Research paper

Applications of parallel synthetic lead hopping and pharmacophore-based virtual screening in the discovery of efficient glycine receptor potentiators

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ABSTRACT

Glycine receptors (GlyRs) are pentameric glycine-gated chloride ion channels that are enriched in the brainstem and spinal cord where they have been demonstrated to play a role in central nervous system (CNS) inhibition. Herein we describe two novel classes of glycine receptor potentiators that have been developed using similarity- and property-guided scaffold hopping enabled by parallel synthesis and pharmacophore-based virtual screening strategies. This effort resulted in the identification of novel, efficient and modular leads having favorable in vitro ADME profiles and high CNS multi-parameter optimization (MPO) scores, exemplified by azetidine sulfonamide 19 and aminothiazole sulfone (ent2)-20.

1. Introduction

Glycine receptors (GlyRs) are pentameric ligand-gated chloride ion channels that mediate synaptic transmission in the adult CNS. These channels are members of the Cys-loop family of ligand-gated ion channels (LGICs), which include GABA A, nACh and 5HT 3 receptors [1]. Unlike the other members of the Cys-loop family, GlyRs are selectively inhibited by strychnine. In humans, three different alpha (GLRA1-3) and one beta (GLRB) gene have been cloned [2]. GlyRα3 is known to play a key role in inflammatory pain via serine phosphorylation that is unique to this member of the family [3], and glycinergic interneurons have been demonstrated to be involved in pain and itch processing [4]. The heteropentameric channel conformation (likely comprised of either three α-subunits and two β-subunits or vice versa) has been reported to be the most physiologically relevant target for neurotransmission modulation with post-synaptic localization due to interactions of the β-subunit with gephyrin at the post-synaptic cell surface [5]. Together, these observations support targeting GlyRα3β channels towards the discovery of novel analgesics.

Although the potential therapeutic benefit of selectively activating GlyRs in order to treat pain has been appreciated for decades, to date there have been limited reports describing small molecule positive modulators of GlyRs [6], with very few of these molecules offering potency, selectivity, and/or ADME (absorption, distribution, metabolism, and excretion) properties favorable for in vivo...
profiling [7,8]. Previously, we described a FLIPR-based HTS strategy targeting hGlyRzβ which resulted in the discovery of a novel class of tricyclic sulfonamides exemplified by early lead 1 (Table 1), which was advanced to high quality proof-of-concept and co-crystallization tools [8,9]. In parallel we wished to identify additional novel, small and modular cores that could deliver a differentiated, and perhaps more ligand efficient lead series, within this unique pharmacophore and binding pocket.

2. Results and discussion

We employed a strategy in which the benzodioxole sulfonamide was initially targeted, as a potency handle, while novel replacements for the tricyclic amine of 1 were sought. We designed a virtual library wherein we enumerated hundreds of commercially available, simple, secondary amines to their derived benzodioxole sulfonamides. The virtual products were evaluated based on similarity (Tanimoto fingerprint or overlay of modeled local minimum conformations) to 1 and selected physicochemical properties. A group of sulfonamides having MW < 450, cLogP < 4, PSA < 100 and significant similarity to 1 were then prepared in parallel library format (Scheme 1 and Table S1). This led to the identification of moderately potent azetidine sulfonamide 2 and pyrrolidine sulfonamide (S)-3, both having high passive permeability and low efflux in Pgp and BCRP-expressing Madin-Darby canine kidney (MDCK) cell lines, and acceptable ligand and lipophilic efficiencies (LE and LipE) [10] (Table 1). The overlay of modeled conformations of leads 1, 2 and (S)-3 is demonstrated by alignment of local minimum conformations of 2 and (S)-3 onto the presumed bioactive conformation of 1 based on docking of 1 onto the pose observed for AM-3607 in GlyR (Fig. 1). As with our previous tricyclic sulfonamide series, we sought to identify quality tool compounds for evaluation in mouse models of pain. Like lead compound 1, azetidine 2 and pyrrolidine (S)-3 suffered from high intrinsic clearance in mouse liver microsomes (MLM Clint = 2180 and > 3000 µL/min-, respectively). Since 2 had improved solubility relative to 1 and comparable efficiency, it was considered an attractive lead with physicochemical properties conducive to oral absorption and CNS penetration.

Hit-to-lead efforts were initiated on azetidine 2 with the goal of improving potency and intrinsic stability in mouse liver microsomes. Alterations to the size of the core ring to a 5-membered pyrrolidine or 6-membered piperidine (3- or 4-substituted) led to substantial potency loss (data not shown). Subsequent SAR studies were therefore conducted with the azetidine core.

A metabolite identification study with azetidine 2 in mouse liver microsomes indicated oxidative and hydrolytic decomposition of the methylene bridge of the benzodioxole. Secondary pathways included O-demethylation, and aryl oxidation on the methoxyphenol moiety. With this information in hand, our efforts focused first on the right-hand-side of the molecule with an aim to improve potency (Table 2). Secondarily, we evaluated the left-hand-side, since sulfonylamination chemistry was amenable to a rapid parallel synthetic approach and we suspected marked improvement in microsomal stability could be attained with simple replacement of the benzodioxole.

We first explored replacement of the methoxy with alternative

### Table 1

Profiles of sulfonamides 1-(S)-3.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Tanimoto fingerprint similarity</th>
<th>hGlyRzβ MaxPot (%)/EC50 (µM)</th>
<th>LE/LipE</th>
<th>MLM CLint (mL/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td>1</td>
<td>68/0.37</td>
<td>0.33/2.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td>1</td>
<td>75/2.3</td>
<td>0.31/2.3</td>
<td>102</td>
</tr>
<tr>
<td>(S)-<strong>3</strong></td>
<td>1</td>
<td>59/4.2</td>
<td>0.31/1.5</td>
<td>2180</td>
</tr>
</tbody>
</table>

*a* Reported data is an average of at least two independent measurements.

* LipE was calculated using EC50 and ACD Daylight V4.81 cLogP.

![Scheme 1. Property-guided parallel synthetic discovery of hits 2 and 3.](image-url)
groups such as H, F, OEt, OCF3, Et, CF3, Cl and CN, but all of these led to substantial potency loss (data not shown). Next, we walked small lipophilic groups around the phenyl ring in order to improve potency and potentially also realize improvement in microsomal stability due to blocking of a metabolic soft spot (4–9). Substitution at C-5 was well tolerated (6, 8, 9) with C-5 chloro (8) affording a six-fold increase in potency but no improvement in intrinsic clearance relative to lead 2.

