Supporting Information

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SI Methods

Cell Culture and Transfections. To generate HEK cells stably expressing EPAC biosensor and the HA tagged mouse dopamine $D2_L$ receptor, HEK-293T cells were first transfected with the EPAC construct and selected to permanently express the EPAC sensor using zeocin (200–400 μg/ml) and then kept in culture with 75 μg/ml of zeocin. These cells were then transfected with HA-$D2_L$R and selected for HA-$D2_L$R expression using hygromycin (300 μg/ml) and sorted by Fluorescence-Activated Cell Sorting (FACS).

For the BRET experiments, 24 h after transfection, the cells were plated in polyD-lysine coated 96-well microplates (Well Assay Plate with clear bottom, Fisher Scientific) at a density of 100,000 cells per well in phenol red free Minimum Essential Medium containing 2% of FBS, 10 mM Hepes, 2 mM L-glutamine. The cells were then cultured for an additional 24 h.

cDNA constructs. Mouse D2 long dopamine receptor was amplified by PCR using a 5′ primer (AAAGATATCATGGATCCACTGAACCTGTCC) containing an E*co* RV restriction site and a 3′ primer (AAAGCCTCAGCAGTGCAGGATCTTCAT) containing a N*ot* I restriction site. PCR was performed on a mouse striatum cDNA library and the PCR product was cloned into a pcDNA3 vector downstream three hemagglutinin tag (HA) which generated amino-terminally HA-tagged $D2_L$R. Expression vector encoding the HA-D2 Long receptor fused to the *Renilla* luciferase (*R*luc) was generated as follows. The full length coding region of mouse $D2_L$R containing the HA tag without a stop codon was amplified by PCR using specific primers with 5′ and 3′ in-frame restriction enzyme sites of X*ho* I and K*pn* I, respectively, and subcloned into humanized ph*R*luc N3 (PerkinElmer) vector. The stop codon was replaced by three arginines to facilitate the natural palmitoylation of cysteine in the C-terminal tail.

The mouse β-arrestin 2 was also amplified from the same cDNA library using a 5′ primer (AAAAAGCTTATGGGAGAAAAACCCGGGAC) containing a H*ind* III restriction site and a 3′ primer (AAAGGGCCCGGCAGAACTGGTCATCACAGT) containing an A*pa* I restriction site and cloned directly into pEYFP N1 mutating the stop codon. All constructs were sequenced to check reading frame and integrity.

The BRET sensor for cAMP was constructed by the modification of an existing FRET-based intramolecular biosensor EPAC (indicator of cAMP using Epac) in which residues 148–881 of Epac1 (exchange proteins activated directly by *c*yclic AMP) were surrounded by eCFP upstream and citrine downstream (19). Using the restriction enzymes B*am*H I and K*pn* I, the eCFP was removed and replaced by a humanized *Renilla* luciferase gene that was PCR amplified from ph*R*luc-C1 (PerkinElmer) and cloned using the same restriction sites to preserve the frame of translation.

Bioluminescence Resonance Energy Transfer Measurement. The BRET signal is determined by calculating the ratio of the light emitted at 515–555 nm over the light emitted at 465–505 nm. Curves were fitted using a non linear regression and log (agonist or inhibitor) vs. response fit equation using GraphPad Prism 5.

To determine the acceptor/donor ratio for titration experiments (Fig. 2*A* and supporting information (SI) Fig. S2), the expression level of each tagged protein was determined by direct measurement of total fluorescence and luminescence from 90,000 cells for each transfection as previously described (28). Briefly, cells were rinsed with PBS and then detached using calcium and magnesium free PBS.

To measure the total fluorescence, 90 μl of cells were distributed in 96-well microplates (Black OptiPlate, PerkinElmer) at a density of approximately 90,000 cells per well. The total fluorescence of cells was measured using a Mithras LB940 instrument with an excitation filter at 485 nm (filter F0251A Chroma), an emission filter at 535 nm (filter F0250A Chroma), and the following parameters: lamp energy, 7000; time, 0.5 s. For each measurement, the mean of duplicate wells was calculated. The total fluorescence was then divided by the background determined in wells containing empty vector transfected cells. To measure the total luminescence, 90 μl of cells corresponding to each transfection were distributed in 96-well microplates (White OptiPlate, PerkinElmer) at a density of approximately 90,000 cells per well. The assay was started by adding the *R*luc substrate to the cell suspension to yield a final concentration of 5 μM. Ten minutes later, readings were collected using a Mithras LB940 instrument. The values were corrected by subtracting the background signal detected when the Receptor-*R*luc construct was expressed alone. To establish the ratio YFP/*R*luc for each transfection, the YFP fluorescent signals were converted into luminescent signals as already described (28).

