Supplemental Data for Molecular Pharmacology article:

Title: Identification of the Functional Binding Site for the Convulsant Tetramethylenedisulfotetramine in the Pore of the α₂β₃γ₂ GABA-A Receptor

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This file contains:
1 Supplemental Figure showing the dominant binding poses of EBOB and picrotoxinin identified by RosettaLigand in the β₃ECD-α₅TMD chimera-based homology model of the α₂β₃γ₂ receptor.

1 Supplemental Figure showing the dominant binding pose of TETS identified by RosettaLigand in the 6HUO-based homology model of the α₂β₃γ₂ receptor in the desensitized state.

1 Supplemental Figure comparing TETS at the T6' ring of the α₂β₃γ₂ receptor in the open state model, the desensitized state model and the closed/resting state model.

1 Supplemental Figure showing the 2D ligand-protein diagram of TETS in the closed/resting state model of the α₂β₃γ₂ GABA_A receptor.

1 Supplemental Figure comparing the picrotoxinin pose observed in the cryo-EM structure of the picrotoxinin-α₁β₅γ₂L complex (6HUG) with the dominant binding pose of picrotoxinin identified by RosettaLigand in the closed/resting state model of the α₂β₃γ₂ GABA_A receptor.
PDB file 1

Homology model of the closed/resting state of the α2β3γ2 receptor based on the α1β3γ2 cryo-EM structure with picrotoxinin bound (6HUG).

PDB file 2

Dominant binding pose of TETS identified by RosettaLigand in the homology model of the closed/resting state of the α2β3γ2 receptor. The extracellular domains are truncated.
Supplemental Figure 1:

A, Dominant binding pose of EBOB identified by RosettaLigand in the pore of β3ECD-α5TMD chimera-based homology model of the α2β3γ2 receptor. B, The two most frequently identified binding poses of picrotoxinin in the same model. Hydrogen bonds are shown in green. Van der Walls interactions are shown in black. The GABA_A receptor is color coded as follows: α2 (blue), β3 (red), γ2 (yellow).
Supplemental Figure 2:

Dominant binding pose of TETS identified by RosettaLigand in the pore of the 6HUO-based homology model of the α₂β₃γ₂ receptor in the desensitized state. Hydrogen bonds are shown in green. The GABAₐ receptor is color coded as follows: α₂ (blue), β₃ (red), γ₂ (yellow).
A open

B desensitized

C closed/resting
Supplemental Figure 3:
A, TETS at the T6' ring of the open state model of the α2β3γ2 GABA_A receptor based on the β3ECD-α5TMD chimera (5O8F). B, TETS at the T6' ring of the desensitized state model of the α2β3γ2 GABA_A receptor based on the α1β3γ2L receptor structure (6HUO). C, TETS at the T6' ring of the closed/resting state model of the α2β3γ2 GABA_A receptor based on the α1β3γ2L receptor structure (6HUG).
Supplemental Figure 4:

2D ligand-protein diagram of TETS in the closed/resting state model of the $\alpha_2\beta_3\gamma_2$ GABA$_A$ receptor. Hydrophobic amino acids are shown in light green, polar amino acids in light blue and hydrogen bonds as purple arrows. A and D chain = $\alpha_2$, B and E = $\beta_3$, C = $\gamma_2$. The diagram was generated in Glide.
Supplemental Figure 5:

Comparison of the picrotoxinin pose observed in the cryo-EM structure of the picrotoxinin-$\alpha_1\beta_3\gamma_2$ complex (6HUG) with the dominant binding pose of picrotoxinin identified by RosettaLigand in the closed/resting state model of the $\alpha_2\beta_3\gamma_2$ GABA$_A$ receptor. Hydrogen bonds are shown in green. The GABA$_A$ receptor is color coded as follows: $\alpha_2$ (blue), $\beta_3$ (red), $\gamma_2$ (yellow).