Computational and Structural Biotechnology Journal 27 (2025) 1335–1341

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

journal homepage: www.elsevier.com/locate/csbj

Short communication

Targeting the inter-monomeric space of TNFR1 pre-ligand dimers: A novel binding pocket for allosteric modulators

Chih Hung Lo [a,b,*]

[a] Department of Biology, Syracuse University, NY 13244, USA
[b] Interdisciplinary Neuroscience Program, Syracuse University, NY 13244, USA

ARTICLE INFO

Keywords:
TNF receptor
TNFR1 signaling
Allosteric mechanism
Conformational change
Structure-activity relationship
Receptor dimerization
Inter-monomeric space
Small molecule
Molecular docking
Drug discovery

ABSTRACT

Tumor necrosis factor (TNF) receptor 1 (TNFR1) plays a central role in signal transduction mediating inflammation and cell death associated with autoimmune and neurodegenerative disorders. Inhibition of TNFR1 signaling is a highly sought-after strategy to target these diseases. TNFR1 forms pre-ligand dimers held together by the pre-ligand assembly domain (PLAD), which is essential for receptor signaling. TNFR1 dimers form the crucial points of interaction for the entire receptor signaling complex by connecting TNF ligand bound trimeric receptors. While previous studies have shown the feasibility of disrupting TNFR1 dimeric interactions through competitive mechanism that targets the PLAD, our recent studies have demonstrated that small molecules could also bind PLAD to modulate TNFR1 signaling through an allosteric mechanism. Importantly, these allosteric modulators alter receptor dynamics and propagate long-range conformational perturbation that involves reshuffling of the receptors in the cytosolic domains without disrupting receptor-receptor or receptor-ligand interactions. In this study, we perform molecular docking of previously reported allosteric modulators on the extracellular domain of TNFR1 to understand their binding sites and interacting residues. We identify the inter-monomeric space between TNFR1 pre-ligand dimers as a novel binding pocket for allosteric modulators. We further conduct pharmacological analyses to understand the bioactivity of these compounds and their interacting residues and pharmacological properties. We then provide insights into the structure-activity relationship of these allosteric modulators and the feasibility of targeting TNFR1 conformational dynamics. This paves the way for developing new therapeutic strategies and designing chemical scaffolds to target TNFR1 signaling.

1. Introduction

Tumor necrosis factor (TNF) receptor 1 (TNFR1) is a transmembrane receptor that regulates inflammatory and cell death signaling pathways [1]. Depending on the cell types, stimulation of TNFR1 by TNF induces IκBα degradation and NF-κB activation in inflammation or triggers caspase activation in cell death [2]. Overactivation of TNFR1 signaling is associated with autoimmune disorders such as rheumatoid arthritis, Crohn's disease, and inflammatory bowel disease as well as neurodegenerative diseases including Alzheimer's disease and multiple sclerosis [3–6]. Currently available treatments of anti-TNF are monoclonal antibodies that block ligand binding to TNFR1. However, anti-TNF suffers from off-target effect by global TNF blockade and inhibit ligand interactions with TNFR2 that is important for immune functions, resulting in adverse side effects [7]. To address these issues, there is an urgent need for the development of receptor-specific therapies that leverage recent advances in understanding the structure and dynamics of TNFR1 [8,9].

A recently resolved crystal structure (PDB: 7KP7) illustrated the coexistence of the TNF trimer and TNFR1 dimers in a ligand-bound receptor signaling network, where each receptor monomer of the receptor-ligand trimeric complex forms dimer with another monomer in a three-fold symmetry [10]. This structure bridges the gap observed in earlier studies, including the ligand-bound receptor monomer (PDB: 1TNR) [11] and the pre-ligand assembled receptor dimer (PDB: 1NCF) [12] in the extracellular domain (ECD) of TNFR1 which is stabilized by the pre-ligand assembly domain (PLAD) [13]. Importantly, it confirms that ligand-bound TNFR1 can still form intact dimers under physiological conditions (Fig. 1**A**). Moreover, this study provides experimental evidence of the formation of receptor-ligand signaling complexes and network [10], offering a revised model of TNFR1 activation that aligns with prior findings [14,15]. Based on this updated model, three primary

* Correspondence to: Department of Biology and Interdisplinary Neuroscience Program, Syracuse University, NY 13244, USA.
E-mail address: clo101@syr.edu.

https://doi.org/10.1016/j.csbj.2025.03.046
Received 18 January 2025; Received in revised form 20 March 2025; Accepted 27 March 2025
Available online 28 March 2025

