Supplementary Information

Conformation guides molecular efficacy in docking screens of activated beta-2 adrenergic G protein coupled receptor

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Methods

Selection of high-ranking molecules for experimental testing. In docking screens top-ranking molecules (~1000) are routinely evaluated manually to select those most likely to be true hits. The trained computational chemist must often integrate several, sometimes contradictory, considerations that are not included in the DOCK scoring function. These considerations have been discussed at length [1,2]. Common reasons to reject a molecule despite a high-ranking DOCK score include:

High internal energy of the molecule

High flexibility of the molecule

Stranded polar groups that do not interact with the protein

Wrong tautomerization state

Wrong protonation state

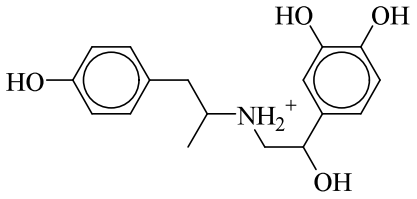
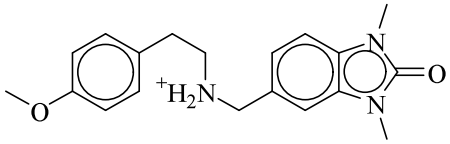
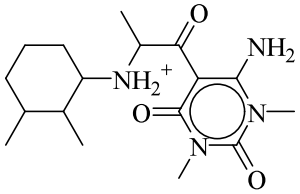
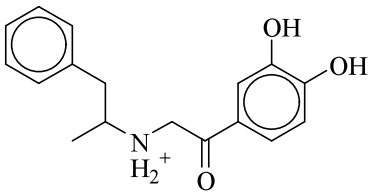
Broken molecules with missing or disconnected atoms

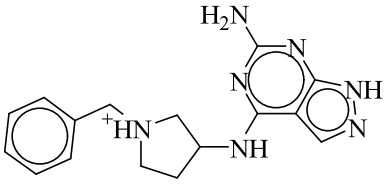
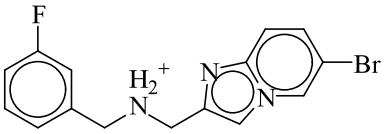
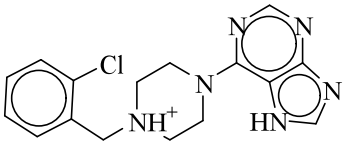
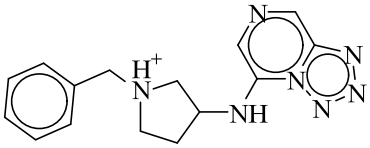
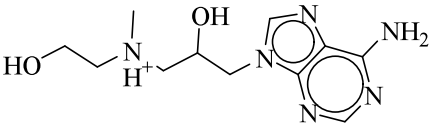
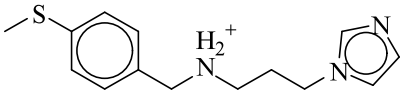
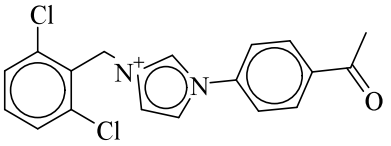
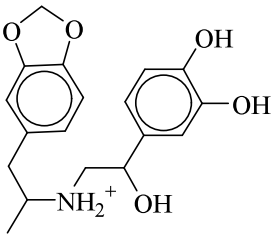
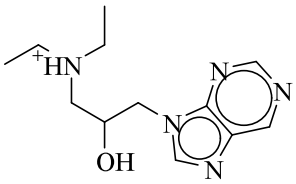
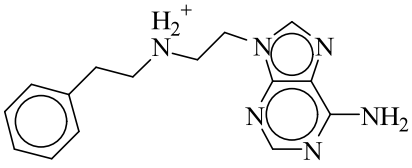
No available vendor found

