**Table S1.**
**5-$HT_{2C}$ Crystallographic Data Collection and Refinement Statistics, Related to Figures 1 and S1.**

| | 5-$HT_{2C}$-ERG | 5-$HT_{2C}$-RIT |
|---|---|---|
| **PDB code** | 6BQG | 6BQH |
| **Data collection** | | |
| Number of crystals | 10 | 11 |
| Space group | P 2 $2_1$ 2 | C 2 2 2 |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 49.84 94.58 145.16 | 88.31 97.05 150.23 |
| Number of reflections measured | 156659 (5711) | 180147 (8921) |
| Number of unique reflections | 12052 (943) | 17076 (1578) |
| Resolution (Å)[a] | 47.3-3.0 (3.1-3.0) | 48.5-2.7 (2.8-2.7) |
| $R_{merge,}$ (%) | 7.8 (40.2) | 8.0 (59.1) |
| Mean $I/\sigma(I)$ | 21.6 (5.0) | 14.3 (2.2) |
| $CC_{1/2}$ | 1 (0.90) | 1 (0.56) |
| Completeness (%) | 83.4 (67.8) | 94.2 (90.2) |
| Redundancy | 13.0 (6.1) | 10.5 (5.7) |
| **Refinement** | | |
| Resolution (Å) | 47.3-3.0 (3.1-3.0) | 48.5-2.7 (2.8-2.7) |
| No. reflections | 12007 (931) | 17051 (1566) |
| $R_{work}/R_{free}$ (%) | 26.1/29.1 | 25.3/27.5 |
| Number of atoms | | |
| Protein | 2976 | 2657 |
| Ligand | 43 (ERG) | 34 (RIT) |
| Lipid and other | 11 | 124 |
| WILSON B ($Å^2$) | 91.6 | 84.7 |
| Average B factors ($Å^2$) | | |
| Overall | 94.7 | 113.7 |
| Receptor | 86.6 | 102.8 |
| BRIL | 118. | 176.7 |
| Ligand | 76.8 | 88.4 |
| Lipid and other | 85.7 | 111.7 |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.003 | 0.003 |
| Bond angles (°) | 0.52 | 0.60 |
| Ramachandran Plot Statistics (%) | | |
| Favored regions | 95.4 | 96.2 |
| Allowed regions | 4.6 | 3.8 |
| Disallowed regions | 0 | 0 |

[a] Values in parentheses are for highest-resolution shell.

Rmerge= Σhkl |I(hkl) - <I(hkl)>|/ Σhkl(hkl), where <I(hkl)> is the mean of the symmetry equivalent reflections of I(hkl).

**Table S2.**

**5-$HT_{2C}$ Mutant Binding Affinities, Related to Figure 2 and Table S7.** Data represent average $K_d/K_i$ and average $pK_d/pK_i$ ±S.E.M. from three independent experiments performed in duplicate and were acquired with 5-$HT_{2C}$ wild-type and mutants expressed in HEKT cells. Bmax was estimated by saturation binding or by homologous competition from three independent experiments. Affinity was assessed by radioligand competition binding performed using [$^3$H]-Mesulergine (0.7 -1.3 nM) to yield $K_d$ or $K_i$ affinity estimates. The $pK_{d/i}$ values were compared using a one-way ANOVA with Dunnett's post-test (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$).

