Hybrid compounds based on the pyrrolidine-2,5-dione scaffold as candidates for new wide spectrum anticonvulsant agents

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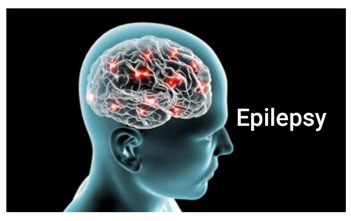


Affects 1% of world population
About one-third of the patients with epilepsy develop resistance to antiepileptic drugs (AEDs)
The prevalence increases with the age

Introduction

In recent years, a considerable interest has been generated in designing new multi-target compounds as they have been proven to be advantageous in the treatment of multifactorial diseases (characterized by complex pathomechanism) and also have been proven to alleviate health conditions linked to drug resistance.[1,2] **Epilepsy, which is recognized as the most common and debilitating neurological disorder, without any doubt, fulfills both the aforementioned criteria**. Keeping the requirements of multi-target drugs in mind and **with the aim of obtaining new highly effective broad-spectrum anticonvulsants**, in the previous studies, we developed integrated hybrid molecules derived from the pyrrolidine-2,5-dione ring.[3-6]

These compounds were designed by applying the fragment-based approach; therefore, they merge on the common structural framework the chemical fragments of three chemically and pharmacologically diversified AEDs such as **ethosuximide** (ETX, effective especially in pentylenetetrazole-induced seizure model (PTZ)), **levetiracetam** (LEV, effective in 6 Hz model), and **lacosamide** (LCS, active in both maximal electroshock (MES) and 6 Hz seizure models). As a result, the process of hybridization yielded hybrid compounds with potent broad-spectrum anticonvulsant activity joining the pharmacological properties of all aforementioned AEDs. (**Fig. 1**.)

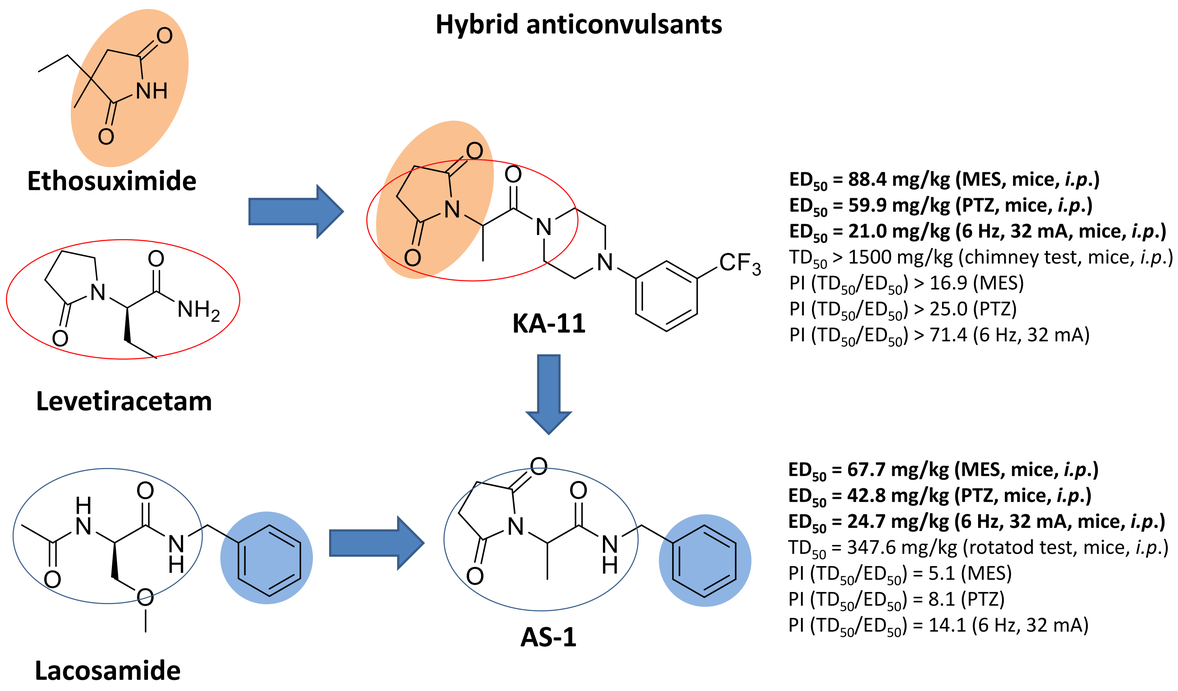


Fig. 1. Structures of selected hybrids.