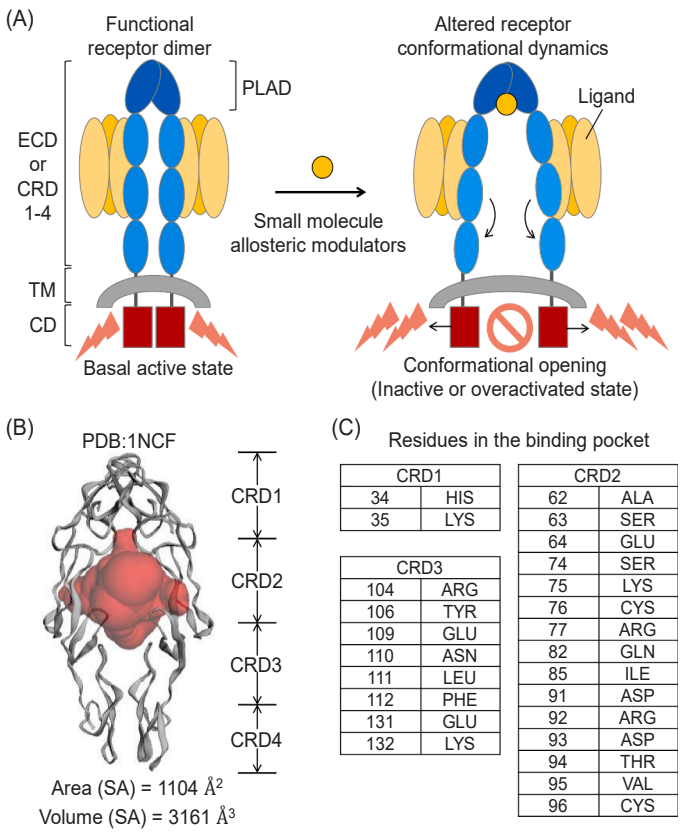


Fig. 1. Inter-monomeric space of TNFR1 pre-ligand dimer as a novel binding pocket. (A) Schematic of small molecule allosteric modulators altering receptor conformational dynamics, resulting in a conformational opening that can either be in an inactive or overactivated state. This also leads to the notion of conformational states of TNFR1 as a molecular switch for receptor function. PLAD: pre-ligand assembly domain, ECD: extracellular domain, CRD: cysteine rich domain, TM: transmembrane domain, and CD: cytosolic domain. (B) Predicted active site or binding pocket on the TNFR1 dimer (PDB:1NCF) labelled with respective CRD1–4, together with the solvent-accessible (SA) area and volume for therapeutic interaction. (C) Common residues in the binding pocket from each chain of the TNFR1 dimer that can form interaction with small molecules.

strategies for modulating the TNFR1/TNF signaling complex have been identified: (i) disrupting receptor-ligand interactions, (ii) interfering with receptor-receptor interactions, and (iii) altering receptor conformational dynamics [8].

While there are several molecules that target the receptor to block ligand binding [16–18], this approach is challenging due to high affinity of the ligand and the extensive binding interface [19,20]. Similarly, strategies targeting TNFR1 dimerization [21–23], a key signaling subunit, face limitations of competitive inhibition with low efficiency. To address these challenges, we have developed noncompetitive or allosteric inhibitors of TNFR1 signaling that could be more effective as they are unencumbered by competition [24,25]. Specifically, we have previously reported a series of allosteric inhibitors including DS41 and DS114 that bind TNFR1 PLAD within the cysteine-rich domain 1 (CRD1), to perturb receptor dynamics through a conformationally active region involving the ligand-binding domain (LBD) [26]. This conformationally active region, centered around the β-hairpin (residues 100–117) of the LBD and a hinge axis (residues 80, 104, 117, 133), facilitates a bending motion of the receptor [14]. Binding of DS41 and DS114 at the PLAD induces long-range perturbations that propagate to the cytosolic domain via torque motion, driving TNFR1 into an open and inactive conformation measurable by fluorescence resonance energy transfer (FRET) biosensors [26,27]. Additionally, we have also identified SB-200646 as a TNFR1 activator that appears to act through similar allosteric mechanism [28]. These allosteric modulators alter receptor dynamics without affecting receptor-receptor or receptor-ligand interactions or their binding affinity, making them true allosteric modulators (Fig. 1A). It is important to note that all biological activity of these compounds have been demonstrated in relevant cell assays [26,28] and our goal is to elucidate their potential binding sites and the associated mechanisms of action in this study.

Here, we use molecular docking and computational analysis to elucidate the binding sites and pharmacological properties of these new allosteric modulators of TNFR1 signaling, in conjunction with the knowledge that we have obtained from experimental observations. We identify the inter-monomeric space between TNFR1 dimers as a key binding pocket for these allosteric compounds where they could induce conformational changes without affecting receptor-receptor or receptor-ligand interactions. In addition, we examine the bioactivity of these chemical scaffolds and their interaction with receptor residues. Furthermore, these allosteric modulators possess promising drug-like properties, good blood-brain barrier (BBB) penetration capabilities, and high intestinal absorption, exemplifying a novel class of modulators of TNFR1 signaling.

2. Materials and methods

2.1. Prediction of active site and binding pocket on TNFR1 dimer

The active site and binding pocket on TNFR1 dimer were predicted using the computed atlas of surface topography of proteins (CASTp) 3.0 server [29]. The target protein of TNFR1 dimer (PDB:1NCF) [12] was uploaded as input to predict the ligand binding sites and the key amino acid residues for interaction on the receptors. CASTp 3.0 locates and measures the geometric and topological properties of protein structures with comprehensive identification of binding pockets and cavities as well as quantification of protein topography.

2.2. Ligand preparation of small-molecule chemical scaffolds for molecular docking

Chemical scaffolds of small-molecule allosteric modulators of TNFR1 signaling are downloaded and subjected to ligand preparation before being used in molecular docking. The two-dimensional (2D) structures of allosteric inhibitors DS41 (Compound ID: 19298144) and DS114 (Compound ID: 16888972) [26] in SDF format are downloaded from ChemBridge Online Chemical Store (www.hit2lead.com). The three-dimensional structures of DS41 and DS114 were generated using Avogadro by continuous optimization of molecular geometry through molecular mechanics under Universal Force Field [30]. The 3D structure of allosteric activator SB-200646 in SDF format was downloaded directly from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The SDF files of these modulators were loaded into Discovery Studio visualizer and first converted into PDB format before ligand preparation. Ligand preparation step was then conducted in AutodockTools 4.2 (ADT4.2) [31] and saved as PDBQT format compatible for subsequent molecular docking using AutoDock Vina [32,33].

