

METHOD FOR TREATMENT OF SEIZURES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Serial No. 63/533,059 filed on August 16, 2023, and of U.S. Provisional Patent Application Serial No. 63/548,345 filed on November 13, 2023. The disclosure of the prior applications are considered part of and are herein incorporated by reference in the disclosure of this application in their entirety.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0002] The present disclosure relates generally to compositions and methods for treating a seizure in a patient.

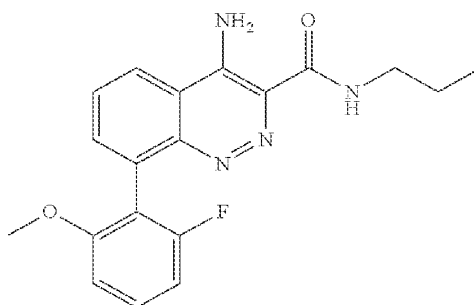
BACKGROUND INFORMATION

[0003] Absence epilepsy is a form of epileptic syndrome where patients show generalized non-convulsive seizures characterized by a brief unresponsiveness to environmental stimuli and cessation of activity. In human, typical absence seizures are associated with bilateral, synchronous and regular spike and wave discharges (SWD). This type of epilepsy presents a specific pharmacology different from the one observed in other types of epilepsies. Therefore, careful evaluation of new antiepileptic drugs in development is needed, as there is a possibility of aggravation of SWD in idiopathic generalized epilepsies and particularly in absence epilepsy.

SUMMARY OF THE INVENTION

[0004] The present invention is based, in part, upon the development of a method of providing anti-seizure effects in a subject by administering a compound BAER-101, which is a selective potentiator of $\alpha 2/3$ -containing GABAA receptors. BAER-101 suppressed seizures in animal models of epilepsy, such as the GAERS animal model of absence seizures, without causing sedation or dizziness.

[0005] In one embodiment, the present invention provides a method of providing anti-seizure effects in a subject including administering to the subject an anti-seizure effective amount of a compound of Formula (I)



Formula (I)

(4-amino-8-(2-fluoro-6-methoxy-phenyl)-N-propyl-cinnoline-3-carboxamide) (also referred to herein as BAER-101 or AZD7325) or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier. In one aspect, the techniques described herein relate to a method, wherein the subject has epilepsy.

[0006] In one aspect, the epilepsy is idiopathic generalized epilepsy, photosensitive epilepsy, or genetic absence epilepsy. In another aspect, the idiopathic generalized epilepsy is childhood absence epilepsy, juvenile absence epilepsy, or Dravet syndrome. In various aspects, the seizures are absence seizures, focal seizures, complex partial seizures, tonic-clonic seizures, grand mal seizures, myoclonic seizures, atonic seizures, audiogenic seizures, or clonic seizures.

[0007] In another aspect, the effective amount is from about 0.1 to 100 mg/kg. In an additional aspect, the effective amount is about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg, about 3.0 mg/kg, about 10 mg/kg, about 30 mg/kg, or about 100 mg/kg.

[0008] In various aspects, the effective amount is administered by oral administration. In one aspect, the compound is administered once, twice, three or four times daily. In another aspect, the compound is administered at least 10 minutes before onset of a seizure episode.

[0009] In one aspect, the method includes administering an additional compound. In an additional aspect, the additional compound is selected from brivaracetam, cannabidiol, carbamazepine, cenobamate, chlordiazepoxide, clobazam, clonazepam, diazepam, eslicarbazepine, ethosuximide, felbamate, fosphenytoin, gabapentin, ganaxolone, lacosamide, lamotrigine, levetiracetam, midazolam, oxcarbazepine, perampanel, phenobarbital, phenytoin, pregabalin, primidone, rufinamide, secobarbital, stiripentol, tiagabine, topiramate, valproate, vigabatrin, or zonisamide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **FIG. 1** is a graph showing a representative EEG tracing showing the well-defined spike-wave discharges that occur spontaneously in the GAERS model during the generalized absence seizure period in these rats.

[0011] **FIG. 2** is a graph showing the number of spike-and-wave discharges (SWDs) per 20 min, in the vehicle condition (top black line), diazepam at 2 mg/kg IP (light grey), and BAER-101 at 0.1 to 100 mg/kg PO (other shades). The arrow indicates the time of compound administration. #, ##, ###, ####: $p < 0.05, 0.01, 0.001$ and 0.0001 as compared to vehicle ($n = 4$ to 12 as per Table 1).

[0012] **FIG. 3** is a bar chart showing the number of SWD over the 80 min after administration, expressed in % of the baseline values, in all conditions. ##, ####: $p < 0.01$ and 0.0001 , as compared to vehicle.

[0013] **FIG. 4** is a graph showing average duration of remaining SWDs per 20 min, in the vehicle condition (top black line), diazepam at 2 mg/kg IP (light grey), and BAER-101 at 0.1 to 3 mg/kg PO (shades of grey). BAER-101 at doses above 3 mg/kg were not considered, as few SWDs durations could be measured. The arrow indicates the administration time. #, ###, ####: $p < 0.05, 0.001$ and 0.0001 as compared to vehicle ($n = 4$ to 12).

[0014] **FIG. 5** is a bar chart showing the average duration of SWDs remaining after administration, over the 80 min after administration, expressed in % of the baseline values. Vehicle, diazepam and BAER-101 doses from 0.1 to 3 mg/kg were considered. Doses of BAER-101 above 3 mg/kg were not considered, as few SWDs could be measured. ###, ####: $p < 0.001$ and 0.0001 , as compared to vehicle.

[0015] **FIG. 6** is a graph showing total time spent in SWDs per 20 min, in the vehicle condition (black), diazepam at 2 mg/kg IP (light grey), and BAER-101 at 0.1 to 100 mg/kg PO (other shades). The arrow indicates the administration. ##, ###, ####: $p < 0.01, 0.001$ and 0.0001 as compared to vehicle ($n = 4$ to 12).

[0016] **FIG. 7** is a bar chart showing the number of SWDs over the 80 min after administration, expressed as a % of the baseline values, in all conditions. ###, ####: $p < 0.001$ and 0.0001 , as compared to vehicle.

[0017] **FIG. 8** is a bar chart showing concentrations of BAER-101 across the six EEG and blood sampling periods of the study.

[0018] **FIG. 9A** is a bar chart showing concentrations of BAER-101 from rat plasma as a function of dose. The bars are means $\pm$ S.D.

[0019] FIG. 9B is a graph showing the relationship of BAER-101 concentration to suppression of SWD in the GAERS model. The points are means.

[0020] FIG. 10 is a timeline for *in vivo* testing of concentrations of BAER-101 and EEG tracings in the GAERS model as described herein (see for example, EXAMPLE 1).

[0021] FIG. 11 is a graph showing the number of spike-and-wave discharges (SWDs) per 20 min, in the vehicle condition (top black line) and diazepam at 2 mg/kg IP (light grey).

