

Supporting Information

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SI Methods

Docking Calculations. All calculations were performed with DOCK3.5.54. Docking spheres were derived based on carazolol atom positions. These spheres may be considered pseudoatom positions that map the negative image of the receptor site; they guide the placement of ligand atoms in the site during docking (1). In the present study, spheres were additionally placed in the pocket extending past the alkane chain of carazolol. After matching, each pose is subjected to 25 steps of minimization with the simplex method. Finally, the binding affinity is calculated by adding up the electrostatic and van der Waals interaction energies and correcting for the desolvation penalty incurred when the ligand is transferred from water into the low-dielectric environment of the protein (2). For efficiency reasons, these energy terms are precalculated and stored on grids.

In the search for novel inhibitors of β_2 -adrenergic receptor (β_2 AR), we docked the lead-like subset of the ZINC 7 database (3) ($-2 < \text{XlogP} < 4$, $150 \text{ g}\cdot\text{mol}^{-1} < \text{molecular mass} < 350 \text{ g}\cdot\text{mol}^{-1}$, number of hydrogen bond donors ≤ 3 , and number of hydrogen bond acceptors ≤ 6). For every ligand, up to 1,000 conformations had been precalculated with the program OMEGA (4). Partial charges had been assigned to every atom with the program AMSOL (5). We note that a search for known β_2 AR agonists by individually comparing the molecules of the subset to the 55 annotated agonists in the WOMBAT database (6) with Pipeline Pilot (7) yielded no exact matches.

Competition Assay. All K_i values were determined based on radioligand displacement assays by using ≈ 60 fmol of ^3H -dihydroalprenolol preincubated with the receptor. Compound stock solutions were prepared in DMSO at a final concentration of 20 mM. Membranes containing the wild-type β_2 AR were prepared from baculovirus-infected Sf9 cells as previously described (8). To initially assess the ability of compounds to bind to the receptor, a single-point competition experiment was performed: identical aliquots of membranes containing ≈ 60 fmol of ^3H -dihydroalprenolol (DHA) binding activity were incubated with 1 nM DHA and 20 μM test compound in 0.5 mL total volume. After 1 h at room temperature with shaking,

binding reactions were filtered by using a Brandel harvester, and filters containing membranes were analyzed for bound DHA by using a Beckman LS6000 scintillation counter. Total possible DHA binding was determined by performing reactions in the absence of test compound, and nonspecific binding was determined by performing reactions in the presence of 10 μM cold alprenolol. All reactions were done in triplicate. Percent displacement was calculated as $[1 - (\text{specific binding with test compound} / \text{specific binding without test compound})]$, where specific binding is the average counts recorded for the competition experiment less the measured nonspecific binding. To obtain competition binding curves, we followed the same protocol as described in ref. 9, using the same membranes as in the single-point competition assay.

Functional Assay. Wild-type unliganded β_2 AR was purified to homogeneity as described previously (9). Purified β_2 AR and tethered- $G_{\alpha s}$ protein (Tet- $G_{\alpha s}$) were mixed in a molar ratio of 1:5 and reconstituted as described in ref. 10. In brief, purified receptor and Tet- $G_{\alpha s}$ were mixed with DOPC lipids supplemented with cholesterol hemisuccinate (0.3 mg/mL and 0.03 mg/mL, respectively) plus reconstitution buffer (20 mM Hepes, pH 7.4, 100 mM NaCl) to 300 μL and placed on ice for 2 h. Vesicles were allowed to form by removing detergent on a Sephadex G-50 (fine) column (25×0.8 cm) using reconstitution buffer.

For [^{35}S]GTP γS binding, vesicles containing reconstituted receptor with Tet- $G_{\alpha s}$ were resuspended in 500 μL of cold binding buffer (75 mM Tris-HCl, pH 7.4, 12.5 mM MgCl $_2$ and 1 mM EDTA) supplemented with 0.05% (wt/vol) BSA, 0.4 nM [^{35}S]GTP γS and GDP (1 μM) with or without ligands. Isoproterenol and ICI 118551 were tested at a final concentration of 10 μM ; all other compounds were tested at a final concentration of 100 μM . Incubations were performed for 30 min at 25 $^\circ\text{C}$ with shaking at 230 rpm. Nonspecific binding was determined in the presence of 100 μM GTP γS and was always $< 0.2\%$ of total binding. Bound [^{35}S]GTP γS was separated from free [^{35}S]GTP γS by filtration through glass fiber filters followed by 3 washes with 3 mL of cold binding buffer. Filter-bound radioactivity was determined by liquid scintillation counting.

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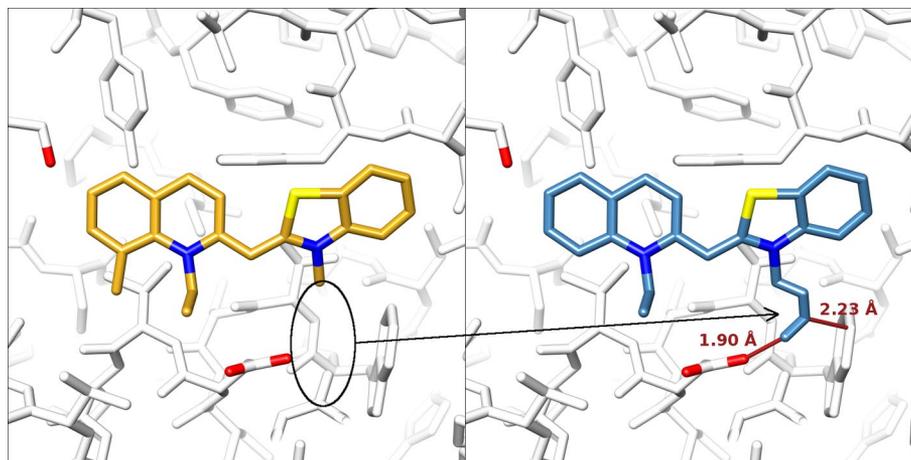


Fig. S1. Comparison of the binding mode of 5, a binder (depicted on the left with golden carbons), with the hypothetical binding mode of 7 (depicted on the right with light-blue carbons). This is the negative control and indeed a nonbinder). The clashes with the protein are drawn as red sticks.