ORIGINAL PAPER

ENHANCING DRUG-TARGET INTERACTIONS THROUGH DUAL-DRUG DOCKING: A COMPUTATIONAL STUDY OF DICLOFENAC-HEPARIN COMPLEXES WITH FACTOR X_A

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Abstract. *This study explores the molecular interactions between two drugs, diclofenac, and heparin, through computational docking to understand the impact of complex formation on binding affinity, orientation, and pharmacological properties. Using molecular modeling and docking simulations, we analyzed how diclofenac and heparin bind individually and as complexes to target receptor Factor Xa, critical in coagulation pathways. Results demonstrate that forming diclofenac-heparin complexes significantly enhances binding affinity, with lower binding energies compared to individual drugs, indicating more stable interactions. Notably, the orientation of the complex (diclofenac_heparin versus heparin_diclofenac) affected the binding site and binding energy, suggesting that sequence and orientation in complex formation are crucial factors in drug-target interaction. Additionally, differences in lipophilicity (logP values) between the complexes suggest that binding orientation may influence bioavailability and membrane permeability. These findings underscore the potential for dual-drug complex formation to enhance pharmacological efficacy, paving the way for optimized drug combinations in therapeutic applications.*

Keywords: *molecular docking, diclofenac, heparin, Factor Xa.*

1. INTRODUCTION

Diclofenac is a widely used nonsteroidal anti-inflammatory drug (NSAID) known for its effectiveness in managing pain and inflammation associated with various conditions such as arthritis, menstrual cramps, migraines, and postoperative pain. Originally introduced in the 1970s, diclofenac functions by inhibiting cyclooxygenase (COX) enzymes, primarily COX-2, thereby reducing the production of prostaglandins—chemical messengers involved in the inflammatory response [1]. Available in various forms, including oral tablets, topical gels, and injections, diclofenac offers versatility for both systemic and localized pain relief [2].

Despite its widespread use and efficacy, diclofenac has been linked to certain risks, particularly gastrointestinal, cardiovascular, and renal complications. The drug's potential side effects have raised concerns and led to regulatory scrutiny, with some countries imposing usage restrictions [3]. Diclofenac's environmental impact has also attracted attention; improper disposal has contributed to environmental contamination, affecting wildlife, particularly vultures in South Asia, where it caused a sharp decline in vulture populations due

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to toxicity [4]. Given its benefits and risks, diclofenac remains a topic of ongoing research to balance its therapeutic value against its potential adverse effects.

Heparin is an anticoagulant medication widely used to prevent and treat blood clots, particularly in patients at risk of venous thromboembolism, pulmonary embolism, and heart attacks. First discovered in 1916 and introduced into clinical use in the 1930s, heparin has since become a mainstay in anticoagulant therapy due to its rapid action and effectiveness in preventing thrombus formation [5,6]. As a glycosaminoglycan, heparin works by enhancing the inhibitory activity of antithrombin III, which in turn reduces the activity of thrombin and factor Xa—two crucial components in the blood clotting cascade [7]. Heparin is administered in two main forms: unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH), each with distinct pharmacokinetic properties, dosing protocols, and clinical applications [8]. While UFH requires regular monitoring and dose adjustments due to its unpredictable response, LMWH offers a more consistent anticoagulant effect and can be administered via subcutaneous injection, making it suitable for outpatient care [6]. Despite its efficacy, heparin use is associated with certain risks, such as bleeding complications and heparin-induced thrombocytopenia (HIT), a potentially severe immune-mediated response to heparin [9]. Therefore, understanding heparin's pharmacology, applications, and potential risks is essential in optimizing its use in clinical practice.

Molecular docking is a computational technique widely used in drug discovery and structural biology to predict the preferred orientation and binding affinity of a small molecule (ligand) to a target biomolecule, such as a protein. By simulating the interaction between molecules, docking helps in understanding and designing new compounds with high specificity and binding efficiency, aiding in the development of drugs, enzyme inhibitors, and therapeutic compounds [10]. The primary goal of molecular docking is to find the optimal conformation of a ligand within a binding site, minimizing the binding energy and thereby predicting the strength and stability of the interaction [11].

