Similarity- and Substructure-Based Development of $\beta_2$-Adrenergic Receptor Ligands Based on Unusual Scaffolds

Denis Schmidt,†‡ Jakub Gunera,† Jillian G. Baker,¶ and Peter Kolb*†‡

Abstract: The $\beta_2$-adrenergic receptor ($\beta_2$AR) is a G protein-coupled receptor (GPCR) and a well-explored target. Here, we report the discovery of 13 ligands, ten of which are novel, of this particular GPCR. They have been identified by similarity- and substructure-based searches using multiple ligands, which were described in an earlier study, as starting points. Of note, two of the molecules used as queries here distinguish themselves from other $\beta_2$AR antagonists by their unique scaffold. The molecules described in this work allow us to explore the ligand space around the previously reported molecules in greater detail, leading to insights into their structure–activity relationship. We also report experimental binding and selectivity data and putative binding modes for the novel molecules.

Keywords: $\beta_2$-adrenergic receptor, similarity searches, docking, SAR-by-catalog

The membrane receptors of the G protein-coupled receptor (GPCR) family are flexible heptahelical bundles trans-ferring signals from the outside to the inside of a cell. This is achieved by a conformational change of the receptor upon binding of a signaling molecule to a cavity located at the extracellular end between the seven helices. GPCRs are expressed in almost all tissues, and it is thus not surprising that approximately 1/3 of present-day drugs interact with a GPCR. Among these receptors, the $\beta_2$-adrenergic receptor ($\beta_2$AR) is considered a prototypical representative and has been investigated for more than 60 years. It was also the first pharmacologically relevant GPCR to succumb to crystallization in 2007. In a previous work, we have identified six ligands (originally labeled 1–6, and referred to as Q1–Q6 in this work to avoid confusion, Chart S1) of the $\beta_2$AR through in silico docking studies, with affinities ranging from 9 nM to 3.2 μM. Notably, these included two molecules (5 and 6 in ref S, denoted as Q5 and Q6, respectively, in the following) that did not follow the classical adrenaline-based scaffold. This was remarkable, as nobody had observed these scaffolds earlier, despite more than six decades of medicinal chemistry in this area. Building upon the discovery of the six ligands, we wanted to expand chemical space around them. In particular, we wanted to investigate the two ligands with unusual scaffolds by employing in silico similarity and substructure searches in the ZINC database. Candidate molecules identified in either way were then docked into the $\beta_2$AR, in order to ascertain that their binding modes were consistent. Here we report the results of this combined ligand- and structure-based screen, which also provides insights into the structure–activity relationship (SAR) of molecules Q5 and Q6 and their derivatives.

The similarity screen among the 8.5 million molecules of the ZINC database resulted in 6363 molecules, which were distributed across the six query molecules as shown in Table S1. From the substructure-based screen, approximately 653 000 hits emerged. Duplicates were removed from both sets. After docking, 5838 and 587 099 molecules remained, respectively, and the top-scoring 500 of each run were visually inspected. After weeding out molecules with artificially inflated scores due to the absence of corrective terms in present-day scoring functions, e.g., unfavorable desolvation contributions or unsatisfied hydrogen-bond donors, during this inspection, we were left with eight and nine molecules from the similarity and substructure searches, respectively. These were acquired from their respective vendors for further experimental testing (Table S5). Three compounds (1, 2, and 3) contained a baryl moiety and a charged amine and thus resembled the classical motif of a $\beta_2$ binder. Indeed, a thorough literature search revealed that these compounds had been described before (Table 1; by the time of selection, these compounds had not been annotated in ChEMBL). To analyze the selectivity of the compounds, we also evaluated them against the closely related $\beta_1$AR. The
Table 1. Affinity ($K_D$ values) and $\beta_2$-Selectivity for Compounds as Measured by $[^3H](-)$ CGP 12177 Whole Cell Binding to CHO-$\beta_1$ and CHO-$\beta_2$ Cells; Values Are Mean $\pm$ SEM of $n$ Separate Experiments

