A multi-targeted *In Silico* approach to select and rank regenerative drugs for managing diabetic wound inflammation signalling through IGFR, TNFR and PPAR -γ pathways

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Abstract. The present research work explores the possibilities of molecular docking to predict and rank the most suitable drugs for diabetic foot ulcer or any degenerate inflammatory wounds among ten different drugs commonly used in wound regeneration. This is achieved by targeting multiple pathways simultaneously in inflammation and wound healing, Tumour necrosis factor Receptor pathway (TNFR) and Peroxisome Proliferator-Activated Receptor -γ (PPAR-γ) for macrophage polarization, inflammation management, collagen deposition etc., and Insulin-like Growth Factor Receptor (IGFR) signalling for wound regeneration. Molecular docking studies were carried out using Swiss Dock which ranked the natural Phyto estrogenic isoflavone Genistein as the best having strong binding affinity to all the receptors TNFR, PPAR-γ, and IGFR; highlighting its potential to regulate inflammation, macrophage polarization, pro angiogenic properties and thus wound regeneration.

Keywords. Wound healing, Genistein, IGFR, TNFR, PPAR-γ, Macrophage polarization, Diabetic foot ulcer, wound regeneration, Swiss dock, Molecular docking.

1. Introduction

Wound regeneration and healing is a complex yet essential physiological process that restores the integrity of damaged tissues following injury. This process involves a series of overlapping phases like haemostasis, inflammation, proliferation, and remodelling which work synergistically in ensuring proper tissue repair [1,2]. —each playing a critical role. However, factors can hinder the healing process, such as infections, chronic inflammatory conditions delay wound healing leading to prolonged recovery and adverse outcomes as it is seen in diabetic foot ulcer. [3]

Molecular docking has become an essential tool in drug design, a computational technique used to predict the interaction of small molecules with target proteins, especially drug binding

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affinity to protein receptors involved in key cellular pathways. Thus, molecular docking provides insights into the therapeutic potential of several drugs saving time, effort and cost compared to conventional drug development strategies [4].

1.1 Molecular targets in wound regeneration Pathways

Wound healing involves the complex yet coordinated action of several molecular pathways [5]. In the initial phase of wound healing, inflammation happens and the release of pro-inflammatory cytokines like tumour necrosis factor (TNF -α) release have a pivotal role as TNF binds to tumor necrosis factor receptor (TNFR) which triggers the activation of a cascade of intracellular signalling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), promoting inflammation mediated immune responses [6].

During the second phase or the proliferation phase growth factors such as insulin-like growth factor 1 (IGF-1) and platelet-derived growth factor (PDGF) are released. These two molecules bind to insulin-like growth factor receptor (IGFR) which stimulates key events in wound regeneration; cell proliferation, migration, and differentiation and thus healing [7]. Another key molecule involved in the process of promoting adipogenesis and tissue repair is peroxisome proliferator-activated receptor -γ (PPARγ) which is activated during proliferation phase, [8] followed by collagen synthesis and deposition happens during remodelling phase under PPARγ regulation which is essential for wound strength and tissue integrity [9]. Moreover, PPARγ signalling also regulates the expression of some selective genes actively involved in tissue remodelling [10].

The interactions between TNFR, IGFR, and PPARγ signalling pathways are complex and coordinated, with TNF-α inhibiting IGFR signalling and IGFR activation reducing TNF-α production [11]. PPAR-γ activation also inhibits TNFR signalling, reducing inflammation and promoting tissue repair [12,13].

1.2 Molecular Docking and *In silico* Drug Discovery

Being a recently developed yet efficient drug discovery strategy, molecular docking is a widely accepted *in silico* process that focuses on the interactions between a drug or ligand to target molecules leading to activation or inhibition of molecular interaction and signalling. This is gaining attention in drug discovery process, as it allows researchers to screen large libraries of compounds cost-effectively and quickly [4]. Swiss dock is an open access molecular docking software which helps to estimate the free energy of binding between proteins and its selective ligands in a binding state [14]

2. Materials and methods

2.1 Protein and Ligand Selection

The target proteins and ligands were selected for the *in silico* molecular drug docking studies using swiss dock open access software. Tumour necrosis factor Receptor (TNFR) and Peroxisome Proliferator-Activated Receptor -γ (PPAR-γ) were selected as targets as they are involved in macrophage polarization, and inflammation management. Insulin-like Growth Factor Receptor (IGFR) was selected as a candidate molecule inducing regeneration and growth.

To begin with, the crystal structures of all the three target molecules were downloaded from the Protein Data Bank (PDB);

IGFR (PDB ID: 1K3A); TNFR (PDB ID: 3MHD); and PPAR-γ (PDB ID: 8HUP). These proteins were reported to have well established roles in inflammation, wound regeneration, and remodelling; thus, known as key molecular targets in wound repair. Protein sequences were then converted to .mol2 format using Open Babel GUI [15].

