

A GABA_A RECEPTOR LIGAND AND THE USE THEREOF IN MEDICINE, IN
PARTICULAR IN THE TREATMENT OF EPILEPSY

Technical field

The present invention relates to a novel compound useful in the treatment of central
5 nervous system diseases and disorders which are responsive to modulation of the
GABA_A receptor complex.

Background

The GABA_A receptors are ligand gated channels which exists in multiple isoforms.
10 Each receptor is a pentameric complex comprising subunits drawn from α_{1-6} , β_{1-3} , γ_{1-3} ,
 δ , ϵ and θ subunit isoforms. The majority of GABA_A receptors present in the CNS
contain two α , two β , and one γ subunit (Mckernan RM. et. al. (1996)). When GABA
binds, GABA_A receptors become selectively permeable to chloride ions. Generally, this
results in an influx of chloride ions into the cell and a hyperpolarization of the cell
15 membrane, thereby inhibiting neuronal signalling. A seizure is a burst of uncontrolled
electrical activity in specific neuronal circuits in the brain.(Johns Hopkins Medicine
(2023)) Some of the most powerful treatment options for aborting seizure activity are a
group of medicines called the benzodiazepines. The mode of action of the
benzodiazepines is non-selective enhancement of the effect of the inhibitory
20 neurotransmitter GABA at GABA_A receptors (Janković SM et al. (2021)) including α_1 ,
 α_2 , α_3 and α_5 containing receptors. It is well accepted that the enhanced effect of
GABA_A α_1 receptors cause the dose-limiting adverse effects of the benzodiazepines,
such as sedation and motoric instability in addition to tolerance development, cognitive
impairment, abuse liability and physical dependence.(Shinotoch, H et al. (1989), Engin,
25 e. (2023).

As epilepsy is considered to be the result in an excitatory-to-inhibitory (E/I) imbalance,
whereby increased excitation or decreased inhibition favors a hyperexcitable state,
positive modulation of GABA_A α_2 - and α_3 receptors are predicted to normalize the E/I
30 imbalance and exert strong antiseizure activity while improved tolerability is anticipated
due to lack of modulation at α_1 containing receptors.

WO 98/34923, EP 0616807, WO 2004/087690, WO 2007/110374, WO 2010/055129,
WO 2010/055131, WO 2010/055132 and WO 2010/055133 describe benzimidazole

derivatives useful in the treatment of central nervous system diseases and disorders, which are responsive to modulation of GABA_A receptor complex.

5 WO 03/086406, WO 03/087099, WO 03/099816 and WO 01/18000 disclose imidazo-pyridine derivatives useful as ligands for GABA receptors.

WO 2000/044752 and WO 99/67245 disclose triazolo-pyridazine derivatives useful as ligands for GABA receptors.

10 These previously presented GABA receptor modulators indicate that small structural differences may have a large impact on the biological activity.

However, many of the previously presented modulators of GABA receptors are associated with unwanted side effects. Thus, there is a strong need for compounds
15 with an optimized pharmacological profile and without the unwanted side effects.

Summary

The present inventors have developed a compound that is a GABA_A $\alpha 2/\alpha 3/\alpha 5$ positive allosteric modulator with selectivity for these three receptor subtypes and with high
20 stability indicating that it will have long half live in vivo. The compound does not have any activity towards GABA_A $\alpha 1$ meaning that it is likely not to induce adverse effects associated with this receptor subtype. The compound demonstrates efficacy in treatment of epilepsy. It acts synergistically in combination with known anti-epileptic compounds.

25 In one aspect, the present invention relates to 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof.

30 Drawings

Figure 1. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) dose dependently protected the mice in 6Hz model of focal epilepsy with minimum effective dose of 1mg/kg (see Example 5 for experimental details).

Figure 2. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) has synergistic effect with levetiracetam in 6Hz model of focal epilepsy (see Example 5 for experimental details).

Figure 3. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) has synergistic effect with valproic acid in 6Hz model of focal epilepsy (see Example 5 for experimental details).

Figure 4. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) dose dependently protected the mice against PTZ induced myoclonic generalized seizures with minimum effective dose of 0.3 mg/kg (see Example 6 for experimental details).

