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Molecular Docking–Based Analysis and Antibacterial Activity Evaluation of Rifaximin

Rifaksimin'in Moleküler Kenetlenme Tabanlı Analizi ve Antibakteriyel Aktivite Değerlendirmesi

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ABSTRACT

Rifaximin, a semi-synthetic antibiotic derived from the rifamycin group, is particularly common in managing gastrointestinal infections. Due to its low systemic bioavailability, the drug predominantly remains localized in the intestines, allowing it to exert its antibacterial effects while minimizing systemic side effects. Rifaximin works by irreversibly binding to the RNA polymerase enzyme in bacteria, inhibiting its function. In this study, the ligand-receptor complex of rifaximin with the RfaH protein of E. coli was examined using molecular docking methods. Molecular docking simulations were performed using the VINA approach in the YASARA program, and findings regarding the rifaximin-RfaH complex were obtained. The findings provide valuable information on the therapeutic potential of rifaximin in inhibiting *E. coli* and its possible applications in clinical settings. The compound's antibacterial, antifungal, and antiviral activities were predicted using AntiBac-Pred, AntiFun-Pred, and AntiVir-Pred. Molecular docking results suggest that rifaximin may exhibit antibacterial potential, based on its binding affinity of -7.792 kcal/mol to the RfaH protein and its interactions with the target. These data suggest that Rifaximin may be effective against *Prevotella disiens* (confidence: 0.9715), *Bacteroides stercoris* (confidence: 0.9685), *Clostridium ramosum* (confidence: 0.9557), and *Porphyromonas asaccharolytica* (confidence: 0.9235).

Keywords: *Rifaximin, E. coli, RfaH protein, molecular docking*

ÖZET

Rifamisin grubundan türetilen yarı sentetik bir antibiyotik olan rifaksimin, özellikle gastrointestinal enfeksiyonların yönetiminde yaygın olarak kullanılmaktadır. Düşük sistemik biyoyararlanımı nedeniyle, ilaç ağırlıklı olarak bağırsaklarda lokalize kalır ve sistemik yan etkileri en aza indirirken antibakteriyel etkilerini göstermesine izin verir. Rifaksimin, bakterilerdeki RNA polimeraz enzimine geri dönüşümsüz olarak bağlanarak işlevini engelleyerek çalışır. Bu çalışmada, rifaksiminin E. coli'nin RfaH proteini ile ligand-reseptör kompleksi moleküler kenetlenme yöntemleri kullanılarak incelenmiştir. Moleküler kenetlenme simülasyonları YASARA programında VINA yaklaşımı kullanılarak gerçekleştirilmiş ve rifaksimin-RfaH kompleksine

ilişkin bulgular elde edilmiştir. Bulgular, rifaksimin›in E. coli›yi inhibe etmedeki terapötik potansiyeli ve klinik ortamlardaki olası uygulamaları hakkında değerli bilgiler sağlamaktadır. Bileşiğin antibakteriyel, antifungal ve antiviral aktiviteleri AntiBac-Pred, AntiFun-Pred ve AntiVir-Pred kullanılarak tahmin edilmiştir. Moleküler kenetlenme modelleri, Rifaximin'in RfaH proteinine -7.792 kcal/mol bağlanma afinitesine ve hedefle etkileşimlerine dayanarak önemli bir antibakteriyel etkiye sahip olacağını göstermektedir. Bu veriler Rifaximin'in *Prevotella disiens* (confidence: 0.9715), *Bacteroides stercoris* (confidence: 0.9685), *Clostridium ramosum* (confidence: 0.9557) ve *Porphyromonas asaccharolytica*'ya (confidence: 0.9235) karşı etkili olabileceğini göstermektedir.

