Pharmacological Profile of the Novel Antiepileptic Drug Candidate Padsevonil: Interactions with Synaptic Vesicle 2 Proteins and the GABA<sub>A</sub> Receptor

Martyn Wood, Veronique Daniels,¹ Laurent Provins, Christian Wolff, Rafal M. Kaminski,² and Michel Gillard

UCB Pharma, Neurosciences Therapeutic Area, Braine l’Alleud, Belgium

Received July 8, 2019; accepted October 10, 2019

ABSTRACT

Padsevonil is an antiepileptic drug (AED) candidate synthesized in a medicinal chemistry program initiated to rationally design compounds with high affinity for synaptic vesicle 2 (SV2) proteins and low-to-moderate affinity for the benzodiazepine binding site on GABA<sub>A</sub> receptors. The pharmacological profile of padsevonil was characterized in binding and electrophysiological experiments. At recombinant SV2 proteins, padsevonil’s affinity for SV2A was greater than that of levetiracetam and brivaracetam (pK<sub>i</sub> 6.5, 5.2, and 6.6, respectively). Unlike the latter AEDs, both selective SV2A ligands, padsevonil also displayed high affinity for the SV2B and SV2C isoforms (pK<sub>i</sub> 7.9 and 8.5, respectively). Padsevonil’s interaction with SV2A differed from that of levetiracetam and brivaracetam; it exhibited slower binding kinetics: dissociation <i>t</i><sub>1/2</sub> 30 minutes from the human protein at 37°C compared with <0.5 minute for levetiracetam and brivaracetam. In addition, its binding was not potentiated by the allosteric modulator UCB1244283. At recombinant GABA<sub>A</sub> receptors, padsevonil displayed low-to-moderate affinity (pIC<sub>50</sub> ≈ 6.1) for the benzodiazepine site, and in electrophysiological studies, its relative efficacy compared with zolpidem (full-agonist reference drug) was 40%, indicating partial agonist properties. In vivo (mice) receptor occupancy studies, padsevonil exhibited SV2A occupancy at low ED<sub>50</sub> (0.2 mg/kg) and benzodiazepine site occupancy at higher doses (ED<sub>50</sub> 36 mg/kg), supporting in vitro results. Padsevonil’s selectivity for its intended targets was confirmed in profiling studies, where it lacked significant effects on a wide variety of ion channels, receptors, transporters, and enzymes. Padsevonil is a first-in-class AED candidate with a unique target profile allowing for presynaptic and postsynaptic activity.

SIGNIFICANCE STATEMENT

Padsevonil is an antiepileptic drug candidate developed as a single molecular entity interacting with both presynaptic and postsynaptic targets. Results of in vitro and in vivo radioligand binding assays confirmed this target profile: padsevonil displayed nanomolar affinity for the three synaptic vesicle 2 protein isoforms (SV2A, B, and C) and micromolar affinity for the benzodiazepine binding site on GABA<sub>A</sub> receptors. Furthermore, padsevonil showed greater affinity for and slower binding kinetics at SV2A than the selective SV2A ligands, levetiracetam, and brivaracetam.

Introduction

Levetiracetam (LEV) was the first antiepileptic drug (AED) shown to exert its therapeutic activity by targeting elements of the synaptic release machinery, namely, through binding to the synaptic vesicle 2A (SV2A) protein (Lynch et al., 2004). SV2A and the other two protein isoforms—SV2B and SV2C—are integral membrane glycoproteins that are present in secretory vesicles of neurons and endocrine cells (Bartholome et al., 2017). The precise function of the proteins remains elusive; however, given their presence in secretory vesicles, it is most likely that they play a role in vesicle exocytosis, an observation substantiated by accumulating evidence (Mendoza-Torreblanca et al., 2013). SV2A knockout mice die in a matter of weeks; however, in vitro recordings of neurons from very young SV2A knockout mice reveal a reduction in the frequency and amplitude of spontaneous inhibitory postsynaptic currents, potentially indicating a negative effect on GABA release from presynaptic neurons (Crowder et al., 1999). Data from animals lacking both SV2A and SV2B suggest that the absence of these proteins leads to presynaptic Ca<sup>2+</sup> accumulation during constrictive action potentials, causing abnormal increases in neurotransmitter release (Janz et al., 1999a). The overall effect is destabilization of synaptic circuits and aberrant neurotransmission (Crowder et al., 1999; Janz et al., 1999a). LEV reduces both inhibitory and excitatory postsynaptic currents in an activity-dependent manner, with the largest effect seen with the highest stimulation frequency (Meehan et al., 2012). Both LEV and...
brivaracem (BRV), a more potent and selective SV2A ligand (Klitgaard et al., 2016), produce frequency-dependent slowing of vesicle exocytosis and recycling, and of synaptic transmission (Meehan et al., 2011, 2012; Yang et al., 2015).