Figure 5. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) has synergistic effect with levetiracetam in the PTZ model of myoclonic generalized epilepsy (see Example 6 for experimental details).

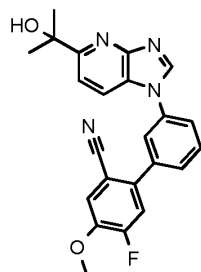
Figure 6. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) has additive effect with retigabine in the maximal electroshock model of tonic clonic generalized epilepsy (see Example 5 for experimental details).

Figure 7. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) effect at GABAA receptor subtypes expressed in *Xenopus* Oocytes. (see Example 3 for experimental details).

Detailed description

In one aspect, the present invention relates to 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1), or a pharmaceutically acceptable salt thereof.

In one aspect, the present invention relates to:



, or a pharmaceutically acceptable salt thereof.

Pharmaceutically Acceptable Salts

The chemical compound of the invention may be provided in any form suitable for the intended administration, including pharmaceutically (i.e. physiologically) acceptable salts. Examples of pharmaceutically acceptable addition salts include, without limitation, non-toxic inorganic and organic acid addition salts such as hydrochloride, hydrobromide, nitrate, perchlorate, phosphate, sulphate, formate, acetate, aconate, ascorbate, benzenesulphonate, benzoate, cinnamate, citrate, embonate, enantate, fumarate, glutamate, glycolate, lactate, maleate, malonate, mandelate, methanesulphonate, naphthalene-2-sulphonate, phthalate, salicylate, sorbate, stearate, succinate, tartrate, toluene-p-sulphonate, and the like. Such salts may be formed by procedures well known and described in the art. Other acids such as oxalic acid, which may not be considered pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining a chemical compound of the invention and its pharmaceutically acceptable acid addition salt.

Examples of pharmaceutically acceptable cationic salts of the compounds of the invention include, without limitation, the sodium, the potassium, the calcium, the magnesium, the zinc, the aluminium, the lithium, the choline, the lysinium, and the ammonium salt, and the like, of the compound of the invention containing an anionic group. Such cationic salts may be formed by procedures well known and described in the art. In the context of this invention the "onium salts" of *N*-containing compounds are also contemplated as pharmaceutically acceptable salts. Preferred "onium salts" include the alkyl-onium salts, the cycloalkyl-onium salts, and the cycloalkylalkyl-onium salts.

Labelled Compounds

The chemical compound of the present invention may be used in its labelled or unlabelled form. In the context of this invention, the labelled compound has one or more atoms replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. The labelling will allow easy quantitative detection of said compound.

The labelled compounds of the invention may be useful as diagnostic tools, radio tracers, or monitoring agents in various diagnostic methods, and for in vivo receptor

imaging. The labelled isomer of the invention preferably contains at least one radionuclide as a label. Positron emitting radionuclides are all candidates for usage. In the context of this invention the radionuclide is preferably selected from ^2H (deuterium), ^3H (tritium), ^{13}C , ^{14}C , ^{131}I , ^{125}I , ^{123}I , and ^{18}F .

5

The physical method for detecting the labelled isomer of the present invention may be selected from Position Emission Tomography (PET), Single Photon Imaging Computed Tomography (SPECT), Magnetic Resonance Spectroscopy (MRS), Magnetic Resonance Imaging (MRI), and Computed Axial X-ray Tomography (CAT), or combinations thereof.

10

Methods of preparation

The chemical compounds of the invention may be prepared by conventional methods for chemical synthesis, e.g. those described in the working examples. The starting materials for the processes described in the present application are known or may readily be prepared by conventional methods from commercially available chemicals.

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In one aspect, the present invention relates to a method for manufacturing 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, comprising the step of reacting 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with 4-fluoro-5-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile. In one embodiment, the reaction is performed in presence of a palladium catalyst and a base. In one embodiment, the palladium catalyst is $\text{Pd}(\text{Dppf})\text{Cl}_2\cdot\text{CH}_2\text{Cl}_2$. In one embodiment, the base is an alkali metal carbonate, such as Na_2CO_3 .

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In one embodiment, the method further comprises the step of reacting 2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with 2-bromo-4-fluoro-1-nitrobenzene to generate 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.

