	PDB ID	Ligand	Nε1/ Hε1 Chemical Shifts (ppm)					
			W29	W32	W129	W143	W246	W268
Antagonists	3EML	ZM241385	0.139 0.188	-0.005 -0.016	0.138 0.128	0.055 0.070	-0.713 -0.480	-0.022 -0.021
	3REY	XAC	0.275 0.327	0.019 0.010	0.118 0.112	0.130 0.156	-1.049 -0.819	-0.030 -0.024
	3RFM	Caffeine	0.233 0.233	0.001 0.008	0.105 0.107	0.093 0.130	-1.020 -0.780	-0.017 -0.017
Agonists	2YDV	NECA	0.340 0.337	0.041 0.030	0.062 0.061	0.149 0.139	-0.506 -0.248	-0.040 -0.041
	3QAK	UK432097	-0.005 -0.003	-0.155 0.026	0.100 0.084	0.110 0.114	-0.478 -0.222	-0.005 -0.003
	4UHR	CGS21680	-0.014 -0.045	-0.070 -0.044	0.060 0.047	0.060 0.110	-0.352 -0.186	-0.051 -0.048
	5G53	NECA + mini Gs	-0.053 -0.115	0.034 -0.124	0.103 0.097	0.121 0.105	-0.178 -0.024	-0.011 -0.009

Table S1 (Related to Figures 2 to 4). Ring Current Shifts for the Tryptophan Indole ^{15}N–^{1}H Signals Calculated from the Crystal Structures.

Ring current shifts were calculated with the program MOLMOL (Koradi et al., 1996) for the tryptophan Nε1 and Hε1 atoms in six crystal structures of $A_{2A}AR$ complexes with ligands of different efficacies (Figures 3E and 4, A–C).

Chemical Shifts of Numbered Signals for $A_{2A}AR$ Variants (ppm)[1]							
Peak Number	$A_{2A}AR$	W29Y	W32Y	W129Y	W143Y	W246F	W268Y
1	6.75 100.97	6.75 100.99	6.74 100.85	6.70 100.87	6.91 101.18	6.74 100.97	6.75 100.94
2	7.59 104.63	7.59 104.68	7.64 104.70	7.64 104.72	7.59 104.63	7.60 104.68	7.60 104.68
3	7.68 105.35	7.68 105.31	7.69 105.35	7.69 105.50	7.69 105.33	7.68 105.33	7.69 105.33
4	7.13 106.15	7.13 106.07	7.14 106.19		7.09 106.07	7.14 106.17	7.14 106.12
5	8.67 108.95	8.67 109.04	8.67 108.94	8.65 108.95	8.63 109.64	8.66 108.95	8.67 108.97
6	9.02 110.39	9.03 110.42	9.02 110.37	8.99 110.76	9.03 110.42	9.03 110.39	9.03 110.44
7	7.80 110.80	7.80 110.78	7.81 110.80	7.80 110.81	7.84 110.89	7.79 110.75	7.80 110.77
8	6.86 110.46	6.87 110.42	6.87 110.39	6.87 110.54	6.91 110.34	6.87 110.44	6.88 110.42
9	7.91 111.64	7.94 112.07	7.91 111.60		7.93 111.62	7.79 110.75	7.92 111.60
10	6.33 111.77	6.32 111.69	6.32 111.76	6.33 111.94	6.31 111.69	6.32 111.72	6.32 111.72
11	8.79 112.89	8.80 112.87	8.80 112.89	8.78 112.87	8.79 112.89	8.85 113.21	8.80 112.89
12	8.25 114.83	8.24 114.90	8.25 114.90		8.24 114.87	8.24 114.90	8.25 114.87
13	8.09 114.65	8.09 114.65	8.10 114.66	8.01 114.58	8.08 114.68	8.09 114.71	8.11 114.65
14	8.98 116.90	9.02 117.04	8.98 116.80		9.00 116.73	7.92 111.66	9.01 116.85
15	8.48 116.71	8.48 116.75	8.48 116.73	8.49 116.78	8.48 116.77	8.49 116.77	8.48 116.78
16	8.31 116.65	8.32 116.68	8.32 116.65	8.30 116.54		8.31 116.66	8.32 116.65
17	8.89 117.81	8.89 117.81	8.89 117.81		8.94 117.69	9.00 116.88	8.89 117.84
18	8.62 117.50	8.62 117.50	8.62 117.50	8.61 117.58		8.63 117.59	8.62 117.57
19	9.81	9.81	9.82		9.80	9.78	9.82

[1] Chemical shifts for ^{1}H (top value in each entry) and ^{15}N (bottom value in each entry).