In an attempt to further boost the potency through conformational bias or engagement of Van der Waals contacts, we methylated the azetidine ring (Table 3) to deliver cis enantiomers 10 and 11 and trans enantiomers 12 and 13 [11]. However, none of the methylated cores were found to offer an improvement in potency.

Finally, we explored the SAR of the left-hand-side sulfonamide moiety using a parallel synthetic, property-guided strategy which focused primarily on obtaining substituted and bicyclic aryl sulfonamides having cLogP < 4 and PSA < 105, towards increasing the probability of achieving decreased oxidative metabolism and retaining low efflux. Selected results are shown in Table 4 (14–19).

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>hGlyRx3β MaxPot (%)/EC50 (µM)a</th>
<th>MLM CLint (µL/min·mg)</th>
<th>LE/LipE b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>75/2.3</td>
<td>2180</td>
<td>0.31/2.3</td>
</tr>
<tr>
<td>4</td>
<td>-/≥25</td>
<td>&gt;3000</td>
<td>-/-</td>
</tr>
<tr>
<td>5</td>
<td>28/21</td>
<td>&gt;3000</td>
<td>0.24/1.2</td>
</tr>
<tr>
<td>6</td>
<td>83/1.2</td>
<td>2890</td>
<td>0.31/2.4</td>
</tr>
<tr>
<td>7</td>
<td>44/6.1</td>
<td>&gt;3000</td>
<td>0.27/1.9</td>
</tr>
<tr>
<td>8</td>
<td>67/0.5</td>
<td>&gt;3000</td>
<td>0.33/2.3</td>
</tr>
<tr>
<td>9</td>
<td>74/3.1</td>
<td>&gt;3000</td>
<td>0.29/1.7</td>
</tr>
</tbody>
</table>

* Reported data is an average of at least two independent measurements.
* LipE was calculated using EC50 and ACD Daylight V4.81 cLogP.

Table 2
SAR of the azetidine aryl ether.

Through this effort, lead compound 19 having retained potency and improved lipophilic efficiency and microsomal stability was identified. The moderate stability in MLM of compound 19 translated to moderate clearance in vivo (CL: 2.3 L/h/kg; Vss: 1.4 L/kg; t1/2: 1.1 h) [12], demonstrating that acceptable clearance can be obtained within this series by optimizing on MLM data.

The functional potentiator activity of 19 was further explored in a Schild shift assay [8,9], where ascending concentrations of 19 increased the potency of glycine in a dose-responsive fashion, resulting in a leftward shift of the glycine dose response curve (Fig. 2).

Comparison of selected data for tricyclic lead 1 and azetidine lead 19 (Table 5) revealed that 19 demonstrated improved metabolic clearance and FaSSIF solubility while maintaining favorable ligand and lipophilic efficiency, passive permeability and low efflux. The overlay of local minimum conformations of 1 and 19 indicates that the scaffolds occupy similar chemical space and suggests that they may occupy the same allosteric binding pocket (Fig. 3). This similarity may explain the observed equivalent potency on hGlyRx3β and hGlyRx1β in both FLIPR functional assays, and the high degree of functional selectivity over other CYS loop receptors observed for lead compound 19 (hGABA 1/2 EC50 > 50 µM; h5HT3A EC50 > 25 µM) [9].

Returning to our tertiary sulfonamide leads overlay of presumed bioactive conformation of 1 with the lowest energy conformations
of 2 and 3 (Fig. 1), we built a pharmacophore model, defining key interactions and imposing a shape-based volume constraint of two times the volume of all overlaid atoms (Fig. 4) [13]. We then used this model to virtually screen a pre-computed multi-conformation database of internal Amgen library compounds (547,226 compounds). All features were required to match, and 4267 hits were returned (0.78%). Upon functional screening in single point (10 mM) in the presence of EC10 Glycine, 17 (0.4%) of the compounds met our functional activity criteria (>50% POC), and were subjected to an internal structural alert protocol which flags compounds with undesired functional groups and potential pan-assay interference compounds [14]. Sulfone hits (±)-20 and (±)-21 had no such predicted liabilities. As such, these hits were resynthesized for confirmation of structure and purity and tested in titration mode to confirm dose-responsive potentiation (EC50 = 5.2 and 3.7, respectively; Table 6). Notably, the dose-responsive potentiator activity of each of (±)-20 and (±)-21 was entirely abolished by the addition of 10 μM strychnine, which is a selective inhibitor of GlyRs (See supporting Information for dose-response curves in the absence and presence of strychnine). This confirms that observed cellular activity is on target. During the resynthesis and chiral resolution of these hits, it was discovered that there is a stereochemical preference of the CF3-alcohol with second eluting enantiomers under chiral HPLC conditions (ent2)-20 and (ent2)-21 having greater activity than the respective first eluting enantiomers, (ent1)-20 and (ent1)-21. Sulfones (ent2)-20 and (ent2)-21 are differentiated relative to our previously described leads (For example, Tanimoto fingerprint similarity of (ent2)-20 relative to 1, 2, 3 = 0.31, 0.26, 0.30, respectively) and represent modular starting points for future optimization. Furthermore, (ent2)-20 and (ent2)-21 have relatively high CNS MPO scores (4.4 and 4.2, respectively), moderate to good solubility and intrinsic stability in MLM. Finally, (ent2)-20 has high passive permeability and low efflux in both Pgp- and BCRP-expressing cell lines.

Having solved the co-crystal structure of advanced tricyclic sulfonamide lead AM-3607 derived from lead 1 in complex with hGlyRα3cryst and glycine [9], we docked azetidine sulfonamide 19 (Fig. 5A) and both enantiomers of sulfone (±)-20 (Fig. 5B, (S)-20 shown) into the observed allosteric binding pocket. In these models, 19 and (S)-20 are hypothesized to make hydrogen bond and Van der Waals interactions with the protein similar to AM-3607. Additionally, (S)-20 may engage Tyr78 in a hydrogen bond interaction through the chiral, tertiary alcohol. In contrast, the modeled (R)-20 does not suggest a direct hydrogen bonding engagement with Tyr78 (data not shown). Novel leads (ent2)-20 and (ent2)-21 and their respective docked models provide unique starting points for further structure-based optimization.

