Targeting Rank1 protein towards the cure of Osteomyelitis using small molecule inhibitors

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ABSTRACT

The study targets RANKL using small molecule inhibitors in order to mitigate bone resorptions and protect immune health. Through protein preparation, ligand optimization, and molecular docking, we were able to analyze different small molecular inhibitors as well as antiviral compounds. Looking for molecular inhibitors and antiviral compounds with topmost docking scores, our results indicated S1774 to be a potential inhibitor with a docking score of -5.035 kcal/mol. In the end, we identified possible inhibitors to mitigate a bone disease like osteomyelitis.

INTRODUCTION

Osteomyelitis typically starts with an infection caused by Staphylococcus aureus. Clinical symptoms of osteomyelitis are displayed through localized bone pain, swelling, fever, and impaired mobility in the affected region.The bacteria S. aureus is known for the apoptosis osteoblast formation, thus inhibiting RANKL/OPG as well.1

Receptor Activator of Nuclear Factor Kappa-B (RANK) is an enzyme receptor located on the surface of osteoclasts. RANKL is the ligand that binds with it, most likely in the form of osteoblasts. When RANK and RANKL bond, it allows osteoclast differentiation to occur, as well as create a pathway that connects bone health with the immune system. To control the activation of RANK/ RANKL, osteoprotegerin (OPG) acts as a decoy receptor. This RANK/RANKL/OPG complex pursues many pathways in the immune system, from the haematopoietic progenitors (considered as immune cells) of both osteoclasts and osteoblasts to T- cell proliferation and dendritic cell (DC) survival. When the T-cell and the dendritic system is weakened by the pathogen, RANK can cause an immunosuppressive phenotype to form (DOI: 10.3389/fimmu.2023.1219895). To summarize, RANKL promotes osteoclast differentiation, while OPG counteracts this process as a decoy receptor. RANK functions as the receptor that drives bone resorption when activated. Ultimately, the infection becomes a much more severe autoimmune disease (DOI: 10.1080/1744666X.2021.1971972).

Currently, bone biopsies and microbial cultures are conducted to identify the underlying inflammatory condition. Most use antibiotics to mitigate the disease; however, if damage is excessive, surgery might be necessary. It is also known that diabetes mellitus and cardiovascular diseases can play a role in encouraging the spread of osteomyelitis (PMID: 34652112).

Recently, advances have been made in the drug Denosumab, a viable RANKL inhibitor. However, this inhibitor isn't sufficient to keep up with the various pathways of RANK/RANKL in the immune system. The lack of consistency in its effects also questions if it is an effective treatment; perhaps it can even cause malignancy (DOI:10.1080/1744666X.2021.1971972). For this reason, it is imperative to seek other molecular inhibitors that can advance what we do know.

In this study, we are looking into small molecule inhibitors for RANK, a receptor for RANKL, as it can help prevent extreme bone resorption and osteoclast differentiation, ultimately leading to the bones being susceptible to osteomyelitis.

MATERIALS AND METHODS

Protein preparation, ligand optimization, and molecular docking, were all performed using Schrödinger Suite 2024-3 (Schrödinger, LLC, New York, NY). From the Protein Data Bank, two crystal structures were selected: 5BNQ (PDB DOI: https://doi.org/10.2210/pdb5bnq/pdb) and 3URF (PDB DOI: https://doi.org/10.2210/pdb3urf/pdb). Using the Protein Preparation Wizard (Schrödinger Suite 2024-3), the protein was prepared with proper bond order assignments, hydrogen additions, and metal ion state corrections were performed, including fixing protonation and oxidation states of metal. Water molecules outside a 5 Å radius of the active site were removed while retaining the rest. Side chain corrections were made based on the local hydrogen-bonding network to determine protonation states of histidine residues. A final energy minimization was conducted using the Optimized Potentials for Liquid Simulations (OPLS4) force field to relieve steric clashes and optimize the geometry of the protein, maintaining the positions of heavy atoms.