The substantial evidence for the role of SV2A in the pathophysiology of epilepsy (Lösch et al., 2016; Ohno and Tokudome, 2017) and the clinical utility of SV2A ligands in the treatment of patients with epilepsy ensured that drug-discovery programs focusing on SV2 ligands continued. One strand of research involved investigating the feasibility of designing a molecule with both presynaptic and postsynaptic activity via interaction with SV2 proteins and the GABA<sub>α</sub> receptor (GABA<sub>α</sub>R), respectively. The rationale for targeting these proteins was based on observations that LEV markedly potentiated the activity of AEDs acting via GABA<sub>α</sub>R transmission, notably benzodiazepines (BZDs), in several animal models resulting in an improved efficacy/safety ratio (Kaminski et al., 2009a). More recent studies have shown that SV2A dysfunction resulting from a missense mutation (L174Q) results in a selective reduction in GABA<sub>α</sub>Rergic transmission, rendering animals carrying the mutation highly susceptible to seizures and markedly facilitating kindling (Tokudome et al., 2016a,b).

In the subsequent rational medicinal chemistry design program, the focus was to develop a single molecule that could target SV2 proteins with high affinity and postsynaptic GABA<sub>α</sub>Rs, specifically the BZD binding site, with lower affinity. Low-to-moderate affinity for this site, coupled with a partial agonist profile, could minimize the potential for the development of tolerance, a phenomenon known to occur with most BZDs (Vinkers and Olivier, 2012; Gravielle 2016). Lead optimization efforts led to the discovery of padsevonil (PSL), an imidazothiadiazole heterocycle coupled to a pyrrolidine moiety (Fig. 1). PSL constitutes a novel chemical class, as indicated by its International Nonproprietary Name, which was approved by the World Health Organization in 2017.

The objectives of the studies reported here are to characterize the interactions of PSL with its intended therapeutic targets and to determine its selectivity for these targets using an array of validated in vitro and in vivo techniques. The pharmacological profile of PSL in nonclinical models of seizures and epilepsy is reported in the accompanying article (Leclerq et al., 2019).

Materials and Methods

Animals

Experimental procedures involving animals were conducted in compliance with the local ethics committee for animal experimentation according to Belgian law. All efforts were made to minimize animal suffering.

Naïve male specific-pathogen-free NMRI mice (Crl:NMRI[Han]; 24–35 g) and male Sprague-Dawley rats (200–300 g) were obtained from Charles River Laboratories (Ecully, France). All animals were housed in a holding room under a 12-hour light/dark cycle with lights on at 06:00 hours. Temperature was maintained at 20–24°C, relative humidity at 40%–70%, and the rate of air replacement at least 15 times an hour. Animals had ad libitum access to standard dry pellet food and tap water.

Radioligands, Drugs, and Chemicals

The following compounds were synthesized at UCB (Braine-l'Alleud, Belgium): PSL ((4R)-4-(2-chloro-2,2-difluoroethyl)-1-[(2-(methoxymethyl)-6-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methyl]pyrrolidin-2-one), LEV (2S-(2-oxo-1-pyrrolidinyl)butanamide), UCB30889 ((S)-2-(4-azidoethyl)-2-oxopyrrolidin-1-yl)butanamide), BRV (2S-2-(4R)-2-oxo-4-propylpyrrolidin-1-yl)butanamide), and UCB1244283 ((4S,5-dimethylphenyl)-N-(2-methoxyethyl)-3-methylbutanamide). For in vitro studies, compounds were dissolved in DMSO, and for in vivo studies, PSL was dissolved in 0.1% Tween 80 in NaCl (0.9%) and LEV in saline.

[3H]PSL (2.55 Ci mmol⁻¹) and [3H]UCB30889 (47 Ci mmol⁻¹) were custom-labeled by Aptuit (Greenwich, CT), and Amersham Biosciences (Amersham, UK), respectively. [3H]Flunitrazepam (80–90 Ci mmol⁻¹) was purchased from GE Healthcare (Gent, Belgium). Human embryonic kidney cell line (HEK)® cells (Flp-In-293) and zeocin were purchased from Life Technologies (Merelbeke, Belgium), fibroblast-like cell line COS-7 cells from European Collection of Authenticated Cell Cultures, Sigma-Aldrich (Bornem, Belgium), complete protease inhibitor cocktail from Roche (Virolvoo, Belgium), and DNAse (deoxyribonuclease I, type II from bovine pancreas) from Sigma-Aldrich. PBS, Dulbecco's modified Eagle's medium, L-glutamine, trypsin, and fetal bovine serum were purchased from Lonza (Verviers, Belgium). All other reagents were of analytical grade and obtained from conventional commercial sources.