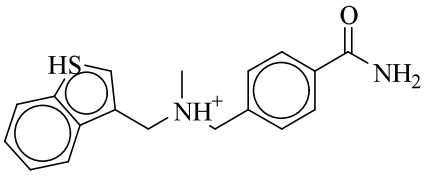
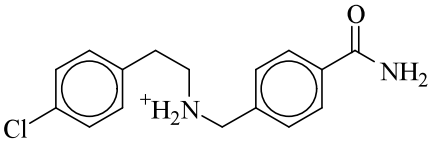
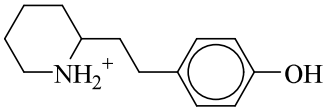
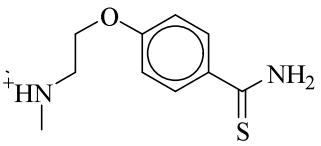
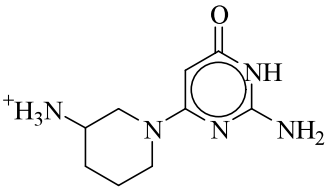
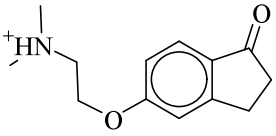
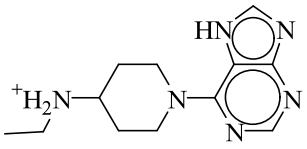
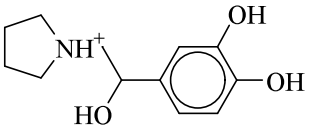
Compound is not novel with respect to known ligands

Compound is not diverse with respect to other compounds picked for testing

Table S1: Compounds tested from virtual screening of the active β2AR structure.

#	ZINC ID	Structure	Tc [a]	Rank active structure	Rank inactive structure
1	C01768066		0.74	57	31320
2	C04993730		0.36	178	86179
3	C47509526		0.32	393	31192
4	C01673161		0.47	451	87426

5	C32581901		0.25	844	135256
6	C55178789		0.24	869	40820
7	C31810657		0.29	958	58285
8	C44974609		0.27	1010	291620
9	C01588041		0.23	966	105841
10	C04453683		0.30	1082	50149
11	C30516172		0.27	1090	73794
12	C01704228		1	1197	9909
13	C01704406		0.22	1201	687679
14	C03999871		0.29	1545	20214

15	C46089688		0.26	2003	118503
16	C35047812		0.38	2311	108426
17	C15635522		0.29	3689	116195
18	C11799337		0.35	28[b]	5242
19	C40457267		0.19	47[b]	30107
20	C01567711		0.26	121[b]	11362
21	C37537197		0.32	157[b]	701
22	C06525260		0.52	397[b]	13100

[a] Tanimoto coefficient (T_c) calculated for all known B2AR ligands in the ChEMBL 15 database

[b] "fragment-like" screen

Table S2: Co-crystal ligand BI-167107 and six additional known partially biased β2AR ligands used for the 2D Similarity Ensemble Approach (SEA).

