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Investigation of the Interaction of ENT Drugs with Target Proteins Using a Molecular Docking Approach

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Abstract: Understanding the interactions of drugs commonly used in the treatment of Ear, Nose and Throat (ENT) diseases at the molecular level is of great importance in terms of increasing treatment efficacy and identifying new therapeutic targets. In this study, five different active drug substances commonly used in the field of ENT (amoxicillin, loratadine, fluticasone and pseudoephedrine) were selected and the binding potentials of these molecules with the relevant biological target proteins (PDB IDs 1ZG4, 3RZE, 1M2Z, 4V7U, 2RH1) were investigated by molecular docking methods. The selected proteins are associated with bacterial resistance mechanisms, allergic responses, inflammation processes and sympathomimetic effects and play important roles in explaining the therapeutic effects of the relevant drugs. It is aimed that the molecular docking results will contribute to the optimization of drug design and current treatment approaches by revealing the structural basis of drug-protein interactions.

Keywords: Molecular docking, ENT pharmacotherapy, drug-protein interaction, therapeutic targets, structure-based drug design

1. Introduction

Ear Nose Throat (ENT) diseases are common clinical conditions that affect a significant part of the upper respiratory tract and occur with infectious, inflammatory and allergic mechanisms. Diseases such as acute and chronic rhinosinusitis, tonsillitis, otitis media, allergic rhinitis and nasal polyposis constitute an important public health problem due to their prevalence in the society and frequent recurrence [1,2]. Antibiotics, glucocorticoids, antihistamines and sympathomimetic agents, which are widely used in the treatment of these diseases, play a critical role in controlling symptoms and preventing disease progression.

However, especially the frequent and inappropriate use of antibiotics has brought about the problem of increasing antibiotic resistance worldwide. The World Health Organization (WHO) defines this situation as a global health threat [3]. Beta-lactamase enzyme-mediated resistance, especially to beta-lactam group antibiotics, creates significant difficulties in the treatment of ENT infections [4]. In addition, the different therapeutic responses observed among individuals to antihistamine drugs used in chronic diseases such as allergic rhinitis indicate that the binding efficiency of these drugs with target receptors varies from person to person [5]. In this context, it is of great importance to analyze the interactions of drugs with target proteins at the molecular level.

Molecular docking is an in silico modeling method that simulates the binding configuration and binding affinity of small molecules to the active sites of target proteins [6]. This technique provides predictions about pharmacodynamic properties by calculating the binding energies and possible interaction types (hydrogen bonds, hydrophobic effects, etc.) of drugs on their biological targets. In addition, the binding affinities of ligands to different protein conformations can be evaluated, contributing to the discovery of new therapeutic targets [7]. In recent years, this technique; Although it is used in many fields such as pharmaceutical chemistry, toxicology and pharmacology, comprehensive molecular docking studies specifically for ENT are limited.

In this study, some pharmaceutical agents commonly prescribed in the field of ENT (amoxicillin, loratadine, fluticasone, azithromycin,

pseudoephedrine) were selected and their binding potentials with target protein structures known to be effective in the clinic (e.g. beta-lactamase, histamine H1 receptor, glucocorticoid receptor, etc.) were evaluated. The crystal structures of these target proteins were obtained from the Protein Data Bank (PDB) database, and ligand molecules were provided via PubChem. The binding sites, affinities and potential inhibitory properties of these molecules were evaluated with a structure-based approach by molecular docking analyses.

Amoxicillin belongs to the beta-lactam antibiotic group, and beta-lactamase production is one of the most common mechanisms of antibiotic resistance [8]. Loratadine is a selective antihistamine used in the treatment of allergic rhinitis and shows its effect via the H1 receptor [5]. Fluticasone is in the class of nasal corticosteroids and performs its anti-inflammatory effects via the glucocorticoid receptor [9]. Pseudoephedrine is used to relieve nasal congestion with its sympathomimetic effect and creates a vasoconstrictor effect by binding to adrenergic receptors [10].

The main purpose of this study is to evaluate the molecular binding potential of amoxicillin, loratadine, fluticasone and pseudoephedrine, commonly used in the field of ENT, with target proteins known to have therapeutic effects, such as beta-lactamase (1ZG4), histamine H1 receptor (3RZE), glucocorticoid receptor (1M2Z) and adrenergic β2 receptor (2RH1), using in silico docking methods. In this context, the findings are expected to provide scientific data on the structural activity of drugs and shed light on drug development studies in the field of ENT in the future.

