Pharmacological fingerprint of antipsychotic drugs at the serotonin 5-HT$_{2A}$ receptor

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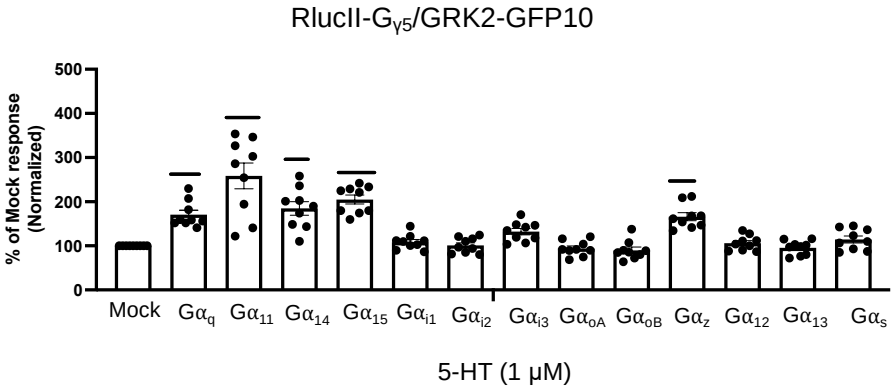
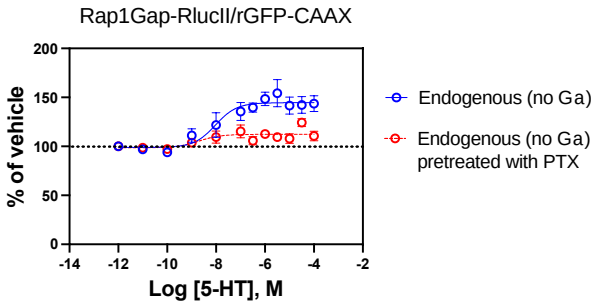
SUPPLEMENTARY INFORMATION (SI)

SUPPLEMENTARY FIGURES:

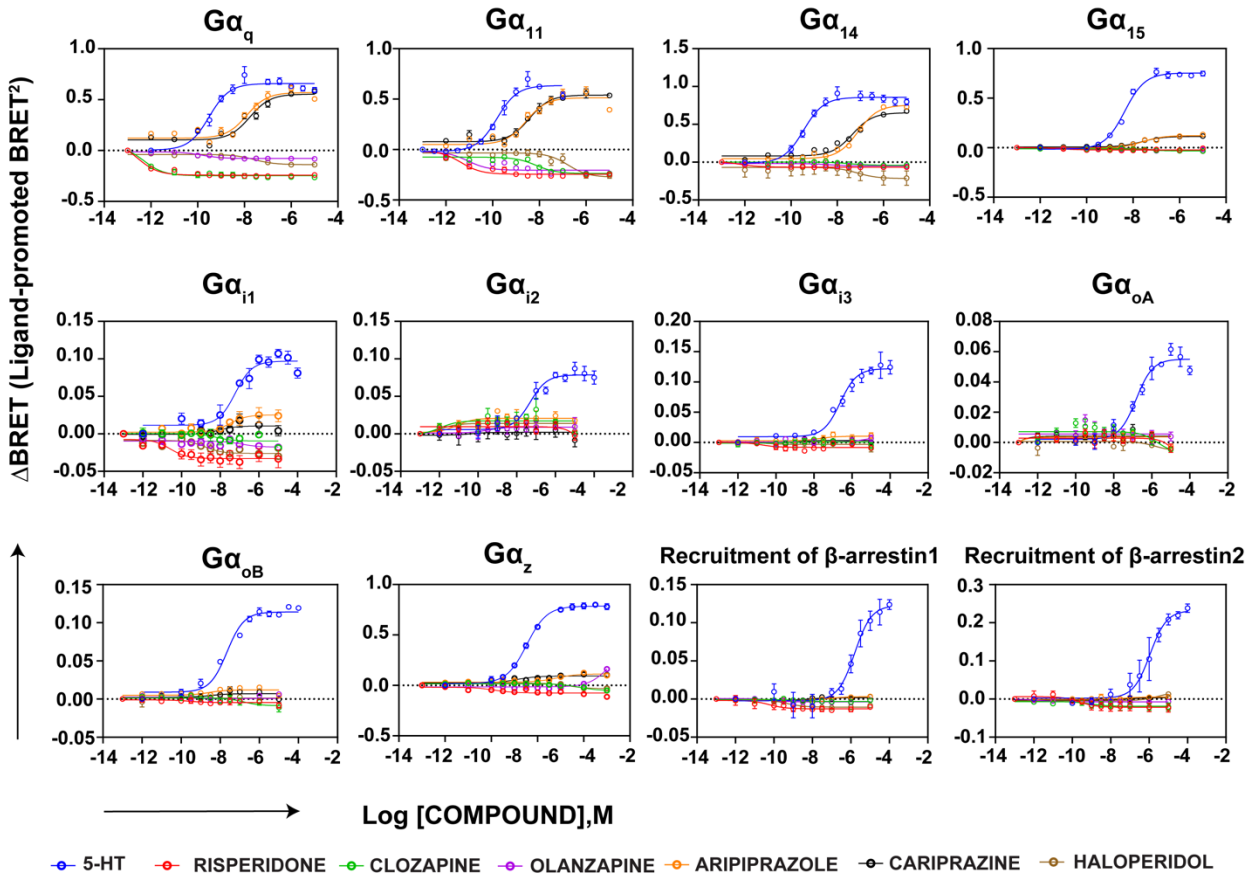
a



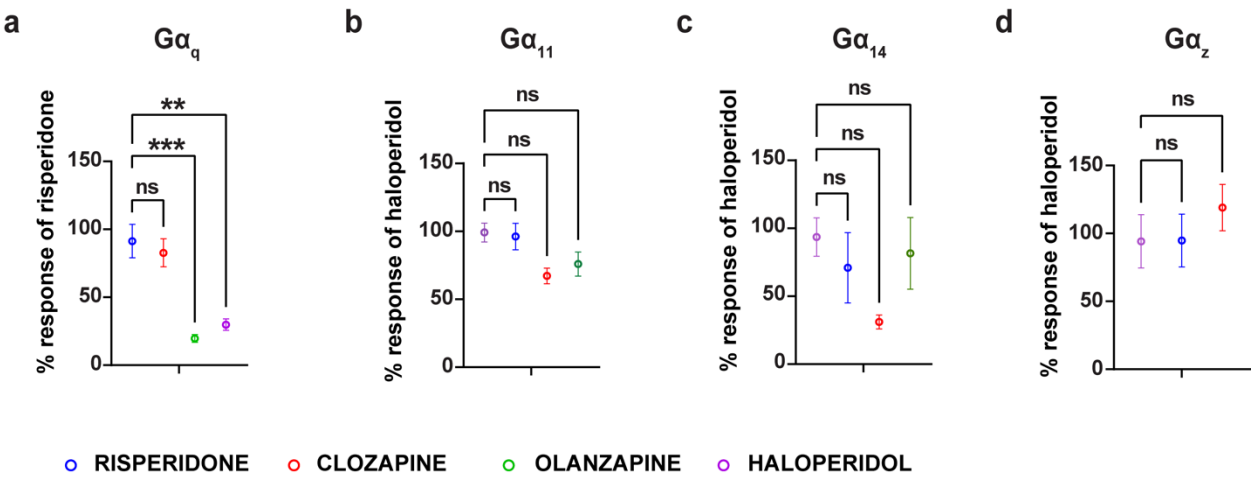
b



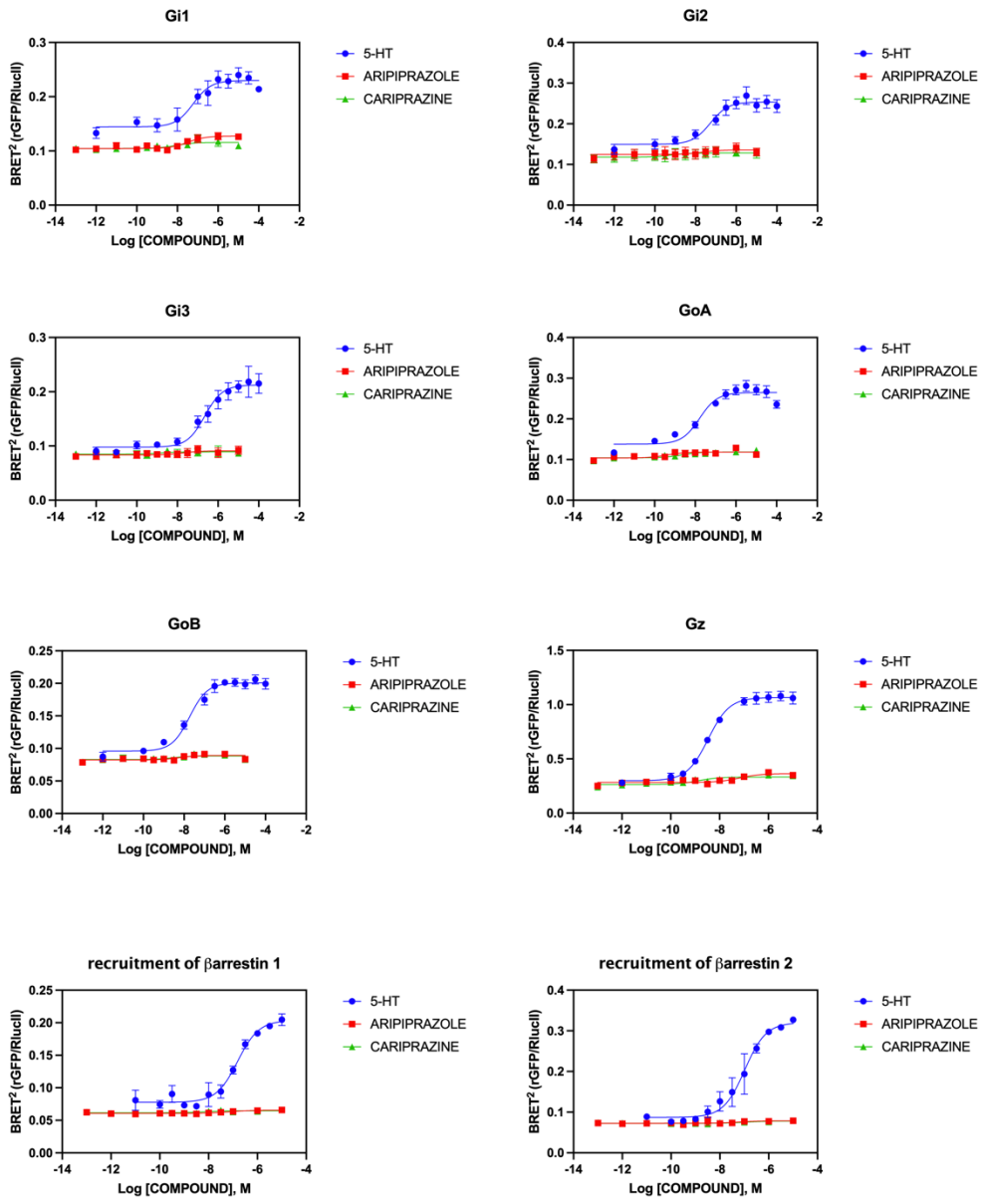
Supplementary Fig. 1: The signaling profile of 5-HT at the 5-HT$_{2A}$ receptor in HEK293 cells. a The complete G protein activation profile of 5-HT (1 μM; 15 min) with HEK293 cells heterologously expressing the untagged 5-HT$_{2A}$ receptor and the biosensor (RlucII-G$_{\gamma5}$/GRK2-GFP10, Gβ_1 and the respective Gα subunits). Results are expressed as BRET ratio (GFP10/RlucII) as % of mock