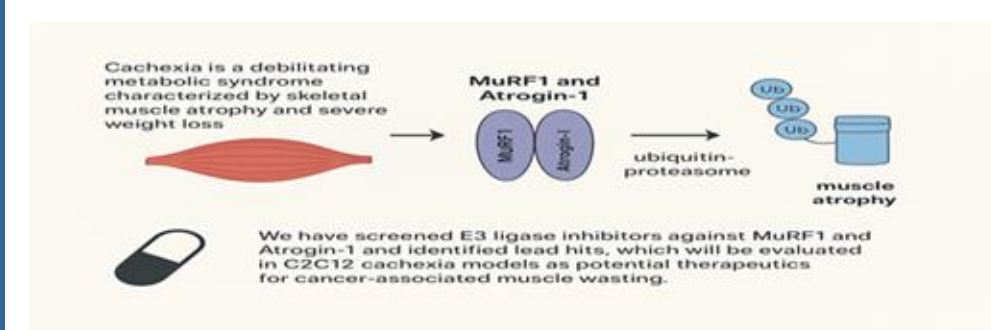


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INTRODUCTION

Cachexia is a multifactorial metabolic syndrome characterized by severe skeletal muscle loss and weight reduction that cannot be reversed by nutritional support. It commonly occurs in chronic diseases such as cancer, AIDS, COPD, and heart failure, significantly increasing morbidity, mortality, and reducing quality of life. Muscle wasting in cachexia is largely driven by activation of the ubiquitin–proteasome pathway, particularly through the muscle-specific E3 ubiquitin ligases MuRF1 and Atrogin-1 (MAFbx). Persistent upregulation of these ligases accelerates protein degradation and promotes progressive muscle atrophy. Because of their central role in muscle catabolism, MuRF1 and Atrogin-1 are promising therapeutic targets. Our study uses computational molecular modeling and docking approaches to identify small-molecule inhibitors that competitively block ligase activity. We are also exploring drug repurposing strategies to identify existing compounds with anti-cachectic potential. Top candidate inhibitors will be validated in C2C12 myotube-based cachexia models to evaluate biological efficacy. These studies aim to support the development of novel therapeutic strategies for cancer-associated cachexia and related neuromuscular conditions.



METHODS

Protein-Ligand Docking Using DiffDock-L by Neurosnap. 3D-structures of E3 ligases (MuRF1 and Atrogin-1) were obtained using UniProt or AlphaFoldDB; SMILES for each drug obtained using PubChem. Using AutoDockTools (ADT), target proteins were prepared for docking by removing water, adding hydrogens, and adding Kollman charges. Docking simulations were performed using DiffDock, a diffusion-based deep learning framework for blind protein–ligand docking. DiffDock predicts binding poses by modeling the reverse diffusion process to iteratively refine ligand conformations within the protein binding pocket without requiring predefined grid boxes. For each protein–ligand pair, multiple poses were generated. DiffDock outputs a confidence score (rank score) for each predicted pose, reflecting the model’s predicted likelihood of correct binding.

