Rational Design of Apigenin Derivatives as Potent Inflammatory Modulators for Improved Diabetic Wound Healing

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Abstract: Chronic diabetic wounds present a significant therapeutic challenge, characterized by ongoing inflammation, disrupted angiogenesis, and excessive remodeling of the extracellular matrix. Flavonoids from grape skins have shown encouraging anti-inflammatory and antioxidant effects; however, their moderate potency restricts their wider therapeutic application. In this research, we utilized a systematic design strategy to create and assess new apigenin derivatives that exhibit enhanced pharmacological properties. A focused library of derivatives was screened against key protein targets involved in diabetic wound pathology, such as NF-κB, VEGFR-2, TGF-β1, and MMP-9, utilizing a combination of *in silico* based modifications and molecular docking techniques. The docking results demonstrated that derivatives showed significantly improved binding affinity in comparison to apigenin, especially with respect to MMP-9, suggesting potential inhibitory activity in the nanomolar to subnanomolar range. Comparable or slightly enhanced binding of VEGFR-2 was noted, alongside similar advancements in the modulation of NF-κB and TGF-β1. The results indicate that by optimizing the structure of apigenin, we can create derivatives that exhibit notably enhanced anti-inflammatory and anti-remodeling properties. The designed molecules effectively address both NF-κB-driven inflammation and MMP-9-mediated matrix degradation, offering a compelling basis for further validation as promising therapeutic candidates in the treatment of chronic diabetic wounds.

Keywords: Diabetes, Wounds, Apigenin, Drug Design, Circular Economy

1. Introduction

Diabetic foot ulcers (DFU) and various chronic wounds stand as significant complications of diabetes mellitus, frequently resulting in infections, hospital admissions, and, in severe cases, limb amputation. In contrast to acute wounds that typically resolve inflammation within a matter of days, diabetic wounds persist in a prolonged state of inflammation. This condition is marked by an excessive infiltration of immune cells, an overproduction of pro-inflammatory cytokines, and a disruption in the

remodeling of the extracellular matrix (ECM). Strategies aimed at therapeutically modulating these processes hold significant clinical relevance [1,2].

Numerous signaling proteins are crucial in the pathology of wounds associated with diabetes. The NF-κB pathway is responsible for the increased expression of inflammatory mediators, VEGFR-2 plays a crucial role in angiogenesis and the formation of blood vessels in tissues, TGF-β1 is key in regulating fibroblast growth and the deposition of extracellular matrix, and MMP-9 is involved in the detrimental breakdown of the extracellular matrix. Although VEGFR-2 signaling is essential for angiogenesis, in diabetic wounds this pathway is often dysregulated, leading to abnormal, leaky, and non-functional vessels; thus, modulation rather than full inhibition of VEGFR-2 activity is a rational therapeutic approach to restore balanced and effective vascularization. Similar can be said for TGF-β1, which means that VEGFR-2 and TGF-β1 should be modulated, not higly inhibited like NF-κB and MMP-9. Flavonoids from grape skins, have garnered significant interest due to their anti-inflammatory, antioxidant, and antimicrobial characteristics, including interactions with aforementioned proteins [1,2]. In our earlier research, showcased at the ATINER Diabetes Microsymposium (2025), we deducted that grape skin extracts abundant in apigenin exhibited notable biological activity pertinent to wound healing, such as antioxidant capacity and initial indications of target modulation [3]. Nonetheless, apigenin itself faces challenges due to its moderate potency, limited bioavailability, and low selectivity. In order to address these limitations, rational drug design was utilized. Through the application of computational drug design, ADMET filtering, and molecular docking techniques, we successfully generated and assessed novel derivatives of apigenin in relation to NF-κB, VEGFR-2, TGF-β1, and MMP-9. The objective was to pinpoint optimized candidates that exhibit improved pharmacological profiles, making them suitable for integration into hydrogel-based wound therapies.

2. Materials and methods

Apigenin was selected as the parent molecule for structural optimization based on previous experimental evidence of its anti-inflammatory and antioxidant properties in diabetic wound models. Novel derivatives were generated using the CReM (Chemically Reasonable Mutations) framework [4], which enables systematic modification of molecular scaffolds by fragment replacement while preserving chemically valid structures. This approach allowed the introduction of substituents that could improve binding affinity, solubility, and overall pharmacological properties without compromising the flavone core. Content radius was set to 3-5, and maximal number of replecments was set at 10 in Scafold mode.The generated derivative library was subjected to rigorous filtering based on absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters. Predictions were performed using ADMETlab 3.0 [5] and SwissADME platforms [6]. Candidate molecules were prioritized according to the following criteria - MW (250-400 Da) suitable for diffusion and incorporation into hydrogel matrices, TPSA (60–110 Å²) optimal for balancing solubility and permeability,

nRot (≤ 7) ensuring conformational stability and hydrogel compatibility, full compliance required with druglikeness criteria, low reactivity, promiscuity, and aggregation potential, and hydrogel incorporation potential: candidates with moderate lipophilicity and hydrogen bonding capacity favored to ensure effective immobilization and controlled release within polymeric networks. Following these criteria, only a subset of derivatives was retained as promising candidates for further docking studies. The final set of apigenin derivatives was evaluated using AMDock, a graphical front-end for AutoDock4Zn/AutoDock4, to explore binding interactions with key protein targets implicated in diabetic wound pathology [7]. The following protein structures were retrieved from the RCSB PDB: NF-κB (ID: 1A3Q), VEGFR-2 (ID: 3V6B), TGF-β1 receptor (ID: 7SXB), and MMP-9 (ID: 2OVX).