condition (in the absence of heterologously expressed Gα subunit) (mean ± SEM; *n* = 3; one-way ANOVA followed by Dunnett's post hoc: ****p* = 0.0002, and *****p* < 0.0001 compared to the mock condition). **b** Concentration response curves showing activation of the endogenous G$\alpha_{i/o/z}$ family (with the EMTA biosensor in HEK293 cells heterologously expressing the untagged 5-HT$_{2A}$ receptor and the biosensor (Rap1Gap-RlucII/rGFP-CAAX) in the absence and presence of pertussis toxin (PTX). The inhibition of the response indicates direct response through the G$\alpha_{i/o}$ family. Results are expressed as BRET^2 (% over vehicle) (mean ± SEM; *n* = 3)



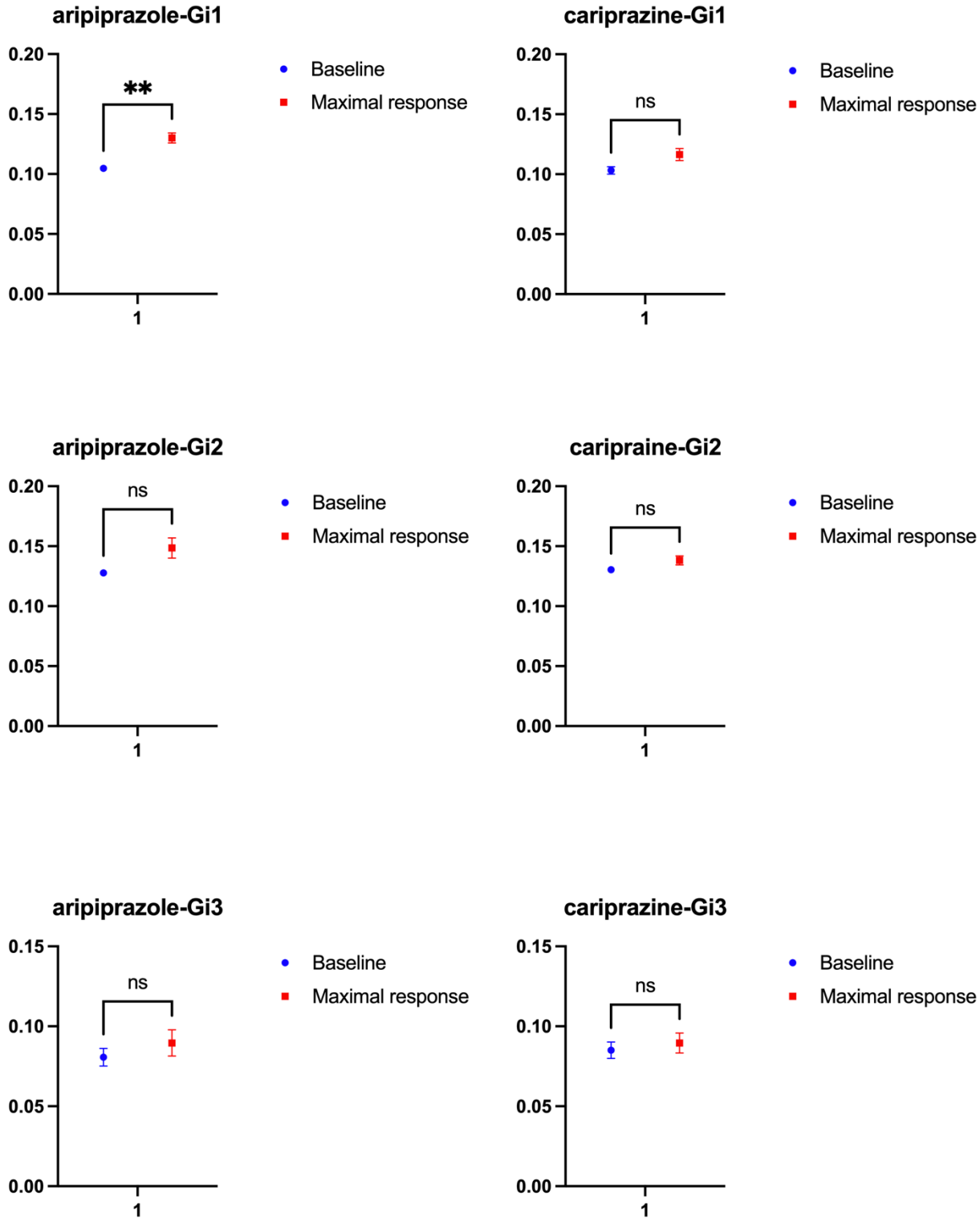
Supplementary Fig. 2: Concentration response curves of 5-HT along with the six antipsychotic drugs to detect the activation or inactivation of the $G\alpha_q$ and $G\alpha_{i/o/z}$ families, and for the recruitment of β-arrestin 1 and β-arrestin 2 using the EMTA biosensors. Results are expressed as ΔBRET (ligand-promoted BRET) (mean ± SEM; *n* = 3).