| | **Bmax** | **Mesulergine** | | **Ergotamine** | | **Ritanserin** | | **5-HT** | |
|---|---|---|---|---|---|---|---|---|---|
| | **±SEM** (pmol/mg protein) | **$K_d$, nM** ($pK_d$ ±SEM) | **$\Delta pK_d$** (mutant - WT) | **$K_i$, nM** ($pK_i$ ±SEM) | **$\Delta pK_i$** (mutant - WT) | **$K_i$, nM** ($pK_i$ ±SEM) | **$\Delta pK_i$** (mutant - WT) | **$K_i$, nM** ($pK_i$ ±SEM) | **$\Delta pK_i$** (mutant - WT) |
| **5-$HT_{2C}$ WT** | 2.33±0.38 | 0.60 (9.23±0.06) | --- | 1.17 (8.96±0.12) | --- | 0.16 (9.81±0.11) | --- | 50.2 (7.30±0.05) | --- |
| **F320L$^{6.44}$** | 1.86±0.26 | 1.19 (8.93±0.06) | -0.30 | 1.46 (8.85±0.08) | -0.11 | 1.43 (8.80±0.07) ** | -0.90 | 28.8 (7.55±0.09) | +0.25 |
| **F223L$^{5.47}$** | 1.26±0.22 | 3.92 (8.43±0.10) *** | -0.80 | 6.14 (8.23±0.10) *** | -0.73 | 3.79 (8.44±0.08) *** | -1.37 | 4,920 (5.35±0.18) *** | -1.96 |
| **I142F$^{3.40}$** | 1.75±0.28 | 2.88 (8.58±0.13) ** | -0.65 | 2.99 (8.60±0.18) | -0.36 | 3.20 (8.50±0.05) *** | -1.31 | 490 (6.33±0.14) *** | -0.97 |
| **F327L$^{6.51}$** | 2.09±0.74 | 1.31 (8.91±0.11) | -0.32 | 0.43 (9.42±0.15) ** | +0.46 | 16.3 (7.85±0.16) *** | -1.95 | 242 (6.64±0.15) * | -0.66 |
| **W324L$^{6.48}$** | 1.05±0.25 | 4.39 (8.40±0.14) *** | -0.83 | 2.58 (8.61±0.09) | -0.35 | 2,920 (5.76±0.31) **** | -4.05 | 22.7 (7.64±0.01) | +0.34 |
| **W324F$^{6.48}$** | 0.94±0.20 | 5.03 (8.46±0.30) *** | -0.77 | 4.29 (8.40±0.11) | -0.56 | 302 (6.57±0.16) *** | -3.24 | 14.8 (7.88±0.22) | +0.58 |
| **W324Y$^{6.48}$** | 1.56±0.37 | 4.51 (8.38±0.12) *** | -0.85 | 2.74 (8.60±0.12) | -0.37 | 27.5 (7.68±0.22) *** | -2.12 | 7.68 (8.13±0.12) ** | +0.83 |
| **N351L$^{7.36}$** | 1.95±0.32 | 0.95 (9.04±0.10) | -0.19 | 1.25 (8.92±0.09) | -0.04 | 0.77 (9.15±0.13) | -0.66 | 135 (6.89±0.13) | -0.41 |
| **N351F$^{7.36}$** | 1.15±0.04 | 0.84 (9.07±0.01) | -0.16 | 1.36 (8.89±0.09) | -0.08 | 0.76 (9.15±0.32) | -0.66 | 55.1 (7.26±0.01) | -0.04 |
| **Y358F$^{7.43}$** | 0.87±0.04 | 0.41 (9.49±0.24) | +0.26 | 0.65 (9.23±0.13) | +0.27 | 0.12 (9.94±0.11) | +0.13 | 201 (6.71±0.12) | -0.59 |
| **Y118A$^{EL1}$** | 1.04±0.21 | 0.82 (9.14±0.16) | -0.09 | 1.02 (9.03±0.13) | +0.07 | 0.29 (9.54±0.01) | -0.26 | 116 (6.94±0.02) | -0.36 |
| **Y118L$^{EL1}$** | 2.01±0.42 | 1.02 (9.00±0.04) | -0.23 | 1.94 (8.76±0.15) | -0.20 | 0.50 (9.34±0.14) | -0.46 | 99.9 (7.04±0.19) | -0.26 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **V135L$^{3.33}$** | 2.51±0.29 | 1.98 (8.76±0.16) | -0.47 | 7.87 (8.14±0.13) ** | -0.82 | 1.45 (8.86±0.11) ** | -0.94 | 690 (6.17±0.07) *** | -1.13 |
| **V135F$^{3.33}$** | 1.85±0.12 | 12.7 (7.98±0.18) *** | -1.25 | 134 (6.89±0.08) *** | -2.07 | 5.52 (8.31±0.15) *** | -1.49 | 8350 (5.08±0.10) *** | -2.22 |
| **V208A$^{EL2}$** | 2.29±0.50 | 1.07 (9.15±0.33) | -0.08 | 2.12 (8.70±0.11) | -0.26 | 0.91 (9.12±0.19) | -0.69 | 158 (6.82±0.12) | -0.48 |
| **V208S$^{EL2}$** | 2.95±0.10 | 0.72 (9.23±0.21) | 0.00 | 1.68 (8.82±0.13) | -0.14 | 0.62 (9.26±0.14) | -0.55 | 99.1 (7.02±0.13) | -0.28 |
| **V208F$^{EL2}$** | 1.87±0.07 | 0.91 (9.05±0.07) | -0.18 | 11.3 (7.98±0.12) *** | -0.98 | 1.22 (8.92±0.03) * | -0.89 | 89.6 (7.07±0.13) | -0.24 |
| **G218A$^{5.42}$** | 2.29±0.21 | 0.56 (9.35±0.22) | +0.12 | 2.28 (8.81±0.27) | -0.15 | 4.04 (8.58±0.28) *** | -1.23 | 586 (6.38±0.38) ** | -0.92 |