[0022] FIG. 12 is a graph showing the number of spike-and-wave discharges (SWDs) per 20 min, in the vehicle condition (top black line), diazepam at 2 mg/kg IP (light grey), and BAER-101 at 3, 10, 30 and 100 mg/kg PO (other shades; lower lines).

DETAILED DESCRIPTION OF THE INVENTION

[0023] The present invention is based on the seminal discovery that the compound AZD7325/BAER-101, which is a selective potentiator of $\alpha 2/3$ -containing GABAA receptors, has potent anti-seizure effects. This discovery is supported by data showing that AZD7325/BAER-101 dose-dependently reduced the number, duration, and total time of spike-wave discharges (SWDs).

[0024] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims.

[0025] The present disclosure provides a method for treatment of seizures in a subject. BAER-101 (formerly AZD7325) is a selective partial potentiator of $\alpha 2/3$ -containing GABAA Receptors (GABAARs) and produces minimal sedation and dizziness. Antiseizure effects in models of Dravet and Fragile X Syndromes have been published. BAER-101 has been administered to over 700 healthy human volunteers and patients where it was found to be safe and well tolerated. To test the extent of the anti-seizure activity of BAER-101, BAER-101 was tested in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model, a widely used and translationally relevant model. GAERS rats with recording electrodes over the frontal and parietal cortices bilaterally were used. Electroencephalography (EEG) signals in freely moving awake rats were analyzed for spike-wave discharges (SWDs). BAER-101 was administered orally at doses of 0.3 to 100 mg/kg and diazepam was used as a positive control using a cross-

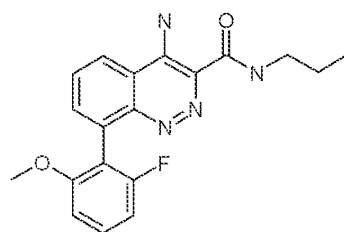
over protocol with a wash-out period between treatments. The number of SWDs was dose-dependently reduced by BAER-101 with 0.3 mg/kg being the minimally effective dose. The duration and total time in SWD were also reduced by BAER-101. Concentrations of drug in plasma achieved a minimally effective dose of 10.1 nM, exceeding the K_i for $\alpha 2$ or $\alpha 3$, but 23 times lower than the K_i for $\alpha 5$ -GABAARs. No adverse events were observed up to a dose 300x the minimally effective dose. The data support the possibility of antiseizure efficacy without the side effects associated with other GABAAR subtypes. This is the first report of an $\alpha 2/3$ -selective GABA PAM suppressing seizures in the GAERS model. The data support the use of BAER-101 in patients with epilepsy.

[0026] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0027] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0028] BAER-101, formerly AZD7325 (Formula (I)), is a positive allosteric modulator (PAM) of g-amino-butyric acid A receptors (GABAARs). It is an N-substituted cinnoline structurally unrelated to the benzodiazepines. BAER-101 has been reported to be potent (~1 nM) and selective for $\alpha 2$ and $\alpha 3$ over other GABAAR subtypes, where it functions as a partial agonist (~15-18% of maximal diazepam response at $\alpha 2$ and $\alpha 3$; ~8% at $\alpha 5$) and a neutral antagonist at $\alpha 1$ subtypes. This compound has been studied in healthy human volunteers and in patients with generalized anxiety disorder where it displayed good tolerability and a relatively low level of sedation and dizziness, side-effects that are often seen with benzodiazepine structured GABAAR PAMs like lorazepam. The compound is disclosed herein as a treatment for epilepsy, as well as for anxiety disorders.

BAER-101 (AZD7325 or AZ12466186), with the structure of Formula (I),



Formula (I)

is a non-benzodiazepine potentiator of $\alpha 2$ and $\alpha 3$ -containing GABAARs with molecular formula C₁₉H₁₉FN₄O₂ and molecular weight 354.38. See also US 7,425,556, which is incorporated herein by reference in its entirety.

[0029] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0030] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, it will be understood that modifications and variations are encompassed within the spirit and scope of the instant disclosure. The preferred methods and materials are now described.

[0031] GABAAR PAMs are anticonvulsant and include the recent addition of ganaxolone into clinical practice for the treatment of seizures in CDKL5 deficiency disorder. While ganaxolone is a neurosteroid which acts on both synaptic and extrasynaptic GABAARs, BAER-101 preferentially potentiates $\alpha 2$ - and $\alpha 3$ -containing GABAARs and is expected to suppress seizures. Darigabat and ENX-101 are $\alpha 2/3/5$ -preferencing GABAAR PAMs being developed for epilepsy. KRM-II-81, like BAER-101, is an $\alpha 2/3$ -selective GABAAR PAM, has robust effects in seizure models and appears to be under development for epilepsy and pain. Preclinical studies have reported antiseizure effects of BAER-101. The role of collybistin association to the $\alpha 2$ -subunit has been investigated using strategies that included specific loss of function mutations. Gabra2-1 mice had enhanced susceptibility to seizures and early mortality. AZD7325 reduced the anxiety and heightened electroencephalography (EEG) δ power of surviving mice. In a mouse model of Dravet syndrome using Scn1a^{+/-} mice, AZD7325 was protective against seizures without notable sedation. Fragile X Mental Retardation Protein (FMRP) is functionally lost in FXS. The potential protective effects of BAER 101 in FMRP-null mice have also been studied, and BAER-101 reduced hyperexcitability in cortical circuits, partially corrected the increased frequency-specific baseline cortical EEG power, reduced susceptibility to audiogenic seizures, and improved novel object recognition memory.

[0032] The rationale for the present study was to demonstrate that BAER-101 reduces seizures in an animal model of generalized seizures where GABA has long been postulated to be a regulating mechanism. The Genetic Absence Epilepsy in Rats from Strasbourg (GAERS) model presents a well-characterized system accepted by those of skill in the art for evaluating

absence seizures in rats using defined electrophysiological readouts. This breeding-derived rat strain exhibits spontaneous generalized cortical seizures in the form of spike-wave discharges (SWDs) during an absence seizure in which there is behavioral arrest (**FIG. 1**).

[0033] Effects of compounds in the GAERS model allow researchers to determine if their compounds are effective in suppressing these generalized seizures. The model has additional utility in predicting seizure exacerbating effects of compounds. Some antiseizure medications are contraindicated for generalized seizures - carbamazepine is one of these anticonvulsants, and also produces increases in SWDs in the GAERS model. The data have predictive validity in patients.