2.3. Protein preparation of TNFR1 dimer for molecular docking

The PDB file of TNFR1 dimer (1NCF) was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) [12]. The TNFR1 dimeric protein was prepared using ADT4.2 by removing unwanted molecules such as water molecules that might interfere with docking. This is followed with the addition of polar hydrogens, which are critical for defining hydrogen bonds and charge distributions. Kollman charges are also assigned to the protein as they

are partial atomic charges required by AutoDock Vina to compute molecular interactions. The final step would be to examine and verify the types, locations, and integrity of amino acid residues present in the protein model. This step ensures that the protein is correctly formatted for molecular docking by identifying any missing, misaligned, or unwanted residues that may affect docking accuracy. Once protein preparation is completed, the file was converted into PDBQT format compatible for docking through the Grid and Macromolecule option.

2.4. Molecular docking of allosteric modulators of TNFR1 signaling

Molecular docking was carried out using AutoDock Vina using the prepared TNFR1 dimer with grid parameters for site-specific docking consisting of 16, 14, and 20 as center for X, Y, and Z and 30, 30, and 30 as size dimension for X, Y, and Z (Fig. S1) with spacing 0.375, energy range 4, and exhaustiveness 8. To execute the docking program, a configuration file containing these parameters was generated for each ligand separately and a command was given in the command prompt to proceed with docking using AutoDock Vina. For each docking experiment, a log file containing the binding affinity (kcal/mol) and an output file containing the dock poses in PDBQT format were generated for each ligand. The complex of the top pose in each output file with the TNFR1 dimer was used for subsequent analysis. A receptor-ligand complex and a two-dimensional interaction map between the chemical scaffold of each allosteric modulator and the TNFR1 dimer was generated using the Discovery Studio visualizer.

2.5. Pharmaceutical properties and druglikeness of allosteric modulators

The pharmaceutical properties and druglikeness of allosteric modulators were predicted by the SwissADME tool [34]. The pharmaceutical analysis as illustrated by a bioavailability radar takes into account parameters including physiochemical properties, lipophilicity, water solubility, pharmacokinetics, druglikness, and medicinal chemistry. Importantly, SwissADME also predicts BBB penetration capability, human intestinal absorption (HIA), and p-glycoprotein (PGP) substrate using the Boiled-Egg method. Druglikeness is also analyzed by various methods include Lipinski's rule of five. These properties will enable further optimization of lead compounds and advancement towards clinical drug candidates.

3. Results

3.1. Inter-monomeric space of TNFR1 pre-ligand dimer as a novel binding pocket

To delineate the mechanism of action of allosteric modulators of TNFR1 signaling, we first examined the potential active sites or binding pockets on the TNFR1 dimer (PDB:1NCF) [12] using the CASTp 3.0 server [29]. The analysis showed that the top predicted binding pocket lies in the inter-monomeric space of TNFR1 pre-ligand dimer, spanning CRD1–3 with a solvent-accessible area of 1104 $Å^2$ and volume of 3161 $Å^3$ (Fig. 1B). From each chain of the TNFR1 dimer, we have also identified the common receptor residues within this binding pocket that are capable of forming interaction with small molecules. These residues include regions in CRD1 (His34-Lys35), CRD2 (Ala62-Glu64, Ser74-Arg77, Gln82, Ile85, and Asp91-Cys96), and CRD3 (Arg104, Tyr106, Glu109-Phe112, and Glu131-Lys132) (Fig. 1C).

3.2. Identification of key interaction between allosteric modulators and TNFR1 dimer

For the pharmacological analysis, we selected known noncompetitive or allosteric modulators of TNFR1 signaling, including allosteric inhibitors DS41 and DS114 [26] as well as allosteric activator SB-200646 [28], for molecular docking. Briefly, both classes of compounds have been reported to bind and perturb TNFR1 conformational dynamics to adopt an open and inactive or open and overactivated state by acting through the conformational active region involving the ligand binding residues. Specifically, DS41 and DS114 have been shown to compete binding site with zafirlukast [22,35], a known competitive inhibitor that target the interface of TNFR1 dimerization, at the PLAD within CRD1 to induce long-range receptor conformational alterations [26]. Importantly, these compounds do not ablate ligand binding or disrupt receptor dimerization. To examine the interaction profiles of these allosteric modulators with TNFR1 dimers, we perform site-specific molecular docking at the region covering the interface of receptor dimerization in CRD1 (Fig. S1).

From our docking analysis, DS41 interacts with TNFR1 dimer with a binding energy of −6.6 kcal/mol. DS41 adopts a L-shape orientation where the cyclopentanecarboxamide group fits into the cavity formed at the receptor dimer interface and interacts with residues His34 and Lys35 of chain A and Lys35 of chain B, predominantly through alkyl interactions (Fig. 2A). The oxygen atom on the cyclopentanecarboxamide group also interacts with Lys35 of chain A and Ser63 of chain B through hydrogen bonding. On the other end of the compound, the benzene ring interacts with Ala62 and Arg92 of Chain B through pi-alkyl interactions. Several other residues including His34, Glu64, Asp93, Thr94, and Val95 also interact with DS41 through van der Waals forces (Fig. 2A).

DS114, which is an analog of DS41 with the cyclopentyl side group replaced by a methoxybenzyl group, interacts with TNFR1 dimer with a binding energy of −6.3 kcal/mol. Although DS114 is similar in structure to DS41 and adopts a similar L-shape orientation, the benzene ring now interacts with the cavity formed by the receptor dimer instead of the methoxybenzamide end (Fig. 2B). The benzene ring interacts with Lys35 and Ser63 of both chains A and B through amide-pi stacked and alkyl interactions. The methoxybenzyl side group now interacts with Arg92, Asp93, and Val95 of chain B through alkyl interaction and hydrogen bonding. Other residues including His34 and Glu64 of chains A and B as well as Ala62 and Asp91 of chain B interact with DS114 through van der Waals forces (Fig. 2B).

