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Use of the X-ray structure of the Beta2-adrenergic receptor for drug discovery

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Abstract—The recently reported X-ray structure of the Beta2-adrenergic receptor, the first reported crystal structure of a ligand-mediated GPCR, is used to explore its utility in computer-aided drug design. Validations were conducted with known beta blockers. This was followed by high-throughput docking studies with proprietary and commercial databases to further validate the X-ray structure's usefulness as a design tool and to explore the potential for discovery of novel chemical classes acting as Beta2 inhibitors. Our results include the finding of ligands with traditional beta-blocker motifs as well as new motifs, thereby serving to both validate the approach and project its usefulness in the finding and design of novel compounds.

The use of X-ray structures in drug discovery is now well established as a powerful means to design and/or screen for new leads and drugs. To date, these approaches have predominantly been applied to soluble proteins whose crystal structures are more amenable to isolation, purification, and crystallization. In contrast to this, while GPCRs play a pervasive role in pharmaceuticals and represent the largest single class of targets for drug discovery and >30% of marketed drugs, the generation of X-ray structures for ligand-mediated GPCRs has eluded investigators for many years. Indeed, the best available X-ray structure of GPCRs, until recently, was that of bovine Rhodopsin.[1,2] Rhodopsin is, however, a light-activated (as opposed to ligand-mediated) GPCR. Thus, while this representative of a Class I GPCR protein provided the opportunity for significant advancements in GPCR homology modeling, there remained the inherent limitations of Rhodopsin in drug discovery: (1) a non-ligand-mediated template and (2) the need for homology models. Such limitations have been difficult to assess. The recent report of the high-resolution crystal structure of the Beta2-adrenergic receptor[3–5] now affords the opportunity for evaluating an actual ligand-mediated GPCR in structure-based design without the above limitations. As discussed by Kobilka and coworkers,[3–5] there are indeed notable differences in the structural features and conformation of the Beta2 receptor compared to that of Rhodopsin as well as unexpected features. For example, there is an alpha helix in the second extracellular loop, ECL2, which was not predicted by models based on Rhodopsin. Compared to Rhodopsin, the Beta2 receptor's extracellular regions of helices I and III sit more outwardly of the ligand-binding site, and other changes are observed in helices I, V, and VI. We also note that the interactions of Corazolol with the protein differ from those of previous homology models for antagonist binding using Beta2 homology models.[6,7] Thus, while both the amino and the hydroxyl groups of the Corazolol side chain each bind to both Asp113 and Asn293, Freddolino et al.[6,7] predict that the hydroxyl of Propranolol interacts with Ser203 and Ser204, and for Butoxamine, a methyl ether oxygen atom on the phenyl ring interacts with Asn293. In the present study, we embark on computational studies using the X-ray structure of the Beta2 receptor for the initiation of drug discovery.

The X-ray structure of the Beta2 receptor includes the cocrystallized ligand Corazolol (Fig. 1), a high affinity inverse agonist (see Fig. 2a). As an initial validation, docking models of the protein were prepared using standard protocols.[8] We used the Glide-XP and GOLD-GoldScore docking tools to dock the *S* isomer of Corazolol into the Beta2 models. The *S* isomer is the more potent isomer as well as the one used to solve

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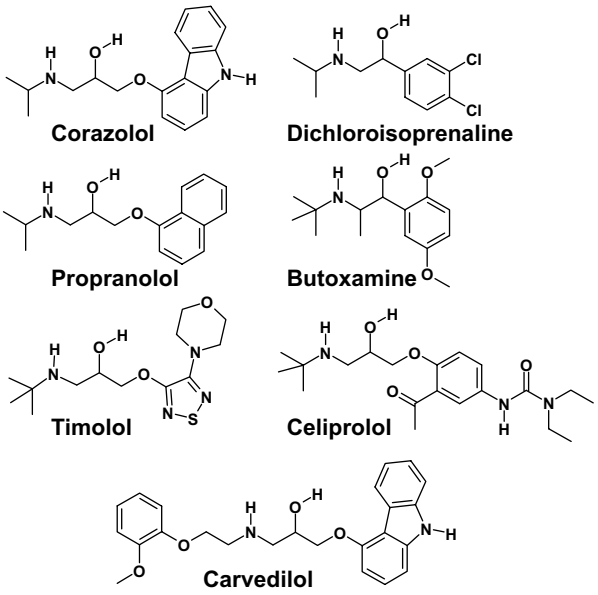