Supplementary Fig. 3: Inverse agonist activity of risperidone, clozapine, olanzapine and haloperidol. a Graph represents the efficacy of the inverse agonist activity at $G\alpha_q$ (**a**), $G\alpha_{11}$ (**b**), $G\alpha_{14}$ (**c**) and $G\alpha_z$ (**d**), normalized with respect to the efficacy of risperidone (**a**, mean ± SEM; *n* = 3; one-way ANOVA followed by Dunnetts's post hoc: compared to risperidone for $G\alpha_q$) or haloperidol (**b-d,** mean ± SEM; *n* = 3-5) (one-way ANOVA followed by Dunnetts's post hoc test compared to risperidone for $G\alpha_q$ and haloperidol for $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_z$). ***p* = 0.0022, ****p* = 0.0008, ns not significant.



Supplementary Fig. 4: Concentration response curves showing the activation of the $G\alpha_{i/o/z}$ family by 5-HT, aripiprazole and cariprazine using the EMTA biosensor in HEK293 cells heterologously expressing the untagged $5\text{-}HT_{2A}$ receptor and the biosensor (Rap1GAP-RlucII/rGFP-CAAX and the respective $G\alpha$ subunits). Results are expressed as BRET ratio (GFP10/RlucII).


aripiprazole-Gi1
0.20
0.15
0.10
0.05
0.00
**
1
