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In silico evaluation of some α7nAChR agonists in apical periodontitis: The role of the cholinergic anti-inflammatory pathway

Emine ERDAG[1*], Dilan KIRMIZI[2], Umut AKSOY[2], Ahmet Ozer SEHIRLI[3]

1 Near East University, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, North Cyprus via Mersin 10, Türkiye
2 Near East University, Department of Endodontics, Faculty of Dentistry, North Cyprus via Mersin 10, Türkiye
3 Near East University, Department of Pharmacology, Faculty of Dentistry, North Cyprus via Mersin 10, Türkiye

ABSTRACT

Activation of the α7 nicotinic acetylcholine receptor, an important part of the cholinergic anti-inflammatory pathway, is considered a target macromolecule in the treatment of many inflammatory disorders. However, there are no molecular studies on the use of α7 nicotinic receptor agonists in apical periodontitis. In this study, we identified some α7 nicotinic acetylcholine receptor agonists that have been previously investigated for use mainly in diseases affecting the central nervous system. The selected ligands were examined in terms of binding affinity and receptor-ligand interactions on α7 nicotinic acetylcholine receptor using the molecular docking method. AutoDock Vina and GROMACS program packages were used in the molecular docking and simulation process. The results showed that B-973B, ABT-107, and GAT107 were the three most effective ligands in receptor binding affinity, respectively. This study explored the potential efficacy of α7 nicotinic acetylcholine receptor agonists in addressing apical periodontitis.

Keywords: α7nAChR, apical periodontitis, cholinergic pathway, inflammation, molecular docking

*Corresponding author: Emine ERDAG
E-mail: emine.erdag@neu.edu.tr
ORCIDs:
Emine ERDAG: 0000-0002-1431-935X
Dilan KIRMIZI: 0000-0003-0483-1736
Umut AKSOY: 0000-0001-7281-508X
Ahmet Ozer SEHIRLI: 0000-0002-5175-9290
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INTRODUCTION

Inflammation resulting from pathological changes in the periapical tissue may result from the immunoinflammatory response of etiological factors such as microorganisms, toxins, harmful metabolic residues, or pulp tissue remaining in the root canal system associated with host infection[1]. Several inflammatory mediators such as cytokines, and growth factors begin to be altered by fibroblasts, endothelial cells, and immune cells with the spread of inflammation in periapical tissue[2]. Destruction of surrounding tissue occurs as part of the defense process, resulting in periapical bone resorption[3]. Host defense triggers an important complex process including diverse components such as cytokines and matrix metalloproteinases (MMPs)[4]. Various cytokines such as interleukins (IL) and TNF-α, affect osteoclast differentiation and activation to initiate bone destruction[2]. In addition, many inflammatory mediators play a role in the bone remodeling process. A hyperinflammatory environment can cause loss of alveolar bone by disrupting the balance between bone formation and destruction. In this context, the effect of host response modulation in the treatment process is considered[5].

Acetylcholine (ACh) is a neurotransmitter that has a significant impact on inflammation by binding to specific receptors on immune and tissue cells. Cells with both vagus nerve and neuronal networks are counted as ACh sources. Non-neuronal-derived ACh is also thought to be involved in the regulation of localized immune responses[6]. It has also been reported by Fujii et al. that oral epithelial cells represent functioning parts of the cholinergic system[7]. It is believed that ACh produced by epithelial cells can regulate periodontal tissue function both locally and within the same cell[6]. ACh is one of the important components of the cholinergic system that binds to nicotinic and muscarinic receptors. These receptors are found in the vast majority of non-neuronal human cells[8].

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channel proteins, and they mediate the influx of Na^+, Ca^{2+}, and K^+ ions. The increase of Ca^{2+} ions in the cell depends on the activation of nAChR. It also affects the activation of protein kinases A and C (PKA and PKC, respectively), Ca^{2+} calmodulin-dependent protein kinase (CaMK), phosphatidylinositol 3-kinase (PI3K), and adenylyl cyclase (AC), which are calcium-sensitive signal transduction protein kinase models. The nAChR structure consists of seven α subtypes; α2, α3, α4, α5, α6, α7, respectively[9].

