Supporting Information

Novel aryloxyethyl derivatives of 1-(1-benzoylpiperidin-4-yl)methanamine as the Extracellular Regulated Kinases 1/2 (ERK1/2) phosphorylation-preferring serotonin 5-HT_{1A} receptor biased agonists with robust antidepressant-like activity

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Functional activity results at 5-HT$_{1A}$ receptors with the SEM values.

Table S1. Functional activity of compounds **10**–**17**, **19**, **20**, **22**, **27** and references at 5-HT$_{1A}$ receptors.

	5-HT$_{1A}$ functional activity							
	ERK		cAMP		β-arrestin		Ca^{2+}	
Compd	E_{MAX}	pEC_{50}	E_{MAX}	pEC_{50}	E_{MAX}	pEC_{50}	E_{MAX}	pEC_{50}
10	77% ± 7.9%	7.88 ± 0.07	91% ± 5.3%	7.10 ± 0.11	99% ± 0.5%	6.35 ± 0.02	74% ± 2.4%	7.07 ± 0.02
11	95% ± 3.5%	7.90 ± 0.08	74% ± 5.1%	7.33 ± 0.06	101% ± 0.5%	6.94 ± 0.11	87% ± 7.5%	7.84 ± 0.06
12	81% ± 6.3%	7.59 ± 0.09	79% ± 2.7%	6.73 ± 0.01	104% ± 2.1%	5.70 ± 0.07	65% ± 3.0%	6.98 ± 0.06
13	83% ± 4.6%	7.76 ± 0.14	86% ± 4.8%	7.26 ± 0.10	99% ± 0.8%	6.58 ± 0.09	52% ± 0.2%	7.13 ± 0.01
14	85% ± 1.3%	6.77 ± 0.02	71% ± 4.7%	5.61 ± 0.11	70% ± 2.9%	5.44 ± 0.03	NT	NT
15	84% ± 2.3%	7.89 ± 0.09	76% ± 7.4%	7.18 ± 0.19	102% ± 2.4%	6.54 ± 0.15	73% ± 9.8%	6.95 ± 0.01
16	92% ± 2.7%	9.10 ± 0.14	90% ± 2.3%	8.09 ± 0.12	96% ± 1.0%	7.98 ± 0.02	52% ± 2.0%	7.39 ± 0.17
17	81% ± 7.2%	9.37 ± 0.19	89% ± 3.3%	8.05 ± 0.09	98% ± 1.0%	7.68 ± 0.11	57% ± 5.8%	7.76 ± 0.05
19	85% ± 0.6%	9.06 ± 0.17	71% ± 7.3%	8.04 ± 0.06	99% ± 0.9%	6.76 ± 0.13	58% ± 1.5%	7.41 ± 0.09
20	99% ± 2.5%	7.62 ± 0.16	100% ± 4.0%	7.32 ± 0.20	101% ± 1.1%	6.87 ± 0.09	98% ± 1.4%	7.34 ± 0.06
22	100% ± 5.2%	8.31 ± 0.17	90% ± 6.4%	7.68 ± 0.08	97% ± 2.8%	7.60 ± 0.17	89% ± 5.3%	7.54 ± 0.20
27	72% ± 8.2%	7.28 ± 0.02	41% ± 1.4%	6.03 ± 0.03	75% ± 3.7%	5.56 ± 0.06	NT	NT
1	100% ± 4.3%	8.33 ± 0.14	92% ± 6.9%	7.22 ± 0.08	98% ± 1.0%	6.71 ± 0.07	60% ± 1.7%	6.61 ± 0.10
8-OH-DPAT	93% ± 3.6%	8.09 ± 0.11	63% ± 2.3%	7.50 ± 0.05	101% ± 1.5%	7.84 ± 0.18	NT	NT
Buspirone	44% ± 5.5%	7.82 ± 0.13	49% ± 5.7%	7.14 ± 0.12	100% ± 1.1%	6.73 ± 0.08	NT	NT
Serotonin	100% ± 0.0	7.48 ± 0.08	100% ± 0.0%	7.51 ± 0.05	100% ± 0.0%	6.89 ± 0.04	100% ± 0.0%	7.30 ± 0.08

E_{max} and EC_{50} values (transformed from pEC_{50} values) used to calculate Bias Factors for compounds 10–17, 19, 20, 22, 27 and references

Table S2. E_{max} and EC_{50} values (transformed from pEC_{50} values) used to calculate Bias Factors for compounds **10**–**17**, **19**, **20**, **22**, **27** and references .