### 3. Chemistry

A general synthetic protocol for sulfonamidation reactions was employed for both library and singleton synthesis. For example, reaction of amines (22a-b or 23) and aryl-sulfonyl chloride (24) in the presence of triethylamine afforded sulfonamides (2, 3, 14–19) in moderate to good yields (Scheme 2).

The chemistry used to access compounds 4–9 and methyl...
substituted azetidine aryl ethers 10–13 is described in Scheme 3. Azetidine alcohols 26a-b were assembled from the reaction of benzo[d][1,3]dioxole-5-sulfonyl chloride (24a) with the appropriate azetidin-3-ol (25a or 25b) in the presence of triethylamine. The cis-trans mixture 26b was then purified by chiral SFC to afford four diastereomers 27–30. Mitsunobu reaction of 26a or 27–30 and the appropriate phenol 31 in the presence of triphenylphosphine and di-ter-butyl azodicarboxylate (DBAD) at 90–140 °C delivered aryl azetidine ether compounds (4–13) in reasonable yields (Scheme 3). The Mitsunobu reactions with the more sterically encumbered 2-methyl azetidin-3-ols (27–30) required elevated temperature (microwave irradiation at 140 °C) (Scheme 3).

N-methyl-indazole 17 was prepared from NH-indazole 16 by reaction with methyl iodide in the presence of sodium hydride. This reaction yielded 17 as the only isomer in 36% yield (Scheme 4).

Table 4
Selected arylsulfonamide SAR in combination with 2-OMe, 5-Cl aryl ether right-hand-side.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>hGlyRz3% MaxPot (%)/EC50 (μM)</th>
<th>MLM CLint (μL/min·mg)</th>
<th>cLogP/PSA</th>
<th>LE/LipE</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>63/0.5</td>
<td>&gt;3000</td>
<td>4.1/74</td>
<td>0.33/2.3</td>
</tr>
<tr>
<td>14</td>
<td>90/0.9</td>
<td>514</td>
<td>3.9/69</td>
<td>0.32/2.1</td>
</tr>
<tr>
<td>15</td>
<td>59/2.3</td>
<td>2210</td>
<td>3.7/84</td>
<td>0.30/1.9</td>
</tr>
<tr>
<td>16</td>
<td>64/0.9</td>
<td>939</td>
<td>3.7/84</td>
<td>0.32/2.3</td>
</tr>
<tr>
<td>17</td>
<td>64/3.3</td>
<td>883</td>
<td>3.5/73</td>
<td>0.28/1.9</td>
</tr>
<tr>
<td>18</td>
<td>79/2.5</td>
<td>263</td>
<td>3.4/74</td>
<td>0.28/2.3</td>
</tr>
<tr>
<td>19</td>
<td>74/0.7</td>
<td>150</td>
<td>2.9/101</td>
<td>1.31/3.2</td>
</tr>
</tbody>
</table>

Fig. 2. Schild data for the lead compound 19.

\(a\) Reported data is an average of at least two independent measurements.

\(b\) LipE was calculated using EC50 and ACD Daylight V4.81 cLogP.
Key interactions: Green - hydrophobic, orange - aromatic, blue - hydrogen bond acceptor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Overlay of modeled local minimum conformations of leads 1 (blue) and 19 (pink). Images generated using PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Pharmacophore model derived from overlay of presumed bioactive confirmation of 1 (blue), with low energy conformations of 2 (orange), and 3 (grey). a Key interactions: Green – hydrophobic, orange – aromatic, blue – hydrogen bond acceptor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Profiles of lead 1 and sulfonamide 19.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>LE/LipE</th>
<th>CNS MPO</th>
<th>pMPO for CNS</th>
<th>MLM CLint (M/min)</th>
<th>MDCK Papp (ave, m/sec)</th>
<th>FaSSIF Sol. (µM)</th>
<th>GlyR3cryst binding KD (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGlyR3β</td>
<td>MaxPot (%)/EC50 (µM)</td>
<td>68/0.37</td>
<td>5.30</td>
<td>0.66</td>
<td>1420</td>
<td>&lt;1</td>
<td>0.07 ± 0.016</td>
</tr>
<tr>
<td>hGlyR3β</td>
<td>MaxPot (%)/EC50 (µM)</td>
<td>108/0.3</td>
<td>3.33/3.8</td>
<td>5.22</td>
<td>0.58</td>
<td>96</td>
<td>0.30 ± 0.08</td>
</tr>
</tbody>
</table>

a Reported data is an average of at least two independent measurements.
b LipE was calculated using EC50 and ACD Daylight V4.81 cLogP.
c Determined by SPR ± SEM.

5. Experimental section

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Microwave-assisted reactions were conducted with a Biotage Initiator. Purifications were performed using standard column chromatography in glass columns or medium pressure liquid chromatography (MPLC) on a CombiFlash Companion (Teledyne Isco) or a Biotage Isolera with prepacked RediSep or Biotage normal-phase silica gel (35–60 µm) columns and UV detection at 254 nm. Preparative reversed-phase high-performance liquid chromatography (HPLC) and high throughput parallel purification were performed with reversed phase preparative LC/MS: Waters auto-purification system; liquid transfer system: Tecan; drying system: Genevac; prep. Column: Xbridge (19 × 100 mm, C18, 10 µm); flow rate: 40 mL/min; general gradient: 5–95% B, 0% additive in both A and B; 10 min gradient; Mobile Phase A: H2O, Mobile Phase B, CH3CN; Additive: TFA or NH4OH. Chiral method development was performed on an analytical Thar SFC/MS. Preparative chiral separations were performed on Thar SFC Prep 80 or SFC Prep 350 instruments. All final compounds were purified to >95% purity as determined by HPLC. Purity (3 min methods only) and reaction analyses were measured using Agilent 1100 Series HPLC systems with UV detection at 254 nm and 215 nm System A: Agilent Zorbax SB-C18 3.0 × 50 mm, 3.5 µm, 5–95% CH3CN in H2O with 0.1% TFA for 3.6 min at 1.5 mL/min or Halo Phenyl-Hexyl, 3 × 50 mm, 2.7 µm, 5–95% CH3CN in H2O with 0.1% TFA for 1.01 min at 2.0 mL/min; System B: Waters
Sensitive to the sensitivity of its probe to changes in chloride and/or iodine described previously. Brie eYFP halide ion sensor were cultured according to protocols. Assay conditions were designed to promote inward thus allowing for a clearer and bigger assay window. Therefore, sensor and induces a more complete quenching of eYFP emission, which is completely absent in cells at baseline. Puromycin was added to the cell media to select for HEK293T- GlyR stable cells co-expressing eYFP. Cells were dispensed into 384-well BioCoat poly-d-lysine-coated plates at a cell density of ~12,000 cells/well using a Multidrop Combi. Plated cells were incubated at 37 °C overnight under the standard cell culture conditions. The next day (~18–24 h after plating), cell media was aspirated from the plated cells and replaced with 25 μL “Low chloride” Assay Buffer (10 mM Hepes Buffer, pH 7.4, 12 mM Na-glucuronate, 5 mM KCl, 2 mM MgCl2, 1 mM CaCl2, 10 mM α-glucose, 160 mM D-mannitol) and equilibrated at room temperature for 30 min. This “low chloride” buffer is designed to minimize the influx of chloride prior to the assay window capture, a higher concentration would lead to an influx of chloride inside the cell down the concentration gradient and would result in a undesirable quenching of the probe prior to the assay window. In these conditions, we attain a stable signal baseline and maintain cell health and integrity. Note that iodine, the anion used for this readout, is delivered simultaneously with glycine to minimize quenching of the signal prior to the assay data acquisition. During the assay, all compounds and glycine were prepared (3.5 × the desired final concentration) in Assay Buffer (10 mM Hepes Buffer, pH 7.4, 12.5 mM Na-glucuronate, 5 mM KCl, 2 mM MgCl2, 1 mM CaCl2, 10 mM α-glucose, 160 mM D-mannitol) and delivered to cells by the FLIPR instrument. Fluorescence emission (using the 470–495 nm/515–575 nm excitation/emission filter set, excitation intensity = 40%, camera gain = 100 and an exposure time of 0.4 s) is measured in real-time to instantaneously detect quenching of eYFP fluorescence in response to the influx of halide ions (iodide) through activated GlyR3β channels. FLIPR kinetic traces were processed using an area under the curve relative to baseline (AUC - BL) algorithm, where the baseline was the first 10 s of the kinetic measurement prior to co-addition of compound with EC_{10} glycine. All measurements proceeded for 120 s post-addition. Responses were subsequently normalized to percent of control (POC) using the maximum achievable glycine response and vehicle (buffer or EC_{10} glycine) as references. Primary screens for GlyR3β potentiators were conducted in duplicate at a single concentration of compound (10 μM) in the

**Table 6**

Profiles of selected functional hits and resolved enantiomers from pharmacophore-based virtual screen.

![Diagram](image)

<table>
<thead>
<tr>
<th></th>
<th>(±)-20</th>
<th>(ent1)-20</th>
<th>(ent2)-20</th>
<th>(±)-21</th>
<th>(ent1)-21</th>
<th>(ent2)-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmpd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hGlyR3β MaxPot (%)/EC_{50} (μM)</td>
<td>32/5.2</td>
<td>15.5/25</td>
<td>33/22</td>
<td>44/3.7</td>
<td>30/12.4</td>
<td>44/2.2</td>
</tr>
<tr>
<td>LE/LipE</td>
<td>0.28/1.8</td>
<td>—</td>
<td>0.28/2.2</td>
<td>0.24/1.7</td>
<td>0.22/1.2</td>
<td>0.25/1.9</td>
</tr>
<tr>
<td>MW, cLogP, PSA</td>
<td>448, 3.5, 79</td>
<td>448, 3.5, 79</td>
<td>448, 3.5, 79</td>
<td>488, 3.7, 71</td>
<td>488, 3.7, 71</td>
<td>488, 3.7, 71</td>
</tr>
<tr>
<td>CNS MPO</td>
<td>4.41</td>
<td>4.41</td>
<td>4.41</td>
<td>3.88</td>
<td>3.88</td>
<td>3.88</td>
</tr>
<tr>
<td>pMPO for CNS</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
<td>0.67</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Solubility FaSSIF (μM)</td>
<td>—</td>
<td>102</td>
<td>130</td>
<td>58</td>
<td>87</td>
<td>66</td>
</tr>
<tr>
<td>MM EL_{05} (μL/min-mg)</td>
<td>133</td>
<td>197</td>
<td>95</td>
<td>131</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MDCK Papp (ave, μm/sec), Pgp ER, BCRP ER</td>
<td>2.9, 2.8</td>
<td>1.4, 0.8</td>
<td>2.9, 2.2</td>
<td>1.1, 1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Reported data is an average of at least two independent measurements.

b LipE was calculated using EC_{50} and ACD Daylight V4.81 cLogP.
presence of EC10 glycine. Counter-screen to ensure on-target activity and removal of fluorescent false positives were performed in parallel in the presence of a high concentration (10 μM) of the potent GlyR antagonist strychnine. Primary hits were selected for dose-response confirmation based on two criteria: 1) the compound can achieve >50% POC levels relative to the maximum achievable glycine response and 2) the response is inhibited >80% in the presence of strychnine. Dose response confirmation of on-target potentiation of GlyR \( \alpha3 \) was performed in the presence and absence of strychnine using 2-fold dilutions of compound diluted sufficiently to generate 22-pt dose response curves. All POC normalized data were then plotted against log[glycine] or log[compound], according to the type of experiment performed, and data were fit to a non-linear regression 4-parameter Hill fit to determine the EC50 from the resulting sigmoidal curve. All curve fitting was performed with the GraphPad Prism 6 software. Alternatively, the modulation of glycine potency by each potentiator was examined using a Schild shift assay in which dose response plates of glycine were prepared at 3.5×/C2 in assay buffer. Glycine EC50 were then measured in the presence of three increasing concentrations of compound.

5.2. ADME

MLM Cl\(_{int}\) and MDCK Papp/ER determinations were conducted as previously described [8,9].

Mouse IV PK: CD1-mice were administered a single dose of 19 (1 mg/kg formulated in dimethyl sulfoxide, 0.5 mL/kg) by bolus injection into the tail vein. Blood samples were collected at 0.05, 0.13, 0.25, 1, 2, 7, and 24 h post-dose and analyzed by LC/MS/MS. PK parameters were calculated with non-compartmental analysis using Small Molecules Discovery Assay Watson® (version 7.0.01, InnaPhase Corp., Philadelphia, PA) software. Mouse work was conducted under an IACUC approved protocol and in an AALAC accredited facility.

5.3. General Procedure A for the parallel synthesis of sulfonamides 2–3 and 14–19 from the reaction of amines (free base or HCl salt or TFA salt) \( \pm \) with sulfonyl chlorides 24

Resealable screw cap reaction vials (8 mL) were placed in a reaction block and each vial was charged with amine 22 or 23 (1 equiv.), triethylamine (3 equiv.) and DMF (0.3 M). The block was then immersed in an ice bath for 10 min. To each mixture at 0°C, was then added aryl sulfonyl chloride 24 (1 equiv.) and the vials were sealed. The block was shaken on a J-KEM Scientific MaxQ 2000 shaker for 16 h at RT. Each mixture was filtered through a Nalgene PTFE 0.2 μm filter, then purified by HT-PP with reversed phase preparative LC/MS: Waters auto-purification system; liquid transfer system: Tecan; drying system: Genevac; prep. Column: Xbridge (19 × 100 mm, C18, 10 μm); flow rate: 40 mL/min; general gradient: 5–95% B, 0.1% additive in both A and B; 10 min gradient; Mobile Phase A: H2O, Mobile Phase B, CH3CN; Additive: TFA or NH4OH.to provide the pure products as solids upon concentration in vacuo.

5.4. General Procedure B: synthesis of azetidin-3-ols 26a, 27–30

To an ice cold solution of azetidin-3-ol hydrochloride 25a or 2-Me azetidin-3-ol hydrochloride 25b (1 equiv.) and triethylamine (3 equiv.) in DMF (0.3 M, for azetidin-3-ol) or ACN (0.3 M, for 2-Me azetidin-3-ol) was added benzo[d][1,3]dioxole-5-sulfonil chloride 24a (1 equiv.) and the mixture was shaken at 0°C for 2 h. The reaction mixture was then diluted with EtOAc (200 mL) and H2O (100 mL). The organic layer was separated, concentrated then

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**Fig. 5.** Structure of hGlyRa3-AM-3607 + Glycine [strychnine [15] and ivermectin [16] sites indicated with arrow] (A) Model of compounds 19 (B) and (S)-20 (C) docked into the AM-3607 binding pocket.a.a Location of the ivermectin binding site is taken from the observed position in GlyRa1, 3JAF.pdb and the strychnine site from hGlyRa3cryst (5CFB.pdb). Coloring for ligand atoms − C: green, O: red, N: blue, F: light blue, Cl: orange. PDB files of the docked models are available in the Supporting Information. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
purified by MPLC (Biotage Isolera One; PuriFlash HP; ramping EtOAc in heptane) to obtain 1-(benzo[d][1,3]dioxol-5-ylsulfonyl) azetidin-3-ol 26a (26% yield) as an oily solid. MS (ESI, positive ion) m/z: 258.0 (M+1) or a mixture of cis and trans isomers 26b (94% crude yield) which were separated by chiral Thar 350 SFC with 30/C250 mm AD-H column with 38.5 mL/min MeOH (20 mM NH₃)þ71 g/min CO₂, 35% co-solvent at 110 g/min. Temp. = 21°C, Outlet pressure = 100 bar, Wavelength = 285 nm, to provide (2S,3R)-1-(benzo[d][1,3]dioxol-5-ylsulfonyl)-2,3-dimethylazetidine 27 (33% yield); and (2R,3R)-1-(benzo[d][1,3]dioxol-5-ylsulfonyl)-2,3-dimethylazetidine 28 (30% yield); (2S,3S)-1-(benzo[d][1,3]dioxol-5-ylsulfonyl)-2,3-dimethylazetidine 29 (5% yield); and (2R,3S)-1-(benzo[d][1,3]dioxol-5-ylsulfonyl)-2,3-dimethylazetidine 30 (3% yield). Relative stereochemistry was assigned by 2D NMR studies. Absolute stereochemistry was randomly assigned.

5.5. General Procedure C: Mitsunobu reactions of alcohols 26a, 27-30 with phenols 4-13.

To an ice cold solution of 1-(benzo[d][1,3]dioxol-5-ylsulfonyl) azetidin-3-ol 26a (1 eq) or 1-(benzo[d][1,3]dioxol-5-ylsulfonyl)-2-methyldiazetidin-3-ol 27-30 (1 equiv.) and phenol 31 (1 equiv.) and PPh₃ (1 equiv.) in THF (0.2 M) was added di-t-butyl
azodicarboxylate (DBAD) (1 equiv.) and the temperature was slowly raised to RT. The reaction mixture was then stirred at 90 °C for 16 h (azetidin-3-ol) or under microwave irradiation at 140 °C for 1 h (2-Me azetidin-3-ol). The mixtures were filtered through a Nalgene PTFE 0.2 μm filter, then purified by HT-PP with reversed phase preparative LC/MPS: Waters autosorption purification; liquid transfer system: Tecan; drying system: Genevac; prep. Column: XBridge (19 × 100 mm, C18, 10 μm); flow rate: 40 mL/min; general gradient: 5–95% B, 0.1% additive in both A and B; 10 min gradient; Mobile Phase A: H2O, Mobile Phase B, CH3CN; Additive: TFA or NH4OH to make the pH approximately 2.

### 1-(1,3-benzodioxol-5-sulfonyl)-3-(2-methoxyphenoxy)azetidine (2):

By Using General Procedure A, reaction of benzyl[d][1,3]dioxole-5-sulfonoyl chloride 24a with 3-(2-methoxyphenoxy)azetidine hydrochloride 22a afforded the title compound (82 mg, 78% yield) as a white solid. MS (ESI, positive ion) m/z: 364.2 [M+1]+. 1H NMR (500 MHz, DMSO-d6) δ ppm 7.29–7.42 (m, 2H), 7.17 (d, J = 8.12 Hz, 1H), 6.78–7.01 (m, 2H), 6.76–6.86 (m, 1H), 6.68 (br d, J = 7.21 Hz, 1H), 6.22 (s, 2H), 4.74–4.84 (m, 4H), 4.16 (dd, J = 5.64, 9.27 Hz, 2H), 3.63 (br dd, J = 4.86, 9.35 Hz, 2H), 3.32 (s, 3H). 13C NMR (126 MHz, DMSO-d6) δ ppm 151.7, 149.1, 142.2, 145.3, 126.4, 124.3, 123.3, 132.7, 114.4, 112.6, 108.6, 101.8, 67.5, 57.7, 55.7. HRMS-ESI (m/z): [M+H]+ calculated for C17H17FNO4S: 350.0862 found 350.0855.