The periodontal ligament fibroblasts express α7nACh receptors[10]. The anti-inflammatory pathway is regulated by the cholinergic system, primarily the alpha7 nicotinic acetylcholine receptor (α7nAChR). This pathway begins with

the distinctive features of inflammation that activate the afferent vagus nerve and the transmission of an informational stimulus to the brain about the inflammation that has occurred. ACh release is increased in inflamed peripheral tissues proximal to the nerve in association with efferent vagal nerve activity. Free AChs bind to α7nAChRs on immune-competent cells and thus play an active role in localized inflammatory processes. Essentially, the leading branch of the vagal nerve reflex is important in ensuring the activation of the immune system[6]. Whether the regulation of the vagal nerve on the periodontal immune response is not known yet. Thus, the anti-inflammatory effect of neuronal ACh, which is vagally released, on immune cells is controversial. There is evidence to suggest that ACh produced outside of neurons plays a role in regulating inflammation in specific areas[11].

Macrophages, monocytes, and B and T immune cells play an important role in the pathogenesis of periodontal disease. The α7nAChR found in immune cells in the periodontium is expressed during periodontal disease. A study observed high amounts of ACh in T cells and monocytes[12]. In another study on α7nAChR deficient (α7nAChR−/−) animals, susceptibility to bacterial lipopolysaccharide (LPS) was noted[13]. In the same study, the production of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) was also observed. This observation proves that the α7nAChR is involved in the regulation of the anti-inflammatory pathway initiated by the cholinergic system and in the control of inflammation in response to pathogens[13]. Several research studies have noted that nicotine possesses the capacity to directly impact periodontal cells through nAChRs, resulting in pathophysiological consequences and the progression of tobacco-related ailments within these cellular structures[14]. In the literature, there is a study reported that inhibited bone destruction and decreased TNF-α expression were observed in animals administered nicotine and that it remarkably decreases synovial inflammation levels[15]. The α7nAChR agonist PHA 543613 was also shown to suppress IL-8 expression that was produced by *Porphyromonas gingivalis*. Although more investigation is required, it is hypothesized that ACh may be involved in the regulation of neutrophil chemotaxis in the periodontium[6].

In light of this information, it has been hypothesized that nicotine suppresses immune response via a cholinergic anti-inflammatory pathway, causing periodontal disease. There are no studies in the literature investigating the effect of nAChR agonists on the pathology of apical periodontitis. The selected ligands have shown efficacy as α7 nAChR agonists in previous clinical trials and many have been used as antidyskinetic agents in the treatment of Parkinson's disease and the treatment of schizophrenia, mainly to improve cognitive

functions[16]. According to the information we obtained from the literature, the selected ligands have generally been tested in disorders affecting the central nervous system. In addition, molecular docking studies of the ligands have not been performed before. Thus, it was aimed to demonstrate their usability as anti-inflammatory agents through the cholinergic anti-inflammatory pathway in the treatment of apical periodontitis and similar endodontic diseases. The cholinergic anti-inflammatory signaling cascade and the activity of α7 nAChR agonists in apical periodontitis were summarized in Figure 1.