[0034] As described in the present disclosure, BAER-101 was given orally to GAERS animals in doses of 3 to 100 mg/kg. When all of these doses were found to suppress SWDs, an overlapping dose range of 0.1 to 3 mg/kg was subsequently explored. BAER-101 suppressed SWDs in the GAERS model with a minimal effective dose of 0.3 mg/kg that resulted in plasma levels that were greater than the K_i for α_2 , but less than the K_i for α_5 GABAARs. When taken together with the antiseizure effects reported in the literature, these data combined with their human translational power, encourage investigation in patients. The GABA PAM, darigabat, suppressed SWDs in the GAERS model and also significantly suppressed seizures in patients with photosensitive epilepsy). The data with BAER-101 provide the first data supporting the antiseizure efficacy of a GABA PAMS selective for the α_2 and α_3 subunit GABAAR constructs.

[0035] The present disclosure shows that BAER-101 suppresses SWDs in the GAERS model of absence seizure. BAER-101 is a potent compound reported in this model (see US 7,425,556 for reference compounds, the content of which is incorporated herein by reference in its entirety) with a minimally effective dose of 0.3 mg/kg, PO. The data show that a GABA PAM is selective for the α_2 and α_3 -subtype GABAARs is active in this model. The pharmacology of BAER-101, which lacks activity at the α_1 -subtype of GABAAR, may be the reason for the low incidence of dizziness and somnolence observed in both animal and human studies with the molecule.

[0036] That potentiation of α_2 and α_3 -containing GABAARs demonstrates anti-absence effects in the GAERS model and is supported by its on-target engagement with these GABAAR subtypes. Drug exposures were dose-dependent for plasma and the concentration of BAER-101 at the minimally effective dose for seizure suppression was 10.1 nM. Since the K_i of BAER-101 at α_2 and α_3 -associated GABAARs of BAER-101 is reported to be about 1 nM and

the K_i at $\alpha 5$ is 230 nM, plasma concentrations of BAER-101 were about 10x the K_i for $\alpha 2$ and $\alpha 3$ but less than 23x the K_i at $\alpha 5$ -containing GABAARs. BAER-101 is reported to be a neutral antagonist at $\alpha 1$ -subtype GABAARs. The selectivity of BAER-10 in vitro and in vivo for $\alpha 2/3$ -subtypes of GABAARs is consistent with its lack of sedation or other adverse events in the present GAERS study and is consistent with the pharmacological profile of KRM-II-81. In healthy human volunteers and anxiety, and FXS patients, BAER-101 demonstrated the low sedative pharmacological effects reported in rats here and elsewhere.

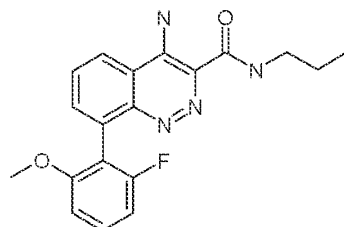
[0037] There are other molecules that have been designed to selectively potentiate $\alpha 2$ and $\alpha 3$ while avoiding activation of $\alpha 1$ with the goal of avoiding sedation and dizziness. Two compounds are currently being developed for epilepsy of this type, darigabat and ENV-101. Darigabat (PF-06372865), an $\alpha 2/3/5$ -preferring GABA PAM was reported to partially suppress SWD in the GAERS model at a minimally effective dose of 1 mg/kg, and was also shown to translate into anti-seizure effects in humans in a photosensitive model of epilepsy in patients at a single dose of 17.5 mg. However, the $\alpha 5$ GABAAR is thought to play a role in synaptic plasticity, cognition, and memory, suggesting that engagement of $\alpha 5$ risks anti-cognitive effects. BAER-101 is the first clinical candidate which is selective for $\alpha 2,3$ and not for $\alpha 1$ or $\alpha 5$, a pharmacology consistent with anti-seizure activity while avoiding side effects common to the GABAA PAM class.

[0038] Absence epilepsy is not fully controlled by current drugs and has a high rate of drug resistance. Absence seizures have been characterized as being dependent on extrasynaptic GABA and antiseizure targets were hypothesized to be thalamic extrasynaptic or GABA transport mechanisms arising from data from a series of elegant studies. The present findings indicate that just an abbreviated subset on synaptic GABAARs is sufficient to suppress absence seizures.

[0039] The GAERS model has pharmacological predictive validity and the efficacy of BAER-101 in generalized seizures of the GAERS model, along with data from other epilepsy models, leads us to believe that BAER-101 will have anti-seizure effects in patients. Darigabat suppressed SWDs in GAERS rats and also completely suppressed generalized seizures in a photosensitive model in patients at a single dose of 17.5 mg. The data in photosensitive epileptic patients appears to be predictive of antiseizure properties in a broad population of patients.

[0040] Based on prior patient safety and tolerability data, coupled with the high potency and full efficacy in the GAERS model, BAER-101 is considered to be a drug suitable for advancing

into human clinical trials for epilepsy. In one embodiment, the present invention provides a method of providing anti-seizure effects in a subject including administering to the subject an



anti-seizure effective amount of a compound of Formula (I)

Formula (I)

(4-amino-8-(2-fluoro-6-methoxy-phenyl)-N-propyl-cinnoline-3-carboxamide) or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier. In one aspect, the techniques described herein relate to a method, wherein the subject has epilepsy.

[0041] In one aspect, the epilepsy is idiopathic generalized epilepsy, photosensitive epilepsy, or genetic absence epilepsy. In another aspect, the idiopathic generalized epilepsy is childhood absence epilepsy, juvenile absence epilepsy, or Dravet syndrome. In various aspects, the seizures are absence seizures, focal seizures, complex partial seizures, tonic-clonic seizures, grand mal seizures, myoclonic seizures, atonic seizures, audiogenic seizures, or clonic seizures.

[0042] The term “effective amount” refers to the amount of a compound, composition, or formulation that is sufficient to treat a condition or disease, to produce desirable effects or results, or to reduce, ease, or arrest symptoms of a condition or disease. “Effective amount” is used interchangeably with the term “therapeutically effective amount”.

[0043] As used herein, the term “treatment” refers to an approach or regimen designed to improve or alleviate symptoms of a disease, sickness, or infirmity. Treatment provides anti-seizure effects. Treatment can lead to reduction in frequency or duration of seizures, improvement of quality of life, or decrease in number, duration, or total time of spike-wave discharges. Providing anti-seizure effects can also occur when symptoms or tests for seizures are nonexistent or improved, or when symptoms or tests for seizures do not worsen, or stabilize.

[0044] In some aspects, the subject is a human. In other aspects, the method of the invention is for veterinary use, such as for non-human mammals, including canine and feline subjects.

[0045] In some aspects, the effective amount is from about 0.1 to 100 mg/kg. In an additional aspect, the effective amount is about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 2.0 mg/kg, about 3.0 mg/kg, about 4.0 mg/kg, about 5.0 mg/kg, about

6.0 mg/kg, about 7.0 mg/kg, about 8.0 mg/kg, about 9.0 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 21 mg/kg, about 22 mg/kg, about 23 mg/kg, about 24 mg/kg, about 25 mg/kg, about 26 mg/kg, about 27 mg/kg, about 28 mg/kg, about 29 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg, or about 100 mg/kg. In one aspect, the effective amount is from about 0.1 mg/kg to about 1 mg/kg, about 1 mg/kg to about 5 mg/kg, about 5 mg/kg to about 20 mg/kg, about 20 mg/kg to about 40 mg/kg, about 40 mg/kg to about 60 mg/kg, about 60 mg/kg to about 80 mg/kg, or about 80 mg/kg to 100 mg/kg.