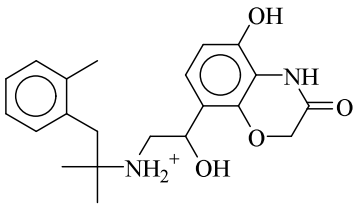
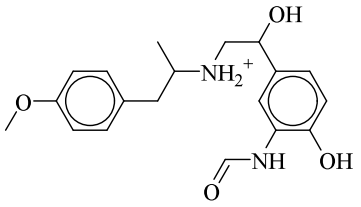
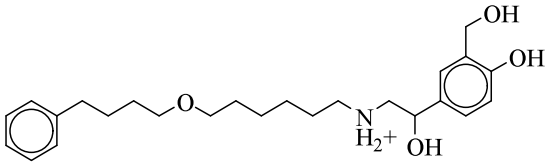
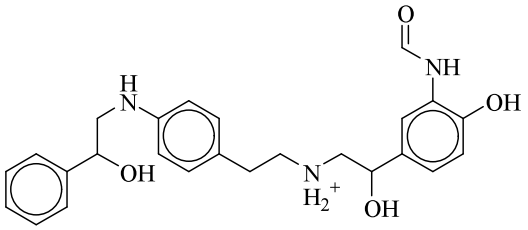
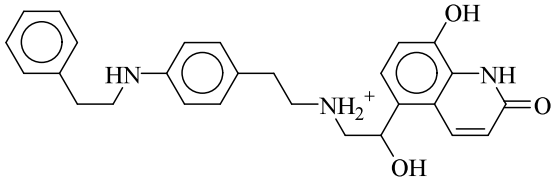
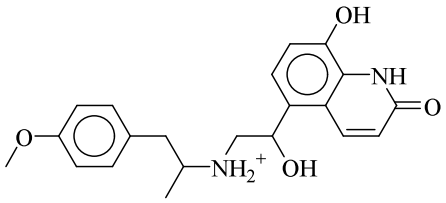
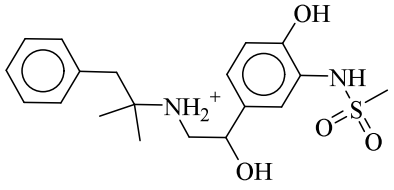
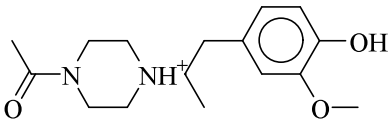
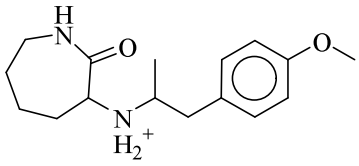
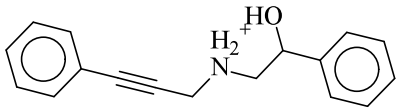
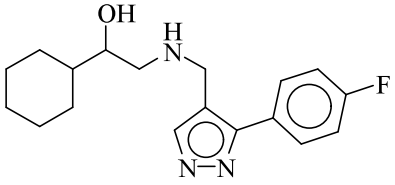
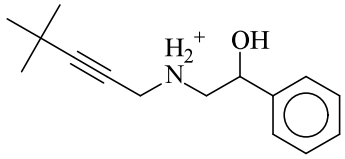
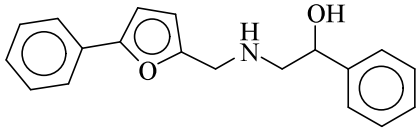
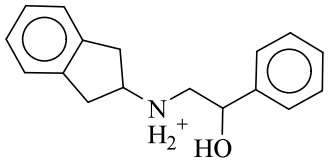
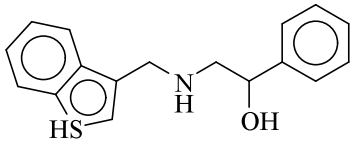
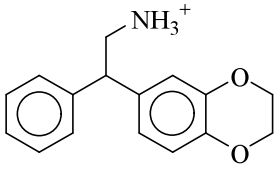
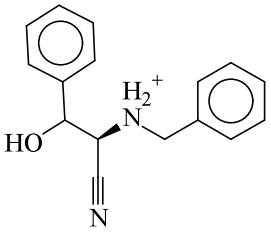
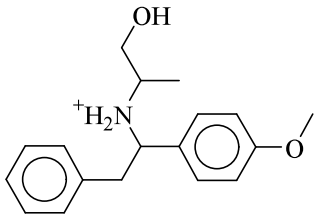
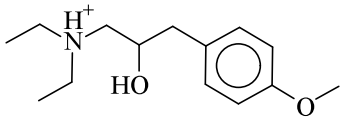
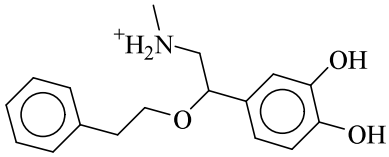