**Table S3.**

**Affinity Estimates for ERG and RIT across Aminergic GPCRs, Related to Figures 4 and 6.** Data represent average $K_i$ and $pK_i$ from at least two independent experiments performed in duplicate. Data were acquired by radioligand competition binding using indicated radioligands to yield $K_i$ affinity estimates. 5-CT, 5-carboxamidotryptamine. LSD, lysergic acid diethylamide. QNB, Quinuclidinyl benzilate. N/A not available.

| Receptor | Radioligand | Ergotamine | | Ritanserin | |
|---|---|---|---|---|---|
| | | $K_i$, nM | $pK_i$ | $K_i$, nM | $pK_i$ |
| **5-HT$_{1A}$** | [$^3$H]WAY100635 | 0.45 | 9.35 | 309 | 6.51 |
| **5-HT$_{1B}$** | [$^3$H]5-CT | 0.17 | 9.77 | 194 | 6.71 |
| **5-HT$_{1D}$** | [$^3$H]5-CT | 0.15 | 9.84 | 36.8 | 7.43 |
| **5-HT$_{1e}$** | [$^3$H]5-HT | 508 | 6.29 | 1228 | 5.91 |
| **5-HT$_{2A}$** | [$^3$H]Ketanserin | 0.70 | 9.16 | 0.22 | 9.66 |
| **5-HT$_{2B}$** | [$^3$H]LSD | 0.29 | 9.54 | 0.14 | 9.87 |
| **5-HT$_{2C}$** | [$^3$H]Mesulergine | 1.65 | 8.78 | 0.24 | 9.61 |
| **5-HT$_4$** | [$^3$H]GR113808 | 354 | 6.45 | >10,000 | <5.00 |
| **5-HT$_{5a}$** | [$^3$H]LSD | 1.63 | 8.79 | 76.6 | 7.12 |
| **5-HT$_6$** | [$^3$H]LSD | 1.14 | 8.94 | 66.9 | 7.17 |
| **5-HT$_{7a}$** | [$^3$H]LSD | 2.46 | 8.61 | 29.6 | 7.53 |
| **D$_1$** | [$^3$H]SCH 23390 | >10,000 | <5.00 | 344 | 6.46 |
| **D$_2$** | [$^3$H]N-methyl Spiperone | 3.50 | 8.46 | 1717 | 5.77 |
| **D$_3$** | [$^3$H]N-methyl Spiperone | 3.22 | 8.49 | 57.7 | 7.24 |
| **D$_4$** | [$^3$H]N-methyl Spiperone | 1.17 | 8.93 | 51.5 | 7.29 |
| **D$_5$** | [$^3$H]SCH 23390 | 2,824 | <5.00 | 163 | 6.79 |
| **$\alpha_{2A}$** | [$^3$H]Rauwolscine | 0.28 | 9.56 | 95.8 | 7.02 |
| **$\alpha_{2B}$** | [$^3$H]Rauwolscine | 2.94 | 8.53 | 1902 | 5.72 |
| **$\alpha_{2C}$** | [$^3$H]Rauwolscine | 1.23 | 8.91 | 269 | 6.57 |
| **$\alpha_{1A}$** | [$^3$H]Prazosin | 10.2 | 7.99 | 80.7 | 7.09 |
| **$\alpha_{1B}$** | [$^3$H]Prazosin | 29.7 | 7.53 | 223 | 6.65 |
| **$\alpha_{1D}$** | [$^3$H]Prazosin | 27.9 | 7.56 | 86.5 | 7.06 |
| **$\beta_1$** | [$^{125}$I]Pindolol | >10,000 | <5.00 | >10,000 | <5.00 |
| **$\beta_2$** | [$^3$H]CGP12177 | 91.2 | 7.04 | 4,600 | <5.00 |
| **$\beta_3$** | [$^3$H]CGP12177 | >10,000 | <5.00 | >10,000 | <5.00 |
| **H$_1$** | [$^3$H]Pyrilamine | >10,000 | <5.00 | N/A | |
| **H$_2$** | [$^3$H]Tiotidine | 271 | 6.57 | 4.5 | 8.35 |
| **H$_3$** | [$^3$H]α-methylhistamine | >10,000 | <5.00 | >10,000 | <5.00 |
| **H$_4$** | [$^3$H]Histamine | >10,000 | <5.00 | >10,000 | <5.00 |