2. Computational Method

In this study, molecular docking analyses were performed via DockingServer [11], a web-based platform. Selected active ingredients (amoxicillin, loratadine, fluticasone and pseudoephedrine) were obtained from the PubChem database and downloaded in mole format, and their 3D structures were optimized via the DockingServer interface. Gasteiger-Marshfield charges were assigned to ligand molecules, and rotatable bond definitions were automatically performed by the system [12]. Energy minimization of ligands was performed using the MMFF94s force field [13]. Target protein structures were obtained from the Protein Data Bank (PDB) database. The selected target proteins were; beta-lactamase targeting for amoxicillin (PDB ID: 1ZG4) [14], Histamine H1 receptor targeting for loratadine (PDB ID: 3RZE) [15], Glucocorticoid receptor targeting for fluticasone (PDB ID: 1M2Z) [16], β2-adrenergic receptor targeting for pseudoephedrine (PDB ID: 2RH1) [17]. Water molecules and cofactors in the protein structures were removed from the system, polar hydrogen atoms were added and missing side chains were completed automatically. Kollman combined loads were also applied to the protein structure.

Docking analyses were conducted using the computational engine based on AutoDock 4.2 algorithm. Lamarckian Genetic Algorithm (LGA) was selected to evaluate the binding affinity between ligand and receptor. Docking process was performed for each ligand over 10 different runs; the maximum number of generations was determined as 27,000 and the population size was set as 150 [18]. The grid box was centered and sized specifically for each target protein to cover the active site. Binding energies (BE, kcal/mol), inhibitory constant (Ki), binding positions and ligand conformations were evaluated. Positions with the lowest binding energy were preferred for visualization and analysis.

3. Results and discussion

3.1. Ligand structures

The four active pharmaceutical ingredients investigated in this study, amoxicillin, fluticasone, loratadine and pseudoephedrine, belong to different pharmacological groups and are widely used in ENT diseases. Optimized three-dimensional structures of these molecules are given in Figure 1. The active pharmaceutical ingredients shown in Figure 1 achieve their therapeutic effects through specific interactions with target proteins. The properties of their molecular structures and binding patterns directly affect the strength and selectivity of these interactions. Amoxicillin is a broad-spectrum antibiotic containing a beta-lactam ring. The beta-lactam group in its structure prevents cell wall formation by forming covalent bonds with enzymes involved in bacterial cell wall synthesis (especially transpeptidases).

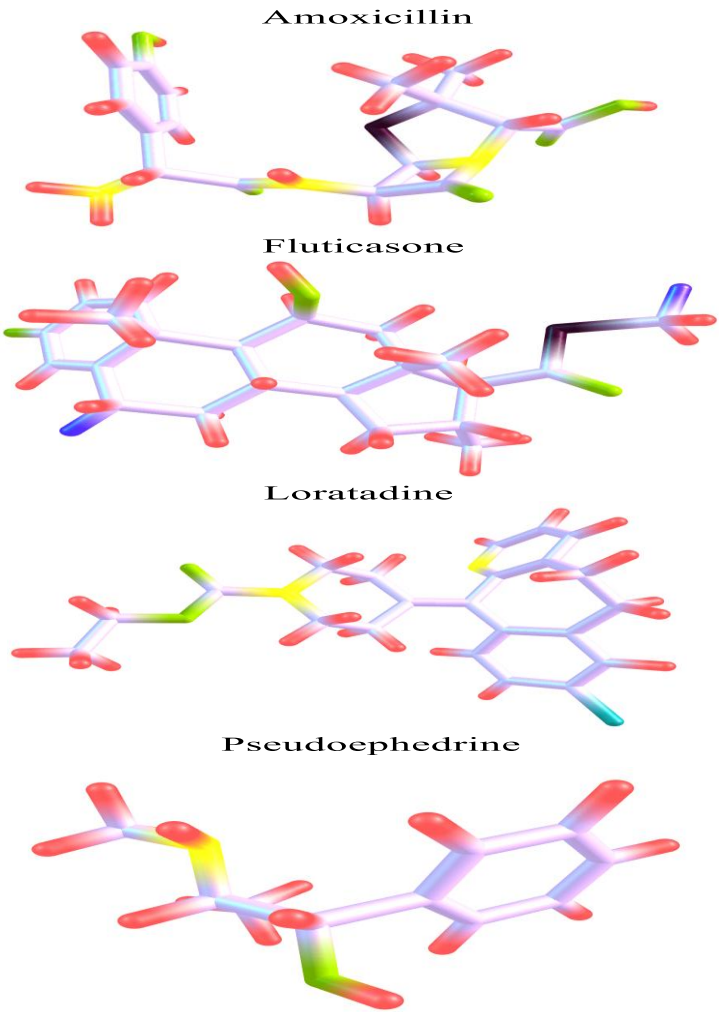

Figure 1. Molecular structures of the examined active pharmaceutical ingredients.