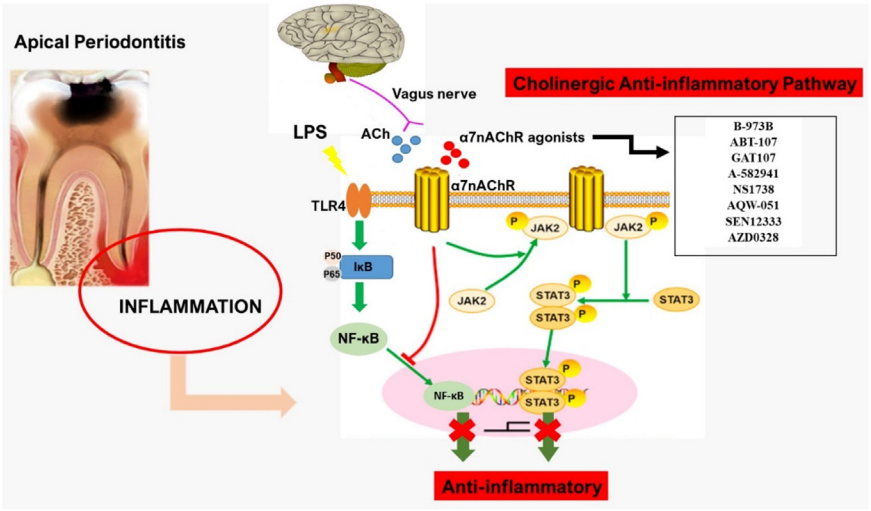

Figure 1. The cholinergic anti-inflammatory pathway and the role of α7 nAChR agonists in apical periodontitis

METHODOLOGY

Selection and preparation of data set

A molecular docking study was performed utilizing the crystallographic structures derived from the ligand binding domain of the α7 nAChR, an acetylcholine binding protein (AChBP) structure derived from *Lymnaea stagnalis* (PDB entry: 5J5I), and some selected α7 nicotinic acetylcholine receptor agonists were performed. The crystal structure of α7 nAChR, obtained from the Protein Data Bank (PDB) was illustrated in Figure 2. Before the docking procedure, we selected some specific α7 nAChR agonists as ligands; ABT-107 (PubChem CID 11151363), AZD0328 (PubChem CID 9794392), AQW-051 (PubChem CID 50914822), A-582941 (PubChem CID 11173546), B-973B (PubChem CID 137319851), NS1738 (PubChem CID 310378), GAT107 (PubChem CID 6554040), and SEN12333 (PubChem CID 45484303) were selected.

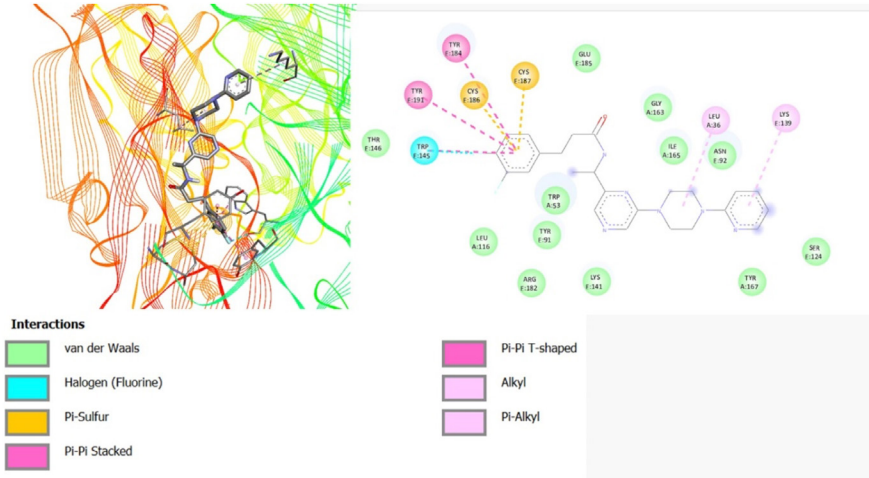

Figure 2. The 2D (on the right) and 3D (on the left) binding interactions of α7 nAChR and B-973B ligand. Green dotted lines; hydrogen bonds, pink lines; hydrophobic interactions, blue lines; halogen bonding, orange lines; electrostatic bonding.

The 2D representations of the selected ligands were obtained from the PubChem database. The ligands were subsequently extracted and converted into mol2 files using OpenBabel software, a preparatory step for molecular docking. Before initiating the molecular docking process, meticulous refinement was conducted on both the three-dimensional (3D) molecular structures of the ligands and the target protein, α7 nAChR. In this refinement procedure, all water molecules and heteroatoms were eliminated from the α7 nAChR structure. Subsequently, polar hydrogens were added, and Gasteiger charges were computed to enhance accuracy.