Ligands were obtained from Selleck, PubChem search, and NCI libraries. To form three-dimensional (3D) conformers and a range of ionization and tautomeric states, each ligand was prepared using the LigPrep tool (Schrödinger Suite 2024-3). Protonation state assignment was performed at pH 7.0 ± 2.0, all possible stereoisomers for each ligand were generated and retained, and energy minimization was conducted using the OPLS4 force field.

Active site analysis was conducted using the SiteMap tool (Schrödinger Suite 2024-3), which identifies potential binding pockets and their properties, including hydrophobicity, hydrogen bond donor/acceptor character, and steric features. The resulting site maps guided the selection of the docking site and highlighted the physicochemical properties essential for ligand binding. Molecular docking was performed using Glide (Schrödinger Suite 2024-3). Receptor grid generation was centered on the native ligand, or, when no ligand was present in the crystal

structure, over residues around the binding site, such as those indicated by SiteMap. Docking calculations were performed using both Standard Precision (SP) and Extra Precision (XP) modes. SP docking was used for large-scale virtual screening to filter non-binding compounds based on a preliminary scoring function, followed by XP docking for a more specific and accurate assessment of top-ranked ligands from the SP screen, accounting for ligand-protein interactions to identify high-affinity binders.

Ligands with higher XP docking scores were analyzed for drug-likeness and ADME (absorption, distribution, metabolism, and excretion) properties using QikProp (Schrödinger Suite 2024-3). QikProp calculates molecular weight, logP, solubility, and blood-brain barrier permeability, assisting in determining oral bioavailability and safety. Ligand interaction analysis was conducted to highlight key binding motifs and residues. Ligand interaction analysis using Maestro noted hydrogen bonds, π-π stacking, cation-π, and hydrophobic interactions between ligands and active site residues.

RESULTS AND DISCUSSION

PDB Structures

The 5BNQ PDB structure, includes homo sapiens RANKL and RANK from mus muscules, a similar bond is formed to the structure 3URF, which displays the bonding of RANKL and OPG (the OPG acting as a decoy receptor).

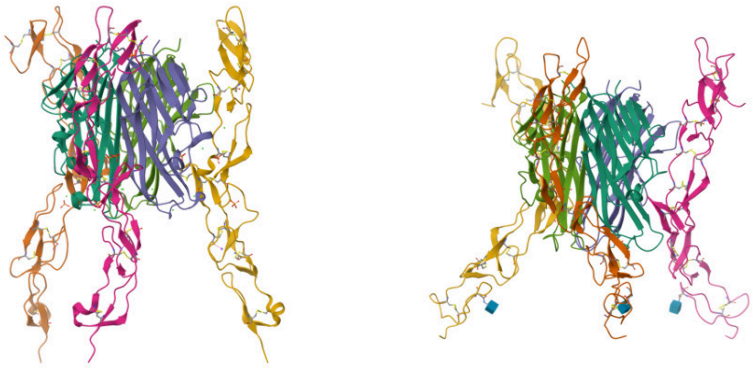


Figure 1: Human RANKL/RANK complex (PDB: 5BNQ) (left); Human RANKL/OPG complex (PDB: 3URF) (right)

In Figure 1 (left), inter-species cytokine is used as an immunogen as an immunization method (TISCAI); this creates an anti-cytokine antibody. RANKL's role is to act as an immunogen to the anti-RANKL immune response. In the end, the antibodies formed prevented osteoclast development from occurring in vitro and osteoporosis in OVX rats. This result is significant, in that this process can be used as an anti-cytokine immunotherapy tactic (DOI:https://doi.org/10.1038/srep14150). Based on the experiment's findings, there is potential in RANKL inhibition, thus supporting studying

small-molecule docking in order to similarly mitigate osteoclast development.

In Figure 1 (right), OPG binds with RANKL to intercept the binding of the RANK/RANKL Axis. OPG forces its decoy receptor functions to hinder the necessary interaction residues that allow RANK/RANKL to bind (DOI: https://doi.org/10.4049/jimmunol.1103387).

