Selective CB2 receptor agonists. Part 1: The identification of novel ligands through computer-aided drug design (CADD) approaches

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Abstract
Computer-aided drug design scaffold hopping strategies were utilized to identify new classes of CB2 agonists when compounds of an established series with low nanomolar potency were challenging to optimize for good drug-like properties. Use of ligand-based design strategies through BI Builder (a tool for de novo design) and PharmShape (a virtual screening software package) approaches led to the discovery of new chemotypes. Specifically, compounds containing azetidine-, proline-, and piperidine-based cores were found to have low nanomolar and picomolar CB2 agonist activities with drug-like properties considered appropriate for early profiling.

Cannabinoids are a group of about 85 distinct compounds found in Cannabis sativa (marijuana) with cannabiol, cannabidiol and Δ9-tetrahydrocannabinol (THC) being the most representative molecules. The therapeutic usage of Cannabis dates back to the ancient dynasties of China and includes applications for various illnesses.1 The physiological effects of cannabinoids are mediated by at least two G-protein coupled receptors, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). CB1 receptors are expressed primarily in the central nervous system and are found also in peripheral tissues including the immune system. CB1 receptors regulate the release of neurotransmitters from the pre-synaptic neurons and are believed to mediate most of the psychotropic effects of cannabis.2 CB2 receptors are predominantly found in the immune system in various cell types, including B cells, dendritic cells, mast cells, microglial cells, monocytes, neutrophils and T cells, suggesting that a wide range of immune functions can be regulated through CB2.3 Small molecule CB2 modulators have been associated with a host of therapeutic indications such as cancer,4 inflammatory and neuropathic pain,5 multiple sclerosis,6 inflammatory settings7 and neurodegenerative diseases.8

Multiple groups, including our own, have pursued different classes of CB2 agonists that have high selectivity over CB1.9,10 GlaxoSmithKline (GW842166X) and Eli Lilly (Example 26) have advanced compounds into human clinical studies for the treatment of pain (Table 1).10 Our in silico approaches have led to the design of additional potent and selective CB2 agonist scaffolds through computer-aided drug design (CADD) efforts.

The two chemotypes of interest to us early in our investigations were the β-sulfonyletacetamides (1)11 and the diazepanes (2),12 both of which displayed CB2 potency (cAMP EC50 <500 nM) and full agonism of the receptor while being selective over CB1 (>100-fold) in an assay measuring the inhibition of cyclic adenosine monophosphate (cAMP) production in forskolin stimulated recombinant CHO cells expressing CB1 and CB2.13 Structure–activity relationships (SAR) common to both compound classes were observed once the left- and right-hand side aryl groups of 1 were incorporated into the diazepane core of 2 to afford compounds such as 3 (Table 2). In general, a broad selection of substituents was tolerated in place of the p-(trifluoromethyl)phenyl groups in 1 and 3, while a narrow selection of right-hand side amines was preferred, with the tert-butylisoxazole being one of our preferred groups.

The potency and selectivity achieved against CB2 in the diazepanes made them an attractive series; however, we had to acknowledge that balancing potency and selectivity with acceptable drug-like properties, such as metabolic stability and permeability, was very difficult despite an intense exploration of SAR.
Hypothesizing that these issues are mainly inherent to the seven-membered core structure, we turned to in silico approaches to identify new CB2 agonist chemotypes for synthesis and profiling with the goal of finding compounds with better overall drug-like properties than the diazepanes.

The parallels in SAR between the two series (e.g., 1 and 3) allowed us to hypothesize that the center parts (the cores) of the β-sulfonylacetamides and the diazepanes were merely scaffolds with the ability to orient their terminal groups into necessary and similar space for bioactivity. To test this hypothesis, the global and low minimum energy conformations of representative molecules from the two series were calculated and analyzed for their similarities.

Compounds 1 and 3 were subjected to multiconformer minimizations using the Torsional Sampling (MCMM) protocol within Schrödinger’s MacroModel. Each of the two chemotypes exhibited two low energy states: an ‘extended’ conformation and a ‘folded’ conformation (Fig. 1). For compounds 1 and 3, the ‘folded’ conformations were energetically favored over the ‘extended’ conformations by 3 and 4 kcal/mol, respectively. The preferences for the ‘folded’ conformations were confirmed with higher order calculations (DFT, B3LYP/6-311+ geometry optimizations). Compounds 2 and 3 were nearly identical in their conformations of the diazepane ring and had similar energy differences between their ‘extended’ and ‘folded’ conformations.