The selected drug candidates for IGFR docking studies are as follows;
Ononin (PubChem ID :442813), Genistein (PubChem ID: 5280961), Curcumin (PubChem ID: 969516), Puerarin (PubChem ID :5281807), Glycitein (PubChem ID:5317750), Biochanin A (PubChem ID: 5280373), Formononetin (PubChem ID: 5280378), Prunetin (PubChem ID: 5281804), Daidzein (PubChem ID: 5281708), and Calycosin (PubChem: 5280448)

The selected drug candidates for TNFR docking studies are as follows;
Genistein (PubChem ID :5280961), Glycitein (PubChem ID:5317750), Daidzein (PubChem ID: 5281708), Homopterocarpin (PubChem ID: 101795), Tectorigenin (PubChem ID: 5281811), Equol (PubChem ID: 91469), Sativanone (PubChem ID: 13886678), and Coumestrol (PubChem ID : 5281707)

The selected drug candidates for PPAR-γ docking studies are as follows;
Genistein (PubChem ID: 5280961), Tectorigenin (PubChem ID: 5281811), Sativanone (PubChem ID: 13886678), Biochanin A (PubChem ID: 5280373), Calycosin (PubChem ID: 5280448), Daidzein (PubChem ID: 5281708), Homopterocarpin (PubChem ID: 101795), Formononetin (PubChem ID: 5280378), Coumestrol (PubChem ID: 5281707), and Equol (PubChem ID: 91469),

2.2 Molecular Docking

Swiss Dock web server, which is based on the EADock DSS algorithm were used for the molecular docking which effectively demonstrated protein-ligand docking and predicted the binding interactions between the ligand and the active site of the proteins.

Initially the following steps were followed for molecular docking:

1. **Protein Preparation**: The PDB structures of the target proteins were downloaded, and cleaned to optimize for docking by removing any non-essential ligands, water molecules, and cofactors.
2. **Ligand Preparation**: PubChem databse is then utilized to obtain the 3D structures of the wound regenerative compounds or ligands and then converted to the '.mol2 format' suitable for further Docking analysis.
3. **Docking Process**: Both the ligands and cellular protein targets were uploaded to Swiss Dock and after that simulations were performed. The results obtained were then ranked based on the drug binding affinity, represented as FF Score - Full Fitness energy scores (kcal/mol). The molecular docked poses with the lowest binding energies were selected for further analysis.

3. Results and Discussion

The binding affinities between the ten ligands selected and the three protein target molecules were evaluated based on their FF Scores (Full Fitness energy scores in kcal/mol) represented in Table 1 and 2. The docking results provided insight into the binding strength and stability of the ligand-protein complexes. A representative figure showing molecular docking of IGFR using a conformer Genistein is given below (Fig 1).

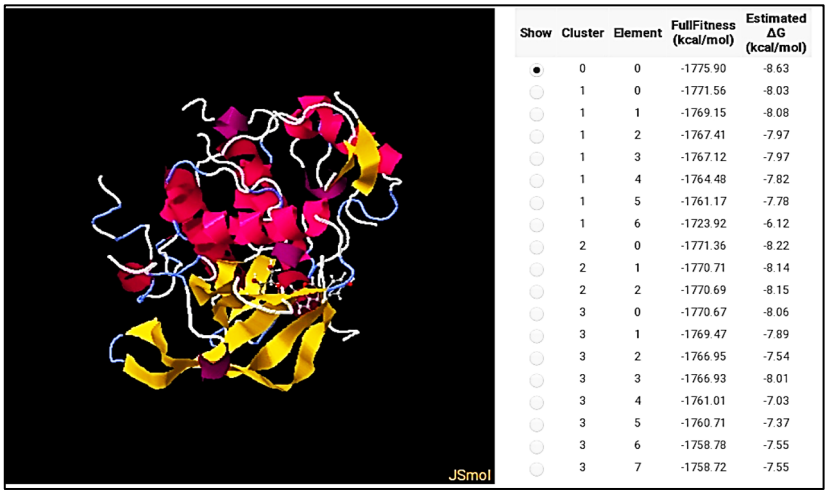

Fig. 1. Molecular docking of IGF1R (1K3A) using conformer 1 Genistein (ID-5280961)

Table 1. Molecular docking results of TNFR with different conformers.

