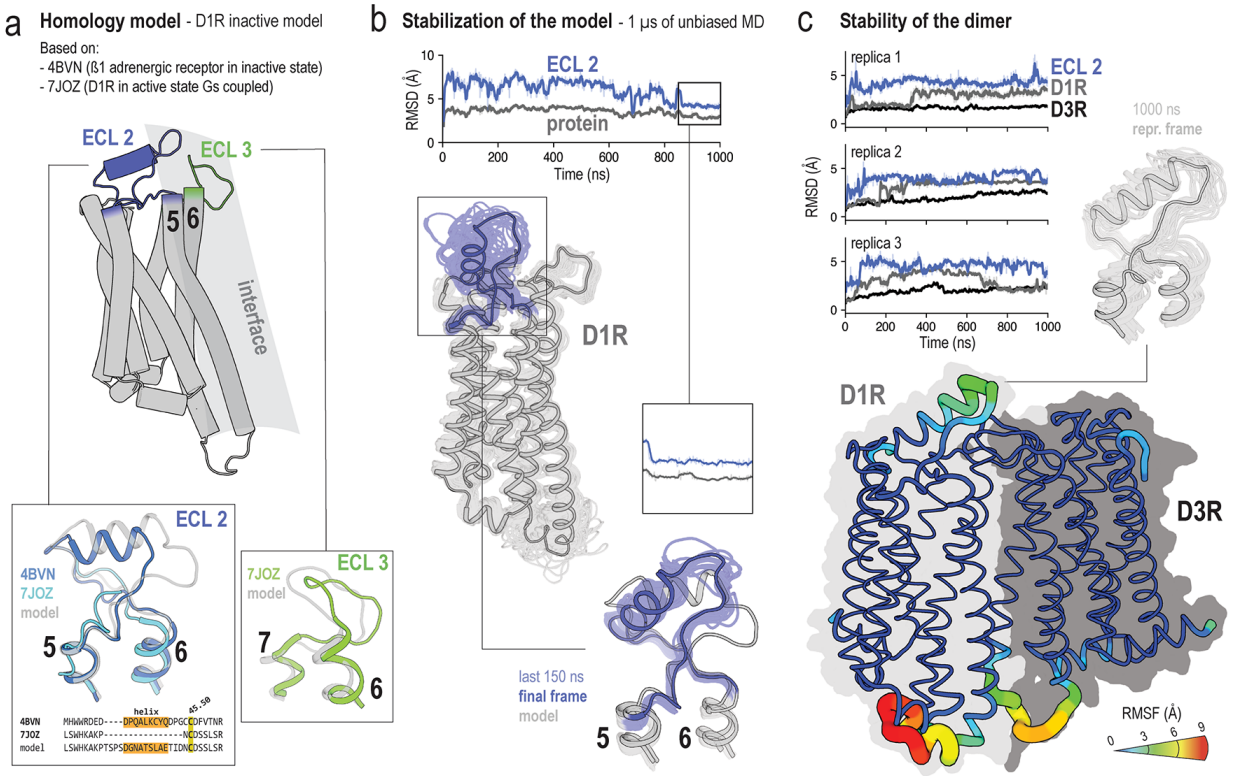
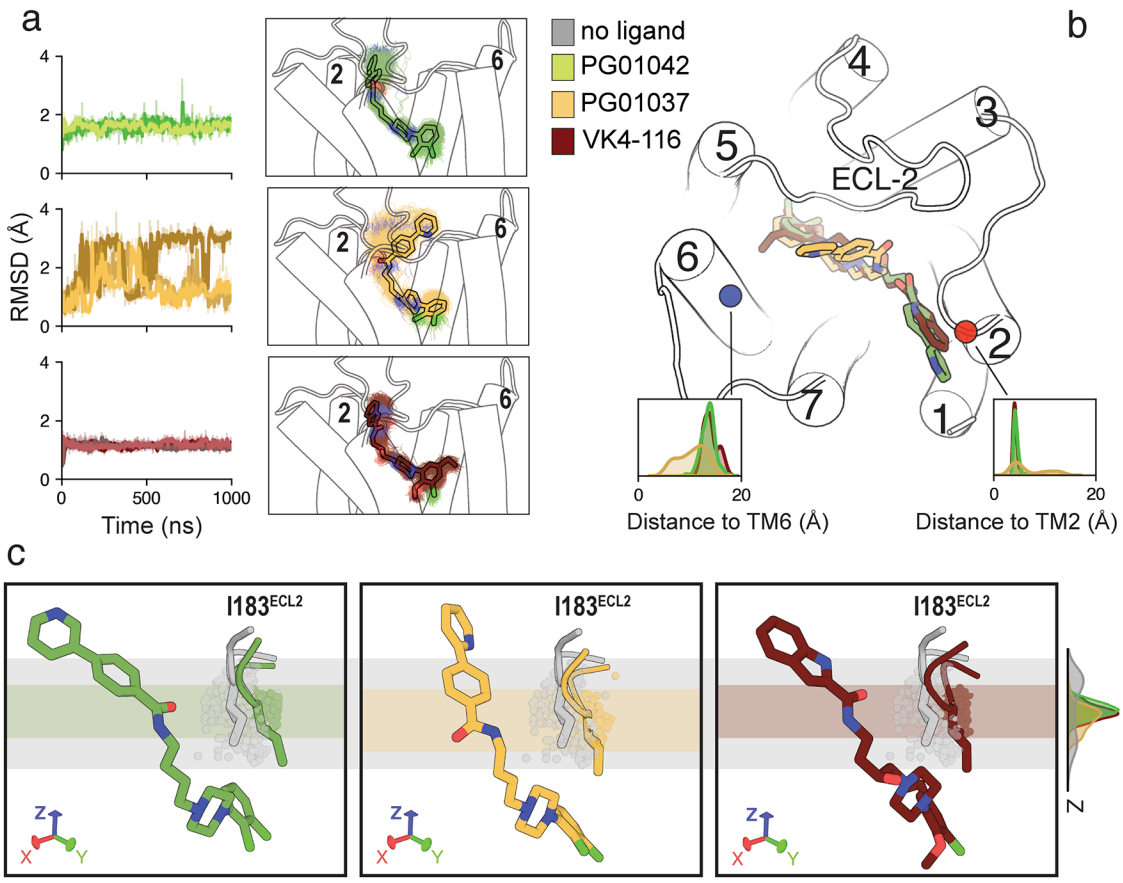
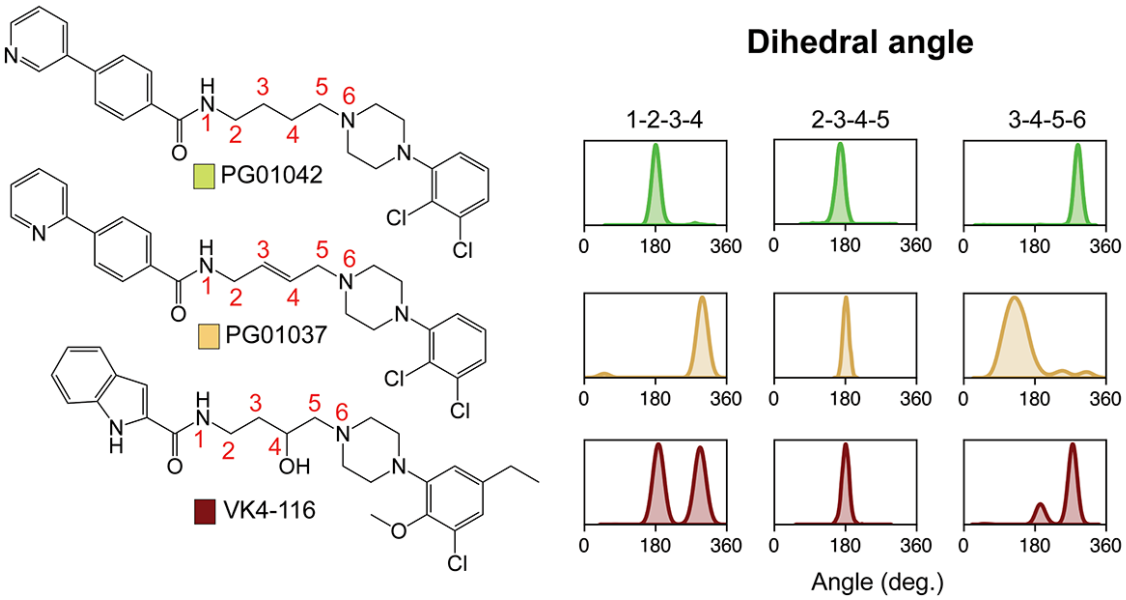
Supplementary Information: Suppl. Figs. 1, 2, 3 and 4



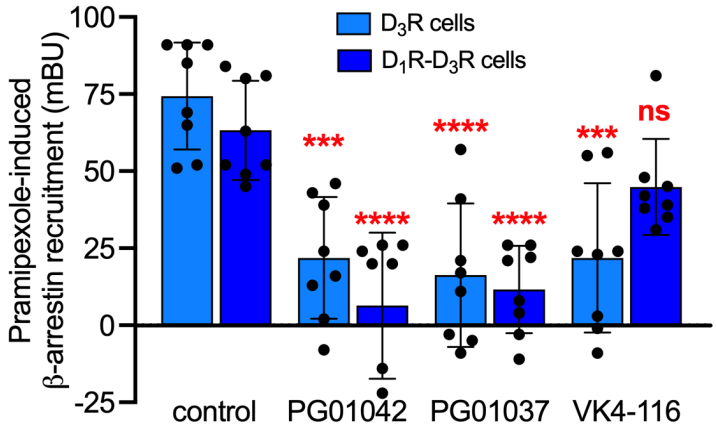
Suppl. Figure 1. Modeling of the D_1R in the D_1R-D_3R heteromer. **a.** The inactive model of D_1R was constructed from the structures of inactive $ß_1$ adrenergic receptor (61% and 42% of sequence similarity and identity, respectively) and active D_1R. ECLs 2 (blue) and 3 (green) are important to remark because they might participate in the TM 5/6 interface. The bottom panel (right) shows ECL 3 in the model (gray) and in the structure of active D_1R (green). However, ECL 2 is not solved in any of the known structures of D_1R and was modeled using the structure of the $ß_1$ adrenergic receptor as template. The bottom panel (left) shows ECL 2 in the model (gray), in the $ß_1$ adrenergic receptor (dark blue), and the solved part in D_1R (light blue). The sequence alignment between D_1R (7JOZ only shows the amino acids solved in the structure) and $ß_1$ adrenergic receptor is also shown. **b.** The stability of this computational model of D_1R was tested in 1 µs unbiased MD simulation. RMSD plots show that, as expected, the modeled ECL 2 is more flexible than the TM domain. However, ECL 2 seems stabilized in the last 150 ns of simulation. Evolution of the structures of D_1R (gray) and ECL 2 (blue) along the MD simulation is shown in the middle panel and a detailed view of the structures of ECL 2 during the last 150 ns is shown in the bottom panel. **c**. A representative structure of the D_1R obtained during the last 150 ns was respawned in three replicas of 1 µs unbiased MD simulation of the D_1R-D_3R heteromer. RMSD values for D_1R and D_3R and for the ECL 2 are shown in the top panel. Average root-mean-square fluctuation (RMSF) of the D_1R-D_3R heteromer is shown in the bottom panel. RMSF accounts for the average fluctuation of the structure along the simulation. A close-up of the ECL 2 is shown for one of the replicas.



Suppl. Figure 2. Molecular dynamic simulations of the D_3R monomer bound to PG01042, PG01037, and VK4-116. a. Rmsd of three replicas of 1 μs unbiased MD simulations. Representative structures (solid sticks) and evolution (lines) of PG01042 (green), PG01037 (orange) and VK4-116 (red) in complex with D_3R (white cylinders, only the initial structure is shown) during the MD simulations. **b.** Comparison of these representative structures in the initial structure of D_3R. Distribution of the distances between the center of mass of the terminal ring in the second pharmacophore unit of these ligands and $N352^{6.58}$ in TM 6 (blue dot) and $G93^{2.68}$ in TM 2 (red dot). PG01042 and VK4-116 remained stable at the ECD near TM 2, whereas PG01037 favors its interaction with TM 6. **c.** Detailed view of I183 of D_3R during MD simulations with no ligand bound (gray) and PG01042 (green), PG01037 (orange) and VK4-116 (red) bound to D3R. Distributions of the Z coordinate (see legend of Fig. 2) corresponding to the C_α atom of $I183^{ECL2}$ during MD simulations. The range of the Z coordinate during the MD simulations are shown by the rectangles, color-coded according to the ligand bound. No significant differences in the position of $I183^{ECL2}$ between ligands are observed.