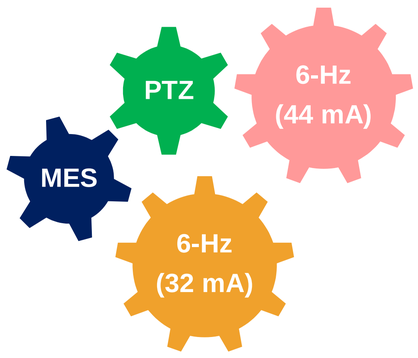
Aim of studies

Considering the anticonvulsant properties of the aforementioned hybrid molecules and **with the aim of obtaining compounds with greater ability to protect against seizures using the MES, 6 Hz (32 mA), and PTZ seizure models, as well as to find substances that are effective in the 6 Hz (44 mA) model of drug-resistant epilepsy**, in this study, we developed a new **AS series** of hybrid compounds with the pyrrolidine-2,5-dione as a core structure. The process of molecular hybridization involved both known AEDs as well as other anticonvulsant active compounds.

Fig. 2. Molecular hybridization.

Anticonvulsant activity

The anticonvulsant activity of hybrid compounds was assessed using the maximal electroshock seizure test (MES), the subcutaneous pentylene-tetrazole seizure test (PTZ), and the 6 Hz seizure model (32 mA and 44 mA), in mice after intraperitoneal injection.



The **AS** hybrids demonstrated broad-spectrum anticonvulsant activity in the preclinical studies (MES, PTZ, 6 Hz 32 mA and 44 mA). The most potent was **AS-34** with following pharmacological parameters (time point of 30 min.).

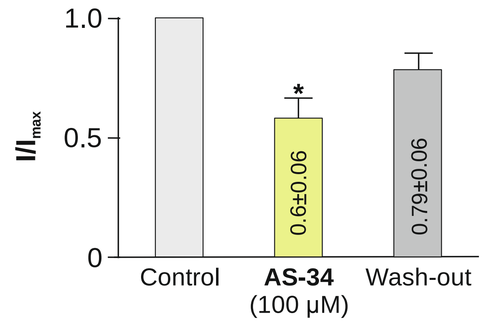
	AS-34	VPA
ED_{50} (MES) (mg/kg)	**40.5**	252.7
ED_{50} (PTZ) (mg/kg)	**50.2**	239.4
ED_{50} (6 Hz, 32 mA) (mg/kg)	**7.6**	130.6
ED_{50} (6 Hz, 44 mA) (mg/kg)	**69.5**	183.1
TD_{50} (rotarod test) (mg/kg)	**> 500**	430.7

AS-34 revealed more potent protection (in each seizure model) and was safer in the rotarod test then broad-spectrum AED - valproic acid (VPA).

Electrophysiological studies

We determined the influence of **AS-34** on fast voltage-gated sodium channels in rat prefrontal cortex pyramidal neurons (at a concentration of 100 µM) using the patch-clamp technique.[7] **AS-34** inhibited significantly maximal amplitude of sodium currents to 0.6±0.06 as compared to control. Importantly, it was possible to obtain partial wash-out (0.79±0.06, n=6), which proves that the effect was reversible. The averaged results are shown in **Fig. 3.**

Fig. 3. Averaged normalized maximal sodium current amplitudes in control, in the presence of AS-34 (*p. < 0.001, ANOVA with Tukey test), and after wash-out. I/Imax (on the vertical axis) means that the currents were normalized to control currents.



In vitro radioligand binding studies

Compound **AS-34** demonstrated broad-spectrum anticonvulsant activity in the preclinical studies, which most likely reflects its multiple sites of action. We performed binding assays for several voltage-gated or ligand-gated channels and GABA-transporter as the most common molecular targets for anticonvulsants. As a result, **AS-34** revealed significant binding toward L-type Ca^{2+} channel (three sites). Furthermore, the L-type calcium ion channel cell-based flux studies revealed that **AS-34** possesses an antagonist activity. Compound **AS-34** did not interact with N-type Ca^{2+} channel, GABA transporter, and notably potassium channel (hERG) at a concentration of 100 µM, thus it has a low risk of cardiac toxicity.

Table 1. *In vitro* binding assays for **AS-34**

binding studies	source	% inhibition of control specific binding[a]
Na^+ channel (site 2)	rat cerebral cortex	38.19
N-type Ca^{2+} (antagonist radioligand)	rat cerebral cortex	1.1
L-type Ca^{2+} (dihydropyridine site, antagonist radioligand)	rat cerebral cortex	**82.3**
L-type Ca^{2+} (diltiazem site, antagonist radioligand)	rat cerebral cortex	**68.5**
L-type Ca^{2+} (verapamil site, antagonist radioligand)	rat cerebral cortex	**57.1**
GABA transporter (antagonist radioligand)	rat cerebral cortex	0.3
GLYT1 (antagonist radioligand)	rat cerebral cortex	23.0
potassium channel (hERG)	human recombinant (HEK-293 cells)	4.54
functional studies		**% inhibition of control agonist response[a]**
L-type Ca^{2+} (antagonist radioligand)	human recombinant (HEK-293 cells)	**85**

[a] Results showing activity higher than 50% are considered to represent significant effects of the test compounds; results showing an inhibition between 25% and 50% are indicative of weak effect; results showing an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level. Binding studies were performed commercially in Cerep Laboratories (Poitiers, France).

References

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