

Structure-based discovery of β_2 -adrenergic receptor ligands

Peter Kolb^{1,†,*}, Daniel M. Rosenbaum^{2,*}, John J. Irwin¹, Juan José Fung², Brian K. Kobilka², and Brian K. Shoichet^{1,‡} [[†]kolb@blur.compbio.ucsf.edu, [‡]shoichet@cgl.ucsf.edu] *contributing equally

¹Department of Pharmaceutical Chemistry, University of California, San Francisco and ²Department of Molecular and Cellular Physiology, Stanford University School of Medicine

Introduction

Aminergic G-protein coupled receptors (GPCRs) have been a major focus of pharmaceutical research for many years. The recently solved x-ray structure of the β_2 -adrenergic receptor (β_2 AR, Fig. 1, [1]) allowed us to investigate the advantages and limitations of structure-based ligand discovery approaches against this and related GPCR targets. The success of such a docking screen might be reflected in the hit rates and the potency of the hits, but also whether the structure is capable of recognizing new chemotypes. In addition, since the β_2 AR was crystallized in complex with an inverse agonist, it is important to determine the efficacy of the ligands.

A beautiful binding site for docking

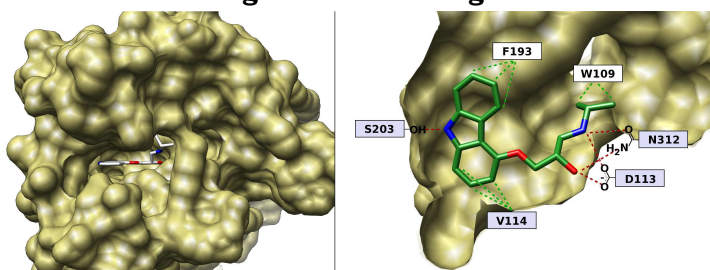


Fig. 2: Top view (left) and side view (right) of the binding site of β_2 AR. It is a narrow and deep cleft, a layout which is emphasized by the polar interactions offered by the residues lining the binding site.

Method

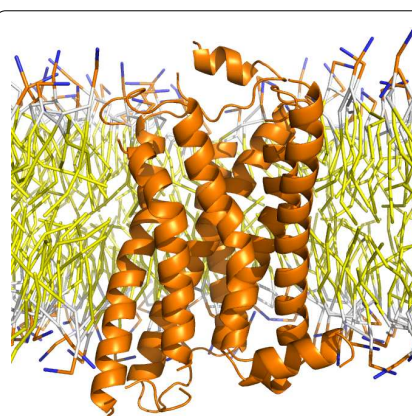
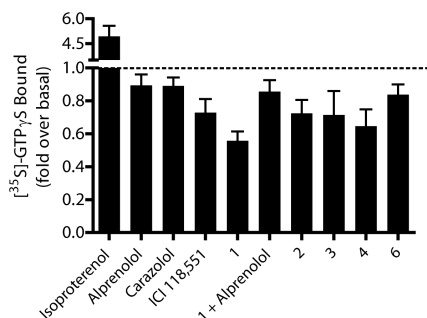
Docking calculations were done with DOCK3.5.54 [2] against the β_2 AR/carazolol complex (Fig. 2), screening the ≈ 1 Million compounds of the “lead-like” subset of ZINC. Compounds were ranked according to electrostatic and van der Waals interaction energies which were corrected for ligand desolvation. Library bias was assessed with the Similarity Ensemble Approach (SEA, [3]). K_i values were determined based on radioligand displacement assays using ³H-dihydroalprenolol.

Results

Twenty-five molecules were chosen after visual inspection of the top 500. Six compounds with percentage of displacement larger than 10% were further characterized to obtain K_i values (Fig. 3). Four compounds (①-④) are similar to carazolol and share a common binding mode, but ⑤ and ⑥ are far away from previously explored chemical space for β_2 AR. Next, we investigated ligand efficacy and found that all compounds are inverse agonists, as is carazolol. ① has an affinity of only 9 nM and is also a potent inverse agonist (Fig. 5). Finally, we quantified library bias and found that available chemical space is strongly biased toward chemotypes recognized by GPCRs (Fig. 4).

Ligand efficacy

Fig. 5: Purified wild-type β_2 AR and Tet- $G_{\alpha s}$ were reconstituted and stimulation of [³⁵S]-GTP γ S binding to Tet- $G_{\alpha s}$ was measured in the presence of the ligands. ① exhibits inverse agonist activity as good or better than ICI 118,551, heretofore the most potent inverse agonist for β_2 AR.



The β_2 -adrenergic receptor

Fig. 1: The β_2 AR inserted into a native-like environment, a bilayer of lipids. Lipid bilayer coordinates from “Database of Inserted Membrane Proteins” at SBCB, Oxford, UK.

Active Compounds

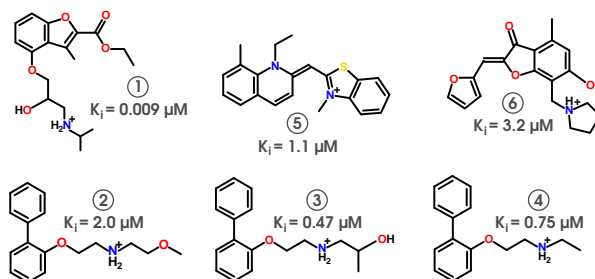


Fig. 3: The six active compounds identified in the docking screen.

Library bias

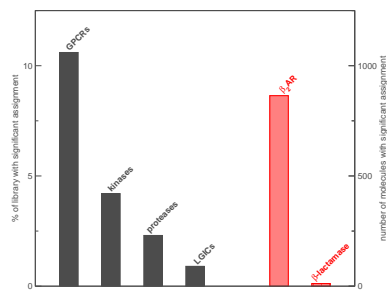


Fig. 4: Comparison of the lead-like subset of ZINC to the annotated ligands of WOMBAT. Left axis: at a confidence level of 10^{-10} , 10.6% are similar to GPCR ligands. This compares with 4.2%, 2.3%, and 0.9% molecules which were assigned to kinases, proteases and LGICs, respectively. Right axis: 864 molecules were assigned to β_2 AR and only 12 to TEM-1.

Discussion

Docking was able to identify six potent ligands of β_2 AR. While four of them resemble typical β_2 AR ligands, two compounds represent novel chemotypes that couldn't have been found with ligand-based methods. Library bias played a role in this screen and it is not surprising that the most potent molecule to emerge resembles adrenergic ligands. Based on the structural “druggability” of aminergic GPCRs, and the general bias in our libraries, structure-based approaches promise to be a fruitful avenue for novel ligand discovery.

References

- [1] CHEREZOV V *et al.* *Science* 318 (2007), 1258 and ROSENBAUM DM *et al.* *Science* 318 (2007), 1266.
- [2] MENG EC, SHOICHET BK, KUNTZ ID. *J. Comput. Chem.* 13 (1992), 505.
- [3] KEISER MJ, ROTH BL, ARMBRUSTER BN, ERNSBERGER P, IRWIN JJ, SHOICHET BK. *Nat. Biotech.* 25 (2007), 197.
- [4] KOLB P, ROSENBAUM DM, IRWIN JJ, FUNG J, KOBILKA BK, SHOICHET BK. *Proc. Natl. Acad. Sci. U.S.A.* 106 (2009), 6843.