On the other hand, allosteric activator SB-200646, being a shorter molecule, adopts a relatively linear orientation while interacting with similar residues with a binding energy of −6.2 kcal/mol. The benzene ring end interacts with His34 (hydrogen bonding) and Glu64 (pi-anion interaction) of chain A and Lys35 (pi-alkyl interaction) of chain B (Fig. 2C). Several hydrogen bonds are also formed between the oxygen atom with Lys35 and nitrogen atom with Ala62 of chain A. The methylindole of the molecule interacts with Arg92 of chain A through pi-alkyl interaction. Other residues including Ser63 of chain A as well as His34, Ser63, and Glu64 of chain B are interacting with SB-200646 through van der Waals forces (Fig. 2C).

3.3. Promising drug-like properties of allosteric modulators of TNFR1 signaling

The careful consideration of pharmacokinetic properties and physiochemical profiles of lead compounds in the early stages of drug discovery will facilitate lead optimization and improve the quality of clinical candidates. We conducted pharmaceutical analysis of DS41 (Figs. 3A and S2), DS114 (Figs. 3B and S3), and SB-200646 (Fig. 3C and S4) using SwissADME [34] with a comparison of the three compounds in a table format (Fig. 3D). All three compounds show good water solubility and high druglikness through Lipinski's rule of five. Importantly, the Boiled-Egg analysis also predicts the compounds to have good BBB penetration capability and high intestinal absorption, with DS41 and SB-200646, but not DS114, being PGP substrates (Fig. 3E). Although all three compounds illustrate good druglike properties, DS114 emerges as the most promising candidate with an optimized bioavailability radar as well as excellent intestinal absorption, BBB penetration capability, and superior biological activity as demonstrated through experiments conducted in the previous study [26]. Overall, our analysis shows that these

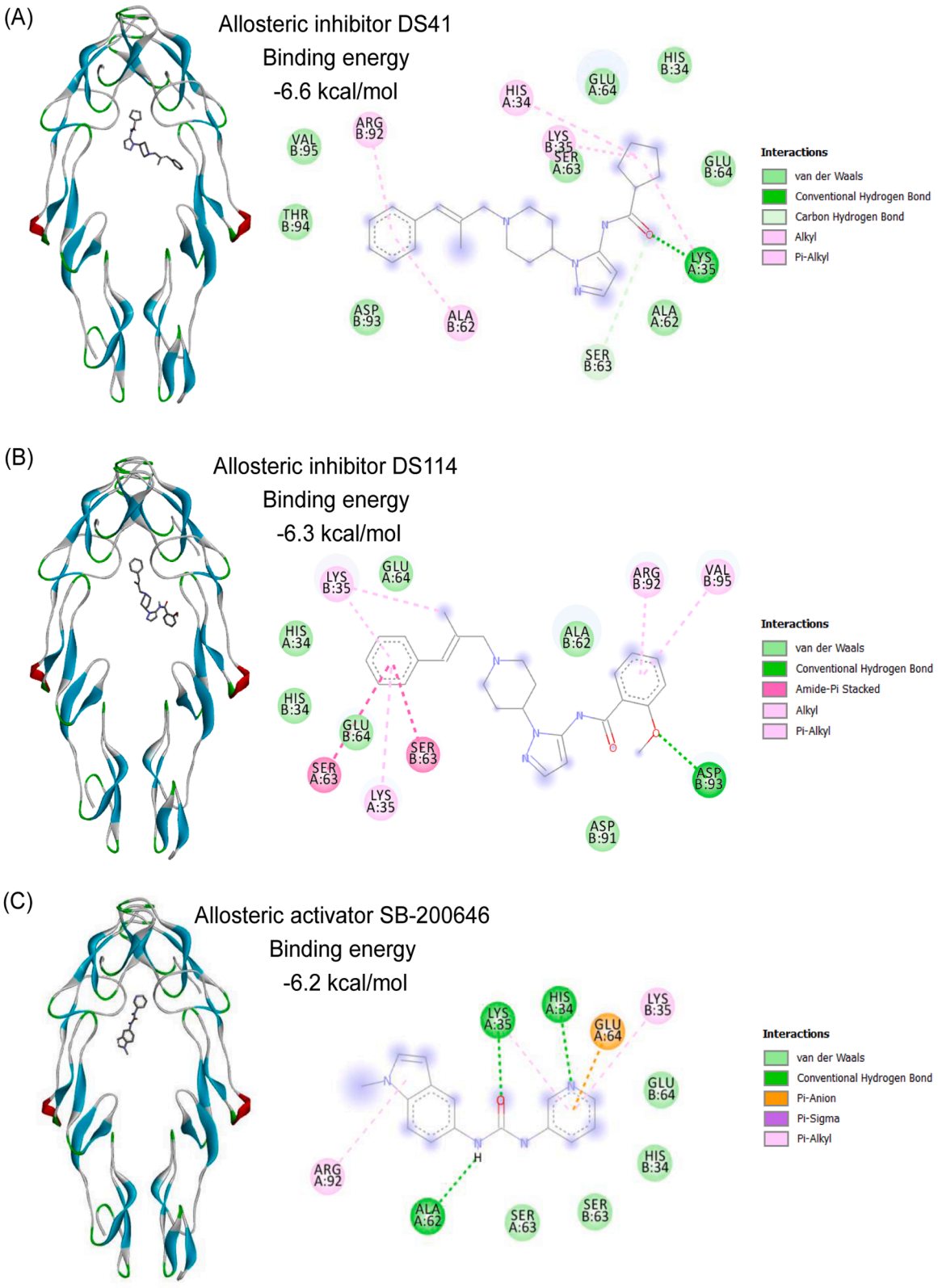
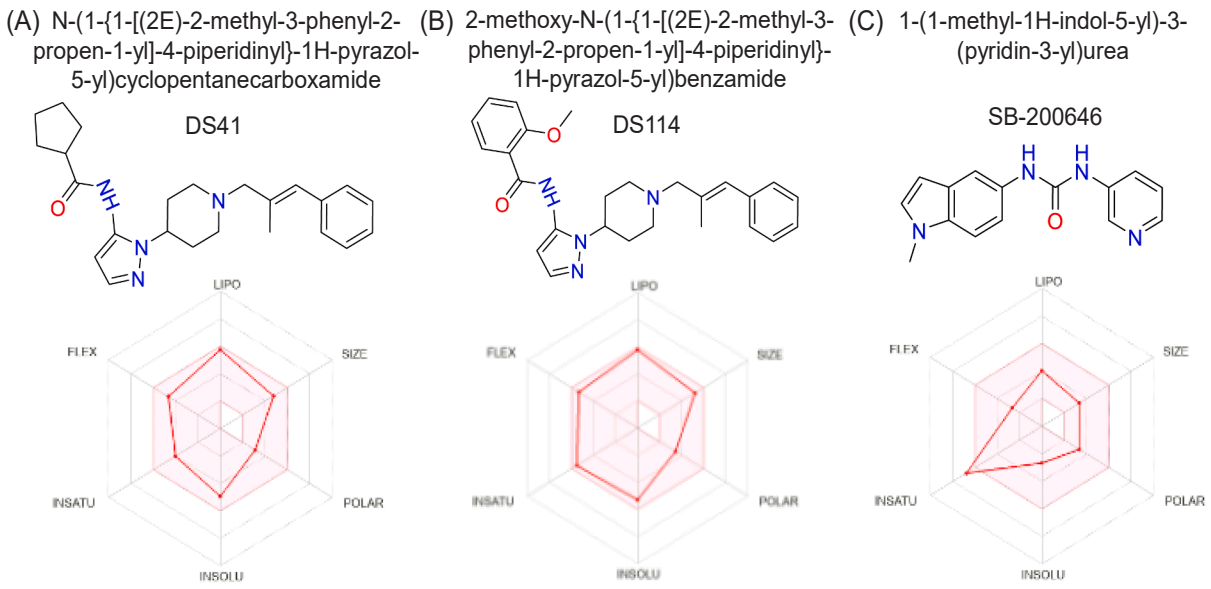