### 1-(1,3-benzodioxol-5-sulfonyl)-3-(2-fluoro-2-methoxyphenoxy)pyrrolidine (3):

By Using General Procedure A, reaction of benzyl[d][1,3]dioxole-5-sulfonoyl chloride 24a with (S)-1-(benzo[d][1,3]dioxol-5-sulfonyl)-3-(2-fluoro-2-methoxy)-2-methylazetidine 23 afforded the title compound (85 mg, 81% yield) as a white solid. MS (ESI, positive ion) m/z: 350.1 [M+1]+. 1H NMR (500 MHz, DMSO-d6) δ ppm 7.19–7.40 (m, 4H), 7.08–7.18 (m, 3H), 6.19 (s, 2H), 3.55–3.69 (m, 1H), 3.33–3.45 (m, 2H), 3.23–3.28 (m, 1H), 3.07–3.18 (m, 1H), 2.08–2.60 (m, 2H), 1.86 (ddt, J = 17.17, 8.12 Hz, 1H). 13C NMR (126 MHz, DMSO-d6) δ ppm 160.3, 151.2, 148.1, 129.3, 128.7, 128.1, 127.4, 124.6, 123.1, 111.4, 108.4, 107.2, 102.5, 52.6, 47.4, 36.6, 30.7. HRMS-ESI (m/z): [M+H]+ calculated for C17H17FNO2S: 350.0862 found 350.0855.

### 1-(1,3-benzodioxol-5-sulfonyl)-3-(3-fluoro-2-methoxyphenylazo)pyrrolidine (4):

By Using General Procedure C, Mitsunobu reaction of 1-(benzo[d][1,3]dioxol-5-sulfonyl)azetidin-3-ol 26a with 3-fluoro-2-methoxyphenol 31a afforded the title compound (45 mg, 52% yield) as a white solid. MS (ESI, positive ion) m/z: 382.1 [M+1]+. 1H NMR (500 MHz, DMSO-d6) δ ppm 7.31–7.41 (m, 2H), 7.17 (d, J = 8.12 Hz, 1H), 6.97 (dt, J = 6.38, 8.32 Hz, 1H), 6.86 (br t, J = 9.00 Hz, 1H), 6.59 (br d, J = 8.33 Hz, 1H), 6.20 (s, 2H), 4.86–4.95 (m, 1H), 4.22 (dd, J = 6.41, 9.62 Hz, 2H), 3.58–3.73 (m, 7H). 13C NMR (126 MHz, DMSO-d6) δ ppm 156.6, 154.6, 151.7, 150.1, 148.2, 136.7, 126.1, 124.4, 123.8, 111.0, 108.5, 102.7, 65.1, 60.7, 57.5. HRMS-ESI (m/z): [M+H]+ calculated for C17H17FNO2S: 382.0761, found 382.0751.

### 1-(1,3-benzodioxol-5-sulfonyl)-3-(4-fluoro-2-methoxyphenylazo)pyrrolidine (5):

By Using General Procedure C, Mitsunobu reaction of 1-(benzo[d][1,3]dioxol-5-sulfonyl)azetidin-3-ol 26a with 4-fluoro-2-methoxyphenol 31b afforded the title compound (28 mg, 30% yield) as a white solid. MS (ESI, positive ion) m/z: 382.0 [M+1]+. 1H NMR (500 MHz, DMSO-d6) δ ppm 7.37–7.39 (m, 2H), 7.17 (d, J = 8.17 Hz, 1H), 6.89 (dd, J = 2.86, 10.61 Hz, 1H), 6.70 (dd, J = 5.61, 8.82 Hz, 1H), 6.61 (dt, J = 2.86, 8.53 Hz, 1H), 6.22 (s, 2H), 4.70–4.88 (m, 1H), 4.12 (dd, J = 6.52, 9.35 Hz, 2H), 3.72 (s, 3H), 3.61 (br dd, J = 4.84, 9.43 Hz, 2H). 13C NMR (126 MHz, DMSO-d6) δ ppm 157.4, 151.6, 150.1, 141.1, 145.1, 126.2, 112.4, 115.3, 108.5, 108.0, 105.6, 102.9, 100.9, 65.9, 57.5, 55.8. HRMS-ESI (m/z): [M+H]+ calculated for C17H17FNO2S: 382.0761, found 382.0751.
methoxyphenol 31e afforded the title compound (12% yield) as a white solid. MS (ESI, positive ion) m/z: 412.2 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ ppm 7.33 (d, J = 1.76 Hz, 1H), 7.39 (dd, J = 18.1, 8.1 Hz, 1H), 7.17 (br s, 1H), 7.20 (d, J = 8.09 Hz, 1H), 6.81−7.05 (m, 2H), 6.78−7.03 (m, 1H), 6.23 (s, 1H), 6.18−6.30 (m, 1H, 472 (dt, J = 2.54, 6.45 Hz, 1H), 4.27 (quin, J = 6.38 Hz, 1H), 4.09 (g, J = 5.22 Hz, 1H), 3.85−3.97 (m, 1H), 3.78 (s, 3H), 3.66 (dd, J = 1.55, 9.95 Hz, 1H), 3.18 (d, J = 5.29 Hz, 3H), 1.17−1.31 (m, 3H).13C NMR (126 MHz, DMSO-d6) δ 151.6, 148.1, 147.9, 146.8, 127.2, 124.1, 124.1, 121.0, 114.0, 113.5, 108.6, 108.0, 102.7, 68.7, 63.6, 55.9, 54.8, 14.6. HRMS-ESI (m/z) [M+H]+ calculated for C18H19ClNO6S: 412.0622, found 412.0612. Relative stereochemistry was confirmed by 2D NMR studies. Absolute stereochemistry was randomly assigned.