	118.05	118.03	118.06		118.10	118.06	118.06
20	8.81 118.94	8.81 118.92	8.82 118.92	8.83 119.00	8.77 119.16	8.82 118.95	8.82 118.95
21	8.78 121.50	8.75 121.41	8.80 121.67	8.77 121.90	8.78 121.53	8.78 121.47	8.79 121.48
22	8.78 122.90	8.79 122.88	8.79 122.90	8.78 123.02	8.78 122.99	8.79 122.94	8.80 122.92
23	8.53 126.70	8.53 126.70	8.53 126.76		8.51 126.90	8.54 126.83	8.54 126.78
24	7.08 118.85	7.07 118.85	7.12 118.73	7.15 118.51	7.08 118.88	7.10 118.83	7.09 118.85
25	6.95 120.17	6.96 120.23	6.94 120.20	6.94 120.04	6.96 120.22	6.95 120.18	6.96 120.22
26	7.30 121.35	7.30 121.40	7.30 121.33	7.30 121.05	7.30 121.40	7.30 121.41	7.30 121.40
27	6.69 122.30	6.70 122.32	6.70 122.29	6.70 122.37		6.69 122.37	6.71 122.34
28	7.02 124.70	7.03 124.68	7.00 124.66	7.01 124.82	7.10 123.82	7.01 124.66	7.02 124.66
29	6.79 126.71	6.80 126.70	6.80 126.71	6.78 126.72	6.83 127.02	6.77 126.44	6.80 126.68
30	9.45 130.83	9.46 130.83	9.46 130.85	9.44 130.85	9.52 130.90	9.47 130.85	9.48 130.87

Table S2 (Related to Figures 1 and S4 to S6). Chemical Shifts of the Signals 1 to 30, which were used to Monitor the Protein Folds of A_{2A}AR Variants.

^{1}H and ^{15}N chemical shifts are listed for A_{2A}AR and the six variants thereof which were used for the assignment of the tryptophan indole ^{15}N–^{1}H signals.

Data collection	
Number of crystals used for data processing	**18**
Space group	***C2 2 2$_1$***
Cell dimensions	
***a, b, c* (Å)**	**39.84, 180.97, 140.57**
Number of reflections processed	**60,178**
Number of unique reflections	**16,063**
Resolution (Å)	**30.0 - 2.50**
R_{sym}	**19.8 (64.8)**
Mean *I/σ(I)*	**6.3 (1.2)**
Completeness (%)	**90.1 (78.7)**
Redundancy	**3.7 (2.3)**
$CC_{1/2}$ in highest shell	**0.581**

Refinement	
Resolution (Å)	**30.0 - 2.50**
Number of reflections (test set)	**797**
R_{work} / R_{free}	**0.219/0.259**
Number of atoms	
Protein	**2,956**
Ligand	**25**
Lipids and waters	**279**
Overall *B* values (Å^2)	
A2a	**50.2**
Bril	**86.3**
Ligand	**40.3**
Lipids and water	**73.1**
RMSD	
Bond lengths (Å)	**0.010**
Bond angles (°)	**1.06**
Ramachandran plot statistics (%)*	
Favored regions	**98.7**
Allowed regions	**1.3**
Disallowed regions	**0.0**

Table S3 (Related to Figure 1). X-ray Diffraction Data Collection and Refinement Statistics.

Highest resolution shell is shown in parentheses. Ramachandran plot statistics were calculated with the program Molprobity (Chen et al., 2010).

