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Conserved Binding Mode of Human β_2 Adrenergic Receptor Inverse Agonists and Antagonist Revealed by X-ray Crystallography

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Abstract: G protein-coupled receptors (GPCRs) represent a large fraction of current pharmaceutical targets, and of the GPCRs, the β_2 adrenergic receptor (β_2AR) is one of the most extensively studied. Previously, the X-ray crystal structure of β_2AR has been determined in complex with two partial inverse agonists, but the global impact of additional ligands on the structure or local impacts on the binding site are not well-understood. To assess the extent of such ligand-induced conformational differences, we determined the crystal structures of a previously described engineered β_2AR construct in complex with two inverse agonists: ICI 118,551 (2.8 Å), a recently described compound (2.8 Å) (Kolb et al, 2009), and the antagonist alprenolol (3.1 Å). The structures show the same overall fold observed for the previous β_2AR structures and demonstrate that the ligand binding site can accommodate compounds of different chemical and pharmacological properties with only minor local structural rearrangements. All three compounds contain a hydroxy-amine motif that establishes a conserved hydrogen bond network with the receptor and chemically diverse aromatic moieties that form distinct interactions with β_2AR. Furthermore, receptor ligand cross-docking experiments revealed that a single β_2AR complex can be suitable for docking of a range of antagonists and inverse agonists but also indicate that additional ligand−receptor structures may be useful to further improve performance for *in-silico* docking or lead-optimization in drug design.

G protein-coupled receptors (GPCRs) are the largest protein family involved in signal transduction across membranes.[1] The β_2 adrenergic receptor (β_2AR) is one of the best characterized members of the GPCR family, for which pharmacologically distinct high-affinity ligands have been described as (i) agonists (compounds activating signaling), (ii) antagonists (blocking agonist signaling), or (iii) inverse agonists (blocking both agonist and basal signaling). The human β_2AR structure has previously been determined in complex with two partial inverse agonists, carazolol ($^{Car}\beta_2$AR-t4l)[2] and timolol ($^{Tim}\beta_2$AR-t4l)[3] and turkey β_1 adrenergic receptor has been determined in complex with the antagonist cyanopindolol.[4] A number of studies have used these structures for *in silico* ligand docking and discovery of new scaffolds of β_2AR ligands.[5−8] Currently, a challenge for rational drug design and docking studies is to ascertain to what degree the conformation of the ligand binding site changes upon interaction with different compounds. To assess the extent of such ligand-induced conformational differences and reveal further details of ligand binding, we determined the X-ray crystal structure of β_2AR in complex with two of the most potent inverse agonists and the well-known antagonist alprenolol.

Using a previously described engineered β_2AR construct,[3] the cocrystal structures of β_2AR-t4l in complex with **1** ($^{ICI}\beta_2$AR-t4l), **2** ($^{2}\beta_2$AR-t4l), and **3** ($^{Alp}\beta_2$AR-t4l) were determined at 2.8, 2.8, and 3.1 Å, respectively (Figure 1; see Supporting Information for experimental details). All three structures show the same overall fold observed for the previous $^{Car}\beta_2$AR-t4l and $^{Tim}\beta_2$AR-t4l structures with an rmsd of ∼0.3 Å (over β_2AR Cα atoms only) between all five reported β_2AR-t4l-ligand structures (Figure 2). Ligand mass spectrometry identification, receptor thermostability analysis, and the crystal structures reported here are consistent with the presence of compound **1**, **2**, and **3** bound in each of the β_2AR-t4l complexes. The electron density shows the compounds bound to the same orthosteric binding site as carazolol and timolol, with minor differences in side chain orientations that reflect specific ligand−receptor interactions (Figure 2).

The binding pocket of β_2AR can be described as a narrow cleft surrounded by mostly hydrophobic residues, with few polar residues located at the 'front' (Asp113$^{3.32}$, Tyr316$^{7.43}$ and Asn312$^{7.39}$) and 'back' (Ser203$^{5.42}$, S207$^{5.46}$ and Asn293$^{6.55}$) of the binding site (Figures 1 and 2). Compounds **2**, **3**, carazolol, and timolol contain an aliphatic oxypropanolamine moiety (compound **1** has a structurally similar oxybutanolamine), referred to as the ligand tail, and chemically and structurally diverse aromatic systems defined as the ligand head groups.

