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IN-SILICO BINDING AFFINITIES OF ALKALOID COMPOUNDS ON NICOTINIC ACETYLCHOLINE RECEPTOR α3β4 FOR SMOKING CESSATION TREATMENT

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Keywords:

Nicotinic acetylcholine receptor α3β4, Alkaloid, *In-silico*, Binding affinity, Molecular docking, Molecular dynamics simulation

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ABSTRACT: Nicotinic acetylcholine receptor α3β4 is considered as a potential target for anti-smoking drug discovery. In this study, *in-silico* approaches, including molecular docking and molecular dynamics simulation were applied to investigate binding affinities of 300 alkaloids into the α3β4 (Pdb id: 6PV8). The docking results showed that most of the alkaloids fitted well into the binding pocket of α3β4. The top hit compounds were A122 (indole alkaloid) and A128 (tropane alkaloid) with their binding affinities of less than -8.0 kcal.mol^{-1} and the interactions with key residue, Trp149. Structures and binding affinities relationships between the indole and tropane compounds with the α3β4 emphasized the important roles of indole backbone and the benzyl substituent at C3 of tropane scaffold in forming the hydrophobic interactions making good binding affinities. Molecular dynamics simulations revealed the potential of A128 to binding stably with the α3β4 during 20 ns. Binding free energy of the complex A128 - α3β4 was calculated based on Molecular Mechanics - Poisson Boltzmann Surface Area (MM-PBSA) method, which also emphasized the importance of electrostatic contacts over *van der* Waals interactions for proper binding. Hence, A128 can be additionally explored by *in-vitro* and *in-vivo* experiments for further confirmation of its smoking cessation treatment.

INTRODUCTION: Smoking cessation is a vital goal to improve health and prolong people's lives in public health worldwide since tobacco smoking causes many deaths and disability in both developed and developing countries [1]. The WHO reported that there are about 8 million people die prematurely each year [2], mostly from smoking-related diseases such as cancer, type 2 diabetes mellitus, heart disease, chronic obstructive pulmonary disease, congenital defects, *etc*., worldwide.

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Despite the harmful consequences related to tobacco use, a few smokers are going to quit smoking, and a small number of people are successful with and/or without nicotine replacement therapies [2].

Nicotine, a tertiary amine alkaloid that is known as a principle addictive substance in tobacco although smoking toxicity is related by other compounds. Nicotine directly binds to different subtypes of the nicotinic acetylcholine receptors (nAChRs) in the central nervous system, including the three most abundant brain subtypes, α4β2, α3β4, and α7 receptors that lead to nicotine addiction [3]. Of which, the α3β4 is a potential target for anti-addiction therapeutics as this nicotinic receptor is expressed focally in brain regions that affect

reward circuits and addiction, especially nicotine addiction [4]. Furthermore, recent studies have demonstrated that AT-1001, the co-crystallized ligand of α3β4 and also the partial α3β4 agonist showed a significant decrease in self-administration of nicotine in rats [5].

In terms of structure, the α3β4 receptor is a pentameric protein formed by combinations of homomeric or heteromeric of two functional subunits α3 and β4 in the order of α3 - β4 - β4 - α3 - β4, which plays a key role in the physiology of both central and peripheral nervous systems. Nicotine binds to the α–β interface to activate the nAChRs and allows cation influx. The diversity of subunit interfaces determines the channel properties leading to the different responses to agonists/ antagonists, desensitization, and downstream signaling and thus, define specialized properties and functions [6]. The α3 subunit transfers a positive charge for the main surface while the β4 subunit provides a negative charge to the complementary surface of the binding site at the interacting position. Each face is formed by three loops of the cavity with loops A-C (the main face) and loops D - F (the complementary face) [4].

Despite the abundant number of smokers worldwide, only a few prescription drugs are used for smoking cessation aid, such as cytisine (Tabex®), varenicline (Chantix®), bupropion (Zyban®) [7]. Notably, cysteine and varenicline are both originally from alkaloids. Cytisine is a pyrrolizidine alkaloid occurring in several plant genera that has been used as the treatment for smoking cessation since 1964 and varenicline is a synthesized compound from cytisine. The binding affinities of cytisine and varenicline were tested *in-vitro* with the K_i values on α4β2 and α7 of 0.17 nM and 4200 nM for cytisine and of 0.06 nM and 322 nM for varenicline, respectively. In addition, varenicline was known to successfully help one of every 11 smokers remain abstinent from tobacco for six months [8].