	5-HT$_{1A}$ functional activity							
	ERK		cAMP		β-arrestin		Ca^{2+}	
Compd	E_{MAX}	EC_{50}	E_{MAX}	EC_{50}	E_{MAX}	EC_{50}	E_{MAX}	pEC_{50}
10	0,77	1.31E-08	0.91	7.96E-08	0.99	4.46E-07	0.74	8.58E-08
11	0.95	1.25E-08	0.74	4.71E-08	1.01	1.15E-07	0.87	1.43E-08
12	0.81	2.55E-08	0.79	1.86E-07	1.04	1.99E-06	0.65	1.04E-07
13	0.83	1.75E-08	0.86	5.44E-08	0.99	2.60E-07	0.52	7.47E-08
14	0.85	1.69E-07	0.71	2.44E-06	0.70	3.61E-06	NT	NT
15	0.84	1.29E-08	0.76	6.62E-08	1.02	2.88E-07	0.73	1.11E-07
16	0.92	7.93E-10	0.90	8.09E-09	0.96	1.05E-08	0.52	4.11E-08
17	0.81	4.29E-10	0.89	8.85E-09	0.98	2.09E-08	0.57	1.72E-08
19	0.85	8.72E-10	0.71	9.10E-09	0.99	1.73E-07	0.58	3.85E-08
20	0.99	2.39E-08	1.00	4.79E-08	1.01	1.36E-07	0.98	4.54E-08
22	1.00	4.94E-09	0.90	2.10E-08	0.97	2.51E-08	0.89	2.86E-08
27	0.72	5.22E-08	0.41	9.25E-07	0.75	2.74E-06	NT	NT
1	1.00	4.71E-09	0.92	6.01E-08	0.98	1.93E-07	0.60	2.47E-07
8-OH-DPAT	0.93	8.17E-09	0.63	3.19E-08	1.01	1.44E-08	NT	NT
Buspirone	0.44	1.51E-08	0.49	7.20E-08	1.00	1.86E-07	NT	NT
Serotonin	1.00	3.28E-08	1.00	3.07E-08	1.00	1.30E-07	1.00	4.99E-08

Radioligand binding assay results and methodology for 5-HT_{2A}, M_1 and H_1 receptors

Table S3. Radioligand binding assay results for 5-HT_{2A}, M_1 and H_1 receptors using [^{3}H]-ketanserin, [^{3}H]-scopolamine and [^{3}H]-pyrilamine, respectively.

	percentage of control specific binding at 1 μM concentration		
Compound	**5-HT_{2A}R**	**M_1R**	**H_1R**
10	23%	10%	0%
11	0%	6%	0%
12	3%	10%	0%
13	12%	0%	0%
14	3%	4%	0%
15	0%	0%	0%
16	2%	0%	0%
17	4%	-1%	0%
18	0%	0%	0%
19	4%	0%	17%
20	44%	0%	40%
21	0%	0%	45%
22	4%	8%	1%
23	0%	6%	0%
24	0%	1%	0%
25	0%	2%	0%
26	0%	0%	0%
27	0%	0%	1%
1	0%	0%	0%
Buspirone	17%	0%	50%
Methiothepin	100%	23%	99%
Mepyramine	–	–	99%
Scopolamine	–	100%	–

Preparation of solutions of test and reference compounds

10 mM stock solutions of tested compounds were prepared in DMSO. Serial dilutions of compounds were prepared in 96-well microplate in assay buffers using automated pipetting system epMotion 5070 (Eppendorf). Each compound was tested in a screening assay at final concentration of 1 μM.

5-HT_{2A} Receptor Binding Assay

Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human 5-HT_{2A} receptor (PerkinElmer). All assays were carried out in duplicates. 50 μL working solution of the tested compounds, 50 μL [^{3}H]-ketanserin (final concentration 1 nM) and 150 μL diluted membranes (7 μg protein per well) prepared in assay buffer (50 mM Tris, pH 7.4, 4 mM $CaCl_2$, 0.1% ascorbic acid) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Mianserin (10 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 60 minutes at 27 °C. The reaction was terminated by rapid filtration through GF/B filter mate presoaked with 0.5% polyethyleneimine for 30 minutes. Ten rapid washes with 200 μL 50 mM Tris buffer (4 °C, pH 7.4) were performed using automated harvester system Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 5 minutes. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software).

