Pharmacological profile of the novel antiepileptic drug candidate padsevonil – characterization in rodent seizure and epilepsy models

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List of nonstandard abbreviations
AED  antiepileptic drug
ADD  afterdischarge duration
BRV  brivaracemt
BZD  benzodiazepine
CBZ  carbamazepine
GAERS  Genetic Absence Epilepsy Rats from Strasbourg
HPD  hippocampal paroxysmal discharges
IP   intraperitoneal
LEV   levetiracetam
LTG   lamotrigine
MES   maximal electroshock
MTLE  mesial temporal lobe epilepsy
PHT   phenytoin
PSL   padsevonil
PTZ   pentylenetetrazol
RTG   retigabine
SC    subcutaneous
SV2   synaptic vesicle protein 2
TI    therapeutic index
TPM   topiramate
VPA   valproate

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ABSTRACT

The antiepileptic drug (AED) candidate, padsevonil, is the first in a novel class of drugs that bind to synaptic vesicle 2 (SV2) proteins, and to the GABA<sub>A</sub> receptor benzodiazepine site, allowing for pre- and postsynaptic activity, respectively. In acute seizure models, padsevonil provided potent, dose-dependent protection against seizures induced by administration of pilocarpine or 11-deoxycortisol, and those induced acoustically or through 6 Hz stimulation; it was less potent in the pentyleneetetrazol, bicuculline, and maximal electroshock models. Padsevonil displayed dose-dependent protective effects in chronic epilepsy models, including the intrahippocampal kainate and GAERS models, which represent human mesial temporal lobe and absence epilepsy, respectively. In the amygdala kindling model, which is predictive of efficacy against focal to bilateral tonic-clonic seizures, padsevonil provided significant protection in kindled rodents; in mice specifically, it was the most potent AED compared with nine others with different mechanisms of action. Its therapeutic index was also the highest, potentially translating into a favorable efficacy and tolerability profile in humans. Importantly, in contrast to diazepam, tolerance to padsevonil’s antiseizure effects was not observed in the pentyleneetetrazol-induced clonic seizure threshold test. Further results in the 6 Hz model showed that padsevonil provided significantly greater protection than the combination of diazepam with either levetiracetam or brivaracetam, both selective SV2A ligands. This observation suggests that padsevonil’s unique mechanism of action confers antiseizure properties beyond the combination of compounds targeting SV2A and the benzodiazepine site. Overall, padsevonil displayed robust efficacy across validated seizure and epilepsy models, including those considered to represent drug-resistant epilepsy.
SIGNIFICANCE STATEMENT

Padsevonil, a first-in-class antiepileptic drug candidate, targets synaptic vesicle 2 (SV2) proteins and the benzodiazepine site of GABA_A receptors. It demonstrated robust efficacy across a broad range of rodent seizure and epilepsy models, several representing drug-resistant epilepsy. Furthermore, in one rodent model, its efficacy extended beyond the combination of drugs interacting separately with SV2 or the benzodiazepine site. Padsevonil displayed a high therapeutic index, potentially translating into a favorable safety profile in humans; tolerance to antiseizure effects was not observed.

KEY WORDS

animal models, anticonvulsants, benzodiazepines, drug tolerance/dependence, GABA receptors, kindling, safety pharmacology, seizures
INTRODUCTION

Epilepsy is one of the most common neurological diseases worldwide, and is associated with a significant healthcare burden (Devinsky et al., 2018; Thijs et al., 2019). For most patients with epilepsy, antiepileptic drugs (AEDs) are the mainstay of therapy, which must be taken on a long-term, often lifelong basis (Trinka et al., 2012; Thijs et al., 2019). Antiepileptic drugs approved in the last decade display good safety and pharmacokinetic profiles, but improved efficacy over first-generation AEDs has not been demonstrated in clinical studies so far (Chen et al., 2018), and approximately a third of patients with epilepsy continue to experience poorly controlled seizures despite treatment, ie, drug-resistant epilepsy (DRE) (Kwan et al., 2010; Kalilani et al., 2018; Chen et al., 2018). Most AEDs were discovered by initial demonstration of their antiseizure activity in simple, classic seizure models, such as the maximal electroshock (MES) and pentylenetetrazol (PTZ) tests, which are highly predictive of clinical efficacy in epilepsy, but not DRE (Löscher et al., 2013).

Polytherapy is a frequent treatment strategy for patients with DRE, since a substantial proportion will require more than one AED to reduce their seizure burden (French and Faught, 2009; Brodie and Sills, 2011). The combination of selected AEDs should allow for synergistic or additive efficacy without any detrimental impact on safety and tolerability (French and Faught, 2009; Brodie and Sills, 2011); however, a nonclinical mechanistic rationale for clinically used AED combinations is often lacking or have not yet translated into superior efficacy.

Levetiracetam (LEV) is an AED that exerts its therapeutic activity primarily by binding to the synaptic vesicle (SV)2A protein (Lynch et al., 2004) and shows a distinctive profile in nonclinical seizure models. While ineffective in standard models used traditionally for AED discovery, such as the MES and PTZ tests, LEV provided protection against seizures in models of acquired and genetic epilepsies (Klitgaard et al., 1998), subsequently translating to broad-spectrum clinical efficacy in humans (Klitgaard and Verdrue, 2007). In the audiogenic seizure and amygdala kindling models, LEV increased the potency of several AEDs and experimental agents that interfere with ligand-gated ion channels, particularly those that enhance GABA-mediated inhibition (Kaminski et al., 2009). Importantly, the increase in potency was devoid of additional
adverse effects (ie, motor impairment) as assessed by the rotarod test; on the contrary, it was associated with an increase in the therapeutic index (Kaminski et al., 2009). Assuming a potential synergistic interaction between ligands that act via SV2 and GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs), a rational medicinal chemistry design program was initiated to develop a single molecular entity that could target both.

