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(54) Title: NOX INHIBITORS FOR PREVENTING EPILEPTIC SEIZURES

(57) Abstract: The present invention relates to a NOX inhibitor compound of formula (I), preferably a NOX2 inhibitor, and a pharmaceutical composition comprising such compound, for use for preventing an epileptic seizure. The invention further concerns such pharmaceutical composition for use for reducing the risks of an apparition, a trigger, an induction, or an occurrence of an epileptic seizure in a subject, preferably suffering from epilepsy.



WO 2020/178267 A1

NOX INHIBITORS FOR PREVENTING EPILEPTIC SEIZURES

FIELD OF THE INVENTION

5 The present invention relates to the field of medicine, in particular to the use of inhibitors of NADPH oxidase (NOX) for preventing and controlling epileptic seizures.

BACKGROUND OF THE INVENTION

10 Epilepsy is one of the most frequent neurological disorder and concerns about 65 million people worldwide. Therefore, a major goal of contemporary epilepsy research is the discovery of therapies that would prevent the development of recurrent seizures in individuals at risk. However, despite the availability of at least 22 anti-seizure drugs, about 30% of patients with epilepsy remain resistant to drug therapy and continue to have seizures. Moreover, the proportion of those not
15 becoming seizure-free despite treatment has not decreased substantially over the past 50 years. In this context, a large number of compounds and molecular targets have been explored.

Dey et al. (Trends Pharmacol Sci., 2016, 37(6), 463-484) report anti-inflammatory small molecules to treat seizures and epilepsy, and mention the possible role of proinflammatory
20 mediators, such as COX-2, PGE₂, IL-1 β , IL-6, HMGB1, TLR4, TNF- α , TGF- β and NOX2 in seizure generation and exacerbation. Particularly, Dey et al. point that NOX2, one of the seven isoenzymes of the NADPH oxidase family, can play central roles in neuroinflammation, neurodegeneration and associated functional deficits in neurological conditions such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, and
25 epilepsy. Accordingly, Dey et al. refer, at particular, to two studies published by Pestana et al. (Neurosciences Letters, 2010, 484, 187-191) and Kim et al. (Brain Research, 2013, 1499, 163-172) using apocynin, as an NADPH oxidase inhibitor, for limiting seizure-induced neuronal death.

More particularly, Pestana et al. have evaluated the role of ROS (Reactive Oxygen Species) in
30 neurodegeneration, and have demonstrated that apocynin decreased the production of ROS induced by epileptic seizures, thereby limiting neurodegeneration. Kim et al. have evaluated the neuroprotective effect of apocynin on epileptic seizure-induced neuronal death and have administrated apocynin in rats in order to suppress the production of ROS. However, such

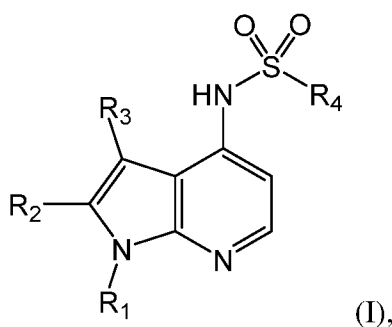
treatments using NOX2 inhibitors, particularly apocynin, are only efficient for treating the side effects or damages caused by epileptic seizures such as neuronal death, only.

Therefore, there is still a need for developing new treatments for preventing and controlling epileptic seizure and/or reducing the risks of triggering an epileptic seizure, which must exhibit a therapeutic effect before a seizure occurs in a patient.

SUMMARY OF THE INVENTION

In this context, the inventors have surprisingly shown that NOX, particularly NOX2, already known as a target for treating epileptic seizure-induced neuronal death, could also be a significant target to avoid the epileptic seizure occurrence in a patient, preferably suffering from epilepsy. More particularly, the inventors have demonstrated that the seizure onset was associated with a rapid release of H_2O_2 resulting from NMDA receptor-mediated activation of NADPH oxidase (NOX). They have shown that NOX blockade prevented the fast H_2O_2 release as well as the DC shift (Direct Current shift) and seizure induction in slices. They have also shown that injection of NOX2 antagonists of formula (I), specifically N-(1-isopropyl-3-(1-methylindolin-6-yl)-1H-pyrrolo[2,3-b]pyridine-4-yl)-1-methyl-1H-pyrazole-3-sulfonamide prevented epileptic seizures.

The present invention thus relates to a compound of formula (I):



for use for preventing an epileptic seizure,

in which:

➤ R_1 represents:

- a hydrogen,
- a (C_1-C_6) alkyl group optionally substituted by at least one radical selected in a group consisting of:

- a cycloalkyl, a heterocycloalkyl, or an aryl, optionally substituted by at least one (C₁-C₆)alkyl group, (C₁-C₆)alkyloxy group, or a halogen,
 - a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group, and
 - 5 ○ a (C₁-C₆)alkyloxy group optionally substituted by a trimethylsilyl group, or
 - a cycloalkyl or a heterocycloalkyl optionally substituted by at least one (C₁-C₆)alkyl group, (C₁-C₆)alkyloxy group, or a halogen;
 - R₂ represents:
 - a hydrogen,
 - 10 • a (C₁-C₆)alkyl group,
 - a halogen, or
 - an indoline or a heteroaryl optionally substituted by a (C₁-C₆)alkyl group;
 - R₃ represents an aryl, a heteroaryl, an indoline, a tetrahydroquinoline, optionally substituted by at least one radical selected in the group consisting of:
 - 15 • a heterocycloalkyl optionally substituted by a (C₁-C₆)alkyl group or a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group,
 - a (C₁-C₆)alkyl group,
 - a (C₁-C₆)alkyloxy group, and
 - a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group;
 - 20 and
 - R₄ represents an aryl or a heteroaryl optionally substituted by at least one radical selected in the group consisting of a (C₁-C₆)alkyl group, a (C₁-C₆)alkyloxy group, a halogen, and a cyano;
- or a pharmaceutically acceptable salt thereof.

25 In a particular embodiment, R₁ represents a (C₁-C₆)alkyl group. Preferably, R₁ represents a methyl, an ethyl, or an isopropyl group, more preferably an isopropyl group.

In a further particular embodiment, R₂ represents a hydrogen.

In a further particular embodiment, R₃ represents an indoline optionally substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

30 In a further particular embodiment, R₄ represents a heteroaryl optionally substituted by a (C₁-C₆)alkyl group. Preferably, R₄ represents a pyrazole optionally substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

A preferred compound for use according to the invention is N-(1-isopropyl-3-(1-methylindolin-6-yl)-1H-pyrrolo[2,3-b]pyridine-4-yl)-1-methyl-1H-pyrazole-3-sulfonamide.

The present invention further relates to a pharmaceutical composition comprising a compound of formula (I) as defined herein, for use for preventing an epileptic seizure. The invention also relates to such pharmaceutical composition for use for reducing the risks of the apparition of an epileptic seizure in a subject, preferably suffering from epilepsy.

In a particular embodiment, the pharmaceutical composition of the invention is administered by oral (*per os*) or parenteral route, preferably by oral route.

In a further particular embodiment, the pharmaceutical composition of the invention is administered at a dose from 0.1 to 500 mg/kg BW preferably from 10 to 200 mg/kg BW, more preferably about 100 mg/kg BW.

In a further particular embodiment, the pharmaceutical composition of the invention is administered once a week, two days a week, four days a week, once a day, preferably once a day. A preferred embodiment of the invention, is a pharmaceutical composition as disclosed herein orally administered at a dose of about 100 mg/kg BW once a day.

In a further particular embodiment, the pharmaceutical composition of the invention further comprises an anti-epileptic drug.

LEGEND OF FIGURES

Figure 1: Extracellular H₂O₂ and glutamate during epileptiform activity induced by 4-AP in hippocampal slices.

A. Glutamate release and H₂O₂ production are associated with interictal spikes. Inset (c) shows power spectral density (PSD) of selected “complex” (black) and “simple” (gray) spikes. **B.** All spontaneous seizures were preceded by a “complex” spike associated with a fast and high amplitude release of H₂O₂. Top: representative traces of field potential recordings (black) and associated extracellular H₂O₂ release (small dash). Inserts below shows field potential traces of selected seizures (black) with superimposed records (below) of LFP (local field potentials), H₂O₂ (small dash) and extracellular glutamate (large dash) corresponded to the seizure onset shown at expanded time scale. Note that the considerable difference in scale creates an illusion of “leading” H₂O₂ signal over the glutamate one. **C.** Dependence of H₂O₂ and glutamate releases versa amplitudes of the first (left) and the second (right) spikes in the complex spike potential. Note that

both glutamate and H₂O₂ peaks were correlated with the amplitude of the second but not the first component in the spike complex. Insets show superimposed traces of field potential and H₂O₂ release, demonstrating strong dependence of this release on the second spike. **D.** Relationship between glutamate and H₂O₂ releases during the same epileptiform spikes. Note the correlation between these values. **E.** Summary histogram of H₂O₂ release amplitudes during epileptiform spikes. Black line indicates a presumable threshold above which seizures are induced.

Figure 2: AMPA/KA receptor, K⁺-Cl⁻ co-transporters or GABAergic transmission blockade does not prevent the DC-shift induction.

A. Superimposed records of field potential and H₂O₂ transients at 100Hz, 1s stimulation of Schaffer collaterals in control ACSF (left) and under 4-AP (induced seizure, middle), and during spontaneous seizure (right). Note that H₂O₂ release triggered by 100Hz stimulation exhibits a biphasic shape characterized by an initial rapid peak (arrows) followed by a delayed, slower and larger release. This second component was absent in case of spontaneous SLEs (Seizure-like Events), therefore was not associated with seizure induction. **B-D.** Neither blockade of AMPA/kainate receptors (B, n = 4) nor blockade of K⁺-Cl⁻ co-transporters (C, n = 3) or inhibitory transmission (D, n = 3) prevented the DC shift associated with rapid high release of H₂O₂. Note in (D) spontaneous seizure preceded by a complex spike with following DC shift despite the blockade of GABAergic transmission.

Figure 3: NOX antagonists ablate fast H₂O₂ production and prevent DC shift and seizure induction.

A-B. Upper traces: Decrease of interictal activity-associated release of H₂O₂ following inhibition of NOX. Superimposed records of field potential (gray) and H₂O₂ release (small dash) during interictal activity induced by 4-AP (left) and following addition of APV (**A**, right) or celastrol (**B**, right). Note that neither APV nor celastrol affected significantly LFP induced by a single stimulation of Schaffer collaterals (insets on the right). Lower traces: LFP and extracellular H₂O₂ transients at 100Hz, 1s stimulation of Schaffer collaterals under 4-AP. Blockade of NMDA receptors by APV (**A**) or of NOX by celastrol (**B**) abolished both the rapid H₂O₂ production (arrows; A: n = 15; B: n = 7) and the associated DC shift.