M_1 Receptor Binding Assay

Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human M_1 receptor. All assays were carried out in duplicates. 50 µL working solution of the tested compounds, 50 µL [^{3}H]-scopolamine (spec. act. 84.1 Ci/mmol, final concentration 0.2 nM) and 150 µL diluted membranes (35 µg protein per well) prepared in assay buffer (PBS, pH 7.4) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Atropine (10 µM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 120 minutes at 27 °C. The reaction was terminated by rapid filtration through GF/A filter mate presoaked with 0.5% polyethyleneimine for 30 minutes. Five rapid washes with 300 µL 50 mM Tris buffer (4 °C, pH 7.4) were performed using 96-well FilterMate harvester (PerkinElmer, USA). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 4 minutes. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 5 (GraphPad Software).

H_1 Receptor Binding Assay

Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human histaminergic H_1 receptor. All assays were carried out in duplicates. 50 µL working solution of the tested compounds, 50 µL [^{3}H]-Pyrilamine (spec. act. 20.0 Ci/mmol, final concentration 1.5 nM) and 150 µL diluted membranes (5 µg protein per well) prepared in assay buffer (50 mM Tris-HCl, pH 7.4; 5 mM $MgCl_2$) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Mepyramine (10 µM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 60 minutes at 27 °C. The reaction was terminated by rapid filtration through

GF/B filter mate presoaked with 0.5% polyethyleneimine for 30 minutes. Five rapid washes with 300 μL 50 mM Tris buffer (4 °C, pH 7.4) were performed using 96-well FilterMate harvester (PerkinElmer, USA). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 4 minutes. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 5 (GraphPad Software).

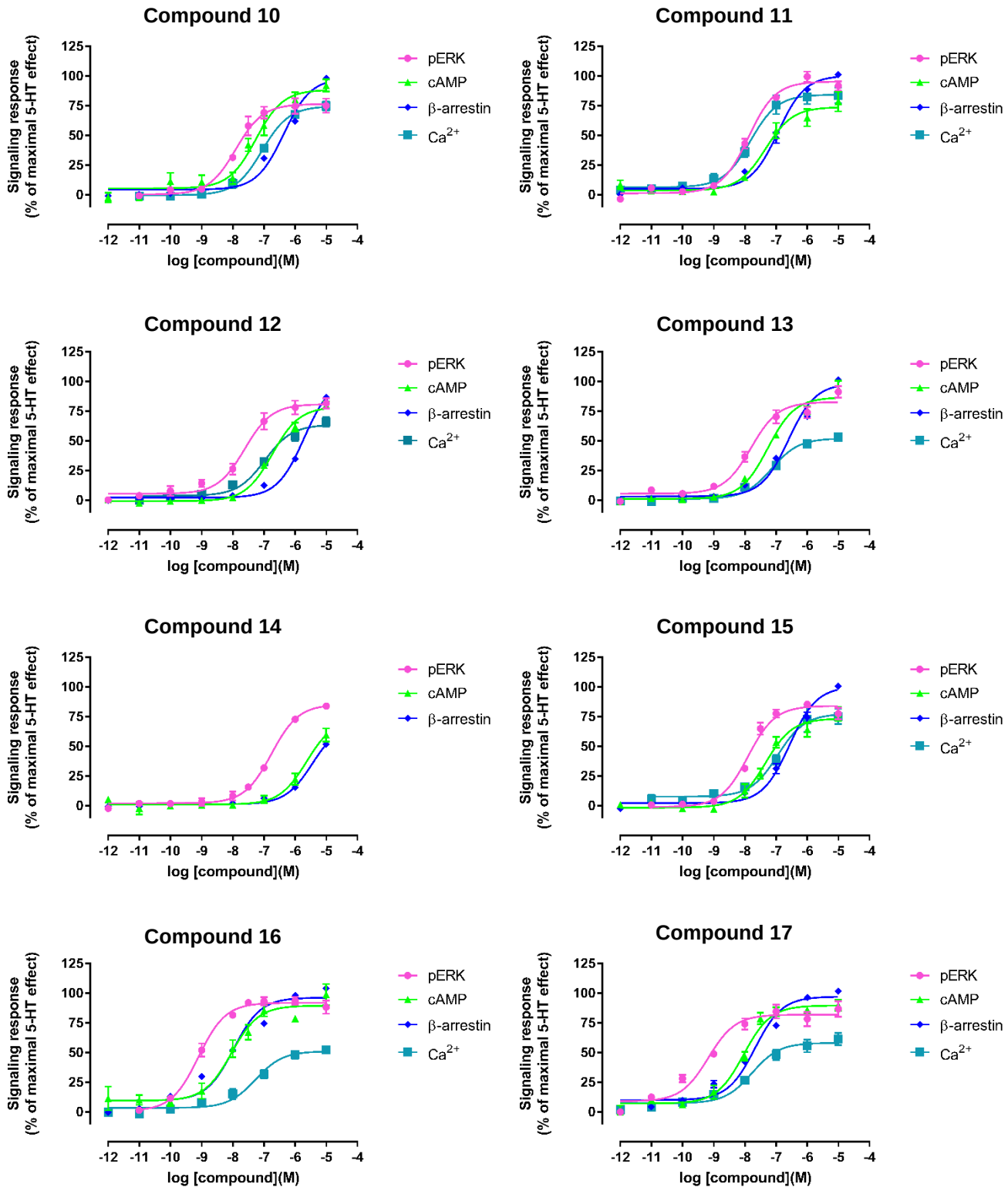
Effect of compound 17 in the forced swimming test in rats (15 and 60 minutes after oral administration).