The outcome of this discovery program was the identification of padsevonil (PSL), the first rationally-designed AED candidate that acts selectively on both pre- and postsynaptic targets. Presynaptically, as a SV2 ligand, PSL displays high affinity (nM) not only for SV2A, but also for the other two protein isoforms, SV2B and SV2C. The latter markedly distinguishes the profile of PSL from that of LEV and brivaracetam (BRV), which are selective SV2A ligands and furthermore, have no established postsynaptic activity. Postsynaptically, as a positive allosteric modulator of GABA<sub>A</sub>Rs, PSL displays low-to-moderate (µM) binding affinity for the benzodiazepine (BZD) site in recombinant human GABA<sub>A</sub>Rs and human and rat brain membrane preparations, where it shows a partial agonist profile. This profile was selected specifically to minimize CNS and respiratory adverse effects, tolerance development and abuse potential typically associated with the use of BZDs that are full agonists (Rundfeldt and Löscher, 2014). The detailed pharmacological and mechanistic profile of PSL is described in the companion paper (Wood et al., 2019). In this report, we describe the activity of PSL in a variety of rodent seizure and epilepsy models and compare its activity with that of mechanistically diverse and clinically used AEDs. We also compare the potential of PSL for development of tolerance with that of the BZD, diazepam (DZP), after chronic dosing in mice.
MATERIALS AND METHODS

Animals
All experiments were conducted in compliance with guidelines issued by the ethics committee for animal experimentation according to Belgian law. Those conducted as part of the murine intrahippocampal kainate model of mesial temporal lobe epilepsy (MTLE) were performed at Synapcell (Grenoble, France); experiments were approved by the European Technology Platform for Global Animal Health and performed in accordance with the European Committee Council directive (2010/63/EU). All efforts were made to minimize animal suffering.

Female, genetically sound-sensitive mice (20–24 g) were derived from a DBA strain from the Laboratory of Acoustic Physiology (Paris, France) and bred in Charles River Laboratories, Italy. Male NMRI mice weighing 20–35 g were used in all other acute electrically-and chemically-induced seizure tests, as well as the rotarod and tolerance tests. Male C57BL/6J mice, weighing 25–34 g, were used for the murine model of amygdala kindling. For the rat model of amygdala kindling, male Sprague-Dawley rats weighing 300–350 g at the initiation of kindling were used. Male Wistar rats of the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) strain were used at a body weight of 280–400 g. Male Sprague-Dawley (200–240 g) rats were used for the rotarod tests. Animals were obtained from Charles River Laboratories, France, and housed in a holding room under a 12-h light-dark cycle with lights on at 06:00. Temperature was maintained at 20–24°C, relative humidity at 40–70% and the rate of air replacement was at least 15 times an hour. Animals had ad libitum access to standard dry pellet food and tap water.

For the intrahippocampal kainate model, male C57BL/6 mice (11 weeks of age) were obtained from Janvier (France) and housed in cages on wood litter for 8 days with free access to food and water until surgery. Animal housing was maintained under artificial lighting from 8:00 to 20:00.

Drugs and chemicals
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), and BRV (2S-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide) were synthesized at UCB (Braine-l’Alleud, Belgium). All other reagents were of analytical grade and obtained from conventional
commercial sources. PSL was dissolved in 10 mM citrate buffer, 1.5% methylcellulose, 0.1% Tween 80, 0.1% silicone antifoam, and LEV and BRV in saline.

Experimental procedures

Murine models of acutely-induced seizures

Methods for the audiogenic, electrically- and chemically-induced seizure models have been previously described in detail (Jackson et al., 1996; Klitgaard et al., 1998; Kaminski et al., 2008; Kaminski et al., 2011; Leclercq and Kaminski, 2015).

For the audiogenic seizures model, mice were placed, one at a time, in a sound attenuated chamber, where audiogenic seizures were induced through application of an acoustic stimulus (85 dB, 10–20 kHz, 30 s). The proportion of mice protected against clonic seizures was used to determine antiseizure activity. This endpoint was chosen because a correlation between SV2A affinity and efficacy against clonic seizures has been previously demonstrated (Kaminski et al., 2008).

For electrically-induced seizures, the MES and 6 Hz models were used. In MES, 50 mA currents were delivered at a constant pulse frequency of 50 Hz and a duration of 0.2 s. The proportion of mice protected against tonic hindlimb extension after stimulation was used to determine antiseizure activity, as well as dose-response. In the 6 Hz model, 44 mA currents were delivered with 0.2 ms monopolar pulses at 6 Hz for a duration of 3 s. After stimulation, mice were observed for 30 s and the duration of immobility (stunned posture) was noted. The proportion showing immobility for <7 s was used as the endpoint for seizure protection, as previously described (Leclercq and Kaminski, 2015).

For the chemically-induced seizure models, pentylenetetrazol (PTZ; 89 mg/kg) and bicuculline (3 mg/kg) were administered subcutaneously (sc), and pilocarpine (373 mg/kg) administered intraperitoneally (ip). In the latter model, the peripheral cholinergic effect was blocked via administration of methylscopolamine (1 mg/kg, ip) 30 min before administration of pilocarpine. The proportion of mice protected against clonic seizures in all four extremities during a 60-min observation period after drug administration was used to determine antiseizure activity. 11-
deoxycortisol (1.0–1.2 mmol/kg) was infused through the lateral tail vein, and protection against generalized seizures during the 60-min observation period after infusion was used to assess antiseizure activity.