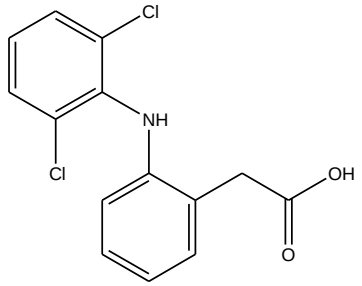
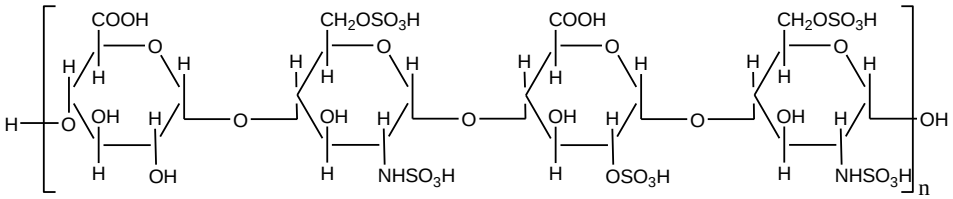
Docking studies involve two key components: a scoring function that estimates the binding affinity, and an algorithm that samples various conformations to identify the most favorable pose. These scoring functions are designed to evaluate the fit between ligand and receptor based on intermolecular forces such as hydrogen bonding, van der Waals interactions, and electrostatic interactions [12, 13]. With advances in computational power and algorithms, molecular docking has become faster and more accurate, enabling virtual screening of large chemical libraries and accelerating early-stage drug discovery [14]. Despite its utility, docking still faces challenges, including accurately predicting protein flexibility and accounting for solvent effects, areas that are currently the focus of extensive research [15].

2. MATERIALS AND METHODS

2.1. MATERIALS

The studied compounds are represented by diclofenac and heparin (Table 1).

Table 1. Structure of the studied compounds [15].

Structure	Compound
Diclofenac	
Heparin	

2.2. METHODS

A computational modeling study was conducted on the molecules heparin and diclofenac using the HyperChem software suite [16]. To investigate their binding interactions with the active site of a target receptor, docking simulations were performed with HEX software [17]. Receptor structures used in these simulations were obtained from the Protein Data Bank (PDB) [18].

To evaluate hydrophobicity (lipophilicity), a key parameter in drug design influencing bioavailability and cell membrane permeability [19], we calculated the partition coefficient (log P) values for heparin and diclofenac. These calculations were performed using HyperChem [15], and the resulting values are summarized in Table 2.

Table 2. The partition coefficient of the studied compounds [15]

Structure	logP (octanol/water)
Diclofenac	-0.21
Heparin	-2.33

3. RESULTS AND DISCUSSION

The initial phase of our research involved using the HyperChem program to perform molecular modeling on compounds heparin and diclofenac. After completing the modeling, we used the Hex 8.0.0 software to assemble these compounds into complexes. In Hex, one compound was designated as the ligand, while the other served as the receptor. The main goal of this analysis was to assess whether the binding order of these two compounds within the complex influenced the results (see Table 3).

Table 3. Docking order and docking energies for compounds heparin and diclofenac [16]

Ligand	Receptor	Energy
diclofenac	heparin	-140.56
heparin	diclofenac	-146.73

The docking results presented in Table 3 indicate the binding energies of two configurations in which diclofenac and heparin interact as ligands and receptors. When diclofenac acts as the ligand and heparin as the receptor, the binding energy is calculated at -140.56 kcal/mol. Conversely, when heparin serves as the ligand and diclofenac as the receptor, the binding energy is slightly lower, at -146.73 kcal/mol.

The difference in binding energies suggests that the sequence of ligand-receptor roles influences the stability of the interaction, with heparin as the ligand resulting in a slightly more stable complex. This could be attributed to the molecular characteristics of heparin, a large, highly negatively charged polysaccharide, which may form stronger interactions when actively binding to diclofenac, a smaller, hydrophobic molecule. Additionally, the structural flexibility and high degree of hydrogen bonding potential in heparin might allow it to adapt its conformation more effectively when acting as the ligand, enhancing binding affinity.

These findings underscore the importance of binding orientation in molecular interactions. Although the energy difference is modest, it could have implications in a biological context, potentially affecting how these molecules behave in vivo. Such insights are valuable for future studies on optimizing drug-receptor interactions, particularly in designing compounds with specific binding preferences to enhance therapeutic efficacy [20]. Further exploration, possibly through molecular dynamics simulations, could provide a deeper understanding of the stability and interaction mechanisms in these complexes.

An essential factor in understanding these interactions is lipophilicity, commonly quantified as the logarithm of the partition coefficient (logP), which indicates a compound's preference for lipid (or octanol) phases over water [21]. LogP values are critical for predicting a molecule’s solubility, permeability, and overall bioavailability. In this study, we analyze the logP values to evaluate their impact on the interactions existing in the complexes formed between diclofenac and heparin.

Table 4. Partition coefficient of diclofenac-heparin complexes [15].

Compound	logP (octanol/water)
diclofenac-heparin	3.76
heparin - diclofenac	4.04

The logP values in Table 4 reveal that the heparin-diclofenac complex (logP = 4.04) is slightly more lipophilic than the diclofenac-heparin complex (logP = 3.76). This variation in lipophilicity suggests that the heparin-diclofenac orientation may exhibit enhanced membrane permeability, making it more likely to traverse lipid bilayers and potentially exhibit distinct bioavailability profiles compared to the diclofenac-heparin complex. The increased lipophilicity of the heparin-diclofenac configuration could improve its ability to penetrate tissues, which is advantageous for certain therapeutic applications requiring deeper tissue delivery.