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In one embodiment, the method further comprises the step of reducing 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol to 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.

In one embodiment, the method further comprises the step of reacting 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with tert-Butyl nitrite and DMSO to generate 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.

- 5 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile may be synthesized as described in Example 1 herein.

Thus, in one aspect, the present invention relates to a method for manufacturing 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, comprises the
10 following steps:

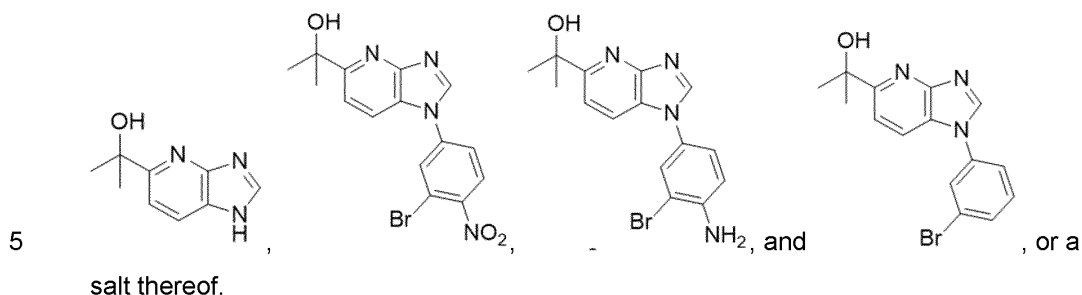
- a. Reacting 2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (3) with 2-bromo-4-fluoro-1-nitrobenzene (4) in the presence of a base to generate 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (5);
- 15 b. Reducing 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (5) to generate 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (6);
- c. Reacting 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (6) with tert-butyl nitrite and DMSO to generate 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (7); and
- 20 d. Reacting 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (7) with 4-fluoro-5-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (10) in presence of a palladium catalyst and a base to generate
25 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (1).

In one aspect, the present invention relates to an intermediate in the synthesis of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile as described herein. In one aspect, the present invention
30 relates to a compound selected from the group consisting of:

- 2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol;
- 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol;
- 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol; and
- 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol,
- 35 or a salt thereof.

In one embodiment, the salt is a pharmaceutically acceptable salt.

In one aspect, the present invention relates to a compound selected from the group consisting of:



The end products of the reactions described herein may be isolated by conventional techniques, e.g. by extraction, crystallization, distillation, chromatography, etc.

Compound-1 may exist in unsolvated as well as in solvated forms with pharmaceutically acceptable solvents such as water, ethanol and the like. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of this invention.

Pharmaceutical compositions

The invention also provides pharmaceutical compositions comprising therapeutically effective amount of Compound-1, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutically acceptable carrier, excipient or diluent.

While the Compound-1 of the present invention for use in therapy may be administered in the form of the raw chemical compound, it is preferred to introduce the active ingredient, optionally in the form of a physiologically acceptable salt, in a pharmaceutical composition together with one or more adjuvants, excipients, carriers, buffers, diluents, and/or other customary pharmaceutical auxiliaries.

In a preferred embodiment, the invention provides pharmaceutical compositions comprising Compound-1, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable carriers, and, optionally, other therapeutic and/or prophylactic ingredients, known and used in the art. The carrier(s) must be

"acceptable" in the sense of being compatible with the other ingredients of the formulation and not harmful to the recipient thereof. Pharmaceutical compositions of the invention may be those suitable for oral, rectal, bronchial, nasal, pulmonal, topical (including buccal and sub-lingual), transdermal, vaginal or parenteral (including cutaneous, subcutaneous, intramuscular, intraperitoneal, intravenous, intraarterial, intracerebral, intraocular injection or infusion) administration, or those in a form suitable for administration by inhalation or insufflation, including powders and liquid aerosol administration, or by sustained release systems. Suitable examples of sustained release systems include semipermeable matrices of solid hydrophobic polymers containing the compound of the invention, which matrices may be in form of shaped articles, e.g. films or microcapsules.