| | | | | | |
|---|---|---|---|---|---|
| **$M_1$** | [$^3$H]QNB | >10,000 | <5.00 | 893 | 6.05 |
| **$M_2$** | [$^3$H]QNB | >10,000 | <5.00 | 1,090 | 5.96 |
| **$M_3$** | [$^3$H]QNB | >10,000 | <5.00 | 1,571 | 5.80 |
| **$M_4$** | [$^3$H]QNB | 5,660 | 5.25 | 1,988 | 5.70 |
| **$M_5$** | [$^3$H]QNB | >10,000 | <5.00 | 1,990 | 5.70 |
| **MOR (μ)** | [$^3$H]DAMGO | >10,000 | <5.00 | >10,000 | <5.00 |
| **KOR (κ)** | [$^3$H]U69593 | >10,000 | <5.00 | 893 | 6.05 |
| **DOR (δ)** | [$^3$H]DADLE | 3,207 | 5.49 | >10,000 | <5.00 |
| **SERT** | [$^3$H]Citalopram | >10,000 | <5.00 | 1,282 | 5.89 |
| **DAT** | [$^3$H]WIN35428 | >10,000 | <5.00 | 569 | 6.24 |
| **NET** | [$^3$H]Nisoxetine | >10,000 | <5.00 | 1,279 | 5.89 |

**Table S4.**

**GPCRome Screening by Tango Examining ERG Agonist Activity (Fold of Basal) at 1, 3, and 10 µM for Adrenergic, Dopamine, Serotonin, and Opioid Receptors, Related to Figures 4 and S5.** Results represent mean ± SEM performed in quadruplicate. Additional details on method, basal activities, and plate arrangement can be found in the methods.