In all experiments, PSL was tested at doses ranging from 0.014 to 181.4 mg/kg. It was administered (10 ml/kg) 30 min before testing, except for audiogenic seizure testing where pre-administration time was 15 min. Testing was initiated in the audiogenic model, before having conducted thorough pharmacokinetics assessment; pre-administration time was subsequently adapted for screening in other models. Each experiment consisted of independent groups of 10–14 mice, with one group receiving vehicle (control) and the others different PSL doses. The experimenter was unaware of the nature of the compound administered.

**Comparative 6 Hz study**

The 6 Hz model was used to compare the protective effect of PSL with that of LEV, BRV and diazepam (DZP), as well as the combination of LEV or BRV with DZP. To allow for a direct, objective comparison, drugs were administered at doses to provide similar in vivo target occupancy. PSL was administered at a dose of 0.17 mg/kg, which is expected to provide 2% and 35% occupancy at the BZD site and SV2A, respectively, based on results of in vivo occupancy studies (Wood et al., 2019). Correspondingly, LEV and BRV were tested at 1.83 and 0.42 mg/kg, respectively, to provide 35% SV2A occupancy, and DZP at 0.017 mg/kg to provide 2% occupancy at the BZD site. All drugs were administered ip 30 min before testing except for LEV, which was administered 60 min before testing. Each experimental arm consisted of 15 or 16 mice.

**Amygdala kindling**

Protocols used for both mouse and rat amygdala kindling experiments have been described previously (Lösch et al., 1986). For the rat model, experiments consisted of five groups of eight fully kindled rats, each group receiving different doses of PSL (0.14–13.9 mg/kg) administered ip (5 ml/kg) 30 min before stimulation with the same supra-maximal current (500 μA, 1 s) used for the induction of kindling. Similarly, six groups of 8–9 mice received different doses of PSL (0.014–13.85 mg/kg) administered ip 30 min before testing with the same supra-maximal stimulation.
current (250 μA, 1 s) used for the induction of kindling. Additionally, similar experiments were conducted in groups of mice receiving BRV, carbamazepine (CBZ), DZP, LEV, lamotrigine (LTG), phenytoin (PHT), topiramate (TPM), retigabine (RTG) or valproate (VPA).

The effects of drugs on three parameters were tested in fully kindled animals. First, as a measure of the drug’s effect on seizure severity, the behavioral effects of the stimulation were scored according to the scale described by Racine, where 0=no reaction, 1= blinking and/or mild facial twitches and chewing, 2=head nodding and/or severe facial clonus, 3=myoclonic jerks of the forelimbs, 4=clonic seizures of the forelimbs with rearing and 5=generalized clonic seizures associated with loss of balance (Racine, 1972). Second, the proportion of animals protected against generalized seizures (scores 3–5) was used to determine the drugs’ ED50 and antiseizure activity. Third, the electroencephalographic effect of the stimulation was determined by measuring the stimulation-induced afterdischarge duration (ADD), defined as an EEG activity with an amplitude at least twice that of the pre-stimulus recording and a frequency >1 Hz.

**Murine intrahippocampal kainate mouse model of mesial temporal lobe epilepsy**

Experiments were performed as previously described (Riban et al., 2002; Duveau et al., 2016). Briefly, male C57BL/6 mice (n=20) were surgically injected with kainate (1 nmol) in the right dorsal hippocampus. Bipolar electroencephalography (EEG) electrodes were implanted into the injected hippocampus, with additional monopolar surface electrodes placed over the frontoparietal cortex and cerebellum. After a 5-week period of epileptogenesis, mice (n=9) displaying hippocampal paroxysmal discharges (HPDs; ≥20/hour) without any generalized seizures were selected. Baseline EEG (20 minutes) was recorded before injection of vehicle (10 mM citrate buffer, 1.5% methylcellulose, 0.1% Tween 80, 0.1% silicone antifoam) or PSL (1, 3, 10, or 30 mg/kg; ip) and recording continued for an additional 90 minutes. Stress caused by handling and drug administration cause a transient decrease in the number of HPDs, as observed reproducibly in vehicle-treated animals. Therefore, the number and duration of HPDs were measured and analyzed for 80 min, after discarding the first 10 min post-drug administration. PSL doses were administered in a randomized crossover manner.
Spike-wave discharges in Genetic Absence Epilepsy Rat from Strasbourg (GAERS)

Four platinum electrodes were implanted bilaterally in the frontal and occipital cortices as described previously (Matagne et al., 2009). After a 2-week recovery period, rats were injected with either vehicle or PSL; EEG was recorded continuously over consecutive 20-min intervals starting 20 mins before, and up to 120 min after drug administration. The cumulative duration of spontaneous spike- and-wave discharges (SWDs) in each 20-min interval was measured by a semi-automatic program. PSL was administered at doses equal to 0.14 mg/kg, 0.43 mg/kg, 1.38 mg/kg and 4.33 mg/kg in a dose volume of 5 ml/kg body weight. Control group received vehicle injection (ip, 5 ml/kg body weight). Eight rats were used in these experiments with a cross-over design in which each animal served as its own control after injection of vehicle.

Tolerance

To determine whether mice developed tolerance to PSL’s antiseizure effects, its impact on the PTZ-induced clonic seizure threshold was tested. For comparison, the tolerance potential of diazepam, a full agonist at the BDZ site, was also evaluated. This test is widely described as a nonclinical tool for assessment of tolerance-like effects of AEDs (Rundfeldt et al., 1995). Briefly, the test consists of two steps; in the first, the PTZ threshold dose for inducing seizures and the ED$_{97}$ of a given AED in providing protection against these PTZ-induced clonic seizures are determined. In the subsequent step, tolerance to the protective effect of the AED after repeated administration is determined.