However, higher lipophilicity may also have trade-offs. It could reduce solubility in aqueous environments, potentially impacting the complex’s distribution in the bloodstream and its overall pharmacokinetics. This balance between lipophilicity and solubility is critical in drug development, as optimal lipophilicity is necessary for achieving efficient absorption while maintaining sufficient solubility for systemic circulation. Differences in the logP values between the two configurations may also influence the complexes' suitability for specific drug delivery routes. For instance, a more lipophilic compound might be better suited for transdermal or oral delivery, where lipid-rich environments dominate, while a less lipophilic one may perform better in aqueous formulations or intravenous applications.

This data highlights the importance of binding orientation and its impact on the physicochemical properties of drug complexes. Understanding these differences is essential for optimizing therapeutic efficacy, as bioavailability and tissue distribution are closely tied to

lipophilicity. Further experimental studies are necessary to validate these computational predictions and explore how these differences in logP values affect pharmacological behavior in vivo. Such studies could provide crucial insights into the design of drug combinations and the development of effective delivery systems tailored to specific therapeutic needs. In the next phase of our study, we present the results of molecular docking simulations between our complexes and a receptor from the Protein Data Bank (PDB): 1fax (for Factor Xa). By incorporating structural data, we aim to elucidate how our complexes interact with the receptor, providing insights into the three-dimensional nature of these interactions and their potential pharmacological implications [22,23].

Table 5. The docking results of the binding energies with Factor Xa [17].

Compound	**Energy**
heparin_diclofenac	-576.86
diclofenac_heparin	-566.76
diclofenac	-433.13
heparin	-290

The docking results in Table 5 reveal the binding energies of heparin, and diclofenac, as well as their complexes with Factor Xa, a key enzyme in the coagulation pathway. Among the configurations tested, the heparină_diclofenac complex exhibits the most favorable binding energy at -576.86 kcal/mol, indicating the highest stability when interacting with Factor Xa [24]. This suggests that when heparin acts as the receptor and diclofenac binds as the ligand, the resulting complex has an enhanced affinity for Factor Xa, likely due to a complementary interaction profile that optimally aligns within the enzyme's active site.

The diclofenac_heparină complex also shows a strong binding affinity, with a binding energy of -566.76 kcal/mol, though it is slightly less stable than the heparină_diclofenac configuration. This difference suggests that the orientation and order of binding—where heparin serves as the receptor for diclofenac rather than the reverse—may allow for a more optimal fit within Factor Xa's active site, leading to slightly stronger intermolecular interactions.

Comparatively, the individual binding energies of diclofenac (-433.13 kcal/mol) and heparin (-290 kcal/mol) with Factor Xa are significantly higher, reflecting weaker interactions when either compound binds to the enzyme alone [25]. This result underscores the advantage of forming a complex between heparin and diclofenac, as the combined structure enhances their binding affinity with Factor Xa beyond what either compound can achieve independently.

These findings highlight the importance of complex formation and binding orientation in modulating interactions with Factor Xa. The heparină_diclofenac complex's superior binding affinity may translate into improved inhibitory activity against Factor Xa, potentially relevant for therapeutic applications targeting blood coagulation pathways [26]. Further analyses, such as molecular dynamics simulations, could provide insights into the molecular basis for the enhanced stability observed in the heparină_diclofenac configuration, supporting the development of optimized drug combinations for anticoagulant therapies.