3. Results and Discussion

A library consisting of 3392 apigenin derivatives was created utilizing the CReM platform, which incorporated chemically plausible modifications to the flavone scaffold. The structures underwent a series of iterative *in silico* ADMET filtering processes utilizing ADMETlab 3.0 and SwissADME. Through a systematic approach that involved the application of progressively stringent criteria the library was refined down to five lead candidates (Figure 1). These candidates exhibit promising pharmacokinetic properties and show potential for integration into hydrogel formulations.

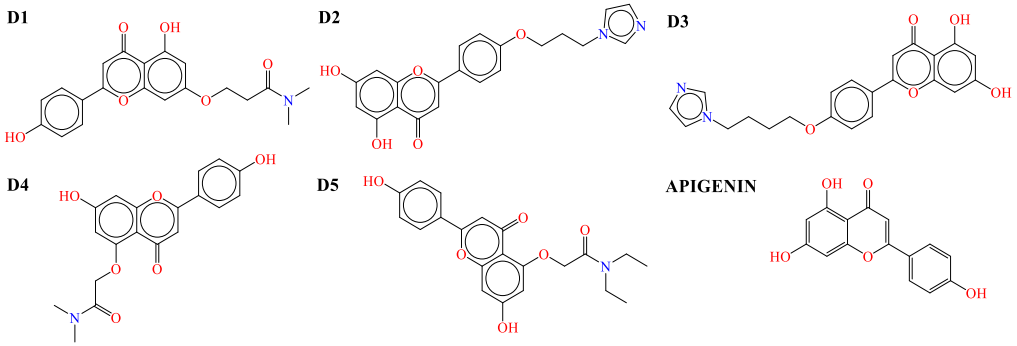

Figure 1. Apigenin derivatives obtained through CReM and filtered through ADMET analysis

In comparison to the parent compound apigenin, the chosen derivatives exhibited enhanced ADMET properties, showcasing improved predicted stability, permeability, and drug-likeness profiles, all while minimizing the potential for off-target effects. The characteristics of these derivatives indicate that they may surpass apigenin in terms of both pharmacological efficacy and formulation applications.

The results from molecular docking validated these predictions, indicating that all five derivatives exhibited stronger interactions with the chosen protein targets than apigenin (Table 1). The derivatives demonstrated a remarkable ability to bind to NF-κB and MMP-9, suggesting an improved potential for anti-inflammatory and anti-remodeling effects. The interactions with TGF-β1 showed notable enhancement, indicating a more effective regulation of fibroblast activity and extracellular matrix deposition, whereas the affinities for VEGFR-2 remained similar or exhibited slight improvement. Collectively, these findings emphasize that thoughtfully engineered apigenin derivatives not only exceed the original molecule regarding anticipated

pharmacokinetic properties but also demonstrate enhanced interactions with crucial molecular targets involved in diabetic wound pathology. Their ability to mitigate chronic inflammation and limit excessive matrix degradation makes them strong candidates for further experimental validation and for integration into hydrogel-based delivery systems aimed at treating diabetic wounds.

Table 1. Parameters describing interactions between derivatives and selected proteins

	D1	D2	D3	D4	D5	Apigenin
NF kB	-9.97/*0.49*	-9.73/*0.74*	-9.63/*0.87*	-9.93/*0.38*	-9.73/*0.74*	-8.74/*0.39*
VEGFR-2	-7.64/*2.51*	-7.26/*4.77*	-7.40/*3.77*	-7.13/*5.94*	-7.40/*3.77*	-7.04/ *6.91*
TGF-β1	-6.31/*23.70*	-6.22/*27.59*	-6.75/*11.28*	-7.59/*2.73*	-6.02/*38.66*	-6.36/*21.78*
MMP-9	-10.56/0.18	-13.84/*0.00*	-14.19/*0.00*	-10.87/*0.11*	-11.25/*0.05*	-7.35/*4.10*

4. Conclusions

This study demonstrates that rationally designed apigenin derivatives possess superior ADMET profiles and enhanced binding affinities compared to the parent compound, particularly toward NF-κB and MMP-9. These properties suggest improved anti-inflammatory and anti-remodeling potential. The identified derivatives represent promising candidates for further validation and possible integration into hydrogel-based therapeutic systems for chronic diabetic wound healing.

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