Net BRET signals were determined by subtracting the BRET signal obtained with cells only expressing *R*luc-tagged $D2_L$R from BRET signals obtained with cells co-expressing both *R*luc-tagged $D2_L$R and YFP-tagged β-arrestin 2. Curves were fitted using a nonlinear regression and one-site binding (hyperbola) fit equation using GraphPad Prism 5.

Fluorescence Microscopy. One day after transfection, HEK293 cells transiently expressing HA-$D2_L$R and β-arrestin2-YFP were seeded onto collagen coated 35 mm Matek glass, coverslip bottomed dishes, and allowed to recover for 24 h. The day of the experiments, cells were starved at 37 °C for 1 h in Minimum Essential Medium containing 10 mM Hepes and 0.1% BSA. They were then pretreated with the different drugs as described in Fig. 5 for 30 min and then quinpirole was added or not in the medium for 30 min. Images were acquired using a Zeiss LSM-510 confocal microscope equipped with a 100× NA 1.4 oil objective using 488 nm excitation for the YFP tag and employing the accompanying fluorescein emission filter set.

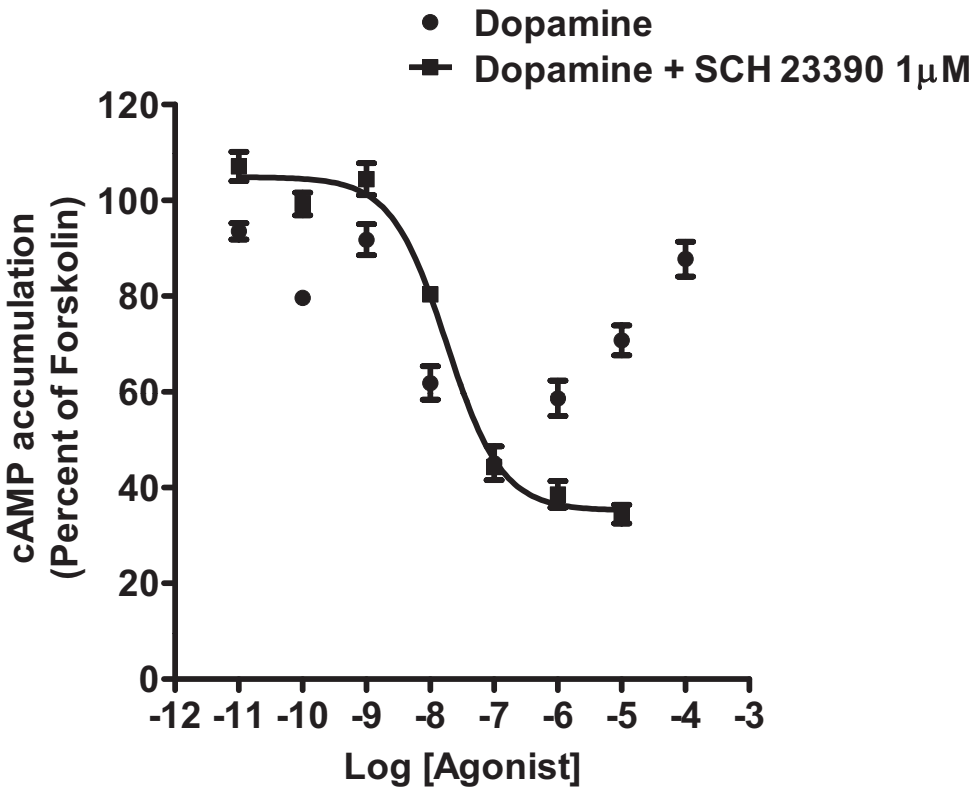
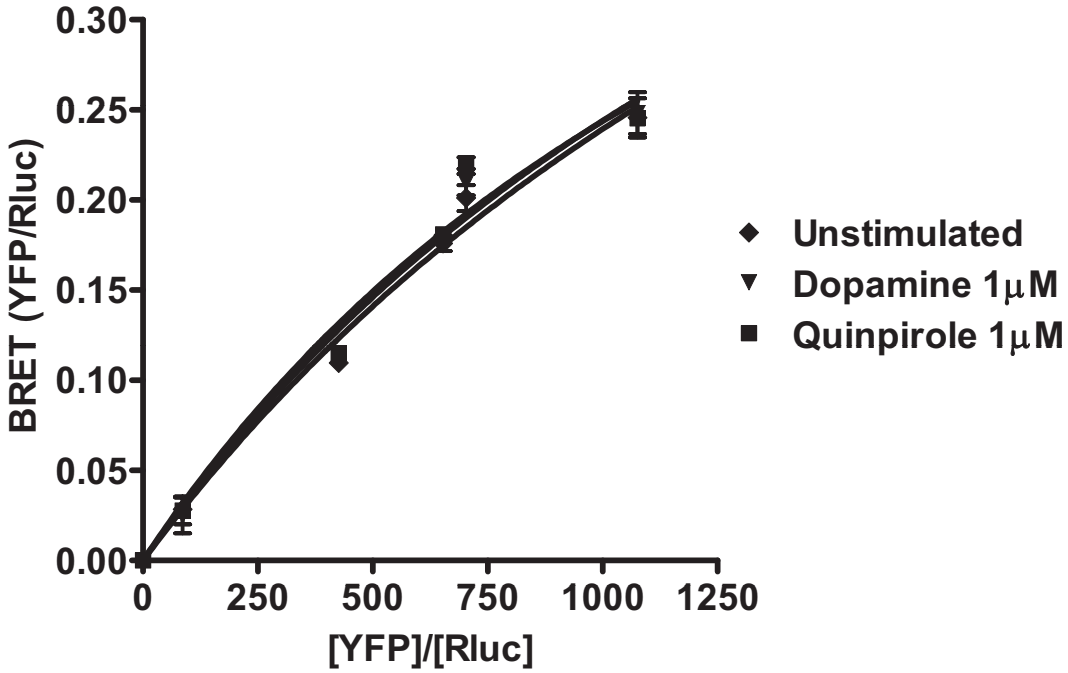


Fig. S1. BRET measurements of cAMP variations induced by Dopamine on dopamine D2L receptor in living cells. HEK cells stably expressing the EPAC biosensor and D2LR were stimulated with dopamine (●). Note the shape of the curve where, at low doses of dopamine, cAMP content in the cells decreases. Starting at 10 μM, cAMP production starts to increase due to the dopamine D1 receptor endogenously expressed in HEK 293 cells. This effect is abolished when cells are pretreated with a specific D1R antagonist SCH23390 (■). cAMP production was normalized to the percentage of forskolin-stimulated cAMP accumulation (set at 100%) and data represent the mean ± SEM of 3 independent experiments each performed in duplicate.



	Unstimulated	Dopamine 1 μM	Quinpirole 1 μM
BRET MAX	0.7903	0.7393	0.6626
BRET 50	2303	2030	1720

Fig. S2. BRET titration curve for the recruitment of YFP alone to dopamine D2L receptor. BRET was measured in cells expressing a fixed amount of *R*luc-tagged D2LR and increasing amounts of YFP as in Fig. 2*A*, treated (with dopamine 1 μM [▼] or quinpirole 1 μM [■]) or not [♦]). BRETmax and BRET50 are presented in the table.

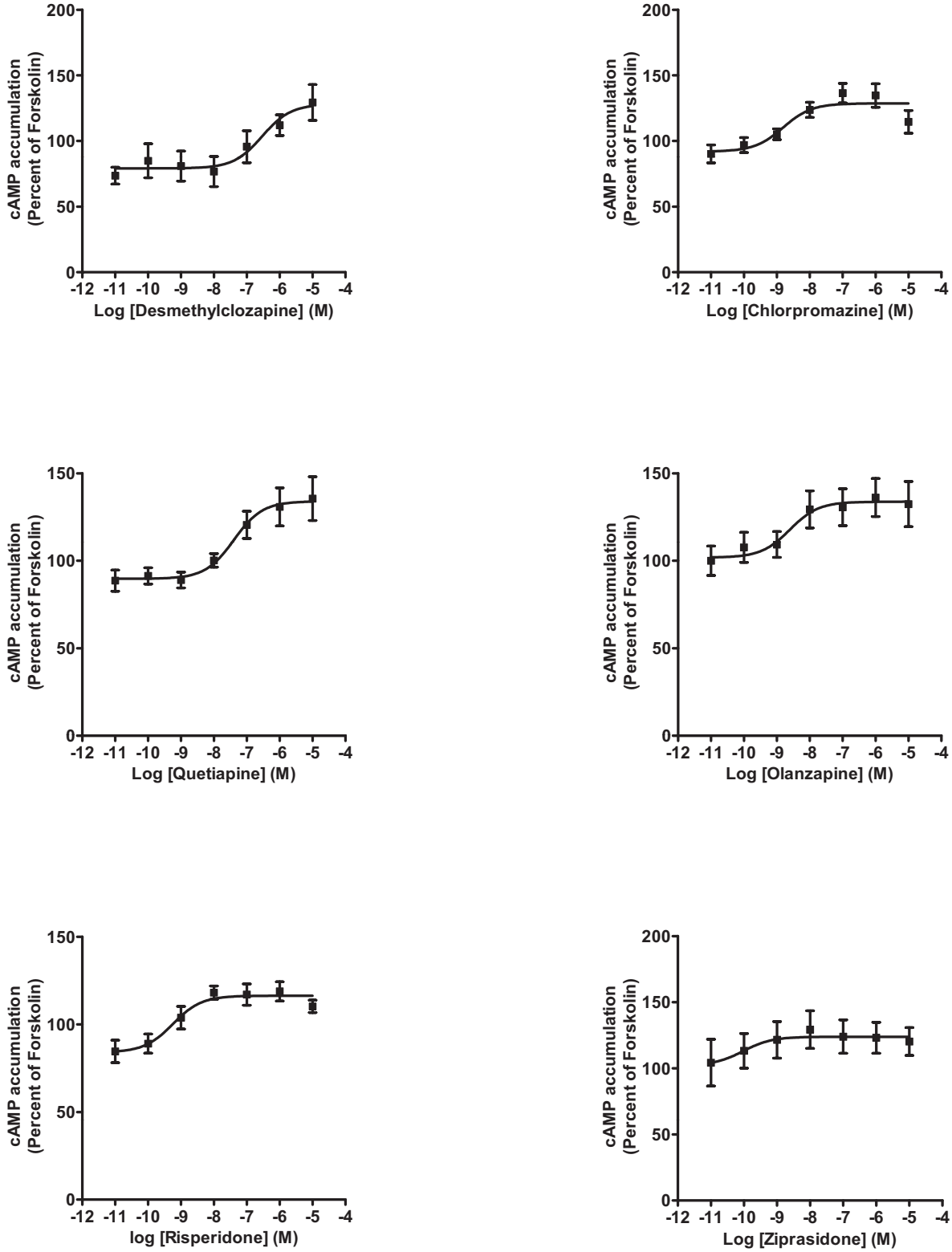


Fig. S3. Intrinsic activity of desmethylclozapine, chlorpromazine, quetiapine, olanzapine, risperidone, and ziprasidone on $G_{i/o}$ activation-$D2_LR$ mediated. HEK 293 cells stably expressing EPAC biosensor and $D2_LR$ were stimulated with different antipsychotics in presence of forskolin (25 μM). cAMP production was normalized to the percentage of forskolin-stimulated cAMP accumulation (set at 100%). Data represent the mean ± SEM of 3 to 5 independent experiments each performed in duplicate.

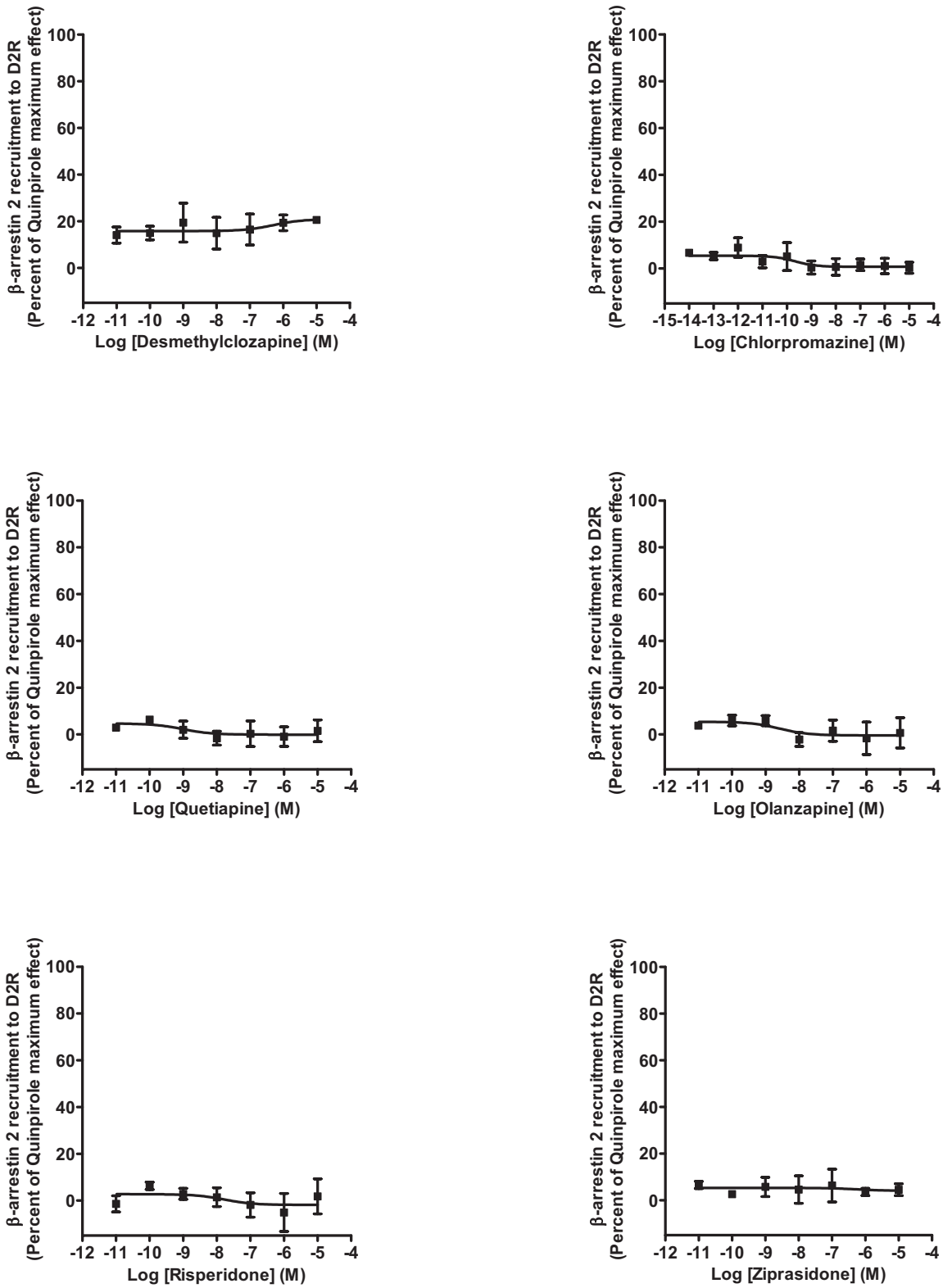


Fig. S4. Concentration-response curve of desmethylclozapine, chlorpromazine, quetiapine, olanzapine, risperidone and ziprasidone for β-arrestin 2 recruitment to $D2_LR$. Cells coexpressing *R*luc-tagged $D2_LR$ and YFP-tagged β-arrestin 2 were stimulated with different antipsychotics. Results are expressed in percent of quinpirole maximum effect. Data represent the mean ± SEM of 3 to 4 independent experiments each performed in duplicate.

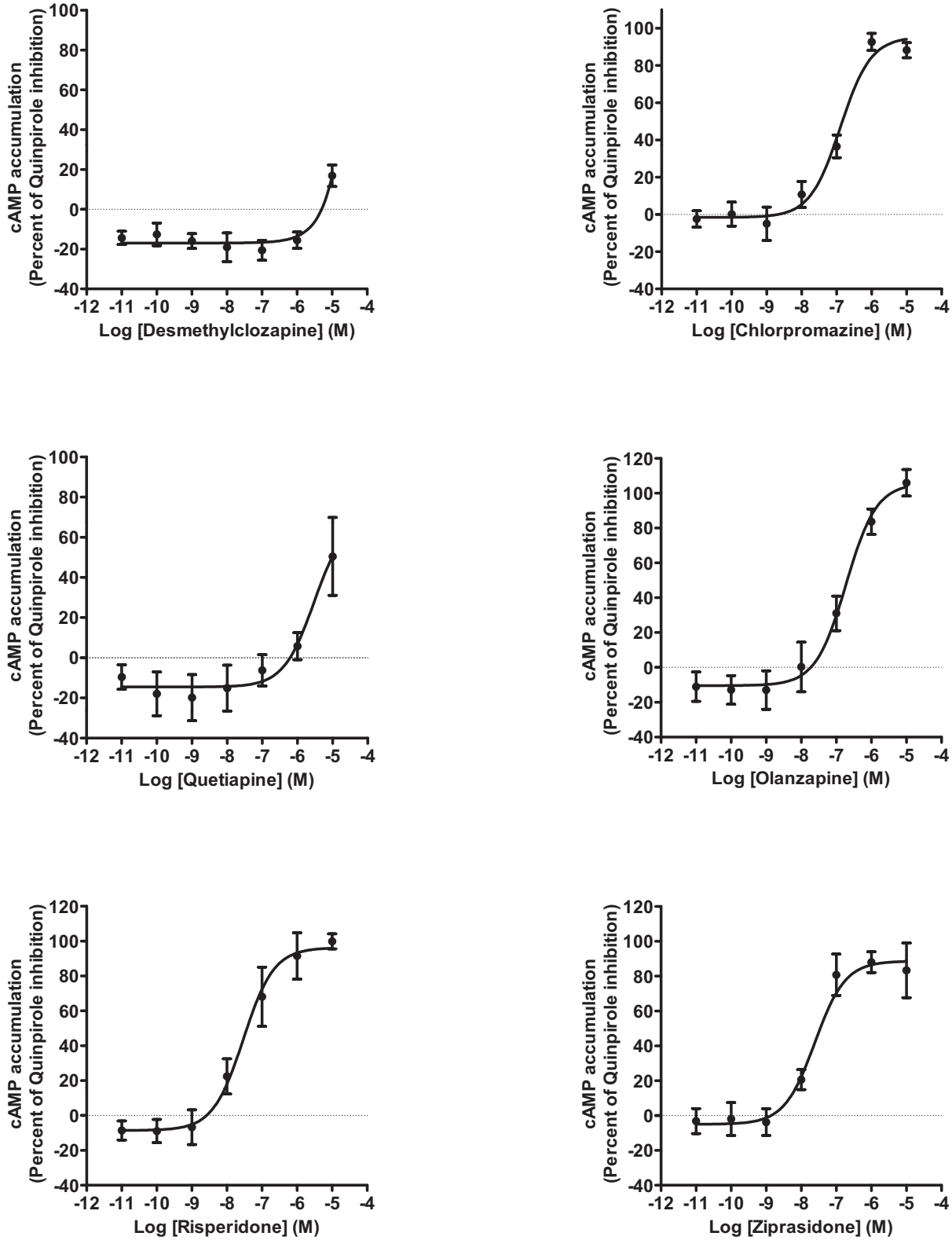


Fig. S5. Antagonist activity of desmethylclozapine, chlorpromazine, quetiapine, olanzapine, risperidone and ziprasidone on $G_{i/o}$ activation induced by quinpirole. Concentration-response curve of different antipsychotics for inhibiting adenylylcyclase inhibition induced by quinpirole. HEK 293 cells stably coexpressing EPAC biosensor and $D2_LR$ were treated with the different antipsychotics and quinpirole (1 μM) in the presence of forskolin (25 μM). Data represent the mean $\pm$ SEM of 3 to 4 independent experiments each performed in duplicate.