| Receptor | 1 µM | 3 µM | 10 µM |
|---|---|---|---|
| $\alpha_{1A}$ | 1.2 ± 0.0 | 2.0 ± 0.2 | 3.0 ± 0.1 |
| $\alpha_{1B}$ | 3.0 ± 0.3 | 4.5 ± 0.5 | 9.8 ± 0.7 |
| $\alpha_{1D}$ | 1.3 ± 0.1 | 1.3 ± .00 | 2.1 ± 0.3 |
| $\alpha_{2A}$ | 42.9 ± 2.3 | 46.8 ± 7.7 | 48.4 ± 2.7 |
| $\alpha_{2B}$ | 63.0 ± 2.6 | 84.3 ± 4.4 | 72.9 ± 8.5 |
| $\alpha_{2C}$ | 188.9 ± 26.7 | 170.3 ± 13.3 | 207.2 ± 5.4 |
| $\beta_1$ | 1.3 ± 0.1 | 2.6 ± 0.1 | 4.8 ± 0.2 |
| $\beta_2$ | 1.3 ± 0.4 | 0.6 ± 0.1 | 0.7 ± 0.1 |
| $\beta_3$ | 2.8 ± 0.5 | 3.5 ± 2.1 | 6.0 ± 0.8 |
| $D_1$ | 1.1 ± 0.1 | 3.9 ± 0.6 | 6.3 ± 0.8 |
| $D_2$ | 55.5 ± 0.9 | 87.6 ± 2.8 | 7.0 ± 0.2 |
| $D_3$ | 182.5 ± 13.5 | 230.9 ± 14.7 | 234.1 ± 9.5 |
| $D_4$ | 21.0 ± 1.3 | 38.1 ± 1.6 | 69.7 ± 3.7 |
| $D_5$ | 5.5 ± 0.4 | 12.3 ± 1.1 | 15.2 ± 0.8 |
| 5-$HT_{1A}$ | 2.7 ± 0.4 | 3.3 ± 0.4 | 3.6 ± 0.5 |
| 5-$HT_{1B}$ | 1.3 ± 0.0 | 1.5 ± 0.1 | 1.6 ± 0.1 |
| 5-$HT_{1D}$ | 1.6 ± 0.1 | 1.8 ± 0.1 | 2.4 ± 0.2 |
| 5-$HT_{1e}$ | 1.1 ± 0.1 | 1.3 ± 0.1 | 1.6 ± 0.1 |
| 5-$HT_{1F}$ | 1.4 ± 0.1 | 1.9 ± 0.1 | 2.1 ± 0.1 |
| 5-$HT_{2A}$ | 2.4 ± 0.1 | 4.0 ± 0.3 | 5.5 ± 0.2 |
| 5-$HT_{2B}$ | 1.3 ± 0.1 | 1.7 ± 0.0 | 2.6 ± 0.1 |
| 5-$HT_{2C}$ INI | 4.4 ± 1.0 | 5.6 ± 0.8 | 8.3 ± 1.9 |
| 5-$HT_{2C}$ VNV | 4.9 ± 0.2 | 6.1 ± 0.5 | 6.9 ± 0.8 |
| 5-$HT_{2C}$ VSV | 8.3 ± 0.6 | 13.1 ± 0.4 | 10.4 ± 1.0 |
| 5-$HT_{2C}$ VGV | 6.2 ± 0.4 | 8.7 ± 0.8 | 6.7 ± 0.2 |
| 5-$HT_4$ | 0.9 ± 0.0 | 0.7 ± 0.0 | 1.0 ± 0.1 |
| 5-$HT_5$ | 1.7 ± 0.1 | 1.5 ± 0.0 | 1.8 ± 0.2 |
| 5-$HT_6$ | 2.7 ± 0.2 | 4.1 ± 0.3 | 6.1 ± 0.5 |
| 5-$HT_7$ | 1.7 ± 0.1 | 2.2 ± 0.2 | 2.7 ± 0.2 |
| DOR (δ) | 1.3 ± 0.2 | 1.5 ± 0.3 | 1.3 ± 0.1 |
| KOR (κ) | 1.2 ± 0.2 | 1.7 ± 0.1 | 2.8 ± 0.2 |
| OPRL1 | 1.7 ± 0.3 | 3.9 ± 0.5 | 26.2 ± 3.7 |
| MOR (µ) | 1.1 ± 0.1 | 2.2 ± 0.2 | 3.6 ± 0.4 |

**Table S5.**

**Binding Properties of [$^3$H]-Mesulergine, RIT and Clozapine to Wild-type and Mutant 5-HT$_{2C}$ in a [$^3$H]-Mesulergine Binding Assay, Related to Figure 6 and Table S7.** The mean $K_d$ ± SEM values for [$^3$H]-mesulergine and the $K_i$ values (with mean $pK_i$ ± SEM values in parentheses) for clozapine and RIT are shown together with the number of independent experiments performed in duplicate that the data are based on (*n*, in brackets). The relative change in the $pK_d$ or $pK_i$ values exhibited by a specific mutant compared to the corresponding values at the WT receptor is given as $\Delta pK_d$ and $\Delta pK_i$, respectively. The $K_d$ values were compared using a one-way ANOVA with Dunnett's post-test, and the $pK_i$ values were compared using a two-way ANOVA with Dunnett's post-test (* = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001).