The amine and hydroxyl groups in the tails of **1**, **2**, and **3** establish a conserved hydrogen bond network with the receptor polar triad Asp113$^{3.32}$, Tyr316$^{7.43}$, and Asn312$^{7.39}$ in the 'front' of the pocket that closely resembles the ligand interactions observed in the $^{Tim}\beta_2$AR-t4l and $^{Car}\beta_2$AR-t4l structures (Figures 1 and 2). The aromatic head groups of the ligands, however, are mostly anchored between the side chains of Val114$^{3.33}$ and Phe290$^{6.52}$ in the 'back' of the binding site, where each compound establishes distinct interactions with β_2AR (Figure 1).

Compared to carazolol, timolol, and compound **2**, the dihydro-indene head group of the inverse agonist compound **1**[9] is smaller and does not contain any polar groups that could accept or donate hydrogen bonds. Also, the $^{ICI}\beta_2$AR-t4l structure shows the additional methyl group in the tail of compound **1** in the vicinity of Phe193$^{5.32}$ and the cyclopentene ring of the dihydro-indene in close proximity to the Phe289$^{6.51}$ and Phe193$^{5.32}$ side chains in the 'back' of the binding site (Figures 1 and 2). Furthermore, **1** has an additional methyl group on the aromatic system, and a comparison between all β_2AR-t4l-ligand structures shows that this compound requires some rearrangements in Ser203$^{5.42}$ (∼1.2 Å compared to other β_2AR-t4l structures) and a slight local shift of ∼0.4 Å in transmembrane helix 5 (TM V) (Figure 2).

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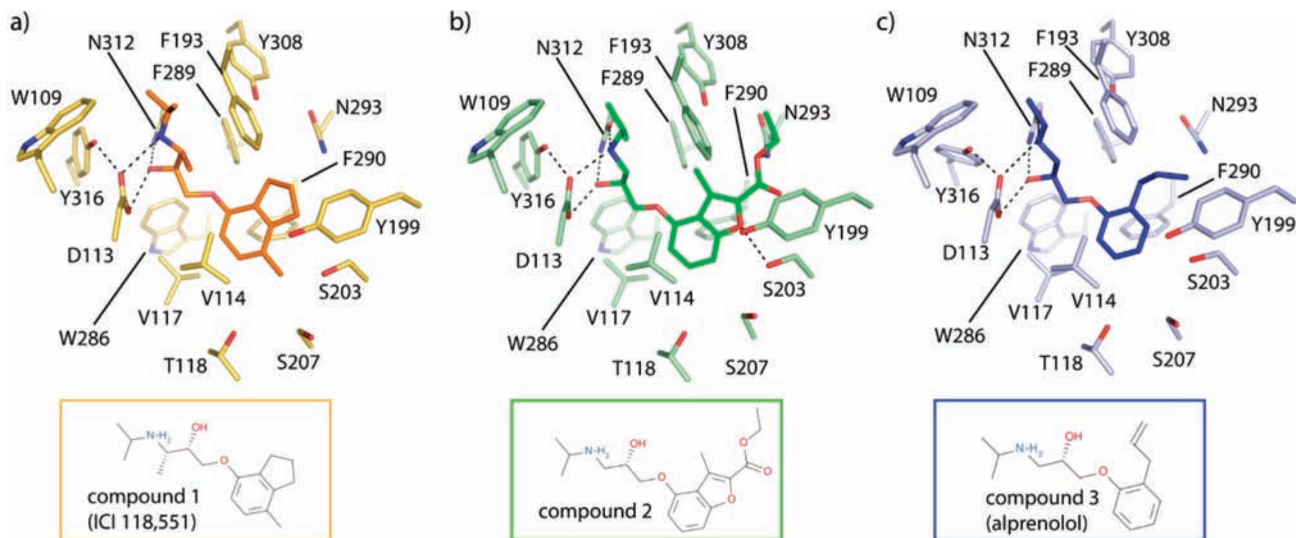