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
<th>$\beta_2$AR $pK_D$</th>
<th>n</th>
<th>$\beta_1$AR $pK_D$</th>
<th>n</th>
<th>$\beta_2$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>5.42 $\pm$ 0.14</td>
<td>5</td>
<td>4.34 $\pm$ 0.07</td>
<td>4</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>5.58 $\pm$ 0.06</td>
<td>6</td>
<td>4.56 $\pm$ 0.06</td>
<td>6</td>
<td>10.5</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>10.45 $\pm$ 0.05</td>
<td>8</td>
<td>9.01 $\pm$ 0.04</td>
<td>5</td>
<td>27.5</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>4.63 $\pm$ 0.07</td>
<td>5</td>
<td>4.01 $\pm$ 0.05</td>
<td>5</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>4.41 $\pm$ 0.08</td>
<td>3</td>
<td>3.59 $\pm$ 0.1</td>
<td>3</td>
<td>6.6</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>4.76 $\pm$ 0.09</td>
<td>5</td>
<td>4.58 $\pm$ 0.03</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7.png" alt="Structure 7" /></td>
<td>4.66 $\pm$ 0.16</td>
<td>5</td>
<td>4.35 $\pm$ 0.04</td>
<td>4</td>
<td>2.0</td>
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<tr>
<td>8</td>
<td><img src="image8.png" alt="Structure 8" /></td>
<td>4.60 $\pm$ 0.11</td>
<td>4</td>
<td>4.33 $\pm$ 0.05</td>
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<td>1.9</td>
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<tr>
<td>9</td>
<td><img src="image9.png" alt="Structure 9" /></td>
<td>4.84 $\pm$ 0.13</td>
<td>4</td>
<td>4.42 $\pm$ 0.11</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>10</td>
<td><img src="image10.png" alt="Structure 10" /></td>
<td>6.05 $\pm$ 0.11</td>
<td>6</td>
<td>5.51 $\pm$ 0.07</td>
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<td>3.5</td>
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<tr>
<td>11</td>
<td><img src="image11.png" alt="Structure 11" /></td>
<td>5.31 $\pm$ 0.12</td>
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<td>4.86 $\pm$ 0.05</td>
<td>5</td>
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<tr>
<td>12</td>
<td><img src="image12.png" alt="Structure 12" /></td>
<td>4.75 $\pm$ 0.12</td>
<td>5</td>
<td>n.c.</td>
<td>4</td>
<td></td>
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<tr>
<td>13</td>
<td><img src="image13.png" alt="Structure 13" /></td>
<td>5.26 $\pm$ 0.06</td>
<td>6</td>
<td>4.45 $\pm$ 0.04</td>
<td>5</td>
<td>6.5</td>
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<td>ICI 118551</td>
<td><img src="image14.png" alt="Structure 14" /></td>
<td>9.61 $\pm$ 0.05</td>
<td>5</td>
<td>6.74 $\pm$ 0.01</td>
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<td>5.84 $\pm$ 0.10</td>
<td>5</td>
<td>8.96 $\pm$ 0.13</td>
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</tbody>
</table>
The efficacy of all compounds was further evaluated in a functional assay. Several of the compounds identified in this work inhibited [3H](−)CGP 12177 whole cell binding (Table 1; see Supporting Information for assay validation and Table S2 for inactive compounds). This assay also demonstrated that compound 3 had very high affinity (pKD 9.01 at β1AR and pKD 10.45 at β2AR) and was therefore 28-fold β2-selective (Figure 1a,c, Table 1). While the remaining compounds had relatively poor affinity in comparison to 3, many of them, e.g., 1, 10, 11, and 13, inhibited [3H](−)CGP 12177 binding to yield measurable affinity values (Figure 1b,d, Table 1).

Next, characteristics of ligands were examined in a functional assay, namely, CRE-gene transcription. The ability of ligands to stimulate a response (intrinsic efficacy) was assessed, but also, given that the affinity of many of the ligands to inhibit [3H](−)CGP 12177 binding were at the very limit of the binding assay, the ability of ligands to inhibit functional responses was also evaluated, thus giving a totally independent measure of affinity from that achieved in the binding assay.

Except for compound 3, no other compound stimulated a measurable response (n = 4–5 for each compound) in this assay (see Supporting Information for more details and assay validation). However, several compounds antagonized the cimaterol response to give a parallel shift of the cimaterol concentration response curve and thus yield measurable KD values (Figure S1, Table S3). For some compounds, e.g., 1, 2, and 13, this gave selectivity values similar to those obtained in the binding assay. For other compounds, e.g., 16 and 17, no rightward shift of the cimaterol response was observed, suggesting no inhibition at the maximum concentration possible (100 μM in each case). For few of the ligands, the highest concentration possible caused a marked fall in CRE-SPAP production to below basal in a manner more consistent with toxicity, cell death, or assay interference, rather than receptor-mediated inverse agonism (see Supporting Information for full details). In these instances, compound concentrations used to inhibit cimaterol responses were reduced until such a time as the reduction in basal was minimal. An example of this was compound 10, which reduced basal at the maximum concentration of 20 μM but not at 2 μM (see Supporting Information for full details).
By reducing the biaryl scaffold to a 2-ethoxy-ethylamine (S6 in Chart S2) for the substructure search, two more substances, 4 and 14, were identified. Compound 4 showed two-digit micromolar affinity, whereas the inhibition by 14 was so weak that no reliable affinity value could be calculated. Interestingly, in 14 the nitrogen matched in the substructure search is the one in the benzoxylic portion, not the exocyclic amine.

Turning to the hits derived from reference molecules Q5 and Q6, we note that they show a much lower Tanimoto similarity of approximately 0.3 and below (when compared to molecules from the ChEMBL database using ECFP4 fingerprints) than the other hits reported in ref 5 (Table S6). This is in line with the fact that these compounds are not based on the classical propanolamine scaffold and underlines the structural novelty of these two scaffolds.