In this study the following known inhibitors were studied to understand their binding strength and docking scores.

Table 1. Known inhibitors of RANKL

Structure	Docking Score	IC_{50} (nM)
Vaccarin	-6.679 kcal/mol	5-64 μM
Pamidronic acid sodium	-6.135 kcal/mol	580 nM
Boldine	-6.004 kcal/mol	400 nM
Pamidronic acid	-5.889 kcal/mol	0.58 μM
Norboldine	-5.700 kcal/mol	27.06 ± 1.04 μM

Note: Pamidronic acid and Norboldine are similar substructures of Boldine and Pamidronic Acid Sodium.

In Table 1, we find the most promising known inhibitors of RANKL to be Vaccarin, Pamidronic acid sodium, and Boldine using MedChem Express search. The docking scores for these compounds range from -6.679 kcal/mol to -6.004 kcal/mol, indicating how strong the structures can bind to RANKL. These scores indicate weak binding. Pamidronic acid and Norboldine have docking scores of -5.889 and -5.700 kcal/mol, respectively.

Table 2. Antiviral Compounds with the Top Docking Scores

Antiviral Compound	Docking Score
S1714	-5.061 kcal/mol
S1774	-5.035 kcal/mol
S1719	-4.920 kcal/mol
S2376	-4.636 kcal/mol

We looked into antiviral compounds as osteomyelitis can be caused by viral infection as well. These antiviral compounds resulted in docking scores in the range of -5.061kcal/mol (the highest) to -4.636 kcal/mol (the lowest). The most promising antiviral compounds are S1714 and S1774, as they have the higher docking scores

compared to the rest.

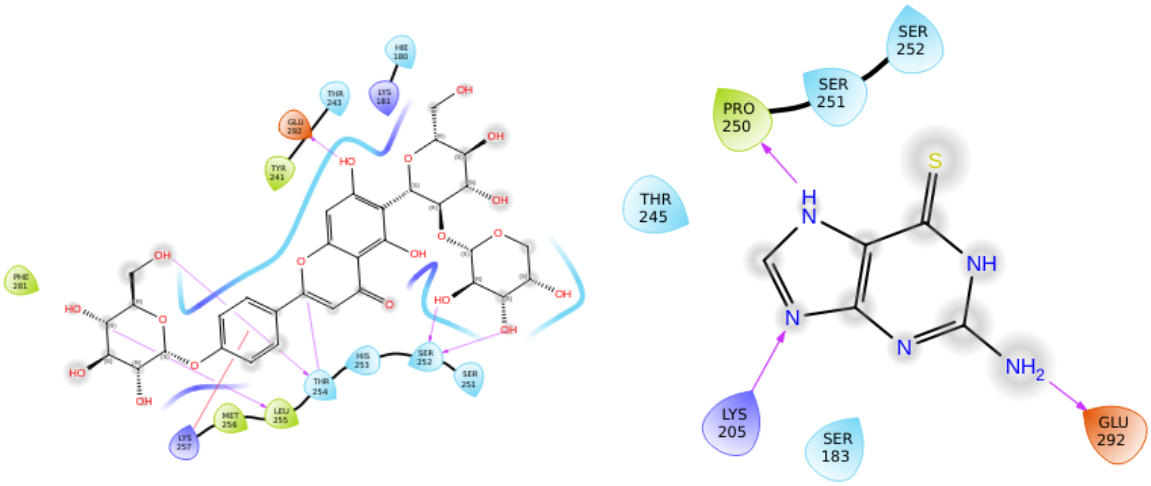


Figure 2: Ligand Interaction of Vaccarin (left), Ligand Interaction of S1774 (right)