Suppl. Figure 3. Distribution of dihedral angles of PG01042, PG01037, and VK4-116 in the simulations of their complexes with D_3R. The dihedral angles are calculated for the chain linking both pharmacophore units. Each plot accounts for the accumulated distribution of the three replicates. Atoms involved in the angles are referenced in red in the 2D structures.



Suppl. Figure 4. Differential effects of PG01042, PG01037, and VK4-116 on pramipexole-induced β-arrestin recruitment in HEK-293T cells transfected with D_3R alone or with D_1R. Results from β-arrestin recruitment experiments in HEK-293T cells transfected with β-arrestin-1-Rluc cDNA (0.5 µg), D_3R-YFP cDNA (1 µg cDNA) with or without D_1R cDNA (1.5 µg cDNA) (D_1R-D_3R cells and D_3R cells, respectively). Cells are treated for 10 min with the D_2-like receptor agonist pramipexole (30 nM) or the D_3R ligands PG01042, PG01037 and VK4-116 (10 nM). Coelenterazine H (5 µM) was added before pramipexole or the selective D_3R ligands for 7 minutes and β-arrestin-1 recruitment was measured by BRET (see Material and Methods). Values are mean ± S.D. (n = 8). ***, **** or **ns**: $p < 0.001$, $p < 0.0001$ or not significant ($p > 0.05$) *versus* corresponding D_3R or D_1R-D_3R cells only treated with pramipexole (control) (one-way ANOVA followed by Dunnett's post hoc comparisons).