Supporting information

Design of novel neurokinin 1 receptor antagonists based on conformationally constrained aromatic amino acids and discovery of a potent chimeric opioid agonist- neurokinin 1 receptor antagonist.

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Non-key compound characterization

Phth-(*1R*)-phenyl-(*4S*)-Aba-Gly-OH 13: Saponification yielded the desired compound (white solid, 98% from ethyl ester.[31] HPLC (standard gradient): t_{Ret} 15.5 min; TLC R_f (EtOAc/MeOH 4:1): 0.73; MS (ESP+) found m/z 441.3 $[M + H]^+$, $C_{26}H_{20}N_2O_5$ requires 440.14, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.86 (m, 2H), 7.72 (m, 2H), 7.56-7.13 (m, 9H), 5.68 (s, 1H), 5.07 (d, 1H, J = 17.4 Hz), 4.87 (dd, 1H, J_1= 13.0 Hz, J_2= 3.0 Hz), 3.77 (t, 1H, J= 13.2 Hz), 3.66 (d, 1H, J= 17.1 Hz), 2.51 (dd, 1H, J_1= 14.0 Hz, J_2= 3.0 Hz); ^{13}C NMR ($CDCl_3$) δ 172.7, 170.6, 138.8, 138.5, 136.8, 134.2, 131.9, 130.5, 129.9, 129.8, 128.7, 127.8, 127.6, 126.4, 123.6, 70.4, 53.6, 53.0, 34.1.

Phth-(1*R*)-phenyl-(4*R*)-Aba-Gly-OH 14: Saponification yielded the desired compound (white solid, 98% from ethyl ester.[31] HPLC (standard gradient): t_{Ret} 15.6 min; TLC R_f (EtOAc/MeOH 4:1): 0.73; MS (ESP+) found m/z 441.3 $[M + H]^+$, $C_{26}H_{20}N_2O_5$ requires 440.14, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.75 (m, 2H), 7.67 (m, 2H), 7.48-7.09 (m, 9H), 5.67 (s, 1H), 5.27 (dd, 1H, J_1= 10.3 Hz, J_2= 4.4 Hz), 4.85 (d, 1H, J= 16.7 Hz), 4.23 (d, 1H, J= 17.0 Hz), 3.91 (dd, 1H, J_1= 17.1, J_2= 10.6 Hz), 3.16 (dd, 1H, J_1= 17.0 Hz, J_2= 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 171.5, 170.8, 170.7, 140.2, 135.3, 135.1, 134.2, 134.1, 131.3, 130.8, 129.2, 128.7, 128.1, 126.8, 126.2, 123.5, 69.5, 53.2, 52.3, 33.3.

Ac-(1*R*)-1-phenyl-(4*S*)-Aba-Gly-NH-3',5'-$(CF_3)_2$-Bn 15: Preparative HPLC yielded the desired compound (white powder, 52%). HPLC (standard gradient): t_{Ret} 17.14 min; TLC R_f (EtOAc): 0.24; MS (ESP+) found m/z 578.2 $[M + H]^+$, $C_{29}H_{25}F_6N_3O_3$ requires 577.18, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.78 (s, 1H,), 7.68 (s, 2H), 7.42-7.04 (m, 12H), 6.83 (br s, 1H), 6.48 (d, 1H, J= 4.6 Hz), 5.81 (s, 1H), 5.31 (s, 2H), 4.95 (d, 1H, J= 15.2 Hz), 4.54-4.34 (m, 1H), 3.72 (d, 1H, J=14.2 Hz), 3.01 (t, 1H, J= 13.3 Hz), 2.86 (m, 1H), 2.05 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 173.6, 170.7, 168.8, 140.7, 138.8, 137.9, 137.2, 132.0, 130.7, 129.9, 129.6, 129.2, 128.9, 127.9, 127.4, 126.7, 126.1, 121.6, 69.9, 55.5, 55.4, 42.6, 35.0, 23.0.