Human cerebral cortex was obtained from Analytical Biologic Services Inc. (Wilmington, DE).

Tissue and Membrane Preparations

Preparation of Membrane Proteins from Rat and Human Cortex

Membrane proteins from rat cortex were prepared as described previously (Fukus et al., 2003). Briefly, after rats were sacrificed by decapitation, brains were removed rapidly and dissected on ice. All subsequent operations were performed at 4°C. Brain tissue, either rat or human cerebral cortex, was homogenized (10% w/v) in 20 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose (buffer A). Homogenates were spun at 30,000g for 15 minutes, and the pellets were resuspended in the same buffer. After incubation at 37°C for 15 minutes, membranes were washed three times using the same centrifugation protocol. Final pellets were resuspended in buffer A at a protein concentration of 10–15 mg ml⁻¹ and stored at −140°C until further use.

Preparation of Membrane Proteins from HEK and COS-7 Cells

Human SV2A, B, and C were expressed in HEK cells and rat GABA<sub>α</sub>R<sub>1</sub> (α1β2γ2; α2β2γ2; α5β2γ2) in COS-7 cells. Cells were subcultured in Dulbecco’s modified Eagle’s medium containing 200 mM L-glutamine and 100 µg ml⁻¹ zeocin, supplemented with 10% fetal bovine serum, and grown in a humidified atmosphere of 5% CO₂ at 37°C. Confluent cells were harvested by trypsinization and pelleted by centrifugation at 150g for 10 minutes at 4°C. The pellet was washed with ice cold PBS using the same centrifugation protocol and homogenized in a buffer containing 15 mM Tris-HCl, 1 mM EDTA, 0.5 mM EGTA, and 2 mM MgCl₂ (pH 7.5) supplemented with complete protease inhibitor cocktail. The homogenate was freeze-thawed twice and equilibrated at 25°C followed by a 10-minute DNase (10 U ml⁻¹) treatment. The solution was centrifuged for 25 minutes at 40,000g and 4°C. Finally, the pellet was resuspended in buffer A at a protein concentration of 5–10 mg ml⁻¹ and stored at −140°C until further use.

Fig. 1. Structure of padsevonil ((4R)-4-(2-chloro-2,2-difluoroethyl)-1-[(2-(methoxymethyl)-6-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methyl]pyrrolidin-2-one) compared with the prototypical SV2 ligand, levetiracetam (2S-(2-oxo-1-pyrrolidinyl)butanamide).
**Radioligand Binding Experiments**

Experiments were performed as previously described (Fuks et al., 2003). For all assays, membrane proteins (100 μg per assay for cortical membrane proteins, 70–125 μg for SV2A, 2–5 μg for SV2B, 40–60 μg for SV2C, and 75–125 μg for rat GABA A R subtypes) were incubated for 120 minutes at 4°C or 60–150 minutes at 37°C in 0.5 or 2 ml of Tris-HCl buffer (50 mM, pH 7.4) containing 2 mM MgCl₂. All glass fiber filters (from Brandel Inc., Gaithersburg, MD) used in the experiments were presoaked in 0.1% polyethyleneimine.

**Competition Binding Experiments.** Increasing concentrations of unlabeled competing drugs were added in the presence of 0.9 or 9 nM [³H]PSL, 1 or 4 nM [³H]UCB30889 or 2 nM [³H]flunitrazepam. At the end of the incubation period, membrane-bound radioligand was recovered by rapid filtration through GF/B filters. Plates were washed rapidly three times with 0.3 ml of ice-cold Tris buffer; the total washing procedure did not exceed 10 seconds.

**Kinetic Experiments.** Specific [³H]PSL binding in association experiments was measured at the indicated times after addition of membrane proteins at 37°C. Dissociation was induced by the addition of 10 μM of unlabeled PSL to the association reaction mixture. Samples were filtered on GFC filters and washed with 5 ml of ice-cold Tris buffer. Total filtration time per sample did not exceed 2 seconds.

**Saturation Binding Experiments.** Membrane proteins were incubated with [³H]PSL at concentrations ranging from 0.05 to 22 nM, and samples were filtered using GF/B filters. Non-specific binding was defined as residual binding observed in the presence of 10 μM unlabeled PSL for [³H]PSL, 1 mM LEV for [³H]UCB30889, or 10 μM diazepam for [³H]flunitrazepam. Radioactivity was determined by liquid scintillation.