[0046] In various aspects, the effective amount is administered by oral, intravenous, intramuscular, subcutaneous, transdermal, or intranasal administration. In another embodiment, the present invention relates to a pharmaceutical composition comprising a compound of the Formula (I). The composition of the present invention is a pharmaceutical composition and comprises an excipient and/or carrier, wherein the excipient and/or carrier is selected from a solvent, diluent, bulking agent, filler, binder, coating, color, disintegrant, flavor, glidant, lubricant, preservative, sorbent, sweetener or vehicle.

[0047] In some aspects, the solvent includes water, ethanol, propylene glycol, polyethylene glycol, glycerol, oil, medium-chain triglyceride, polyethylene glycol, ethyl acetate, triacetin, mineral oil, acetic acid, benzyl alcohol, cetyl alcohol, dimethyl sulfoxide, dimethylacetamide, and N-methyl-2-pyrrolidone. In one aspect, the diluent includes hydroxypropyl beta cyclodextrin (HPbCD), water, saline, ethanol, glycerol, propylene glycol, sorbitol, and polyethylene glycol. In other aspects, the bulking agent or filler includes hydroxypropyl beta cyclodextrin (HPbCD), lactose, microcrystalline cellulose, mannitol, cellulose, starch, sodium starch glycolate, pregelatinized starch, dicalcium phosphate, calcium sulphate, colloidal silicon dioxide, magnesium trisilicate, talc, magnesium carbonate, magnesium oxide, titanium dioxide, StarLac, croscarmellose sodium, crospovidone, citric acid, sodium bicarbonate, polysaccharides, lactose, glucose, galactose, starch, and sucrose. In various aspects, the binder includes xanthan gum, tragacanth, acacia gum, alginate, pectin, methylcellulose, carboxymethylcellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, polyvinylpyrrolidone, polyethylene glycol, poloxamer, sorbitol, glycerin, propylene glycol, gelatin, chitosan, bentonite, and magnesium aluminum silicate. In various aspects, the disintegrant includes croscarmellose sodium, sodium starch glycolate, crospovidone, calcium silicate, starch, starch derivatives, alginic acid, polacrillin potassium citric acid,

microcrystalline cellulose, and silicon dioxide. In another aspect, the glidant includes polysorbate 80, sodium lauryl sulfate, cetylpyridinium chloride, methylcellulose, hydroxyethylcellulose, bentonite, veegum, alginate, glycerin, propylene glycol, and polyethylene glycol. In some aspects, the lubricant includes dimethicone, simethicone, glyceryl monostearate, sorbitan esters, polyethylene glycol 400, cetyl alcohol, stearyl alcohol, mineral oil, castor oil, glycerin, propylene glycol, polysorbates, sodium lauryl sulfate, lecithin, wax, and stearic acid.

[0048] In some aspects, the carriers include buffers such as phosphate, citrate, and other organic acids. In other aspects, the carriers include antioxidants such as ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol.

[0049] In various aspects, the composition is formulated in crystalline, powder, granular, compacted solid, liquid, solution, tablet, capsule, granule, suspension, elixir, syrup, emulsion, cream, gel, droplet, mist, vapor or spray form. In various aspects, the compound is administered via intracutaneous, subcutaneous, intravenous, intralesional, intraperitoneal, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, transdermal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal, oral, sublingual buccal, rectal, vaginal, nasal ocular administrations, as well infusion, inhalation, and nebulization routes.

[0050] In one aspect, the effective amount is administered by oral administration. In one aspect, compound is administered once, twice, three or four times daily. In another aspect, the compound is administered at least 10 minutes before onset of a seizure episode. In one aspect, the compound is administered from about 1 to about 120 minutes before onset of a seizure episode. In another aspect, the compound is administered from about 1, about 5, about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, or about 120 minutes before onset of a seizure episode. In some aspects, the compound is administered from about 1 to about 10 minutes, about 10 to about 20 minutes, about 20 to about 30 minutes, about 30 to about 40 minutes, about 40 to about 50 minutes, about 50 to about 60 minutes, about 60 to about 70 minutes, about 70 to about 80 minutes, about 80 to about 90 minutes, about 90 minutes to about 100 minutes, about 100 to about 110 minutes, or about 110 to about 120 minutes before onset of a seizure episode.

[0051] In one aspect, the effective amount is administered for one week to 52 weeks or more. In another aspect, the effective amount is administered for at least one week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks or at least 8 weeks. In further aspects, the effective amount is administered for at least 1 months, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, or at least 12 months.

[0052] In one aspect, the method includes administering an additional compound. In an additional aspect, the additional compound is selected from brivaracetam, cannabidiol, carbamazepine, cenobamate, chlordiazepoxide, or another GABAAR potentiator. In some aspects, the additional compound is clobazam, clonazepam, diazepam, eslicarbazepine, ethosuximide, felbamate, fosphenytoin, gabapentin, ganaxolone, KRM-II-81, lacosamide, lamotrigine, levetiracetam, midazolam, oxcarbazepine, perampanel, phenobarbital, phenytoin, pregabalin, primidone, rufinamide, secobarbital, stiripentol, tiagabine, topiramate, valproate, vigabatrin, or zonisamide.

[0053] Treatment with the claimed method provides an anti-seizure effect to the patient. An anti-seizure effect occurs when the number, duration, or total time of spike-wave discharges is reduced. In other aspects, the frequency, duration, or total time of spike-wave discharges, as measured in an EEG, are reduced, providing an anti-seizure effect to the patient. In one aspect, the frequency, duration, or total time of SWDs are reduced by about 5 to about 100%, compared to the frequency, duration or total time of SWDs before treatment. In some aspects, the frequency, duration, or total time of SWDs are reduced by about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% compared to the frequency, duration or total time of SWDs before treatment.

[0054] In some aspects, frequency, intensity, length of seizures, or a combination thereof, are reduced after administration of the claimed method. In some aspects, frequency, intensity, length of seizures, or a combination thereof are reduced by about 5 to about 100% compared to the frequency, intensity, length of seizures, or a combination thereof before treatment. In some aspects, the frequency, intensity, length of seizures, or a combination thereof are reduced by about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about

70% to about 80%, about 80% to about 90%, or about 90% to about 100% compared to the frequency, intensity, length of seizures, or a combination thereof before treatment.