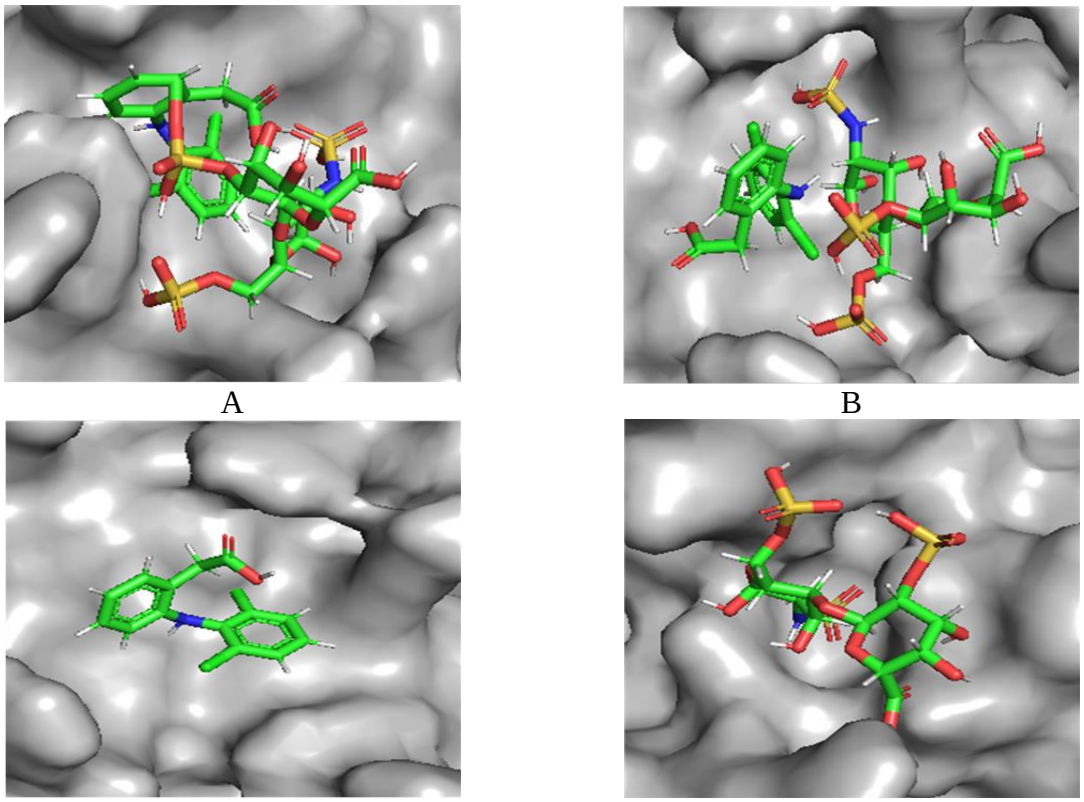


Figure 1. Docking Images of the factor 1a receptor with A diclofenac_heparin complex, B heparin_ diclofenac complex, C diclofenac, D heparin [27].

Fig. 1 provides a detailed visual representation of the docking poses of various diclofenac-heparin configurations with the Factor Xa receptor, highlighting critical differences in their interaction patterns. Specifically, the diclofenac_heparin complex (Fig. 1A) and the heparin_diclofenac complex (Fig. 1B) exhibit distinct binding sites on the Factor Xa receptor compared to those occupied by the individual compounds diclofenac (Fig. 1C) and heparin (Fig. 1D). These observed differences in docking positions underline the significant structural and spatial rearrangements that occur when diclofenac and heparin are combined into complexes. Such shifts in binding location are particularly important as they influence the binding energies, which reflect the strength and stability of the interactions. The altered binding sites may also suggest potential changes in the complexes' pharmacodynamic and pharmacokinetic properties, such as receptor specificity, interaction dynamics, and functional activity. These findings underscore the importance of evaluating the binding orientations of multi-drug complexes, as they may lead to synergistic or modified therapeutic effects distinct from those of the individual components. The change in binding site likely alters the interactions between the receptor and each complex, influencing factors such as hydrogen bonding, van der Waals forces, and hydrophobic contacts. As a result, the complexes exhibit distinct binding energies depending on their orientation and location on the receptor surface [28]. For example, the diclofenac_heparin complex may experience different stabilizing interactions compared to the heparin_diclofenac complex due to the spatial arrangement of functional groups relative to the active or allosteric sites on Factor Xa.

These findings suggest that the flexibility in binding site location and orientation can lead to variability in binding affinities. This variability is important in understanding how complex formation affects pharmacological interactions, as different binding sites and energies may influence the efficacy and specificity of Factor Xa inhibition. Further

investigation into the structural details of each binding site could provide insights into optimizing the orientation and interaction profiles for enhanced therapeutic outcomes.

4. CONCLUSIONS

This study provides a comprehensive analysis of the molecular interactions between diclofenac, and heparin, and their complexes with Factor Xa receptor. The findings highlight that the formation and orientation of diclofenac-heparin complexes significantly impact binding energies and binding site locations, ultimately affecting the stability and affinity of these interactions. Complex formation resulted in lower binding energies compared to individual compounds, underscoring the potential benefits of these configurations for enhanced binding specificity and stability.

Additionally, the differing logP values between diclofenac_heparin and heparin_diclofenac complexes indicate that binding orientation influences lipophilicity, potentially affecting the pharmacokinetic profiles of these complexes. These variations in binding energy, site location, and lipophilicity suggest that optimized diclofenac-heparin complexes could provide therapeutic advantages by improving target specificity, enhancing bioavailability, and potentially reducing dosage requirements.

Overall, this study advances our understanding of diclofenac-heparin interactions and lays the groundwork for further exploration into complex formation as a strategy for optimizing drug-receptor interactions. Future studies may build on these findings by investigating the in vivo implications of these complexes and refining docking methodologies to better predict therapeutic outcomes.

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