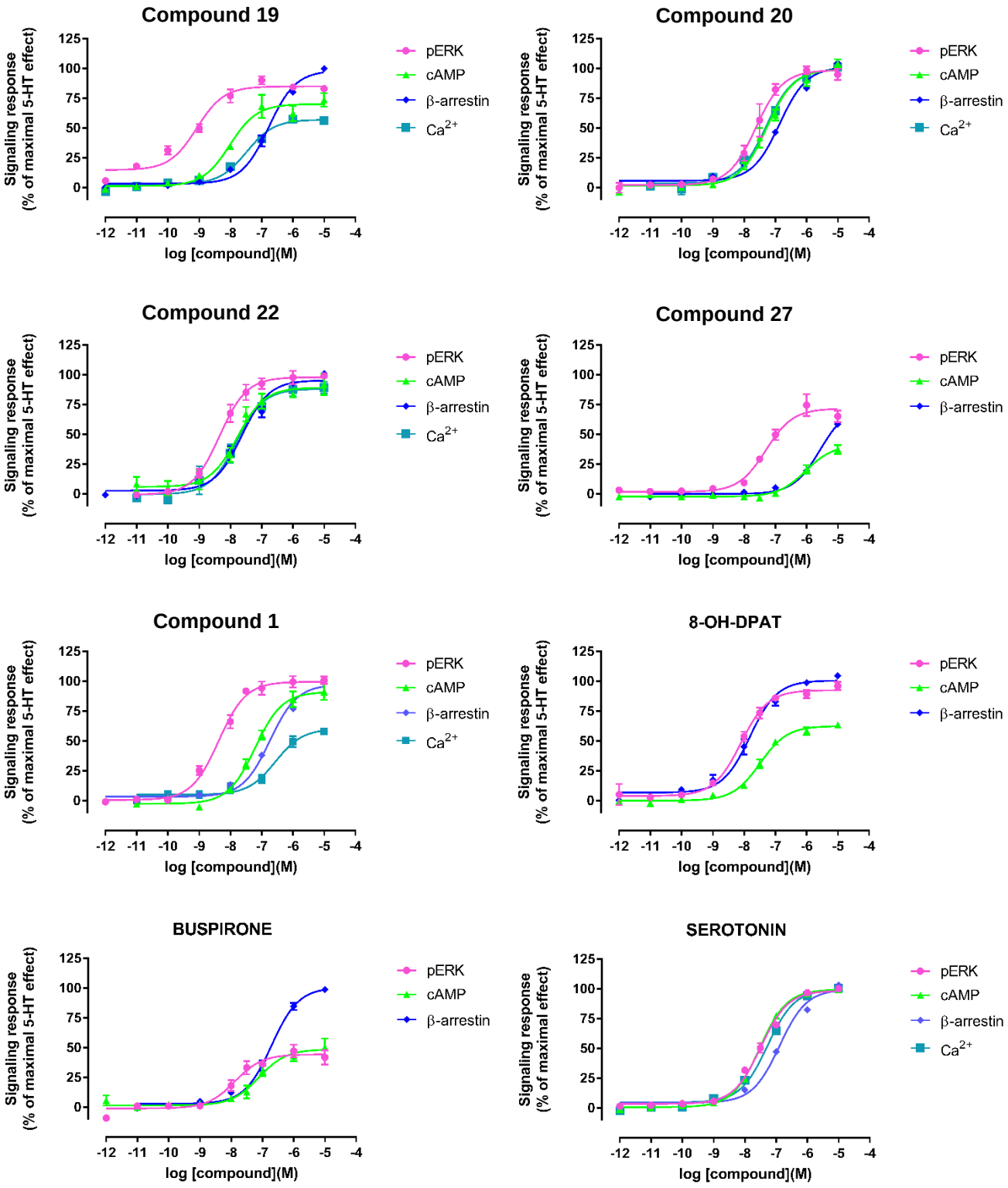
Table S4. Effect of compound **17** in the forced swimming test in rats (15 and 60 minutes after oral administration).