[0055] In some aspects, a change in the frequency of seizures experienced by a patient is quantified by maintaining a detailed seizure diary, where patients or caregivers record each seizure event. The data collected over a period, such as several months, are then compared to the baseline seizure frequency established prior to the initiation of treatment. A statistically significant reduction in the number of seizures per unit of time is indicative of a positive treatment outcome. A reduction in the length of time of a seizure is quantified by using a stopwatch or electronic timing device by an observer at the time of the seizure, or retrospectively estimated by the patient or caregiver immediately after the event. Providing an anti-seizure effect to the patient occurs when the severity of seizures is reduced. In one aspect, severity or duration of seizures is assessed using standardized scales such as the Chalfont Seizure Severity Scale, the National Hospital Seizure Severity Scale, the Liverpool Seizure Severity Scale, the Quality of Life in Epilepsy Inventory, the Seizure Frequency Scale, or other scales. These scales take into account factors such as the level of consciousness during the seizure, injuries sustained, and postictal symptoms. In some aspects, after administration of the claimed method, an anti-seizure effect is a reduction in the severity scale.

[0056] Presented below are examples discussing methods of providing anti-seizure effects contemplated for the discussed applications. The following examples are provided to further illustrate the embodiments of the present invention but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLES

EXAMPLE 1

A Phase 2- Ready Potentiator of $\alpha 2/3$ -Containing GABA_A Receptors Potently and Fully Blocks Seizures in Rats with Genetic Absence Epilepsy

[0057] **Rationale.** GABAA receptor potentiators are in various stages of development for the treatment of patients with epilepsy. BAER-101 (formerly AZD7325) is a selective potentiator of $\alpha 2/3$ -Containing GABA_A Receptors (GABAARs) with a lower liability for sedation, dizziness, and ataxia than that of non-selective potentiators. Prior published data have confirmed antiseizure effects of BAER-101 in genetic models of Dravet Syndrome and Fragile X Syndrome. The aim of the present study was to determine efficacy in a model of generalized

seizures. The Genetic Absence Epilepsy in Rats from Strasbourg (GAERS) model is widely accepted for evaluating novel antiseizure medications and is translatable to patients.

[0058] **Methods.** Fourteen adult male GAERS were maintained and used in a facility approved and monitored by the animal care and use controls in France in accord with all ethical guidelines. Recording electrodes were implanted under general anesthesia using stereotaxic methods. Four active monopolar electrodes were positioned over the frontal and parietal cortices, on both sides of the brain. The approximate coordinates for the frontal cortex were: AP +2 mm, ML +/- 3 mm (from bregma as reference). The approximate coordinates for the parietal cortex were: AP -7 mm, ML +/- 3 mm. A fifth electrode was placed on the cerebellum as reference. EEG signals were analyzed to count spike-wave discharges (SWDs).

[0059] BAER-101 was given orally in doses of 3-100 mg/kg and subsequently, in doses of 0.1-3.0 mg/kg. Diazepam was used as a positive control, administered intraperitoneally (2 mg/kg, IP). A cross-over protocol was used for each rat and minimal wash-out period of 7 days occurred between treatments. EEG recordings were collected on freely moving and awake animals for 40 min pre-administration (baseline period) and 90 min post-administration using SYSTEMPLUS EVOLUTION™ (MICROMED®). EEG recordings were subsequently analyzed offline and quantified blindly by an expert on our proprietary platform (CUE®), to identify SWD

[0060] **Results.** The number of SWDs was dose-dependently reduced in rats given BAER-101. When the data were summarized over the observation period, 0.3 mg/kg was found to be the minimal effective dose. The duration and total time in SWD were also significantly reduced by BAER-101.

[0061] BAER-101 produced anti-seizure effects with a minimal effective dose of 0.3 mg/kg, PO in GAERS rats confirming the antiseizure effects of this compound. The data are expected to translate into patients with seizures. BAER-101 has already been in over 700 patients with generalized anxiety disorders and Fragile X Syndrome where it was safe and tolerable. Overall, the data encourage clinical study in patients with epilepsy.

[0062] This study aimed at evaluating test compound AZD7325 on spike-and-wave discharges (SWD) in the genetic absence epilepsy rat from Strasbourg (GAERS). AZD7325 was evaluated at doses ranging from 0.1 to 100 mg. AZD7325 was evaluated in treatment of epilepsy in the GAERS model of absence epilepsy. The study used 14 adult male GAERS rats that were obtained from the breeding colony of INSERM, Grenoble Institute of Neurosciences, France. The rats were implanted with electrodes for SWD recording using EEG. The collected

data included spike-and-wave discharge (SWD). AZD7325 was administered at 0.1, 0.3, 1, 3, 10, 30 and 100 mg/kg, PO, with diazepam at 2 mg/kg administered intraperitoneally as a reference compound. The study concluded that AZD7325 reduced the occurrence of SWD dose-dependently in the GAERS model.

[0063] Genetic absence epilepsy rat from Strasbourg (GAERS) is a selectively inbred strain of Wistar rats displaying spontaneous SWD. For the last twenty years, the GAERS has become a reference model for absence epilepsy, since these rats were shown to present behavioral, electrophysiological and pharmacological features of absence seizures. Cortical EEG recording is characterized by SWD lasting 17-25 sec, with a recurrence of 45-60 seizures per hour. SWDs start and end abruptly and are associated with a behavioral arrest lasting the time of the discharge. As in human patients, SWD in rats are suppressed by antiepileptic drugs such as valproate, ethosuximide and levetiracetam, whereas other antiepileptic drugs have no efficacy or can even aggravate SWD, particularly carbamazepine, phenytoin, and pregabalin.

[0064] This genetic model offers therefore a high predictivity for identifying anti-absence as well as pro-epileptic effect for AED in development or other compounds acting on the CNS.

[0065] This study was performed following local Standard Operating Procedures.

Materials and methods

Compound preparations

[0066] Test compound AZD7325 was formulated at the appropriate concentrations, for a dose volume of 5 ml/kg. Diazepam was formulated for a dose volume of 10 ml/kg.

AZD7325

[0067] AZD7325 was formulated at 0.02 to 20 mg/ml, for a dosing volume of 5 ml/kg, in a 20% hydroxypropyl beta cyclodextrin (HPbCD) in water.

[0068] The vehicle was prepared in advance and kept at 4°C for a maximum of 4 weeks. HPbCD (batch A0434679, ref. 297561000, FISCHER SCIENTIFIC™, France) was weighed, and transferred in 50% of the final volume of water for injection (Cooper, France). The mixture was stirred until complete dissolution, then the volume was adjusted to the final volume.

[0069] A first solution of AZD7325 was prepared at 20 mg/ml (phase 1) or 0.6 mg/kg (phase 2). AZD7325 material was weighed in a glass vial. Then the appropriate volume of vehicle was added little by little while stirring. The mixture was vortexed, then sonicated for 15 min. The resulting formulation was a clear solution.