Figure 4: Fast H₂O₂ release and DC shift magnitudes depend on the efficacy of cellular cytoplasmic antioxidant defense.

A. Interictal activity-induced fast H_2O_2 release is increased following replacement of glucose for pyruvate in ACSF (see H_2O_2 release records above). In pyruvate-ACSF, mitochondria have sufficient fuel for generation of ATP, however intracellular cytoplasmic pentose phosphate pathway dependent antioxidant defense is diminished due to the absence of glucose (see schematic). **B.** The DC shift is significantly enhanced in pyruvate-ACSF ($n = 12$). Graph on the right demonstrates summary of the DC shift amplitude in glucose-ACSF and pyruvate-ACSF solutions.

Figure 5: Long-lasting hippocampal seizure activity induced in an anesthetized rat by intracerebroventricular 4-AP injection ($7\mu\text{g}/1\mu\text{L}$).

Corresponding dynamics of seizure frequency and duration (right). Injection of 4-AP induced seizure activity stable for at least 2 hours.

Figure 6: NOX inhibition reduces seizure activity in vivo. A-C. LFP recordings in hippocampus of anesthetized rats.

Intracerebroventricular injection of 4-AP induced regular ictal discharges (red, see also Fig. 5; $n = 13$) that were suppressed by application of NMDA antagonist APV (**A**; $4\mu\text{g}/1\mu\text{L}$; $n = 4$) or by inhibition of NOX with GSK2795039 (**B**; $45\mu\text{g}/1\mu\text{L}$; $n = 5$) or celastrol (**C**; $72\mu\text{g}/2\mu\text{L}$; $n = 4$). (**Aa**) and (**Bb**) demonstrate long-lasting field recordings (gray) with the inserted black traces representing field recordings low-pass filtered at 1Hz, that shows clearly ultra-slow baseline deviations during seizure activity. (**C**) also demonstrates extracellular H_2O_2 release (small dash) and distribution of H_2O_2 peak amplitudes. **D.** Summary of *in vivo* experiments. The DC line index represents the low-frequency deviation from the LFP baseline (see Methods).

Figure 7:

A. Long-lasting hippocampal seizure activity induced in an anesthetized rat by intracerebroventricular kainate injection. **B.** Inhibition of kainate-induced seizures by celastrol ($72\mu\text{g}/2\mu\text{L}$; $n = 3$). Upper trace demonstrates long-lasting field recordings (gray) with an inserted black trace representing field recording low-pass filtered at 1Hz, clearly showing ultra-slow baseline deviations during seizure activity.

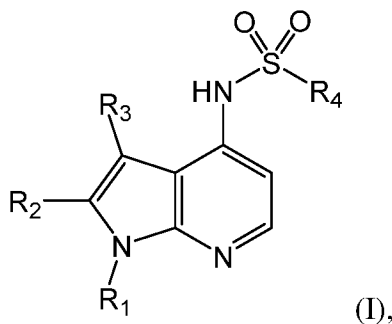
Figure 8: Concurrent combination of several events is required for seizure induction.

A. Both spontaneous and induced seizures are associated with tissue swelling. **B.** Schematic representation of events during seizure induction.

DETAILED DESCRIPTION OF THE INVENTION

Compounds

5 The present invention thus relates to a compound of formula (I):



for use for preventing an epileptic seizure,

wherein:

➤ R₁ represents:

- 10
- a hydrogen,
 - a (C₁-C₆)alkyl group optionally substituted by at least one radical selected in a group consisting of:
 - a cycloalkyl, a heterocycloalkyl, or an aryl, optionally substituted by at least one (C₁-C₆)alkyl group, (C₁-C₆)alkyloxy group, or a halogen,
 - 15 ○ a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group, and
 - a (C₁-C₆)alkyloxy group optionally substituted by a trimethylsilyl group, or
 - a cycloalkyl or a heterocycloalkyl optionally substituted by at least one (C₁-C₆)alkyl group, (C₁-C₆)alkyloxy group, or a halogen;

20 ➤ R₂ represents:

- a hydrogen,
- a (C₁-C₆)alkyl group,
- a halogen, or
- an indoline or a heteroaryl optionally substituted by a (C₁-C₆)alkyl group;

25 ➤ R₃ represents an aryl, a heteroaryl, an indoline, a tetrahydroquinoline, optionally substituted by at least one radical selected in the group consisting of:

- a heterocycloalkyl optionally substituted by a (C₁-C₆)alkyl group or a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group,

- a (C₁-C₆)alkyl group,
- a (C₁-C₆)alkyloxy group, and
- a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group; and

- 5 ➤ R₄ represents an aryl or a heteroaryl optionally substituted by at least one radical selected in the group consisting of a (C₁-C₆)alkyl group, a (C₁-C₆)alkyloxy group, a halogen, and a cyano;

or a pharmaceutically acceptable salt thereof.

- 10 According to the present invention, the terms below have the following meanings:

The terms mentioned herein with prefixes such as for example C₁-C₃ or C₁-C₆ can also be used with lower numbers of carbon atoms such as C₁-C₂, or C₁-C₅. If, for example, the term C₁-C₃ is used, it means that the corresponding hydrocarbon chain may comprise from 1 to 3 carbon atoms, especially 1, 2 or 3 carbon atoms. If, for example, the term C₁-C₆ is used, it means that the
15 corresponding hydrocarbon chain may comprise from 1 to 6 carbon atoms, especially 1, 2, 3, 4, 5 or 6 carbon atoms.

- The term “alkyl” refers to a saturated, linear or branched aliphatic group. The term “(C₁-C₃)alkyl” more specifically means methyl, ethyl, propyl, or isopropyl. The term “(C₁-C₆)alkyl” more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl or hexyl. In
20 a preferred embodiment, the “alkyl” is a methyl, an ethyl, or an isopropyl, more preferably a methyl or an isopropyl.

- The term “alkyloxy” or “alkoxy” corresponds to the alkyl group as above defined bonded to the molecule by an -O- (ether) bond. (C₁-C₃)alkyloxy includes methoxy, ethoxy, propyloxy, and isopropyloxy. (C₁-C₆)alkyloxy includes methoxy, ethoxy, propyloxy, isopropyloxy, butyloxy,
25 isobutyloxy, *tert*-butyloxy, pentyloxy and hexyloxy.

The term “halogen” corresponds to a fluorine, a chlorine, a bromine, or an iodine atom.

- The term “cycloalkyl” corresponds to a saturated or unsaturated mono-, bi- or tri-cyclic alkyl group comprising between 3 and 20 atoms of carbons. It also includes fused, bridged, or spiro-connected cycloalkyl groups. The term “cycloalkyl” includes for instance cyclopropyl, cyclobutyl,
30 cyclopentyl, and cyclohexyl. The term “cycloalkyl” may also refer to a 5-10 membered bridged carbocyclyl.

The term “heterocycloalkyl” corresponds to a saturated or unsaturated cycloalkyl group as above defined further comprising at least one heteroatom such as nitrogen, oxygen, or sulphur atom. It also includes fused, bridged, or spiro-connected heterocycloalkyl groups. Representative

heterocycloalkyl groups include, but are not limited to 3-dioxolane, benzo [1,3] dioxolyl, azetidiny, oxetanyl, pyrazolinyl, pyranyl, thiomorpholinyl, pyrazolidinyl, piperidyl, piperazinyl, 1,4-dioxanyl, imidazolinyl, pyrrolinyl, pyrrolidinyl, piperidinyl, imidazolidinyl, morpholinyl, 1,4-dithianyl, pyrrolidinyl, oxazoliny, oxazolidinyl, isoxazolinyl, isoxazolidinyl, thiazolinyl, thiazolidinyl, isothiazolinyl, isothiazolidinyl, dihydropyranyl, tetrahydro-2H-pyranyl, tetrahydrofuranly, and tetrahydrothiophenyl. The term “heterocycloalkyl” may also refer to a 5-10 membered bridged heterocycloalkyl.

The term “aryl” corresponds to a mono- or bi-cyclic aromatic hydrocarbons having from 6 to 12 carbon atoms. For instance, the term “aryl” includes phenyl, biphenyl, or naphthyl.

The term “heteroaryl” as used herein corresponds to an aromatic, mono- or poly-cyclic group comprising between 5 and 14 atoms and comprising at least one heteroatom such as nitrogen, oxygen or sulphur atom. The terms “fused arylheterocycloalkyl” and “fused arylcycloalkyl” correspond to a bicyclic group in which an aryl as above defined is bounded to a heterocycloalkyl or a cycloalkyl as above defined by at least two carbons. In other terms, the aryl shares a carbon bond with the heterocycloalkyl or the cycloalkyl. Examples of such mono- and poly-cyclic heteroaryl group, fused arylheterocycloalkyl, and “fused arylcycloalkyl” may be: pyridinyl, thiazolyl, thiophenyl, furanyl, pyrrolyl, pyrazolyl, imidazolyl, triazolyl, tetrazolyl, benzofuranly, thianaphthalenyl, indolyl, indolinyl, quinolinyl, isoquinolinyl, benzimidazolyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, triazinyl, thianthrenyl, isobenzofuranly, chromenyl, xanthenyl, phenoxanthinyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indoliziny, isoindolyl, indazolyl, purinyl, quinoliziny, phtalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, carbazolyl, β -carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, indolinyl, isoindolinyl, oxazolidinyl, benzotriazolyl, benzoisoxazolyl, oxindolyl, benzoxazolinyl, benzothienyl, benzothiazolyl, isatinyl, dihydropyridyl, pyrimidinyl, s-triazinyl, oxazolyl, or thiofuranly. A preferred heteroaryl group is a pyrazolyl. A preferred fused arylcycloalkyl group is an indolinyl.

The expression “substituted by at least” means that the radical is substituted by one or several groups of the list.

The “pharmaceutically salts” include inorganic as well as organic acids salts. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, maleic, methanesulfonic and the like. Further examples of pharmaceutically inorganic or organic acid

addition salts include the pharmaceutically salts listed in J. Pharm. Sci. 1977, 66, 2, and in Handbook of Pharmaceutical Salts: Properties, Selection, and Use edited by P. Heinrich Stahl and Camille G. Wermuth 2002. In a preferred embodiment, the salt is selected from the group consisting of maleate, chlorhydrate, bromhydrate, and methanesulfonate. The “pharmaceutically salts” also include inorganic as well as organic base salts. Representative examples of suitable inorganic bases include sodium or potassium salt, an alkaline earth metal salt, such as a calcium or magnesium salt, or an ammonium salt. Representative examples of suitable salts with an organic base includes for instance a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine. In a preferred embodiment, the salt is selected from the group consisting of sodium and potassium salts.

In a particular embodiment, R₁ represents a (C₁-C₆)alkyl group. Preferably, R₁ represents a methyl, an ethyl, or an isopropyl group, more preferably an isopropyl group.

In a further particular embodiment, R₂ represents a hydrogen.

In a further particular embodiment, R₃ represents an indoline optionally substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

In a further particular embodiment, R₄ represents a heteroaryl optionally substituted by a (C₁-C₆)alkyl group. Preferably, R₄ represents a pyrazole optionally substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

A preferred compound for use according to the invention is a compound of formula (I) in which:

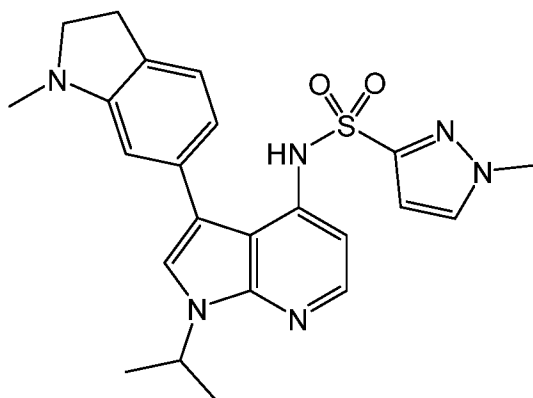
- R₁ represents a (C₁-C₆)alkyl group, preferably an isopropyl;
- R₂ represents a hydrogen,
- R₃ represents an indoline, preferably substituted by a (C₁-C₆)alkyl group, preferably a methyl; and
- R₄ represents an heteroaryl, preferably a pyrazole, preferably substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

In a more preferred embodiment, a compound for use of formula (I) is N-(1-isopropyl-3-(1-methylindolin-6-yl)-1H-pyrrolo[2,3-b]pyridine-4-yl)-1-methyl-1H-pyrazole-3-sulfonamide.

N-(1-isopropyl-3-(1-methylindolin-6-yl)-1H-pyrrolo[2,3-b]pyridine-4-yl)-1-methyl-1H-pyrazole-3-sulfonamide, also called herein GSK2795039, has been used by Hirano et al. (Antioxidants & Redox Signaling, 2015, 23, 358-374) to demonstrate inhibition of the NOX2 enzyme *in vivo* and

the therapeutic potential of such compound in diseases, such as paw inflammation and acute pancreatitis.

GSK2795039 has the following formula:



5

The compounds of formula (I) for use according to the present invention may be prepared by any synthetic routes currently used and known from a skilled person. Preferably, the compounds of formula (I) are prepared using protocols of synthesis disclosed by WO 2012/170752.

10 Application

The present invention relates to a compound of formula (I) as above defined, or a pharmaceutically acceptable salt thereof, for use for preventing an epileptic seizure. The invention further relates to a pharmaceutical composition comprising a compound of formula (I) as above defined, or a pharmaceutically acceptable salt thereof, for use for preventing an epileptic seizure.

As used herein, the terms “prevention”, “prevent”, or “preventing” refer to any act, such as the use of a compound of formula (I) according to the invention, intended to avoid the apparition, the trigger, the induction, or the occurrence of an epileptic seizure in a subject. Such terms also mean the reduction of the risks of an apparition, a trigger, an induction, or an occurrence of an epileptic seizure in a subject. The epileptic seizure can be a focal or generalized seizure. Accordingly, treatments of the invention are applied before the epileptic seizure occurs. Thus, such treatments do not include the curative treatments applied in a subject to fix the damages after a seizure occurs, such as for instance, treating seizure-induced neuronal death. The treatment of the invention can therefore delay the epileptic seizure onset.

As used herein, the terms “subject”, “individual” or “patient” are interchangeable and refer to an animal, preferably to a mammal, even more preferably to a human. In a particular embodiment, the subject is a subject suffering from epilepsy or a subject having potential to trigger an epileptic seizure or a subject at risks for developing an epileptic seizure. In a preferred embodiment, the patient has already been the subject of at most three, two or one seizures. The treatment of the invention is more particularly suitable for such patient which presents therefore a long-term risk of further seizures.

In a further aspect, the invention relates a pharmaceutical composition comprising a compound of formula (I) as above defined, or a pharmaceutically acceptable salt thereof, for use for reducing the risks of the apparition of an epileptic seizure in a subject, preferably suffering from epilepsy.

A further object of the invention is a use of a compound of formula (I) as above defined, or a pharmaceutically acceptable salt thereof, for the manufacture of a pharmaceutical composition for preventing an epileptic seizure. A further object of the invention is a use of a compound of formula (I) as above defined, or a pharmaceutically acceptable salt thereof for reducing the risks of the apparition of an epileptic seizure in a subject, preferably suffering from epilepsy.

The present invention also concerns a method for reducing the risks of an apparition or a trigger of an epileptic seizure in a subject, comprising administering an effective amount of a compound of formula (I) or a pharmaceutical salt thereof, or a pharmaceutical composition as defined herein in said subject, preferably suffering from epilepsy.

The present invention further concerns a method for avoiding an apparition or a trigger of an epileptic seizure in a subject, comprising administering an effective amount of a compound of formula (I) or a pharmaceutical salt thereof, or a pharmaceutical composition as defined herein in said subject, preferably suffering from epilepsy.

As shown by the examples, the present invention relies to the inhibition of NOX enzyme, especially NOX2, to prevent epilepsy seizure, avoiding thereby a patient to trigger a seizure.

The present invention thus further concerns a method for inhibiting NOX enzyme, especially NOX2 in a subject, preferably suffering from epilepsy, comprising administering an effective amount of a compound of formula (I) or a pharmaceutical salt thereof, or a pharmaceutical composition as defined herein in said subject, , thereby reducing the risks and/or avoiding an

apparition or a trigger of an epileptic seizure in said subject. In a preferred embodiment, the inhibition of NOX2 by a compound of formula (I) is a direct inhibition of NOX2.

The present invention further concerns a method for blocking the H₂O₂ release and/or the DC shift in a subject, preferably suffering from epilepsy, comprising administering an effective amount of
5 a compound of formula (I) or a pharmaceutical salt thereof, or a pharmaceutical composition as defined herein in said subject, thereby reducing the risks and/or avoiding an apparition or a trigger of an epileptic seizure in said subject.

As used herein, the terms “quantity,” “amount,” and “dose” are used interchangeably herein and
10 may refer to an absolute quantification of a molecule.

As used herein, the term “effective amount” refers to a sufficient quantity of a compound of formula (I), or a pharmaceutically acceptable salt thereof, or of a pharmaceutical composition that prevents, reduces the risks and/or avoids an apparition, a trigger, an induction, or an occurrence of
15 an epileptic seizure in a patient. It is obvious that the quantity to be administered can be adapted by the man skilled in the art according to the subject to be treated, etc. In particular, doses and regimen of administration may be function of the nature, the stage, the frequency, and the severity of the epileptic seizures to be treated, as well as of the weight, the age and the global health of the subject to be treated, as well as of the judgment of the doctor.

20 In a particular embodiment, the pharmaceutical composition of use according to the invention comprises a pharmaceutically acceptable carrier and/or at least one excipient.

As used herein, the term "excipient or pharmaceutically acceptable carrier" refers to any ingredient except active ingredients that is present in a pharmaceutical composition. Its addition may be
25 aimed to confer a particular consistency or other physical or gustative properties to the final product. An excipient or pharmaceutically acceptable carrier must be devoid of any interaction, in particular chemical, with the active ingredients.

In a particular embodiment, the pharmaceutical composition as defined herein comprises a
30 compound of formula (I) in a dose from 0.1 to 500 mg/kg BW preferably from 10 to 200 mg/kg BW, more preferably about 100 mg/kg BW. A particular object of the invention is thus a pharmaceutical composition for use as disclosed herein, in which said composition is administered at a dose from 0.1 to 500 mg/kg BW preferably from 10 to 200 mg/kg BW, more preferably about 100 mg/kg BW. As used herein, the term “BW” means bodyweight.

In a particular aspect, the compounds and the pharmaceutical compositions for use of the invention can be administered once a week, two days a week, four days a week, once a day, preferably once a day. Treatments of the invention may be applied during one week, several weeks such as 2, 3, 4, and 5 weeks, one month, several months such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 months, one year, 2, 3, 4, 5 years, and up to the death of the subject.

In a preferred embodiment, the pharmaceutical composition for use according to the invention is administered once a week, two days a week, four days a week, once a day, preferably once a day.

The administration route can be topical, transdermal, oral, rectal, sublingual, intranasal, intrathecal, or parenteral (including subcutaneous, intramuscular, intraperitoneal, intracerebroventricular, intravenous and/or intradermal). In a preferred aspect, the administration route is oral. In a further preferred aspect, the administration is parenteral, preferably intracerebroventricular. The pharmaceutical composition is adapted for one or several of the above-mentioned routes.

The pharmaceutical composition can be formulated as solutions in pharmaceutically compatible solvents or as emulsions, suspensions or dispersions in suitable pharmaceutical solvents or vehicles, or as pills, tablets or capsules that contain solid vehicles in a way known in the art. Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient. Every such formulation can also contain other pharmaceutically compatible and nontoxic auxiliary agents, such as, e.g. stabilizers, antioxidants, binders, dyes, emulsifiers or flavoring substances. The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof. The pharmaceutical compositions are advantageously applied by injection or intravenous infusion of suitable sterile solutions or as oral dosage by the digestive tract. Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature.

In a further preferred embodiment, the pharmaceutical composition for use according to the invention is orally administered at a dose of about 100 mg/kg BW once a day, preferably once a day.

5 A further particular object of the invention concerns a pharmaceutical composition for use according to the invention, further comprising an anti-epileptic drug. As used herein, an anti-epileptic drug includes drugs for treating epilepsy as well as drug for preventing an epileptic seizure. In a preferred embodiment, the anti-epileptic drug is an anti-seizure drug. An anti-epileptic drug includes any medicines currently used for preventing and/or treating an epileptic seizure. As
10 an example of an anti-epileptic drug without limitation, it may be cited Acetazolamide, Brivaracetam, Carbamazepine, Clobazam, Clonazepam, Eslicarbazepine acetate, Ethosuximide, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, Oxcarbazepine, Perampanel, Phenobarbital, Phenytoin, Piracetam, Pregabalin, Primidone, Rufinamide, Sodium valproate, Stiripentol, Tiagabine, Topiramate, Valproic acid, Vigabatrin, and Zonisamide.

15 Further aspects and advantages of the present invention will be described in the following examples, which should be regarded as illustrative and not limiting.

EXAMPLES

20

MATERIAL AND METHODS

Animals

All animal protocols and experimental procedures were approved by the INSERM Ethics
25 Committee for Animal Experimentation (#30-03102012).

Tissue slice preparation

Ex-vivo local field potential (LFP) recordings were performed on brain slices from P21-56 OF1 male mice (Charles River Laboratories, France). A mouse anaesthetized with isoflurane was
30 decapitated; the brain was rapidly removed from the skull and placed in the ice-cold ACSF. The ACSF solution consisted of (in mmol/L): NaCl 126, KCl 3.50, NaH₂PO₄ 1.25, NaHCO₃ 25, CaCl₂ 2.00, MgCl₂ 1.30, and dextrose 5, pH 7.4. ACSF was aerated with 95% O₂/5% CO₂ gas mixture. Sagittal slices (350 μm) were cut using a tissue slicer (Leica VT 1200s, Leica Microsystem, Germany). During cutting, slices were submerged in an ice-cold (< 6°C) solution consisting of (in

mmol/L): K-gluconate 140, HEPES 10, Na-gluconate 15, EGTA 0.2, NaCl 4, pH adjusted to 7.2 with KOH. Slices were immediately transferred to a multi-section, dual-side perfusion holding chamber with constantly circulating ACSF and allowed to recover for 2h at room temperature (22°C-24°C).

5

Synaptic stimulation and field potential recordings

Slices were transferred to a recording chamber continuously superfused (10 ml/min) with ACSF (33-34°C) with access to both slice sides. Schaffer collateral/commissures was stimulated using the DS2A isolated stimulator (Digitimer Ltd, UK) with a bipolar metal electrode. Stimulus current was adjusted using single pulses (40-170 μ A, 200 μ s, 0.15 Hz) to induce a LFP of about 50% maximal amplitude. LFPs were recorded using glass microelectrodes filled with ASCF, placed in stratum pyramidale of CA1 area and connected to the ISO DAM-8A amplifier (WPI, FL). Synaptic stimulation consisted of a stimulus train (200 μ s pulses) at 100 Hz lasting 1s.

Oxygen, glutamate and H₂O₂ measurements

A Clark-style oxygen microelectrode (Unisense Ltd, Denmark) was used to measure slice tissue pO₂, while extracellular H₂O₂ was measured with Null sensor (Sarissa Biomedical, Coventry, UK). Extracellular glutamate was measured using enzymatic microelectrodes (Sarissa Biomedical, Coventry, UK) connected to a free radical analyzer TBR4100 (Word Precision Instruments Ltd, UK).

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Pharmacology

Antagonists of NMDA receptors, (2R)-amino-5-phosphonopentanoate (APV) and kynurenic acid; antagonist of AMPA/kainate receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and GABAA receptors, bicuculline, were purchased from Tocris Bioscience (Bio-Techne Ltd, UK); 4-aminopyridine (4-AP) and Celastrol were purchased from Sigma-Aldrich (Sigma-Aldrich Chimie S.a.r.l.); GSK2795039 from MedChemExpress Europe.

25

In Vivo Experiments

19 mature Wistar male rats (300-400g) were used for *in vivo* experiments. Animals were anesthetized with pentobarbital (35mg/kg) supplemented with xylazine (10 mg/kg). Body temperature was kept at 37°C using a heating pad. Rats were placed in a stereotaxic frame, scalped and holes for electrodes and guide cannula were drilled. Extracellular nichrome electrode ($\varnothing$ 25 μ m) was implanted in hippocampal CA1 field (stereotaxic coordinates AP=-3.2mm, L= 2mm,

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H=3.5 mm), guide cannula for drug injection was implanted just above lateral ventricle (AP=0.8mm, L= 1.7mm, H=2.3 mm). Indifferent electrode was screwed into occipital bone. In experiments with H₂O₂ sensor, additional cranial window (Ø 2mm) above contralateral hippocampus was drilled and sensor was dipped in hippocampus (3.5mm) using stereotaxic manipulator. After surgical preparation, the cortex was kept under saline to prevent drying. Stereotaxic frame was transferred to electrophysiological setup and recordings started. LFPs were amplified (Grass Instrument, U.S.A.), filtered (high-pass filter 0.01 Hz, digitized at 5 kHz), and stored using DataPack2k software (RUN Technologies, USA). H₂O₂ sensor (Sarissa probe) was polarized +500mV and recorded using potentiostat (Diamond Electrotech). Following 10 min recordings of control activity either 7µg of 4-AP in 1µL saline or 0.1µg of kainate (KA) in 2µL was injected i.c.v. using Hamilton syringe to induce seizure activity. After induction of stable seizure activity (at least 1 seizure episode per 5 min, from 30 to 60 min after 4-AP/KA injection) drugs were injected i.c.v. (APV 4µg in 1µL saline, GSK2795039 45µg in 1µL DMSO, celastrol 72µg in 2µL DMSO).

For spontaneous seizure detection, automated computer analysis of LFP recordings was used with the following selection limits: seizure threshold 7*RMS (root mean square) of baseline; number of oscillations in a scanning window (5 s) exceeding the threshold no less than 10; minimal seizure duration 10 s; time interval between seizures no less than 3 s.

For low frequency (DC) shift analysis representative episodes 300s long in each experiment in control, after 4-AP/KA and after drug injection were chosen. LFPs were filtered from 0 to 1 Hz, the average LFP amplitude was set as zero and the negative component of LFP was reflected against zero. DC line index was calculated as an area (integral) under resulting curve normalized to control values.

Statistical analysis and signal processing

Group measures were expressed as means ±SEM. Statistical significance was assessed using the Wilcoxon's signed paired test and the Wilcoxon Rank-Sum test. Significance level was set at p<0.05. Correlation between two variables was calculated using Pearson's correlation coefficient (r). Signal analysis was performed using the IgorPro software (WaveMetrics, Inc., USA) with custom developed macros.

RESULTS

Glutamate-induced activation of NOX triggers seizures in hippocampal slices

Experiments in acute brain slices were performed using a 4-AP seizure model (n = 36) with results reproduced using bicuculline (n = 3) and low-Mg (0.1 mM; n = 12) seizure models.

Enhanced efficacy of both excitatory and inhibitory transmissions induced by 4-AP resulted in hippocampal network hyperexcitability manifested as interictal activity (Fig. 1A). Interictal discharges displayed two major patterns defined by the inventors either as “simple” or “complex” spikes (Fig. 1A: (a)-complex, (b)-simple). The complex spikes normally consisted of two or more closely spaced voltage peaks, while a “simple” spike was one peak only. All recorded spontaneous seizure-like events (SLEs) were preceded by a complex spike that was followed by DC shift (Fig. 1B, insets: arrows). Simultaneous field (black), extracellular glutamate (large dash) and H₂O₂ (small dash) measurements (Fig. 1B, lower traces) revealed that the complex spikes were associated with an especially high and fast production of H₂O₂ as well as an augmented release of glutamate; both glutamate and H₂O₂ peaks were correlated with the amplitude of the second but not the first spike in the complex (Fig. 1C). Accordingly, the spike-induced H₂O₂ production was positively correlated with the release of glutamate (Fig. 1D). The distribution of H₂O₂ peaks induced by interictal activity (Fig. 1E) revealed a presumable threshold value (black line) for subsequent DC shift and SLE initiation.

Taken together, these results suggest that SLE induction requires an interictal event of a specific profile - the complex spike - associated with suprathreshold release of H₂O₂. To investigate such a causal link, spontaneous SLEs were replicated using a 100Hz, 1s stimulation of Schaffer collaterals under 4-AP application. Spontaneous SLEs were observed in 44% of experiments, while synaptic stimulation successfully induced SLEs in each slice. In control ACSF, such stimulation normally resulted in an initial, rapid and small-amplitude production of H₂O₂ followed by a delayed (a few seconds) and a much larger but slow increase in extracellular H₂O₂ concentration (Fig. 2A, left, small dash trace). Since the last component was characteristic for the stimulation of Schaffer collaterals but was absent during spontaneous SLEs (Fig. 2A, right), its origin is likely related to the intracellular (mitochondrial) generation of reactive oxygen species (ROS) during en-masse activation of Schaffer collaterals. Therefore, it was focused primary attention on the initial fast H₂O₂ transient presumably directly related to SLE induction. It was also attempted to elucidate the origin of DC shift that is an inherent feature of SLEs.

Glutamate spillover during synaptic activity could potentially activate extra-synaptic receptors and thus induce secondary slow network depolarization resulting in the observed DC shift. However, application of CNQX (40 μ M), a potent antagonist of AMPA/KA receptors, inhibited the interictal activity but affected neither the fast H₂O₂ release nor the DC shift induced by synaptic stimulation (Fig. 2B). Indeed, CNQX prevents activation of postsynaptic AMPA/KA receptors, thus excluding the contribution of these receptors to the DC-shift. However, CNQX does not affect the presynaptic glutamate release during Schaffer collaterals stimulation, and presumably exactly this glutamate activates fast H₂O₂ release with a consequent induction of the DC-shift (see below).

Recent reports suggest KCC2 co-transporters as providers of enhanced extracellular K⁺ responsible for the DC shift during seizure initiation. However, application of a cation-chloride co-transporter's antagonist bumetanide (50 μ M) failed to reduce H₂O₂ release and to prevent neither the DC shift nor SLE induction by stimulation (n = 3; Fig. 2C). Note that bumetanide is not specific for the NKCC1 co-transporter only and at higher concentrations (50 μ M) also blocks KCC2. Finally, inhibition of GABAergic transmission by bicuculline (20 μ M) did not block spontaneous SLEs (n = 3; Fig. 2D) indicating that network synchronization by GABAergic neurotransmission is not mandatory for the SLE initiation.

Thus, neither activation of extrasynaptic AMPA/KA receptors nor GABAergic transmission turned to be primary players in the SLE onset. Therefore, the role of fast H₂O₂ release in the SLE initiation was investigated.

It was supposed that rapid H₂O₂ production is underlain by the activation of NADPH oxidase (NOX), as NOX's unique biological function is to generate ROS; NOX is expressed in membranes of both neurons and astrocytes and the NMDA receptor-dependent signaling is one of NOX activation pathways. Indeed, in the experiments a rise in extracellular glutamate preceding the SLE onset could be sufficient to activate NMDA receptors possessing high affinity to this neurotransmitter. Therefore, it was tested the effects of NMDA or NOX antagonists on SLE generation. NMDA receptor blockade by APV did not prevent the interictal activity nor significantly affected synaptic transmission (Fig. 3A). Meanwhile, under APV rapid H₂O₂ production was substantially decreased during interictal events and was ablated at same stimulus that normally induced SLEs (Fig. 3A, arrows). Importantly, APV prevented both the subsequent DC shifts and SLEs (in 14 of 15 experiments). Similarly, application of celastrol (40 μ M), a potent NOX antagonist, affected neither the interictal activity nor synaptic transmission (Fig. 3B), while

the fast H₂O₂ production was inhibited during interictal events and was almost eliminated at 100Hz stimulation. Celastrol also blocked the DC shifts and SLEs in 6 of 7 experiments.