Figure 1. Structural comparison of the ligand binding sites in the (a) $^{ICI}\beta_2$AR-t4l, (b) $^{2}\beta_2$AR-t4l, and (c) $^{Alp}\beta_2$AR-t4l crystal structures. The ligands **1** (ICI 118,551), **2**, and **3** (alprenolol) are colored in darker shades of orange, green, and blue, respectively, while residues around the binding site are colored in lighter shades and labeled. Hydrogen bonds are depicted as black dotted lines. Chemical structures of compounds are shown in boxes.

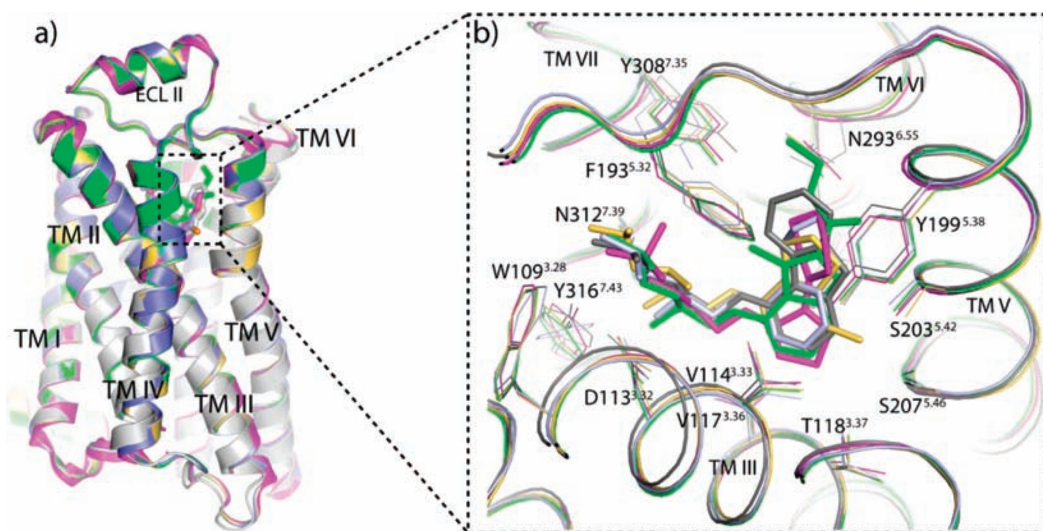

Figure 2. Conserved overall fold of the $^{ICI}\beta_2$AR-t4l, $^{2}\beta_2$AR-t4l, and $^{Alp}\beta_2$AR-t4l structures compared to $^{Tim}\beta_2$AR-t4l and $^{Car}\beta_2$AR-t4l. (a) Superimposition of all β_2AR-t4l crystal structures determined to date (t4l omitted): $^{ICI}\beta_2$AR-t4l (yellow), $^{2}\beta_2$AR-t4l (green), $^{Alp}\beta_2$AR-t4l (blue), $^{Tim}\beta_2$AR-t4l (magenta), and $^{Car}\beta_2$AR-t4l (gray). (b) Close view of the ligand binding site showing the conserved binding of the hydroxy-amine motif and the differences in the aromatic system moieties. Compounds are shown as sticks, and surrounding residue side chains are shown as lines. Superscripts indicate the Ballesteros−Weinstein numbering convention.

The structure of $^{2}\beta_2$AR-t4l provides further structural insights into the binding mode of the strong inverse agonist compound **2**.[6] The geometry adopted by compound **2** in the active site of the $^{2}\beta_2$AR-t4l structure overlaps well with that of carazolol in the $^{Car}\beta_2$AR-t4l structure, and we also observe a hydrogen bond between the side chain of Ser203$^{5.42}$ (TM V) and the benzofuran oxygen of compound **2** (Figures 1 and 2). In addition, the ethyl-carboxylate moiety extends toward Asn293$^{6.55}$ and allows for an additional hydrogen bond interaction between the ethoxy oxygen and the amine group of Asn293$^{6.55}$ side chain in TM VI (Figure 2). A comparison between the available crystal structures of β_2AR-ligand complexes reveals that compound **2** is the only ligand that connects TM V and VI through hydrogen bond networks. Other than a few minor differences, the compound **2** pose in the $^{2}\beta_2$AR-t4l structure is similar to that predicted by Kolb et al.[6] with an rmsd of ∼0.9 Å.