Construct	Primer Sequence
A_{2A}AR[W29Y] Forward	ATCCTGGGCAATGTGCTGGTGTGCTACGCCGTGTGGCTCAACAGCAACCTG
A_{2A}AR[W29Y] Reverse	CAGGTTGCTGTTGAGCCACACGGCGTAGCACACCAGCACATTGCCCAGGAT
A_{2A}AR[W32Y] Forward	AATGTGCTGGTGTGCTGGGCCGTGTACCTCAACAGCAACCTGCAGAACGTC
A_{2A}AR[W32Y] Reverse	GACGTTCTGCAGGTTGCTGTTGAGGTACACGGCCCAGCACACCAGCACATT
A_{2A}AR[W129Y] Forward	GCTAAGGGCATCATTGCCATCTGCTACGTGCTGTCGTTTGCCATCGGCCTG
A_{2A}AR[W129Y] Reverse	CAGGCCGATGGCAAACGACAGCACGTAGCAGATGGCAATGATGCCCTTAGC
A_{2A}AR[W143Y] Forward	ATCGGCCTGACTCCCATGCTAGGTTACAACAACTGCGGTCAGCCAAAGGAG
A_{2A}AR[W143Y] Reverse	CTCCTTTGGCTGACCGCAGTTGTTGTAACCTAGCATGGGAGTCAGGCCGAT
A_{2A}AR[W246F] Forward	ATTGTGGGGCTCTTTGCCCTCTGCTTCCTGCCCCTACACATCATCAACTGC
A_{2A}AR[W246F] Reverse	GCAGTTGATGATGTGTAGGGGCAGGAAGCAGAGGGCAAAGAGCCCCACAAT
A_{2A}AR[W268Y] Forward	CCCGACTGCAGCCACGCCCCTCTCTACCTCATGTACCTGGCCATCGTCCTC
A_{2A}AR[W268Y] Reverse	GAGGACGATGGCCAGGTACATGAGGTAGAGAGGGGCGTGGCTGCAGTCGGG
A_{2A}AR[D52N] Forward	TTTGTGGTGTCACTGGCGGCGGCCAACATCGCAGTGGGTGTGCTCGCCATC
A_{2A}AR[D52N] Reverse	GATGGCGAGCACACCCACTGCGATGTTGGCCGCCGCCAGTGACACCACAAA
A_{2A}AR[G5A] Forward	GATGACGATAAGATGCCCATCATGGCTTCCTCGGTGTACATCACGGTGGAG
A_{2A}AR[G5A] Reverse	CTCCACCGTGATGTACACCGAGGAAGCCATGATGGGCATCTTATCGTCATC
A_{2A}AR[G69A] Forward	CCCTTTGCCATCACCATCAGCACCGCTTTCTGCGCTGCCTGCCACGGCTGC
A_{2A}AR[G69A] Reverse	GCAGCCGTGGCAGGCAGCGCAGAAAGCGGTGCTGATGGTGATGGCAAAGGG
A_{2A}AR[G114A] Forward	ATCCGCATCCCGCTCCGGTACAATGCTTTGGTGACCGGCACGAGGGCTAAG
A_{2A}AR[G114A] Reverse	CTTAGCCCTCGTGCCGGTCACCAAAGCATTGTACCGGAGCGGGATGCGGAT
A_{2A}AR[G118A] Forward	CTCCGGTACAATGGCTTGGTGACCGCTACGAGGGCTAAGGGCATCATTGCC
A_{2A}AR[G118A] Reverse	GGCAATGATGCCCTTAGCCCTCGTAGCGGTCACCAAGCCATTGTACCGGAG
A_{2A}AR[G158A] Forward	AAGGAGGGCAAGCAACACTCCCAGGCTTGCGGGGAGGGCCAAGTGGCCTGT
A_{2A}AR[G158A] Reverse	ACAGGCCACTTGGCCCTCCCCGCAAGCCTGGGAGTGTTGCTTGCCCTCCTT
A_{2A}AR[G160A] Forward	GGCAAGCAACACTCCCAGGGCTGCGCTGAGGGCCAAGTGGCCTGTCTCTTT
A_{2A}AR[G160A] Reverse	AAAGAGACAGGCCACTTGGCCCTCAGCGCAGCCCTGGGAGTGTTGCTTGCC
A_{2A}AR[G218A] Forward	CAGATGGAGAGCCAGCCTCTGCCGGCTGAGCGGGCACGGTCCACACTGCAG
A_{2A}AR[G218A] Reverse	CTGCAGTGTGGACCGTGCCCGCTCAGCCGGCAGAGGCTGGCTCTCCATCTG

Table S4 (Related to STAR Methods section). Primers used in this study.

Plasmid: human A_{2A}AR in pPIC9K vector
Plasmid: human A_{2A}AR[W29Y]
Plasmid: human A_{2A}AR[W32Y]
Plasmid: human A_{2A}AR[W129F]
Plasmid: human A_{2A}AR[W143Y]
Plasmid: human A_{2A}AR[W246F]
Plasmid: human A_{2A}AR[W268Y]
Plasmid: human A_{2A}AR[G5A]
Plasmid: human A_{2A}AR[G69A]
Plasmid: human A_{2A}AR[G114A]
Plasmid: human A_{2A}AR[G118A]
Plasmid: human A_{2A}AR[G158A]
Plasmid: human A_{2A}AR[G160A]
Plasmid: human A_{2A}AR[G218A]
Plasmid: human A_{2A}AR[D52N]
Plasmid: human A_{2A}AR[D52N, W246F]
Plasmid: human A_{2A}AR with BRIL inserted into ICL3

Table S5 (Related to STAR Methods section). Plasmids used in this study.