Supporting Information for

Novel Positive Allosteric Modulators of the Human α7 Nicotinic Acetylcholine Receptor [†]

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Method

To give insight into the interaction between PAMs and the agonist/competitive antagonist site at different AChR interfaces, compound 2 (representative of compounds with PAM activity) was docked to the agonist site at the hα7/α7, hα3/β4 and hα4/β2 interfaces, respectively. Then, 1-ns molecular dynamics simulations were performed to investigate the stability of the complexes. For comparison, (+)-epibatidine was docked to the agonist site at the hα7/α7 interface and molecular simulations were also conducted. To shorten the time used in the searching of the molecular conformations, simulations were performed at high temperature (450K). Since we only want to observe the stability of the ligands, the Cα atoms were constrained to preserve the 3D structure of the protein.

Results

PAMs do not Bind to the AChR Agonist Sites. The dynamics simulation results show that (+)-epibatidine binds to the hα7/α7 interface in a steady manner, whereas compound 2 moves away from its initial position at the hα7/α7, hα3/β4 and hα4/β2 agonist sites (Figs. S1B-E). The larger RMSD values of compound 2 at the different agonist sites (~0.4-0.8 nm) compared to that for (+)-epibatidine (~0.2 nm) at the hα7/α7 interface (Fig. S1A) also indicate that these PAMs are unstable when binding to the agonist sites.

Hydrogen Bonds are important for PAM Activity. More hydrogen bonds are found in the hα7 AChR-compound 2 complex (Fig. S2A) than that found in the complex between the hα3β4 or hα4β2 AChR and compound 2 (Figs. S2B,C). Two hydrogen bonds are formed in the hα7-PAM complex, one between the oxygen atom from the carboxyl group of compounds 2-4 and the Asn104 side chain at the (-) face and the other between the nitrogen of the compound's amide group and the Lys84 backbone at the (+) face (Fig. S2A). In the hα3β4-ligand complex, however, the nitrogen atom forms a hydrogen bond with the side chain of the homologous residue Trp83 at the (+) face, whereas there is no hydrogen bond with the (-) face (Fig. S2B). In the case of the hα4β2 AChR, the determined distances between the oxygen atom

of the amide linkage and the backbone oxygen and the side chain nitrogen of the homologous residue Arg84 at the (+) face are both in the range of forming hydrogen bonds, whereas hydrogen bonds with the (-) face are not found (Fig. S2C).

To compare the hydrogen bond interactions of compounds 2-4 with that of compound 1 at the hα7/α7 interface, the distances between the hydrogen bond donors and acceptors were calculated. The distance for each hydrogen bond for compounds 2-4 is ~3.30 Å and ~2.80 Å, respectively, whereas these distances are longer for compound 1 (compare Figs. S2A,D). The docking results of compound 1 show that although the benzene ring is aligned with that of compounds 2-4 (compare Figs. S2A,D), its amide group is shifted away from this alignment because of steric hindrance mediated by the methyl group located between the amide and benzene groups (Fig. 1). As a result of this structural shifting, the distance between the nitrogen of the amide group and the Asn104 side chain is augmented to 6.33 Å whereas that between the oxygen of the carboxyl group and the Lys84 side chain is enlarged to 3.08 Å (Fig. S2D). Hence, although compound 1 is structurally similar to compounds 2-4 (see Fig. 1), the hydrogen bond interaction between the amide group and Asn104 that are important for the PAM activity elicited by compounds 2-4 is very improbable to take place by compound 1. However, compound 1 may still form a hydrogen bond with the (+) face.

The PAM site in the hα7 AChR is larger than that in the hα3β4/hα4β2 AChRs. In the hα7 AChR, the furan ring of compounds 2-4 is positioned between the side chains from His102 and Lys84 (Fig. S2A). However, in hα3β4/hα4β2 AChRs, α7-His102 is mutated to β2/β4-Tyr104. Since Tyr104 has a larger side chain compared to that for His102, the space between Tyr104 and Lys84/Arg84 in the hα3/β4 and hα4/β2 interfaces is smaller (compare Figs. S2A-C). Supporting this structural difference, the calculated distance between the mass centers of the α7-His102 and α7-Lys84 side chains is 9.27 Å, whereas the distance between the α4-Arg84/α3-Lys84 and the respective β2-Tyr104/β4-Tyr104 side chains are 8.09 Å and 6.28 Å, respectively. The smaller volume in the hα3β4/hα4β2 AChR binding pockets precludes

the fitting of the furan ring in this domain, and consequently, these compounds cannot produce any PAM activity on these AChRs.

