Supporting Information for

Molecular Docking Screening using Agonist-Bound GPCR Structures: Probing the A_{2A} Adenosine Receptor

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Supporting Information Inventory:

Table S1. Modified partial charges for residues Asn253$^{6.55}$, Ser277$^{7.42}$, and His278$^{7.43}$. For each residue, partial charges for all atoms are shown for the default AMBER parameters, the modified values used to increase the side chain dipole moment, and the difference between the two. The average docking energies for reference A_{2A}AR agonists improved by 7 kcal/mol for modified grids. For example, the value for adenosine decreased from –25.7 to –31.5 kcal/mol after the modification to the electrostatic potential grids.

Atom name	Default partial charge	Modified partial charge	Δ(partial charge)
Asn253$^{6.55}$			
N	-0.520	-0.520	
CA	0.217	0.217	
C	0.526	0.526	
O	-0.500	-0.500	
CB	0.003	0.003	
CG	0.675	0.675	
ND2	-0.867	-0.867	
OD1	-0.470	-0.870	-0.4
HN	0.248	0.248	
HND2	0.344	0.544	+0.2
HND1	0.344	0.544	+0.2
Ser277$^{7.42}$			
N	-0.520	-0.520	
CA	0.292	0.292	
C	0.526	0.526	
O	-0.500	-0.500	
CB	0.194	0.194	
OG	-0.550	-1.350	-0.8
HN	0.248	0.248	
HOG	0.310	1.110	+0.8
His278$^{7.43}$			
N	-0.520	-0.520	
CA	0.219	0.219	
C	0.526	0.526	
O	-0.500	-0.500	
CB	0.060	0.060	
CG	0.089	0.089	
CD2	0.145	0.545	+0.4
ND1	-0.444	-0.444	
CE1	0.384	0.784	+0.4
NE2	-0.527	-1.327	-0.8
HN	0.248	0.248	
HND	0.320	0.320	

Table S2. 2D structure and pharmacological properties of meperidine, which was selected as reference opioid receptor agonist.

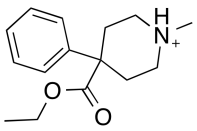
Name(s)	Structure	Pharmacological profile
Meperidine (Phetidine, Demerol)		$\underline{IC_{50}}$ values:[1] μ-OR = 315 nM κ-OR = 2,370 nM δ-OR = >10,000 nM Synthetic opiate agonist from the phenylpiperidine class of drugs[2]

Table S3. Quantification of the number of top-ranked known agonists and antagonist in docking screens using the ZINC database as background. Based on the predicted scores for known agonists (63) and antagonists (357), we quantified how many of these ligands would have been ranked in the top 0.3% of the screened library of commercially available compounds.

	2YDO	**2YDV**	**3QAK**	**3EML**
# agonists	24	8	9	0
% agonist set	38.1%	12.7%	14.3%	0.0%
# antagonists	49	45	5	37
% antagonist set	13.1%	12.0%	1.3%	9.9%
ratio (# agonists) / (# antagonists)	*0.5x*	*0.2x*	*1.8x*	*0.0x*
ratio (% agonist set) / (% antagonist set)	*2.9x*	*1.1x*	*10.7x*	*0.0x*

Table S4. Radioligand binding data for predicted ligands at the A_1, A_{2A}, and A_3 human AR subtypes.