| | [$^3$H]-Mesulergine | | Clozapine | | Ritanserin | |
|---|---|---|---|---|---|---|
| | **$K_d$±SEM (nM) [*n*]** | **$\Delta pK_d$ (mutant - WT)** | **$K_i$ (nM) ($pK_i$ ±SEM) [*n*]** | **$\Delta pK_i$ (mutant - WT)** | **$K_i$ (nM) ($pK_i$ ±SEM) [*n*]** | **$\Delta pK_i$ (mutant - WT)** |
| **5-HT$_{2C}$ WT** | 0.64 ±0.04 [*6*] | --- | 9.8 (8.01±0.08) [*5*] | --- | 0.40 (9.39±0.09) [*3*] | --- |
| **S138C$^{3.36}$** | 0.31±0.03 [*6*] | 0.32 | 2.6 (8.59±0.07) [*5*] **** | 0.58 | 0.25 (9.60±0.03) [*3*] | 0.20 |
| **V208T$^{EL2}$** | 0.70±0.06 [*7*] | -0.03 | 12 (7.91±0.13) [*4*] | -0.10 | 0.91 (9.04±0.03) [*4*] * | -0.35 |
| **G218S$^{5.42}$** | 3.21±0.24 [*6*] **** | -0.69 | 24 (7.62±0.04) [*4*] ** | -0.39 | 24 (7.62±0.04) [*3*] **** | -1.77 |
| **S219T$^{5.43}$** | 0.35±0.02 [*6*] | 0.27 | 13 (7.87±0.05) [*4*] | -0.14 | 1.3 (8.90±0.06) [*3*] *** | -0.50 |
| **N331A$^{6.55}$** | 0.57±0.04 [*6*] | 0.06 | 3.4 (8.48±0.10) [*4*] *** | 0.46 | 0.57 (9.25±0.05) [*3*] | -0.15 |
| **L350G$^{7.35}$** | 1.26±0.13 [*7*] * | -0.28 | 6.2 (8.21±0.11) [*4*] | 0.20 | 1.5 (8.82±0.04) [*4*] **** | -0.57 |
| **N351A$^{7.36}$** | 0.91±0.06 [*7*] | -0.15 | 15 (7.84±0.09) [*5*] | -0.17 | 0.88 (9.06±0.07) [*4*] * | -0.34 |
| **V354N$^{7.39}$** | 2.37±0.28 [*6*] **** | -0.56 | 27 (7.56±0.06) [*5*] **** | -0.45 | 170 (6.76±0.05) [*3*] **** | -2.63 |
| **V208T$^{EL2}$/V354N$^{7.39}$** | 2.78±0.18 [*6*] **** | -0.64 | 32 (7.50±0.03) [*4*] **** | -0.51 | 350 (6.45±0.05) [*3*] **** | -2.94 |
| **G218S$^{5.42}$ /V354N$^{7.39}$** [a] | 18.0±6.3 [*4*] [b] *** | -1.31 | 230 (6.65±0.03) [*4*] **** | -1.36 | ~10,000 (~5) [*3*] | ~ -4.39 |

[a] The data for G218S $^{5.42}$ /V354N $^{7.39}$ were obtained in another round of binding experiments than those for the other receptors in the table. Thus, the statistical analysis for this mutant have been made by comparing these data to other mutants from this round (not show) using a two-way ANOVA with Dunnett's post-test. The $\Delta pK_d$ and $\Delta pK_i$ values for this double mutant were calculated using the WT receptor data obtained in that round (mianserin $K_d$ ± SEM: 0.66 ± 0.02 nM, clozapine $pK_i$ ± SEM: 8.00 ± 0.05; RIT $pK_i$ ± SEM: 9.25 ± 0.17)

[b] The $K_d$ value for G218S $^{5.42}$ /V354 N$^{7.39}$ was found to be higher than the maximal [$^3$H]-mesulergine concentration used in the saturation binding experiments. Thus, this $K_d$ value should be considered an approximation extracted from the fitted specific binding curve.

**Table S6.**

**5-$HT_{2C}$ Mutant Lorcaserin Binding Affinities, Related to Figure 6 and Table S7.** Data represent average $K_i$ and average $pK_i$ ±S.E.M. from three independent experiments performed in duplicate and were acquired with 5-$HT_{2C}$ wild-type and mutants expressed in HEKT cells. Affinity was assessed by radioligand competition binding performed using [$^3$H]-Mesulergine (0.9 -3.2 nM) to yield $K_i$ affinity estimates. The $pK_i$ values were compared using a one-way ANOVA with Dunnett's post-test (* = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001).

| | **Lorcaserin** | |
|---|---|---|
| | **$K_i$, nM ($pK_i$ ±SEM)** | **$\Delta pK_i$ (mutant - WT)** |
| **5-$HT_{2C}$ WT** | 445 (6.35±0.03) | --- |
| **V135L$^{3.33}$** | 3981 (5.40±0.02)* | -0.95 |
| **S138C$^{3.36}$** | 282 (6.60±0.15) | +0.24 |
| **V208T$^{EL2}$** | 450 (6.45±0.23) | +0.10 |
| **G218A$^{5.42}$** | 616 (6.24±0.11) | -0.11 |
| **G218S$^{5.42}$** | 6310 (5.20±0.10)** | -1.15 |
| **S219T$^{5.43}$** | 156 (6.88±0.17) | +0.53 |
| **W324Y$^{6.48}$** | 27 (7.83±0.34)*** | +1.48 |
| **F327L$^{6.51}$** | 686 (6.19±0.11) | -0.17 |
| **N331A$^{6.55}$** | 117 (6.97±0.14) | +0.62 |
| **L350G$^{7.35}$** | 1186 (5.93±0.06) | -0.42 |
| **N351A$^{7.36}$** | 208 (6.82±0.26) | +0.46 |
| **V354N$^{7.39}$** | 23 (7.71±0.18)*** | +1.35 |
| **Y358F$^{7.43}$** | 1166 (6.21±0.30) | -0.14 |
| **V208T$^{EL2}$/V354N$^{7.39}$** | 3890 (5.41±0.22)* | -0.94 |
| **G218S$^{5.42}$ /V354N$^{7.39}$** | 27 (7.66±0.22)*** | +1.30 |

**Table S7.**

**The List of 5-$HT_{2C}$ Primers Sequences for Site-Direct Mutagenesis Studies, Related to Figures 2, 3, 6, Tables S2, S5 and S6**.

| Name | Sequence 5'-3' |
|---|---|
| C360N_reverse | GGATTGATTCCTGAATTCACGTAGCCAAT |
| C360N_forward | ATTGGCTACGTGAATTCAGGAATCAATCC |
| F320L_reverse | TCAGGAGCACAAAAAACACAATTCCCAAAACTTTAGATGCTTTTCG |
| F320L_forward | GTGCTCCTGATTATGTGGTGCCCGTTCTTCATCAC |
| F223L_reverse | GAATAAACAAGGCCACGAAAGATCCAATCAGCAC |
| F223L_forward | GCCTTGTTTATTCCGCTCACAATTATGGTGATTACCTACTG |
| I142F_reverse | GGTGCATGAAACTTGCGGTAGAAAACAACACGTCC |
| I142F_forward | CGCAAGTTTCATGCACCTGTGTGCAATTAGCCTG |
| F327L_reverse | GATGAAGAGCGGGCACCACATAATCAGAAACACAAAAAAC |
| F327L_forward | CCGCTCTTCATCACCAACATCCTTAGCGTTCTCTG |
| W324L_reverse | CGGGCACAGCATAATCAGAAACACAAAAAACACAATTCCCAAAACTTTAGATG |
| W324L_forward | GATTATGCTGTGCCCGTTCTTCATCACCAACATCC |
| W324F_reverse | GCAGAACATAATCAGAAACACAAAAAACACAATTCCCAAAACTTTAG |
| W324F_forward | TATGTTCTGCCCGTTCTTCATCACCAACATCC |
| W324Y_reverse | GCAGTACATAATCAGAAACACAAAAAACACAATTCCCAAAACTTTAG |
| W324Y_forward | TATGTACTGCCCGTTCTTCATCACCAACATCC |
| N351L_reverse | CAAACACCAAGAGGAGCTTCTCCATGAGCTTCTGATTAC |
| N351L_forward | GCTCCTCTTGGTGTTTGTCTGGATTGGTTACGTTTGCAG |
| N351F_reverse | CAAACACGAAGAGGAGCTTCTCCATGAGCTTCTGATTAC |
| N351F_forward | CTCCTCTTCGTGTTTGTCTGGATTGGTTACGTTTGCAG |
| Y358F_reverse | AAACGAAACCAATCCAGACAAACACGTTGAGGAG |
| Y358F_forward | TGGTTTCGTTTGCAGCGGGATCAACCCTC |
| Y118A_reverse | CACGGCATCGTACAGGATTGCCAGGAGGGAC |
| Y118A_forward | GATGCCGTGTGGCCGTTGCCCCG |
| Y118L_reverse | CACACAAGATCGTACAGGATTGCCAGGAGGGAC |
| Y118L_forward | GTACGATCTTGTGTGGCCGTTGCCCCG |
| V135L_reverse | CAACAAGTCCAGGGATATCCAGACCGGGC |
| V135L_forward | GACTTGTTGTTTTCTACCGCAAGTATCATGCACCTG |
| V135F_reverse | CAAGAAGTCCAGGGATATCCAGACCGGGC |
| V135F_forward | GACTTCTTGTTTTCTACCGCAAGTATCATGCACCTG |
| V208A_reverse | CAACGCGCAGGTTGTGTTGTTAACAAAGACTTTCTCTTC |
| V208A_forward | CTGCGCGTTGAACGACCCCAACTTCGTGCTG |
| V208S_reverse | CAAGCTGCAGGTTGTGTTGTTAACAAAGACTTTCTCTTC |
| V208S_forward | CTGCAGCTTGAACGACCCCAACTTCGTGCTG |
| V208F_reverse | CAAGAAGCAGGTTGTGTTGTTAACAAAGACTTTCTCTTC |
| V208F_forward | CTGCTTCTTGAACGACCCCAACTTCGTGCTG |

| | |
|---|---|
| G218A_reverse | AAGATGCAATCAGCACGAAGTTGGGGTCGTTC |
| G218A_forward | GATTGCATCTTTCGTGGCCTTTTTTATTCCGCTC |
| S138C_reverse | CATGATGGACGCTGTGCAAAATAAAACATCTAAAG |
| S138C_forward | CTTTAGATGTTTTATTTTGCACAGCGTCCATCATG |
| V208T_reverse | TTTGGGTCGTTGAGCGTGCACGTCGTGTTG |
| V208T_forward | CAACACGACGTGCACGCTCAACGACCCAAA |
| G218S_reverse | GAAAGCTACGAAGGACGAAATAAGAACGAAATT |
| G218S_forward | AATTTCGTTCTTATTTCGTCCTTCGTAGCTTTC |
| S219T_reverse | GAAAGCTACGAAGGTCCCAATAAGAACG |
| S219T_forward | CGTTCTTATTGGGACCTTCGTAGCTTTC |
| N331A_reverse | GAACAGACAGAATAGCGGTAATGAAAAATGG |
| N331A_forward | CCATTTTTCATTACCGCTATTCTGTCTGTTC |
| L350G_reverse | AACAAACACATTCCCAAGCTTTTCCATG |
| L350G_forward | CATGGAAAAGCTTGGGAATGTGTTTGTT |
| N351A_reverse | CCAAACAAACACAGCCAGAAGCTTTTCC |
| N351A_forward | GGAAAAGCTTCTGGCTGTGTTTGTTTGG |
| V354N_reverse | CATAGCCAATCCAATTAAACACATTCAGAAG |
| V354N_forward | CTTCTGAATGTGTTTAATTGGATTGGCTATG |