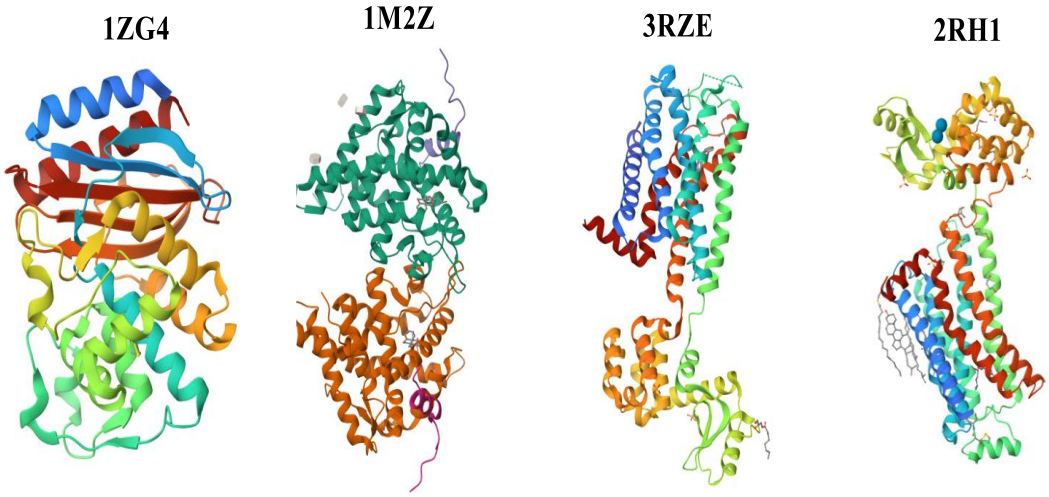

Figure 2. Target protein structures.

However, beta-lactamase enzymes produced by bacteria (PDB ID: 1ZG4) can cleave this ring and render the antibiotic ineffective. Therefore, modeling the interaction of amoxicillin with beta-lactamase is critical for understanding the development of resistance. Fluticasone is a synthetic corticosteroid structurally reinforced with fluorine atoms. The molecule binds to the glucocorticoid receptor (PDB ID: 1M2Z) with high affinity, activating anti-inflammatory gene expression and inhibiting inflammatory cytokine production. The lipophilic regions and hydrogen bonding groups in its structure allow it to establish stable interactions with the receptor. This interaction explains its effectiveness especially in diseases such as allergic rhinitis and nasal polyposis. Loratadine is a selective H1 antihistamine with a tricyclic structure. Thanks to its aromatic ring systems and heteroatom-containing regions, it binds to the histamine H1 receptor (PDB ID: 3RZE) via hydrophobic and π-π interactions. This binding competitively prevents histamine from binding to the receptor, thus blocking an important component of the allergic response. Pseudoephedrine is a sympathomimetic agent effective on the adrenergic system. Due to its structure, it binds to the adrenergic β2 receptor (PDB ID: 2RH1) and constricts the vascular smooth muscles in the nasal mucosa and reduces congestion. While the amine group of the molecule can establish ionic interactions, the hydroxyl group can establish stable contact with the receptor site via hydrogen bonds.

3.2. Molecular docking poses

The most suitable binding positions obtained as a result of molecular docking analyses reveal in detail the location of each active drug substance in the active site where it binds to the target protein and the interaction patterns. The binding positions of amoxicillin with beta-lactamase (1ZG4), fluticasone with glucocorticoid receptor (1M2Z), loratadine with histamine H1 receptor (3RZE) and pseudoephedrine with β2-adrenergic receptor (2RH1) are visualized on three-dimensional structures in Figure 3.

The binding poses given in Figure 3 clearly show the placement of the ligands in the active site of the relevant proteins and the specific interactions they establish with the surrounding amino acid residues. The docking results obtained as a result of the binding of the ligands to the target proteins are given in Table 1. The binding energies (BE), inhibitor constants (Ki), van der Waals and hydrogen bonds (SE), electrostatic energy (EE), total interaction energies (T.I.E) and interaction surfaces (IS) values of each ligand-protein complex were examined.

Table 1. Molecular docking results of ligand–protein complexes

Complex	**BE (kcal/mol)**	**Ki (μM)**	**IE (kcal/mol)**	**EE (kcal/mol)**	**T.I.E (kcal/mol)**	**IS**
Amoxicillin-1ZG4	-6.57	24.08	-7.81	-0.96	-7.77	605.18
Fluticasone -1M2Z	-6.16	30.57	-7.05	-0.07	-7.12	593.53
Loratadine-3RZE	-7.75	15.36	-6.11	-0.34	-6.45	813.36
Pseudoephedrine-2RH1	-5.27	137.48	-4.72	-1.19	-5.92	434.91

Binding energy (BE), given in the docking results, represents the free energy change of the complex formed by binding of the ligand to the target protein. More negative values indicate stronger binding affinity [19]. Inhibitory Constant (Ki) quantifies the ability of the ligand to inhibit the protein. A low Ki value reflects a high inhibitory potential. The Ki value is derived from BE [20]. It is the sum of the dissociation energies of secondary interactions (SE), such as van der Waals interactions and hydrogen bonds, from solution. It is determinant for the stability of ligand-protein binding [21]. The electrostatic energy indicates the electrostatic attraction or repulsion energy between the ligand and the receptor. Negative values indicate attractive force [12].

The total interaction energy includes the sum of all interactions. It provides information about the overall stability of the complex. The interaction surface represents the surface area where the ligand makes contact with the protein. A larger surface

area means more extensive contact and potentially more stable binding [22].