In recent years, a few studies applying *in-silico* methods have shown the inhibitory potential of alkaloids on α3β4 receptors for anti-smoking effect. For example, three compounds excreted from *Aristotelia chilensis,* namely aristoteline, aristo-quinoline, and aristone [9], had the capacity of inhibiting and binding selectively on α3β4 receptor [9]. Besides, some alkaloids such as nalmefene, and naltrexone, morphine derivatives, are in phase II clinical trials for smoking cessation activities [10]. Therefore, this study aimed to give more insight into *in-silico* binding affinities of alkaloids on nicotinic acetylcholine receptor α3β4, through molecular docking and molecular dynamics simulation in order to identify anti-addiction compounds assisting in smoking cessation treatment. Subsequently, the binding energy of the best ligand was calculated using the Molecular Mechanics - Poisson Boltzmann Surface Area (MM-PBSA) method.

MATERIALS AND METHODS:

Molecular Docking: Molecular docking was applied to investigate the binding affinities of 300 alkaloids on the nicotinic acetylcholine receptor α3β4. The crystal structures of human α3β4 receptor in complex with two different active ligands, nicotine and AT-1001 were available on Protein Data Bank (http://www.rcsb.org) with PDB codes: 6PV7 and 6PV8. As AT-1001 was known as a partial agonist of α3β4 receptor related to anti-smoking activity, the structure of α3β4 and AT-1001 complex (PDB code: 6PV8 with the resolution of 3.87 Å) was chosen for molecular docking study. The receptor α3β4 was prepared by separating the extracellular domain along with the binding pocket from the experimental structure due to the large structure with more than 1900 amino acids and saved as pdbqt file by AutoDock Tools - 1.5.611 **Fig. 1**.

Ligands for docking were 300 alkaloids (A1 to A300) derived from PubChem. The ligands were converted to 3D structures and minimized energy using ChemDraw Ultra 12.012 and saved as pdbqt file by AutoDock Tools - 1.5.611. Molecular docking was subsequently carried out by using AutoDock Vina with the following parameters of grid box: center_x, y, z of 147.642; 109.982; and 102.485 (Å), respectively and size_x, y, z of 22 x 22 × 22 (Å 3), respectively. The docking results were analyzed based on three criteria, including the binding mode with the best conformation of ligands, binding affinity ($kcal.mol^{-1}$), and the interactions of ligands and key residues at the active site of the α3β4 for anti-smoking activities.