RANK	CONFORMER	PubChem ID	TNFR Binding Energy Estimated ΔG(kcal/mol)	RELATIVE STABILITY (Based on binding energy)
1	Genistein	5280961	-6.91	Highly stable
2	Glycitein	5317750	-6.88	Moderately stable
3	Daidzein	5281708	-6.86	Moderately stable
4	Homopterocarpin	101795	-6.85	Moderately stable
5	Tectorigenin	5281811	-6.81	Moderately stable
6	Equol	91469	-6.80	Moderately stable
7	Sativanone	13886678	-6.58	Moderately stable
8	Coumestrol	5281707	-6.47	Moderately stable

Table 2. Molecular docking results of PPAR -γ with different conformers.

RANK	CONFORMER	ID	PPAR -γ Binding Energy Estimated ΔG (kcal/mol)	RELATIVE STABILITY (Based on bindingenergy)
1	Genistein	5280961	-7.96	Highly stable
2	Tectorigenin	5281811	-7.92	Highly stable
3	Sativanone	13886678	-7.79	Highly stable
4	Biochanin A	5280373	-7.68	Moderately stable
5	Calycosin	5280448	-7.68	Moderately stable
6	Daidzein	5281708	-7.60	Moderately stable
7	Homopterocarpin	101795	-7.56	Moderately stable
8	Formononetin	5280378	-7.51	Moderately stable
9	Coumestrol	5281707	-7.41	Moderately stable
10	Equol	91469	-7.37	Moderately stable

Table 3. Molecular docking results of IGFR with different conformers.

RANK	CONFORMER	ID	IGFR Binding Energy Estimated ΔG(kcal/mol)	RELATIVE STABILITY (Based on binding energy)
1	Genistein	5280961	-8.63	Highly stable
2	Curcumin	969516	-8.43	Highly stable
3	Ononin	442813	-8.35	Moderately stable
4	Puerarin	5281807	-7.78	Moderately stable
5	Glycitein	5317750	-7.27	Moderately stable
6	Biochanin A	5280373	-7.23	Moderately stable
7	Formononetin	5280378	-7.22	Moderately stable
8	Prunetin	5281804	-7.15	Moderately stable
9	Daidzein	5281708	-7.13	Moderately stable
10	Calycosin	5280448	-7.10	Moderately stable

The protein IGF1R - 1K3A, TNFR and PPAR -γ and chemical conformers were docked with Swissdock using default parameters. The values were obtained in terms of energy full fitness and ΔG Kcal/mol. Different docking poses were screened based on ΔG Kcal/mol value; the appropriateness of the docked pose means lowest ΔG Kcal/mol energy. For IGFR docking, the best binding score or ΔG Kcal/mol was found with GENISTEIN (PubChem ID 5280961) followed by CURCUMIN (PubChem ID 969516) about -8.63 Kcal/mol, and -8.43 Kcal/mol respectively. So, the docking studies based on the binding energy of individual conformers with IGF1R- 1K3A in 10 different poses have revealed that the compounds GENISTEIN and CURCUMIN are having the least binding energy. The docking results with other molecular targets indicated that Genistein exhibits relative binding affinity with TNFR (PDB ID:3MHD) and PPAR -γ (PDB ID: 8HUP). Genistein docked with TNFR yielded a moderately favorable binding score of (-6.91), suggesting an interaction and potential

modulation of TNFR related signaling pathways. Similarly, Genistein showed significant binding affinity with PPAR -γ, with a docking score (-7.96) indicating robust interaction and potential implications for PPAR -γ activity. These findings underscore Genistein's potential as a modulator of all the three major receptors involved in wound healing and regeneration, IGFR, PPAR -γ and TNFR highlighting its relevance in therapeutic strategies targeting these pathways.

4. Conclusion

In conclusion, the present molecular Docking study successfully demonstrated the degree of binding energies of selected chemical conformers against three molecular targets IGFR, PPAR -γ and TNFR which are playing pivotal role in regulating signaling events within the cell, ultimately leading to various cellular responses important for wound cell regeneration, angiogenesis and healing**.** Among the compounds tested, Genistein can be considered as the lead compound to be suggested as the best drugs to be used in the drug formulations for wound healing *in vitro* and *in vivo* experimentations and nanodrug formulations as it activate Cellular Responses Mediated by IGFR which primarily include cell proliferation and migration where IGF-1/IGF-IR pathway stimulates the proliferation and migration of various cell types essential for wound healing, including fibroblasts (collagen producers), keratinocytes (resurface the skin), and endothelial cells (form new blood vessels). Genistein is also having significant interaction with PPAR-γ and TNFR making it noble candidate molecule in regulating macrophage polarization as part of regeneration of wound tissue, collagen deposition and remodeling. So Genistein is shown to be the best stable drugs in the biological system compared to other conformers used in this study for the development of drugs for degenerate and resistant wounds like diabetic wounds which deserve multitargeted approach or regulation of multiple pathways involved in wound healing and regeneration.

5. Declarations

Ethics approval and consent to participate: No animal or human experimentation. Only *in silico* methods are used
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