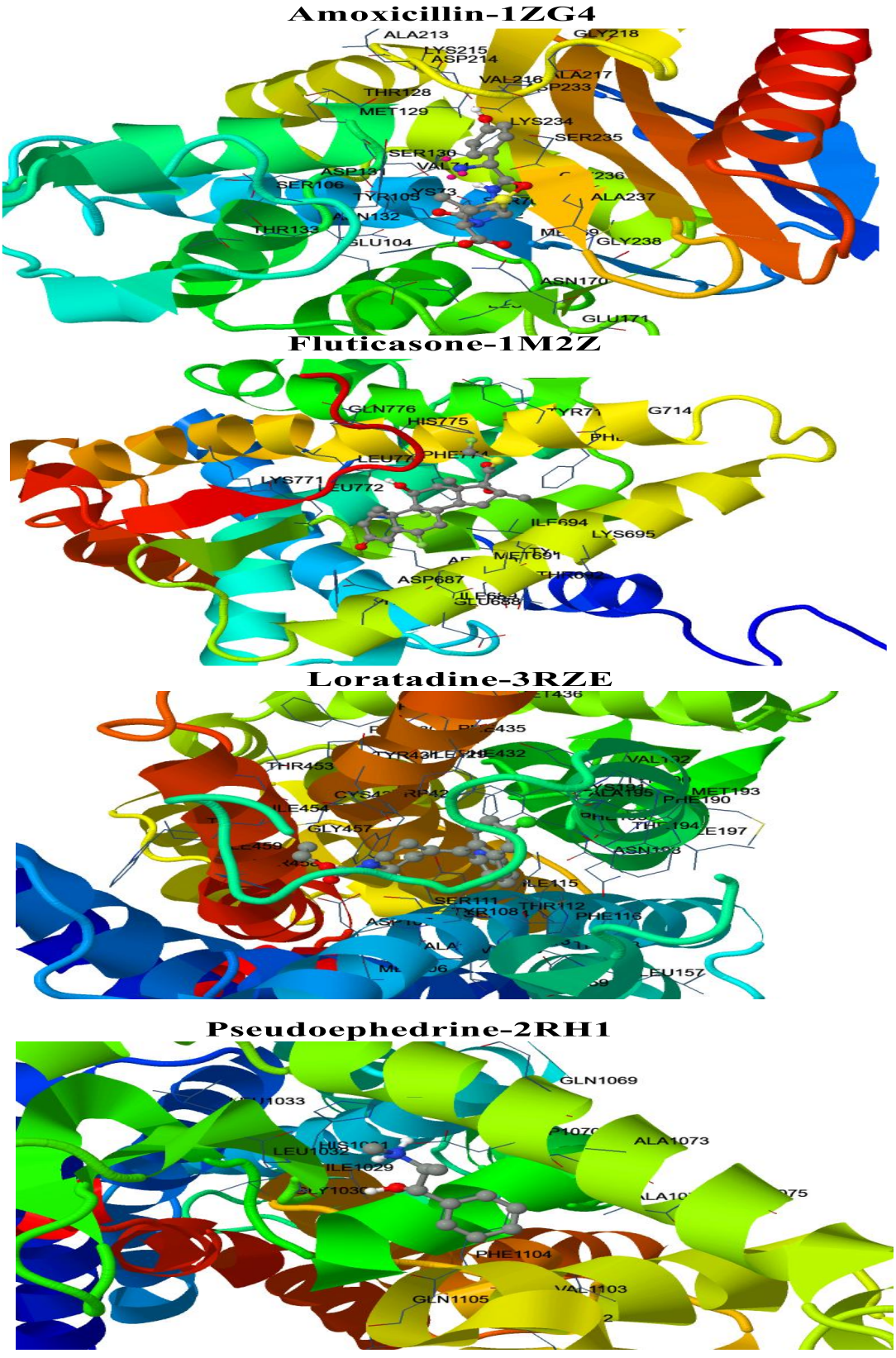

Figure 3. Docking poses between the studied ligands and target proteins.

According to the results obtained within the scope of the study, the complex formed by loratadine with histamine H1 receptor exhibited the strongest interaction with a binding energy of -7.75 kcal/mol and an inhibitory constant of 15.36 μM. In addition, the large interaction surface of 813.37 Å² shows that loratadine fits into the receptor binding pocket with high conformity. These findings support the clinical efficacy of loratadine in inhibiting allergic reactions at the molecular level (support the clinical efficacy of loratadine in inhibiting allergic reactions at the molecular level [5]. The interaction of amoxicillin with beta-lactamase enzyme reveals a moderate binding strength with a binding energy of -6.57 kcal/mol and a Ki value of 24.08 μM. However, the relatively high value of the interaction surface as 605.18 Å² shows that the molecule forms a stable complex with the target protein. This is especially valuable for the mechanistic understanding of beta-lactamase-mediated antibiotic resistance [4]. When the fluticasone molecule is evaluated with the glucocorticoid receptor based on the binding energy of -6.16 kcal/mol and the inhibitory constant of 30.57 μM, it exhibits a weaker binding affinity profile than loratadine, but a significant profile in terms of overall binding stability. The lipophilic regions of the molecule and the potential for hydrogen bonding provide a stable interaction with the receptor [23]. Pseudoephedrine shows the lowest binding energy to the β2-adrenergic receptor with -5.27 kcal/mol and the highest Ki value with 137.48 μM. At the same time, having a narrow interaction surface of 434.92 Å² reveals that it binds weakly to the receptor and has limited inhibitory potential. This result suggests that although the molecule shows symptomatic relieving effects, it does not offer a strong binding profile at the receptor level [24]. When evaluated in general, loratadine has the strongest interaction profile in the study due to its high affinity and large contact surface with the target receptor. While amoxicillin and fluticasone exhibit significant binding in terms of structural stability, pseudoephedrine shows a weaker binding strength. These findings provide the opportunity to comparatively evaluate the molecular activity levels of these agents commonly used in ENT diseases.

3.3. Ligand and protein interactions

The analysis of specific interactions established by ligands with target proteins provides structural and functional information beyond binding affinity in molecular docking studies. These interactions generally occur through forces such as hydrogen bonds, hydrophobic contacts, electrostatic interactions, and π-π stacking between aromatic surfaces. In Figure 4, the binding modes of amoxicillin, fluticasone, loratadine, and pseudoephedrine molecules with 1ZG4, 1M2Z, 3RZE, and 2RH1 proteins, respectively, are presented with two-dimensional interaction diagrams. In the visual representations, the specific contact points where the ligands are positioned in the receptor active sites and establish with the surrounding amino acid residues are detailed. The binding mode of each complex, interaction types, and contacted amino acid residues are systematically given in Table 2.

Table 2. Types of ligand-protein interactions

Complex	H-bond	Halogen bond	Polar	Hydrophobic
Amoksisilin-1ZG4	ALA237	-	SER70	-
			ASN170	
Flutikazon-1M2Z	-	PHE774	ARG690	MET691
				ASP687
Loratadin-3RZE	SER111	LYS191	TYR458	TRP428
		THR194	ASP107	TYR108
				PHE435
				TRP158

				ILE115
				PHE432
				ILE454
Pseudoefedrin-2RH1	ASP107	-	-	PHE110
	HIS103			VAL110
				ALA107

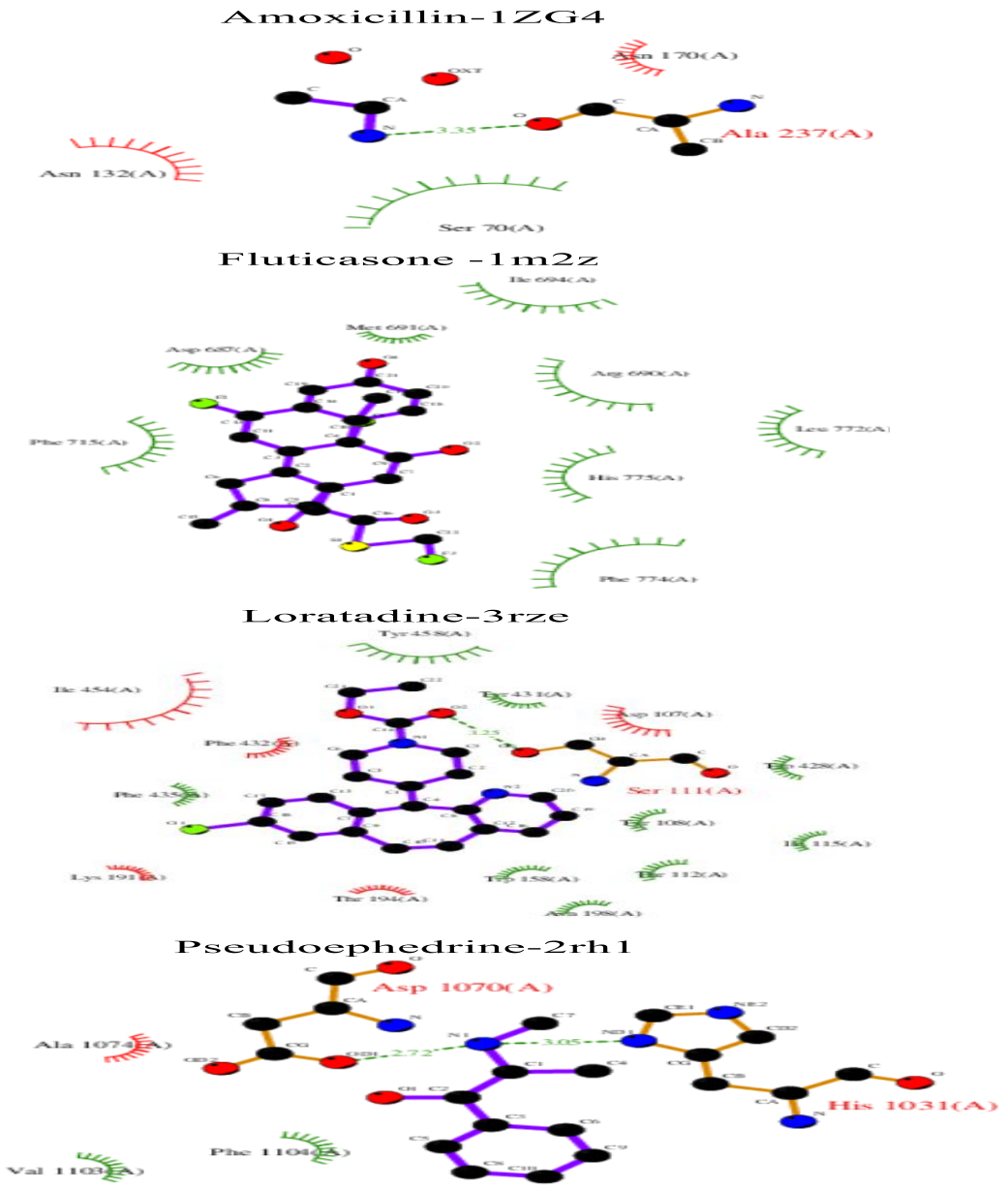

Figure 4. Interaction modes between ligands and target proteins.

The data presented in Table 2 reveal that each ligand exhibits marked differences in the type and intensity of binding interactions with the target protein. These interactions determine not only the binding energies but also the specificity and activity against biological targets [25,26]. In the amoxicillin–1ZG4 complex, the ligand is observed to form a hydrogen bond (ALA237) and exhibit a

polar interaction (SER70, ASN170). These interactions increase the binding stability at the active site of the beta-lactamase enzyme and form the basis of the inhibitory effect of the antibiotic against the enzyme. In particular, the hydrogen bond with ALA237 is significant in terms of contact with the catalytic site, which directly contributes to the cleavage of the beta-lactam ring [4]. In the fluticasone–1M2Z complex, halogen bond (PHE774), polar (ARG690) and hydrophobic (MET691, ASP687) contacts are present, indicating that the ligand is specifically and stably located in the glucocorticoid receptor binding pocket.

Halogen bonds increase the binding specificity of fluorine-containing corticosteroids in particular and provide selective interaction with the receptor [27]. Loratadine–3RZE complex exhibits the broadest interaction profile. The ligand establishes hydrogen bonds (SER111), halogen bonds (LYS191), multiple polar interactions (TYR458, ASP107) and rich hydrophobic contacts (TRP428, TYR108, PHE435, TRP158, ILE115, PHE432, ILE454). This dense contact network explains the high affinity of loratadine for the H1 receptor and strengthens the molecular basis of its antihistamine activity [5]. In the pseudoephedrine–2RH1 complex, a limited number of hydrogen bonds (ASP107, HIS103) and hydrophobic interactions (PHE110, VAL110, ALA107) were observed. This binding model is consistent with the low binding affinity with the adrenergic β2 receptor and indicates that pseudoephedrine should be used at higher doses for symptomatic effect [28]. This interaction profile reveals that the molecule is weaker in terms of selectivity and binding stability. Loratadine provides the most comprehensive binding profile in terms of interaction diversity and number, forming the basis of high specificity and affinity. Fluticasone exhibits specific binding with halogen and polar interactions, while amoxicillin presents a classical inhibitory profile based mainly on hydrogen bonds. Pseudoephedrine is the compound with the lowest pharmacological activity at the molecular level with its weaker and limited interaction network. These structural analyses provide important information supporting the pharmacodynamic effects of drugs and structural optimization opportunities for potential therapeutic targets.

4. Conclusions

In this study, the binding potentials of four different drug active ingredients (amoxicillin, fluticasone, loratadine and pseudoephedrine) widely used in the treatment of Ear, Nose and Throat (ENT) diseases with therapeutic target proteins were evaluated using molecular docking methods. Each drug was paired with specific target proteins known to be effective in the clinic (1ZG4, 1M2Z, 3RZE, 2RH1) and the binding energies (BE), inhibitory constants (Ki), van der Waals and hydrogen bonds (SE), electrostatic energy (EE), total interaction energies (T.I.E), interaction surfaces (IS) values and binding modes of these complexes were analyzed in detail. The findings revealed that loratadine showed the strongest binding energy (-7.75 kcal/mol) and the lowest Ki value (15.36 μM) with histamine H1 receptor. This situation shows that loratadine binds to the target with high affinity and may be effective even at low doses. While fluticasone and amoxicillin exhibited intermediate binding profiles, pseudoephedrine exhibited a more limited inhibitory potential compared to other molecules with the weakest binding energy and the highest Ki value. Ligand-protein interaction analyses showed that each complex formed different numbers and types of bonds. Especially loratadine exhibited a binding network enriched with numerous hydrogen bonds, polar and hydrophobic interactions. Fluticasone, on the other hand, was directed to specific binding sites with halogen bonds and cation-π interactions, which supported its high selectivity with the glucocorticoid receptor. While amoxicillin established stable interactions with classical beta-lactam targets via hydrogen bonds, pseudoephedrine binding to the receptor was limited in terms of both energy level and interaction diversity. These results demonstrate that the molecular docking approach is an effective tool for predicting the binding efficiency, structural specificity and dosage requirements of drugs. The findings of the study provide a better understanding of the target interaction profiles of current drugs used in the treatment of ENT diseases; at the same time, they provide a structural basis for next-generation drug development studies. Supporting these in silico data with future experimental and clinical validation studies is of great importance for pharmacotherapeutic optimization.

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