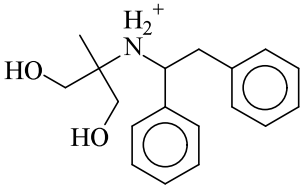
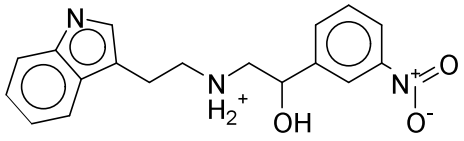
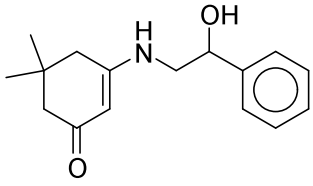
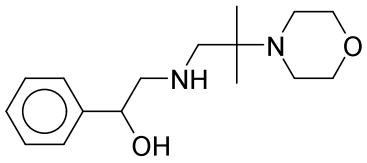
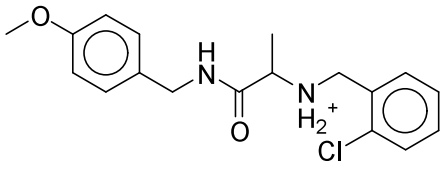
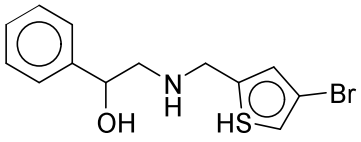
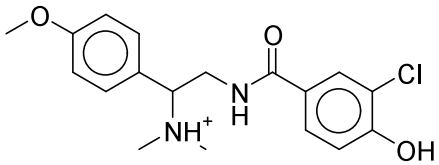
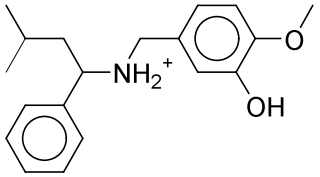
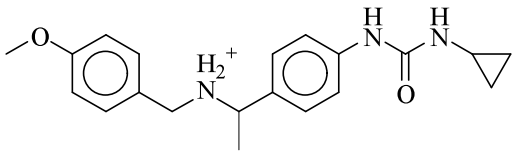
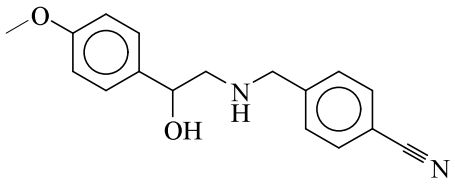
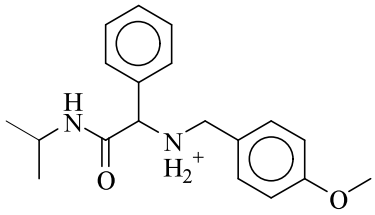
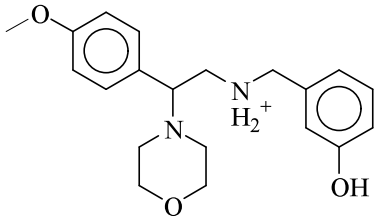
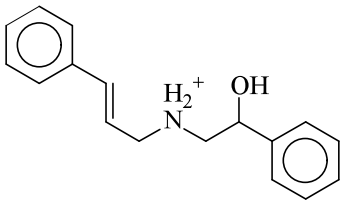
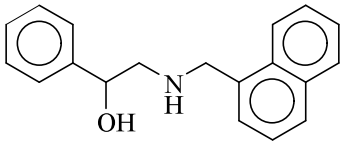
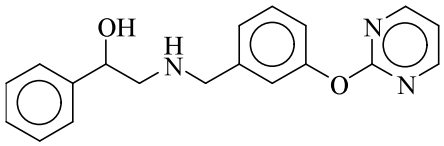
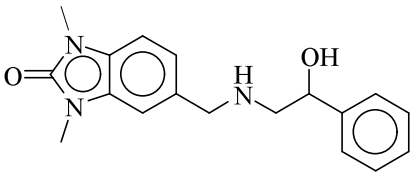
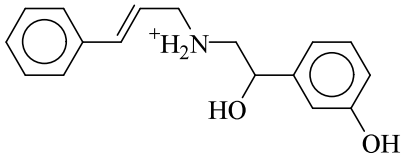
#	Structure
BI167107	
Formoterol	
Salmeterol	
TD3227	
THRX144877	
Carmoterol	
Zinterol	