For the first step, an iv infusion of PTZ (5 mg/ml) was administered into the tail vein of freely moving mice and the time to the three stages of seizures (twitch, clonic and tonic) was noted. Padsevonil, DZP or vehicle was administered ip (10 ml/kg) 30 minutes before PTZ infusion to determine the dose that increased the PTZ threshold dose by 97% (ED$_{97}$). Different treatments were randomly distributed within each group of mice (6, 8 or 10 mice per group for PSL, and 6, 10 or 11 mice per group for DZP experiments) with injections at 5 min intervals. In the second step, mice were administered with the previously selected PSL/DZP dose (ED$_{97}$) or vehicle, twice daily for 4 consecutive days (n=12 each group). On day 5, they were treated with PSL/DZP or vehicle 30 min before assessment of their respective seizure threshold, following iv infusion of PTZ. There were four experimental groups, as described in Table 1.
Rotarod

The impact of PSL on motor activity was evaluated using the rotarod test in both mice and rats using previously described protocols (Klitgaard et al., 1998). Animals were trained and only those able to remain on the rod for at least 60 s in three consecutive trials were used in the tests. In mice, PSL was administered ip (10 ml/kg) 30 min before testing; one group (control) received vehicle and the others PSL doses 4.3–77.9 mg/kg (n=10 each group). In rats, PSL was administered ip (5 ml/kg) 30 min before testing; one group (control) received the vehicle and the others PSL doses 4.3–43.3 mg/kg (n=8 each group). The median tolerated dose, at which toxicity, or impairment of motor coordination occurs in 50% of animals (TD50) was calculated and used for determining the therapeutic index (TI) of PSL. The TI is defined as ratio between doses producing motor impairment (TD50) and doses providing protection against seizures (ED50). To compare the TI of PSL with that of other AEDs in amygdala kindling model, the TD50 of the following drugs was also determined in naïve mice: BRV, CBZ, DZP, LEV, LTG, PHT, TPM, RTG and VPA.

Data analysis

Unless otherwise noted, ED50 and its associated 95% confidence intervals (CI) were calculated using a non-linear fitting of the dose-response curve with GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA). In the 6 Hz comparative study, Fisher’s exact test was used for statistical comparisons of the number of animals protected with PSL and with the combinations of LEV or BRV with DZP using GraphPad Prism (as above).

Amygdala kindling

Significant differences between compound and vehicle in the median behavioral seizure score, in protection against generalized seizures, and in the ADD were evaluated with Wilcoxon signed rank test, Fisher’s exact test and Mann-Whitney U-test, respectively. All statistical analyses were performed with GraphPad Prism (as above).

Intrahippocampal kainate model

Statistical analyses were performed with GraphPad Prism version 7 using two-way analysis of variance (ANOVA) for repeated measures, with the factors time and compound dose (with
repeated measures applying only on the time factor), followed by Bonferroni’s multiple comparison test.

*Spike-wave discharges in GAERS*
For each treatment, the mean cumulative duration of SWDs (± SEM) was calculated for each 20-min interval. Results for each 20-min interval were compared with those of vehicle treatment using a two-way analysis of variance (ANOVA) with repeated measures, followed by a post hoc Bonferroni multiple comparison test (p<0.05), using GraphPad Prism. Due to the high variability of the responses observed in each 20-min interval for different rats, data were further analyzed using the cumulative duration of SWDs covering the total post-drug observation period (120 min). This allowed application of non-linear regression curve fitting of the results and estimation of the protective ED$_{90}$.

*Tolerance*
The effective dose increasing the PTZ threshold by 97% (ED$_{97}$) was calculated using a non-linear fitting of individual values of the dose-response curve (SAS/STAT$^R$ Software version 9.1). A one-way ANOVA followed by a Tukey multiple comparison test were performed with individual calculated doses of PTZ inducing clonic seizures in the four groups of mice; statistically significant differences between (chronic vehicle + test compound ED$_{97}$ dose) and (chronic test compound ED$_{97}$ dose + test compound ED$_{97}$ dose) were used for assessing development of tolerance.
RESULTS

Murine models of acutely-induced seizures

Administration of PSL provided potent, dose-dependent protection against seizures induced by 6 Hz stimulation, an acoustic stimulus and a bolus dose of pilocarpine (ED$_{50}$ 0.16, 0.17 and 0.19 mg/kg, respectively). The potency of PSL in these three models was greater than that of LEV and BRV (ineffective in the pilocarpine model) (Table 2). PSL also provided dose-dependent protection against clonic seizures induced by a bolus dose of PTZ. Its potency in this model was higher than that of BRV, while LEV was ineffective. In the 11-deoxycortisol model, PSL provided dose-dependent and almost complete protection against seizures; at the highest dose tested (43.3 mg/kg), 90% of animals were protected. Brivaracetam was ineffective in this model, while LEV provided only limited protection at the highest doses tested. PSL showed low potency against seizures induced by a bolus of bicuculline, while LEV and BRV were ineffective in this model. The lowest potency was seen in the MES model (ED$_{50}$ 92.8 mg/kg). The lack of activity or low potency in this model was also observed with LEV and BRV.

