

Assembly of a π - π stack of ligands in the binding site of an acetylcholine binding protein

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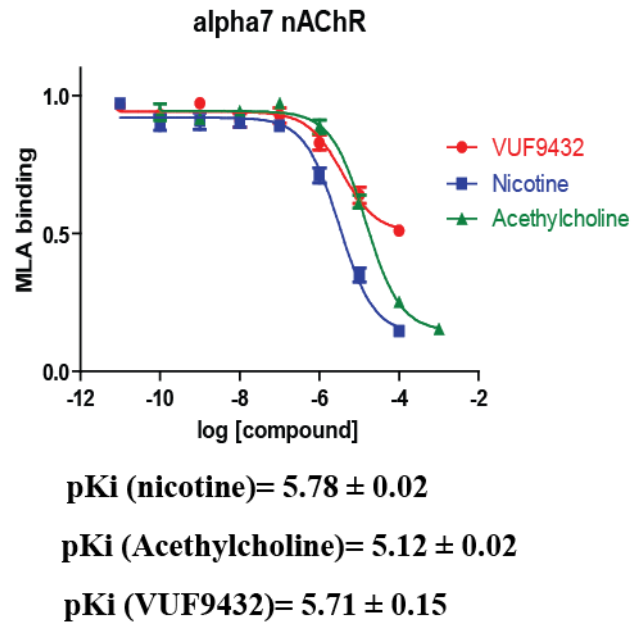
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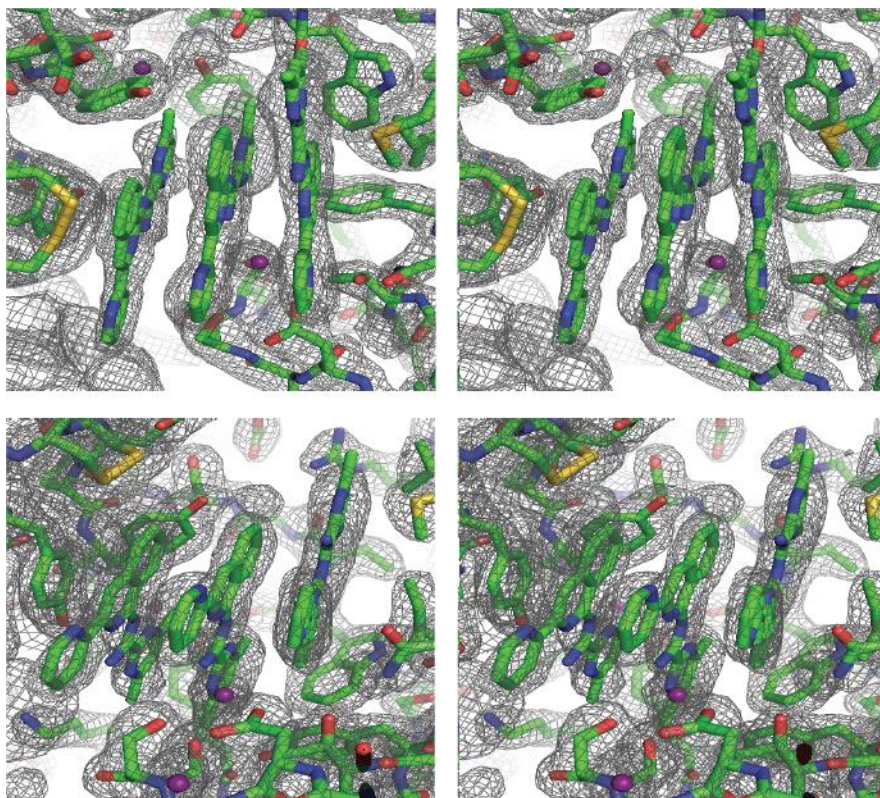
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SUPPLEMENTARY FIGURES