Table S3: Compounds tested for biased agonism to the β2AR, selected from a 2D chemical similarity search.

#	ZINCID	Structure	T_c [a]
SEA01	C00214190		0.28
SEA02	C04022531		0.33
SEA03	C04810679		0.38
SEA04	C53945771		0.31
SEA05	C04850887		0.39
SEA06	C19872322		0.34
SEA07	C00118342		0.35
SEA08	C19924993		0.34
SEA09	C00085165		0.29

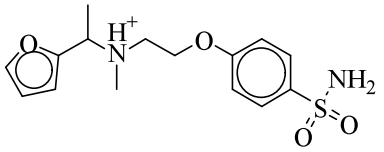
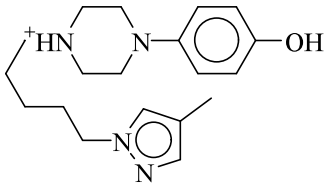
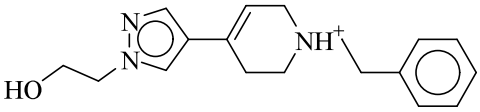
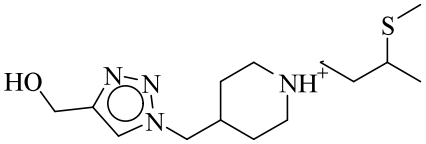
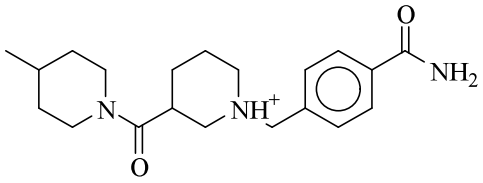
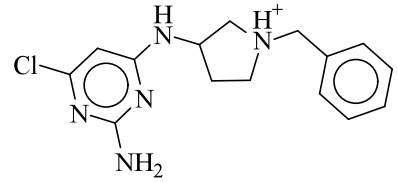
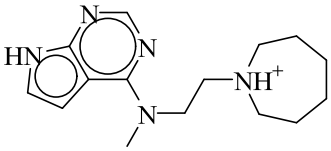
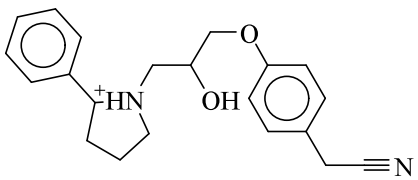
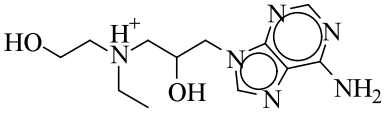
SEA10	C01871693		0.33
SEA11	C01667040		0.36
SEA12	C01668555		0.33
SEA13	C01593542		0.48
SEA14	C01594041		0.28
SEA15	C01820508		0.70
SEA16	C04786375		0.32
SEA17	C22030114		0.38
SEA18	C07987502		0.31

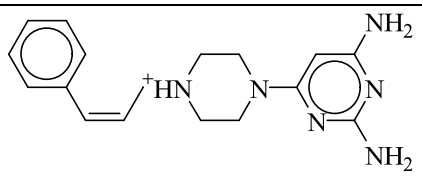
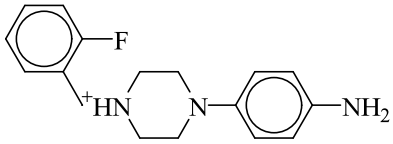
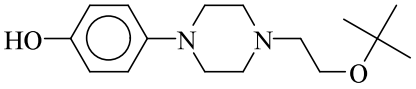
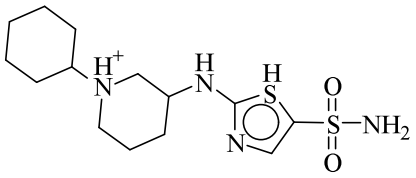
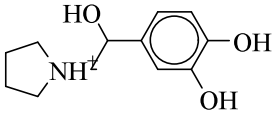
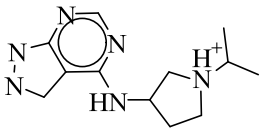
SEA19	C35112775		0.36
SEA20	C60634384		0.35
SEA21	C60844264		0.30
SEA22	C47725577		0.33
SEA23	C37749077		0.40
SEA24	C56142982		0.32
SEA25	C60818125		0.31
SEA26	C06703797		0.37

SEA27	C16477523		0.41
SEA28	C13731635		0.34
SEA29	C32616882		0.35
SEA30	C08728035		0.57

[a] Tanimoto coefficient (T_c) calculated for all known β2AR ligands in the ChEMBL 15 database

Table S4: Compounds tested from virtual screening of the active DRD2 model.

#	ZINC ID	Structure	T_C [a] DRD2	Rank active model	Rank inactive structure
23	C69067842		0.29	181	20673
24	C65513278		0.40	735	18882
25	C65435880		0.49	2026	11238
26	C20864202		0.28	2079	11808
27	C58405280		0.37	3211	278164
28	C40051228		0.48	5965	27188
29	C65403691		0.36	7633	91253
30	C54993535		0.42	11254	165851
31	C05499507		0.21	11758	24380

32	C65461088		0.5	13340	55040
33	C04244028		0.46	14406	41726
34	C67715357		0.48	15416	848018
35	C68768373		0.30	15608	62989
36	C06525260		0.52	827 [b]	12256
37	C69000627		0.27	883 [b]	8896

[a] Tanimoto coefficient (T_c) calculated for all known DRD2 ligands in the ChEMBL 15 database