Figure 1. Structures of potent Beta2 ligands.

the X-ray structure. Figure 2b shows these docked structures compared to the X-ray structure, in what are predicted to be the most favored binding modes. Both the Glide and GOLD approaches produce Corazolol binding poses that have striking overlaps with the ligand in the X-ray structure. The key hydrogen bonds and salt bridges are maintained, where the amine and hydroxyl groups are interacting with Asp113 and Asn293. Also, the aromatic carbazole group overlaps strikingly well with its X-ray counterpart. Finally, the isopropyl side chains sit in the same location with both docking protocols. We note that we found a very similar binding mode, having the same interactions, for the *R* isomer of Corazolol, which is slightly more favored by the Glide-XP docking protocol, but the *R* isomer's ab initio energy is 2 kcal/mol higher than the *S* isomer when both are frozen in their binding structures. Below, we continued with Glide and will be conducting a similar study with GOLD.

While the ability to reproduce the binding mode observed in the X-ray structure is a necessary validation step, we are also concerned that the model be validated with other known ligands. To further assess the use of the X-ray model for docking, we therefore studied six known beta blockers (i.e., Propranolol, Timolol, Butoxamine, Dichloroisoprenaline, Celiprolol, and Carvedilol) to ensure that they bind in a reasonable manner. Figure 3 depicts our predicted binding mode for each of these ligands using Glide-XP. For each of these ligands, the binding mode is very compelling when compared to that of Corazolol. In each case, the aminoethyl-hydroxyl segments overlap well. The aromatic/hydrophobic regions overlap well with that of the carbazole group of Corazolol. The overall hydrophobic nature of this region is clear from these overlaps. In the case of Celiprolol, the phenyl ring rotates away from what would be the topologically corresponding ring of Corazolol, thereby having the acetyl portion, instead of the more hydrophobic *N*,*N*-diethylurea group, overlap with the rest of the carbazole moiety. It is clear from the X-ray structure that, were the phenyl group of Celiprolol to be positioned by a topology corresponding to Corazolol, the para position of the phenyl ring would be close to the backbone of Ser203 and Ser204 of helix 6, thereby colliding with the *N*,*N*-diethylurea group. From examination of the X-ray structure, we also note that the carbazole NH of Corazolol, which is 3.3 Å from the OH of S203 in the X-ray structure, is not represented by a corresponding NH in these analogs, suggesting that this proton-donating interaction is not required. As seen in the Timolol structure, the Corazolol-carbazole counterpart need not be purely aromatic. Finally, each of these examples overlaps nicely on Corazolol's isopropyl moiety with the corresponding hydrophobic tail. Taken together, the results for these known ligands support the use of this X-ray-based model for drug discovery.

The above examples illustrate the ability of the model to explain the binding modes of known ligands when studied individually. A valuable approach in drug discovery where X-ray structures are available is the use of the protein structure for in silico screening of large libraries