Cpd	ZINC code	SMILES	PDB code active[a]	K_i (nM) or *%inh @ 10 μM*[b]		
				A_1AR	A_{2A}AR	A_3AR
13	C71708091	c1ccc2c(c1)c(c[nH]2)c3cc(c(c(n3)N)C#N)c4ccc(o4)CO	2YDO	1,520 ± 60	1,290 ± 280	128 ± 17
14	C67559048	COc1ccccc1OCc2cc(n[nH]2)C(=O)N3CC[C@H](C3)O	2YDO	*10 ± 5%*	*18 ± 7%*	*17 ± 5%*
15	C71738699	c1cc(oc1CO)c2c3c(nc(c2C#N)N)-c4cc[nH]c4CC3	2YDO	217 ± 63	37.2 ± 7.8	378 ± 129
16	C67714485	Cc1ccc(o1)c2cc(c(c(n2)N)C#N)c3ccc(o3)CO	2YDO	122 ± 27	175 ± 30	22.1 ± 8
17	C71764951	c1cc(cc(c1)O)c2c3c(nc(c2C#N)N)-c4cc[nH]c4CC3	2YDO	37.7 ± 3.8	15.9 ± 4.7	513 ± 110
18	C71575632	c1cc(oc1CO)c2c3c(nc(c2C#N)N)CCOC3	2YDO	783 ± 216	390 ± 50	3,790 ± 1330
19	C71771533	CCn1c(c(c(n1)C)c2cc(c(c(n2)N)C#N)c3ccc(cc3)O)C	2YDO	*54 ± 2%*	1,670 ± 154	2,900 ± 112
20	C71750186	c1ccc2c(c1)c(c[nH]2)c3cc(c(c(n3)N)C#N)c4c[nH]cn4	2YDV	1,310 ± 20	760 ± 50	412 ± 61
21	C67894633	c1cn(c([nH+]1)c2cnc(nc2)NC[C@H]3CCCO3)CCO	3QAK	*11 ± 3%*	*4 ± 3%*	*18 ± 5%*
22	C69872248	c1c[nH]c2c1c(ncn2)N3CCC[C@H]3Cn4cncn4	2YDO	*7 ± 3%*	*15 ± 4%*	*13 ± 2%*
23	C08345951	c1ccc(cc1)c2c3cc(cnn3c(n2)N)C(=O)c4ccccc4O	2YDO	63.1 ± 31.9	262 ± 73	365 ± 58
24	C72275965	COc1ccc(cc1)CCNc2cc(ncn2)N3CCC[C@H]3CO	3QAK	*16 ± 2%*	*11 ± 4%*	*40 ± 5%*
25	C75128452	c1cc(ccc1CNc2cc(ncn2)N3CCC[C@@H]3CO)F	3QAK	*6 ± 4%*	*24 ± 9%*	*23 ± 4%*
26	C08735001	C[C@@H](C(=O)N)Sc1nnc(s1)NCc2ccccc2	3QAK	*5 ± 2%*	*9 ± 5%*	*15 ± 4%*
27	C02616094	CN1Cc2cc(cnc2NC1=O)C(=O)c3cc(ccc3O)OC	3QAK	*18 ± 2%*	*25 ± 8%*	*42 ± 3%*
28	C72806511	c1cc(ccc1OCc2nc([nH]n2)SCCCO)Cl	2YDV	*6 ± 2%*	*8 ± 8%*	*15 ± 1%*
29	C84852928	Cc1cccc(c1)Nc2nnc(s2)SCC3(COC3)CO	3QAK	*11 ± 4%*	*22 ± 7%*	*26 ± 4%*
30	C00197243	Cc1c2c(n(n1)c3ccccc3)OC(=C([C@@H]2c4ccc(cc4)O)C#N)N	2YDO	*11 ± 1%*	*28 ± 10%*	*12 ± 7%*
31	C05026096	Cc1ccc(cc1C)Nc2nc(c(c(n2)NCCO)[N+](=O)[O-])N	2YDO	*38 ± 1%*	3,110 ± 260	363 ± 81
32	C20102586	Oc1ccc(Cl)cc1C(=O)c1cnc2NNC(=O)c2c1	2YDO	*12 ± 1%*	*0 ± 0%*	*24 ± 4%*

[a] The PDB code of the crystal structure used in the docking screen.

[b] Data is expressed as mean ± standard error resulting from three independent experiments.

Table S5. Functional assays for discovered ligands. Data shows the effect of 10 μM compound concentration on intracellular cAMP levels. An additional control experiment for compound **23**, which demonstrates that the observed increase in cAMP is not mediated by the A_{2A}AR, is also shown.

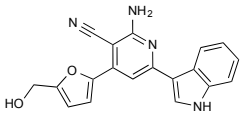
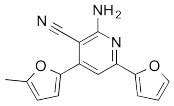
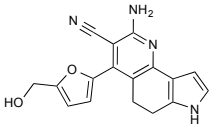
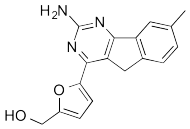
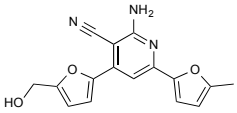
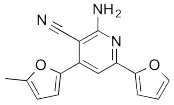
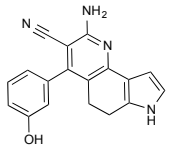
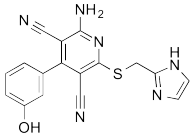
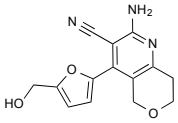
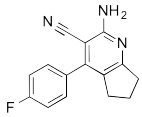
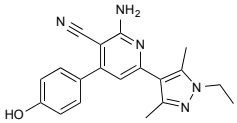
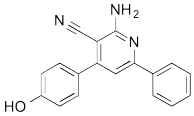
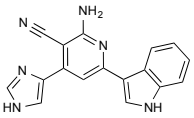
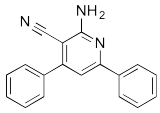
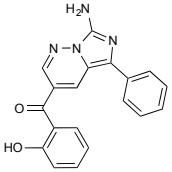
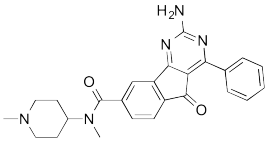
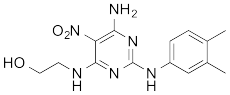
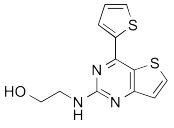
Compound	**Relative cAMP levels @ 10μM**[a,b]	**EC_{50} (μM)**[b]	
Cells	*CHO + A_{2A}AR*	*CHO*	*CHO + A_{2A}AR*
forskolin	100%	3.8 ± 0.4	0.9 ± 0.1
CGS21680	79.8 ± 13.5%	N.E.[c]	0.009 ± 0.001
23	32.4 ± 5.4 %	25.5 ± 4.0	16.4 ± 2.7
13	4.0 ± 7.8 %	-	-
15	-22.6 ± 10.7 %	-	-
16	-0.3 ± 2.9 %	-	-
17	-4.7 ± 2.1 %	-	-
18	4.6 ± 9.3 %	-	-
19	6.5 ± 2.8 %	-	-
20	7.9 ± 3.5 %	-	-
31	12.5 ± 0.7 %	-	-