The effect of UCB1244283, a positive allosteric modulator of SV2A, on PSL binding to SV2A was also evaluated. Studies were performed at 4°C according to the protocol described by Wood and Gillard (2017).

**In Vivo Receptor Occupancy.** The protocol developed by Li et al. (2006) was followed using NMRI mice instead of rats. Animals received vehicle (TWEEN/saline) or PSL (10 ml/kg body weight), administered intraperitoneally, followed 27 minutes later by tail-vein injection of [³H]PSL, 1 mM LEV for [³H]UCB30889, or 10 μM diazepam for [³H]flunitrazepam. Radioactivity was determined by liquid scintillation.

The effect of UCB1244283, a positive allosteric modulator of SV2A, on PSL binding to SV2A was also evaluated. Studies were performed at 4°C according to the protocol described by Wood and Gillard (2017).
observed at low doses and BZD site occupancy at higher doses, indicating a greater PSL affinity for SV2A than the BZD site. In the dose-range administered intraperitoneally in mice, PSL ED_{50} for SV2A occupancy was 0.4 μmol/kg (0.2 mg/kg; pED_{50} 6.4 mol/kg), whereas that of BZD site occupancy was markedly higher at 72 μmol/kg (36 mg/kg; pED_{50} 4.14 mol/kg) (Fig. 2).

### Binding Characteristics at SV2 Isoforms

Detailed characterization of PSL binding to the three SV2 isoforms was performed using [3H]PSL, the tritium-radiolabeled compound.

**Kinetic Experiments.** Binding kinetics of [3H]PSL were determined on human recombinant SV2 isoforms expressed in HEK cells and in human and rat cortex at 37°C (Fig. 3; Table 2). [3H]PSL association kinetics were monophasic at all protein sources. Dissociation kinetics were also monophasic for the recombinant SV2 isoforms but more complex in human and rat cortex, although no clear separation of the phases was evident.

**Saturation Binding Experiments.** Saturation binding curves of [3H]PSL on human recombinant SV2A/B/C and on human and rat cortex proteins were compatible with the labeling of a homogeneous population of binding sites at 37°C (Fig. 4). Corresponding affinities and B_{max} values are given in Table 3. The affinity of [3H]PSL was similar for all protein sources, and the K_d value obtained for recombinant human SV2A corresponded with the K_d obtained for the unlabeled compound using [3H]UCB30889 (Table 1).

UCB1244283 is a positive allosteric modulator of SV2A that increases the binding of BRV and LEV to the protein (Daniels et al., 2013). Use of the modulator (at 30 μM and at 4°C) had no effect on the B_{max} for [3H]PSL binding to the human SV2A protein in HEK membranes, but it reduced the affinity from 2.3 ± 0.2 nM (n = 3) to 3.90 ± 0.5 nM (P < 0.05, Student’s t test).

**Competition Experiments.** Competition experiments were performed on all human recombinant SV2 isoforms expressed in HEK cells and on human and rat cortex proteins using several SV2A ligands, as well as diazepam (Fig. 5). These experiments were performed with 0.9 nM [3H]PSL. Data were analyzed using sigmoidal dose-response fits with variable slope, and the resulting pIC_{50} values were transformed into pK_i values (Table 4). The affinity of the selective SV2A ligands—LEV, UCB30889, and BRV—was very low for both SV2B and SV2C labeled with [3H]PSL, resulting in incomplete competition curves at the concentration range used. This finding is in line with previous reports, demonstrating the selectivity of LEV and BRV for SV2A (Noyer et al., 1995; Gillard et al., 2011). Competition with PSL led to a complete displacement of [3H]PSL binding on all protein sources. For all human recombinant SV2 isoforms, the Hill slopes of the curve fits were not different from unity, and the obtained K_i values for PSL agreed with the K_d values of the radiolabeled compound.

In human and rat cortex, the competition curves of LEV, BRV, and UCB30889 fitted on the data using a sigmoidal dose-response curve with variable slope were shallower than those for PSL. The corresponding Hill coefficients ranged from −0.8 to −0.7 and reached significant difference from unity for LEV in both human and rat cortex (P < 0.01) and for UCB30889 and BRV in rat cortex (P < 0.01) (Table 4). The pronounced shallow profile of these competition curves allowed analysis of data with a model describing the binding to two independent populations of binding sites. At a radioligand concentration of 0.9 nM, the result fit with 85% of the binding sites having an affinity similar to that on recombinant human SV2A, and the remaining sites displaying an affinity in the low millimolar range. The latter proportion of binding sites most likely represents the SV2B component, given the very low presence of SV2C in the cortex.

### Effects in Selectivity Studies

Selectivity of PSL for its therapeutic targets was determined using radioligand binding, receptor activation and electrophysiological studies.