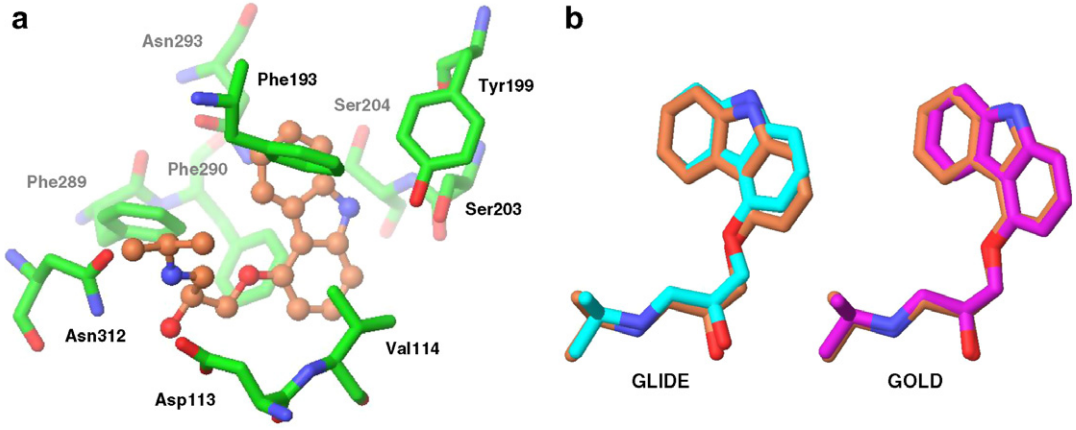

Figure 2. (a) X-ray structure of Corazolol in the Beta2-adrenergic binding site. (b) Overlap of the X-ray structure of Corazolol in the binding site (in brown) compared to the docked structures of Glide (left) and GOLD (right).

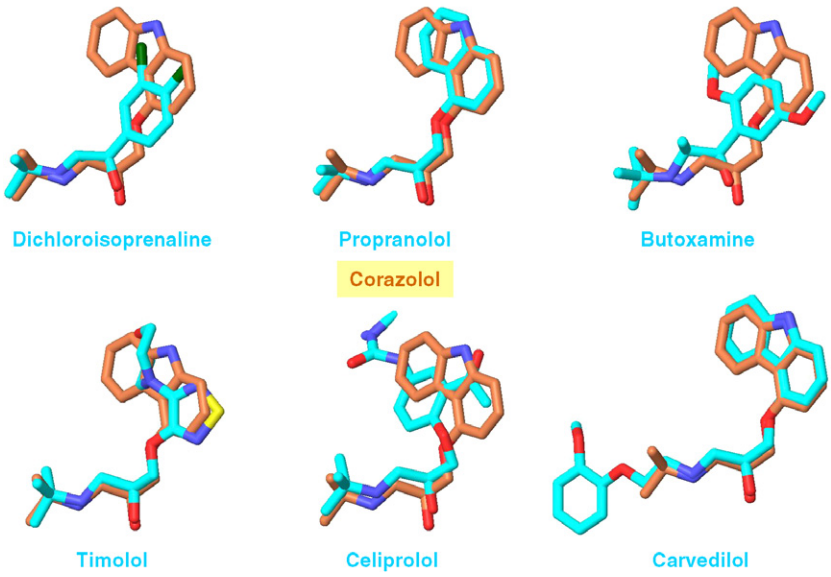

Figure 3. Overlap of docking results with the X-ray structure of bound Corazolol with docked structures of Dichloroisoprenaline, Propranolol, Butoxamine, Timolol, Celiprolol, and Carvedilol.

of compounds via high-throughput docking (HTD). The Beta2 X-ray structure now opens the door to the use of these same approaches for this GPCR target without having to employ a homology model. We performed a test study of this approach using a library of approximately four million commercially available compounds (see below). We first conducted a similar study, as a preliminary test, using a tenfold smaller selection from an in-house compound collection. The test of the effectiveness of an HTD approach lies in its ability to provide effective ranking of active ligands in a large database of compounds. In the top 30 (0.008%) scoring compounds of the proprietary database resulting from the present HTD study using Glide, both Corazolol and Carvedilol (see Fig. 1) were found. Indeed, in the top 100 compounds there were 11 compounds which were either known beta blockers or very closely related structures.

An important advantage for the role of structure-based design studies is the potential to find new chemical classes for a given target. With the above results supporting the ability of the HTD to identify active compounds, we now explore the ability of the model to provide chemical leads that are structurally different from the traditional motif. In a preliminary analysis of this, we show in Figure 4a the overlapped structures of the top 100 scoring ligands in the HTD of the proprietary database. These 100 structures have highly similar docking scores, which suggest that the predicted binding abilities of the 100 structures are not distinguishable. In Figure 4a, we see that many of these top scorers significantly overlap with the ligand, Corazolol, in the X-ray structure. We also see that a significant number of the ligands occupy a space complementary to that of Corazolol. As traditional beta blockers were found in the absence of X-ray structures, the beta blockers evolved around a common motif. It is therefore likely that they do not sample and/or capture all of the binding opportunities offered by the protein. To help understand the potential of finding ligands that probe these other regions of the protein, we extracted from the 100 structures those ligands that have the amino-alkyl-hydroxyl motif; see Figure 4b. We see that a significant number of the residual compounds still occupy the same region of space. This suggests that new chemical classes could be found which interact with the same region as traditional inhibitors. To further assess how critical is the occupancy of this region and how divergent new chemical classes can be, in Figure 4c, we also removed all compounds (by visual inspection) with significant structural overlap with this region (40 compounds). Strikingly, we find that removal of those compounds that do not have any significant overlap with the amino-alkyl-hydroxyl moiety leaves approximately half of the compounds. In the inverted U-shaped overlaps of Figure 4c, the lower right-hand side corresponds to the carbazole portion of Corazolol, while the lower left portion corresponds to the isopropyl chain of Corazolol. In addition to these 2 hydrophobic regions, there is a densely populated region, as shown at the top of Figure 4c. Figure 4d and e shows these same overlaps with the 7TM region represented by a ribbon diagram. It is clear that this upper region provides interactions with the extracellular loops ECL2 and ECL3. For GPCRs which bind endogenous peptides, this extracellular region is where the primary binding is believed to occur. It is therefore quite reasonable to find that binding in this region can play an important role. The observation that a good fraction of the top-scoring ligands bind in this EC region and the 2 hydrophobic pockets described above, but without occupying the amino-ethyl-hydroxyl core, demonstrates that some of the top-scorers represent very different chemical classes compared to the traditional beta blockers. This observation may provide a source for novel compounds.

We have repeated the above HTD study using a database of compounds that is approximately an order of