NMDA-NOX signaling role in SLE induction was further confirmed by brief direct application of NMDA onto CA1 stratum radiatum under 4-AP: NMDA-induced DC shifts and SLEs were observed (not shown; n = 3; see also) and were also prevented by celastrol. It was concluded, therefore, that NOX activation via NMDA receptor signaling triggers a cascade of events initiating the DC shift and the subsequent SLE.

Direct involvement of ROS in the process of SLE initiation and DC shift generation was further confirmed by the experiments with substitution of ACSF glucose with pyruvate that weakened the cytoplasmic antioxidant defense system (see schematics in Fig. 4A). In pyruvate-ACSF, the fast H₂O₂ production during interictal events was significantly enhanced (Fig. 4A). Moreover, the DC shift during both the induced and spontaneous SLEs was increased (Fig. 4B), presumably due to higher extracellular K⁺ concentration.

Acute seizures in vivo are prevented by NOX antagonists

To verify the results obtained in slices acute seizures were induced in anesthetized rats by intracerebroventricular injection of 4-AP. Epileptic discharges started shortly (a few minutes) following 4-AP injection and lasted for more than two hours without decrementing (Fig. 5). The efficacy of three NOX antagonists possessing different mechanisms of action APV, celastrol and GSK2795039 was tested on inhibition of seizures (Fig. 6). All three antagonists strongly reduced seizure activity (Fig. 6D). Importantly, GSK2795039 is a novel small molecule selective direct inhibitor of NOX2 with efficiency demonstrated *in vivo* and with brain bioavailability demonstrated at oral administration. Therefore, it possesses significant therapeutic potential for the development of anti-seizure medication.

To ensure that the anti-ictogenic effects of NOX antagonists are not specific for the 4-AP-induced seizures only, as an alternative seizure activity was induced by i.c.v. injection of kainate (KA). KA has been widely used to induce acute brain seizures via activation of KA receptors expressed in both neurons and astrocytes. In all experiments (n=6), KA induced stable seizure activity (Fig. 7A). In three rats, celastrol was i.c.v. injected following the kainate-induced seizure activity: the average number of seizures under KA was (9.6 ± 1.85)/hour (average duration 27.6 ± 3.07 s); in all these rats seizure activity was completely abolished by application of celastrol (Fig. 7B). It

was concluded therefore that the NOX activation likely plays a dominating role in the onset of variety of acute seizures.

CONCLUSIONS

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The inventors have therefore demonstrated that the glutamate-induced and NMDA-mediated activation of NOX is the main trigger for seizures that are typical for focal epilepsies. Importantly, NOX2 upregulation has been reported in surgical hippocampal specimens from a patient suffering from pharmacoresistant seizures. Although elucidation of the subsequent process of actual seizure generation is a matter of future studies, the available data allow to propose the following sequence of events (Fig. 8B): NOX-induced oxidative stress transiently inhibits Na/K-ATPase, restricting the uptake of extracellular K⁺ (increased by several mM at the very beginning of seizure initiation) as well as the efflux of intracellular Na⁺, thus giving rise to focal network depolarization. The depolarization-induced additional K⁺ release as well as astrocytic glutamate release (e.g. Fig. 8A) may further increase this depolarization. In addition, it was observed significant tissue (astrocytic) swelling coincident at seizure onset with the sentinel spike (Fig. 8A) that can augment the extracellular K⁺/glutamate concentrations even further by reducing extracellular volume. Together, these events presumably underlie the DC shift. Pyramidal cell firing during the repolarization phase of the DC shift may underlie the network synchronization and following ictal discharges.

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Considering the origin of complex spikes, interictal events initiating seizures may likely be induced by initial intensive spiking of interneurons. Indeed, in slices under 4-AP, a brief (30ms) optogenetic interneuron stimulation evoked the appearance of interictal-like events and suggested that activation of interneurons recruits and entrains pyramidal cells; some of these stimuli also induced ictal events. *In vivo* animals with acute 4-AP application, optogenetic interneuron stimulation during the interictal phase readily induced seizures both in neocortex and hippocampus. Single-unit recordings revealed that the powerful ictogenic effect of interictal interneuron activation probably resulted from rebound firing enhancement of pyramidal neurons following the optogenetic interneuron stimulation. In chronic epileptic animals, interneurons were preferentially recruited during spontaneous interictal activity in the CA1 region, as well as before and during ictal events. In patients with mesial–temporal lobe epilepsy during the onset of L VF seizures in hippocampus, inhibitory neurons dramatically increased their firing rate prior to an increase in excitatory neuron firing. The general conclusion made from these data implies the dominating role of interneuron firing for the onset of both interictal and ictal events with the

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delayed pyramidal cell firing as the result of post-inhibition rebound excitation. In the present case, one possible scenario is that the complex spikes represent the initial interneuron firing (first peak) with the second peak representing the delayed pyramidal cell firing with subsequent NOX activation and corresponding release of H₂O₂. Correlation of glutamate (as well as H₂O₂) release with the second peak amplitude but not with the first one validates such an assumption (Fig. 1C). Indeed, NOX2 is mainly expressed in pyramidal cells and microglia while considering interneurons, available (although very limited) data report highly low (almost below sensitivity threshold) and much smaller than in pyramidal cells NOX2 expression. Worth noting is that the “complex” (consisting of several peaks) spikes in the presence of bicuculline was observed. However, inhibition of GABAergic transmission does not itself prevent the interneuron firing; in addition, there is no evidence that these spikes have the same origin as those observed in the presence of GABAergic transmission. As suggested, enhanced pyramidal cell excitability due to the absence of inhibition may be involved in these spontaneous events.

Considering the relevance of the NOX antagonists utilized in the inventor’s study for a clinical application, NMDA receptor antagonists, e.g., dizocilpine or ketamine, inhibited seizures in experimental animals evoked by pentetrazole, pilocarpine, maximal electroshock or sensory stimulation. However, NMDA receptor blockade can affect multiple brain functions and therefore chronic administration of NMDA receptor inhibitors, APV or its analogs, would likely lead to severe side effects. The therapeutic usefulness and anti-inflammatory properties of celastrol have been studied in several inflammatory diseases, including rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, inflammatory bowel disease, osteoarthritis, allergy and skin inflammation. However, despite the therapeutic potential of celastrol, clinical applications are limited by low water solubility, reduced oral bioavailability, narrow window of dosage, and adverse side effects. In contrast, GSK2795039 is not cytotoxic at concentrations efficacious for NOX2 inhibition. It has been shown that GSK2795039 is orally available and can be measured in the blood and central nervous system, suggesting that it can cross the blood–brain barrier. It fully inhibited NOX2 enzyme activity *in vivo* following systemic dosing in mice, while GSK2795039 effects were reversible since 24 h after administration. GSK2795039 was well tolerated by rodents, with no obvious adverse effects following 5 days of oral administration.

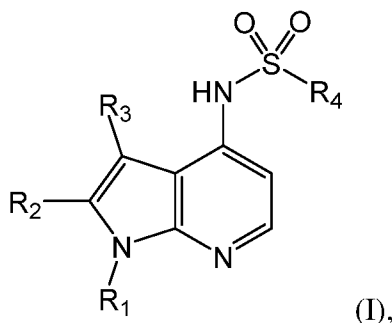
While considering the principal role of NOX-induced oxidative stress in seizure initiation, it is also important to note a failure of exogenous antioxidants to affect the fast H₂O₂ release. Indeed, as it was demonstrated previously, neither Tempol nor apocynin (potent antioxidants) significantly

affected the fast H₂O₂ transient, indicating that anti-seizure drug development should focus either on the enhancement of endogenous antioxidant defense and/or on direct antagonists of NOX. In addition, direct application H₂O₂ to quiescent slices either in ACSF perfusate or as short-lasting puffs failed to replicate the effects of NOX activation (the DC shift), indicating that a concurrent combination of several events, e.g. glutamate release and spillover inducing NOX activation and excessive release of K⁺ (see Fig. 8B) might be required for seizure induction.

In summary, the inventors have revealed a specific trigger mechanism for seizure onset as well as ways to block it and thus to prevent seizures, with major implications for epilepsy treatment. Such mechanism relies on the inhibition of NOX2 enzyme by compounds of formula (I) of the invention, specifically GSK2795039.

CLAIMS

1. A compound of formula (I):



5 for use for preventing an epileptic seizure,

wherein:

➤ R₁ represents:

- a hydrogen,
- a (C₁-C₆)alkyl group optionally substituted by at least one radical selected in a group consisting of:
 - a cycloalkyl, a heterocycloalkyl, or an aryl, optionally substituted by at least one (C₁-C₆)alkyl group, (C₁-C₆)alkyloxy group, or a halogen,
 - a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group, and
 - a (C₁-C₆)alkyloxy group optionally substituted by a trimethylsilyl group, or
- a cycloalkyl or a heterocycloalkyl optionally substituted by at least one (C₁-C₆)alkyl group, (C₁-C₆)alkyloxy group, or a halogen;

➤ R₂ represents:

- a hydrogen,
- a (C₁-C₆)alkyl group,
- a halogen, or
- an indoline or a heteroaryl optionally substituted by a (C₁-C₆)alkyl group;

➤ R₃ represents an aryl, a heteroaryl, an indoline, a tetrahydroquinoline, optionally substituted by at least one radical selected in the group consisting of:

- a heterocycloalkyl optionally substituted by a (C₁-C₆)alkyl group or a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group,
- a (C₁-C₆)alkyl group,
- a (C₁-C₆)alkyloxy group, and

- a -NR_aR_b unit with R_a and R_b being independently a hydrogen or a (C₁-C₆)alkyl group; and

➤ R₄ represents an aryl or a heteroaryl optionally substituted by at least one radical selected in the group consisting of a (C₁-C₆)alkyl group, a (C₁-C₆)alkyloxy group, a halogen, and a cyano;

or a pharmaceutically acceptable salt thereof.

2. A compound for use according to claim 1, wherein R₁ represents a (C₁-C₆)alkyl group.

3. A compound for use according to claim 1 or 2, wherein R₁ represents a methyl, an ethyl, or an isopropyl group, preferably an isopropyl group.

4. A compound for use according to any one of claims 1 to 3, wherein R₂ represents a hydrogen.

5. A compound for use according to any one of claims 1 to 4, wherein R₃ represents an indoline optionally substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

6. A compound for use according to any one of claims 1 to 5, wherein R₄ represents a heteroaryl optionally substituted by a (C₁-C₆)alkyl group.