In radioligand studies, PSL at 10 μM lacked significant effects (>50%) on a wide variety of molecular targets, including ligand-gated and G-protein–coupled receptors, ion channels, transporters, and enzymes. The only significant effect was at the BZD binding site of the GABA_A receptors and NK2 receptor in mouse cortex 30 minutes after intraperitoneal administration. In receptor activation assays, PSL at concentrations up to 30 μM failed to elicit release of any of the tested cytokines from human peripheral blood mononuclear cells, indicating a lack of activity on TLR 2 and 4. In electrophysiological studies, PSL at 10 μM had no significant effect on any of the classic AED targets such as voltage-gated ion channels or glutamatergic receptors.

### Activity at Recombinant GABA_A Receptors

In the recombinant human α1β2γ2 GABA_A subtype, application of GABA resulted in activation of Cl⁻ currents

---

**Table 1.** Affinity of padsevonil for sites labeled with [3H]UCB30889 (SV2A) and [3H]flunitrazepam (benzodiazepine site of the GABA_A receptor) in human and rat cortex at 37°C. Results are the mean ± S.D. of at least three independent experiments. Data from competition curves were analyzed by nonlinear regression using a sigmoidal dose-response model with variable slope.

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Tissue</th>
<th>pIC_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]UCB30889</td>
<td>Rat cortex</td>
<td>8.43 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>Human cortex</td>
<td>8.97 ± 0.15</td>
</tr>
<tr>
<td>[3H]flunitrazepam</td>
<td>Rat cortex</td>
<td>5.50 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Human cortex</td>
<td>5.80 ± 0.17</td>
</tr>
</tbody>
</table>
with an EC\textsubscript{50} of 15 \textmu M (data not shown). To evaluate the effects of drugs on these GABA-evoked Cl\textsuperscript{−} currents, an agonist concentration corresponding to its EC\textsubscript{20} (5 \textmu M) was selected.

PSL, in a concentration range of 1 nM–30 \textmu M, when added alone for 5 minutes during preincubation, did not significantly change basal Cl\textsuperscript{−} currents compared with control (DMSO 0.5%, data not shown) but potentiated Cl\textsuperscript{−} currents evoked by 5 \textmu M GABA. Potentiation of GABA-induced Cl\textsuperscript{−} currents was dose-dependent with an EC\textsubscript{50} of 137 nM and a maximal effect reaching 167\% (Fig. 6).

The relative efficacy of PSL in potentiating GABA\textsubscript{A}R Cl\textsuperscript{−} currents was compared with that of zolpidem, a reference drug included in each experiment. Zolpidem is a full agonist at the BZD site and potentiates GABA \textit{EC\textsubscript{20}} with a maximal efficacy at 1 \mu M. The relative efficacy of PSL at 10 \mu M was 44\% \pm 16\% compared with the maximal response to zolpidem defined as 100\%.

**Discussion**

PSL was developed in a rational drug-discovery program initiated to develop a molecule with a novel mode of action aimed at the treatment of patients with drug-resistant epilepsy. Results of experiments described here confirm that PSL is a high-affinity pan-SV2 ligand, and it acts as a low-affinity partial agonist at the BZD site of GABA\textsubscript{A}Rs. Profiling studies also confirmed the lack of effect on typical AED targets, such as ion channels and glutamate receptors, as well
as a variety of other central nervous system targets, including ligand-gated and G-protein–coupled receptors, transporters and enzymes.

PSL is the first ligand that interacts with all three SV2 isoforms. In saturation and competition studies, [3H]PSL displayed nanomolar affinity for SV2A, SV2B, and SV2C. This finding was in contrast to LEV and BRV, both displaying marked selectivity for SV2A and interacting with SV2B and SV2C only at concentrations 100-fold higher than that at which they interacted with SV2A. This finding was also consistent with the lack of binding of a LEV derivative and of [3H]BRV in mouse brain tissue lacking SV2A (Lynch et al., 2004; Gillard et al., 2011). The high affinity of [3H]PSL for all isoforms was also reflected in kinetic data. [3H]PSL dissociation kinetics were monophasic at human recombinant SV2 isoforms but more complex in human and rat cortex, most likely owing to the labeling of a relatively low fraction of SV2B and SV2A at 0.9 nM [3H]PSL. At this concentration, relatively more SV2A binding sites are labeled since [3H]PSL has a 4-fold higher affinity for SV2A (Kd 1.5 nM) than for SV2B (Kd 6.3 nM). Labeling of only a small proportion of SV2B in both human and rat cortex is supported by results of competition experiments; SV2C labeling was not anticipated given its restricted expression in the cortex (see the following). Results with LEV and BRV demonstrated labeling of approximately 85% of the binding sites with affinities similar to that at human recombinant SV2A. Finally, both SV2A and SV2B labeling in the cortex is supported by the observation that the Bmax values for [3H]PSL were approximately twice as high as those observed for SV2A-selective ligands (Gillard et al., 2003, 2011). Results do not allow an accurate prediction of the SV2A/B ratio in the cortex because differences in the labeling of both isoforms in native versus recombinant expression systems cannot be excluded. Overall, results are consistent with the interaction of PSL with all three SV2 isoforms and suggest that it may be a useful ligand in further characterization of these proteins.