[b] "fragment-like" screen

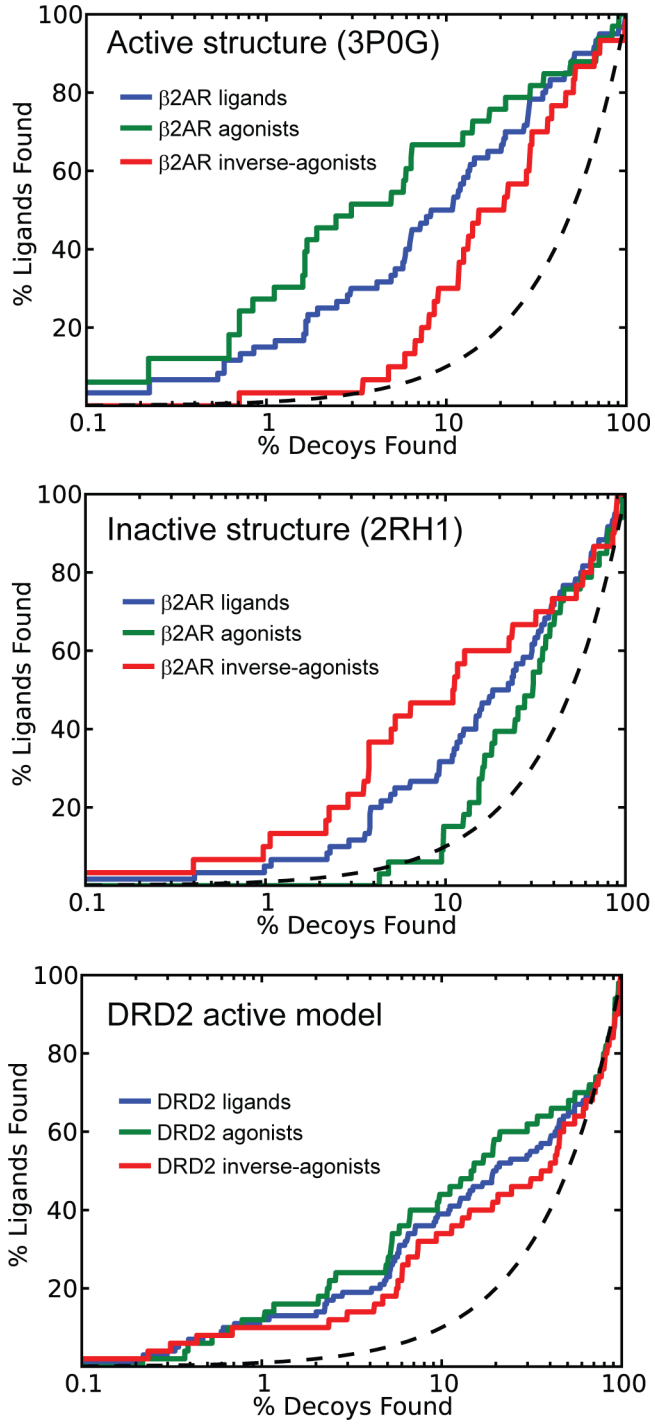


Figure S1: Retrospective enrichment of known β2AR ligands from a set of computationally matched decoys. (Top) Retrospective enrichment using the β2AR active structure, with 60 known ligands (blue), of which 30 are agonists (green) and 30 are inverse-agonists (red). The docked set also contains 2400 computationally generated matched decoys. (Middle) Retrospective enrichment using the inactive β2AR structure with the same set of 60 known ligands and 3840 decoys. (Bottom) Retrospective enrichment using the active DRD2 model with 100 known ligands (blue), of which 50 are known agonists (green) and 50 are known antagonists (red), from 6400 computationally matched decoys.