Comparative 6 Hz study

The protective effect of PSL in the 6 Hz model was compared with that of LEV, BRV and DZP alone, and with the combinations of LEV or BRV with DZP at doses expected to provide similar occupancy at SV2A (35%) or the BZD site (2%). PSL protected a greater proportion of mice than LEV, BRV and DZP alone or in combination (Figure 1). The difference in the protection offered by PSL and that of the LEV/DZP and BRV/DZP combinations was statistically significant (p=0.021 and p=0.0008, respectively, Fisher’s exact test). The difference in the protection provided by BRV and the BRV/DZP combination or the LEV and LEV/DZP combination was not significant (p=0.4 and p=0.145, respectively).

Amygdala kindling

The protective effect of PSL against seizures was evaluated in fully kindled animals using three parameters. In rats, PSL provided dose-dependent and complete protection against focal to bilateral seizures (secondary generalized seizures). The reduction in the proportion of rats displaying generalized seizures at doses of 2.4, 4.3 and 13.9 mg/kg was statistically significant,
with 100% of animals protected at the highest dose (Figure 2, right panel). The ED$_{50}$ was estimated to be 2.43 (2.41–2.46) mg/kg. Significant, dose-dependent reductions in the median seizure severity score and ADD were also observed with PSL, starting from a dose of 2.4 mg/kg.

In mice, just as in rats, PSL significantly reduced the proportion of animals with focal to bilateral seizures and the median seizure severity score starting from a dose of 1.4 mg/kg (Figure 2, left panel). Based on the proportion of mice protected from focal to bilateral seizures (secondary generalized seizures), the ED$_{50}$ was estimated to be 1.2 (0.43–3.40) mg/kg. PSL also reduced the ADD, but only at the highest dose tested (13.9 mg/kg); at lower doses an increase was observed, with the increase (40%) at the 1.38 mg/kg dose being statistically significant.

The TI of PSL in kindled mice was 9.8, which was relatively high compared with that of BRV and VPA, 2.8 and 1.2, respectively (Table 3). Other AEDs tested in this model displayed only partial protection against generalized seizures; therefore, it was not possible to calculate their TI.

**Intrahippocampal kainate model**
PSL administration (1, 3, 10, or 30 mg/kg) resulted in dose-dependent and statistically significant reductions in the number of HPDs compared with vehicle or baseline, between 30 and 70 minutes after administration. PSL 10 and 30 mg/kg doses were associated with significant reductions in the number of HPDs from 10 to 30 minutes after administration (Figure 3, top panel). Dose-dependent effects of PSL were also observed when the cumulated duration of HPDs was calculated, with all PSL doses associated with significant reductions compared with vehicle 50–70 minutes after administration. Maximal effects were observed with 10 and 30 mg/kg doses after 10–30 minutes (Figure 3, bottom panel).

**Spike-wave discharges in GAERS**
PSL (0.14–4.33 mg/kg) produced a dose-related suppression in spontaneous SWDs, which was statistically significant from the 0.43 mg/kg dose – the suppression was almost complete at a dose of 4.33 mg/kg (Figure 4). The effect was apparent in the first 20-min test interval and persisted throughout the recording period (up to 120 min). Treatment with PSL also resulted in a
dose-dependent reduction in the cumulative duration of spontaneous SWDs recorded over the 120 min post-drug period (ED$_{50}$ 0.87 mg/kg).

**Tolerance**

Having established the PTZ threshold dose for inducing clonic seizures, PSL and DZP were tested. Both drugs increased the seizure threshold in a dose-dependent manner; the ED$_{97}$ of PSL was 15.9 mg/kg and that of DZP 2.1 mg/kg.

Animals that were treated twice daily for 4 days with vehicle, PSL or DZP at the calculated ED$_{97}$ dose, were injected again on day 5 with the same dose before assessment of the seizure threshold following iv PTZ infusion. Treatment with PSL (15.9 mg/kg) caused a significant increase in the PTZ threshold dose with a similar magnitude in both groups (mice chronically treated with vehicle or drug). The difference in the mean doses of PTZ that induced seizures in mice treated chronically with vehicle and those treated chronically with PSL was not statistically significant (Figure 5).

Diazepam (2.1 mg/kg) also caused a significant increase in the PTZ threshold dose for clonic seizures in both groups, but with a much lower magnitude in mice chronically treated with DZP, reflecting development of tolerance to its antiseizure effects. The mean dose of PTZ inducing clonic seizures in mice treated chronically with the vehicle was comparable to the mean dose calculated in mice treated chronically with DZP (Figure 5).

**Rotarod**

Administration of PSL resulted in a dose-dependent impairment in the performance of both mice and rats in the rotarod test; TD$_{50}$ values were 11.8 (9.2–15.2) mg/kg and 24.4 (15.0–39.7) mg/kg, respectively. Using these, and ED$_{50}$ values determined in various models, the TI of PSL was calculated. PSL had a TI of 28 in the GAERS and 10 in the rat amygdala kindling models. In mice, the TI was calculated to be 69 in the audiogenic, 62 in the pilocarpine-induced, 74 in the 6 Hz-induced and 2.5 in the PTZ-induced seizure tests. As noted above, the TI in the murine amygdala kindling model was 9.8.
DISCUSSION

PSL is the first in a novel class of drugs that bind to SV2 proteins and the BZD site on GABA\(_A\)Rs. As shown in studies reported here, this pre- and postsynaptic activity results in a distinct pharmacological profile across a wide range of seizure and epilepsy models representing focal and generalized epilepsy in humans.

