Supporting Information

Design, synthesis and structure-activity relationships of highly potent 5-HT_3 receptor ligands

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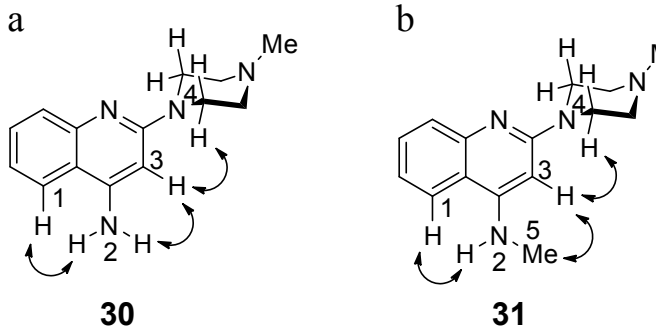

Figure S1: NOESY couplings that were observed using 2D ^{1}H NMR measurements on compounds **30** and **31**. (a) The correct regio isomers of compound **30**. The ^{1}H NMR signal at 6.16 ppm (s, 1H) can be ascribed to proton 3 since it couples to both the multiplet of 4 at 3.72-3.63 ppm (m, 4H) as well as to the signal of the aniline protons of 2 at 4.51 ppm (br s, 2H). Furthermore, the aniline proton of 2 at 4.51 ppm (br s, 2H) couples with the aromatic proton of 1 at 7.43-7.31 ppm (m, 1H). In addition, there is no coupling observed between the signals of the aromatic proton of 1 7.43-7.31 ppm (m, 1H) and the multiplet of 4 at 3.72-3.63 ppm (m, 4H). (b) The correct regio isomers of compound **31**. The ^{1}H NMR signal at 5.92 ppm (s, 1H) can be ascribed to proton 3 since it couples to both the multiplet of 4 at 3.79-3.69 ppm (m, 4H) as well as to the signal of the methylaniline protons of 5 at 3.00 ppm (d, 3H). Furthermore, the aniline proton of 2 at 4.89 ppm (br s, 1H) couples with the aromatic proton of 1 at 7.52-7.43 ppm (m, 2H). Interestingly, there is no coupling observed between proton 1 and the methylaniline protons of 5. In addition, no coupling is observed between aniline proton 2 and aromatic proton 3 suggesting that the rotamer drawn above is preferred. Finally, there is no coupling observed between the signals of the aromatic proton of 1 7.43-7.31 ppm (m, 1H) and the multiplet of 4 at 3.72-3.63 ppm (m, 4H).

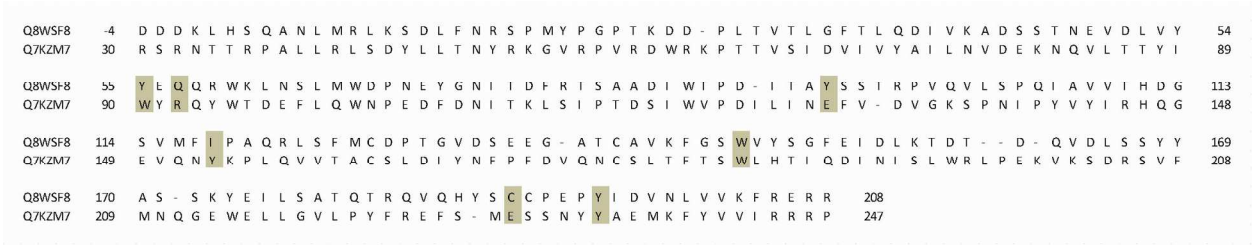

Figure S2: Sequence alignment for *Ac*-AChBP (Q8WSF8) and 5-HT$_{3A}$R (Q7KZM7). 5-HT$_3$AR residues shown in Figure 3 and their corresponding residues in *Ac*-AChBP are highlighted.

Table S1: Cross-target pharmacology of compound 22 at a concentration of 0.1 µM.

Receptor	% Inhibition of Control Specific Binding		
	1ste	2nd	Mean
GABAA1 (α1,β2,γ2)[a]	8.5	4.6	6.6
Glycine[b]	4.1	17.0	10.5
nACh (α4β2)[a]	8.3	-4.4	1.9
nACh (α7)[a]	30.7	31.9	31.3
5-HT_1A[a]	11.9	-1.8	5.0
5-HT_1B[b]	-4.4	-11.8	-8.1
5-HT_1D[b]	7.7	-1.2	3.3
5-HT_2A[a]	4.0	9.4	6.7
5-HT_2B[a]	42.8	43.4	43.1
5-HT_2C[a]	-3.1	-10.0	-6.5
5-HT_4E[a]	-2.9	1.8	-0.5
5-HT_6[a]	-5.1	6.3	0.6
5-HT_7[a]	-3.3	-6.8	-5.0

[a] Human receptor; [b] Rat receptor; [c] Results are expressed as a percent inhibition of control specific binding obtained in the presence of compound **22**. Results showing an inhibition < 15% considered non binding.