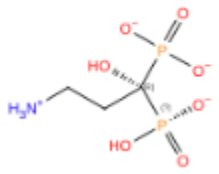
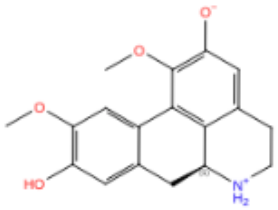
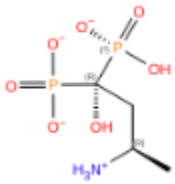
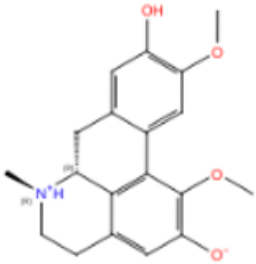
Figure 2 (left) displays the hydrogen bonds with residues Glu292, Ser252, Leu255, Thr254 and Lys257. Many of the polar residues are either hydrogen bond donors or acceptors. The nonpolar amino acids cause hydrophobic, noncovalent interactions which stabilize the ligand inside the protein.

Figure 2 (right) displays hydrogen bonding to residues Pro250, Glu292 and Lys205 of the receptor. Polar amino acids act as either donors or acceptors to stabilize its position in the binding site. Acidic residues, such as Glutamic acid, provide stability with polar groups on the ligand. The nonpolar residues create hydrophobic, noncovalent bonds around S1774, supporting the molecule within the protein.

Though Vaccarin has the top docking score, it is also a large inhibitor, containing about 89 atoms, making it harder for it to be used as an effective prevention method. In conclusion, we must look for the next compound with not only a good docking score, but also less complex and of a reasonable size.

Our aim in the study conducted was to find new small molecule inhibitors for RANK in the effort to prevent bones from becoming susceptible to viral bone diseases like osteomyelitis. Looking at both small molecules (Table 1) and antiviral compounds (Table 2), we were able to find possible inhibitors to mitigate the worsening conditions of antiviral bone diseases.

Table 3: QikProp results of compounds in this study

Title	Structure	docking score	MW	QPlogS	QPlogBB
Compound 1		-5.889	235.07	0.495	-2.181
Compound 2		-5.7	313.35	-2.205	-0.264
Compound 3		-5.596	249.09	0.304	-1.871
Compound 4		-5.57	327.37	-2.774	-0.165

In **Table 3**, Compounds 2 & 4 are the top small molecule inhibitors from this study. We used Qikprop to calculate critical properties for drug use: absorption, distribution, metabolism, and excretion. The log BB values indicate the permeability of the molecules in crossing the blood-brain barrier. Qikprop BB values between -1.0 to 0.5 tend to be more ideal in permeability to the central nervous system.

Compound 4 has a value of -0.165, and Compound 2 is -0.253 for QlogBB. Thus, these Qikprop values are ideal in permeability.

The QikProp results indicate these small molecules as promising for effective drug delivery. As they have the best balance of docking and ADME; there is high binding,

high oral absorption, and a low PSA. Compound 2 is also present with strong bonding, good absorption, and CNS penetrant. However, these molecules have slightly less absorption than Compound-4.

While these small molecules in Figure 5 display that RANKL can be inhibited, there are some limitations due to the complexity of the known inhibitors. Vaccarin, for example, has a stronger binding score but is too large to be used as a drug. However, Pamidronic acid sodium and Boldine, along with our top small molecules, are smaller, though they have slightly lower binding scores than Vaccarin. It is through these less complex inhibitors that we may find potential drug applications to inhibit RANKL.

CONCLUSION

The study found potential small molecules and antiviral compounds, including Compound-4 and S1774, that can inhibit RANKL and mitigate osteoclast-mediated bone resorption. With experimental validation and optimization, these promising inhibitors of RANKL may provide a foundation for the development of targeted therapeutics to prevent or treat osteomyelitis.

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Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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ABBREVIATIONS

ADME: Absorption, Distribution, Metabolism, and Excretion; CNS: Central Nervous System; DC: Dendritic Cell; OPG: Osteoprotegerin; PSA: Polar Surface Area; QPlogBB: QikProp predicted brain/blood partition coefficient; QPlogS: QikProp predicted aqueous solubility; RANK: Receptor Activator of Nuclear Factor Kappa-B; RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand

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