The MES and PTZ tests, considered gold standards for early detection of antiseizure activity, are used for screening candidate compounds (Bialer and White, 2010; Klitgaard, 2005). LEV is inactive in both models, while BRV, a more potent and selective SV2A ligand than LEV shows weak activity in both models (Matagne et al., 2008). Similarly, PSL showed activity in both models, but its potency, while greater than that of BRV, was also relatively weak. PSL’s effect was greater in the PTZ than in the MES test, which is likely to be mediated partially via the BZD site, since BZDs show high potency in this model (Löscher et al., 2011). PSL also showed relatively low potency in the bicuculline test, where typical BZDs are active, but not abecarnil, a partial agonist at the BZD site (Turski et al., 1990); consequently, low activity was expected, since both LEV or BRV are inactive in this test, and PSL shows a partial agonist profile.

PSL provided potent, dose-dependent protection against seizures induced in sound-sensitive mice, a genetic model of generalized epilepsy. BRV is active in this model, while LEV shows lower potency, correlating with their SV2A binding affinity (Matagne et al., 2008; Kaminski et al., 2008). PSL also provided strong protection against pilocarpine-induced clonic seizures, where in contrast to the audiogenic model, BRV is ineffective, while LEV shows relatively high potency. Among acute models, PSL displayed the highest potency in the 6 Hz model (ED\(_{50}\) 0.16 mg/kg), used as a test for protection against drug-resistant focal seizures, since many older (eg, CBZ, phenobarbital, PHT) and newer AEDs (eg, felbamate, LTG, tiagabine, TPM) fail to fully protect animals (Barton et al., 2001). This model was also used to compare the efficacy of PSL against LEV, BRV, DZP and LEV/DZP and BRV/DZP combinations. Importantly, for this comparison, doses calculated to provide similar SV2A and BZD site occupancy were used for the SV2 ligands and DZP, 35% and 2%, respectively. Given that LEV and BRV require 80% SV2A for antiseizure activity in nonclinical models (Gillard et al., 2011), low level occupancy was selected...
in these experiments to further differentiate PSL activity. Protection offered by PSL, even at 35% SV2A occupancy was almost 70% and significantly greater than that provided by either LEV or BRV in combination with DZP. These observations suggest that PSL’s antiseizure properties are due to a differentiated mode of action that provides greater protection than co-administration of an SV2A ligand and a BZD. Furthermore, the interaction of PSL with SV2B and SV2C may also contribute to enhanced antiseizure effects.

The 11-deoxycortisol model is also considered to represent drug-resistant seizures (Kaminski et al., 2011). LEV offers only partial protection at the highest doses, while phenytoin, carbamazepine and valproate are ineffective; BRV has also proven to be ineffective. Padsevonil, however, demonstrated robust efficacy, providing dose-dependent protection with an ED50 of 10 mg/kg. 11-deoxycortisol induces paroxysmal epileptiform network activity and seizures by significantly reducing GABAergic neurotransmission, which may explain why many AEDs, but not PSL, fail to suppress seizures (Kaminski et al., 2011).

The intrahippocampal kainate model displays many features of human MTLE (Ribau et al., 2002, Pernot et al., 2011). Unilateral injection of kainate in the dorsal hippocampus results in neuronal loss, mossy fiber sprouting, and dispersion of granule cells, followed by spontaneous and recurrent HPDs observed on EEG (Suzuki et al., 1995; Mitsuya et al., 2009). Focal seizures remain frequent and stable during the animal’s life, and importantly, resistant to most AEDs (Ribau et al., 2002, Duveau et al., 2016), as in human MTLE (Engel et al., 1997). PSL displayed dose-dependent protective effects, with almost complete and long-lasting inhibition of HPDs at the highest dose (30 mg/kg).

The GAERS model is considered predictive of human absence epilepsy (Danobe et al., 1998; van Luijjielaar et al., 2002). LEV has a weak effect in this model, while BRV suppresses spontaneous SWDs with complete inhibition at the highest dose (67.9 mg/kg), which again correlates with their affinity for SV2A (Matagne et al., 2008; Kaminski et al., 2008). PSL showed higher potency than BRV and markedly suppressed spontaneous SWDs with almost complete inhibition at the highest dose (4.33 mg/kg), providing further evidence for PSL’s broad spectrum of activity against both focal and generalized seizures.
AED activity in the amygdala kindling model is predictive of efficacy against focal to bilateral tonic-clonic seizures in the clinical setting (Löscher and Schmidt, 1988). Electrographic and behavioral symptoms of seizures are initially localized at the site of stimulation, but rapidly evolve to bilateral activity, with seizures increasing in length and severity upon repeated stimulation (Löscher et al., 2011, White et al., 2003). In the rat model, PSL significantly reduced the proportion of animals displaying seizures, with 100% of animals protected at the highest dose. PSL also reduced the seizure severity score and the ADD, indicating effects on both local seizure discharge and seizure spread, or evolution to bilateral seizures. PSL was substantially more potent than LEV and BRV; while BRV significantly reduces the ADD at only high doses, LEV has no effect (Klitgaard et al., 2016). PSL’s effects in the mouse kindling model mirrored those in the rat model, with one exception; it reduced the ADD only at the highest dose. The reduction in ADD at the 13.9 mg/kg dose and the increase at the 1.38 mg/kg dose were both statistically significant, somewhat similar to the effects of low BRV doses (Matagne et al., 2008).

In the mouse model, PSL was the most potent compared with nine other mechanistically different AEDs. It was only possible to determine the ED50 of BRV and VPA since the others failed to provide full protection at high doses. Results were also used to compare the TI of AEDs, a measure of the margin between antiseizure and adverse effects, expressed by the ratio between doses producing adverse effects and seizure protection (TD50/ED50); the greater the TI, the greater the separation between toxic and therapeutic doses. In mice, PSL TD50 was 12 mg/kg and the ED50 1.2 mg/kg, resulting in a TI of 10. In comparison, the TI of BRV and VPA were 3 and 1, respectively. Since the protective ED50 of the remaining AEDs could not be determined, due to limited efficacy, their TI could not be calculated. Overall, these findings indicate that PSL has full efficacy in the kindling model, displaying a high TI, potentially translating into higher efficacy and improved tolerability in humans.