Table S2: Reference Compounds.

Receptor	Compound	IC_{50} (M)	K_i (M)	n_H
$GABA_A$ (α1β2γ2)				
	Muscimol	7.2E-08	4.8E-08	0.9
Glycine				
	Strychnine	9.5E-09	8.6E-09	0.7
nACh (α4β2)				
	Nicotine	4.3E-09	1.4E-09	0.9
nACh (α7)				
	Nicotine	2.0E-10	1.5E-10	0.6
5-HT_1A				
	8-OH-DPAT	3.7E-10	2.3E-10	1.1
5-HT_1B				
	Serotonin	7.8E-09	4.8E-09	0.8
5-HT_1D				
	Serotonin	1.4E-09	4.7E-10	1.0
5-HT_2A				
	(±)DOI	5.0E-10	3.7E-10	0.6
5-HT_2B				
	(±)DOI	6.6E-09	3.3E-09	0.8
5-HT_2C				
	(±)DOI	6.6E-10	5.9E-10	0.9
5-HT_4E				
	Serotonin	3.4E-07	1.1E-07	0.8
5-HT_6				
	Serotonin	1.7E-07	7.9E-08	0.9
5-HT_7				
	Serotonin	3.1E-10	1.1E-10	1.1

The IC_{50} values (concentration causing a half-maximal inhibition of specific binding) and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using the Hill equation:

$$Y = D + [\frac{A - D}{1 + (C/C_{50})^{nH}}]$$

where Y = specific binding, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration and n_H = slope factor. This analysis was performed using Hill software (Cerep) and validated by comparison with SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.). The inhibition constants (K_i) were calculated using the Cheng Prusoff equation:

$$K_i = \frac{IC_{50}}{(1 + L/K_D}$$

where L = concentration of radioligand in the assay, and K_d = affinity of the radioligand for the receptor.

Table S3: Binding assays

Receptor	Source	Ligand	Conc.	Kd	Non Specific	Incubation
GABAA1 (α1,β2,γ2)	Human recombinant (CHO cells)	[^{3}H]muscimol	15 nM	30 nM	Muscimol (10 μM)	120 min RT
Glycine	Rat spinal cord	[^{3}H]strychnine	2 nM	20 nM	Strychnine (100 μM)	15 min 0°C
nACh (α4β2)	Human recombinant (SH-SY5Y cells)	[^{3}H]cytisine	0.6 nM	0.3 nM	Nicotine (10 μM)	120 min 4°C
nACh (α7)	Human recombinant (HEK-293 cells)	[^{3}H]epibatidine	3 nM	5.8 nM	Nicotine (3mM)	120 min 4°C
5-HT$_1$A	Human recombinant (HEK-293 cells)	[^{3}H]8-OH-DPAT	0.3 nM	0.5 nM	8-OH-DPAT (10 μM)	60 min RT
5-HT$_1$B	Rat cerebral cortex	[^{125}I]CYP (+ 30 μM isoproterenol)	0.1 nM	0.16 nM	Serotonin (10 μM)	120 min 37°C
5-HT$_1$D	Rat recombinant (CHO cells)	[^{3}H]serotonin	1 nM	0.5 nM	Serotonin (10 μM)	60 min RT
5-HT$_2$A	Human recombinant (HEK-293 cells)	[^{125}I](±)DOI	0.1 nM	0.3 nM	(±)DOI (1 μM)	60 min RT
5-HT$_2$B	Human recombinant (CHO cells)	[^{125}I](±)DOI	0.2 nM	0.2 nM	(±)DOI (1 μM)	60 min RT
5-HT$_2$C	Human recombinant (HEK-293 cells)	[^{125}I](±)DOI	0.1 nM	0.9 nM	(±)DOI (1 μM)	60 min 37°C
5-HT$_4$E	Human recombinant (CHO cells)	[^{3}H]GR 113808	0.3 nM	0.15 nM	Serotonin (100 μM)	60 min 37°C
5-HT$_6$	Human recombinant (CHO cells)	[^{3}H]LSD	2 nM	1.8 nM	Serotonin (100 μM)	120 min 37°C
5-HT$_7$	Human recombinant (CHO cells)	[^{3}H]LSD	4 nM	2.3 nM	Serotonin (10 μM)	120 min RT

K_d = affinity of the radioligand for the receptor.