The overlays of the ‘extended’ conformations of 1 and 3 showed the near-matching positions of the left-hand side aryl groups when the tert-butyl isoxazoles were overlaid directly on one another. The overlay of the ‘folded’ conformations of 1 and 3 showed even more remarkable similarities in the positioning and overlap of the left-hand side aryl rings within the two molecules (Fig. 1). Energetically, and through a more complete overlay, the in silico approaches sparked the initial hypothesis that the ‘folded’ conformations of 1 and 3 would be characteristic of the bioactive conformations for these chemotypes against the CB2 receptor.

To help determine if one of these two conformations was responsible for bioactivity, compound 4, the piperizine analog of diazepane 2, was synthesized. Based on multiconformer minimizations, 4 did not adopt the ‘folded’ conformation of 2 within the realm of the calculations. Conversely, 2 and 4 exhibited an excellent overlay of their ‘extended’ conformations (not shown). Experimentally, 4 was found to have a CB2 cAMP EC50 >20,000 nM (Table 3) while 2 measured a CB2 cAMP EC50 of 140 nM, thereby providing the first experimental evidence that these compounds are not binding through their ‘extended’ conformations to CB2.

The theoretical and experimental data combined to give credence to the hypothesis that the ‘folded’ conformations were the bioactive conformations for these chemotypes. Consequently, two internally developed in silico methods were utilized to help identify new core scaffold replacements that could mimic the space and pharmacophore requirements of the bioactive ‘folded’ conformation of the β-sulfonylacetamides and the diazepanes: BI Builder (a tool for de novo design)15 and PharmShape (a virtual screening software package).16 Though 1 and 3 were used in concert throughout the journey to new chemotypes, 1 was selected as the primary template for the two ligand-based CADD approaches.

The amino tert-butylisoxazole of 1 was fixed as an activity anchor within BI Builder. The software grew fragments incrementally from the amino group of the tert-butylisoxazole to match the amide carbonyl (hydrogen bond acceptor), gem-dimethyl (hydrophobic), and phenyl (aromatic) pharmacophore features (Fig. 2a). The pharmacophore model that was applied to PharmShape virtual screening was slightly different, using only the phenyl and isoxazoyl aromatic pharmacophores and the amide carbonyl hydrogen bond acceptor (Fig. 2b). In both cases, the shape of the ‘folded’ overlay of 1 and 3 was used as the primary filter for obtaining hit sets.

BI Builder returned 388 hits (molecular weight ≤450 amu) when the protocol was restricted to two fragment growth steps. PharmShape searches of our compound database and of external

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB2 EC50 (nM)</th>
<th>CB1 EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.77</td>
<td>420 (545-fold)</td>
</tr>
<tr>
<td>2</td>
<td>1.36</td>
<td>&gt;20,000 (&gt;145-fold)</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>1000 (714-fold)</td>
</tr>
</tbody>
</table>

Table 3

4

CB2 EC50 > 20 μM

Figure 1. Low energy conformations of 1 (orange) and 3 (maroon) overlaid (a) in their ‘extended’ conformations and (b) in their ‘folded’ conformations.
Compounds selected to test the 'folded' conformation binding hypothesis

<table>
<thead>
<tr>
<th>Target</th>
<th>CB2 EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.23 nM</td>
</tr>
<tr>
<td>6</td>
<td>&gt;20 μM</td>
</tr>
</tbody>
</table>

Figure 2. Pharmacophore models with shape restriction: (a) BI Builder approach and (b) PharmShape virtual screen.

Figure 3. Representation highlighting the key trajectories that 'high priority' cores needed to replicate on template 1 from which each of the aromatic groups was projected toward its aryl pharmacophore point.

Figure 4. Pictorial in silico workflow to identify ‘high priority’ cores.
isomer. (S)-proline compounds 5, 8, and 10 registered full agonism with picomolar potencies against CB2 while maintaining selectivity over CB1 (>100-fold). (R)-Proline analogs 7 and 9 showed only partial agonism (<70% efficacy). 11 was unique in that this (R) enantiomer had attractive data for both CB2 and CB1. As it appeared to be the outlier in the series, further SAR designs were based on the (S)-proline core.