Anahtar kelimeler: *Rifaksimin, E. coli, RfaH protein, moleküler kenetlenme*

INTRODUCTION

Antibiotics are pharmaceutical compounds used to treat bacterial infections by inhibiting the growth of microorganisms, often helping to eliminate or halt their reproduction. These drugs exert their effects by targeting essential bacterial processes, thereby controlling infections and supporting the immune system (Van Vlem et al., 1996). Rifaximin (C43H51N3O11) is an important drug commonly used in antibiotic treatments, especially for gastrointestinal infections (Scarpignato et al., 2005). It was first discovered in 1980 by Alfa Wassermann and has since been widely used in various clinical settings. Rifaximin is a semi-synthetic antibiotic from the rifamycin class (Calanni et al., 2014). Derivatives like rifaximin, rifampin, rifabutin, rifapentine, and rifalazil inhibit bacterial DNA-dependent RNA polymerase, thereby suppressing bacterial RNA synthesis. This mechanism classifies them as broad-spectrum antibiotics. Rifaximin is notable for its low systemic bioavailability, which keeps the drug localized in the intestines and minimizes systemic side effects (Hirota, 2016).

Rifamycins target RNA polymerase, the enzyme that enables bacteria to replicate their genetic material. Rifampin (RIF), for example, binds deeply within the RNA exit tunnel of this enzyme. This binding inhibits the elongation of RNA chains and causes premature termination of mRNA synthesis (Campbell et al., 2001). Previous experiments have shown that RIF stops mRNA chain elongation by creating steric hindrance after forming the second phosphodiester bond during transcription (McClure et al., 1978). RIF attaches to the RpoB enzyme through hydrogen bonds formed by hydroxyl groups on its naphthol ring and interacts with hydroxyl groups at positions C21 and C23 on the ansa bridge. These molecular interactions position RIF optimally to target RNA polymerase (McClure et al., 1978; Surette et al., 2021). Rifaximin, when taken orally, is only minimally absorbed into the body, allowing the drug to remain mainly in the intestines, where it exerts its effects. This drug works by inhibiting bacterial RNA synthesis, specifically by binding to the β-subunit of the DNA-dependent RNA polymerase enzyme. This enzyme plays a vital role in RNA production, and its inhibition prevents bacteria from replicating and surviving (Gillis et al., 1995).

The clinical use of rifaximin is especially important in treating various gastrointestinal diseases, including irritable bowel syndrome (IBS) and hepatic encephalopathy. IBS is a chronic condition affecting millions worldwide and significantly lowers quality of life. Rifaximin provides an effective option for reducing symptoms in IBS patients (Pimentel et al., 2009). Hepatic encephalopathy, a

serious condition caused by liver failure, impacts brain function and mental states. Rifaximin helps improve mental status in these patients by targeting ammonia-producing bacteria in the intestines, thus lowering the toxic effects of high ammonia levels in the brain (Ojetti et al., 2009). It exhibits strong inhibitory activity against several microorganisms, such as Streptococcus, Escherichia coli, Shigella, Salmonella, Bacillus cereus, Moraxella catarrhalis, Haemophilus influenzae, Haemophilus ducreyi, Bacteroides bivius-disiens, and Pseudomonas (Hoover et al., 1993). Due to its irreversible binding to RNA polymerase, rifaximin permanently inhibits bacterial RNA synthesis, which enhances its effectiveness against infections and contributes to its long-lasting therapeutic effects (Jiang et al., 2005). In pathogenic bacteria like E. coli and K. pneumoniae, the RfaH protein interacts with RNA polymerase and ribosomes to activate virulence genes. These genes are crucial for producing protective and structural components such as capsules, cell walls, and pili, which increase the bacteria's ability to cause disease (Hustmyer et al., 2022; Ashraf et al., 2024; Svetlov et al., 2018).

Rifaximin is a promising option for inhibiting RfaH, further reducing the bacteria's pathogenicity. Rifaximin's interaction with RfaH and its inhibitory effects on E. coli can be seen through molecular dynamics simulations supported by molecular docking studies. The crystal structure of RfaH (PDB: 2OUG) has been crucial in understanding specific binding sites and molecular interactions, offering insights into how rifaximin may influence bacterial virulence by targeting the RfaH protein effectively. The 2OUG structure serves as the main reference in the RfaH protein simulations, ensuring accurate analysis of its structure and behavior (Seifi et al., 2021). This structure, stored in the Protein Data Bank (PDB) with the ID 2OUG, depicts RfaH with its N-terminal and C-terminal domains close together in a tight, folded form. This domain-closed state is vital for the protein's function because it regulates bacterial gene expression. The 2OUG structure helps clarify the spatial arrangement of the protein's domains and provides critical information for scientists studying RfaH's molecular activity (GC et al., 2014).