7. A compound for use according to any one of claims 1 to 6, wherein R₄ represents a pyrazole optionally substituted by a (C₁-C₆)alkyl group, preferably a methyl group.

8. A compound for use according to any one of claims 1 to 7, wherein said compound is N-(1-isopropyl-3-(1-methylindolin-6-yl)-1H-pyrrolo[2,3-b]pyridine-4-yl)-1-methyl-1H-pyrazole-3-sulfonamide.

9. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8, for use for preventing an epileptic seizure.

10. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 8, for use for reducing the risks of the apparition of an epileptic seizure in a subject, preferably suffering from epilepsy.

11. A pharmaceutical composition for use according to claim 9 or 10, wherein said composition is administered by oral or parenteral route, preferably by oral route.

5 12. A pharmaceutical composition for use according to any one of claims 9 to 11, wherein said composition is administered at a dose from 0.1 to 500 mg/kg BW preferably from 10 to 200 mg/kg BW, more preferably about 100 mg/kg BW.

10 13. A pharmaceutical composition for use according to any one of claims 9 to 12, wherein said composition is administered once a week, two days a week, four days a week, once a day, preferably once a day.

14. A pharmaceutical composition for use according to any one of claims 9 to 13, wherein said composition is orally administered at a dose of about 100 mg/kg BW once a day.

15 15. A pharmaceutical composition for use according to any one of claims 9 to 14, wherein said composition further comprises an anti-epileptic drug.

