

# Mutant Cycle Analysis with Modified Saxitoxins Reveals Specific Interactions Critical to Attaining High Affinity Inhibition of hNa<sub>v</sub>1.7

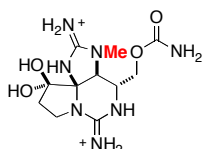
## Supplemental Appendix

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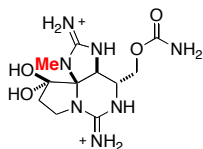
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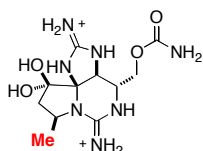
### Characterization data for synthetic toxins:



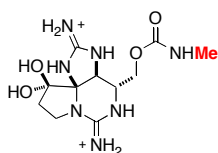
**Compound 2.** <sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz) δ 4.63 (s, 1H), 4.36–4.32 (s, 1H), 4.08 – 3.97 (m, 2H), 3.82 (t, *J* = 10.0 Hz, 1H), 3.60 (q, *J* = 9.4 Hz, 1H), 3.06 (s, 3H), 2.44 (dd, *J* = 14.0, 8.0 Hz, 1H), 2.39–2.29 (m, 1H) ppm. HRMS (ES<sup>+</sup>) calcd for C<sub>11</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub> 314.1571 found 314.1571 (MH<sup>+</sup>).



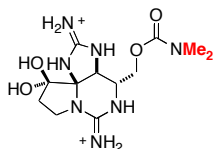
**Compound 3.** <sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz) δ 4.74 (s, 1H), 4.26 (dd, *J* = 11.7, 8.6 Hz, 1H), 4.15–4.01 (m, 2H), 3.79–3.77 (m, 1H), 3.69 (q, *J* = 9.5 Hz, 1H), 2.96 (s, 3H), 2.59–2.35 (m, 2H) ppm. HRMS (ES<sup>+</sup>) calcd for C<sub>11</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub> 314.1571 found 314.1571 (MH<sup>+</sup>).



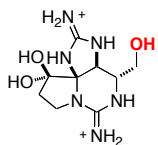
**Compound 4.** <sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz) δ 4.40 (d, 1H, *J* = 6.9 Hz), 4.30–4.28 (m, 2H), 4.16–4.09 (m, 1H), 3.79–3.75 (m, 1H), 2.66 (dd, 1H, *J* = 14.2, 7.9 Hz), 2.10 (dd, 1H, *J* = 14.7, 8.9 Hz), 1.47 (d, 3H, *J* = 6.5 Hz) ppm. HRMS (ES<sup>+</sup>) calcd for C<sub>11</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub> 315.1644 found 315.1614 (MH<sup>+</sup>).



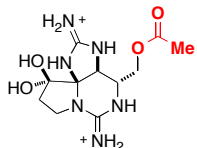
**Compound 5.** <sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz) δ 4.75 (s, 1H), 4.38–4.27 (m, 1H), 4.03 (dd, *J* = 11.8, 5.2 Hz, 1H), 3.89–3.77 (m, 1H), 3.59 (td, *J* = 10.0, 8.3 Hz, 1H), 2.72 (s, 1H), 2.50–2.39 (m, 1H), 2.39–2.30 (m, 1H) ppm. HRMS (ES<sup>+</sup>) calcd for C<sub>11</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub> 314.1571 found 314.1576 (MH<sup>+</sup>).



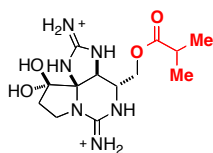
**Compound 6.**  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz)  $\delta$  4.32 (ddd,  $J = 11.5, 9.2, 1.7$  Hz, 1H), 4.08 (ddd,  $J = 11.6, 5.2, 1.7$  Hz, 1H), 3.87 (ddt,  $J = 9.0, 5.3, 1.5$  Hz, 1H), 3.85–3.79 (m, 1H), 3.63–3.55 (m, 1H), 2.94 (s, 2H), 2.90 (s, 3H), 2.44 (ddd,  $J = 14.0, 8.1, 1.8$  Hz, 1H), 2.36 (ddd,  $J = 13.8, 10.8, 9.1$  Hz, 1H) ppm. HRMS ( $\text{ES}^+$ ) calcd for  $\text{C}_{12}\text{H}_{23}\text{N}_7\text{O}_4$  328.1728 found 328.1725 ( $\text{MH}^+$ ).



**Compound 7.**  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz)  $\delta$  4.74 (s, 1H), 3.81 (t,  $J = 10.0$  Hz, 1H), 3.74–3.61 (m, 3H), 3.61–3.53 (m, 1H), 2.42 (dd,  $J = 14.1, 8.0$  Hz, 1H), 2.35 (dt,  $J = 13.8, 9.9$  Hz, 1H) ppm. HRMS ( $\text{ES}^+$ ) calcd for  $\text{C}_9\text{H}_{18}\text{N}_6\text{O}_3$  257.1357 found 257.1362 ( $\text{MH}^+$ ).



**Compound 8.**  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz)  $\delta$  4.76 (s, 1H), 4.37 (dd,  $J = 11.7, 9.1$  Hz, 1H), 4.10 (dd,  $J = 11.7, 5.4$  Hz, 1H), 3.89–3.79 (m, 2H), 3.63–3.56 (m, 1H), 2.47–2.31 (m, 3H), 2.14 (s, 2H) ppm. HRMS ( $\text{ES}^+$ ) calcd for  $\text{C}_{11}\text{H}_{20}\text{N}_6\text{O}_4$  299.1462 found 299.1467 ( $\text{MH}^+$ ).



**Compound 9.**  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz)  $\delta$  4.35 (dd,  $J = 11.6, 8.8$  Hz, 1H), 4.11 (dd,  $J = 11.6, 5.7$  Hz, 1H), 3.89 (ddd,  $J = 8.8, 5.7, 1.3$  Hz, 1H), 3.83 (td,  $J = 10.1, 1.9$  Hz, 1H), 3.59 (td,  $J = 10.0, 8.1$  Hz, 1H), 2.68 (h,  $J = 7.0$  Hz, 1H), 2.44 (ddd,  $J = 14.1, 8.2, 2.0$  Hz, 1H), 2.36 (dt,  $J = 14.2, 10.0$  Hz, 1H), 1.17 (dd,  $J = 7.0, 1.2$  Hz, 5H) ppm. HRMS ( $\text{ES}^+$ ) calcd for  $\text{C}_{13}\text{H}_{24}\text{N}_6\text{O}_4$  327.1775 found 327.1776 ( $\text{MH}^+$ ).

