SUPPLEMENTARY MATERIAL

DISCOVERY OF NOVEL α4β2 NEURONAL NICOTINIC RECEPTOR MODULATORS THROUGH STRUCTURE-BASED VIRTUAL SCREENING

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CONTENT

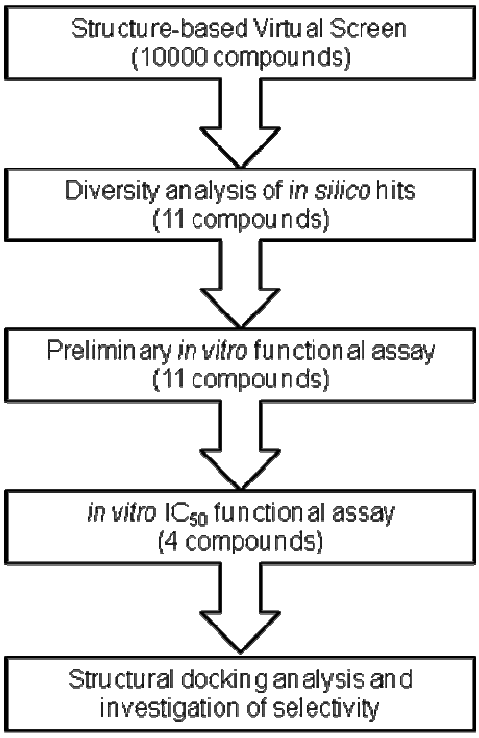

Figure S1: The schematic representation of the workflow described in the manuscript.

Table S1. Chembridge IDs for virtual screening hits and negative controls

Compound	ChemBridge ID
1	7995652
2	9042047
3	9006145
4	7992142
5	9058912
6	7995406
7	9010069
8	9040046
9	9025009
10	9030802
11	9055804
N1	7974335
N2	9052807
N3	7961836

Compounds 1-11 represent the molecules tested for experimental activity in the calcium accumulation assay as selected from the top-ranking molecules of the virtual screening protocol. Compounds N1-N3 are negative controls.

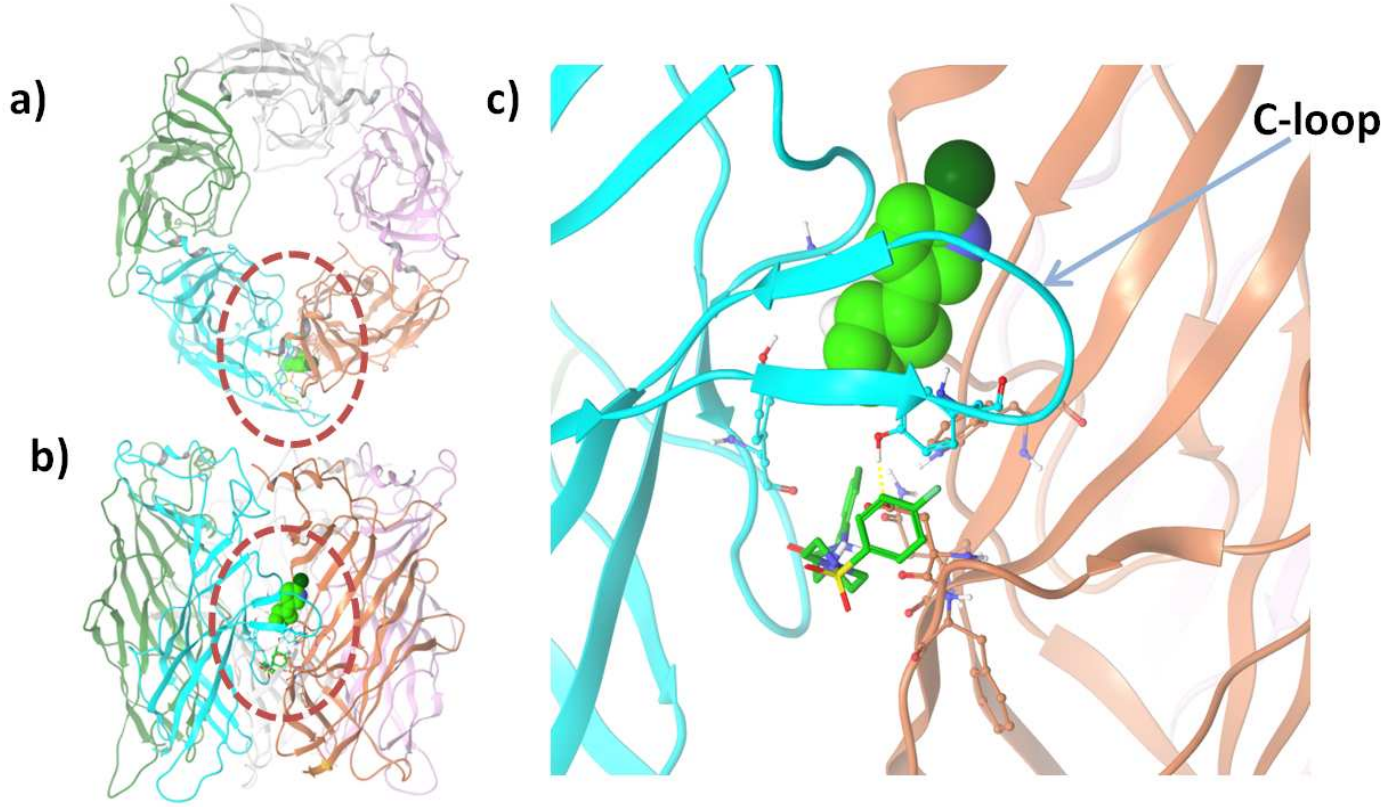

Figure S2: Putative allosteric binding site of the hit compounds on the hα4β2 nAChR extracellular domain. **A.** Top view of the extracellular domain of the nAChR. **B.** Side view. **C.** Zoomed in side-view of the binding pocket. Epibatidine is displayed as space filling representation (green carbon) whereas the docked conformation of compound **2** is in stick representation (green carbon). The monomer chains are differentially colored (α4=cyan, β2=light orange).

Model Building, Molecular Dynamics, and AutoDock Docking

Human α4β2 nAChR extracellular domain homology models were built in an iterative fashion with MODELLER9v1 based on PDB IDs: 1UW6, 2BYR, 2BJ0, and 2QC1 as previously described.[1, 2] The virtual screening template was prepared by docking the agonist epibatidine to multiple receptor conformation as extracted from a molecular dynamics (MD) simulation. The MD simulation used the Amber ff99 force field and Sander algorithm with explicit water solvation (15 Å buffer of water around all sides of the protein).[3] Sodium ions were added to achieve charge neutrality for simulation which used the particle mesh Ewald method to treat electrostatic interactions. After a 500 steps of steepest descent and 1500 steps of conjugate gradient minimization, the system was heated from 0 to 300 K over 200 ps while all protein atoms were restrained in place. Finally, a 5 ns production run at constant temperature and pressure (300 K, 1 atm) was carried out. Receptor conformations were extracted at regular 200 ps intervals to which epibatidine was docked with AutoDock4. AutoDock ligands were assigned Gasteiger charges then docked 100 individual times; the docking results were clustered by all-atom RMSD with a tolerance of 2 Å. The conformation to which epibatidine

docked most similarly to the crystallographic position (PDB ID: 2BYQ) was used as the virtual screening template.

Calcium Accumulation Assay

For the calcium accumulation assays, HEK tsA201 cells stably expressing either hα4β2 nAChRs or hα3β4 nAChRs were used with either fluo-4AM or Calcium 5. An assay previously reported was used with slight modifications.[4, 5] Plated cells were incubated at 37ºC, 5% CO_2, in DMEM supplemented with 10% fetal bovine serum, 10 mM L-glutamine, 0.7 mg/ml G418, 100 units/ml penicillin, 100 µg/ml streptomycin, and 100 µg/ml zeocin. Cells were used for experiments at ~100% confluency, typically 24 to 48 hrs after plating. Using fluo-4AM, cells were washed (100 µl) with HEPES-buffered Krebs (HBK) solution, and incubated (protected from light) for 30 minutes at 37ºC followed by 30 minutes at 24^{o}C with 40 µl of HBK containing 2 µM fluo-4-AM solution, 2.5 mM probenecid, and 0.05% pluronic F-127. When using the Calcium 5 probe, the cells were loaded with Calcium 5 (50% of manufacturer's recommendation) for 1 hour protected from light. Fluo-4-AM and pluronic F-127 were dissolved in DMSO (100% and 20% w/v, respectively), resulting in a final DMSO concentration of <0.1%. After loading the cells with fluo-4-AM, the cells were washed (1X) and 80 µl of HBK were added to each well. The plates were then placed into a fluid handling integrated fluorescence plate reader (FlexStation, Molecular Devices, Sunnyvale, CA). Fluo-4 fluorescence was read at excitation of 494 nm and emission of 520 nm from the bottom of the plate, and changes in fluorescence were monitored at ~0.7 second intervals. Calcium 5 fluorescence was read at an excitation of 485 nm and emission of 525 nm. Probenecid (2.5 mM) was included in all of solutions once the cells were loaded with fluo-4 to prevent its leakage from the cells. Probenecid was not used with Calcium 5. Functional responses were quantified by first calculating the net fluorescence changes (the difference between control sham-treated and control agonist-treated groups). Net peak (maximum) fluorescence values during the third treatment period for both the control-agonist treatment group and the antagonist (with agonist) treatment group were determined. Results were expressed as a percentage of control-agonist groups.

References

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