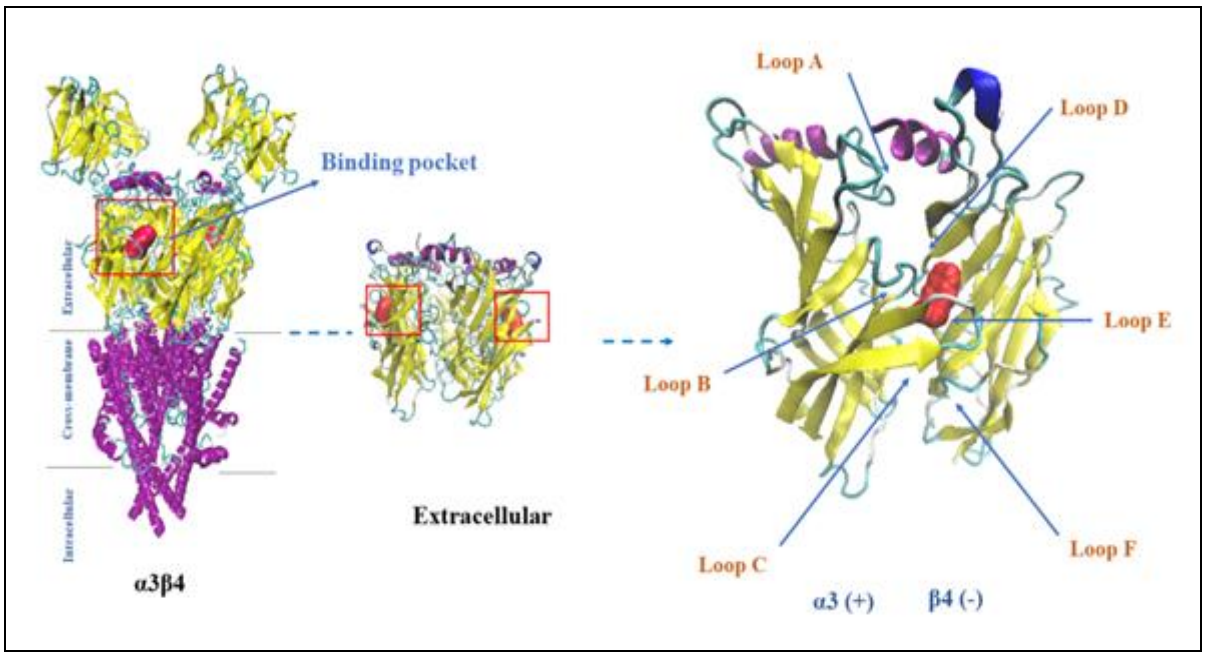

FIG. 1: THE EXTRACELLULAR DOMAIN OF α3β4 RECEPTOR OBTAINED FROM THE PROCESSING STAGES (PDB CODE: 6PV8)

Molecular Dynamics Simulations: The top docking candidates (A122, A128) obtained from AutoDock Vina docking, which was hoped to be the most active one, were subjected to molecular dynamics simulations (MDs) using GROMACS 2020.2 [13] with CHARMM22 force field. MDs were to investigate the stability and flexibility of the complex of protein-ligand under biological conditions. The two complexes of protein α3β4 and ligands A122, A128 were immersed in a dodecahedron water box (1 nm thick) of TIP3P water molecules, and periodic boundary conditions were also applied in all steps. The systems were neutralized with the Na^+ and Cl^- ions and run the steepest-descent energy minimization process to remove potential bad interactions from the initial structures for 20,000 steps. System minimization was followed by the equilibration, in which the heavy atoms in two chains were restrained, and the solvent molecules with counterions were allowed to move during the 1,000 ps (or 1 ns) and to gradually heat the system under NPT conditions at 300 $^{\circ}K$ (with keeping a number of particles (N), system pressure (P), and temperature (T) constant).

These equilibrated structures were run molecular dynamics simulations for 20 ns. MDs results were evaluated by the values of root-mean-square-deviation (RMSD) and root-mean-square-fluctuation (RMSF) to evaluate the stability of the protein-ligand docking complex.

Molecular Mechanics Poisson-Boltzmann Surface Area: The free binding energy (ΔG) of the protein and best ligand complex was measured by three factors, including the gas-phase free energy (ΔE_{MM}), solvation free energy ($\triangle G_{solv}$) and the change in the system entropy (-TΔS), which can be calculated by Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) module[15], as expressed in the following Equation:

$$\Delta G = \Delta E_{MM} + \Delta G_{solv} - T\Delta S \quad (1)$$

In the present study, MM-PBSA calculations were carried out using the GROMACS 18.1 tool to calculate the interaction energy between the ligand and the residues of the protein active site.

RESULTS AND DISCUSSION:

Molecular Docking: Molecular docking was conducted to identify the best candidate among the 300 alkaloids in terms of their binding affinities and binding interactions into the receptor α3β4. The docking results showed that there were 254 compounds fitted well into the binding pocket of α3β4 receptor with their binding affinities ranged between -8.4 and -0.2 $kcal.mol^{-1,}$ whereas 46 remaining ligands could not enter into the cavity due to their large bulky structures. The hit compounds were A122 (indole alkaloid) and A128 (tropane alkaloid) because of their good docking affinities (lower than -8.0 $kcal.mol^{-1}$) and the

interactions with some key residues, namely Trp149 on loop B; Cys192 and Cys193 on loop C; and Leu121 on loop E[16].