Following this structural refinement, the 3D conformation of α7 nAChR was saved as a PDBQT file. For the subsequent molecular docking analysis, AutoDock Vina and Discovery Studio Visualizer programs were employed to facilitate the extraction of diverse docking poses, as well as the calculation of binding affinities (ΔG) and the generation of ligand-receptor interaction profiles for the selected ligands. To comprehensively evaluate the performance of the chosen agonists on α7 nAChR, the docking study was carried out separately. Consistency was maintained throughout all docking procedures by employing a grid box with dimensions of 30 × 30 × 30 × 30 Å.

Molecular dynamics simulations

Utilizing the GROMACS molecular dynamics tool[17], we conducted molecular dynamics simulations. To simulate the protein-ligand complexes, GROMOS96 force field parameters were employed. To maintain charge neutrality within the protein-ligand complex, sodium ions (Na^+) and chloride ions (Cl) were added. Additionally, a simple point-charged water model was utilized to replicate water molecules.

The simulations were executed at a temperature of 310 K and a pressure of 1 bar, spanning a duration of 100 nanoseconds. This simulation protocol was consistent with a previously published methodology[18]. Throughout the simulations, we monitored protein-ligand interactions and assessed root-mean-square deviation (RMSD) characteristics to pinpoint the optimal conformations.

RESULTS and DISCUSSION

While the selected α7nAChR agonists have been extensively studied in diseases affecting the central nervous system, their application and potential efficacy in modulating inflammatory responses in apical periodontitis represent an unexplored and novel area of research. Our study is the first to investigate these ligands in this context, highlighting a new potential therapeutic pathway for a condition with a distinct inflammatory profile.

Table 1. Critical amino acid residues of α7 nAChR active site interacting with selected agonists, and their binding affinities

Name of Agonist	Binding Affinity, ΔG (kcal/mol)	Critical Amino Acid Residues
B-973B	-9.8	LEU36, TRP53, TYR91, LEU116, LYS139, LYS141, TRP145, THR146, GLY163, TYR167, ARG182, TYR184, GLU185, CYS186, CYS187, TYR191
ABT-107	-9.1	TRP53, LEU116, LYS139, LYS141, THR146, TYR167, ARG182, TYR184
GAT107	-8.9	TRP53, TYR91, LYS141, TRP145, ARG182, TYR184, CYS186, CYS187, TYR191
A-582941	-8.7	LEU116, TRP145, GLY163, TYR167, TYR184, CYS186, CYS187, TYR191
NS1738	-8.5	TYR91, CYS125, LYS139, ILE165, TYR167
AQW-051	-8.4	LEU36, TRP53, LEU106, TRP145, TYR184, GLU185, CYS186, CYS187, TYR191
SEN12333	-8.2	LEU36, TRP53, TYR184, CYS186, CYS187, TYR191
AZD0328	-7.4	ASP17, PRO79, SER81, TRP84, VAL85, LEU104

All ligands exhibited favorable binding affinities within the range of -9.8 to -7.4 kcal/mol. Table 1 provides a comprehensive summary of specific information concerning the interacting amino acid residues, types of interactions, and the binding affinities of the agonists. The binding affinity results derived from the ligands are presented in Table 1. The docking outcomes revealed that both ligands bound to their respective target receptors primarily through interactions involving hydrogen bonding and electrostatic bonds (Figure 3, Figure 4, and Figure 5). In this study, 3D and 2D receptor-ligand binding interactions obtained from molecular docking showed that all ligands interacted with amino acid residues which play critical roles in the sub- and complementary loops in receptor recognition and signal transduction. The findings indicated that B-973B exhibited the highest binding affinity (-9.8 kcal/mol) among the ligands. In addition, B-973B manifested bonding interactions with the receptor among all ligands tested.