To explore the antivirulence effect of rifaximin on antibiotic-resistant bacteria and to characterize its interaction with Escherichia coli RfaH, molecular docking simulations were conducted.

1. MATERIALS AND METHODS

Molecular docking is a computational method used to predict the best position of one molecule relative to another, forming a stable complex with minimal energy. This technique aims to analyze interactions between molecules and determine the most efficient and stable structure (Lengauer et al., 1996). These methods are highly valuable in drug development, elucidating molecular recognition mechanisms, and understanding biological targets (Naqvi et al., 2018). In this study, the crystal structure of the RfaH transcription factor at 2.1 Å resolution (PDB ID: 2OUG) was used as the target protein in the docking analysis (Belogurov et al., 2007). The target protein from the Protein Data Bank (PDB ID: 2OUG) was prepared for docking by removing heteroatoms and water molecules and adding polar hydrogen atoms. Kollman charges on RfaH were then calculated and assigned to better represent its native chemical structure. Additionally, the target protein was optimized through energy minimization using the NOVA force field (Krieger et al., 2002) in the YASARA software (Krieger et al., 2014). The initial molecular structure of Rifaximin was obtained from PubChem (CID 6436173) and further optimized using YASARA with the

NOVA force field (Krieger et al., 2002).

Molecular docking was performed using the VINA method (Trott et al., 2010) in the YASARA program (v22.9.24). Default settings were used for all other parameters. The docking simulations employed a semi-flexible approach, allowing the ligand to be flexible in its conformation while the target protein remained rigid, ensuring accurate ligand-protein interaction calculations.

2. RESULTS AND DISCUSSIONS

The interaction of Rifaximin with RfaH (PDB ID: 2OUG) was examined through docking simulations to explore its possible therapeutic mechanisms. **Figure 1** shows the interactions between docked rifaximin and the residues of the 2OUG target protein. The docking results showed that Rifaximin interacted with TYR5 and PRO61 amino acid residues of RfaH through hydrogen bonding and hydrophobic interactions, respectively. Rifaximin formed a hydrogen bond with TYR5 at 2.04 Å and participated in an alkyl interaction with PRO61 at 4.3 Å. The binding energy was computed as -7.792 kcal/mol.