Thus, alkaloids belonging to two scaffolds, indole, and tropane backbone were taken for docking analysis. The structures of indole alkaloids and their binding affinities, along with their binding interactions with the α3β4 receptor, were displayed in **Fig. 2**. As the results, the structure – binding affinities relationship between the indole compounds with the α3β4 were related to two rings (A) and (B) of the indole backbone, which was able to form hydrophobic bonds (π - π) with the indole ring of residue Trp149. Additionally, substituted C13 of A122 (-8.4 kcal.mol^{-1}) created the π - sulfide bond with Cys198 on loop C and fitted better into the receptor than other compound A226 (-7.5 kcal.mol^{-1}). The intermolecular closure of substituent groups at C13 could make the main backbone not be able to fully enter the binding cavity and lower the docking affinity (A64, A74, A81). Therefore, the structure of the substituent at C13 played an integral role in the binding ability of ligands and the α3β4 receptor.

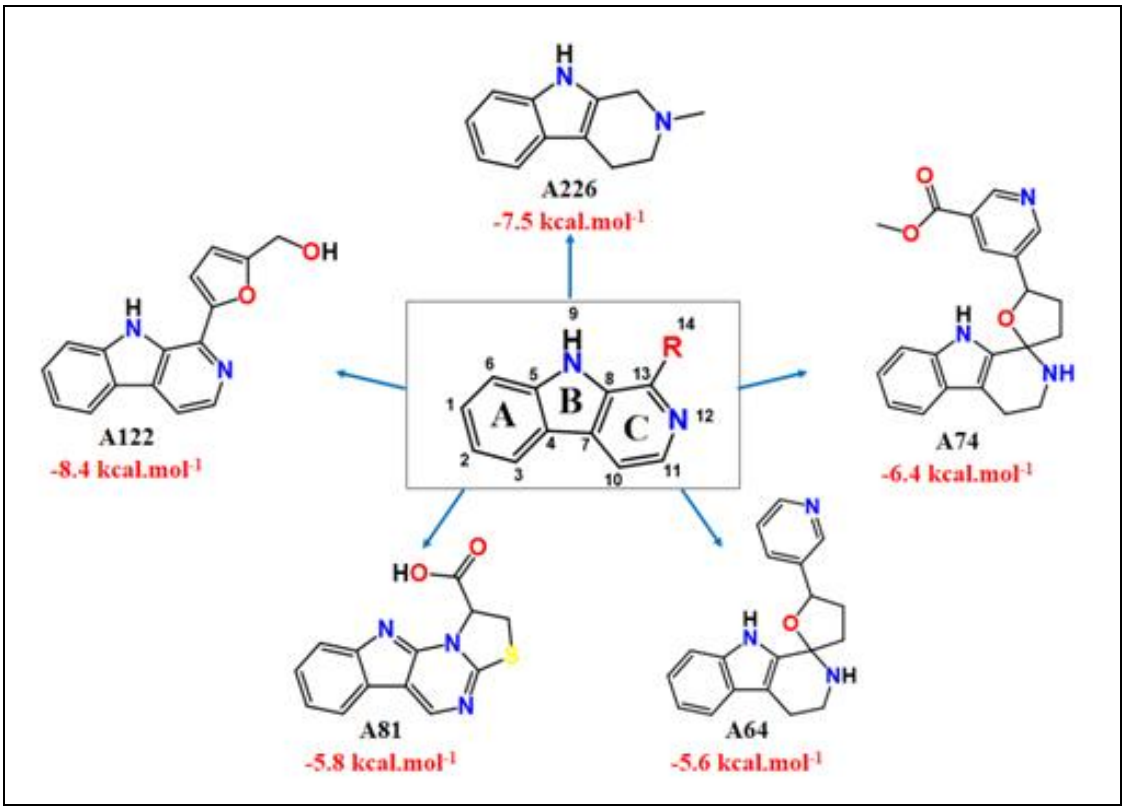

FIG. 2: INDOLE ALKALOIDS AND THEIR BINDING AFFINITIES INTO THE α3β4 RECEPTOR (PDB: 6PV8)