Starting from the benzothiazole-based compound Q5, six molecules were identified with benzothiazole (5, 10, 11, 15) and benzimidazole (16, 17) motifs. Of these, all benzothiazole-containing molecules except 15 show affinity toward the β1AR in the micromolar range. Docking poses indicate that the orientation of the benzothiazole ring is comparable to the one of Q5, with a polarized methyl group interacting with Asp113\(^{3-12}\) (Figures S5 and S6). The benzimidazole compounds 16 and 17 show no activity in our assay. These compounds might be more sterically hindered in the vicinity of the positively charged nitrogen atom, in particular compound 16.

Furthermore, the different polarity of the ring system, owing to the variation of the heteroatoms, might render the predicted interaction with Asp113\(^{3-12}\) less likely.

Six additional compounds could be identified on the basis of the parent molecule Q6. All these molecules (6, 7, 8, 9, 12, and 13) share a benzofuran-based moiety, independent of whether they originated from the substructure or the similarity search. This moiety, namely, a 3-oxo-4-methyl-6-hydroxy-benzofuran, is present in the parent molecule Q6, too, and can thus be considered a “stable scaffold” in terms of SAR. All molecules display affinity, with \(pK_D\) values varying between 5.26 and 4.6.

Interestingly, 8, which is the weakest affinity of this set, differs from 7 only by a methoxy group, which is absent in 8. This methoxy group could act as an acceptor, which is also present in all remaining molecules of this series as (benzo)-furan or methoxy group. The role of this group is not clearly evident from the docking predictions, but an interaction with Thr195\(^{SC2}\) seems to be the most likely explanation (Figures S5 and S6). Furthermore, the docking poses indicate a binding mode of this scaffold, which resembles the key interactions seen in biaryl-based compounds. The benzofuran scaffold forms interactions with Phe193\(^{SG}\), Phe289\(^{SG}\), Phe290\(^{SG}\), and Val114\(^{SG}\). The hydroxy group at position 6 forms an additional hydrogen bond to Asp113\(^{3-12}\), while the ketone serves as acceptor for a hydrogen bond from Ser203\(^{SG}\). A second aromatic moiety is attached at position 2, interacting with Tyr199\(^{SG}\), Tyr308\(^{SG}\), and, presumably, Thr195\(^{SC2}\). An increased size of the aromatic system appears to be detrimental for affinity (methoxyphenyl in 13 vs benzofuran in 9). The charged amine in the pyrrolidine moiety is expected to form a salt bridge with Asp113\(^{3-12}\).

We have elaborated on six previously identified novel binders 236 of the β1AR through SAR-by-catalog. Using similarity and substructure searches followed by a docking assessment of the interactions of each compound and the receptor, 13 ligands of the β1AR were verified experimentally. Ten of these molecules are indeed novel ligands for the receptor, while the remaining 241
three turned out to have been described before. Based on this data, several conclusions can be drawn.

First, the benzofuran scaffold of compound Q5 and the benzothiazole scaffold of compound Q6 in ref 5 indeed constitute novel chemotypes with derivatization potential for this receptor. Especially the benzofuran series showed a consistent SAR that is in agreement with the predicted binding modes. This study can thus also provide retrospective evidence that the predicted binding modes are indeed very likely correct.

The affinities of the novel compounds are not comparable with those of highly optimized adrenaline- or biaryl-based scaffolds. The latter are exemplified by Q1 with an affinity of 9 nM and 3 with its pKᵢₒ of 10.74. However, the novel compounds can serve as unprecedented starting points for further optimization.

Second, that the combination of similarity- and substructure-based searches with protein-structure-based docking constitutes a powerful combination. This is manifest in the quite high hit rate (more than 75% of the molecules bind with an affinity below 100 µM) and the fact that we (re)discovered a molecule with an affinity of only 35 pM. This compound is also known as bipanol or berlafenone, an antiarrhythmia drug.

In terms of selectivity, most of the compounds displaying an affinity are mildly selective toward the β₂AR. Again, 3 takes the lead here at 28-fold selectivity for the β₂AR. While other compounds such as 1 and 2 still have at least 10-fold preference toward the β₂AR, all values are far below 100-fold. For some receptors, however, the novel scaffolds can be optimized. It is also encouraging to have confirmed that unbiased computational methods can present us with novel molecules, even for target proteins as well-investigated as the β₂AR.

EXPERIMENTAL PROTOCOLS

Substructure queries (Chart S2) were manually derived from the original hits. Substructure and similarity searches were run on the ZINC database and docked to the β₂AR (PDB 2RH1), as previously described. [TH(—)]CGP 12177 whole cell binding and CRE-SPAP production assays were run using CHO-K1 cells expressing either the human β₂AR or the human β₂AR as previously described. See Supporting Information for detailed descriptions of experimental procedures.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.b600363.

Tables of similar compounds, SMILES codes for all compounds, detailed experimental methods, Supplementary Figures and Charts (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES