why RA's broader TREM2 engagement translates to superior docking energetics suitable for manuscript emphasis.

Collectively, these interaction profiles suggest that both RA and SalB can engage the apical TREM2 Ig-domain loops through networks of hydrogen bonds, electrostatics, and hydrophobic contacts, with RA forming a more extensive and energetically favorable interface under the present docking conditions.

DISCUSSION

This present *in silico* study provides an initial ligand-receptor of RA and SalB to the apical CDR-like loops of the human TREM2 ectodomain. In the study, RA exhibited a more favorable MolDock Score and interaction energy than SalB, supported by appreciable network of hydrogen bonds, electrostatic interactions with ARG52 and ARG62, and hydrophobic contacts with residues, such as VAL63 and ASP104 in the apical pocket. SalB, by contrast, showed moderate affinity characterized by a prominent salt bridge, several hydrogen bonds, but fewer π-type contacts and mild internal strain, resulting in less favorable overall docking energetics compared with RA. These findings suggest a modest energetic preference for RA over SalB as a candidate TREM2-binding ligand under the present computational

Table 2: Interaction profile of ligands in TREM2

Ligand	Types	Donor (chemistry)	Acceptor (chemistry)
Rosmarinic acid (PubChem CID:5281792)	Electrostatic attractive charge	Protein ARG62:NH1 (Positive)	Ligand:O2 (Negative)
	Conventional hydrogen bond	Protein ARG52:NH1 (H-Donor)	Ligand:O4 (H-Acceptor)
	Conventional hydrogen bond	Protein ARG62:NE (H-Donor)	Ligand:O2 (H-Acceptor)
	Conventional hydrogen bond	Protein SER81:OG (H-Donor)	Ligand:O7 (H-Acceptor)
	Conventional hydrogen bond	Protein TYR108:OH (H-Donor)	Ligand:O8 (H-Acceptor)
	Conventional hydrogen bond	Ligand:O3 (H-Donor)	Protein GLN61:O (H-Acceptor)
	Conventional hydrogen bond	Ligand:O7 (H-Donor)	Protein ASP104:OD1 (H-Acceptor)
	Conventional hydrogen bond	Ligand:O8 (H-Donor)	Protein ASP104:OD1 (H-Acceptor)
	Carbon Hydrogen Bond	Protein ARG52:CD (H-Donor)	Ligand:O4 (H-Acceptor)
	Pi-Donor Hydrogen Bond	Protein GLN61:NE2 (H-Donor)	Ligand (Pi-Orbitals)
	Pi-Donor Hydrogen Bond	Protein THR82:OG1 (H-Donor)	Ligand (Pi-Orbitals)
	Pi-Sigma	Protein VAL63:CG1 (C-H)	Ligand (Pi-Orbitals)
	Pi-Alkyl	Ligand (Pi-Orbitals)	Protein VAL63 (Alkyl)
Salvianolic acid B (PubChem CID:6451084)	Salt bridge	Protein ARG52:NH2 (H-donor)	Ligand:O13 (H-acceptor)
	Conventional hydrogen bond	Protein ARG52:NH1 (H-donor)	Ligand:O11 (H-acceptor)
	Conventional hydrogen bond	Protein ARG62:N (H-donor)	Ligand:O5 (H-acceptor)
	Conventional hydrogen bond	Protein ASN79:ND2 (H-donor)	Ligand:O8 (H-acceptor)
	Conventional hydrogen bond	Protein TYR108:OH (H-donor)	Ligand:O11 (H-acceptor)
	Conventional hydrogen bond	Protein TYR108:OH (H-donor)	Ligand:O12 (H-acceptor)
	Conventional hydrogen bond	Ligand:O11 (H-donor)	Protein HIS103:O (H-acceptor)
	Conventional hydrogen bond	Ligand:O11 (H-donor)	Ligand:O7 (H-acceptor)
	Conventional hydrogen bond	Ligand:O15 (H-donor)	Protein ASP104:OD1 (H-acceptor)
	Carbon hydrogen bond	Protein ARG52:CD (H-donor)	Ligand:O10 (H-acceptor)
	Carbon hydrogen bond	Protein VAL63:CA (H-donor)	Ligand:O3 (H-acceptor)
	Carbon hydrogen bond	Protein ASP104:CA (H-donor)	Ligand:O11 (H-acceptor)
	Pi-Donor hydrogen bond	Ligand:O15 (H-donor)	Ligand (Pi-orbitals)
	Pi-Alkyl	Ligand (Pi-orbitals)	Protein ARG62 (Alkyl)
	Pi-Alkyl	Ligand (Pi-orbitals)	Protein VAL63 (Alkyl)

conditions, while emphasizing that docking scores are relative ranking metrics rather than direct surrogates for binding free energy.[5,19]