Fig. 2. Molecular docking of allosteric modulators of TNFR1 signaling reveals key interacting chemical groups and receptor residues. Top docked pose and two-dimensional interaction map for allosteric inhibitors (A) DS41 and (B) DS114 and allosteric activator (C) SB-200646 on TNFR1 dimer (PDB:1NCF).

allosteric modulators of TNFR1 conformations exhibit promising drug-like properties and are important chemical scaffolds for subsequent therapeutic development to modulate the TNFR1 signaling pathway.

4. Discussion

In this study, we described the inter-monomeric space of TNFR1 pre-ligand dimer as a novel binding pocket for allosteric modulators that act



(D)

Pharmacological properties			
Compound	**DS41**	**DS114**	**SB-200646**
MW (g/mol)	392.54	430.54	266.30
TPSA (Å^2)	50.16	59.39	58.95
Log P	3.86	3.99	1.85
Water solubility	Moderate	Moderate	High
GI absorption	High	High	High
BBB permeant	Yes	Yes	Yes
Druglikeness	Yes	Yes	Yes

(E)

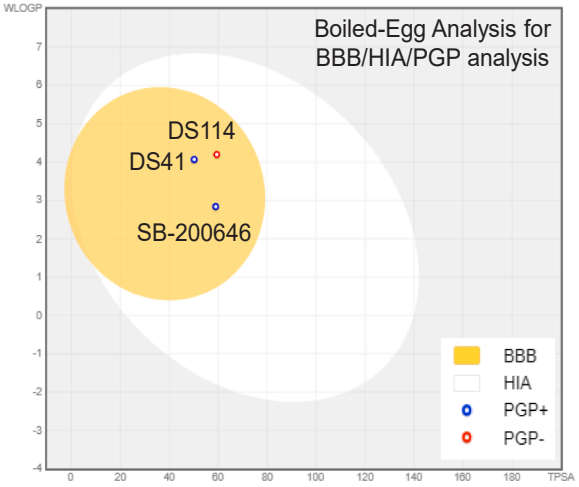


Fig. 3. Pharmacokinetic properties and physiochemical analysis of allosteric modulators of TNFR1 signaling. (A-C) Chemical name, structures, and bioavailability radar obtained from SwissADME for allosteric inhibitors (A) DS41 and (B) DS114 and allosteric activator (C) SB-200646. (D) Comparison of the pharmacological properties across DS41, DS114, and SB-200646 with descriptions of molecular weight (MW), total polar surface area (TPSA), lipophilicity (Log P), water solubility, gastrointestinal (GI) adsorption, blood-brain barrier (BBB) permeability, and druglikeness. (E) Boiled-Egg analysis showing good human intestinal absoprtion (HIA) and the BBB penetration capability of DS41, DS114, and SB-200646, and an indicator on whether they are P-glycoprotein (PGP) substrates.

through perturbing receptor dynamics and conformational states. We provide the first proof-of-concept evidence suggesting the feasibility of noncompetitive or allosteric compounds binding at the inter-monomeric space proximal to the cavity formed by the TNFR1 dimeric interface. Importantly, binding at this position and interacting with His34 and Lys35 of CRD1 is consistent with experimental findings showing that the allosteric modulators are interacting at the PLAD to induce long-range receptor conformational changes [22,26,28]. As receptor dimerization is formed by interactions of several residues including Gln17, Lys19, Cys33, His34, Lys35, Gly36, Gln48, and Asp49 [22], the interaction of the allosteric modulators with His34 and Lys35 may not be sufficient to disrupt receptor-receptor interaction as they are still being held together by other interactions, consistent with previous experimental observations that these allosteric modulators do not disrupt PLAD-PLAD or ECD-ECD interactions [26,28]. Furthermore, binding of allosteric modulators at this inter-monomeric space does not interfere with receptor regions such as Arg77-Gly81 and W107–Q113 that form the LBD [14,36,37], which is also consistent with these compounds not ablating ligand binding experimentally [26,28].