(2S,3R)-1-(benzo[d][1,3]dioxol-5-ylsulfanyl)-3-(5-chloro-2-methoxyphenoxy)-2-methylazetidine (12): Using General Procedure C, Mitsunobu reaction, reaction of (2S,3R)-1-(benzo[d][1,3] dioxol-5-ylsulfanyl)-2-methylazetidin-3-ol 27 with 5-chloro-2-methoxyphenol 31e afforded the title compound (11% yield) as an off-white solid. m/z: 412.2 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ 7.36 (d, J = 8.10−10 Hz, 1H), 7.32 (s, 1H), 7.17 (d, J = 8.10 Hz, 1H), 6.94−7.01 (m, 2H), 6.81 (d, J = 2.34 Hz, 1H), 6.22 (d, J = 2.47 Hz, 2H), 4.58 (J = 5.71 Hz, 1H). The reaction mixture was purified with Gilson RP-HPLC ramping ACN in H2O (25−95%, 0.1% TFA) to afford the title compound (55 mg, 36% yield) as a white solid. MS (ESI, positive ion) m/z: 408.1 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ ppm 8.23−8.46 (m, 2H), 7.74−7.99 (m, 2H), 6.88−7.01 (m, 2H), 6.60−6.80 (m, 1H), 4.74−4.87 (m, 1H), 4.08−4.27 (m, 5H), 3.53−3.65 (m, 5H).13C NMR (126 MHz, DMSO-d6) δ 148.0, 145.9, 140.8, 134.6, 125.2, 125.0, 124.0, 123.7, 122.9, 121.6, 114.2, 113.5, 110.7, 65.4, 57.4, 55.7, 35.7. HRMS-ESI (m/z) [M+H]+ calculated for C18H19ClNO6S: 408.0785, found 408.0772. Structure was confirmed by HMBC and ROESY NMR.

5-((3-(5-chloro-2-methoxyphenoxy)-1-azetidinyl)sulfonyl)-1-methyl-1H-indazole (16): Using General Procedure A, reaction of 1H-indazole-5-sulfonoyl chloride 24d with 3-(5-chloro-2-methoxyphenoxy)azetidin-3-ol afforded the title compound (20 mg, 17% yield) as a white solid. MS (ESI, positive ion) m/z: 392.0 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ ppm 8.30−8.42 (m, 2H), 7.72−7.83 (m, 2H), 7.53−7.66 (m, 2H), 6.85−6.97 (m, 2H), 6.64−6.76 (m, 1H), 4.74−4.87 (m, 1H), 4.13−4.24 (m, 2H), 3.54−3.64 (m, 5H). HRMS-ESI (m/z) [M+H]+ calculated for C18H19ClNO6S: 394.0617, found 394.0623.

5-((3-(5-chloro-2-methoxyphenoxy)-1-azetidinyl) sulfonyl)-1-methyl-1H-indazole (17): A solution of 16 (146 mg, 0.371 mmol) in DMF (1.48 mL) was cooled in an ice water bath prior to the addition of NaH (32.62 mg, 0.82 mmol). The resulting mixture was stirred for 10 min at 0 °C then 1 h at RT. The resulting solution was cooled in an ice water bath and MeI (0.077 mL, 1.22 mmol) was added. The resulting solution was stirred at RT for 10 min and then at 60 °C for 16 h. The reaction mixture was purified with Gilson RP-HPLC: MS (ESI, positive ion) m/z: 408.1 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ ppm 8.23−8.46 (m, 2H), 7.74−7.99 (m, 2H), 6.88−7.01 (m, 2H), 6.60−6.80 (m, 1H), 4.74−4.87 (m, 1H), 4.08−4.27 (m, 5H), 3.53−3.65 (m, 5H).13C NMR (126 MHz, DMSO-d6) δ 148.0, 145.9, 140.8, 134.6, 125.2, 125.0, 124.0, 123.7, 122.9, 121.6, 114.2, 113.5, 110.7, 65.4, 57.4, 55.7, 35.7. HRMS-ESI (m/z) [M+H]+ calculated for C18H19ClNO6S: 408.0785, found 408.0772. Structure was confirmed by HMBC and ROESY NMR.

5-((3-(5-chloro-2-methoxyphenoxy)-1-azetidinyl)sulfonyl)-1-methyl-1H-benzimidazole (18): Using General Procedure A, reaction of 1-methyl-1H-imidazole-5-sulfonoyl chloride 24e with 3-(5-chloro-2-methoxyphenoxy)azetidin-3-ol afforded the title compound (24 mg, 20% yield) as a white solid. MS (ESI, positive ion) m/z: 408.0 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ ppm 8.38−8.53 (m, 1H, 8.04−8.15 (m, 1H), 7.83−7.96 (m, 1H), 7.69−7.81 (m, 1H), 6.86−6.99 (m, 2H), 6.65−6.77 (m, 1H), 4.73−4.86 (m, 1H), 4.09−4.24 (m, 2H), 3.86−3.99 (m, 3H), 3.53−3.66 (m, 5H).13C NMR (126 MHz, DMSO-d6) δ 148.0, 147.6, 145.9, 142.8, 137.8, 126.2, 124.1, 121.2, 121.6, 120.3, 114.3, 111.5, 111.4, 111.5, 65.5, 57.4, 55.7, 31. HRMS-ESI (m/z) [M+H]+ calculated for C18H19ClNO6S: 408.0780, found 408.0779.

