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Structure-Based Evaluation of Gemin D as a Natural HNE Inhibitor: Toward Novel Therapeutic Strategies for Foot Health in Diabetes

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A B S T R A C T

Foot ulcers represent a major complication of diabetes, contributing to impaired mobility, infection, and increased mortality. Human neutrophil elastase (HNE), a serine protease involved in chronic inflammation, plays a key role in tissue degradation and delayed wound healing. Natural polyphenols such as gemin D have emerged as promising candidates for topical anti-protease therapy. In this study, molecular docking simulations were performed to evaluate the binding affinity and interaction profile of gemin D with HNE using the high-resolution crystal structure (PDB ID: 1PPF). The docking grid was centered on the co-crystallized inhibitor site, and gemin D exhibited a binding affinity of –8.1 kcal·mol^{-1}. Key hydrogen bonds were identified with HIS57, VAL99, ARG177, SER214, VAL216, GLY218, and GLY219, along with hydrophobic contacts at PHE192 and VAL216. These results support the hypothesis that gemin D may act as a non-covalent, reversible inhibitor of HNE and warrant further in vitro and ex vivo studies to validate its therapeutic potential in diabetic foot ulcer management.

Keywords: Human Neutrophil Elastase (HNE), Diabetic Foot Ulcer, Molecular Docking, Gemin D

1. Introduction

Maintaining optimal foot health is imperative for preserving mobility and overall quality of life. Chronic wounds of the lower extremity persist as a significant cause of morbidity among individuals with diabetes. According to global estimates, 19–64% of individuals diagnosed with diabetes will develop a foot ulcer during their lifetime. Moreover, the 5-year mortality rate following a severe ulcer or lower-limb amputation approaches 45–60% (Chen, Sun, Gao, & Ran, 2023; Parveen, Hussain, Anwar, Elagib, & Kausar, 2025). Among the biochemical drivers that convert an acute lesion into a chronic, non-healing diabetic foot ulcer (DFU) is an imbalance between proteolytic burden and tissue repair. In particular, human neutrophil elastase (HNE), a serine protease released from degranulating neutrophils and NETs (neutrophil extracellular traps), accumulates at persistently inflamed wound beds (Grinnell & Zhu, 1996; Ribeiro et al., 2024; Yang et al., 2020). HNE displays broad substrate specificity, degrading structural and regulatory extracellular matrix (ECM) components, including elastin, fibronectin, laminin, and proteoglycans. In addition, HNE can inactivate growth factors or their receptors indirectly, thereby impairing granulation and re-epithelialization. Elevated levels of HNE activity in wound exudates have been observed to correlate with delayed wound closure, increased bioburden, and an elevated risk of recurrent infection, particularly in cases involving Staphylococcus aureus or Pseudomonas aeruginosa in patients with diabetic foot ulcers (DFUs) that are polymicrobial in nature. This phenomenon is referred to as a "protease–antiprotease imbalance," which occurs when endogenous inhibitors are overwhelmed or proteolytically degraded. Therefore, modulating HNE is a rational adjunct strategy for restoring a pro-healing microenvironment (Lobmann, Zemlin, Motzkau, Reschke, & Lehnert, 2006; Suleman, 2016; Yang et al., 2020). Although small-molecule synthetic elastase inhibitors have been described, clinical translation into topical foot ulcer management is constrained by issues of cytotoxicity, instability or insufficient selectivity. Consequently, phenolic and flavonoid natural compounds with reported anti-inflammatory or protease-modulating properties are under investigation as safer, formulation-friendly candidates. Nevertheless, for many such phytochemicals, atomistic binding mode data against HNE remain limited or purely predictive, leaving a gap in structure-guided prioritization (Jakimiuk, Gesek, Atanasov, & Tomczyk, 2021; Pereira et al., 2022). Despite growing interest in natural elastase inhibitors, structural insights into their binding to human neutrophil elastase (HNE) remain limited. Here, we performed molecular docking of gemin D, a phenolic compound with reported anti-inflammatory properties, using the high-resolution crystal structure of HNE (PDB ID: 1PPF). This study aims to generate preliminary structure-based evidence supporting gemin D as a potential competitive inhibitor and to inform future experimental validation.

2. Materials and Methods

2.1 Molecular docking

Molecular docking analyses were conducted to investigate the interaction between gemin D and human neutrophil elastase (HNE). The crystal structure of HNE (PDB ID: 1PPF, 1.84 Å) was obtained from the Protein Data Bank, while the ligand structure was retrieved from PubChem and optimized using

Avogadro with Gasteiger charges. Protein preparation, including hydrogen addition and charge assignment, was performed using BIOVIA Discovery Studio and AutoDockTools v4.2.6. Docking was carried out in AutoDock Vina v1.1.2 with an exhaustiveness of 32. The protocol was validated by re-docking the native inhibitor and evaluating RMSD (Baloglu et al., 2025; Yildirim et al., 2025). Binding site interactions were examined via PLIP, and molecular visualizations were generated in PyMOL v2.5.8 (Angeles Flores et al., 2024; Cetiz et al., 2024).