Moreover, the structure-binding affinity relationships between the tropane scaffold and the α3β4 receptor were illustrated in **Fig. 3**. The benzyl substituent (-$CH_2C_6H_5$) at C3 existing in the majority of alkaloid compounds had good binding affinities (less than -7.0 kcal.mol^{-1}) and formed hydrophobic interactions with Trp149. However, when replacing the benzyl group by another, there was a significant decline in the binding affinity (A149 with -6.3 kcal.mol^{-1}) due to the failure of forming hydrophobic bond with the important residue Trp149. Although R (chemical substituent) can be at C5 or C8 of the azabicyclic ring, R at C5 demonstrated better docking results than it at the C8 site. This was because R at C5, for example, A128 (-8.1 kcal.mol^{-1}), made the compound clamp-like, suitable for the wide-binding cavity, which was able to interact with most of the key amino acids of α3β4 binding pocket. When R was at C8, A46 (-7.8 kcal.mol^{-1}), the compound was longer, and it pushed the part of the structure out of the binding pocket and reduced the ligand possibility of interacting with the amino acids of α3β4. Replacing R with a smaller group such as acetyl (-COOH) on A93 (-6.9 kcal.mol^{-1}) did not create the interaction with Trp149 due to the increasing distance.

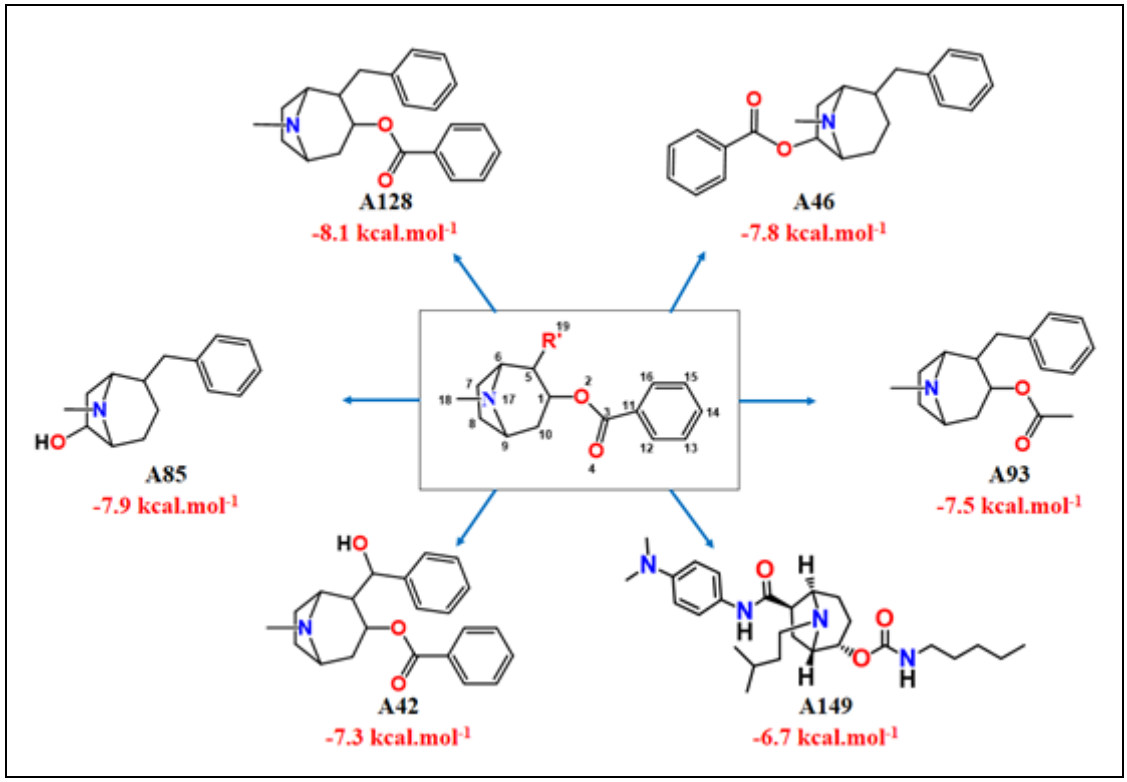

FIG. 3: SOME TROPANE ALKALOIDS AND THEIR BINDING AFFINITIES INTO THE α3β4 (PDB: 6PV8)

Molecular Dynamics Simulations: In protein-ligand docking, the protein was kept rigid, and the ligand was flexible, thus to better understand the flexibility of two best docking ligand (A122, A128) and protein complexes under biological conditions, MDs of these two complexes were conducted for 20ns. MDs results were evaluated on the RMSD and RMSF values of protein and ligands. The RMSD values were calculated for all frames in the MDs trajectory.