Ac-(1*R*)-1-phenyl-(4*S*)-Aba-Gly-NMe-3',5'-$(CF_3)_2$-Bn 16: Preparative HPLC yielded the desired compound (white powder, 40%). HPLC (standard gradient): t_{Ret} 17.53 min; TLC R_f (EtOAc): 0.18; MS (ESP+) found m/z 592 $[M + H]^+$, $C_{30}H_{27}F_6N_3O_3$ requires 591.20, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.79 (s, 1H), 7.69 (s, 2H), 7.44-7.19

(m, 9H), 6.85 (d, 1H, *J*= 5.4 Hz), 5.86 (s, 1H), 5.19 (d, 1H, *J*= 16.4 Hz), 4.68 (s, 2H), 4.35 (m, 1H), 3.72 (d, 1H, *J*= 15.7 Hz), 3.16 (t, 1H, *J*= 13.3 Hz), 3.00 (s, 3H), 2.95 (m, 1H), 2.07 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 172.9, 171.1, 168.5, 139.5, 138.1, 137.3, 132.3, 131.7, 130.2, 130.0, 129.4, 128.7, 128.0, 127.6, 127.2, 126.7, 126.4, 121.6, 69.8, 55.3, 51.4, 51.0, 35.1, 34.9, 23.0

Ac-(1*R*)-1-phenyl-(4*R*)-Aba-Gly-NMe-3',5'-(CF_3)$_2$-Bn 17: Preparative HPLC yielded the desired compound **17** (white powder, 49%). HPLC (standard gradient): t_{Ret} 17.70 min; TLC R_f (EtOAc): 0.35; MS (ESP+) found m/z 592 $[M + H]^+$, $C_{30}H_{27}F_6N_3O_3$ requires 591.20, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.85 (s, 1H), 7.70 (s, 2H), 7.44-7.09 (m, 9H), 6.70 (d, 1H), 5.71 (s, 1H), 4.86 (m, 1H), 4.92 (d, 1H, *J*= 16.0 Hz), 4.76 (d, 1H, *J*= 16.0 Hz), 4.49 (d, 1H, *J*= 15.2 Hz), 4.38 (d, 1H, *J*= 15.2 Hz), 3.41 (dd, 1H, J_1= 17.5 Hz, J_2= 4.9 Hz), 2.92 (m, 1H), 3.03 (s, 3H), 1.93 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 172.5, 169.3, 168.4, 141.2, 139.4, 135.5, 134.1, 132.3, 131.9, 130.7, 129.1, 128.4, 128.0, 126.4, 121.4, 69.0, 52.5, 50.9, 49.7, 36.1, 35.0, 23.0

Boc-Tic-NH-3',5'-(CF_3)$_2$-Bn: After flash chromatography (EtOAc/Hexane 1:1) the desired compound was obtained (yellow solid, 90%). HPLC (standard gradient): t_{Ret} 18.96 min; TLC R_f (EtOAc/hexane 1:1): 0.82; MS (ESP+) found m/z 503 $[M + H]^+$, $C_{24}H_{24}F_6N_2O_3$ requires 502.17, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.72 (s, 1H), 7.48 (s, 2H), 7.24-7.11 (m, 4H), 4.77 (m, 1H), 4.60-4.18 (m, 4H), 3.31 (m, 1H), 3.09 (m, 1H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 172.3, 155.2, 140.6, 133.6, 132.1, 131.6, 129.3, 127.9, 127.6, 127.0, 126.9, 126.0, 121.3, 81.6, 45.2, 45.0, 42.7, 38.8, 28.4

Ac-Tic-NH-3',5'-(CF_3)$_2$-Bn 22: Compound **22** was purified by preparative HPLC (white powder, 90%). HPLC (standard gradient): t_{Ret} 16.29 min; TLC R_f (EtOAc/hexane 1:1): 0.12; MS (ESP+) found m/z 445 $[M + H]^+$, $C_{21}H_{18}F_6N_2O_2$ requires 444.13, ^{1}H NMR 250 MHz ($CDCl_3$): δ 7.72 (br s, 1H, NH), 7.39 (s, 1H), 7.54 (s, 2H), 7.30-7.08 (m, 4H), 5.12 (t, 1H, *J*= 6.0 Hz), 4.60-4.46 (m, 3H), 4.33 (d, 1H, *J* = 6.0 Hz), 3.37 (dd, 1H, J_1= 15.4 Hz, J_2= 5.6 Hz), 3.04 (m, 1H, J_1= 16.0 Hz, J_2= 6.5 Hz), 2.28 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 172.9, 171.2, 140.8, 133.4, 132.1, 131.5, 128.3, 128.2, 127.9, 127.4, 127.1, 125.7, 121.3, 53.9, 47.3, 42.5, 30.4, 21.9