[0070] Lower concentrations for each phase were then prepared using serial dilutions from the initial concentrated solution described above. An appropriate volume of AZD7325 solution

was measured, and then the appropriate volume of vehicle was added. The mixture was vortexed.

[0071] All formulations were then aliquoted in appropriate volumes for one EEG recording. Aliquots were protected from light and stored at 4°C for a maximum duration of 10 days. One formulation was used for two EEG recordings.

[0072] When formulations were stored at 4°C before use, they were left at room temperature for 10-15 minutes before use. Then they were placed in an ultrasound bath for 15 minutes. All formulations were still clear solutions.

Diazepam

[0073] Diazepam (Diazepam TVM, ROCHE®, batch F0015-05) was diluted down to 0.2 mg/ml using saline (0.9% NaCl), for a dose volume of 10 ml/kg. Diazepam was prepared immediately before administrations.

Animal model

[0074] 14 adult male GAERS were obtained from INSERM, Grenoble Institute of Neurosciences, Grenoble, France and delivered to the laboratory at the age of ~3 months. Animals were first housed in groups in cages on wood litter with free access to food and water until surgery. Animal housing was maintained under artificial lighting between 7:30 am to 7:30 pm in a controlled ambient temperature ($22 \pm 2^\circ\text{C}$) and relative humidity.

[0075] At the time of experiments (compound testing), animals were aged 4 to 5 months old. They were fully naïve and had not been submitted to any experimentations before the protocol described hereafter.

Electrode implantation

[0076] Electrodes for SWD recording were implanted in all 14 rats, under general anesthesia (isoflurane, 2% in oxygen) using stereotaxic methods.

[0077] Animals first received an injection of the analgic drug buprenorphine (0.05 mg/kg), about 2 hours before induction of anesthesia. After anesthesia induction, rats were placed in the stereotaxic frame. The body temperature was monitored and maintained constant during the surgery. An ophthalmic gel was placed on the eyes to avoid the drying of the cornea. The depth of anesthesia was controlled before starting the surgery and the breathing rate and the cardiac rhythm were visually controlled during the surgery. The incision zone was first cleaned, and betadine was applied. The skin was incised, and the skull cleaned. Five monopolar electrodes were positioned over the frontal and parietal cortices, in both sides of the brain. The

approximate coordinates for the frontal cortex were: AP +2 mm, ML +/- 3 mm (from bregma as reference). The approximate coordinates for the parietal cortex were: AP -7 mm, ML +/- 3 mm. The rats were equipped with a female connector fixed on the skull to allow chronic EEG recordings. A fifth electrode was placed on the cerebellum as reference.

[0078] After surgery, animals were maintained in individual cages. Within 8 hours from surgery, animals received a second injection of buprenorphine (0.05 mg/kg). Animals were left in their home cage for at least one week of recovery.

Animal selection

[0079] Two weeks after electrode implantation, a one-hour preliminary EEG was recorded on all animals, in control conditions. EEG signals were analyzed, to count SWD.

From the 14 implanted GAERS, all 14 animals were found valid for EEG experiments, according to quality criteria of signal-to-noise ratio and number of SWD per hour: they all presented a satisfying signal-to-noise ratio, and a sufficient number of SWD (above 20 per hour). 12 animals were randomly selected for the pharmacological experiments. The 2 remaining animals were kept in a reserve pool.

Compound testing in EEG recordings

[0080] Acute doses of AZD7325 were administered and evaluated during EEG recordings grouped in 2 phases.

Compound administrations

[0081] All compound conditions were administered PO, in a 5 ml/kg dose volume except diazepam which was administered intraperitoneally (IP) at 10 ml/kg dose volume.

Phase 1

[0082] The initial study plan with BAER-101 included the following 6 conditions:

[0083] Vehicle (20% HPβCD in water) PO, intraperitoneal diazepam at 2 mg/kg, BAER-101 at 100 mg/kg PO, BAER-101 at 30 mg/kg PO, BAER-101 at 10 mg/kg PO, and BAER-101 at 3 mg/kg PO. Compound conditions were administered in a cross-over protocol, following the rules: each animal received each condition, in a random order and in separate recording sessions, two animals received each condition in each recording session, and a minimal wash-out period of 7 days was allowed between each administration.

[0084] An intermediate check on study results after the second EEG of this cross-over protocol indicated that compound doses all produced substantial effects on EEG. The cross-over was interrupted, and doses were changed in phase 2 to a lower overlapping dose set.

[0085] An intermediate check on study results after the second EEG of this cross-over protocol indicated that compound doses were too high and were adjusted. The cross-over was interrupted, and doses were changed in phase 2.

Phase 2

[0086] Four EEG recordings were completed with the following set of compound conditions: Vehicle (20% HPbCD in water) PO, intraperitoneal diazepam 2 mg/kg, and BAER-101 (0.1, 0.3, 1, and 3 mg/kg, PO). Compound conditions were still administered in a cross-over protocol, following the rules: each animal received 4 different conditions from the 6 indicated above, in a random order and in separate recording sessions, animals having received the vehicle, diazepam or BAER-101 at 3 mg/kg during phase 1 were not ascribed again to these conditions, and a minimal wash-out period of 7 days was allowed between each administration.

[0087] The final administration table after phases 1 and 2 are described in the **Table 1**.

[0088] **Table 1:** Administration table during the 6 EEG recordings, indicating which compound condition was administered to which animal (in line) during which recording (in columns).

	EEG 1		EEG 2		EEG 3	
	comp.	dose (mg/kg)	comp.	dose (mg/kg)	comp.	dose (mg/kg)
23SC0633	vehicle	N/A	diazepam	2	AZD7325	0.1
23SC0634	AZD7325	10	diazepam	2	AZD7325	3
23SC0635	AZD7325	3	AZD7325	100	vehicle	N/A
23SC0636	AZD7325	3	AZD7325	30	AZD7325	1
23SC0637	vehicle	N/A	AZD7325	10	diazepam	2
23SC0638	AZD7325	30	AZD7325	100	diazepam	2
23SC0639	AZD7325	100	AZD7325	30	AZD7325	0.1
23SC0641	diazepam	2	AZD7325	3	AZD7325	0.3
23SC0643	AZD7325	100	AZD7325	10	AZD7325	3
23SC0644	AZD7325	30	vehicle	N/A	AZD7325	1
23SC0645	AZD7325	10	vehicle	N/A	AZD7325	0.3
23SC0646	diazepam	2	AZD7325	3	vehicle	N/A
	EEG 4		EEG 5		EEG 6	
	comp.	dose (mg/kg)	comp.	dose (mg/kg)	comp.	dose (mg/kg)
23SC0633	AZD7325	0.3	AZD7325	3	AZD7325	1
23SC0634	vehicle	N/A	AZD7325	1	AZD7325	0.3
23SC0635	AZD7325	0.3	AZD7325	0.1	diazepam	2
23SC0636	AZD7325	0.1	diazepam	2	vehicle	N/A