The results indicated that when A122 bound to α3β4 receptor, protein RMSD values showed a significant change in the first 9 ns before reaching stable state **Fig. 4A**, compared to A128- α3β4 complex, the protein RMSD exhibited a slight fluctuated of around 0.2 nm during 20 ns MDs **Fig. 4C**. Furthermore, both A122 and A128 ligand RMSD values changed by below 0.1 nm showing that these substances bound stably with protein at binding pocket during MDs process **Fig. 4B** & **4D**.

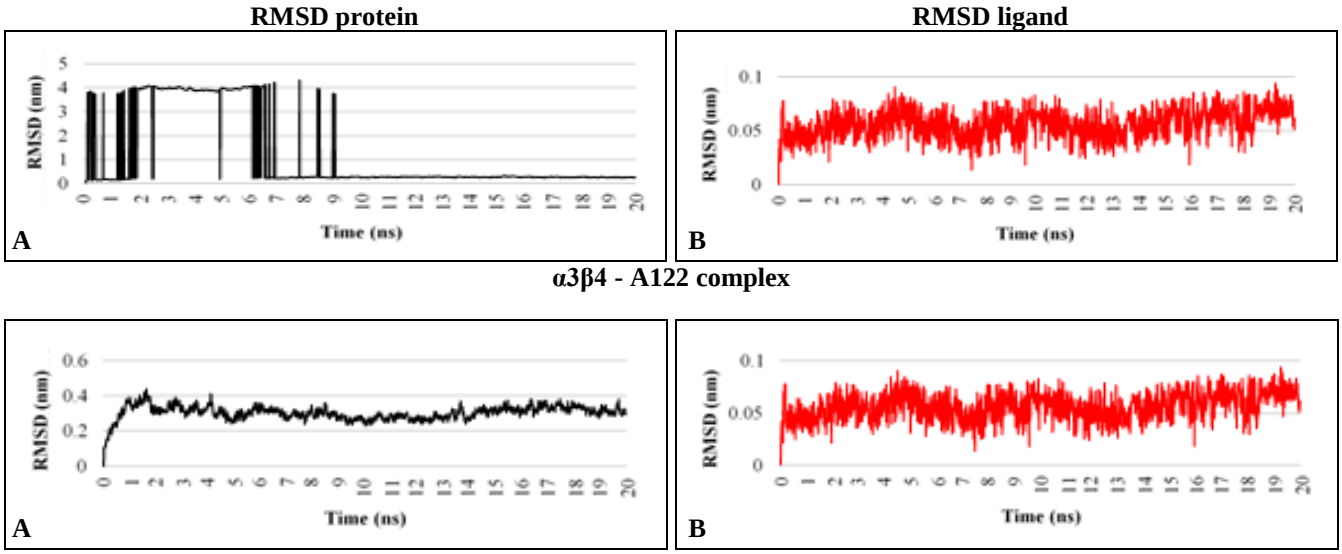

FIG. 4: ROOT-MEAN-SQUARE DEVIATION (RMSD) VALUES OF TWO BEST DOCKING COMPLEXES, INCLUDING RMSDs OF PROTEIN α3β4 (A) IN BLACK COLOR AND RMSDS OF LIGAND A122 AND A128 (B) IN RED COLOR

The RMSF value is another indication of flexibility, and the higher the value, the higher the degree of flexibility. Both A122 and A128 presented stable bonds with amino acids in the binding cavity, such as loop A-C on α3, loop D-F on β4, that was why residues in the binding pocket had less flexibility with the lower RMSF value when binding with ligands than those in apoprotein (protein only). In particular, compound A128 had better interactions described in the much lower RMSF value, which was almost not deviated and kept under 0.01 nm during MDs progress. This proved that complex A128-protein was the best stable one.

Furthermore, the best ligand A128 and α3β4 were selected to calculate the free binding energy of the complex in terms of the MM-BPSA method. This ligand-bound well to the receptor with the binding energy of -206.088 ± 23.351 kcal.mol$^{-1,}$ and v*an der* Waals energy played the most important role among the others **Table 1**.