PSL was designed to exert its therapeutic activity via two distinct mechanisms: as a SV2 ligand, and as a partial agonist at the BZD site of the GABA\(_A\)R. Partial agonism was selected based on evidence suggesting that the likelihood of developing tolerance to therapeutic effects is lower compared with full agonists (Miller et al., 1990; Serra et al., 1994; Rundfeldt et al., 2014). Clinical evidence supports these observations. Clobazam, a BZD and partial agonist, has been used
successfully for the treatment of patients with Lennox-Gastaut syndrome (Faulkner, 2015; Gauthier and Mattson, 2015). Results of a long-term trial demonstrated sustained seizure control at stable dosages over a 3-year period (Conry et al., 2014; Gidal et al., 2016). Another partial agonist, abecarnil, has shown efficacy in the treatment of patients with photosensitive epilepsy without development of tolerance (Kasteleijn-Nolst Trenité et al., 2016). To evaluate PSL’s tolerance potential, the PTZ-induced clonic seizure threshold test was used, where the ability of AEDs to increase the seizure threshold is assessed after acute, and twice daily PTZ infusion for 4 days at the ED$_{97}$ dose (Rundfeldt et al., 1995). Under both regimens, PSL increased the threshold for PTZ-induced seizures to the same extent, indicating that tolerance was not developed; in contrast, DZP showed significant loss in its ability to increase the threshold.

The precise role of SV2A in synaptic transmission, and how ligand binding translates into antiseizure activity remain to be fully elucidated, yet the strength of ligands’ antiseizure activity correlates with their binding affinity – BRV’s greater affinity for SV2A over that of LEV translated into superior antiseizure activity in animal models (Matagne et al., 2008; Kaminski et al., 2008). In turn, PSL’s affinity for SV2A has been shown to be greater than that of BRV (Wood et al., 2019). PSL’s additional actions on SV2B and SV2C, and the GABA$_{A}$R BZD site, have resulted in a nonclinical profile that differs substantially from that of other AEDs. Additional evidence from the present studies suggest that the pre- and postsynaptic mechanism of action confers enhanced antiseizure properties beyond the combination of compounds targeting SV2A and the BZD site. PSL’s highly differentiated antiseizure profile suggests a robust therapeutic benefit, an observation supported by results of a Phase IIb proof-of-concept trial (Muglia et al., 2017).
CONFLICTS OF INTEREST
All authors are current or former employees of UCB Pharma.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1
Protective effect of padsevonil (0.17 mg/kg) in the 6 Hz model compared with that of levetiracetam (1.83 mg/kg), brivaracetam (0.42 mg/kg), and diazepam (0.017 mg/kg), as well as the combination of diazepam with levetiracetam or brivaracetam. The drugs were administered at doses associated with similar in vivo SV2A (35%) and benzodiazepine site (2%) occupancies (comparisons were made using Fisher's exact test).

Figure 2
Effect of padsevonil on seizure parameters recorded after supra-threshold stimulation in fully kindled rats (right panel) and mice (left panel). Control recordings were performed 48 h before testing with padsevonil. Values are mean ± standard error of mean for afterdischarge duration. Comparisons between drug and control in protection against generalized seizures, seizure severity score and afterdischarge duration were evaluated with Wilcoxon signed rank test, Fisher's exact test and Mann-Whitney U-test, respectively, with *indicating statistically significant differences (p<0.05).

Figure 3
Padsevonil (PSL) activity in the murine intrahippocampal kainate model – effect on the mean number (top panel) and the mean cumulated duration of hippocampal paroxysmal discharges (HPDs; bottom panel). Values are mean ± standard error of the mean (n=9); comparisons are *p<0.05, **p<0.01, ***p<0.001, **** p<0.0001 vs baseline (Bonferroni's multiple comparison test).

Figure 4
Padsevonil (PSL) activity in the GAERS model – effect on the duration of spontaneous spike-and-wave discharges. Values are mean ± standard error of the mean (n=8 per group), with * indicating statistically significant difference with respective time point in vehicle-treated group (p<0.05 Bonferroni's multiple comparison test).
Figure 5
Effect of chronic (4 days) treatment with padsevonil (PSL; 15.9 mg/kg) or diazepam (DZP; 2.1 mg/kg) on the pentylentetrazol (PTZ)-induced seizure threshold. On the fifth day, PSL increased the threshold to the same extent in animals that had been treated chronically with vehicle or PSL. In contrast, there was a significant decrease in the ability of DZP to increase the threshold in animals that had been treated chronically with DZP, indicating development of tolerance. Development of tolerance assessed based on statistically significant differences between (chronic vehicle + test compound ED$_{97}$ dose) and (chronic test compound ED$_{97}$ dose + test compound ED$_{97}$ dose) using a one-way ANOVA followed by a Tukey multiple comparison test.
**Table 1**

Experimental groups in the pentylenetetrazol-induced clonic seizure threshold test.

|                  | Days 1 – 4               | Day 5                                      |
|------------------|--------------------------|--------------------------------------------|
| **Group 1**      | Vehicle twice daily      | Vehicle administered 30 mins before iv PTZ |
| **Group 2**      | Vehicle twice daily      | PSL/DZP at ED$_{97}$ administered 30 mins before iv PTZ |
| **Group 3**      | PSL/DZP twice daily at ED$_{97}$ | Vehicle administered 30 mins before iv PTZ |
| **Group 4**      | PSL/DZP twice daily at ED$_{97}$ | PSL/DZP at ED$_{97}$ administered 30 mins before iv PTZ |