From the docking analysis, both DS41 and DS114 have similar binding energies, although the potency of DS114 is about 3-fold more than DS41 in cell-based assays [26]. This could partly be explained by more interactions that DS114 made with the receptor residues and the orientation of the small molecule in terms of how well it adheres to the TNFR1. On the other hand, DS41 appears to interact strongly at both ends of the small molecules, with the receptor and the central portion of the molecule appearing to be not essential in the binding, making it not adhering well with the receptor. This might explain its consistent allosteric inhibitory mechanism but with the lack of biological potency. On the other hand, SB-200646 has a slightly weaker docking score than DS41 and DS114 potentially due to the lack of interaction with the receptor monomer at the end of the L-shape orientation to stabilize its binding conformation. Altogether, this suggests that for small molecules to exhibit strong interaction at this novel binding pocket, there is a need for more polar groups or ring structures at two ends as well as an optimized central connecting group that provide good adherence of the molecule to the receptor.

We propose that the allosteric modulators binding at this new cavity

are potentially important in triggering the long-range receptor perturbation. This is because the L-shape conformation of the small molecules allows one end to anchor at the cavity formed by the receptor dimer interface, while the other end interacts with the receptor, stabilizing it toward the center of the dimer. This could potentially perturb the conformationally active region where a hinge axis involving the ligand binding residues has been suggested [26,28,38]. Stabilizing the receptor towards the center of the dimer could result in a torsion at this hinge axis which would lead to a thrust to the receptor to adopt an open conformation. It is important to mention that the feasibility of the inter-monomeric space as potential binding sites is supported by our recent study, which demonstrated that a peptide-based allosteric inhibitor can bind to the CRD2–3 region within the TNFR1 dimer, and this interaction induces a conformational change, shifting the receptor into an open and inactive state [38]. The feasibility of allosteric modulation of TNFR1 signaling is further supported by an allosteric small molecule inhibitor called F002 [39]. In contrast to the receptor conformational rearrangement proposed in our study, F002 acts through perturbation of W107, a key ligand binding residue, which might reduce ligand binding affinity or abolish ligand binding [39].

It is also important to note that both allosteric inhibitors and activators could interact at this novel binding pocket to perturb receptor dynamics leading to either inhibitory or overactivated conformational states, acting through the conformationally active region of the receptor that involves the ligand binding residues. The differentiation on how these receptor perturbations lead to an inhibitory or overactivated states remains unknown, although experimental evidence has suggested that this lies in either decreased or increased recruitment of downstream signaling molecules, respectively [26,28]. This suggests that the conformational changes of the receptor might not be in a two-dimensional manner, but a three-dimensional receptor conformational opening that changes how the receptor could interact with downstream signaling molecules with altered affinity or different spatial orientations. The need to take into account three-dimensional receptor or protein conformation changes is supported by several other studies [40–43].

This study provided insights into a conserved binding site for allosteric modulators of TNFR1 signaling. This would enable further optimization of the existing compounds to enhance their potency and biological effects. While there is some existing experimental evidence, the exact binding sites and the pharmacological properties of these compounds should be further determined experimentally. The discovery of these compounds being able to cross the BBB opens avenues for their potential use as therapeutic agents in neurological disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and glioblastoma [44–46] or diseases that arise from pathological body-brain interactions [47,48]. This new druggable region involving inter-monomeric space between receptor dimer could be applicable to other TNF receptors and membrane proteins in general [49,50]. Furthermore, this could open future opportunities in virtual screening [51] or experimental high-throughput screening efforts such as using FRET-based biosensors with superior sensitivity [27,52] to discover new and more effective noncompetitive or allosteric modulators of TNF receptor signaling for treatment of autoimmune and metabolic disorders, cancers, and neurodegenerative diseases.

Funding

C.H.L. is supported by a start-up grant from the Department of Biology at Syracuse University.

Author contributions

C.H.L. conducted the active site prediction and molecular docking analysis, generated the figures, and wrote the manuscript.

Data statement

All data required to evaluate the conclusions of the paper are present in the main text or the Supplementary Materials of the paper.

CRediT authorship contribution statement

Chih Hung Lo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The author declares no conflict of interest.

Acknowledgements

The author thanks Dr. Jialiu Zeng from the Department of Biomedical and Chemical Engineering at Syracuse University for proofreading the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2025.03.046.