Boc-(4*S*/*R*)-Aia-Gly-OMe 26a/b: After flash chromatography (EtOAc/*c*-hexane 1:4) the desired compound was obtained with a yield of 33% (yellow foam). HPLC (standard gradient): t_{Ret} 13.93 min; TLC R_f (CH_2Cl_2:EtOAc 1:1): 0.4; MS (ESP+) found m/z 388 $[M+H]^+$, 332 $[M+2H-t\text{-}Bu]^+$, 288 $[M+2H-Boc]^+$, $C_{20}H_{25}N_3O_5$ requires 387.2, ^{1}H NMR 250 MHz ($CDCl_3$): δ 8.05 (s, 1H) 7.26-7.37 (M, 4H), 5.91 (d, 1H, *J*= 7.3 Hz), 5.12-5.18 (m, 1H), 5.26 (d, 1H, *J*= 17.1 Hz), 4.57 (d, 1H, *J*= 17.5 Hz), 4.04 (d, 1H, *J*= 2.5 Hz), 3.97 (d, 1H, *J*= 3.3 Hz), 3.68 (s, 3H), 3.32 (dd, 1H, J_1= 13.6 Hz, J_2= 2.1 Hz), 2.82 (t, 1H, *J*= 14.1 Hz), 1.48 (s, 9H). ^{13}C NMR 63 MHz ($CDCl_3$) δ 173.1, 169.6, 155.0, 139.4, 134.7, 132.2, 131.8, 128.5, 127.8, 127.6, 122.9, 121.6, 120.8, 120.0, 118.4, 110.6, 109.8, 79.9, 52.4, 51.1, 50.1, 46.4, 29.7, 28.9

Boc-(4S/R)-Aia-Gly-NMe-3',5'-$(CF_3)_2$Bn 27a/b: After flash chromatography (EtOAc/*c*-hexane 1:4) the desired compound was obtained with a yield of 83% (yellow foam). HPLC (standard gradient): t_{Ret} 19.33 min; TLC R_f (EtOAc): 0.47; MS (ESP+) found m/z 613 $[M+H]^+$, $C_{29}H_{30}F_6N_4O_4$ requires 612.2, ^{1}H NMR 250 MHz ($CDCl_3$) δ 8.22 (s, 1H), 7.03-7.79 (M, 7H), 5.92 (d, 1H, *J*= 6.9 Hz), 5.26 (d, 1H, *J*= 17.1 Hz), 5.10-5.20 (m, 1H), 4.72 (d, 1H, *J*= 17.5 Hz), 4.70 (d, 1H *J*= 15.6 Hz), 4.46 (d, 1H *J*= 15.6 Hz), 4.18 (d, 1H, *J*= 17.3 Hz), 4.10 (d, 1H, *J*= 16.3 Hz), 3.34 (dd, 1H, J_1= 15.8 Hz, J_2= 2.3 Hz) 2.92 (s, 3H), 2.82 (t, 1H, *J*= 14.1 Hz), 1.48 (s, 9H). ^{13}C NMR 63 MHz ($CDCl_3$) δ 28.4, 29.1, 34.9, 46.2, 49.9, 50.8, 51.1, 79.8, 139.3, 134.7, 132.3, 131.8, 128.5, 127.9, 127.6, 122.8, 121.7, 120.8, 120.1, 118.4, 110.7, 109.8, 155.2, 168.7, 173.1

Boc-(4S/R)-Tcc-NMe-3',5'-$(CF_3)_2$Bn 31a/b: After flash chromatography (EtOAc/*c*-hexane 1:4) the desired compound was obtained with a yield of 78% (yellow foam). HPLC (standard gradient): t_{Ret} 20.33 min; TLC R_f (EtOAc): 0.62; MS (ESP+) found m/z 556 $[M+H]^+$, 456 $[M-Boc]^+$, $C_{27}H_{27}F_6N_3O_3$ requires 555.2, ^{1}H NMR 250 MHz ($CDCl_3$) δ 7.96 (s, 1H), 7.77 (s, 1H), 7.62 (s, 2H), 7.25-7.47 (M, 5H), 4.88-4.92 (m, 3H), 4.86 (s, 2H), 4.32 (d, 1H, *J*= 15.3 Hz), 3.25-3.30 (m, 1H), 3.17 (s, 3H), 3.13-3.17 (m, 1H), 2.02 (s, 9H). ^{13}C NMR X MHz ($CDCl_3$) δ 173.5, 155.3, 136.5, 128.2, 127.5, 126.8, 126.4, 124.1, 122.4, 121.9, 121.6, 119.8, 117.8, 111.0, 104.4, 50.8, 49.6, 41.6, 35.3, 28.3, 22.9