Baseline
Maximal response
cariprazine-Gi1
0.20
0.15
0.10
0.05
0.00
ns
1
Baseline
Maximal response
aripiprazole-Gi2
0.20
0.15
0.10
0.05
0.00
ns
1
Baseline
Maximal response
caripraine-Gi2
0.20
0.15
0.10
0.05
0.00
ns
1
Baseline
Maximal response
aripiprazole-Gi3
0.15
0.10
0.05
0.00
ns
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Maximal response
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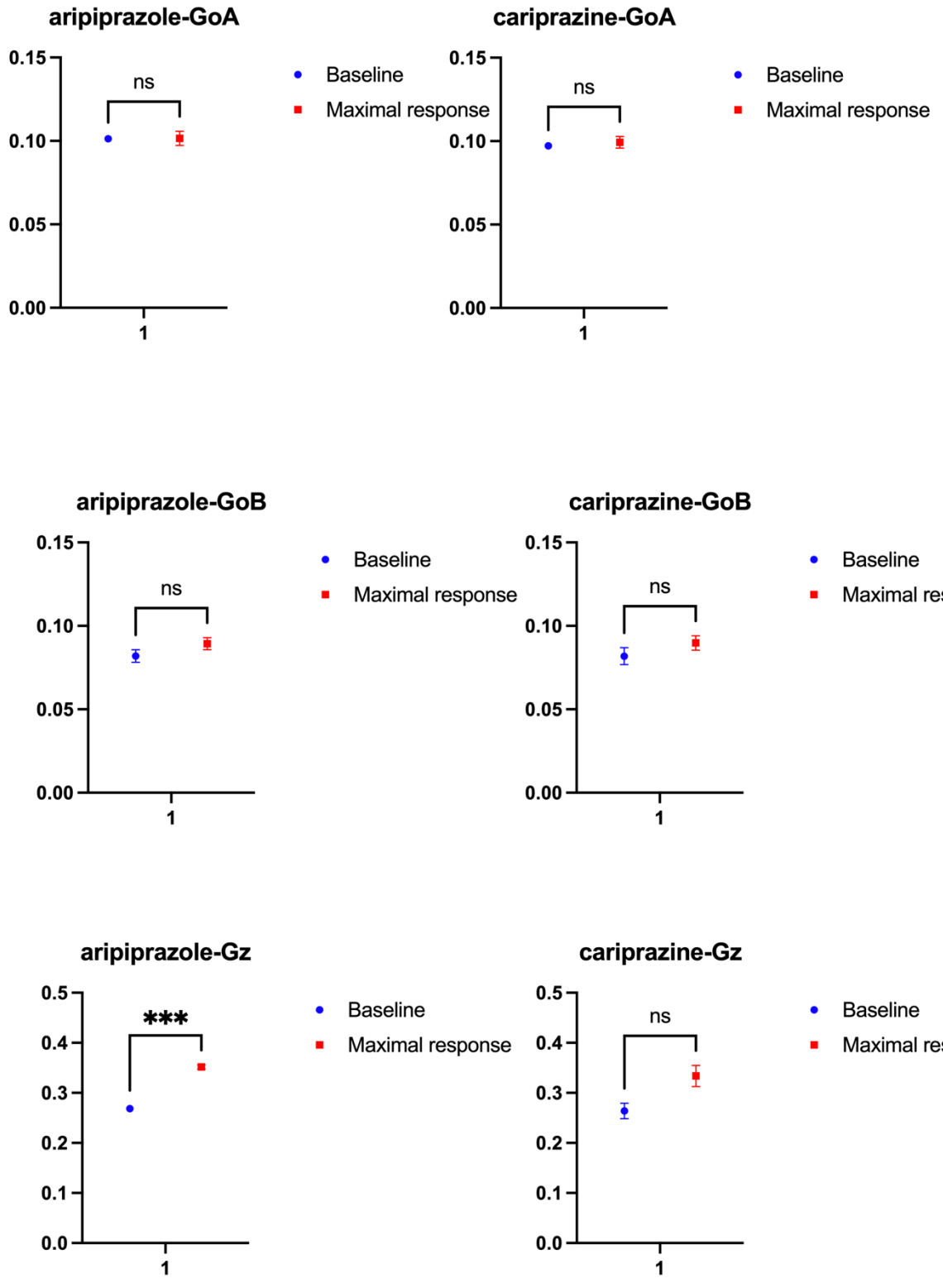
0.10
0.05
0.00
ns
1
Baseline
Maximal response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0.10
0.05
0.00
ns
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Baseline
Maximal response
cariprazine-GoA
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0.10
0.05
0.00
ns
1
Baseline
Maximal response
aripiprazole-GoB
0.15
0.10
0.05
0.00
ns
1
Baseline
Maximal response
cariprazine-GoB
0.15
0.10
0.05
0.00
ns
1
Baseline
Maximal response
aripiprazole-Gz
0.5
0.4
0.3
0.2
0.1
0.0
***
1
Baseline
Maximal response
cariprazine-Gz
0.5
0.4
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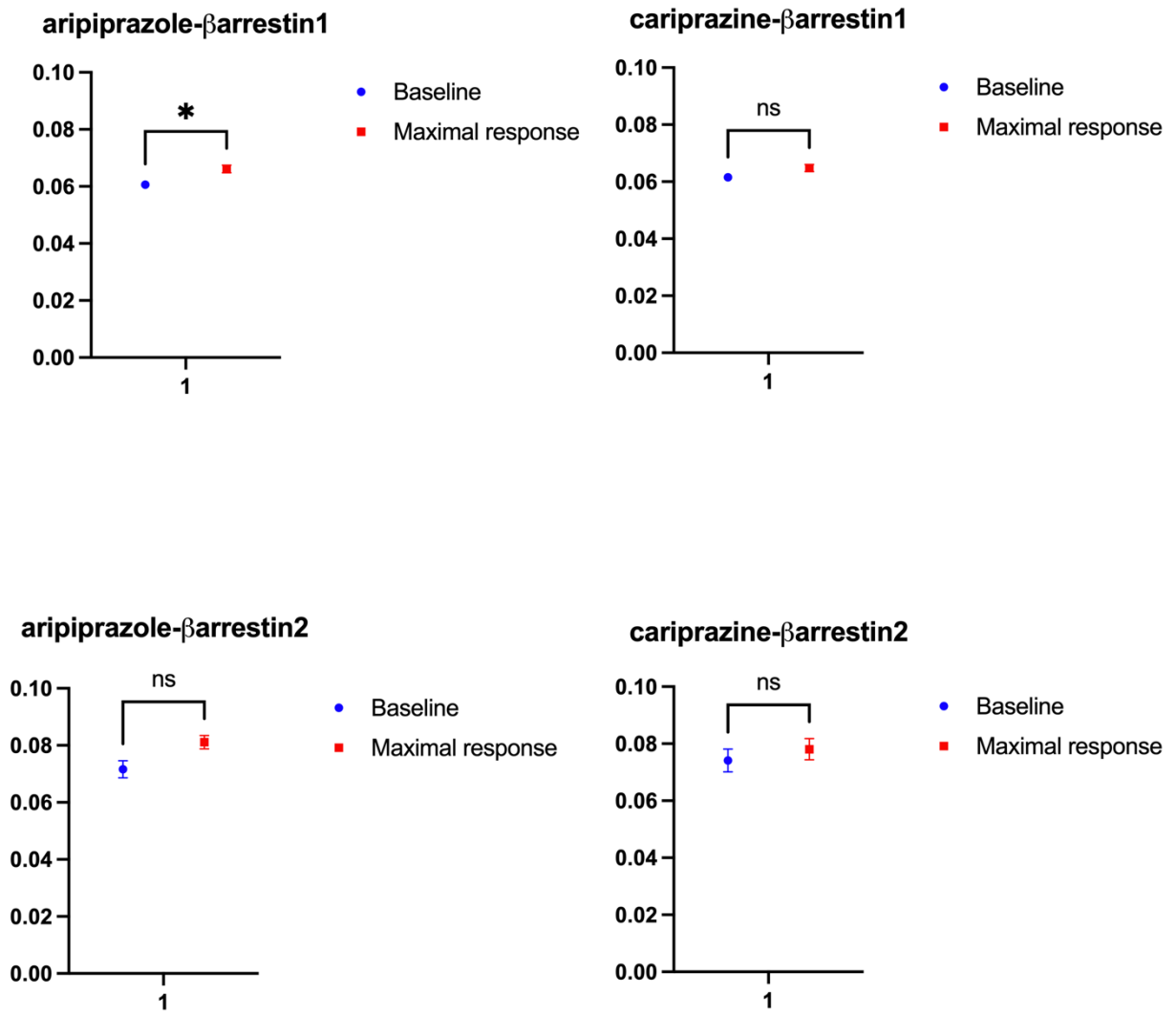
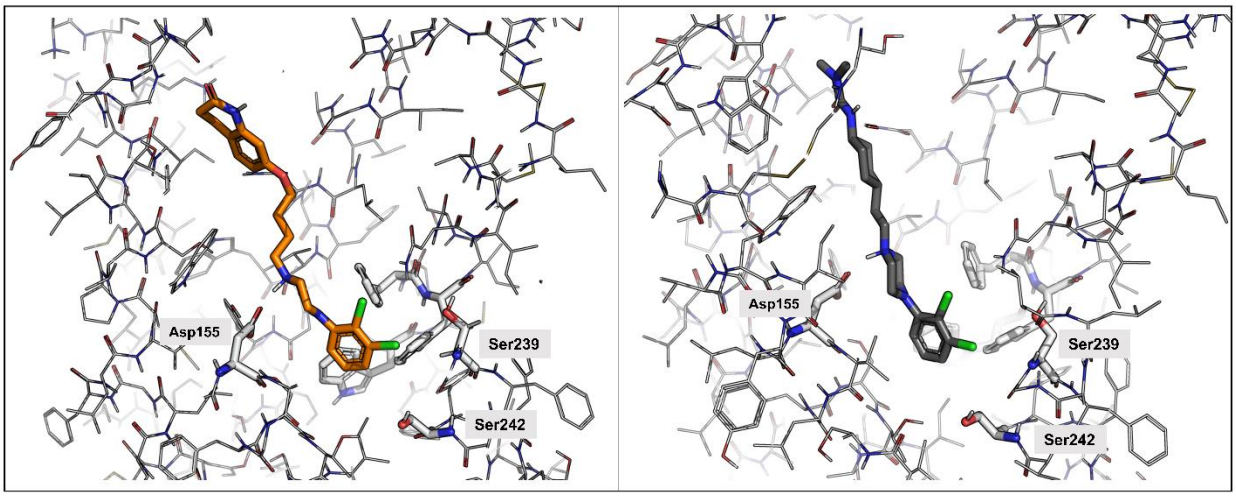
ns
1
Baseline
Maximal response