Compounds 5, 10, and 12 were designed to probe the torsion angle between the proline ring and the left-hand side aryl ring. The initial modeling showed that the p-[(trifluoromethyl)phenyl] rings of 5 and 1 were orthogonal to one another (Fig. 4). The 5-[(trifluoromethyl)-2-pyrilidyl replacement in 10 was added to decrease the torsion angle, versus, 5, while the 3-chloro-5-[(trifluoromethyl)-2-pyrilidyl group in 12 was added to increase the torsion angle, versus, 5 to better match the torsion of the template aryl group. Surprisingly, both 10 and 12 were slightly more potent than 5 with respect to CB2, indicating that this binding region in the protein has no preference for a specific rotamer. Furthermore, tolerance of this pocket for additional bulk (the Cl substituent in 12) was demonstrated. CB1 selectivity for pyridine compound 10 (757-fold) was in line with its phenyl counterpart (5, 1335-fold), though the CB1 selectivity window for the chloropyridine analog (12, 88-fold) was less desirable.

Additional modeling suggested that an α-methyl group on the proline core would overlay perfectly with one of the methyls of the gem-dimethyl group in the β-sulfonylacetamide template, thus making the α-methyl proline core a target for synthesis (Table 6). When this methyl group was incorporated into 5 to afford 13, the CB2 activities of the two compounds were equipotent. However the CB1 cAMP EC50 of 13 dropped to 15 nM. Additional methylated analogs (14 and 15) likewise saw the boost in CB1 potency resulting in the loss of selectivity for CB2; henceforth, SAR was deprioritized in the α-methyl proline series.

With the initial success of the prolines, we returned to the ligand-based scaffold hopping results to two homologous ring systems of the proline core: the azetidine and the piperidine cores. While the initial overlay of the proline core was interpreted to have the best trajectories toward the terminal aryl pharmacophores (compare Figs. 4, 5a and 5b), the azetidine and piperidine cores also presented themselves as 'high priority' scaffolds. Based on the slight differences in the trajectories between the prolines, azetidines, and piperidines and the template, one may expect to observe differences in the SAR between the series. Indeed, azetidine 16 lost 16-fold activity against CB2, while piperidine 17 maintained potency, versus, proline 10 (Table 7). With their low nanomolar/picomolar activities toward CB2 and high selectivities over CB1, all three compounds were deemed reasonable starting points for further studies.

Additional profiling of compound 10 alongside its direct analogs with the β-sulfonylacetamide and diazepane cores (18 and 19, respectively) for stability in human liver microsomes (HLM) and aqueous solubility highlighted that the new proline core

### Table 5
Initial SAR on the proline scaffold

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R’/S</th>
<th>CB2 EC50 (nM)</th>
<th>CB1 EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>S</td>
<td>R/R’</td>
<td>0.23</td>
<td>307 (1335 fold)</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td></td>
<td>99</td>
<td>&gt;20,000 (&gt;200 fold)</td>
</tr>
<tr>
<td>8</td>
<td>S</td>
<td>R</td>
<td>0.19</td>
<td>2600 (13684 fold)</td>
</tr>
<tr>
<td>9</td>
<td>R</td>
<td></td>
<td>50</td>
<td>&gt;20,000 (&gt;400 fold)</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>R</td>
<td>0.07</td>
<td>53 (757 fold)</td>
</tr>
<tr>
<td>11</td>
<td>R</td>
<td></td>
<td>33</td>
<td>&gt;20,000 (&gt;600 fold)</td>
</tr>
<tr>
<td>12</td>
<td>S</td>
<td>R</td>
<td>0.08</td>
<td>7 (88 fold)</td>
</tr>
</tbody>
</table>

* Partial agonist.

### Table 6
α-Methyl SAR on the proline scaffold

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R’/S</th>
<th>CB2 EC50 (nM)</th>
<th>CB1 EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>H</td>
<td>Me</td>
<td>0.23</td>
<td>307 (1335 fold)</td>
</tr>
<tr>
<td>13</td>
<td>Me</td>
<td></td>
<td>0.29</td>
<td>15 (52 fold)</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>Me</td>
<td>0.19</td>
<td>2600 (13684 fold)</td>
</tr>
<tr>
<td>14</td>
<td>Me</td>
<td></td>
<td>0.21</td>
<td>54 (257 fold)</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>Me</td>
<td>0.07</td>
<td>53 (757 fold)</td>
</tr>
<tr>
<td>15</td>
<td>Me</td>
<td></td>
<td>0.04</td>
<td>0.5 (12 fold)</td>
</tr>
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</table>