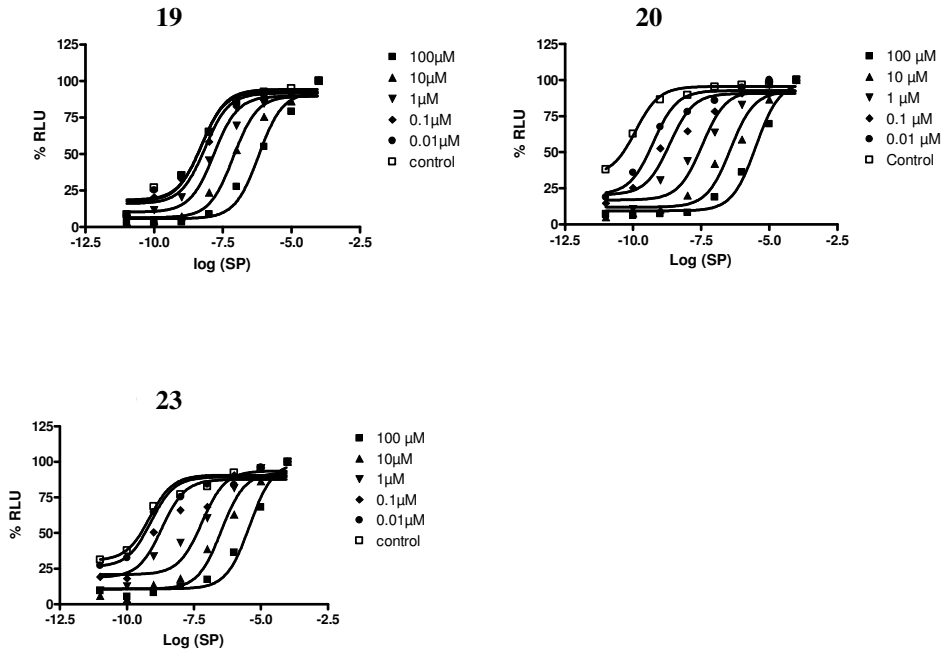
Boc-(4S)-Aia-3',5'-$(CF_3)_2$Bn 29: After flash chromatography (EtOAc/*c*-hexane 1:3) the desired compound was obtained with a yield of 42% (yellow foam). HPLC (standard gradient): t_{Ret} 19.38 min; TLC R_f (EtOAc:*c*-hexane 1:3): 0.17; MS (ESP+) found m/z 542 $[M\text{-}t\text{Bu}+H]^+$, $C_{27}H_{27}F_6N_3O_3$ requires 555.2, ^{1}H NMR 250 MHz ($CDCl_3$) δ 7.85 (s, 1H), 7.07-7.80 (M, 7H), 5.91 (d, 1H, *J*= 7.3 Hz), 5.12 (d, 1H, *J*= 16.9 Hz), 5.12-5.22 (m, 1H), 4.87 (d, 1H, *J*= 15.8 Hz), 4.78 (d, 1H, *J*= 15.8 Hz), 3.90 (d, 1H, *J*= 17.2 Hz), 3.35 (dd, 1H, J_1= 14.2 Hz, J_2= 4.5 Hz), 2.90 (t, 1H, *J*= 14.2 Hz), 1.45 (s, 9H). ^{13}C NMR 63 MHz ($CDCl_3$) δ 173.1, 155.4, 139.3, 134.7, 132.3, 131.8, 128.5, 127.9, 127.6, 122.8, 121.7, 120.9, 120.1, 118.3, 110.7, 109.8, 80.1, 51.4, 51.0, 45.2, 29.0, 28.4.

Ac-(4S/R)-Aia-Gly-NMe-3',5'-$(CF_3)_2$Bn 28a/b: Preparative HPLC yielded the desired compound with a yield of 52% (white powder). HPLC (standard gradient): t_{Ret} 17.43 min; TLC R_f (EtOAc): 0.15; MS (ESP+) found m/z 388 $[M+H]^+$, 332 $[M+2H\text{-}t\text{-Bu}]^+$, 288 $[M+2H\text{-Boc}]^+$, $C_{26}H_{24}F_6N_4O_3$ requires 554.2, ^{1}H NMR 250 MHz ($CDCl_3$) δ 8.28 (s, 1H), 7.81 (s, 1H), 7.67 (s, 2H), 7.02-7.34 (M, 5H), 5.38 (m, 1H), 4.79 (d, 1H, *J*= 15.9 Hz), 4.79 (d, 1H, *J*= 15.9 Hz), 4.78 (d, 1H, *J*= 15.3 Hz), 4.47 (d, 1H, *J*= 15.3 Hz), 4.18 (d, 1H, *J*= 17.2 Hz), 4.06 (d, 1H, *J*= 15.9 Hz), 3.32 (dd, 1H, J_1= 15.70 Hz, J_2= 4.20 Hz), 2.98 (s, 3H), 2.80 (t, 1H, *J*= 12.60 Hz), 2.12 (s, 9H). ^{13}C NMR 125 MHz ($CDCl_3$) δ 170.2, 168.6, 139.3, 134.6, 132.1, 131.9, 128.5, 127.8, 127.6, 122.9, 121.7, 120.8, 120.1, 118.3, 110.7, 109.8, 51.4, 50.7, 50.5, 46.9, 35.3, 28.6, 23.6.