3. Results and Discussion

3.1 Molecular docking result

In this structure-based in silico investigation, the binding interactions of gemin D—a phenolic natural compound with reported anti-inflammatory activity—were evaluated against human neutrophil elastase (HNE), a serine protease linked to chronic wound pathogenesis. The docking simulations were performed using the high-resolution co-crystallized structure of HNE (PDB ID: 1PPF, 1.84 Å), where the binding site was defined based on the position of the native peptidyl chloromethyl ketone inhibitor. The docking grid was centered at X = 35.958, Y = 15.029, Z = 53.690 with box dimensions of $40 \times 40 \times 40$ Å, and docking was executed in AutoDock Vina with an exhaustiveness of 32. Gemin D displayed a top binding affinity of –8.1 $kcal \cdot mol^{-1}$, occupying the same inhibitor-bound cavity. The predicted binding pose involved hydrogen bonds with HIS57, VAL99, ARG177, SER214, VAL216 (dual), GLY218, and GLY219, and hydrophobic interactions with PHE192 and VAL216 (Fig. 1). These residues cluster within the active cleft adjacent to the substrate recognition subsites, supporting a plausible competitive inhibitory mechanism. Despite lacking a covalent warhead, gemin D forms a multivalent non-covalent network via its polyphenolic scaffold. These findings provide structural justification for further enzymatic validation and highlight gemin D as a candidate for topical HNE modulation in foot ulcer therapy.

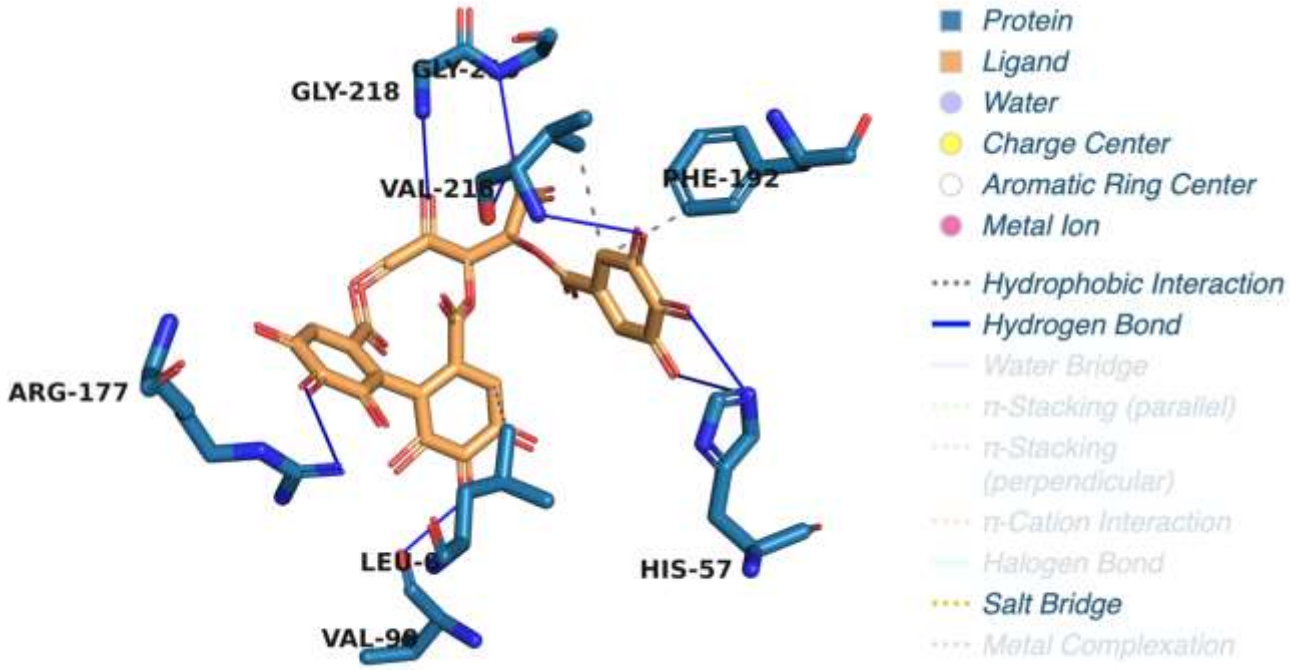

Fig. 1. Binding pose of gemin D in the human neutrophil elastase.

5. Conclusion

This molecular docking study provides structural insights into the interaction between gemin D and human neutrophil elastase (HNE), a key enzymatic contributor to impaired wound healing in diabetic foot ulcers. Gemin D demonstrated favorable binding energy and established a network of hydrogen bonds and hydrophobic interactions within the active site pocket defined by the co-crystallized inhibitor. These findings suggest that gemin D may serve as a competitive, non-covalent modulator of HNE activity. Given its natural origin and predicted affinity, gemin D holds promise as a candidate for topical therapeutic development targeting protease imbalance in chronic diabetic wounds. Experimental validation is recommended to confirm its inhibitory potential and pharmacological relevance.

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Conflict of interest: The authors declare no competing interests.

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Data Availability Data will be made available on request.

Code Availability: Not applicable

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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