DZP=diazepam; iv=intravenous; PSL= padsevonil; PTZ=pentylenetetrazol
Table 2
Potency of padsevonil and selective SV2A ligands in murine models of acutely-induced seizures.

| Model                | Padsevonil ED50 (mg/kg) | Brivaracetam ED50 (mg/kg) | Levetiracetam ED50 (mg/kg) |
|----------------------|-------------------------|---------------------------|---------------------------|
| 6 Hz                 | 0.16 (0.1–0.2)          | 4.4^a                     | 19.2^a                    |
| Audiogenic           | 0.17 (0.1–0.2)          | 2.4^a                     | 30^a                      |
| Pilocarpine          | 0.19 (0.1–0)            | NE^a                      | 7.1^b                     |
| Pentylenetetrazol    | 4.8 (1.7–10.8)          | 30^a                      | NE^a                      |
| 11-deoxycortisol     | 9.9 (4.6–21.0)          | NE^c                      | 540^c                     |
| Bicuculline          | 27.3 (17.2–43.1)        | NE^b                      | NE^b                      |
| Maximal electroshock | 92.8 (74.3–115.9)       | 113^a                     | NE^a                      |

NE=not effective
All drugs were administered intraperitoneally 15 mins before testing in the audiogenic seizures model, 30 mins in others
*Minimally active dose, defined as the lowest dose providing statistically significant protection against seizures
^a Klitgaard H, et al (2016). Epilepsia 57:538–48
^b Klitgaard H, et al (1998). Eur J Pharmacol 353:191–206
^c Kaminski RM, et al (2011). Neuropharmacol 60:1098–1108.
Table 3
Comparison of the therapeutic index of padsevonil and nine other antiepileptic drugs with different mechanisms of action determined in the mouse amygdala kindling model and rotarod test. Other than padsevonil, brivaracetam and valproate, the ED50, and therefore, the therapeutic index of the other antiepileptic drugs could not be calculated.

|                | Amygdala kindling ED50 (mg/kg) | MAD (mg/kg) | MTD (mg/kg) | Rotarod test TD50 (mg/kg) | Therapeutic index |
|----------------|--------------------------------|-------------|-------------|--------------------------|------------------|
| Padsevonil     | 1.2 (0.4–3.4)                  | 1.4 | 60          | 13.9 | 100                  | 12 (9–15)        | 9.8 |
| Brivaracetam   | 68 (39–118)                    | 134 | 90          | 212 | 91                   | 195 (133–245)    | 2.8 |
| Levetiracetam  | –                              | 540 | 60          | 540 | 60                   | 1389 (962–2041)  | –   |
| Valproate      | 239 (169–338)                  | 250 | 56          | 400 | 89                   | 298 (201–418)    | 1.2 |
| Phenytoin      | –                              | >70 | 0           | 70 | 0                    | 129 (76–194)     | –   |
| Carbamazepine  | –                              | 56 | 89          | 56 | 89                   | 36 (27–48)       | –   |
| Lamotrigine    | –                              | >56 | 33          | 56 | 33                   | 20 (13–27)       | –   |
| Diazepam       | –                              | 3 | 89          | 3 | 89                   | 3 (2–4)          | –   |
| Topiramate     | –                              | >300 | 25        | 300 | 25                  | 249 (150–357)    | –   |
| Retigabine     | –                              | 15 | 100         | 15 | 100                  | 12 (8–18)        | –   |

*Protection against focal to bilateral tonic-clonic seizures
MAD=minimally active dose, defined as the lowest dose providing statistically significant protection against focal to bilateral tonic-clonic seizures
MTD=maximal tested dose, defined as the highest dose tested in the amygdala kindling model
Figure 1

The figure shows the proportion of mice protected by different treatments. The treatments include Brivaracetam, Diazepam, Levetiracetam, and Padsevonil. The treatments are compared with and without Diazepam. The graph indicates that Padsevonil has a significantly higher proportion of mice protected compared to the other treatments, with a p-value of <0.05.
Figure 2

The figure shows three main panels, each depicting the relationship between dose (in mg/kg) and either the proportion of protected animals, median seizure severity score, or afterdischarge duration (in s) before and after drug administration. The panels are labeled as follows:

- **Proportion of protected animals (%)**
  - Bars represent different doses: 0.014, 0.14, 1.4, 4.3, and 13.9 mg/kg.
  - Post-drug bars are shaded blue, and pre-drug bars are shaded turquoise.
  - Asterisks indicate significant differences.

- **Median seizure severity score**
  - Similar to the proportion of protected animals, with bars for doses 0.014, 0.14, 1.4, 4.3, and 13.9 mg/kg.
  - Post-drug bars are blue, and pre-drug bars are turquoise.
  - Asterisks indicate significant differences.

- **Afterdischarge duration (s)**
  - Bars represent doses 0.014, 0.14, 1.4, 4.3, and 13.9 mg/kg.
  - Post-drug bars are blue, and pre-drug bars are turquoise.
  - Asterisks indicate significant differences.
Figure 4

The diagram shows the duration of spike-and-wave discharges (s) over time (minutes) for different treatment conditions. The x-axis represents time in minutes, while the y-axis represents the duration of spike-and-wave discharges. Lines with error bars indicate the variability across different treatment conditions, including Vehicle, PSL 0.14 mg/kg, PSL 0.43 mg/kg, PSL 1.38 mg/kg, and PSL 4.33 mg/kg. Statistically significant differences are indicated by asterisks.
Figure 5