23SC0637	AZD7325	1	AZD7325	0.3	AZD7325	3
23SC0638	AZD7325	3	vehicle	N/A	AZD7325	0.1
23SC0639	diazepam	2	vehicle	N/A	AZD7325	3
23SC0641	AZD7325	1	AZD7325	0.1	vehicle	N/A
23SC0643	vehicle	N/A	AZD7325	0.3	diazepam	2
23SC0644	AZD7325	3	diazepam	2	AZD7325	0.1
23SC0645	diazepam	2	AZD7325	3	AZD7325	1
23SC0646	AZD7325	0.1	AZD7325	1	AZD7325	0.3

EEG recordings

[0089] The day of the recording session, the 12 selected animals were placed in a recording chamber and connected to their cable. A habituation period (~30 min) was allowed before the EEG recording session. EEG recordings were collected on freely moving animals for 40 minutes pre-administration (baseline period) and 90 minutes post-administration using SYSTEMPLUS EVOLUTION™ (MICROMED®). Animals were maintained in quiet wakefulness state during the recording session. An experimenter was monitoring the animals and was maintaining wakefulness by gently stimulating the animals when needed.

Data analysis

[0090] EEG recordings were subsequently analyzed offline and quantified blindly by an expert on the proprietary platform, to identify SWD (Error! Reference source not found. 1). The SWD occurs spontaneously, with clear-cut start and stop well defined over the basal EEG activity

[0091] SWD were analyzed during a 40 min baseline period (immediately before compound administration), and for a period of 80 min between 10 and 90 min after compound administration. The first 10 min immediately after administration are not considered since the administration process disturbs strongly the occurrence of SWD.

[0092] Data are expressed as mean $\pm$ SEM. For each animal and administration, data were computed for number, average duration and cumulated duration of SWD per 20 min periods. Post-treatment data were also pooled over the 80 min duration of the post-treatment period, and normalized in % for baseline values, providing a single data point measuring the percentage of inhibition for each animal and each dose. ED50 was calculated from these data, using a non-linear regression in GRAPHPAD PRISM™ v9.5.

Statistics

[0093] Statistical analyses were performed using GRAPHPAD PRISM™ v9.5. The significance level was set at $p < 0.05$.

[0094] Absolute data measured per 20 min periods were analyzed using a two-way ANOVA with the compound condition and the time from administration as factors. As the crossover design was not fully completed, not all animals received all doses of AZD7325. Consequently, measures were considered repeated within the time factor. When significant, ANOVA was followed by paired comparisons using a Dunnett test, with comparisons versus the second baseline period, versus vehicle, and versus diazepam.

[0095] Pooled data over the whole post-treatment period were analyzed using a one-way ANOVA, with the compound condition as factor. When significant, the ANOVA was followed by paired comparisons using a Dunnett test, versus the vehicle condition.

Plasma Concentrations of BAER-101

[0096] Drug concentrations were determined for the purpose of correlating with pharmacodynamic effects.

Blood samples for PK measurements

[0097] For the purpose of determining plasma drug concentrations, blood was sampled from each animal. Directly after each EEG recordings, blood was collected via tail sampling on each animal from the crossover for analysis and plasma collection.

Compound conditions

[0098] A sample was collected at the end of each EEG from each phase. All animals included in EEG were sampled. The administration table is provided in Table 1.

Sample collections

[0099] Plasma samples were collected from each animal at the end of each recording sessions (i.e., 6 recordings in total) about 2 hours after compound administration. The tail vein was punched with a needle, and blood was collected directly into a SARSTEDT® MINIVETTE® POCT system. Blood samples were then transferred into a 0.2 ml PCR tube. All tubes were kept on ice for less than 30 min before centrifugation. The centrifugation was conducted at 3000 g, for 10 min, at 4°C. The resultant plasma, at least 25 µl was collected and transferred into a labelled tube and stored at -80°C until shipment.

Bioanalytical Methods

[0100] Plasma was analyzed for concentrations of BAER-101 by INOTIV® (West Lafayette, IN, USA) by LC/MS. LC was a SCIEX® EXION®. A SCIENX® AD multiplate

was employed. MS detection was by AB SCIEX® 5500 TRIPLE QUAD_4®. MS analysis for BAER-101 (Q1/Q3 Masses: 355.000/296.100 Da) used labetalol as an internal standard (Q1/Q3 Masses: 329.20/294.20 Da).

[0101] The UPLC method made use of a WATERS™ HSS T3 column (2.7 x 50 mm). Elution was by gradient 0.6 mL/min with Mobile Phase A = 0.1% formic acid in water and Mobile Phase B = 90:10 CAN:MeOH.

Bioanalytical Data Statistics

[0102] Concentrations were evaluated for each experimental phase and at each dose level. The mean $\pm$ SEM concentration of BAER-101 at each dose was determined and assessed for dose-dependence by ANOVA and Dunnett's test using the concentration of BAER-101 at 0.1 mg/kg as the control.

[0103] Although different doses of AZD7325 were evaluated in separate phases, all results from both phases were combined, to provide a better overview of the effects of AZD7325 on SWDs. Resulting from the dose change after interruption of phase, the number of data in each compound conditions were uneven (Table 2).

[0104] **Table 3:** final number of animals having completed the compound conditions during the study.

Compound condition	Number of Animals
Vehicle PO	n = 12
Diazepam at 2 mg/kg IP	n = 12
AZD7325 at 0.1 mg/kg PO	n = 7
AZD7325 at 0.3 mg/kg PO	n = 6
AZD7325 at 1 mg/kg PO	n = 6
AZD7325 at 3 mg/kg PO	n = 12
AZD7325 at 10 mg/kg PO	n = 4
AZD7325 at 30 mg/kg PO	n = 4
AZD7325 at 100 mg/kg PO	n = 4

Vehicle

[0105] Oral (PO) administration with the vehicle composed of 20% HP β CD in water had no significant effect on the occurrence of SWD. The number and the duration of SWD remained unchanged after administration, as compared to baseline. For example, the number of SWD during the 80 min post-treatment period remained stable at 101.8 ± 7.5 % of the baseline value.

Diazepam

[0106] Diazepam was administered at 2 mg/kg intraperitoneally.

Number of SWD

[0107] Diazepam induced a significant reduction of the number of SWD, immediately after administration. From an average frequency of SWD at 25.6 ± 1.1 SWD per 20 min during baseline, the epileptic activity was reduced down to 1.2 ± 1.1 SWD during the first 20 min period after treatment (10 to 30 min after administration). This reduction remained stable for the whole post-treatment period, up to 90 min after administration. The average effect of SWD over the 80 min after treatment was 93.1 ± 6.5 % inhibition from baseline.

Average duration of SWD

[0108] The average duration of remaining SWD was not accurately measured, as two animals were still showing continuously epileptic events after treatment. In the other 10 animals, SWD were totally inhibited for at least 70 min after administration.

