Supporting Information

Extensive rigid analogue design maps the binding conformation of potent *N*-benzylphenethylamine 5-HT_{2A} serotonin receptor agonist ligands

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A) Receptor alignment used for homology modeling.

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B2AR   DEVWVVGMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMG 83
       +        ++ +++++  + GN+LVI A++  ++LQ  TNYF+ SLA AD+++G
5HT2A  HLQEKNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAIADMLLG 124

B2AR   LAVVPFGAAHILM-KMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFAITS 137
         V+P     IL    W   +  C  W  +DVL  TASI  LC I++DRY AI +
5HT2A  FLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQN 179

B2AR   PFKYQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINCYANETCCD 192
       P  +    ++ KA + I+ VW +S   S             Q+    +    +C
5HT2A  PIHHSRFNSRTKAFLKIIAVWTISVGISMPIPVFG-----LQDDSKVFKEG-SCL 228

B2AR   FFTNQAYAIASSIVSFYVPLVIMVFVYSRVFQEAKRQL/KFCLKEHKALKTLGII 274
          +  + +  S VSF++PL IMV  Y    +  +++  +    E KA K LGI+
5HT2A  LADDN-FVLIGSFVSFFIPLTIMVITYFLTIKSLQKEA/QSISNEQKACKVLGIV 328

B2AR   MGTFTLCWLPFFIVNIVHVI-QD--N-LIRKEVYILLNWIGYVNSGFNPLIYCR- 328
          F + W PFFI NI+ VI ++  N  +   +  +  WIGY++S  NPL+Y
5HT2A  FFLFVVMWCPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLF 383

B2AR   SPDFRIAFQELL-CL 342
       +  +R AF   + C
5HT2A  NKTYRSAFSRYIQCQ 398

   Identities = 91/289 (31%)
   Positives = 148/289 (51%)
   Gaps = 14/289 (5%)
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This sequence alignment is presented and scored in the style of BLAST (Basic Local Alignment Search Tool) output (http://blast.ncbi.nlm.nih.gov/Blast.cgi).[1] Conserved residues appear between the receptor sequences as one-letter residue codes. Plus ('+') signs correspond to residues with similar properties. Gaps are denoted with '-' signs, and a truncated region within the sequence (replaced by a T4 lysozyme in the of the β_2 adrenergic receptor X-ray structure),[2] is represented by a '/' sign. A value of 31% sequence homology was found for this alignment of these regions of the receptor, which increased to 51% when including similar residues.

- Protein AC/IDs from PIR:[3, 4]

β_2AR = P07550

5-HT$_{2A}$ = P28223

- Disulfide Bonds:

β_2AR: Cys106-Cys191; Cys184-Cys190

5-HT$_{2A}$: Cys148-Cys227; Cys349-Cys353

B) Refinement of the homology model and general docking and post-processing procedures.

The 5-HT_{2A} homology model used for our docking studies was based on our previously detailed *in silico*-activated β_2 adrenergic receptor.[5] The procedure for generating this model was similar to that performed for the D_1 receptor,[5] using Modeller 9 version 2,[6,7] with a few differences. The ligand *R*-DOB (Figure S1) was used during the homology model refinement stages, and was docked into the receptor (with flexible sidechains) using GOLD. 10ns of MD simulations, with position restraints on both protein and ligand, were performed, followed by 42 ns of simulation with no position restraints, but with two distance restraints between the two molecules: one for the critical salt bridge, and another for the known hydrogen bond between the 5-methoxy group of the ligand and Ser239 in TM5.

At this point, the protein-ligand distance restraints were removed and an MD simulation was performed, with the inclusion of point charges as lone pair substitutes (0.47 Å from the heavy atom at tetrahedral angles).[8] During a timeframe of 20 ns, both polar interactions were stable. The output structure was energy minimized and used for docking ligands, and the docking poses were refined by energy minimization and molecular dynamics. After equilibration was observed (by plateauing of the RMSD vs. time curve of the protein heavy atoms), an output frame was energy minimized and used for evaluation.

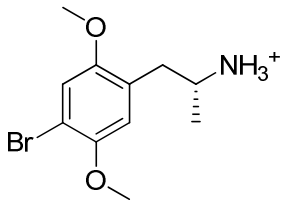

Figure S1. Structure of *R*-DOB (2,5-dimethoxy-4-bromoamphetamine), the agonist ligand used during the equilibration of the 5-HT_{2A} homology model.

C) References.

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