The roles of SV2B and SV2C in the pathophysiology of epilepsy remain unknown. Few reports have addressed the role of SV2B since its distribution in the mammalian brain partly overlaps with that of SV2A (Bajjalieh et al., 1993, 1994). SV2B−/− mice do not show phenotypic abnormalities, and electrophysiological studies on cultured neurons from these mice do not reveal any obvious effects on neurotransmission (Janz et al., 1999a; Chang and Südhof, 2009; Venkatesan et al., 2012). In a recent study, SV2C expression was strongest in the basal ganglia and more restricted in the cortex of rodent, rhesus macaque, and human brain (Dunn et al., 2019), consistent with results from previous rodent studies (Janz and Südhof, 1999b; Dardou et al., 2011). In the human brain, SV2C was highly colocalized with the GABA transporter in the striatum, substantia nigra, and ventral tegmental area (Dunn et al., 2019). SV2C expression was weak or absent in the hippocampus of autopsy controls, but it increased in biopsies from patients with temporal lobe epilepsy owing to mesial temporal sclerosis; in contrast, SV2A and SV2B expression

![Image](https://i.imgur.com/3QG.png)

**Fig. 4.** Saturation binding curves of [3H]padsevonil on human cortex (left panel) and on human recombinant SV2C (right panel) expressed in HEK cells. Membrane preparations were incubated with increasing concentrations of [3H]padsevonil for 150 minutes at 37°C. Non-specific binding (NSB) represents residual binding in the presence of 10 μM of padsevonil. Specific binding = total binding – NSB. Data are representative of three independent experiments.
levels were decreased (Crèvecœur et al., 2014). Since PSL has high affinity for all three SV2 isoforms, it could be postulated that binding to SV2B/C, in addition to SV2A, may contribute to broader, more sustained antiseizure effects.

PSL differs from SV2A-selective ligands not only in its affinity for SV2B and SV2C, but also in its interaction with SV2A, as suggested by the slow kinetics of [3H]PSL and the effect of the modulator, UCB1244283. BRV and LEV have

### Table 3

<table>
<thead>
<tr>
<th>SV2A</th>
<th>SV2B</th>
<th>SV2C</th>
<th>Rat Cortex</th>
<th>Human Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$ (nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.53 ± 0.15</td>
<td>6.34 ± 1.67</td>
<td>2.40 ± 0.16</td>
<td>2.73 ± 1.04</td>
<td>2.11 ± 0.84</td>
</tr>
<tr>
<td>$B_{max}$ (pmol/mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>109.9 ± 3.2</td>
<td>52.8 ± 7.8</td>
<td>23.8 ± 4.6</td>
<td>27.3 ± 9.2</td>
<td>9.3 ± 1.6</td>
</tr>
</tbody>
</table>

Fig. 5. Affinity of compounds for sites labeled with [3H]padsevonil in human recombinant SV2A/B/C proteins and on rat and human cortex. A concentration range of the compounds was incubated with 0.9 nM [3H]padsevonil for 150 minutes at 37°C. B0 is the binding of [3H]padsevonil in the absence of any competing compound. Data represent the mean ± S.D. of at least three independent experiments. Data were normalized to the B0 condition and fitted using a nonlinear regression model describing competitive binding to one (straight line) or two (dotted line) binding sites.
crease [3H]PSL binding but reduced its affinity for SV2A, binding capacity. The modulator did not significantly in-
duces that specific conformation could seemingly increase
the presence of an allosteric modulator that stabilizes or
selective binding of some ligands to only one conformation,
-facing conformations (Quistgaard et al., 2016), and with
superfamily, SV2 can adopt different inward and outward
however, as a putative transporter of the major facilitator
transmembrane transporters (MFTs), SV2 may play a key
role in anticonvulsant action (Schuster and Rinne, 2011; Ono
et al., 2009). In functional studies, PSL lacked any direct effect
on recombinant α1β2γ2 GABAARs, but it potentiated the
affinity of low GABA concentrations (EC20, giving around
20% of the maximum GABA response) with a pEC50 of
approximately 7.0 (100 nM). This observation is in keeping
with the known mechanism of action of many BZDs, positive
allosteric modulators of GABAARs that do not open the Cl−
channel on binding, but increase the affinity for channel
gating by GABA (Sigel and Steinmann, 2012). PSL
acts as a partial agonist. PSL was designed specifically to act
as a partial agonist in an effort to minimize the potential for
tolerance. PSL has a high pEC50 of approximately 6.7 (100 nM).
Table 4 shows that PSL is more potent on human SV2A than
rat SV2A, which is consistent with previous reports (Wood
et al., 2009b; Gillard et al., 2011), it is plausible that such a high-
affinity and long-lasting interaction could also lead to improved
antiseizure efficacy.