[0109] These effects of diazepam at 2 mg/kg IP are standard in the GAERS model, and match reference data.

AZD7325

[0110] AZD7325 was evaluated at doses ranging from 3 to 100 mg/kg PO during phase 1. After evaluation of results from the first 2 recordings with these doses, a near complete inhibition of SWD was obtained with all doses. Doses were thus reassessed and reduced down to doses ranging from 0.1 to 30 mg/kg PO in 4 additional recordings.

[0111] Of note, no gross behavioral modifications or adverse effects were observed after the administration of AZD7325, at any dose included in this study.

Number of SWD

[0112] AZD7325 induced a dose-dependent inhibition of SWD. When considering the number of SWD per 20 min periods after administrations (Error! Reference source not found. 2), AZD7325 induced a significant reduction of events at all doses above 1 mg/kg PO, as compared to both the baseline and the vehicle. The effect was immediate, as it was already significant during the first measurement time-point, 10 to 90 minutes after administration. And the effect remained stable over the whole post-treatment period, as it was still significant 70 to 90 minutes after administration.

[0113] When post-treatment data were pooled over the 80 minutes and expressed in percentage of baseline (**FIG. 3**), the SWD inhibition was significant for all doses above 0.3 mg/kg, as compared to the vehicle. The ED50 was estimated at 0.51 mg/kg (95% CI: 0.47 to 0.56).

[0114] The average duration of remaining SWD was measured with doses of AZD7325 up to 3 mg/kg. Above that dose, few animals have been tested, and almost no SWD remained, preventing any accurate measure of SWD duration.

[0115] At 1 and 3 mg/kg, AZD7325 induced a significant reduction of the average duration of SWD, as compared to the vehicle (FIG. 4). For example, SWD lasted on average for 15.9 ± 0.8 s during the 80 min after vehicle administration, and they were reduced to 8.1 ± 0.8 s after treatment with AZD7325 at 3 mg/kg (FIG. 5). The effect was though less stable than the reduction of the number of SWD: at the end of recordings, 70 to 90 min after administrations, the average duration of SWD in all AZD7325 conditions were returned to values similar to the vehicle condition (FIG. 4).

Total time in SWD

[0116] The total time spent in SWD is a combined parameter, affected by both the number of remaining SWD and their duration. As both measures were significantly reduced by AZD7325, the total time in SWD was significantly reduced by AZD7325.

[0117] AZD7325 induced a significant reduction of the total time as compared to the vehicle, at all doses above 1 mg/kg (Error! Reference source not found.). The reduction was immediately obtained, 10 to 30 min after administration, with all these doses. And the reduction was still significant at the end of recordings, 70 to 90 min after administration.

[0118] Pooled data over the 80 min post-treatment period showed a dose-dependent effect, with a significant reduction as compared to vehicle at all doses above 0.3 mg/kg (FIG. 7). At doses above 1 mg/kg, the effect of AZD7325 was not significantly different from the one of diazepam at 2 mg/kg IP. The ED₅₀ for AZD7325 was calculated at 0.47 mg/kg (95% CI: 0.41 to 0.55).

[0119] AZD7325 is effective at inhibiting SWD in the GAERS model of absence seizures. Doses above 0.3 mg/kg PO can significantly reduce the number and/or the duration of SWD, with an ED₅₀ around 0.5 mg/kg. No adverse effects were observed at doses up to 100 mg/kg.

Drug Concentrations

[0120] Concentrations of BAER-101 in plasma of the rats were consistent across the EEG and blood sampling periods and showed dose-dependence (FIG. 8). The dose of orally administered BAER-101 was proportionally related to the concentrations of BAER-101 achieved in rat plasma (FIG. 9A) - ($F_{6,17} = 732.5$, $p < 0.0001$). The concentration of BAER-101 in plasma was significantly associated with suppression of the number of SWD (FIG. 9B).

[0121] FIG. 10-12 provide further data from our GAERs model investigation showing the surprising potency of BAER-101. In the model, dosing started out with 3 higher doses which showed efficacy. Accordingly, lower doses were used to provide a full a dose response.

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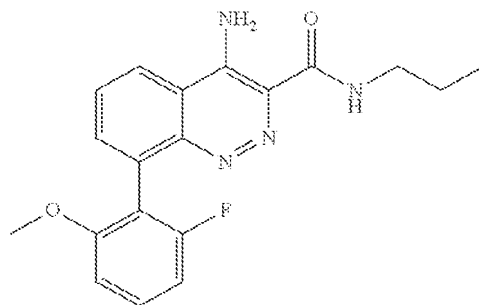
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- [0153] Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. A method of providing anti-seizure effects in a subject comprising administering to the subject an anti-seizure effective amount of a compound of Formula (I)



Formula (I)

2. (4-amino-8-(2-fluoro-6-methoxy-phenyl)-N-propyl-cinnoline-3-carboxamide) or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.
3. The method of claim 1, wherein the subject has epilepsy.
4. The method of claim 2, wherein the epilepsy is idiopathic generalized epilepsy, photosensitive epilepsy, or genetic absence epilepsy.
5. The method of claim 3, wherein the idiopathic generalized epilepsy is childhood absence epilepsy, juvenile absence epilepsy, or Dravet syndrome.
6. The method of claim 1, wherein the seizures are absence seizures, focal seizures, complex partial seizures, tonic-clonic seizures, grand mal seizures, myoclonic seizures, atonic seizures, audiogenic seizures, or clonic seizures.
7. The method of claim 1, wherein the effective amount is from about 0.1 to 100 mg/kg.
8. The method of claim 6, wherein the effective amount is about 0.1 mg/kg, about 0.3 mg/kg, about 1.0 mg/kg, about 3.0 mg/kg, about 10 mg/kg, about 30 mg/kg, or about 100 mg/kg.
9. The method of claim 6 or 7, wherein the effective amount is administered by oral administration.
10. The method of claim 1, wherein the compound is administered once, twice, three or four times daily.

10. The method of claim 1, wherein the compound is administered at least 10 minutes before onset of a seizure episode.
11. The method of claim 1, further comprising administering an additional compound.
12. The method of claim 11, wherein the additional compound is selected from
brivaracetam, cannabidiol, carbamazepine, cenobamate, chlordiazepoxide, clobazam,
clonazepam, diazepam, eslicarbazepine, ethosuximide, felbamate, fosphenytoin,
gabapentin, ganaxolone, lacosamide, lamotrigine, levetiracetam, midazolam,
oxcarbazepine, perampanel, phenobarbital, phenytoin, pregabalin, primidone,
rufinamide, secobarbital, stiripentol, tiagabine, topiramate, valproate, vigabatrin, or
zonisamide.
13. The method of claim 1, wherein the subject is a human or non-human mammal.
14. The method of claim 13, wherein the subject is a canine or feline subject.