		15 minutes after p.o. administration	**60 minutes after p.o. administration**
Treatment	**Dose (mg/kg)**	**Immobility time (s) (mean ± SEM)**	
Vehicle (water)	0	252.50 ± 12.19	212.63 ± 17.1
Compd **17**	0.04	212.33 ± 23.53; ns	174.75 ± 10.2; ns
	0.16	189.67 ± 10.47; p<0.05	145.43 ± 13.9; p<0.01
	0.63	76.17 ± 17.11; p<0.00001	67.75 ± 12.5; p<0.00001
	2.5	2.38 ± 0.46; p<0.00001	6.86 ± 2.3; p<0.00001
		$F^a(4,29)=63.220$; p<0.0000001	$F^a(4,33)=43.736$; p<0.000001
Vehicle (water) + vehicle	0 + 0	253.20 ± 11.70	207.14 ± 16.2
Compd **17** + vehicle	**2.5** + 0 (15 minutes)/ **0.16** + 0 (60 minutes)	2.38 ± 0.46; p<0.001 vs veh p<0.001 vs WAY p<0.001 vs WAY+ Compd **17**	114.00 ± 9.4 p<0.001 vs veh p<0.001 vs WAY + Compd **17**
WAY-100635 + vehicle	0.63 + 0	260.43 ± 8.65; ns vs veh p<0.001 vs NLX204 p<0.001 vs WAY+NLX204	260.43 ± 8.7 p<0.05 vs veh p<0.001 vs WAY + Compd **17**
WAY-100635 + Compd **17**	0.63 + **2.5** (15 minutes)/ 0.63 + **0.16** (60 minutes)	142.38 ± 17.57; p<0.001 vs veh p<0.001 vs WAY p<0.001 vs Compd **17**	210.00 ± 12.7 p<0.01 vs WAY p<0.001 vs Compd **17**
		$F^b(1,27)=31.616$; p<0.0001	$F^b(1,24)=3.1208$; NS

Compound **17** was given p.o. 15 min or 60 min, while WAY-100635 was injected s.c. 30 min or 75 min, respectively before the test. Values represent the mean ± SEM of the immobility time during 5-min test session compared to: the respective vehicle group ([a]one-way ANOVA followed by Bonferroni's post hoc test – when only one drug was given); or the respective groups ([b] two-way ANOVA followed by Newman-Keuls post hoc test, when two drugs were injected); N=6-8

Dose-response curves for functional activity.




Compound 19
Compound 20
Compound 22
Compound 27
Compound 1
8-OH-DPAT
BUSPIRONE
SEROTONIN
Signaling response (% of maximal 5-HT effect)
Signaling response (% of maximal effect)
log [compound](M)
pERK
cAMP
β-arrestin
Ca2+