[a] Increase in cAMP levels at a compound concentration of 10 μM.

[b] Data is expressed as mean ± standard error resulting from three independent experiments.

[c] No effect.

Table S6. 2D similarities of the discovered ligands to the closest ChEMBL compounds. Each discovered ligand is shown together with the closest annotated AR compound from the ChEMBL15 database. The Tanimoto similarity coefficient (T_c), calculated with ECFP4 fingerprints, is also indicated for each compound pair.

Compound ID	Structure	T_c [a]	Closest ChEMBL [b]
13		0.45	
15		0.38	
16		0.68	
17		0.51	
18		0.40	
19		0.58	
20		0.45	
23		0.32	
31		0.30	

[a] Tanimoto coefficient of molecular similarity calculated with ECFP4 fingerprints.

[b] Closest molecule annotated as AR ligand in ChEMBL15 database.

Table S7. List of compounds from top-ranked complexes that are predicted to have ligand-receptor interactions similar to agonist-bound crystal structures of the A_{2A}AR. The top 50,000 ranked complexes in docking screens for each of the A_{2A}AR agonist-bound structures (PDB codes 2YDO, 2YDV and 3QAK) were filtered according to the establishment of a double hydrogen bond with Asn253$^{6.55}$, and polar contacts to both Ser277$^{7.42}$ and His278$^{7.43}$ with two different hydrogen bonding atoms. Heteroatom distance cutoff for a hydrogen bond was set to 3.5 Å.

#	Rank[a]	ZINC ID
2YDO		
1	49	C67896118
2	58	C67642542
3	283	C67967614
4	322	C67982301
5	503	C67786606
6	507	C67802867
7	566	C20906941
8	646	C67713073
9	668	C20905254
10	851	C67726538
11	932	C20428995
12	1,210	C67694675
13	1,330	C22978383
14	1,415	C32692837
15	1,419	C67674127
16	1,967	C67783349
17	2,551	C21038636
18	3,053	C53357683
19	3,223	C72582255
20	3,369	C78991781
21	3,645	C20494508
22	3,708	C67855415
23	3,901	C12377095
24	4,679	C84405954
25	5,904	C59018757
26	6,329	C79916271
27	6,504	C59018508
28	7,495	C00134008
29	8,271	C78485552
30	9,312	C81783517
31	10,347	C71748784
32	11,745	C77840936
33	11,903	C79453266
34	12,086	C81238528
35	14,142	C06122390
36	16,465	C20906238
37	18,048	C15657781[b]
38	18,072	C47707534
39	18,547	C20479767
40	23,369	C81763365
41	26,670	C67840882
42	33,273	C83323908[b]
43	37,185	C67920483
44	37,747	C82060213
45	37,789	C17009153
46	40,786	C06341871
47	42,845	C76760826
48	44,639	C22782433
2YDV		
49	5,328	C15657781[c]
50	3,090	C79682059[d]
51	10,521	C32091424[d]
52	17,531	C83291127
53	17,922	C83291128
54	16,730	C83323908[c]
55	38,132	C78485551
56	42,086	C67847751
3QAK		
57	26,120	C32091424[c]
58	28,194	C73970936
59	35,396	C05523326
60	37,878	C79682059[c]

[a] Docking rank in screens of 6.7 million commercially available compounds against three agonist-bound structures of the A_{2A}AR.

[b] Also selected from the screen on PDB structure 2YDV.

[c] Also selected from the screen on PDB structure 2YDO.

[d] Also selected from the screen on PDB structure 3QAK.

Supplemental references

(1) Poulain, R.; Horvath, D.; Bonnet, B.; Eckhoff, C.; Chapelain, B.; Bodinier, M. C.; Deprez, B. From Hit to Lead. Combining Two Complementary Methods for Focused Library Design. Application to Mu Opiate Ligands. *J. Med. Chem.* **2001**, *44* (21), 3378-90.

(2) Bryant, B. J.; Knights, K. M.; Salerno, E., *Pharmacology for Health Professionals*. Elsevier Australia: Chatswood, N.S.W., 2010.