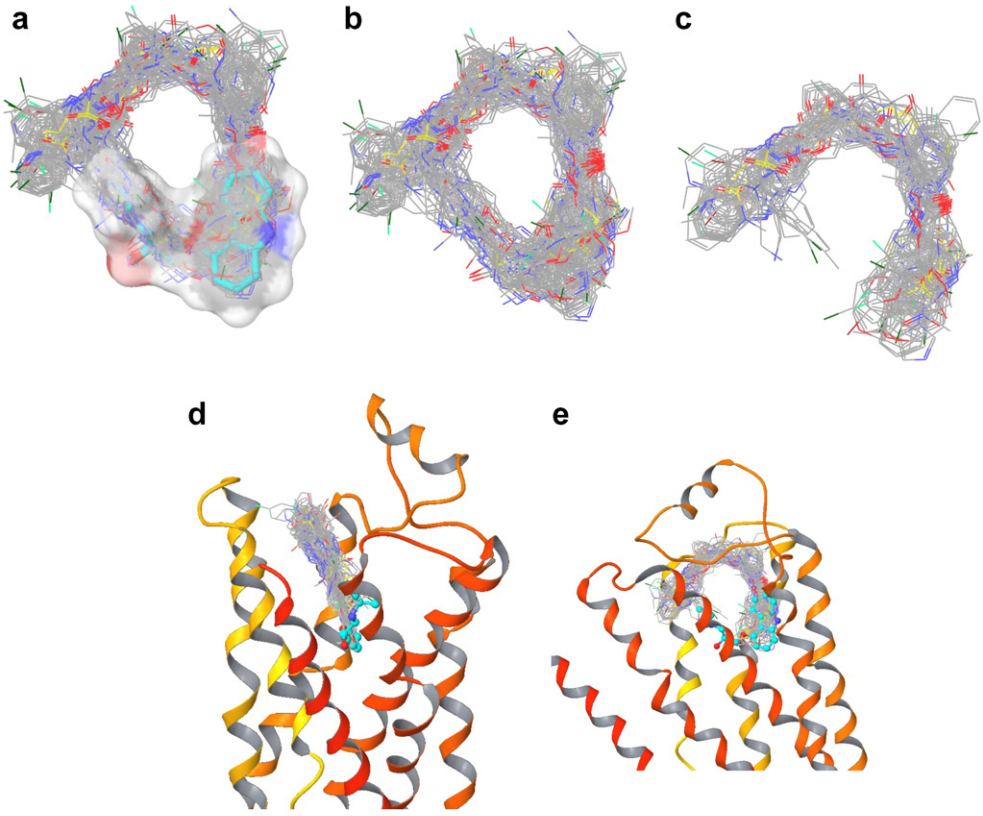

Figure 4. (a) Overlay of the top 100 Glide docking hits from a database of proprietary compounds and Corazolol from the X-ray structure. The gray surface is that of Corazolol. (b) Same as (a) after removal of compounds with the traditional beta blocker alkyl motif. (c) Same as (b) after removal of all compounds with atoms in the region of the traditional beta blocker alkyl motif. (d and e) Same as (c) showing a ribbon diagram of the protein. (d and e) Rotated 90° around the vertical axis with respect to each other.

magnitude larger, that is, 4 million compounds. This serves as a more stringent evaluation of the potential for extracting ligands that are expected to bind well to the Beta2 receptor based on historical data. This is also an opportunity to sample a more diverse space to identify novel compounds. A comparison of the overlapping of the top-scoring compounds (not shown) is similar to that depicted in Figure 4 for the smaller, proprietary database. In these hits as well, we find structures that are both different from traditional beta blockers and diverse with respect to each other. Thus, here too, the examples span different binding motifs. While we do not expect all of the compounds in the top-scoring few hundred compounds, to be potent inhibitors, the results do indicate that promising new directions are made available through the use of the recently published X-ray structure. Testing of these compounds is planned and will be reported separately.

The long-awaited, first X-ray structure of the ligand-mediated GPCR, the Beta2-adrenergic receptor, has recently become available. We have examined herein the viability of its use for computer-aided drug design. Validation of the docking model has been provided by the ability to (i) reproduce the binding mode of the cocrystallized ligand, Corazolol, (ii) predict compelling binding modes for known antagonists, and (iii) extract known antagonists from HTD studies. Analyses of the hits from HTD studies indicate the ability to find diverse chemical leads that differ from historic beta-antagonist motifs. Moreover, it appears that the recently published, high-accuracy structure of the Beta2-adrenergic receptor has ushered in the era of structure-based drug design for GPCRs.

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8. The Glide docking studies were conducted with the Schrödinger software suite.[9] Hydrogen atoms were first added to the coordinates of the X-ray structure of the Beta2-adrenergic receptor. Hydrogen atoms that appeared to be pointed in unfavorable directions were initially adjusted by hand. This was followed by a slight relaxation of all the coordinates using the Protein Preparation module. For the high-throughput docking studies, the faster high-throughput virtual screening (HTVS) mode was used to extract 50,000 structures. These were then subjected to a second HTD round using the standard precision (SP) mode. In the case of the library of commercial compounds, the top-scoring 5000 structures were then evaluated using the extra precision (XP) mode. For the individual ligand studies, the XP mode was used. Version 8 of Schrödinger's software suite was used for all the XP studies and version 7 for all the other studies. For GOLD, the protein preparation step was the same as that for Glide (above), except that relaxation was excluded. Docking was performed with the GOLD software package[10] from CCDC.[11] The GoldScore parameters were used for scoring. Ab initio calculations were performed with the Jaguar module from Schrödinger.[9] The B3LYP density functional method was used with the 6-311G** basis set. The proprietary and commercial databases were prepared for docking by using CORINA[12] to convert 2D coordinates to 3D structures, add hydrogen atoms, generate stereoisomers, and sample ring conformations. Schrödinger's LigPrep module[9] was used to adjust protonation states to pH 7.
9. Glide v4.0 with Maestro v7.5, Glide v4.5 with Maestro v8.0, and Jaguar v7.0 Schrödinger, LLC:2005, Portland, US.
10. Gold version 3.2 CCDC. www.ccdc.cam.ac.uk.
11. CCDC (Cambridge Crystallographic Data Centre), 12 Union Road, Cambridge CB2 1EZ, UK. Tel.: +44 1223 336408.
12. CORINA Molecular Networks. www.molecular-networks.de; Henkestraβe 91, 91052 Erlangen, Germany. Tel.: +49 9131 815668.