Ac-(4*S/R*)-Tcc-NMe-3',5'-$(CF_3)_2$Bn 32a/b: Preparative HPLC yielded the desired compound (white powder, 66%). HPLC (standard gradient): t_{Ret} 15.97 min; TLC R_f (EtOAc): 0.27; MS (ESP+) found m/z 498 $[M+H]^+$, 241 $[M\text{-}NMeCH_2C_6H_4(CF_3)_2]^+$, $C_{24}H_{21}F_6N_3O_2$ requires 497.2, ^{1}H NMR 250 MHz ($CDCl_3$) δ 7.99 (s, 1H), 7.77 (s, 1H), 7.56 (s, 2H), 7.12-7.49 (M, 5H), 5.11 (d, 1H, *J*= 14.6 Hz), 4.81 (d, 1H, *J*= 14.9 Hz), 4.75 (d, 2H, *J*= 15.6 Hz), 4.47 (d, 1H, *J*= 15.6 Hz), 3.30 (dd, 1H, J_1= 15.90 Hz, J_2= 7.30 Hz), 3.23 (s, 3H), 3.20 (t, 1H, *J*= 15.9 Hz), 2.37 (s, 3H). ^{13}C NMR 125 MHz ($CDCl_3$) δ 173.7, 172.6, 139.1, 136.6, 128.2, 127.5, 126.8, 126.3, 124.1, 122.4, 121.9, 121.6, 119.9, 117.8, 111.0, 104.5, 51.0, 48.4, 43.4, 35.7, 22.7, 21.8.

Ac-(4S)-Aia-3',5'-$(CF_3)_2$Bn 30: Preparative HPLC gave the desired compound with a yield of 65% (white powder).. HPLC (standard gradient): t_{Ret} 15.99 min; TLC R_f (EtOAc): 0.18; MS (ESP+) found m/z 484 $[M+H]^+$, $C_{23}H_{19}F_6N_3O_2$ requires 483.2**,** ^{1}H NMR 250 MHz ($CDCl_3$) δ 8.02 (s, 1H), 7.72 (s, 1H), 7.56 (s, 2H), 7.33 (s, 1H, *J*= 6.9 Hz), 7.02-7.27 (M, 4H), 5.34-5.36 (m, 1H), 4.99 (d, 1H, *J*= 17.0 Hz), 4.88 (d, 1H, *J*= 15.7 Hz), 4.72 (d, 1H, *J*= 15.7 Hz), 3.95 (d, 1H, *J*= 17.1 Hz), 3.30 (dd, 1H, J_1= 15.6 Hz, J_2= 4.3 Hz), 2.84 (t, 1H, *J*= 14.2 Hz), 2.21 (s, 3H). ^{13}C NMR 63 MHz ($CDCl_3$) δ 172.6, 171.6, 138.8, 134.7, 132.6, 132.3, 132.0, 131.8, 128.1, 127.7, 126.2, 124.0, 122.9, 121.9, 120.2, 119.7, 118.2, 116.0, 113.7, 110.8, 109.0, 51.5, 50.3, 45.1, 28.1, 22.8.

Figure S1: SP-induced aequorin luminescence in the presence of antagonists **19**, **20** and **23**.



Agonist (substance P) induced bioluminescence data were obtained by means of an aequorin-based functional assay on NK1R-expressing CHO cells, in the absence (control) or presence of antagonist (compounds **23**, **19** and **20** were tested at concentrations ranging from 10^{-8} to 10^{-4} M, *cf.* the legend to the right of each graph). Substance P (SP) concentrations are indicated in a logarithmic scale on the X-axis. Data are expressed as a percentage of the maximal luminescence (% RLU, relative light units) which was obtained with 10^{-4} M SP in the absence of any antagonist. Increasing antagonist concentrations clearly result in a rightward shift of the dose-response curve for SP, indicating that all three compounds act as competitive antagonists of the NK1 receptor.