Postsynaptically, PSL displayed low-to-moderate (micromo-
lar) affinity for the BZD site of GABAARs in human and rat
brain membranes with no interspecies difference in potency.
Results were similar in recombinant GABAAR subtypes with
α1, α2, and α5 subunits coexpressed with β2 and γ2 subunits.
These subunits were chosen because they have been suggested
to mediate many of the BZD’s pharmacological effects (DHulst
et al., 2009). In functional studies, PSL lacked any direct effect
on recombinant α1β2γ2 GABAARs, but it potentiated the
effects of low GABA concentrations (EC20, giving around
20% of the maximum GABA response) with a pEC50 of
approximately 7.0 (100 nM). This observation is in keeping
with the known mechanism of action of many BZDs, positive
allosteric modulators of GABAARs that do not open the Cl−
channel on binding, but increase the affinity for channel
gating by GABA (Sigel and Steinmann, 2012). PSL’s relative
efficacy compared with zolpidem was 44%, indicating that it
acts as a partial agonist. PSL was designed specifically to act
as a partial agonist in an effort to minimize the potential for
induction of tolerance. BZDs have potent antiseizure effects;
however, their long-term use is limited by sedation, tolerance,
and the risk of dependence (Riss et al., 2008; Rudolph and
Knoflach, 2011; Ochoa and Kilgo., 2016). In epilepsy, tolerance
is associated with a loss of antiseizure efficacy, a progressive
increase in seizure frequency and severity, and an increased
risk of withdrawal seizures, even if AEDs are kept at constant

---

**TABLE 4**
pK values and Hill slopes of selective synaptic vesicle protein (SV2A ligands and diazepam for sites labeled with 0.9 nM [3H]padsevonil on recombinant human SV2A/B/C and on human and rat cortex at 37°C. Data are presented as mean ± S.D. (n = 3–10) and were obtained from nonlinear regression analysis of untransformed raw data using a sigmoidal dose-response model with variable slope. Missing values could not be calculated because of incomplete competition curves as a result of compound solubility limits.

<table>
<thead>
<tr>
<th></th>
<th>Levetiracetam</th>
<th>Brivaracetam</th>
<th>UCB30889</th>
<th>Padsevonil</th>
<th>Diazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV2A</td>
<td>5.2 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>6.7 ± 0.2</td>
<td>8.5 ± 0.1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>SV2B</td>
<td>3.1 ± 0.4</td>
<td>&lt;4</td>
<td>-4</td>
<td>7.9 ± 0.1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>SV2C</td>
<td>3.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.8 ± 0.2</td>
<td>8.5 ± 0.2</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>Human cortex</td>
<td>5.3 ± 0.3</td>
<td>6.4 ± 0.2</td>
<td>6.7 ± 0.2</td>
<td>8.7 ± 0.1</td>
<td>5.9 ± 0.4</td>
</tr>
<tr>
<td>Rat cortex</td>
<td>5.0 ± 0.1</td>
<td>6.4 ± 0.1</td>
<td>6.6 ± 0.2</td>
<td>8.7 ± 0.1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Hill slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human cortex</td>
<td>-0.71 ± 0.03</td>
<td>-0.68 ± 0.05</td>
<td>-0.74 ± 0.12</td>
<td>-0.99 ± 0.21</td>
<td>ND</td>
</tr>
<tr>
<td>Rat cortex</td>
<td>-0.76 ± 0.03</td>
<td>-0.74 ± 0.26</td>
<td>-0.79 ± 0.15</td>
<td>-0.94 ± 0.13</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.