Supplementary Fig. 5 Graphs comparing the baseline and maximal response [mean ± SEM; unpaired t test (two-tailed)] from Supplementary Fig. 2 of aripiprazole and cariprazine respectively at $G\alpha_{i1}$: (mean ± SEM; n = 3;: $^{**}p$ = 0.0072 for aripiprazole), $G\alpha_{i2}$: (mean ± SEM; n = 3), $G\alpha_{i3}$: (mean ± SEM; n = 3), $G\alpha_{oA}$: (mean ± SEM; n = 3), $G\alpha_{oB}$: (mean ± SEM; n = 4), $G\alpha_{z}$: (mean ± SEM; n = 3; $^{***}p$ = 0.0005 for aripiprazole), recruitment of β-arrestin1: (mean ± SEM; n = 3; $^{*}p$ = 0.0451 for aripiprazole) and β-arrestin2: (mean ± SEM; n = 3).



Supplementary Fig. 6: Alternative poses for aripiprazole (left) and cariprazine (right), obtained from docking studies.

SUPPLEMENTARY TABLES:

Supplementary Table 1: $logEC_{50}$ and $logEC_{80}$ of 5-HT at the different G protein pathways and for recruitment of the β-arrestins

Pathway	$logEC_{50}$ ± SEM	$logEC_{80}$ ± SEM
$G\alpha_q$	-9.49 ± 0.14	-8.89 ± 0.14
$G\alpha_{11}$	-9.81 ± 0.09	-9.20 ± 0.09
$G\alpha_{14}$	-9.47 ± 0.07	-8.86 ± 0.07
$G\alpha_{15}$	-8.35 ± 0.05	-7.75 ± 0.05
$G\alpha_{i1}$	-7.26 ± 0.21	-6.66 ± 0.21
$G\alpha_{i2}$	-7.66 ± 0.22	-7.06 ± 0.22
$G\alpha_{i3}$	-8.02 ± 0.24	-7.41 ± 0.24
$G\alpha_{oA}$	-7.79 ± 0.19	-7.19 ± 0.19
$G\alpha_{oB}$	-7.65 ± 0.10	-7.05 ± 0.10
$G\alpha_z$	-8.472 ± 0.05	-7.87 ± 0.05
β-arrestin1 recruitment	-6.812 ± 0.09	-6.21 ± 0.09
β-arrestin2 recruitment	-7.020 ± 0.15	-6.42 ± 0.15

Supplementary Table 2: Inverse agonist activity of antipsychotics at the G protein pathways

	Risperidone	Clozapine	Olanzapine	Haloperidol
$G\alpha_q$	-12.56 ± 0.14	-12.31 ± 0.21	-9.81 ± 0.22	-7.58 ± 0.21
$G\alpha_{11}$	-11.35 ± 0.18	-8.16 ± 0.19	-11.06 ± 0.25	-6.77 ± 0.21
$G\alpha_{14}$	-12.20 ± 0.28	-7.45 ± 0.30	-8.35 ± 0.34	-7.13 ± 0.84
$G\alpha_z$	-9.86 ± 0.36	-6.17 ± 0.29	NA	-6.70 ± 0.49
$G\alpha_{i1}$	-10.61 ± 0.42	-8.24 ± 0.70	-6.87 ± 0.81	-8.77 ± 0.29

$logEC_{50}$ (± SEM)

NA: no activity

Supplementary Table 3: Partial agonist activity of aripiprazole and cariprazine at the G protein pathways (normalized as % response of 5-HT)

	Aripiprazole		Cariprazine	
	$logEC_{50}$	E_{max}	$logEC_{50}$	E_{max}
$G\alpha_q$	-7.87 ± 0.15	67.74 ± 4.97	-7.70 ± 0.13	67.99 ± 4.29
$G\alpha_{11}$	-8.56 ± 0.13	70.75 ± 4.97	-8.42 ± 0.08	70.72 ± 4.97
$G\alpha_{14}$	-6.94 ± 0.10	73.28 ± 3.61	-7.72 ± 0.09	68.10 ± 3.05
$G\alpha_{15}$	-7.59 ± 0.09	16.29 ± 0.71	-7.61 ± 0.08	13.93 ± 0.53
$G\alpha_{i1}$	-7.89 ± 0.37	26.30 ± 3.70	-8.91 ± 0.59	12.72 ± 3.42
$G\alpha_{i2}$	-7.73 ± 0.27	17.15 ± 1.93	-8.92 ± 0.22	11.72 ± 2.50
$G\alpha_{i3}$	-7.47 ± 0.26	21.85 ± 2.90	-7.76 ± 0.32	16.05 ± 2.57
$G\alpha_{oA}$	-9.37 ± 0.37	11.59 ± 2.90	-8.56 ± 0.46	11.59 ± 2.44
$G\alpha_{oB}$	-8.43 ± 0.38	6.64 ± 1.46	-8.17 ± 0.74	4.32 ± 2.16
$G\alpha_z$	-7.18 ± 0.30	10.64 ± 1.68	-8.90 ± 0.21	9.02 ± 0.88
βarrestin1	NA	NA	NA	NA
βarrestin2	NA	NA	NA	NA

$logEC_{50}$ (± SEM)

E_{max} (% ± SEM)

NA: no activity

Supplementary Table 4a: The potency ($logIC_{50}$) of the six antipsychotics for the inhibition of the 5-HT (EC80)-mediated activation of the different G protein pathways and recruitment of β-arrestin1 and β-arrestin2

