Supplementary Material (Supplementary Figure Legends and Tables)

Supplementary Figure 1. Alignment of the human D2S and D3 receptor primary amino acid sequence. The putative transmembrane regions are indicated in gray. Underlined amino acids in bold lettering represent the different regions that were swapped between D2S and D3 receptor to generate the various chimeric receptors. The amino acids in the box represent the region that was specifically swapped in the D2S-D3IL2TM4EC2 chimeric receptor. Amino acids that are identical (:) or similar (.) between the two receptor subtypes are also indicated.

Supplementary Figure 2. Structural models of D2S (orange), D3 wild type (blue) and D2 D3IL123TM4EC2 chimera (cyan) represented as cartoons are superimposed. The D2 D3IL123TM4EC2 chimera show significant structural homology to D3 wild type receptor than the original D2S receptor.

Supplementary Figure 3. Structural superposition of D3-D187A mutant activated by quinpirole (red), D3-D187A activated by PD128907 (blue) and D3-C147K activated by quinpirole (orange) illustrated in cartoon models show that they stabilize in close non-tolerance inducing conformations with rmsd ranging between 1.79Å and 2.05Å.

Supplementary Figure 4. Root mean squared deviations of PD128907-activated wild type D3 receptor (blue), PD128907-activated D3-C147K mutant (black) and PD128907-activated D3-D187A mutant during the 25ns long molecular dynamics simulation is plotted using VMD trajectory analysis module. Structural super positioning of the receptor models are shown in Figure 8.

Supplementary Figure 5. Root mean squared deviation (rmsd) measured in Å between PD128907-induced wild type D3 receptor conformation (tolerance inducing) and PD128907-induced D187A mutant D3 receptor conformations (tolerance non-inducing) is plotted along the D3 sequence. The regions that are significantly different between the tolerance inducing

and non-inducing conformations are indicated by black open braces and correspond to IL2-TM4-EC2-TM5 and EC3 regions of the D3 receptor.

Supplementary Table 1.

Construct Name	Quinpirole-induced GIRK response (pA/pF)	PD128907-induced GIRK response (pA/pF)
Wild type D3	6.1 ± 0.5[a]	6.4 ± 0.65[b]
D3 D187A	5.8 ± 0.21	7.8 ± 0.66
D3 C147K	6.3 ± 0.6	8.1 ± 1.1
D3 H354L	0[c]	3.9 ± 0.5[d]
D3-D2IL2	9.1 ± 0.77	9.3 ± 1.7
D3-D2IL23T	10.6 ± 0.97	8.7 ± 1.1
Wild type D2	9.2 ± 0.85	11 ± 1.03
D2-D3IL2	8.9 ± 0.69	7.9 ± 1.0
D2-D3IL23T	10.0 ± 1.3	10.5 ± 1.1
D2-D3IL123T	9.2 ± 1.4	12.1 ± 1.6
D2-D3IL123T TM4	10.3 ± 1.04	9.02± 0.59
D2-D3IL123TTM4EC1	8.1 ± 0.96	8.9 ± 1.6
D2-D3IL123TTM4EC2	7.9 ± 0.64	8.0 ± 0.66

The acute 100 nM quinpirole[a]- and 100 nM PD128907[b]-induced current density in AtT20-cells transfected with wild type human D3 receptor was significantly different than all other receptors, $P<0.01$, ANOVA, post-hoc, Holm-Sidak test. D3 H354L mutant receptor did not elicit GIRK response to 100 nM quinpirole[c] and its GIRK response to 100 nM PD128907[d] was significantly reduced, $P<0.01$, Student's t-test.

Supplementary Table 2

Chimeric construct*	Rmsd compared to wild type D2 receptor (Å)	Rmsd compared to wild type D3 receptor (Å)
D2D3IL1	0.8	2.9
D2D3IL2	1.2	2.5
D2D3TM4	1.8	1.96
D2D3TM4EC1	1.2	2.3
D2D3IL123TM4EC1	1.1	2.0
D2D3IL123TM4EC2	2.1	1.48

* IL3 loop in both D3 and D2 receptors was not included in the root mean squared deviation (rmsd) calculations as they were modeled as truncated regions with constraints.

Rmsd computed for the various chimeric receptors were compared to wild type D2 and D3 receptors. Only main chain atoms were used for computing the rmsd.

Supplementary Table 3

Construct Name	PD128907-induced GIRK response (pA/pF)
D2-D3IL123T TM4 EC2	8.0 ± 0.66
D2-D3IL123T TM4 EC2(P178Q)	8.4 ± 1.2
D2-D3IL123T TM4 EC2(V180E)	9.5 ± 0.7
D2-D3IL123T TM4 EC2(S182I)	7.8 ± 2.2
D2-D3IL123T TM4 EC2(S184A)	14.8 ± 1.9[a]
D2-D3IL123T TM4 EC2(D187A)	7.8 ± 0.77
D2-D3IL123T TM4 EC2(D187E)	8.7 ± 0.85
D2-D3IL123T TM4 EC2(D187L)	9.1 ± 1.1
D2-D3IL2 TM4 EC2	5.9 ± 1.1

The acute 100 nM PD128907[a]-induced current density in AtT20-cells transfected with the D2-D3IL123T TM4 EC2(S184A) chimeric/mutant receptor was significantly different than all other receptors, $P<0.05$, ANOVA, post-hoc, Holm-Sidak test.