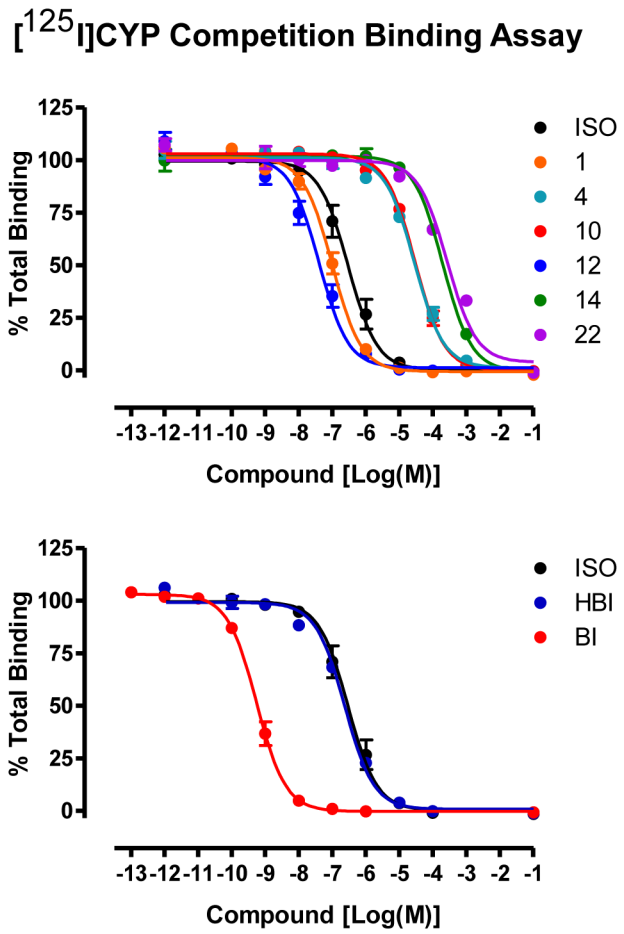


Figure S2: Binding assays for the β2AR hits predicted by virtual screening, measured by radioligand displacement of [^{125}I]cyanopindolol. (Top) Dose-response competition curves for the six β2AR agonists discovered in the virtual screen. (Bottom) Control compounds Isoproterenol (ISO), hydroxybenzylisoproterenol (HBI), and BI-167107 (BI). Each data point represents mean ± S.E. obtained from three independent experiments done in duplicates. Dose-dependent competition curves for each compound were obtained using the nonlinear iterative curve-fitting computer program Prism.