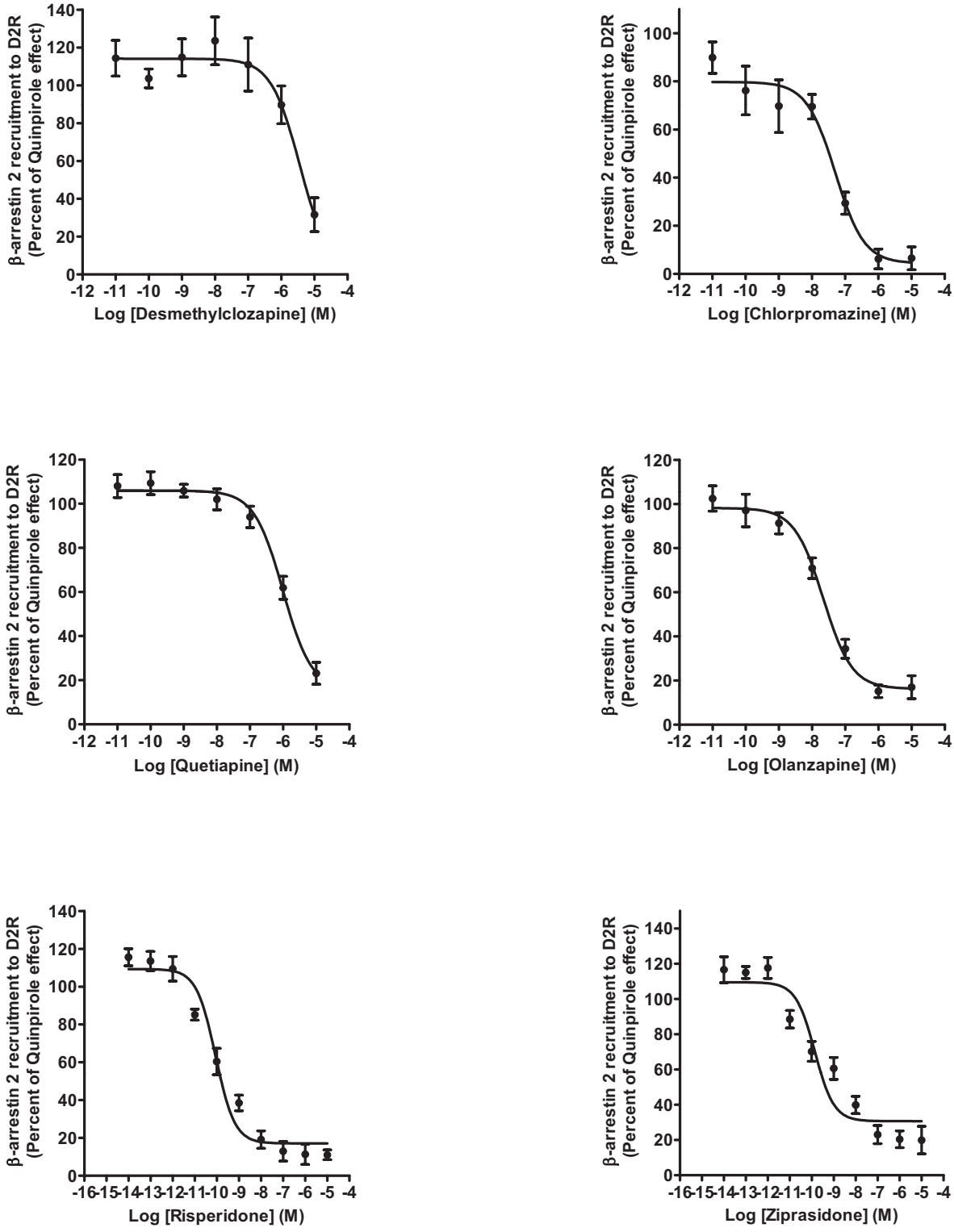


Fig. S6. Antagonist activity of desmethylclozapine, chlorpromazine, quetiapine, olanzapine, risperidone and ziprasidone on β-arrestin 2 recruitment to $D2_LR$ induced by quinpirole. BRET was measured in cells coexpressing *R*luc-tagged $D2_LR$ and YFP-tagged β-arrestin 2. Cells were pretreated with the different antipsychotics as described in *Methods* section, and then stimulated with quinpirole (1 μM). Data are expressed as the percentage of quinpirole maximum effect (1 μM).