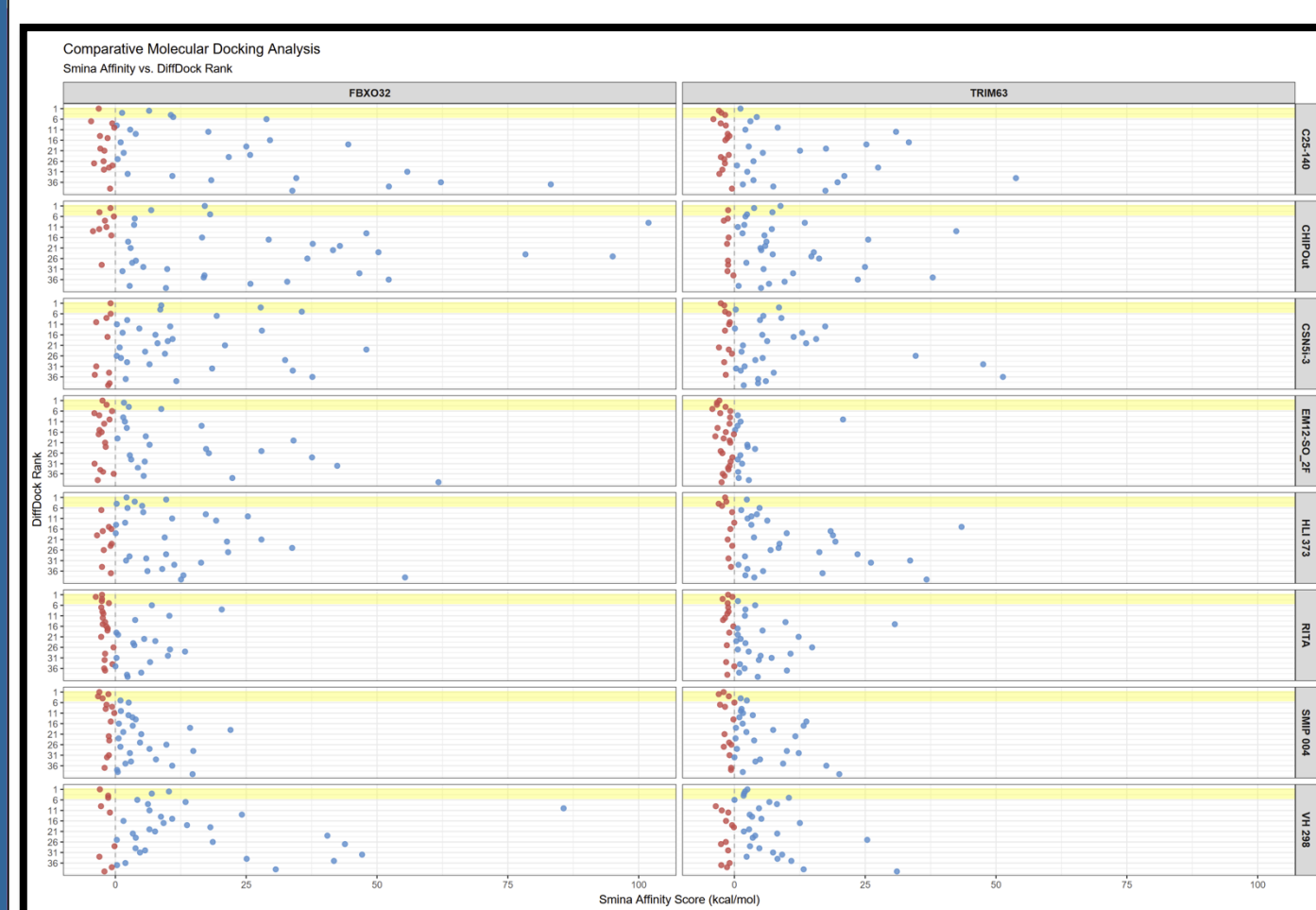


Fig 1. Molecular docking pose rank computed using DiffDock and the associated smina affinity score between Atrogin1 and MuRF1 and inhibitors. Top-5 docking ranks highlighted in yellow.