References

[1] Gough P, Myles IA. Tumor necrosis factor receptors: pleiotropic signaling complexes and their differential effects. Front Immunol 2020;11. https://doi.org/10.3389/fimmu.2020.585880.
[2] Preedy MK, White MRH, Tergaonkar V. Cellular heterogeneity in TNF/TNFR1 signalling: live cell imaging of cell fate decisions in single cells. Cell Death Dis 2024;15(3):202. https://doi.org/10.1038/s41419-024-06559-z.
[3] Siegmund D, Wajant H. TNF and TNF receptors as therapeutic targets for rheumatic diseases and beyond. Nat Rev Rheuma 2023;19(9):576–91. https://doi.org/10.1038/s41584-023-01002-7.
[4] van Loo G, Bertrand MJM. Death by TNF: a road to inflammation. Nat Rev Immunol 2023;23(5):289–303. https://doi.org/10.1038/s41577-022-00792-3.
[5] Plantone D, Pardini M, Righi D, Manco C, Colombo BM, De Stefano N. The role of TNF-α in Alzheimer's disease: a narrative review. Cells 2024;13(1). https://doi.org/10.3390/cells13010054.
[6] Asimakidou E, Reynolds R, Barron AM, Lo CH. Autolysosomal acidification impairment as a mediator for TNFR1 induced neuronal necroptosis in Alzheimer's disease. Neural Regen Res 2024;19(9).
[7] Evangelatos G, Bamias G, Kitas GD, Kollias G, Sfikakis PP. The second decade of Anti-TNF-a therapy in clinical practice: new lessons and future directions in the COVID-19 era. Rheumatol Int 2022;42(9):1493–511. https://doi.org/10.1007/s00296-022-05136-x.
[8] Lo CH. TNF receptors: structure-function relationships and therapeutic targeting Strategies. Biochim Biophys Acta BBA Biomembr 2025;1867(1):184394. https://doi.org/10.1016/j.bbamem.2024.184394.
[9] Fischer R, Kontermann RE, Pfizenmaier K. Selective targeting of TNF receptors as a novel therapeutic approach. Front Cell Dev Biol 2020;8. https://doi.org/10.3389/fcell.2020.00401.
[10] McMillan D, Martinez-Fleites C, Porter J, Fox D, Davis R, Mori P, et al. Structural insights into the disruption of TNF-TNFR1 signalling by small molecules stabilising a distorted TNF. Nat Commun 2021;12(1):582. https://doi.org/10.1038/s41467-020-20828-3.
[11] Banner DW, D'Arcy A, Janes W, Gentz R, Schoenfeld H-J, Broger C, et al. CrystaL structure of the soluble human 55 Kd TNF receptor-human TNFβ complex: implications for TNF receptor activation. Cell 1993;73(3):431–45. https://doi.org/10.1016/0092-8674(93)90132-A.
[12] Naismith JH, Devine TQ, Brandhuber BJ, Sprang SR. Crystallographic evidence for dimerization of unliganded tumor necrosis factor receptor *. J Biol Chem 1995;270(22):13303–7. https://doi.org/10.1074/jbc.270.22.13303.
[13] Chan FK-M, Chun HJ, Zheng L, Siegel RM, Bui KL, Lenardo MJ. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science 2000;288(5475):2351–4. https://doi.org/10.1126/science.288.5475.2351.
[14] Lewis AK, Valley CC, Sachs JN. TNFR1 signaling is associated with backbone conformational changes of receptor dimers consistent with overactivation in the R92Q TRAPS mutant. Biochemistry 2012;51(33):6545–55. https://doi.org/10.1021/bi3006626.