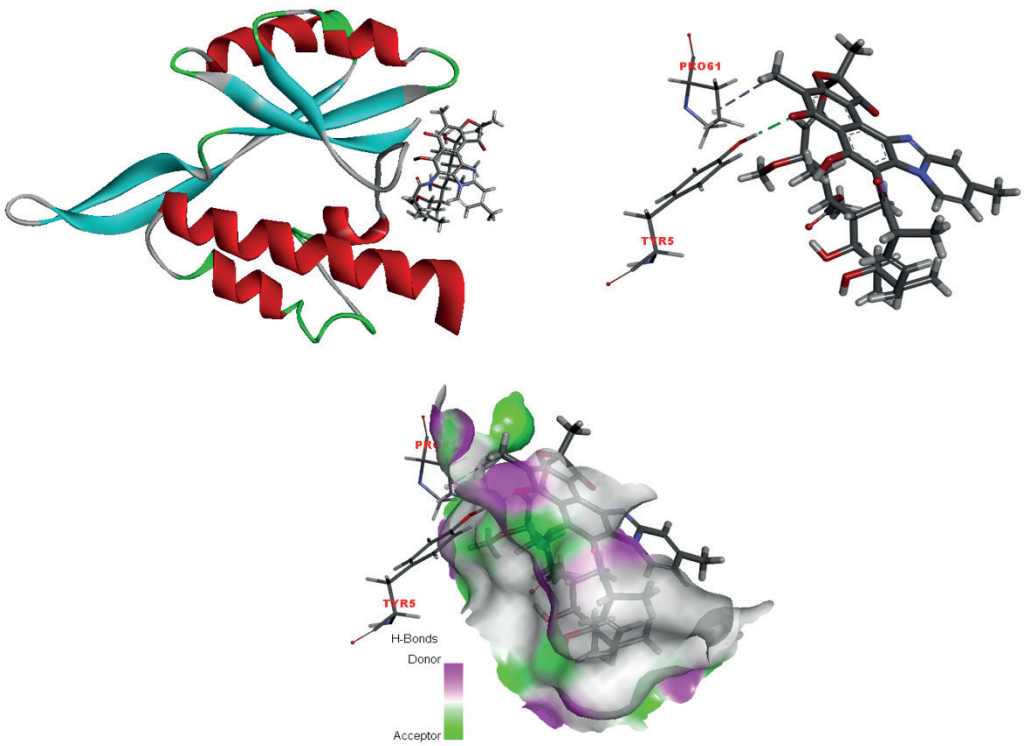

Figure 1. Molecular docked model of rifaximin with RfaH. The interactions between rifaximin and RfaH are labeled using colored dashed lines ($\Delta E_{binding}$ = -7.792 kcal/mol)

Additionally, molecular docking analyses were conducted to investigate the interactions between rifaximin and the RNA polymerase (PDB ID: 5UHB) and *E. coli* RNAP (PDB ID: 4KMU) receptors. The binding energies were calculated as -10.358 and -9.773 kcal/mol, respectively.

3.1. Antibacterial, Antifungal, and Antiviral Activities

The antibacterial, AntiBac-Pred, AntiFun-Pred, and AntiVir-Pred (Pogodin et al., 2019; Brown et al., 2016; antifungal, and antiviral activities of the compound were predicted using Gaulton et al., 2017; Fourches et al., 2015; Pogodin et al., 2015; Pogodin et al., 2018; Filimonov et al., 2014). The probability scores of Rifaximin are presented in **Table 1**. According to these findings, Rifaximin may be effective against *Prevotella disiens* (confidence: 0.9715), *Bacteroides stercoris* (confidence: 0.9685), *Clostridium ramosum* (confidence: 0.9557), and *Porphyromonas asaccharolytica* (confidence: 0.9235).

Table 1. Probability scores of the rifaximin.

Predicted antibacterial targets		
Name	CHEMBL ID	Confidence
Prevotella disiens	CHEMBL612235	0.9715
Bacteroides stercoris	CHEMBL614750	0.9685
Clostridium ramosum	CHEMBL614971	0.9557
Porphyromonas asaccharolytica	CHEMBL615058	0.9235
Predicted antifungal activity		
Rhizopus oryzae	CHEMBL612306	0.2925
Absidia corymbifera	CHEMBL612369	0.2542
Predicted inhibition of viral proteins		
Virus	Protein target	Confidence
Woolly monkey sarcoma virus	Simian sarcoma virus Pol protein	0.0139

CONCLUSION

The docking simulations were used to explore the biological activity of Rifaximin in its most stable conformation. Since the RfaH protein of *E. coli* is a key target for antibacterial drugs, docking simulations of Rifaximin with RfaH were performed to estimate its antibacterial effectiveness. Based on a binding affinity of -7.792 kcal/mol to RfaH, these simulations suggest that Rifaximin has a notable antibacterial effect. In addition, *in silico* predictions of antibacterial, antifungal, and antiviral activities of Rifaximin were made using AntiBac-Pred, AntiFun-Pred, and AntiVir-Pred. The results showed that Rifaximin could be effective against *Prevotella disiens* (confidence: 0.9715), *Bacteroides stercoris* (confidence: 0.9685), *Clostridium ramosum* (confidence: 0.9557), and *Porphyromonas asaccharolytica* (confidence: 0.9235).

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