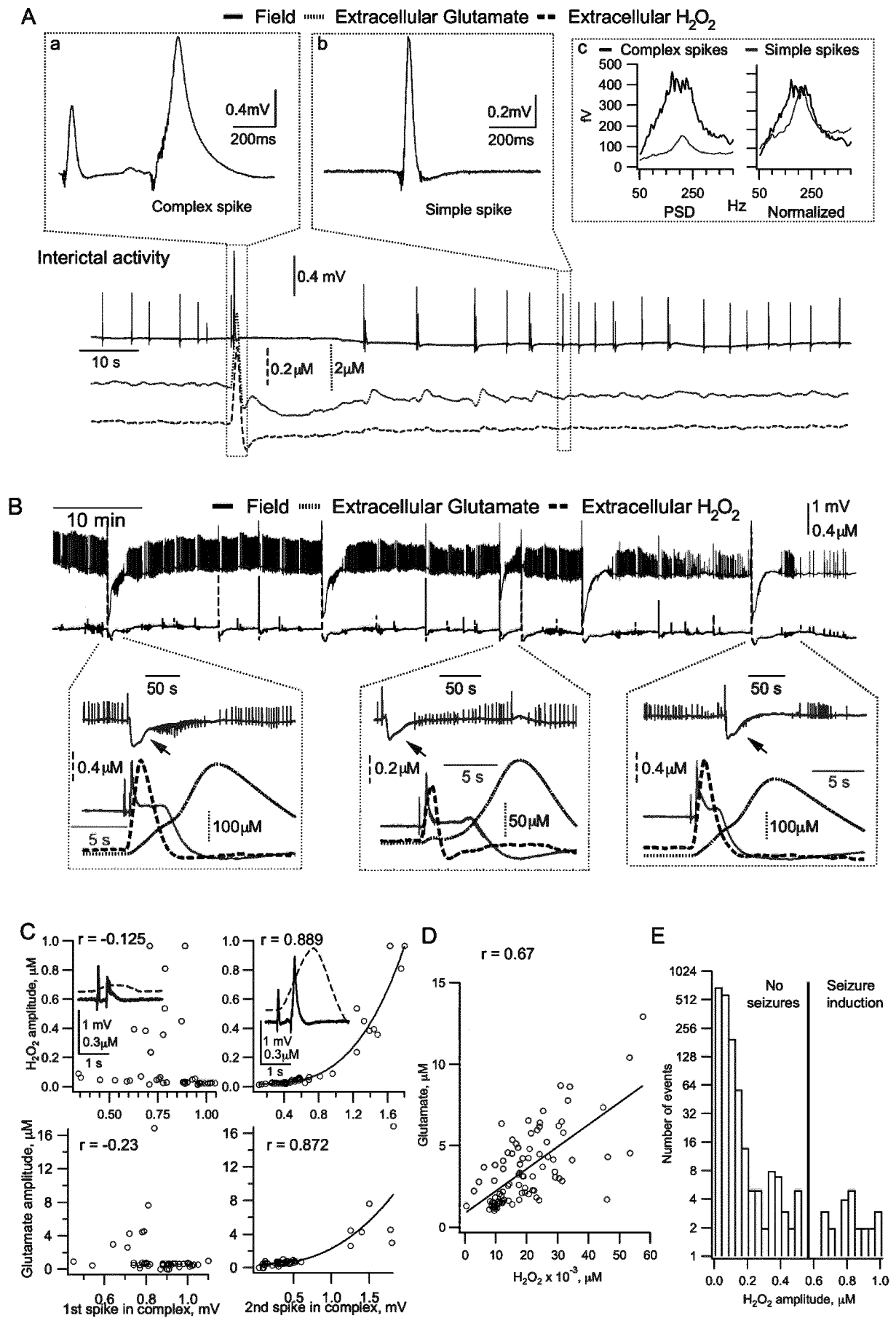


FIGURE 1

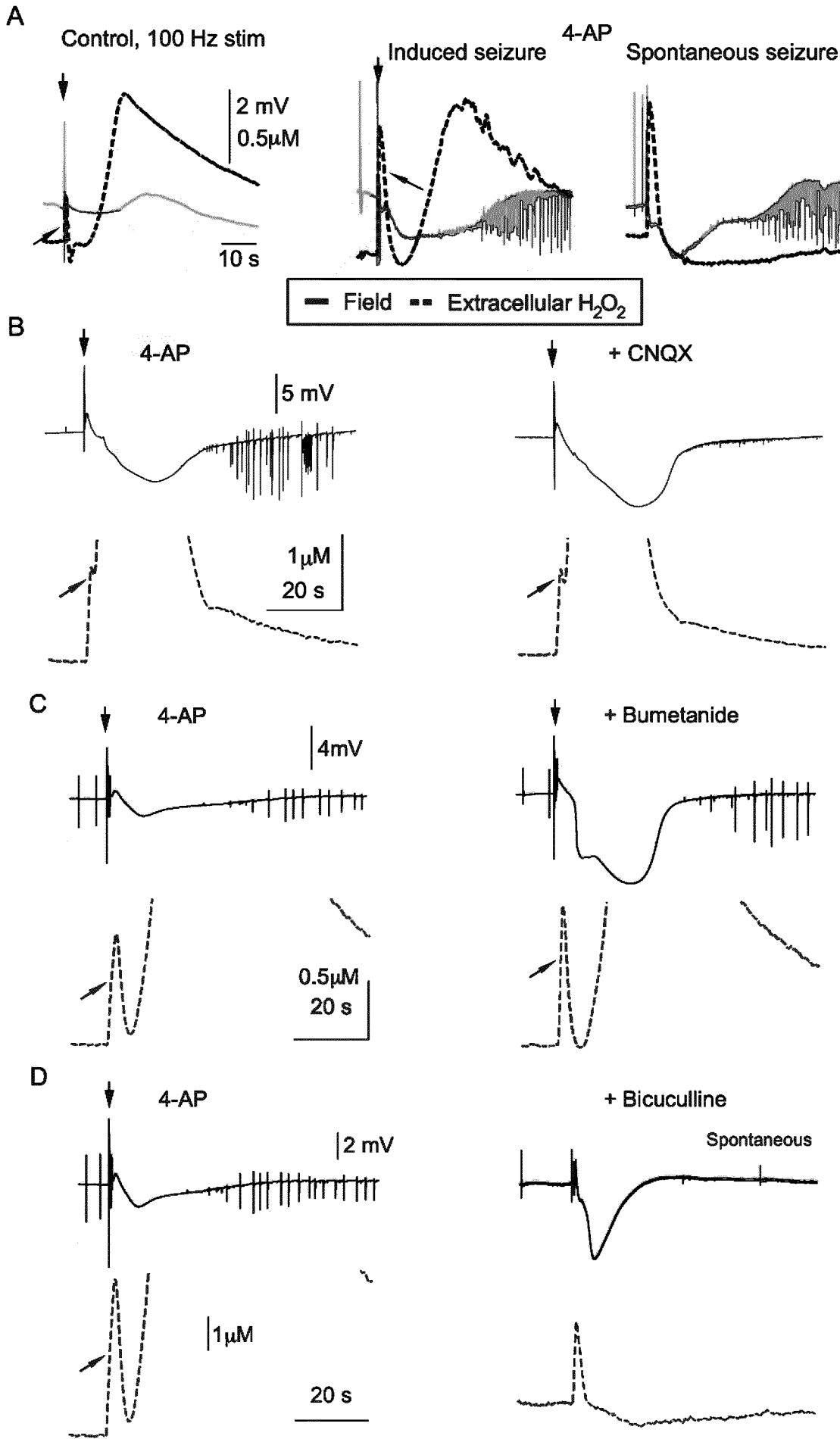


FIGURE 2

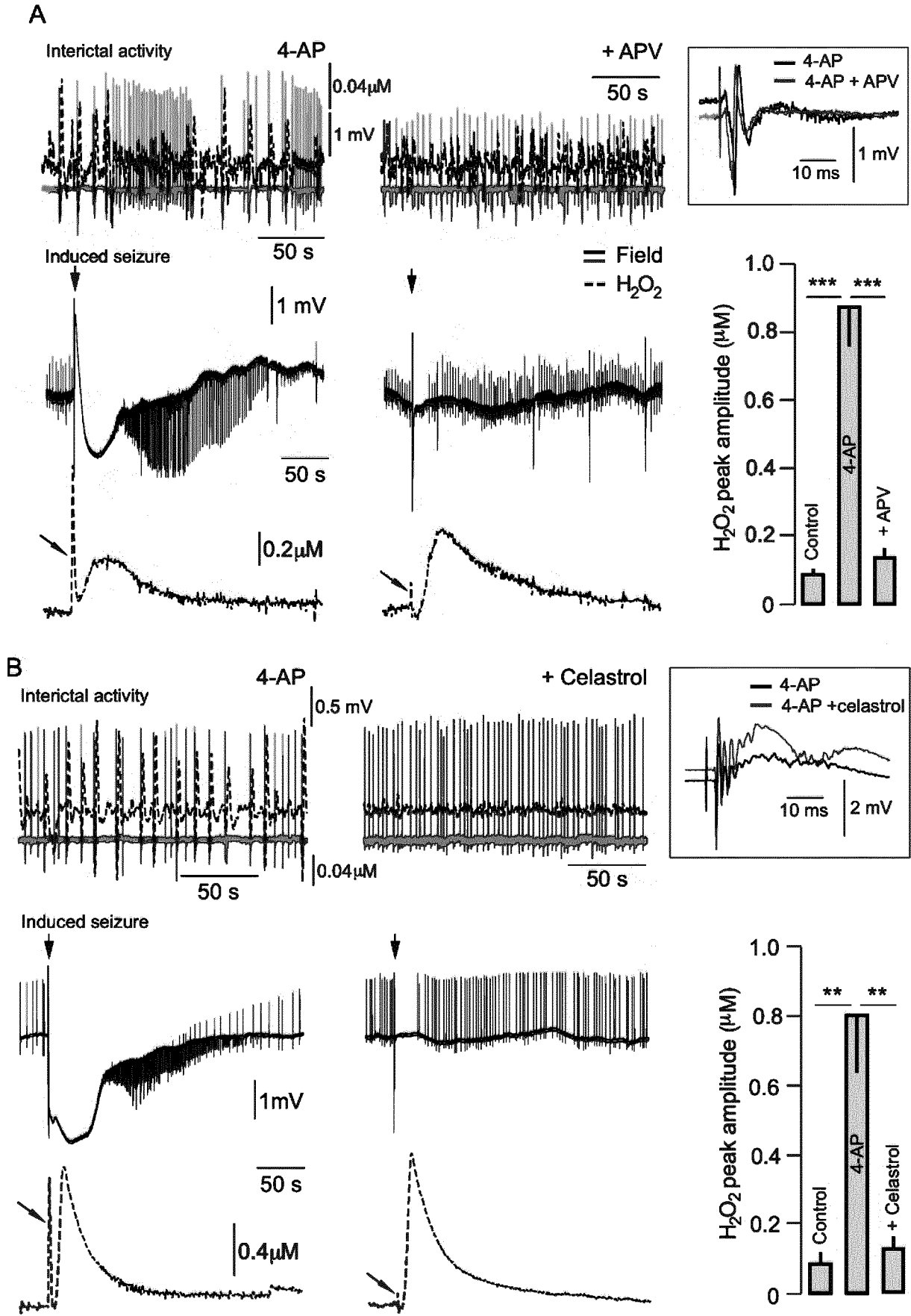


FIGURE 3

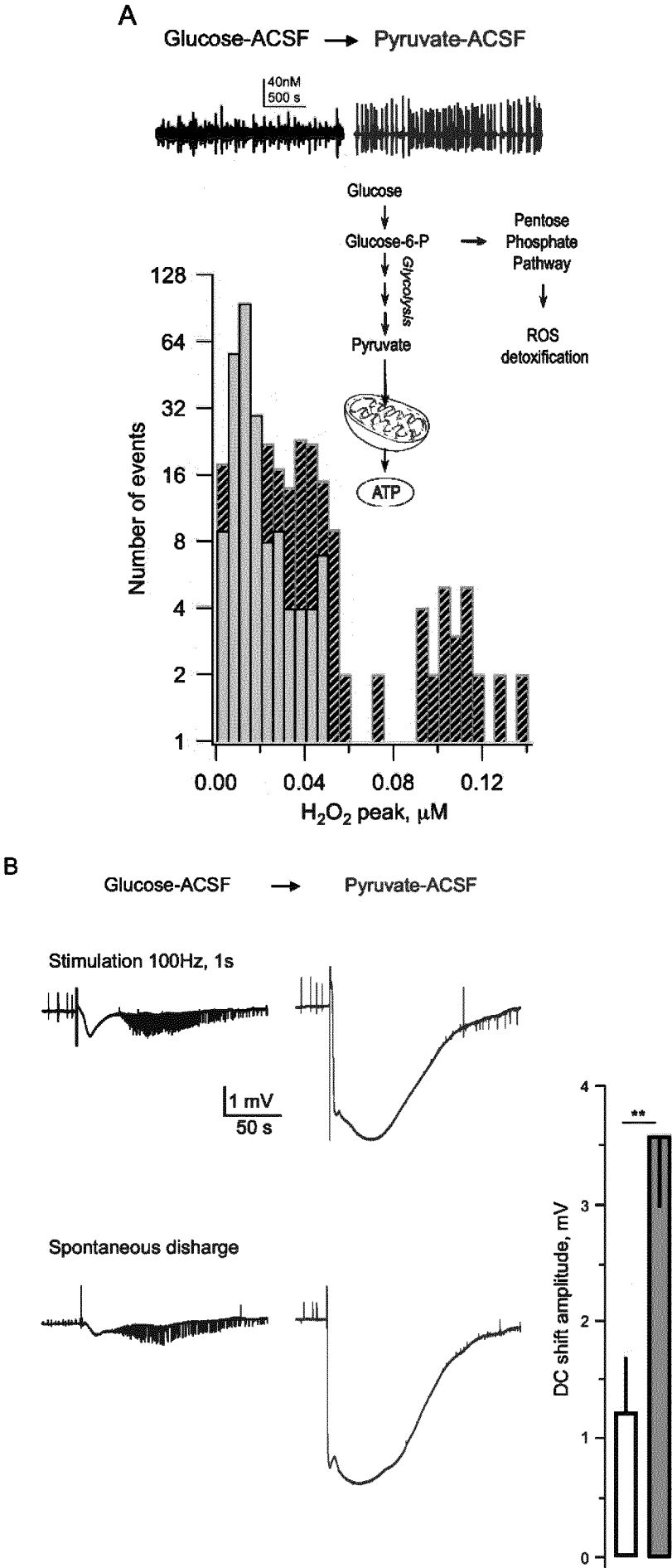


FIGURE 4

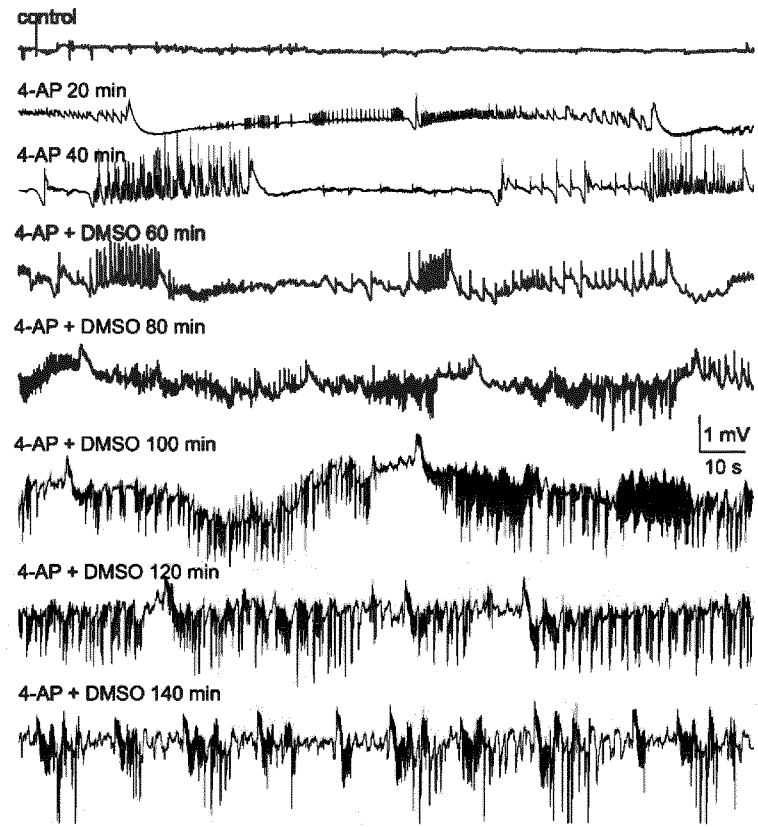
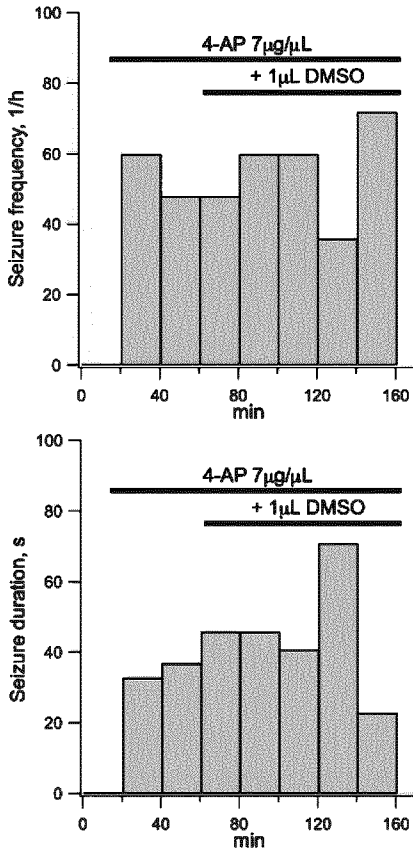