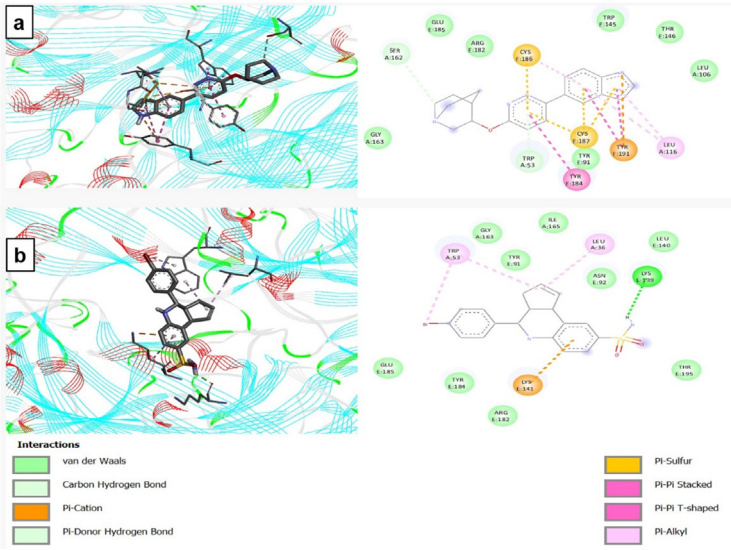

Figure 3. The 2D and 3D binding interactions of α7 nAChR with the ligands ABT-107 (a) and GAT107 (b). Green dotted lines; hydrogen bonds, pink lines; hydrophobic interactions, orange lines; electrostatic bonding.

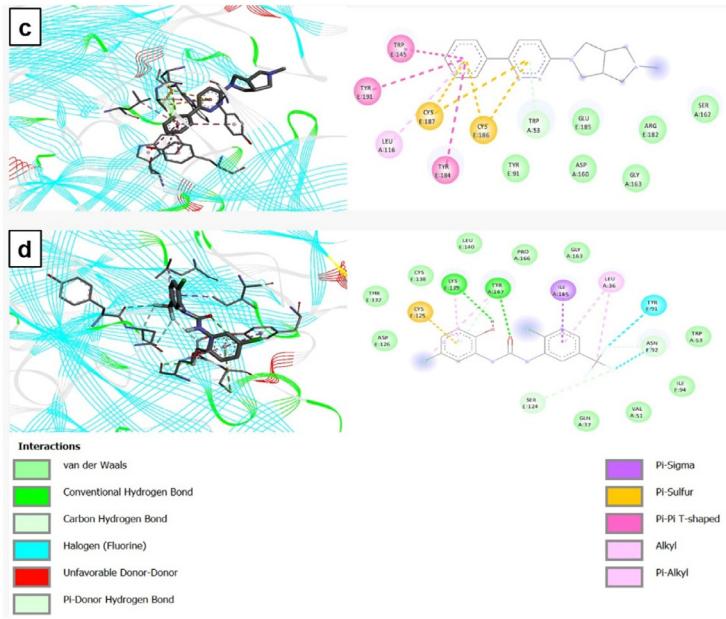

Figure 4. The 2D and 3D binding interactions of α7 nAChR and the ligands A-582941 (c) and NS1738 (d). Green dotted lines; hydrogen bonds, pink/purple lines; hydrophobic interactions, blue lines; halogen bonding, orange lines; electrostatic bonding.

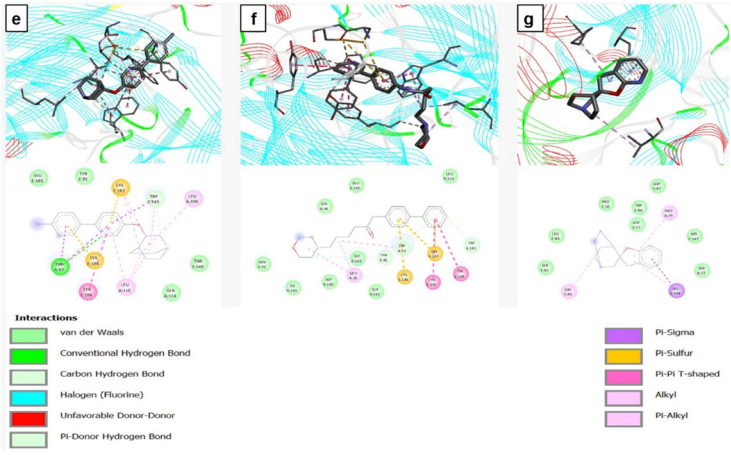

Figure 5. The 2D and 3D binding interactions of α7 nAChR and the ligands AQW-051 (e), SEN12333 (f), and AZD0328 (g). Green dotted lines; hydrogen bonds, pink/purple lines; hydrophobic interactions, blue lines; halogen bonding, orange lines; electrostatic bonding.