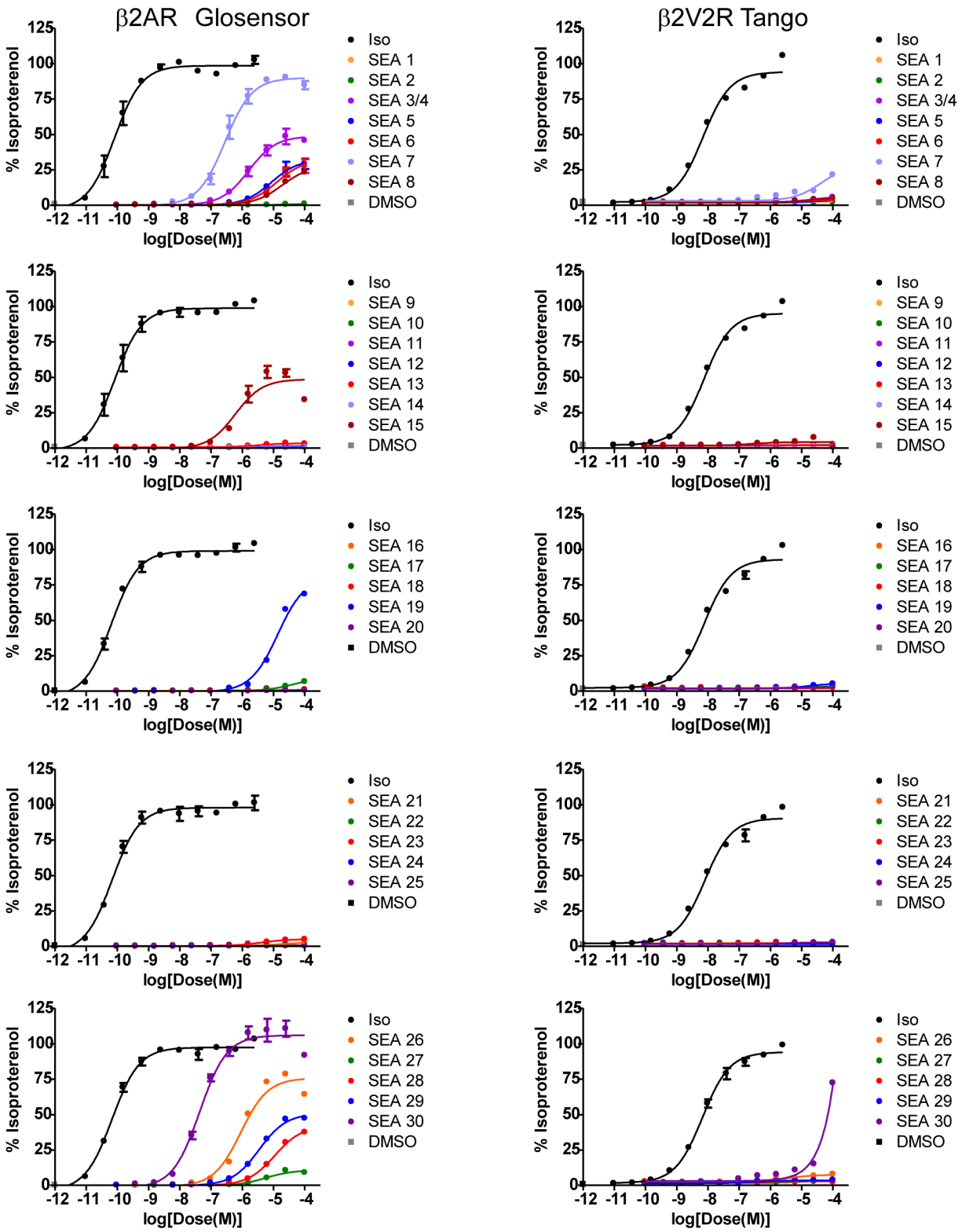


Figure S3: Dose-response curves for 30 compounds predicted by 2D chemical similarity to be partially β-arrestin biased agonists. (Left) Measurement of cAMP formation using the GloSensor assay. (Right) Measurement of β-arrestin recruitment using the Tango assay. Each data point represents mean ± S.E. and dose response curves for each compound were obtained from three independent experiments done in duplicates..

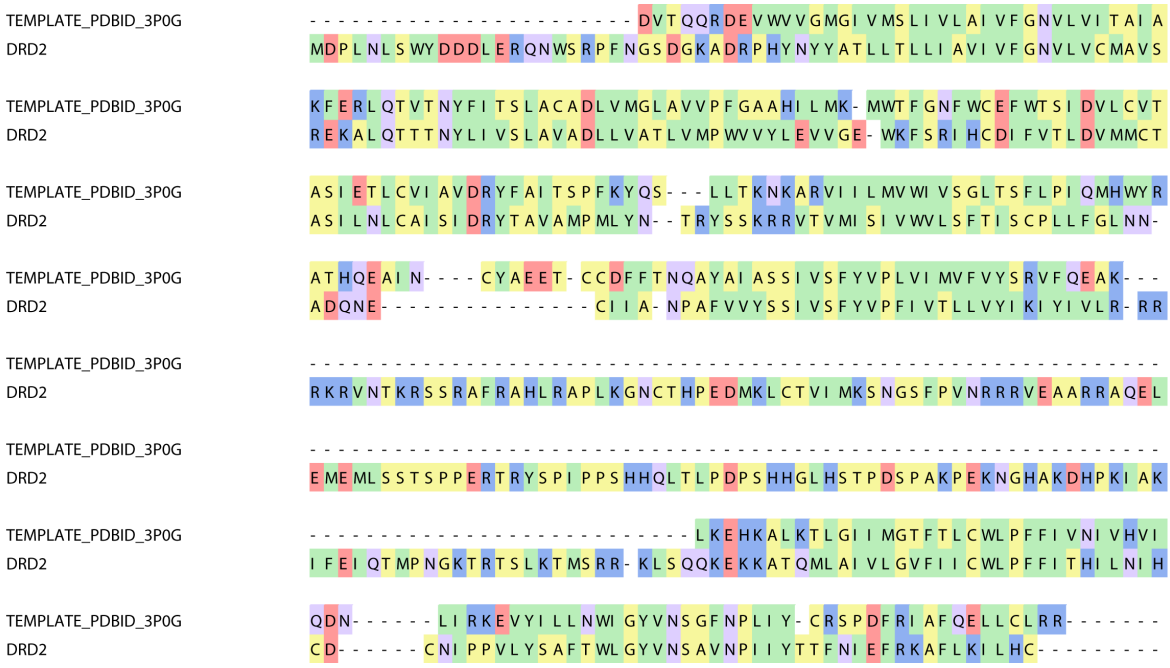


Figure S4: Sequence alignment of dopamine D2 receptor to the template β2AR (PDBID 3P0G)

References

1 Carlsson, J. *et al.* Ligand discovery from a dopamine D3 receptor homology model and crystal structure. *Nature chemical biology* **7**, 769-778 (2011).

2 Mysinger, M. M. *et al.* Structure-based ligand discovery for the protein-protein interface of chemokine receptor CXCR4. *Proc Natl Acad Sci U S A* **109**, 5517-5522 (2012).