FIGURE 5



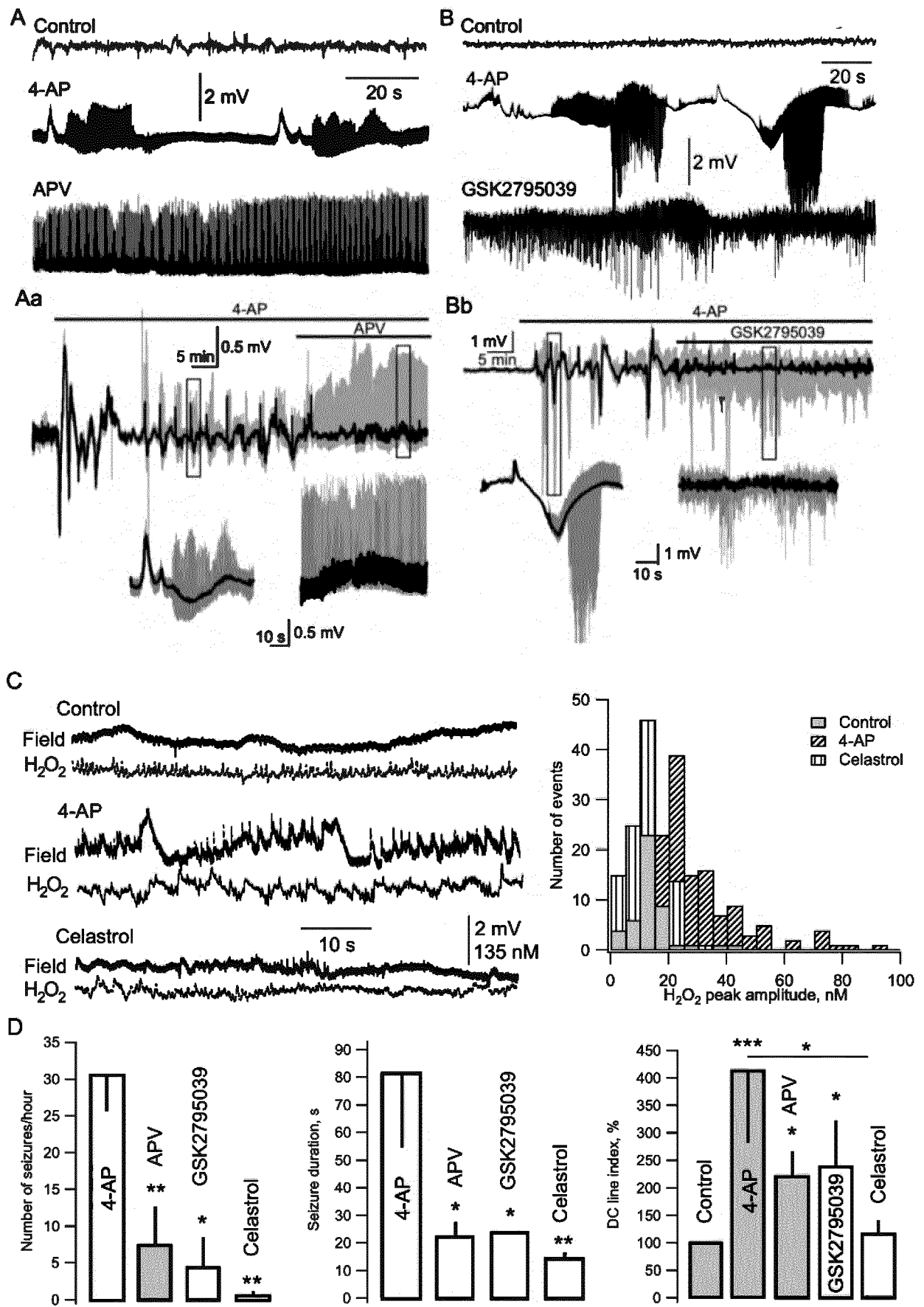


FIGURE 6

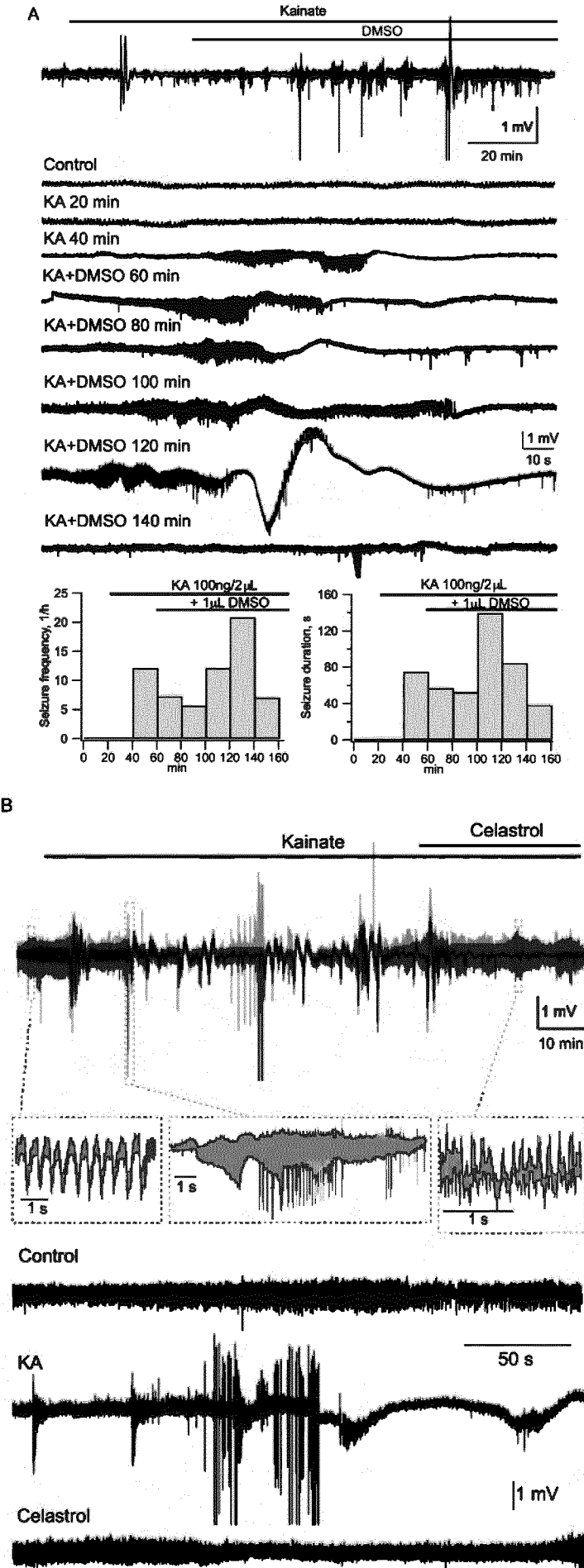


FIGURE 7

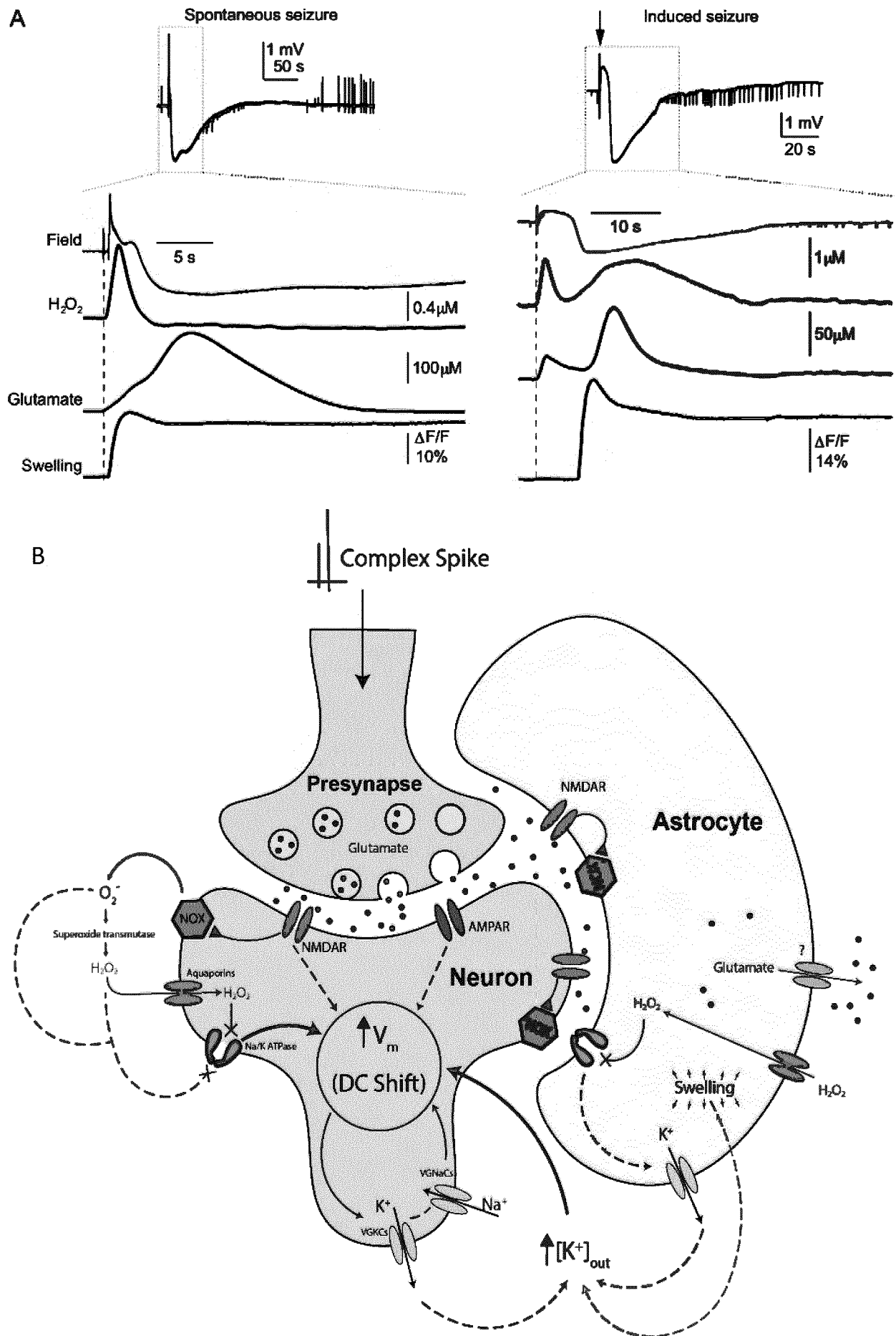


FIGURE 8

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/055504

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/437 A61P25/08 C07D471/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HUANG WAN-YU ET AL: "NADPH oxidases as potential pharmacological targets against increased seizure susceptibility after systemic inflammation.", JOURNAL OF NEUROINFLAMMATION 12 MAY 2018, vol. 15, no. 1, 12 May 2018 (2018-05-12), page 140, XP002793502, ISSN: 1742-2094 page 4, right-hand column, last paragraph - page 10, left-hand column, paragraph 1 -----	1-15
Y	US 2017/071902 A1 (TAKEMOTO DAISUKE [JP] ET AL) 16 March 2017 (2017-03-16) claims 1, 4, 5, 14, 15 ----- -/-	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

23 April 2020

Date of mailing of the international search report

12/05/2020

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Authorized officer

Collura, Alessandra

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/055504

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HIRANO KAZUFUMI ET AL: "Discovery of GSK2795039, a Novel Small Molecule NADPH Oxidase 2 Inhibitor.", ANTIOXIDANTS & REDOX SIGNALING 10 AUG 2015, vol. 23, no. 5, 10 August 2015 (2015-08-10), pages 358-374, XP002793503, ISSN: 1557-7716 the whole document	1-15
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A	----- NAYERNIA ZEYNAB ET AL: "New insights on NOX enzymes in the central nervous system.", ANTIOXIDANTS & REDOX SIGNALING 10 JUN 2014, vol. 20, no. 17, 10 June 2014 (2014-06-10), pages 2815-2837, XP002793504, ISSN: 1557-7716 the whole document table 1	1-15
A	----- PESTANA R R F ET AL: "Reactive oxygen species generated by NADPH oxidase are involved in neurodegeneration in the pilocarpine model of temporal lobe epilepsy", NEUROSCIENCE LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 484, no. 3, 5 November 2010 (2010-11-05), pages 187-191, XP027322229, ISSN: 0304-3940 [retrieved on 2010-08-21] cited in the application the whole document figures 1, 3	1-15

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Information on patent family members

International application No

PCT/EP2020/055504

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