The postulated PAM sites at the hα7 AChRs are not probable at the hα3β4 and hα4β2 AChRs. Compound 2 was first docked to the three potential domains for PAMs: (1) the extracellular domain of the hα3/β4 (or hα4/β2) subunit interface, (2) the extracellular-transmembrane junction of hα3 (or hα4) subunit and (3) the transmembrane domain of the hα3 (or hα4) subunit. After docking compound 2 to the three potential PAM sites at the hα3β4 and hα4β2 AChRs, 10-ns molecular dynamics simulations were performed on the ligand-receptor complexes to evaluate their stability. The calculated RMSD values at the ha3/β4 interface and at the extracellular-transmembrane junction of the hα3 subunit are ~0.4-0.5 nm, whereas that at the hα3 transmembrane domain is about 0.6 nm (Fig. S3). The RMSD values for the PAM sites on the hα4β2 AChR are ~0.4-0.8 nm (Fig. S4). These values indicate that compound 2 is unstable when it binds to each one of these potential PAM sites on the hα3β4 and hα4β2 AChRs.

FIGURE S1: Molecular dynamics simulations of compound 2 at the agonist/competitive antagonist site on different AChR subunit interfaces and of (+)-epibatidine at the hα7/α7 interface. (A) RMSD values for compound 2 bound to the agonist/competitive antagonist site at the hα7/α7 (red line), hα3β4 (blue line), and hα4β2 (green line) interfaces, and for (+)-epibatidine bound to the hα7/α7 interface (black line). Location of (B) (+)-epibatidine and (C) compound 2 before (blue) and after (green) 1-ns dynamics simulations at the hα7/α7 interface, and of compound 2 at the (D) hα3β4 and (E) hα4β2 interfaces, respectively. The protein is shown as ribbons, whereas compound 2 and (+)-epibatidine are shown as sticks. The primary [i.e., (+) face] and complementary [i.e., (-) face] components of the extracellular domain are shown in yellow and cyan, respectively.

FIGURE S2: Different conformations of compound 2 (orange) (Class 1) at the (A) hα7/α7, (B) hα3/β4, and (C) hα4/β2 subunit interfaces, and different conformations of (D) compound 1 (green), (E) compound 6 (Class 2) (green), and (F) compound 9 (Class 3) (green) at the hα7/α7 subunit interface. The primary [i.e., (+) face] and complementary [i.e., (-) face] components are shown in yellow and cyan, respectively, whereas the oxygen, nitrogen, and hydrogen atoms are shown in red, blue, and white, respectively. The distance (in Å) between hydrogen bond donors and acceptors forming (full lines) and not forming (broken lines) hydrogen bond interactions are labeled. The natural mutation hα7-His105 to hα4/hα3-Tyr105 and the number of hydrogen bonds are important for receptor specificity.

FIGURE S3: Molecular dynamics simulations of compound 2 at the three postulated PAM sites on the hα4β2 AChR. (A) RMSD values for compound 2 when it binds to the hα4/β2 subunit interface domain (black line), to the transmembrane domain (red line), and to the extracellular-transmembrane junction (blue line). Location of compound 2 before (blue) and after (green) 10-ns dynamics simulations when is bound to (B) the subunit interface domain, (C) the transmembrane domain, and to (D) the extracellular-transmembrane junction. The protein is shown as ribbons, whereas the compound 2 is shown as stick.

The primary [i.e., (+) face] and complementary [i.e., (-) face] components of the subunit interface are shown in yellow and cyan, respectively, whereas the transmembrane segments are shown in fuchsia. The molecular dynamics results on the hα3β4 AChR are similar to that for the hα4β2 AChR.

Figure S1:

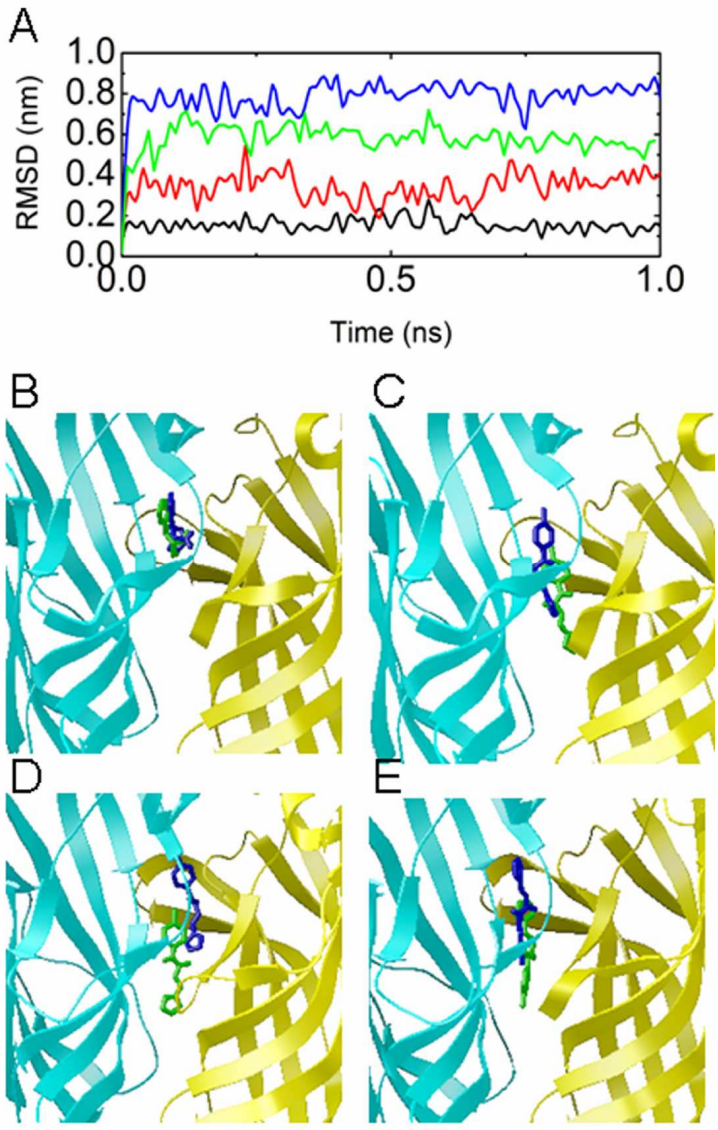

Figure S2:

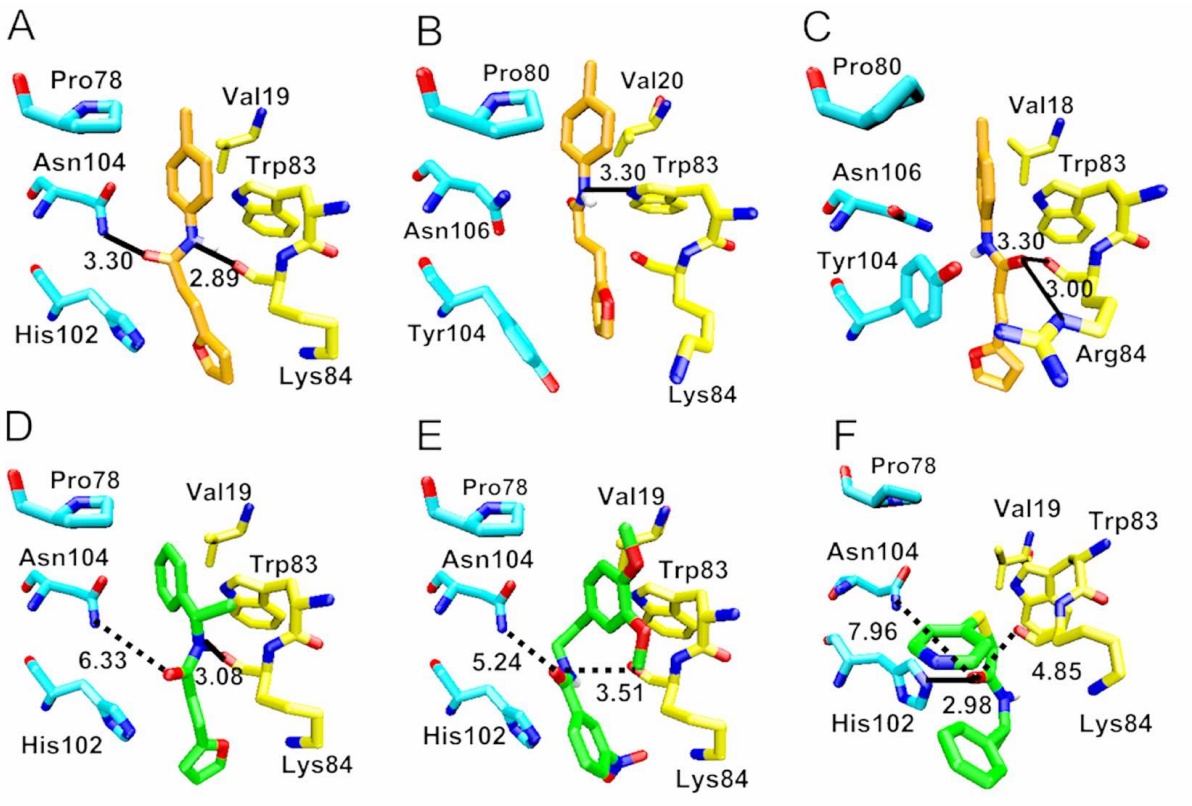
A
Pro78
Val19
Asn104
Trp83
3.30
2.89
His102
Lys84
B
Pro80
Val20
3.30
Trp83
Asn106
Tyr104
Lys84
C
Pro80
Val18
Asn106
Trp83
Tyr104
3.30
3.00
Arg84
D
Pro78
Val19
Asn104
Trp83
6.33
3.08
His102
Lys84
E
Pro78
Val19
Asn104
Trp83
5.24
3.51
His102
Lys84
F
Pro78
Val19
Trp83
Asn104
7.96
4.85
2.98
His102
Lys84

Figure S3