TABLE 1: BINDING FREE ENERGY (ΔG) OF A128 WITH THE α3β4 BY USING MM-BPSA CALCULATION INCLUDING THREE TYPES OF ENERGIES CALCULATED BY MOLECULAR MECHANICS (ΔE_{MM}), *VAN DER* WAALS ENERGY (ΔE_{vdW}) AND ENERGY FROM POLAR SOLVATION ($\Delta G_{polarsolvat}$)

ΔE_{MM} (kJ/mol)	ΔE_{vdW} (kJ/mol)	$\Delta G_{polarsolvat}$(kJ/mol)	ΔG(kJ/mol)
-114.383 ± 20.679	182.183 ± 35.149	105.607 ± 53.757	-206.088 ± 23.351

Combination of molecular docking, molecular dynamics simulations, and free binding calculation, there was compatibility among these results. Ligand A128 **Fig. 5** mimicked the co-crystalized ligand (AT-1001, the agonist compound of α3β4 receptor) in forming the interactions with the key residues of α3β4 binding pocket, which resulted in the best binding affinity (-8.2 kcal.mol^{-1}).

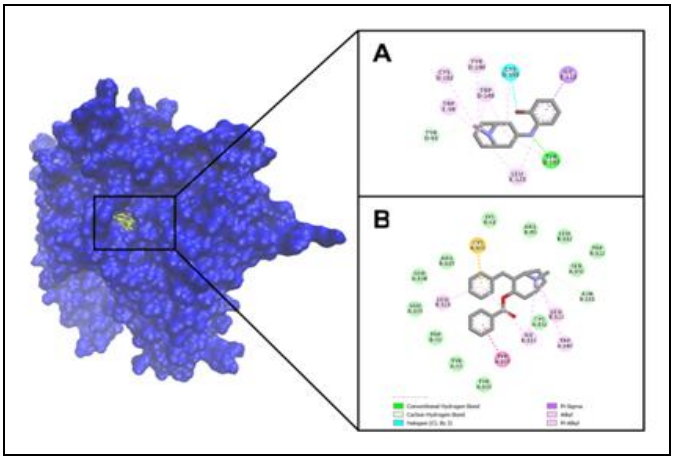

FIG. 5: INTERACTION OF AT-1001 (A) AND A128 (B) WITH PROTEIN α3β4 (PR_03) INCLUDES HYDRO-PHOBIC BONDS WITH Trp149, Tyr190, Cys192, Cys193 (CHAIN A) AND Ile113, Leu121, Leu123 (CHAIN B)

To be more detailed, the azabicyclic ring of A128 constituted the π - π interaction with the indole ring of Trp149 (α3) and the phenyl ring of the ligand created π – sulfide bond with sulfur atom of Cys193 on loop C. Moreover, the π – π interaction with Tyr197 and the π - alkyl bond with the aromatic ring of Ile113 also increased the ligand docking affinity. Comparing with AT-1001, it presented some similarities when interacting with Trp149, Tyr190, Cys192, Cys193 (Chain A), and Ile113, Leu121, Leu123 (Chain B) by hydrophobic bonds **Fig. 5.** On the other hand, owing to these stable interactions, the A128-α3β4 complex showed good RMSD, RMSF values, and free binding energy, which represented a durable binding state.

CONCLUSION: In this study, the molecular docking, MDs, and free binding energy calculation were performed to identify the potential alkaloid compound as well as to explore the binding mode of α3β4 nicotinic acetylcholine receptor with 300 alkaloids downloaded from PubChem. The best ligand conformation was selected based on the binding affinity, interaction with key residues Trp149. The conclusion achieved from the docking analysis was that compounds belonging to indole backbone (A122) and tropane backbone (A128) had the highest binding affinities with the α3β4 nicotinic acetylcholine receptor. Subsequently, MDs was carried out to get insight into the binding stability of A122-α3β4 and A128-α3β4 complexes. The RMSD, RMSF values revealed that A122-α3β4 complex was highly stable as compared to the remaining complex. The free binding energy of A128 to α3β4 receptor was-206.088 ± 23.351 kcal.mol^{-1}. Despite a few reports on the medicinal usage of alkaloids for smoking cessation, there is no *in-silico* study investigating a large number of alkaloids on PubChem along with predicting for smoking cessation effect of tropane scaffold. In particular, A128 was identified as a potential hit compound for anti-smoking drug development.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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