### Table 7
Comparison of representative compounds bearing the new cores

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB2 EC50 (nM)</th>
<th>CB1 EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1.15</td>
<td>1400 (1217 fold)</td>
</tr>
<tr>
<td>10</td>
<td>0.07</td>
<td>53 (757 fold)</td>
</tr>
<tr>
<td>17</td>
<td>0.09</td>
<td>250 (2778 fold)</td>
</tr>
</tbody>
</table>
could offer a better balance of properties than the diazepanes (Table 8). The early proline SAR showed that this series was ready for further SAR exploration and optimization. The synthetic approaches and additional SAR of the proline and piperidine scaffolds will be available in later publications. \(^{19,20}\)

In summary, our CB2 agonist program required a new agonist series to replace the diazepanes, a compound class lacking a balance of potency and acceptable drug-like properties. The diazepanes and a second distinct series, the \(\beta\)-sulfonfylacetamidines, were analyzed through computational and experimental approaches in search of the bioactive conformation common to these chemical series. Once the ‘folded’ conformation of these chemotypes was determined to be responsible for their bioactivity, the necessary pharmaceophore models were derived for the in silico studies from experimental data. Additionally, a shape restriction derived from the ‘folded’ conformations of 1 and 3 was used in ligand-based CADD approaches toward the identification of new cores to replace the diazepane scaffold. BI Builder and PharmShape methodologies led to the identification of three new ‘high priority’ cores. Elaboration of these cores with preferred substituents allowed for the synthesis and validation of the proline-, azetidin-, and piperidine-based series as viable chemotypes for our CB2 agonist program. Analogs containing the new proline scaffold exhibited picomolar CB2 activity, high selectivity over CB1, and initial drug-like profiling that inspired further optimization within the series.

Acknowledgments

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Supplementary data

Supplementary data (the coordinates for the models in Figures 1, 4, and 5) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.033. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

13. (a) CB2 and CB1 CAMP assays: CHO cells expressing human CB2 or CB1 (Euroscreen) were plated at a density of 5000 cells per well in 384-well plates and incubated overnight at 37 °C. After removing the media, the cells were treated with test compounds diluted in stimulation buffer containing 1 mM IBMX, 0.25% BSA and 10 μM forskolin. The assay was incubated for 30 min at 37 °C. Cells were lysed and the cAMP concentration was measured using DiscoverX XS+ cAMP kit, following the manufacturer’s protocol. The maximal amount of cAMP produced by forskolin compared to the level of cAMP inhibited by 200 nM CP-55940 is defined as 100%. The EC50 value of each test compound was determined as the concentration at which 50% of the forskolin-stimulated CAMP synthesis was inhibited using a four-parameter logistic model. (b) Reproducibility of the cAMP assays is assessed using the control compound CP-55940 which is tested twice on every assay plate to rule out any plate artifacts. Efficacy at CB1 and CB2 is expressed as a percentage relative to the efficacy of CP-55940. Each compound is tested in triplicate at least two times with individuals dilutions from the stock. The results reported in the table are the mean values of the measurements and individual values for the compounds and do not differ by more than a factor of three from the mean.
17. **HLM assay**: Test compounds were incubated in Duplicate Matrix MultiScreen mintubes (Matrix Technologies, Hudson, NH) with liver microsomes (Xenotech, Lenexa, KS). Each assay is performed in 50 mM potassium phosphate buffer, pH 7.4, and 2.5 mM NADPH. Compounds were tested at a final assay concentration of 1.0 μM. The protein concentration in the reaction mix was 1 mg/mL. Compounds were preincubated for 5 min at 37 °C and the metabolic reactions were initiated by the addition of NADPH. Aliquots of 80 μL were removed from the incubation mix at 0, 5 and 30 min after the start of the reaction for screening data. Each aliquot was added to 160 μL acetonitrile for extraction by protein precipitation. These samples were mixed for 1 min by vortexing, and a volume of the mixture was filtered through wells in 0.25 mm glass fiber filter plates by centrifugation at 3000 rpm for 5 min. Sample extracts were analyzed by LC–MS–MS to determine parent compound levels. Percent loss of parent compound was calculated from the peak area at each time point to determine the half-life for test compounds ($t_{1/2}$, min).

18. **Solubility assay**: Compound solubility was measured at pH 4.5 and pH 7.4 buffers. 6 μL of a 10 mM DMSO stock solution was spiked into pH 4.5 and 7.4 buffers targeting 50–200 μM final concentrations in buffers in duplicate deep well plates. The DMSO content was 0.5%. The samples were incubated for 16–18 h, filtered and analyzed using a UV spectrophotometer. The spectra of samples and reference are scanned from 230 to 500 nm. Solubility is measured by taking the ratio of area under the curve of reference to sample.