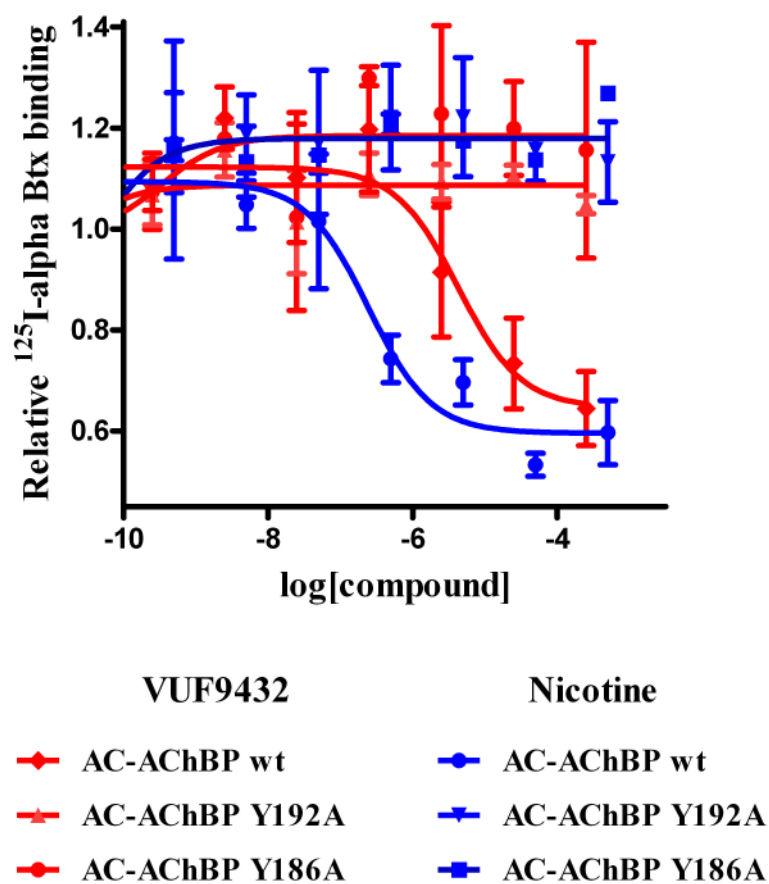
Supplementary Figure S1: Binding of VUF9432 to alpha 7 nAChR. Displacement of [³H] methyllycaconitine with VUF9432 (red curves), nicotine (blue curves) and acetylcholine (green curves) on alpha 7 nAChR expressing membranes. Data are the mean +/- s.e.m. of 3 experiments and are reported below the panel.



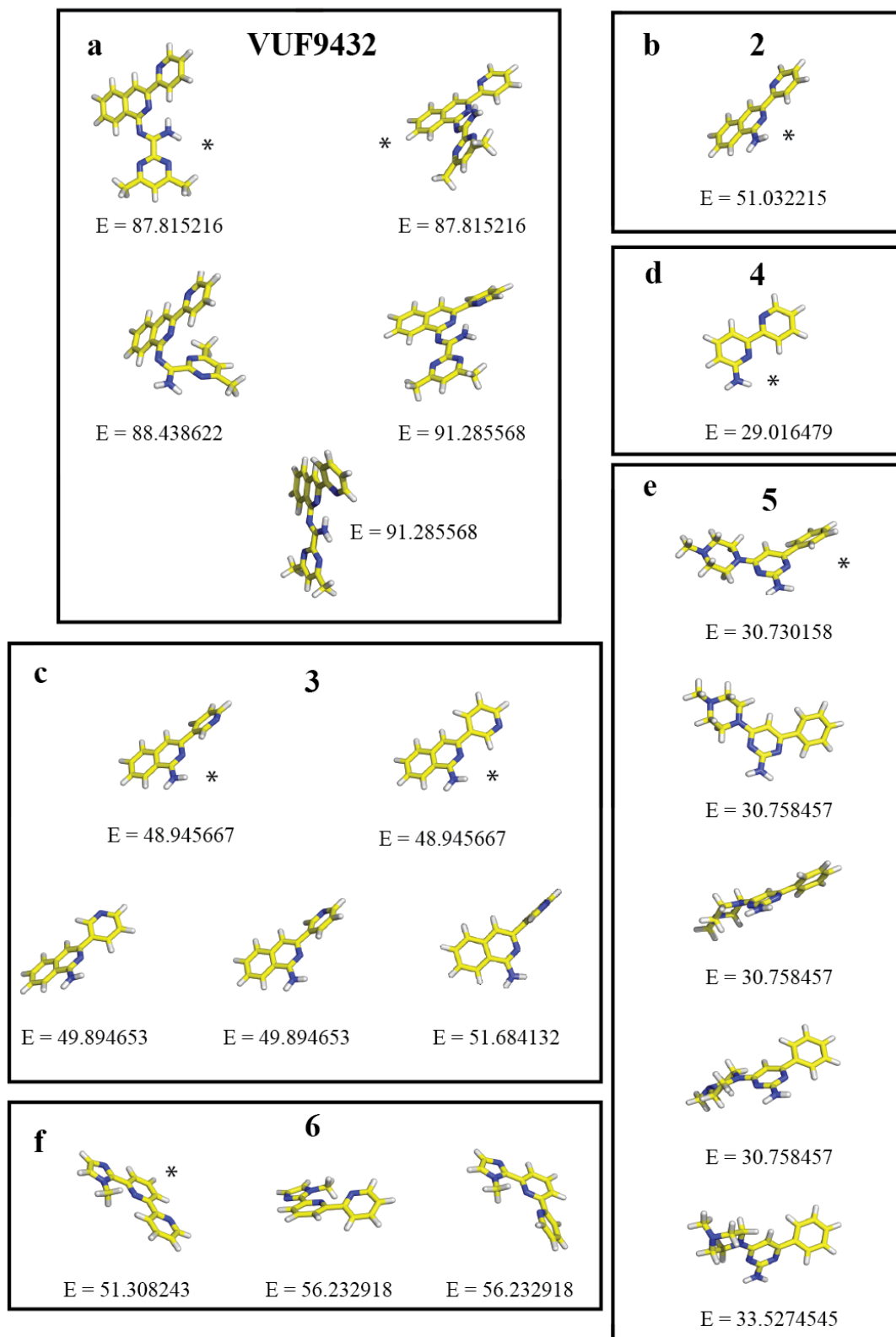
Supplementary Figure S2: Electron density map and final model structure. Shown are two views of the refined *2Fo-Fc* electron density map around the VUF9432-binding site of AChBP. The map is contoured at 1.0σ . The model structure is shown as sticks. Carbon atoms are in green, nitrogens in blue, oxygens in red and sulphurs in yellow. Water molecules are shown as spheres in magenta.

	Surface (Å ²)	B.S.A. (Å ²)	C-loop (Å)
Nicotine	393	419	8.6
Imidacloprid	574	571	8.8
VUF9432 (double stack)	652	655	15.8
Tubocurarine	1578	1560	13.8
VUF9432 (triple stack)	960	959.9 ± 4.8	17.0 ± 0.3
ImI	1163	1164	17.4
PnIA	1471	1468	17.2

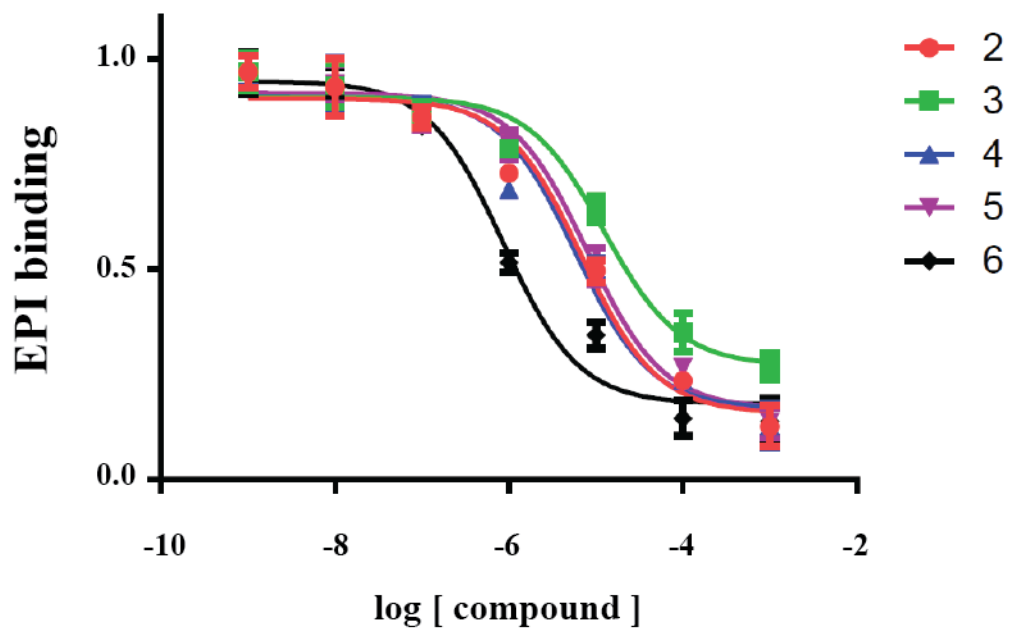
Supplementary Figure S3: Comparison of ligand binding site properties for a series of ligands. Ligand surface area, ligand buried surface area and C-loop opening for AChBP-ligand complex are compared to show that the self assembly of VUF9432 stacks resemble known ligands in their binding site opening.



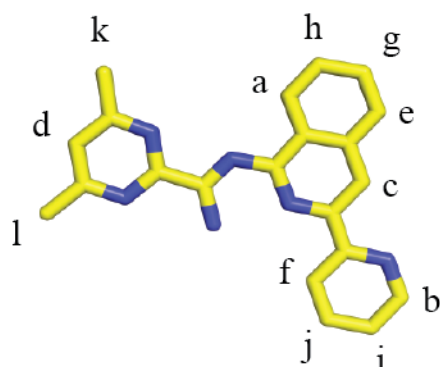
Supplementary Figure S4: VUF9432 binding to AChBP depends on Y186 and Y193. Displacement of [α - 125 I] bungarotoxin with VUF9432 (red curves), nicotine (blue curves) on purified Ac-AChBP wt, Y186A and Y192A showing inability of VUF9432 to displace the bound toxin from the mutants.



Supplementary Figure S5: Conformations of VUF9432 and the original fragment hits. Conformations and rotational freedom of VUF9432 and compounds **2-6**. Stochastic conformation sampling in gas was performed for the indicated ligands. Energy scores are indicated in KJ/mol. Most stable conformation is indicated with * . Only the closest iso-energetic conformations are shown.



Supplementary Figure S6: Displacement of [³H] epibatidine with derivatives 2-6 on Ac-AChBP (purified protein). Data are the mean +/- s.e.m. of 3 experiments. pKi are reported in figure 4.



VUF9432 : ^1H NMR (500 MHz, DMSO): **a** δ 8.82 (s, 1H), **b** 8.74 (s, 1H), **c** 8.61 (m, 1H), **d** 8.48 (s, 1H), **e** 8.25 (s, 1H), **f** 8.04 (d, 1H), **g** 7.98 (s, 1H), **h** 7.77 (t, 1H); **i** 7.66 (t, 1H), **j** 7.45 (d, 1H), **k** (or **l**) 2.57 (s, 3H), **l** (or **k**) 2.50 (s, 3H);

^{13}C NMR (125 MHz, DMSO): **a** δ 126.96 , **b** 149.97 , **c** 120.59 , **d** 123.99 ,
e 123.96 , **f** 137.97 , **g** 131.12 , **h** 126.96, **j** 123.99 , **k** 24.20, **l** 24.20;

UV/Vis: λ_{max} 300 nm;

HRMS (m/z): $[\text{M}]^+$ calc. for $\text{C}_{21}\text{H}_{19}\text{N}_6$, 355.200; found, 355.1656;

Supplementary Figure S7: NMR and spectral data on VUF9432. ^1H NMR, ^{13}C NMR, UV-VIS and HRMS data of VUF9432.

SUPPLEMENTARY METHODS

Chemicals - VUF9432, VUF5954, VUF11370 (ref 61), VUF6141, VUF14476 (ref 62) and VUF10460 (ref 63) are part of the proprietary VU compound library. The stability and the structure of VUF9432 was validated using NMR (refer to Supplementary Fig. S7) and HRMS.

SUPPLEMENTARY REFERENCES

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