5-((3-(5-chloro-2-methoxyphenoxy)-1-azetidinyl)sulfonyl)-1,3-benzoxazol-2(3H)-one (19): Using General Procedure A, reaction of 2-oxo-2,3-dihydro-1,3-benzoxazole-6-sulfonoyl chloride 24f with 3-(5-chloro-2-methoxyphenoxy)azetidin-3-ol afforded the title compound (35 mg, 35% yield) as a white solid. MS (ESI, positive ion) m/z: 409.0 [M+1]−.1H NMR (500 MHz, DMSO-d6) δ ppm 12.08 (s, 1H). 1H NMR (500 MHz, DMSO-d6) δ ppm 12.08 (s, 1H). 1H NMR (500 MHz, DMSO-d6) δ ppm 12.08 (s, 1H).
for 2 h. The reaction mixture was filtered through an SPE frit and purified on a Waters auto-purification system; liquid transfer system: Tecan; drying system: Genevac; prep. Column: Xbridge (19 × 100 mm, C18, 10 μm); flow rate: 40 mL/min; general gradient: 5–95% B, 0.1% additive in both A and B; 10 min gradient; Mobile Phase A: H2O, Mobile Phase B, CH3CN; Additive: NH4OH. 0.1% NH4OH in ACN and water as mobile phase to obtain desired compound (±)-20 as a light yellow solid; HRMS-ESI (m/z) [M+H]+ calculated for C21H28F6N2O3S2: 449.1035, found 449.1040; 1H NMR (600 MHz, DMSO-d6) δ 7.91–7.94 (m, 1H), 7.49–7.56 (m, J = 8.64 Hz, 2H), 7.86–7.91 (m, J = 8.64 Hz, 2H), 6.94 (s, 1H), 2.54 (s, 2H), 2.54 (s, 1H), 1.71 (s, 3H), 1.15 (s, 3H). 13C NMR (126 MHz, DMSO-d6) δ ppm 170.3, 145.8, 141.7 (q, J= 36.5 Hz), 141.10, 128.0, 126.0, 125.6 (q, J= 128.0 Hz), 126.6, 125.5 (q, J= 126.6 Hz), 122.9 (br, s, 1H), 1.71 (s, 3H). The reaction mixture was filtered through an SPE frit and purified on a Waters auto-purification system; liquid transfer system: Tecan; drying system: Genevac; prep. Column: Xbridge (19 × 100 mm, C18, 10 μm); flow rate: 40 mL/min; general gradient: 5–95% B, 0.1% additive in both A and B; 10 min gradient; Mobile Phase A: H2O, Mobile Phase B, CH3CN; Additive: NH4OH. 0.1% NH4OH in ACN and water as mobile phase to obtain desired compound (±)-20 as a light yellow solid; HRMS-ESI (m/z) [M+H]+ calculated for C21H28F6N2O3S2: 449.1035, found 449.1040; 1H NMR (600 MHz, DMSO-d6) δ 7.91–7.95 (m, J = 8.65 Hz, 2H), 7.86–7.90 (m, J = 8.64 Hz, 2H), 6.94 (s, 1H), 2.54 (s, 2H), 2.54 (s, 1H), 1.71 (s, 3H), 1.15 (s, 3H). 13C NMR (126 MHz, DMSO-d6) δ ppm 170.3, 145.8, 141.7 (q, J= 36.5 Hz), 141.10, 128.0, 126.6, 125.6 (q, J= 128.0 Hz), 126.6, 125.5 (q, J= 126.6 Hz), 122.9 (br, s, 1H), 1.71 (s, 3H). 

5.6. Pharmacophore modeling and virtual screening

Conformational ensembles were generated for 1, 2, and (S)-3 using the modeling package MOE [13] and the molecular mechanics minimum conformations were flexibly aligned to the presumed bioactive conformation of 1. The resulting aligned conformations were minimized after alignment to ensure local minimum conformations using MMFF94 and Generalized Born implicit solvation. The bioactive conformation was derived from the co-crystal structure of hGlyRa3 with the structurally related potentiatior [35,3aS,9bS]-2-(1,3-benzodioxol-5-ylsulfonyl)-3,5-dimethyl-1,2,3,3a,5,9b-hexahydro-4H-pyrrrolo[3,4-c][1,6]naphthyridin-4-one, PDB: 5TIO [9]. A consensus pharmacophore was generated using the Pharmacophore Editor in MOE. The neighborhood distance tolerance threshold was set to 1.75, and one hundred percent consensus was required. The resulting pharmacophore was generalized by removing the hydrogen bond acceptor elements on the benzoazole ring. A Ligand Shape volume constraint was imposed set at two-times the volume of the overlaid atoms. Coordinates for the pharmacophore elements can be found in the Supplementary Information. The resulting pharmacophore was searched against a pre-computed multi-conformation database of internal Amgen library compounds (547,226 compounds) using MOE. All features were required to match, and 4267 hits were returned (0.78%). 17 (0.4%) of the compounds met our functional activity criteria (>50% POC in the single point assay), and were subjected to an internal structural alert protocol which flags compounds with undesired functional groups and potential pan-assay interference compounds. Compounds (±)-20 and (±)-21 had no such predicted liabilities.

5.7. Ligand docking

Compound 19 and both enantiomers of 20 were flexibly docked into the novel allosteric potentiator pocket of 5TIO.pdb [9] using Glide SP [18,19]. Top-scoring poses were visually inspected and compared to the key interactions of x-ray bound conformation of [35,3aS,9bS]-2-(1,3-benzodioxol-5-ylsulfonyl)-3,5-dimethyl-1,2,3,3a,5,9b-hexahydro-4H-pyrrrolo[3,4-c][1,6]naphthyridin-4-one. The best scoring pose was further optimized in the pocket; the ligand and residues within 4.5 Å of the ligand were allowed to relax using the AMBER10-EHT force field and Generalized Born implicit solvation as implemented in MOE, while the remainder of the receptor was held fixed. The GlideScore of (S)-20 was more favorable than for the (R) enantiomer and chosen as the active model. Inspection of the poses suggested that the (S) enantiomer may be preferred as the hydroxyl group is better positioned to interact with Tyr78 rather than buried against Leu83.

Author contributions

All authors have contributed and have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Abbreviations

μW, microwave; Clact, intrinsic clearance; MLM, mouse liver microsomes; MDRI, multidrug resistance protein 1, MDCK, Madin-Darby canine kidney; BCRP, breast cancer resistance protein; Papp, apparent passive permeability; LE, ligand efficiency; LipE, lipophilic efficiency; GABA, gamma-aminobutyric acid; nACH, nicotine
acetylcholine; 5-HT, 5-hydroxytryptamine; SnAr, nucleophilic aromatic substitution; POC, percent of control, MPO, multiparameter optimization; pMPO, probabilistic multiparameter optimization; FaSSIF, fasted state intestinal fluid.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.05.036.

References


[10] LipF was calculated using EC50 and ACD Daylight V4.81 cLogP.


[12] CD1-mice dosed i.v. at 1 mg/kg, formulated in dimethyl sulfoxide, 0.5 mL/kg.

[13] CD1-mice dosed iv. at 1 mg/kg, formulated in dimethyl sulfoxide, 0.5 mL/kg.