The conformational deviations in the receptor and the stability of the ligand in the receptor binding pocket were evaluated. A molecular dynamics (MD) simulation investigation was conducted to explore the interaction between the α7nAChR receptor and the most potent compound, B-973B ligand, which exhibited promising outcomes in the prior molecular docking analysis. The findings from this simulation reveal that the fluctuations observed throughout the 100 ns simulation period remain within the acceptable range of 1 to 3 Å.

Macrophages play an important role in the periodontal immune system by strengthening the defense against pathogens. Through the secretion of ACh, α7nAChR regulates the transmission of activation states and immunological responses of macrophages[6]. In the literature, several investigations have documented the utilization of macrophages for the identification of α7nAChR[19]. These studies have additionally revealed that the activation of macrophages plays a crucial role in modulating signaling pathways, resulting in the inhibition of NF-κB nuclear translocation and the subsequent reduction in the transcription of proinflammatory cytokines[19]. Additionally, it was discovered in research that modest dosages of nicotine had an inhibitory effect on the production of TNF-α and macrophage inflammatory protein 1 alpha (MIP-lα) in LPS-activated human peripheral monocytes[20]. All these states are moderated by 7nAChR, which in turn inhibits the NF-κB transcription factor by stopping the phosphorylation of IκBa (inhibitor of nuclear factor of kappa light polypeptide gene enhancer in B-cells, alpha)[20]. In addition, in some in vitro and in vivo studies in the literature, it has been observed that the JAK-2/STAT-3 pathway is also stimulated in α7nAChR activation. The release of STAT-3 proinflammatory cytokines is inhibited by the binding of nicotine to α7nAChR and activation of α7nAChR[21]. Activation of STAT-3 by the IL-10 receptor mediates the anti-inflammatory effect of IL-10. Studies have reported that only the specific down-regulation of proinflammatory cytokines can be induced by the activation of α7nAChR since it does not inhibit the production of IL-10[22].

Evidence on IgG-secreting B cells that have a role in the periodontal disease progression despite the effect of immune response on disease[23]. Under inflammatory conditions, T cells promote the differentiation of Th2 cells[24]. Th2 cells have anti-inflammatory responses and immune suppressive associated with periodontal disease[24]. While IL-17 production in CD4+ T cells in the blood is inhibited by Nicotine (nAChR agonist) through α7nAChR, α-bungarotoxin (α −BTX) provides the balance by showing an antagonistic effect[25]. The α7nAChR can differentiate into Treg cells, affect their immunosuppressive functions and nicotine increases the evidence of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and forkhead box 3 protein (FoxP3) and increases improvement of Treg cells into the central nervous system (CNS)[26].

Side effects occur in people who are addicted to cigarettes due to their continuous and high-dose exposure to nicotine throughout their lives. As a consequence of the location of nicotine in the central nervous system, it targets nicotinic acetylcholine receptors. In addition, there are non-neuronal nicotine binding sites in peripheral blood cells. Detailed examination of these regions has been proposed as an approach to understanding the pharmacological mechanisms in the tobacco addiction state[27]. The effects of tobacco on periodontal tissues and the causality of smoking with periodontitis have been reported by many researchers[27-29]. As a result of the inhibition of β-defensin by nicotine-induced *Porphyromonas gingivalis* LPS, it causes the progression of periodontal disease[29]. The increased IL-8 releases induced by *P. gingivalis* LPS or TNF-a decreases with nicotine activation[29]. This information implies that nicotine has a direct proinflammatory impact when present in the context of periodontal disease[6]. In previous studies, it has been observed that nicotine up-regulates IL-1β expression in periodontal cells[6, 29].

The ligand-binding core and regions of the α7 nAChR structure that play an important role in the analysis of signal transduction are covered with α7 residues[30]. Therefore, it is also critical for the recognition of ligand binding to the receptor. Recognition of ligand binding to the receptor is mediated by residues in loops A-C, the main subunit of the receptor, and loops D-E of the subunits, respectively[16,30]. Some critical amino acids in loops A and B of the main subunits are responsible for ligand binding and recognition. These amino acids are TYR91 in loop A and TRP145 in loop B. In loop C, the amino acids TYR184 and TYR191 play an important role in the transition to complementary subunits[16]. TRP53 in loop D, GLN114 in loop E, and LEU116 in loop E of the subunits enable ligand recognition mode and intra-unit communication inside the binding pocket. The ligand binding region of the receptor is surrounded by residues that are unique to the α7 subunit located throughout the C loop[16]. When the agonist binds to the receptor, these residues adopt a different conformation[30]. Conformational changes in the A, B, and C loops of the receptor during ligand binding led to the rearrangement of residues in the ligand binding site. The most important of these changes are the shifts of TYR91, TRP145, and TYR184. Also, TYR191 and TRP53 are the primary stabilizing residues[16].

The results obtained in our study showed that the selected ligands interact with these critical amino acid residues through different bonds. In particular, ligand B-973B, which was found to have the most effective binding affinity, interacted strongly with TYR184 and TYR191 via π- π stacked and π- π T-shaped interactions. Furthermore, TRP145 amino acid residue was observed to bind

with fluorine atoms in the ligand structure via halogen bonding. In addition, it is believed that the different interactions of the tested compounds with TRP53 and LEU116 amino acids, which are important in ligand recognition and signal transduction, as well as different types of bonding with ARG182 and GLU185 amino acids in the loop C region of the receptor, may affect the affinity of the α7-selectively bound agonist.

In general, as seen from the two-dimensional interactions, stabilizing interactions between the receptor and ligands were generally thought to consist of a π-cation bonding between the ligands and TRP145 amino acid residue due to the presence of an indole ring in its structure. The presence of the carbonyl functional group in the main chain of the amino acid TRP145 and the -OH group carried by TYR91 leads to the formation of hydrogen bonds between the ligands and the amino acid residues, which may constitute a component of stabilizing interactions. It is also concluded that extensive van der Waals contacts between the aliphatic part of the azabicyclo moiety and TYR184, CYS186, CYS187, and TRP145 in ligands such as ABT-107, AZD0328, or NS6784 may enhance the interactions.

The limitation in *silico* studies is to make calculations about the effects of chemical substances using computer simulations and present scientific data with estimated values. However, from a future perspective, preliminary data obtained from the *in silico* study can be evaluated and supported for use in *in vitro* and *in vivo* studies. Therefore, this molecular docking study may shed light on future *in vitro* and *in vivo* studies for apical periodontitis.

In summary, the binding affinity values obtained from the agonists selected as a result of the molecular docking study were compared. B-973B ligand, which was found to have the most effective binding affinity among them and showed a stable receptor-ligand relationship as a result of MD simulation, may be effective in reducing inflammation in the treatment of apical periodontitis and similar endodontic diseases. The results obtained may shed light on the use of the α7nAChR-mediated cholinergic anti-inflammatory pathway in apical periodontitis with effective α7nAChR agonists, mainly B-973B. In addition, this study may contribute to the design and synthesis of new α7nAChR agonists with halogen-substituted pyrazine propanamide derivatives similar to the molecular structure of B-973B, especially for use in LPS-induced apical periodontitis.

STATEMENT OF ETHICS

Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

Emine Erdag: Design, Acquisition of Data, Analysis of Data, Drafting of the Manuscript. Dilan Kirmizi: Design, Acquisition of Data, Drafting of the Manuscript. Umut Aksoy: Supervision. Ahmet Ozer Sehirli: Supervision. All authors read and approved the final manuscript.

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