Unlike compound **1**, **2**, timolol, and carazolol, which contain at least one cyclic system other than the aromatic ring, the allylbenzene head group of the antagonist compound **3**[10] is smaller and contains only a short prop-1-ene attached to the benzene group. Although the $^{Alp}\beta_2$AR-t4l structure has been determined at 3.1 Å resolution and therefore decreased confidence in the ligand placement (see Supporting Information), there is sufficient electron density detail to orient the prop-1-ene chain of **3** in the same location as the cyclic system present on the other four compounds (Figure 2).

Although we observe a conserved binding mode for the β-hydroxy-amine motif on the ligand tails, a common feature among the 'classical' scaffold of β_2AR ligands with inverse agonist, antagonist, or full/partial agonist activities,[5] all β_2AR-t4l-ligand crystal structures show distinct interactions between the head groups of the ligands and the receptor (Figures 1 and 2). While the aromatic moieties of all compounds are anchored by strong hydrophobic interactions in the binding cleft, specific hydrogen bonds are also established by substituent moieties in compound **2**, timolol, and carazolol.

Recently performed large scale docking and virtual screening studies[6,11] suggest that the ${}^{Car}\beta_2$AR-t4l structure is highly efficient in screening for a wide range of antagonists and inverse agonists, though certain changes in the binding pocket may still be required for optimal binding of high affinity agonists. Since almost identical conformations were found for the ligand binding site in all five β_2AR-t4l structures, we set out to investigate whether a single complex structure could be suitable for docking a range of antagonists and inverse agonists.[6,11] To test this hypothesis, we performed cross-docking experiments where each of the five ligands was docked into each β_2AR-t4l structure. The results (see Supporting Information) show excellent accuracy of docking pose predictions (rmsd < 1 Å) and high binding scores (ICM Score < −30 kJ/mol) for the docked compounds. The exception is compound **1**, which cross-docks poorly into all other crystal structures, mostly because of its exocyclic methyl group, which cannot be optimally accommodated within the slightly smaller pockets of the other structures. Overall, these results support the applicability of different β_2AR-ligand structures for docking and virtual screening of antagonists and inverse agonists. Substantially better binding scores for self-docking (except for compound **2**), however, suggest that additional ligand−receptor structures can further improve the performance of *in silico* docking and can be particularly valuable for rational drug design at lead optimization stages.

Minimal structural differences between the three complexes reported here indicate that the ligands studied exert only a minor local impact on the structure of the receptor. The most conserved region is the 'front' part of the orthosteric binding pocket of the receptor, and therefore it is unlikely to be associated with distinct pharmacological properties of antagonists and inverse agonists. Instead, differences in specific interactions between the ligand and receptor TMs III, V, and VI that take place through the aromatic ring system appear to define the pharmacologic effects. Note that agonists, characterized by a distinctly shorter "tail" and multiple polar substituents in the aromatic system, are likely to introduce other changes in the β_2AR binding pocket associated with activation of the receptor, although the degree of these changes are yet to be structurally observed.

The result that β_2AR bound to pharmacologically distinct ligands (antagonists and inverse agonists) have virtually identical backbone conformations in the crystal structures suggests that the conformational changes capable of modifying signaling properties are very small, beyond the resolution of the obtained data. Alternatively, the major effect of inverse agonists, antagonists, and extrapolated to agonists on β_2AR is not on modifying a specific conformation with large conformational changes, but on minor structural changes and a significantly larger contribution from receptor dynamics. The answer to this intriguing problem should likely arrive from a combination of crystallography with techniques sensitive to dynamics, such as NMR,[12] EPR,[13] and HDX.[14]

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Supporting Information Available: Supplementary experimental procedures, crystallographic data, structural figures, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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