[15] Chan FK-M. Three is better than one: pre-ligand receptor assembly in the regulation of TNF receptor signaling. Cytokine 2007;37(2):101–7. https://doi.org/10.1016/j.cyto.2007.03.005.
[16] Carter PH, Scherle PA, Muckelbauer JA, Voss ME, Liu R-Q, Thompson LA, et al. Photochemically enhanced binding of small molecules to the tumor necrosis factor receptor-1 inhibits the binding of TNF-α. Proc Natl Acad Sci 2001;98(21):11879–84. https://doi.org/10.1073/pnas.211178398.
[17] Chen S, Feng Z, Wang Y, Ma S, Hu Z, Yang P, et al. Discovery of novel ligands for TNF-α and TNF receptor-1 through structure-based virtual screening and biological assay. J Chem Inf Model 2017;57(5):1101–11. https://doi.org/10.1021/acs.jcim.6b00672.
[18] Saddala MS, Huang H. Identification of novel inhibitors for TNFα, TNFR1 and TNFα-TNFR1 complex using pharmacophore-based approaches. J Transl Med 2019;17(1):215. https://doi.org/10.1186/s12967-019-1965-5.
[19] Wootten D, Christopoulos A, Sexton PM. Emerging paradigms in GPCR allostery: implications for drug discovery. Nat Rev Drug Discov 2013;12(8):630–44. https://doi.org/10.1038/nrd4052.
[20] Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. Nature 2007;450(7172):1001–9. https://doi.org/10.1038/nature06526.
[21] Deng G-M, Zheng L, Ka-Ming Chan F, Lenardo M. Amelioration of inflammatory arthritis by targeting the pre-ligand assembly domain of tumor necrosis factor receptors. Nat Med 2005;11(10):1066–72. https://doi.org/10.1038/nm1304.
[22] Lo CH, Vunnam N, Lewis AK, Chiu T-L, Brummel BE, Schaaf TM, et al. An innovative high-throughput screening approach for discovery of small molecules that inhibit TNF receptors. SLAS Discov Adv Sci Drug Discov 2017;22(8):950–61. https://doi.org/10.1177/2472555217706478.
[23] Wang X, Guo F, Zhang Y, Wang Z, Wang J, Luo R, et al. Dual-targeting inhibition of TNFR1 for alleviating rheumatoid arthritis by a novel composite nucleic acid nanodrug. Int J Pharm X 2023;5:100162. https://doi.org/10.1016/j.ijpx.2023.100162.
[24] Schön A, Madani N, Smith AB, Lalonde JM, Freire E. Some binding-related drug properties are dependent on thermodynamic signature. Chem Biol Drug Des 2011;77(3):161–5. https://doi.org/10.1111/j.1747-0285.2010.01075.x.
[25] Schön A, Lam SY, Freire E. Thermodynamics-based drug design: strategies for inhibiting protein–protein interactions. Future Med Chem 2011;3(9):1129–37. https://doi.org/10.4155/fmc.11.81.
[26] Lo CH, Schaaf TM, Grant BD, Lim CK-W, Bawaskar P, Aldrich CC, et al. Noncompetitive inhibitors of TNFR1 probe conformational activation states. Sci Signal 2019;12(592):eaav5637. https://doi.org/10.1126/scisignal.aav5637.
[27] Lo CH, Schaaf TM, Thomas DD, Sachs JN. Fluorescence-Based TNFR1 Biosensor for Monitoring Receptor Structural and Conformational Dynamics and Discovery of Small Molecule Modulators. In: Bayry J, editor. The TNF Superfamily: Methods and Protocols. New York, NY: Springer US; 2021. p. 121–37. https://doi.org/10.1007/978-1-0716-1130-2_9.
[28] Lo CH, Huber EC, Sachs JN. Conformational states of TNFR1 as a molecular switch for receptor function. Protein Sci 2020;29(6):1401–15. https://doi.org/10.1002/pro.3829.
[29] Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res 2018;46(W1):W363–7. https://doi.org/10.1093/nar/gky473.
[30] Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J Cheminf 2012;4(1):17. https://doi.org/10.1186/1758-2946-4-17.
[31] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 2009;30(16):2785–91. https://doi.org/10.1002/jcc.21256.
[32] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31(2):455–61. https://doi.org/10.1002/jcc.21334.
[33] Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. J Chem Inf Model 2021;61(8):3891–8. https://doi.org/10.1021/acs.jcim.1c00203.
[34] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7(1):42717. https://doi.org/10.1038/srep42717.
[35] Vunnam N, Yang M, Lo CH, Paulson C, Fiers WD, Huber E, et al. Zafirlukast is a promising scaffold for selectively inhibiting TNFR1 signaling. ACS Bio Med Chem Au 2023;3(3):270–82. https://doi.org/10.1021/acsbiomedchemau.2c00048.
[36] Murali R, Zhang H, Cai Z, Lam L, Greene M. Rational design of constrained peptides as protein interface inhibitors. Antibodies 2021;10(3). https://doi.org/10.3390/antib10030032.
[37] Zhang J, Zhao H, Zhou Q, Yang X, Qi H, Zhao Y, et al. Discovery of cyclic peptide inhibitors targeted on TNFα-TNFR1 from computational design and bioactivity verification. Molecules 2024;29(21). https://doi.org/10.3390/molecules29215147.
[38] Zeng J, Loi GWZ, Saipuljumri EN, Romero Durán MA, Silva-García O, Perez-Aguilar JM, et al. Peptide-based allosteric inhibitor targets TNFR1 conformationally active region and disables receptor–ligand signaling complex. Proc Natl Acad Sci 2024;121(14):e2308132121. https://doi.org/10.1073/pnas.2308132121.
[39] Murali R, Cheng X, Berezov A, Du X, Schön A, Freire E, et al. Disabling TNF receptor signaling by induced conformational perturbation of Tryptophan-107. Proc Natl Acad Sci 2005;102(31):10970–5. https://doi.org/10.1073/pnas.0504301102.
[40] Zacharias M. Accounting for conformational changes during protein–protein docking. Theory Simul Macromol Assem 2010;20(2):180–6. https://doi.org/10.1016/j.sbi.2010.02.001.
[41] Halakou F, Kilic ES, Cukuroglu E, Keskin O, Gursoy A. Enriching traditional protein-protein interaction networks with alternative conformations of proteins. Sci Rep 2017;7(1):7180. https://doi.org/10.1038/s41598-017-07351-0.
[42] Katritch V, Kufareva I, Abagyan R. Structure based prediction of subtype-selectivity for adenosine receptor antagonists. High Resolut 2011;60(1):108–15. https://doi.org/10.1016/j.neuropharm.2010.07.009.
[43] Mollaei, P.; Farimani, A.B. A Machine Learning Method to Characterize Conformational Changes of Amino Acids in Proteins. bioRxiv 2023, 2023.04.16.536913. https://doi.org/10.1101/2023.04.16.536913.
[44] Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxid Med Cell Longev 2015;2015(1):610813. https://doi.org/10.1155/2015/610813.
[45] Asimakidou E, Tan JK, Zeng J, Lo CH. Blood–brain barrier-targeting nanoparticles: biomaterial properties and biomedical applications in translational neuroscience. Pharmaceuticals 2024;17(5). https://doi.org/10.3390/ph17050612.
[46] McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J Neuroinflamm 2008;5(1):45. https://doi.org/10.1186/1742-2094-5-45.
[47] Asimakidou E, Saipuljumri EN, Lo CH, Zeng J. Role of metabolic dysfunction and inflammation along the liver–brain axis in animal models with obesity-induced neurodegeneration. Neural Regen Res 2025;20(4).
[48] Zeng J, Cheong LYT, Lo CH. Therapeutic targeting of obesity-induced neuroinflammation and neurodegeneration. Front Endocrinol 2025;15. https://doi.org/10.3389/fendo.2024.1456948.
[49] Vunnam N, Lo CH, Grant BD, Thomas DD, Sachs JN. Soluble extracellular domain of death receptor 5 inhibits TRAIL-induced apoptosis by disrupting receptor–receptor interactions. J Mol Biol 2017;429(19):2943–53. https://doi.org/10.1016/j.jmb.2017.08.009.
[50] Vunnam N, Young MC, Liao EE, Lo CH, Huber E, Been M, et al. Nimesulide, a COX-2 inhibitor, sensitizes pancreatic cancer cells to TRAIL-induced apoptosis by promoting DR5 Clustering. Cancer Biol Ther 2023;24(1):2176692. https://doi.org/10.1080/15384047.2023.2176692.
[51] Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc 2016;11(5):905–19. https://doi.org/10.1038/nprot.2016.051.
[52] Lo CH. Recent advances in cellular biosensor technology to investigate Tau oligomerization. Bioeng Transl Med 2021;6(3):e10231. https://doi.org/10.1002/btm2.10231.