Interpreting these docking results can be corroborated with existing biological data on SalB and RA in microglial and neurodegenerative models. SalB is well established as a neuroprotective polyphenol that attenuates neuroinflammation by inhibiting the NLRP3 inflammasome, switching microglial polarization from M1 to M2 phenotypes, and reducing production of pro-inflammatory mediators, such as caspase-1 and interleukin-1β.[7,10,11] In high-fat diet and palmitic acid models, SalB improves cognitive outcomes and reduces neuroinflammation in hippocampal tissue and BV2 microglial cells by modulating the Trem2/Dap12 pathway, resulting in lowering of mRNA levels of TNF-α and IL-6.[8] However, no biophysical studies to date have demonstrated direct binding of SalB to TREM2; instead, docking and functional work have focused on other targets, including BACE1, SIRT1, and ischemia-related signaling pathways.[9,13] Our observation of only moderate docking scores and a relatively simpler interaction network for SalB at TREM2 is therefore consistent with a model in which SalB influences TREM2-dependent outcomes predominantly through indirect mechanisms – such as upstream suppression of lipotoxic or inflammatory stimuli, promotion of M2 polarization, or modulation of parallel signaling pathways that converge on TREM2/DAP12 rather than through strong, direct occupation of the TREM2 ligand-binding pocket.[7,8,20]

RA similarly exerts robust microglia-modulating and neuroprotective effects, including suppression of LPS-induced M1 activation, promotion of M2 phenotypes via the PDPK1/Akt/HIF pathway, and attenuation of neuroinflammation *in vivo*.[14] In Alzheimer's-like models, particularly when combined with tacrine, RA increases hippocampal TREM2 expression, reduces Aβ plaque burden, and supports a shift from Iba-1-positive M1 to Arg-1-positive M2 microglia, suggesting that RA indirectly enhances TREM2-mediated phagocytosis and neuroprotection.[15] At the same time, RA has been shown to bind other protein targets, such as human transferrin and TRPC1 with measurable affinities, inducing conformational changes relevant to iron homeostasis and Ca^{2+} signaling, respectively.[16,17] Notably, there are no docking or biophysical studies demonstrating direct RA-TREM2 interactions in the present literature. Our docking results, which indicate that RA can form an extensive hydrogen-bond and electrostatic network[16,17] in TREM2's apical basic pocket, thus raise the hypothesis that RA could directly engage TREM2 under certain conditions; however, this should be regarded as a putative interaction until independently validated by biophysical and functional assays.[5,6]

The specific interaction pattern observed for RA is notable in light of the known structural biology of TREM2. TREM2's ectodomain binds anionic lipids, ApoE, and heparan sulfate through basic and hydrophobic patches on its V-type Ig domain, with residues, such as ARG62 and neighboring hydrophobic residues contributing to key binding hotspots.[2,18,21] In our docking, RA engaged ARG52 and ARG62 through electrostatic and hydrogen-bond contacts and formed additional interactions with GLN61, SER81, THR82, VAL63, and ASP104, creating a polyvalent network reminiscent of the multivalent contacts described for heparan sulfate and other anionic ligands.[18] This convergence suggests that anionic polyphenols with appropriately spaced hydroxyl and carboxyl groups may be able to exploit the same basic/hydrophobic landscape used by endogenous TREM2 ligands, at least at the level of local interaction motifs.[5,18] By contrast, the more limited π-stacking and slightly strained conformation of SalB in the same region may explain its less favorable predicted affinity relative to RA, despite SalB's potent microglial effects through TREM2-related pathways *in vivo*.[8,10]

Several important limitations of the present work must be emphasized. First, all docking simulations were carried out on a single crystallographic conformation of the soluble TREM2 ectodomain (PDB 6YYE), without explicit modeling of the cell membrane, full glycosylation, or lipidic microenvironment, all of which are known to influence TREM2 conformation and ligand access.[18] Second, a single docking engine (MVD) and scoring framework were used; although MVD has performed well in benchmarks, cross-validation with alternative docking platforms and rescoring schemes would increase confidence in pose ranking.[5,19] Third, docking scores, even when decomposed into interaction and internal energy components, do not directly correspond to experimental binding free energies and should be interpreted as relative metrics for prioritizing ligands and interaction motifs rather than quantitative measures of affinity.[19] Finally, no molecular dynamics simulations were performed to assess conformational flexibility, water-mediated interactions, or long-timescale stability of the predicted complexes, and no biophysical or cell-based experiments (e.g., surface plasmon resonance, microscale thermophoresis, or TREM2-dependent microglial assays) have yet been used to test the predictions arising from this docking study.[6,22]

Despite these limitations, the present analysis generates several testable hypotheses and a structural framework for future work. The RA pose suggests that small anionic polyphenols with specific stereochemistry and functional group topology can establish multivalent interactions with the apical basic pocket of TREM2, centered on ARG52/ARG62 and neighboring residues, potentially modulating TREM2 signaling if direct engagement occurs in a cellular context.[14,15,18] SalB, while displaying only moderate docking affinity in this pocket, remains highly relevant as a TREM2-pathway modulator at the systems level, likely

acting through indirect mechanisms that ultimately impact TREM2/DAP12-driven microglial phenotypes.[7,8] Future studies should therefore include (i) molecular dynamics simulations of RA-TREM2 and SalB-TREM2 complexes to evaluate pose stability and solvent effects, (ii) free-energy calculations to better quantify relative binding strengths, and (iii) experimental validation using purified TREM2 ectodomains and microglial models with intact and disrupted TREM2 signaling.[6,22]

CONCLUSION

In conclusion, our docking results support RA as a higher-ranked candidate TREM2 binder than SalB under the present computational conditions, while also aligning with the existing literature that both polyphenols modulate TREM2-related microglial functions largely through indirect, pathway-level mechanisms. These findings provide a structural starting point for biophysical and functional studies aimed at defining and exploiting small-molecule modulation of TREM2 in neuroinflammatory and neurodegenerative disease.

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Source of Support: Nil. **Conflicts of Interest:** None declared.