---

extremely fast kinetics: BRV’s dissociation t1/2 is 20 seconds
at 37°C (Gillard et al., 2011), and that of LEV is 30 seconds at
25°C (Noyer et al., 1995). [3H]PSL dissociation t1/2 from human
SV2A was 30 minutes at 37°C. This slower off-rate
was also seen in rat and human cortex, suggesting a predom-
nance of SV2A in these tissues. The SV2A positive allosteric
modulator, UCB1244283, increases both BRV and LEV bind-
ing, but by different mechanisms; an increase in the binding
capacity for LEV and an increase in binding affinity and
capacity for BRV (Wood and Gillard 2017). Allosteric modu-
lation is typically associated with an increase in binding affinity;
however, as a putative transporter of the major facilitator
superfamily, SV2 can adopt different inward and outward
facilitating conformations (Quistgaard et al., 2016), and with
selective binding of some ligands to only one conformation,
the presence of an allosteric modulator that stabilizes or
induces that specific conformation could seemingly increase
binding capacity. The modulator did not significantly in-
crease [3H]PSL binding but reduced its affinity for SV2A, suggest-
ing that SV2A may exist in multiple conformational
states that can be stabilized by the SV2A modulator and that the
three ligands—[3H]LEV, [3H]BRV, and [3H]PSL—interact

differently with it. Although LEV and BRV exert their therapeu-
getic activity via interaction with SV2A (Matagne et al., 2008),
their effect on the protein remains unknown. Therefore, it is
difficult to predict the functional consequences of the slow off-
rates and the novel mechanism of interaction of PSL with SV2A.

Given the evidence that antiseizure potency correlates with
SV2A binding affinity (Noyer et al., 1995; Kaminski et al., 2008,
2009b; Gillard et al., 2011), it is plausible that such a high-
affinity and long-lasting interaction could also lead to improved
antiseizure efficacy.

**Fig. 6.** Padsevonil potentiates GABA-mediated Cl− currents in CHO cells stably expressing human α1β2γ2 GABAAR receptor subunits (left panel) by acting as a partial agonist at the benzodiazepine site of the receptor (right panel; bar represents S.D.). Zolpidem, a full agonist, displays a maximal response at 1 μM defined as 100%.
Padaslovinita Interactions with its Therapeutic Targets

9 maintenance doses (Riss et al., 2008; Ochoa and Kilgo, 2016). Traditional BZDs such as DZP act as full agonists at the BZD site; partial agonists, however, with lower intrinsic efficacy, could potentially be associated with a lower likelihood of tolerance (Rundfeldt and Löscher, 2014). Approaches to reduce the limitations of BZDs have focused on the development of subtype-selective agents or those with low intrinsic efficacy (Rudolph and Knoflach, 2011; Rundfeldt and Löscher, 2014).

In vivo binding data indicate 50% SV2A and BZD site occupancy at 0.2 and at 36 mg/kg, respectively, supporting in vitro results, with 100-fold lower PSL potency at the BZD site compared with SV2A. In the accompanying report, PSL is shown to be active in a variety of nonclinical seizure and epilepsy models in the 0.1–10 mg/kg dose range (Leclerq et al., 2019). This finding corroborates observations that moderate-to-high SV2 occupancy is associated with antiseizure activity and that low-level BZD site occupancy potentiates this effect (Kaminski et al., 2009a).

In conclusion, PSL is a first-in-class AED candidate with a presynaptic and postsynaptic mechanism of action. Studies described here have shown that PSL displays high affinity for the three SV2 isoforms, ranging from 1.5 to 6.3 nM, and low-to-moderate affinity for the GABAAR BZD site, where it acts as a partial agonist, and that this profile is maintained in vivo. Furthermore, the interaction of PSL with SV2A differs from that of LEV and BRV in that it displays a markedly slower dissociation rate, and its activity is not potentiated by an SV2A modulator. These additional properties may contribute to the highly active profile of PSL in nonclinical seizure and epilepsy models, including those where the SV2A-selective ligand, LEV and BRV, show limited or no activity (Leclerq et al., 2019).

Acknowledgments

We thank John Lambert, David Urbain, Marielle Martini, and Veronique Declerq (UCB Pharma, Braine l’Alleud, Belgium) for skillful technical assistance; Barbara Pelgrims (UCB Pharma, Brussels, Belgium) for overseeing the development of the manuscript; and Azita Tofighy for providing writing support, funded by UCB Pharma.

Authorship Contributions

Participated in research design: Wood, Daniels, Wolff, Gillard. Conducted experiments: Daniels.

Contributed new reagents or analytic tools: Provins. Performed data analysis: Wood, Daniels, Wolff, Gillard.

Wrote or contributed to the writing of the manuscript: Wood, Wolff, Kaminski, Gillard.

References


Address correspondence to: Michel Gillard, UCB Pharma, Neurosciences Therapeutic Area, Chemin du Foriester, 1420 Braine l’Alleud, Belgium. E-mail: michael.gillard@ucb.com