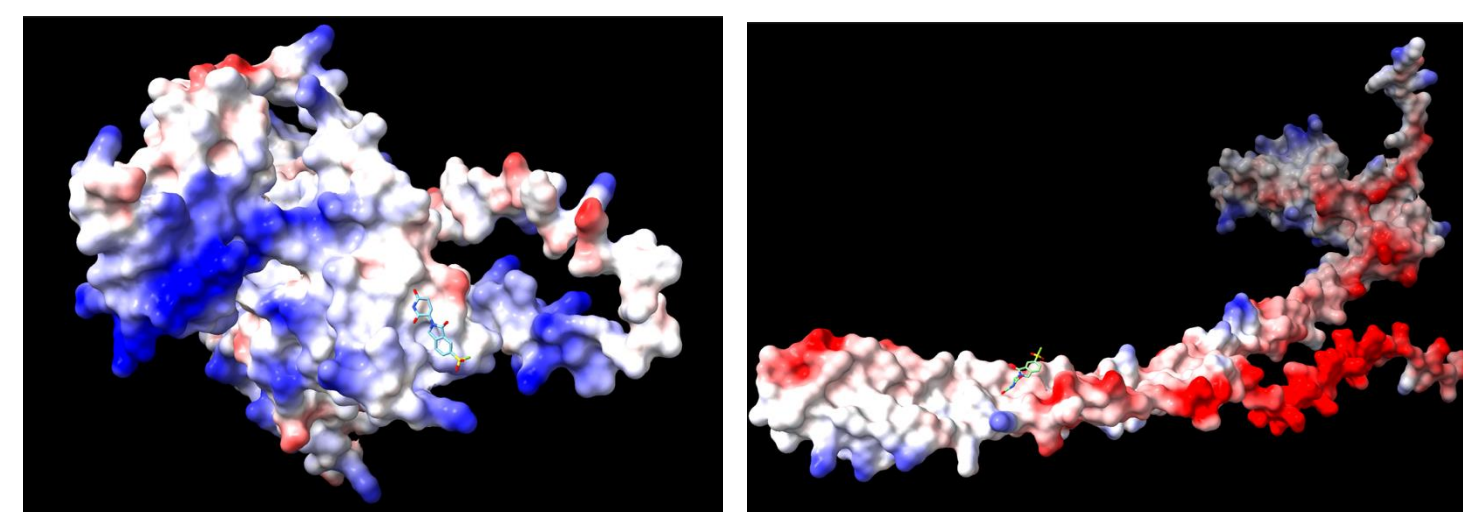


Fig. 2. 3D binding orientation of docked ligands within the MaFBx and MuRF 1 binding site.

RESULTS

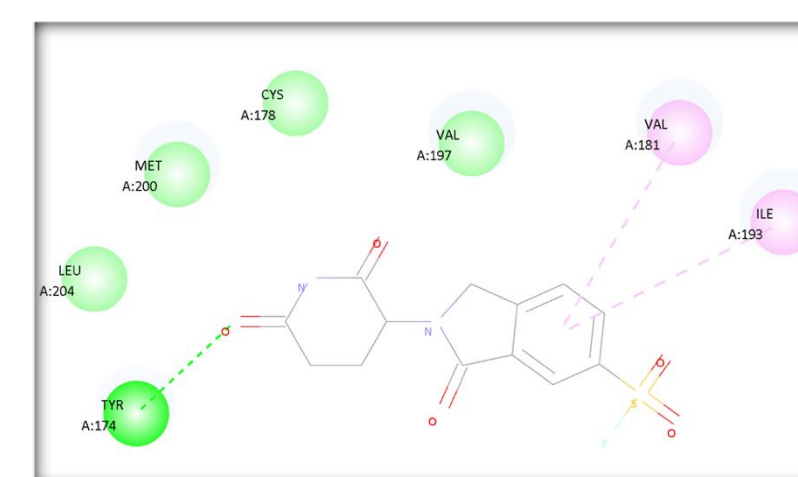
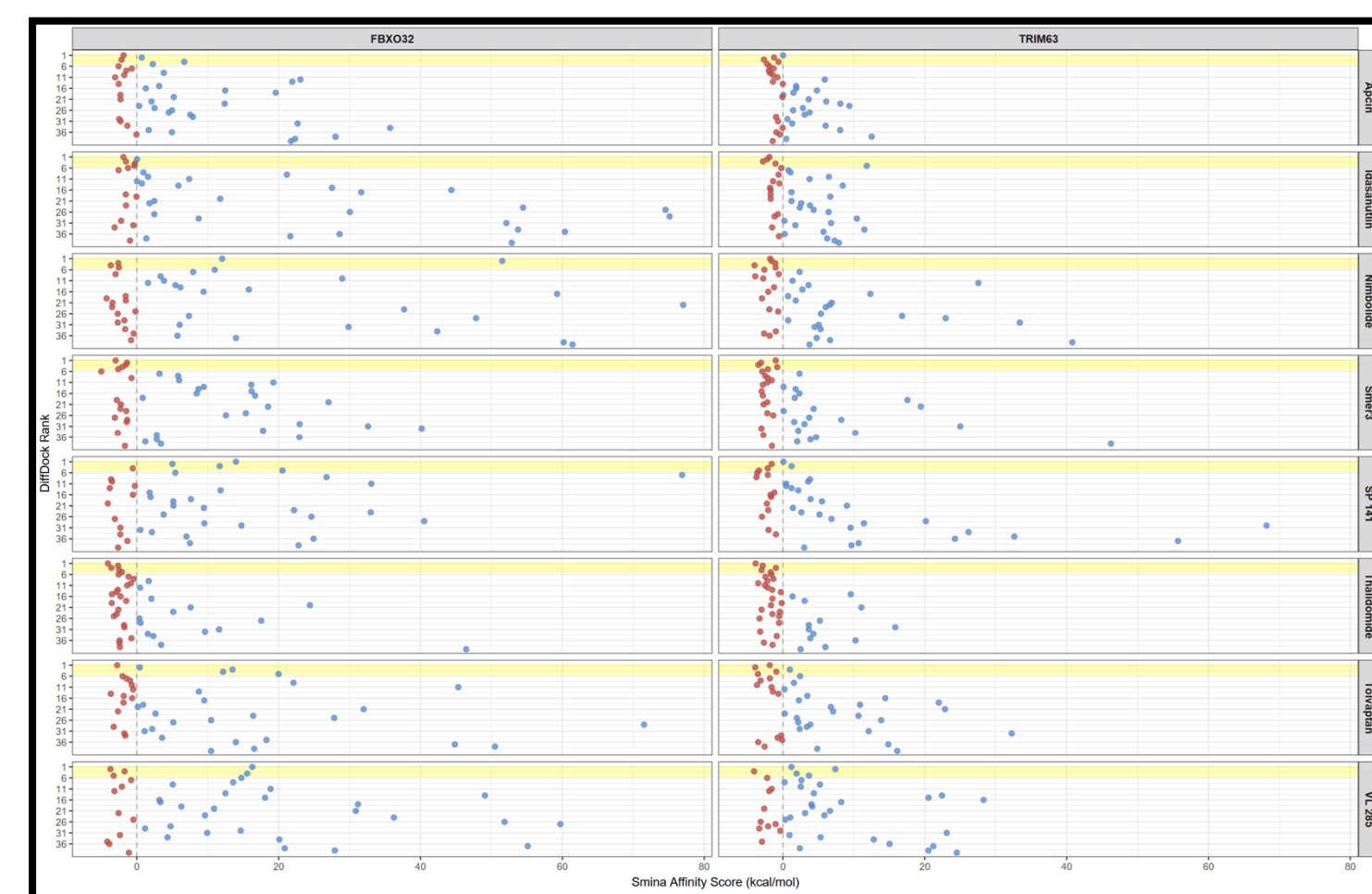
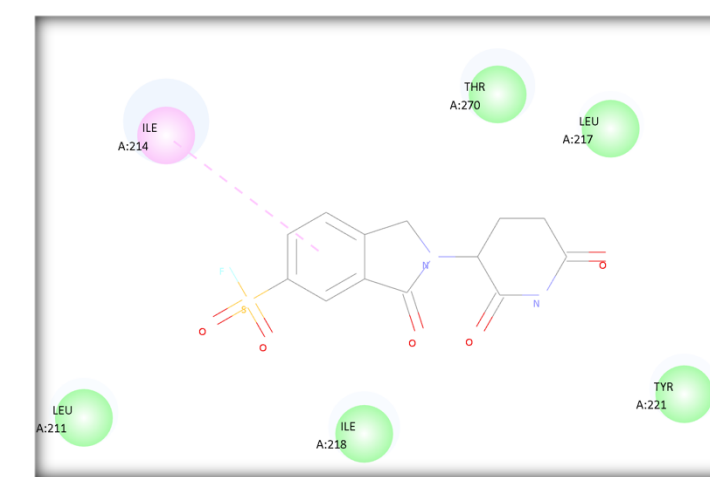


Fig. 3. 2D interaction diagrams of lead compound with Atrogin 1 and MuRF1 showing hydrogen bonding and hydrophobic contacts.



Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Alkyl

CONCLUSION

In this study, we performed computational docking of multiple reported E3 ubiquitin ligase inhibitors against the muscle-specific ligases Atrogin-1 (MAFbx) and MuRF1 to identify potential modulators of muscle protein degradation. Several compounds demonstrated favorable binding interactions within the predicted active or substrate-recognition regions of both ligases. Notably, compounds such as SMER3 and thalidomide exhibited strong predicted binding affinities and high-confidence docking poses, suggesting potential inhibitory activity against Atrogin-1 and MuRF1. These findings support the feasibility of repurposing known E3 ligase–targeting compounds for modulation of muscle atrophy pathways. Future work will expand our in-silico screening to include additional small molecules to identify candidates with improved binding strength and specificity. Top-performing compounds will be evaluated experimentally in vitro using C2C12 myotube-based cachexia models to assess their ability to attenuate muscle atrophy–associated molecular markers and functional outcomes. Together, this integrated computational and experimental strategy advances the identification of candidate therapeutics targeting muscle wasting in cancer-associated cachexia.

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