	RISPERIDONE	CLOZAPINE	OLANZAPINE	ARIPIPRAZOLE	CARIPRAZINE	HALOPERIDOL
Gq	-10.27 ± 0.05	-8.01 ± 0.07	-9.06 ± 0.06	NA	NA	-6.42 ± 0.08
G11	-9.58 ± 0.08	-7.53 ± 0.08	-8.29 ± 0.08	NA	NA	-6.22 ± 0.11
G14	-11.46 ± 0.13	-7.90 ± 0.12	-8.77 ± 0.12	NA	NA	-6.48 ± 0.15
G15	-9.17 ± 0.07	-6.47 ± 0.10	-7.52 ± 0.09	logIC50 > 10 uM	logIC50 > 10 uM	logIC50 > 10 uM
Gi1	-9.44 ± 0.28	-5.94 ± 0.28	-7.05 ± 0.29	-5.71 ± 0.28	-6.13 ± 0.26	-6.45 ± 0.33
GoB	-12.01 ± 0.29	-7.52 ± 0.16	-8.56 ±0.15	-6.35 ± 0.16	-6.74 ± 0.16	-6.31 ± 0.20
Gz	-10.03 ± 0.13	-7.02 ± 0.15	-8.46 ± 0.21	-6.40 ± 0.19	-6.42 ± 0.24	-6.34 ± 0.19
βarrestin1	-8.42 ± 0.09	-7.29 ± 0.09	-8.22 ± 0.07	-5.97 ± 0.16	-6.32 ± 0.10	-7.74 ± 0.07
βarrestin2	-8.64 ± 0.10	-7.56 ± 0.11	-8.47 ± 0.08	-6.06 ± 0.11	-6.45 ± 0.07	-7.83 ± 0.09

NA: no activity

$logIC_{50}$ (± SEM)

Supplementary Table 4b: The efficacy (% inhibition) of the six antipsychotics for the inhibition of the 5-HT (EC80)-mediated activation of the different G protein pathways and recruitment of β-arrestin1 and β-arrestin2

	RISPERIDONE	CLOZAPINE	OLANZAPINE	ARIPIPRAZOLE	CARIPRAZINE	HALOPERIDOL
Gq	113.80 ± 2.53	101.70 ± 3.21	109.60 ± 3.02	NA	NA	134.50 ± 5.52
G11	157.70 ± 6.25	157.40 ± 6.84	154.60 ± 5.50	NA	NA	175.90 ± 10.71
G14	115.80 ± 6.89	102.70 ± 6.17	102.00 ± 5.62	NA	NA	124.10 ± 9.20
G15	93.38 ± 3.20	105.00 ± 5.24	98.75 ± 4.51	logIC50 > 10 uM	logIC50 > 10 uM	logIC50 > 10 uM
Gi1	58.21 ± 7.67	97.59 ± 16.42	79.78 ± 11.97	81.08 ± 16.91	84.84 ± 12.48	62.88 ± 10.18
GoB	66.55 ± 11.70	81.07 ± 6.73	54.86 ± 3.82	105.70 ± 8.27	97.86 ± 7.66	98.32 ± 10.19
Gz	87.39 ± 4.97	96.01 ± 7.35	63.75 ± 6.12	88.97 ± 8.43	73.31 ± 8.67	107.00 ± 10.43
βarrestin1	105.90 ± 4.36	89.36 ± 3.53	97.99 ± 3.28	101.00 ± 10.03	104.60 ± 6.03	114.30 ± 3.67
βarrestin2	101.50 ± 4.79	98.90 ± 4.82	93.39 ± 3.34	100.30 ± 6.43	100.20 ± 3.78	117.40 ± 4.69

NA: no activity

% inhibition (± SEM)

Supplementary Table 5: The equilibrium dissociation constant (logK_B) of the antipsychotics for the different pathways calculated based on the modified Cheng-Prusoff equation

	RISPERIDONE	CLOZAPINE	OLANZAPINE	ARIPIPRAZOLE	CARIPRAZINE	HALOPERIDOL
Gq	-10.27 ± 0.05	-8.01 ± 0.07	-9.06 ± 0.06	NA	NA	-6.42 ± 0.08
G11	-9.58 ± 0.08	-7.53 ± 0.08	-8.29 ± 0.08	NA	NA	-6.22 ± 0.11
G14	-11.46 ± 0.13	-7.90 ± 0.12	-8.77 ± 0.12	NA	NA	-6.48 ± 0.15
G15	-9.17 ± 0.07	-6.47 ± 0.10	-7.52 ± 0.09	logIC_{50} > 10 µM	logIC_{50} > 10 µM	logIC_{50} > 10 µM
Gi1	-9.44 ± 0.28	-5.94 ± 0.28	-7.05 ± 0.29	-5.71 ± 0.28	-6.13 ± 0.26	-6.45 ± 0.33
GoB	-12.01 ± 0.29	-7.52 ± 0.16	-8.56 ±0.15	-6.35 ± 0.16	-6.74 ± 0.16	-6.31 ± 0.20
Gz	-10.03 ± 0.13	-7.02 ± 0.15	-8.46 ± 0.21	-6.40 ± 0.19	-6.42 ± 0.24	-6.34 ± 0.19
βarrestin1	-8.42 ± 0.09	-7.29 ± 0.09	-8.22 ± 0.07	-5.97 ± 0.16	-6.32 ± 0.10	-7.74 ± 0.07
βarrestin2	-8.64 ± 0.10	-7.56 ± 0.11	-8.47 ± 0.08	-6.06 ± 0.11	-6.45 ± 0.07	-7.83 ± 0.09

NA: no activity