Compound-1, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of pharmaceutical compositions and unit dosages thereof. Such forms include solids, and in particular tablets, filled capsules, powder and pellet forms, and liquids, in particular aqueous or non-aqueous solutions, suspensions, emulsions, elixirs, and capsules filled with the same, all for oral use, suppositories for rectal administration, and sterile injectable solutions for parenteral use. Such pharmaceutical compositions and unit dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed. Compound-1 can be administered in a wide variety of oral and parenteral dosage forms. It will be obvious to those skilled in the art that the following dosage forms may comprise, as the active component, either Compound-1 or a pharmaceutically acceptable salt thereof.

For preparing pharmaceutical compositions from Compound-1, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavouring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packaged tablets, capsules, and
5 powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

A therapeutically effective dose refers to that amount of active ingredient, which
10 ameliorates the symptoms or condition. Therapeutic efficacy and toxicity, e.g. ED₅₀, may be determined by standard pharmacological procedures in cell cultures or experimental animals. The dose ratio between therapeutic and toxic effects is the therapeutic index and may be expressed by ratio between plasma levels resulting in therapeutic effects and plasma ratios resulting in toxic effects. Pharmaceutical
15 compositions exhibiting large therapeutic indexes are preferred.

The dose administered must of course be carefully adjusted to the age, weight and condition of the individual being treated, as well as the route of administration, dosage form and regimen, and the result desired, and the exact dosage should of course be
20 determined by the practitioner.

The actual dosage depends on the nature and severity of the disease being treated, and is within the discretion of the physician, and may be varied by titration of the dosage to the particular circumstances of this invention to produce the desired
25 therapeutic effect. However, it is presently contemplated that pharmaceutical compositions containing of from about 0.1 to about 10.000 mg of active ingredient per individual dose, preferably of from about 1 to about 1000 mg, most preferred of from about 10 to about 500 mg, are suitable for therapeutic treatments. The active ingredient may be administered in one or several doses per day. A satisfactory result can, in
30 certain instances, be obtained at a dosage as low as 0.1 µg/kg i.v. and 1 µg/kg p.o. The upper limit of the dosage range is presently considered to be about 10 mg/kg i.v. and 100 mg/kg p.o. Preferred ranges are from about 0.1 µg/kg to about 10 mg/kg/day i.v., and from about 1 µg/kg to about 100 mg/kg/day p.o.

Biological activity

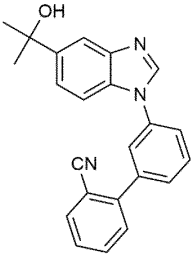
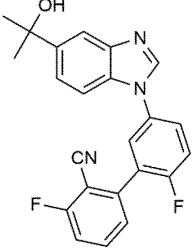
5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile is a GABA_A $\alpha 2/\alpha 3/\alpha 5$ positive allosteric modulator.

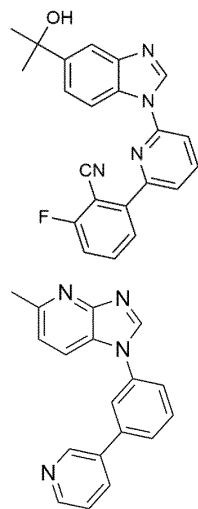
- 5 As demonstrated in Examples 3 and 4, relatively small structural differences between two molecules may have a large impact on the biological activity.

- 10 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile modulates $\alpha 3\beta 3\gamma 2$ -containing GABA_A receptors with an efficacy of at least 49% (see Example 3).

- 15 Further, 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile has a high metabolic stability measured in hepatocytes with QH of less than 25% which describes clearance scaled to an intact whole liver divided by liver blood flow (see Example 4).

The following compounds have been included as comparative compounds in Examples 3 and 4:

Structure	Name
	2-[3-[5-(1-hydroxy-1-methyl-ethyl)benzimidazol-1-yl]phenyl]benzonitrile (A)
	2-fluoro-6-[2-fluoro-5-[5-(1-hydroxy-1-methyl-ethyl)benzimidazol-1-yl]phenyl]benzonitrile (B)



2-fluoro-6-[6-[5-(1-hydroxy-1-methyl-ethyl)benzimidazol-1-yl]-2-pyridyl]benzonitrile (C)

5-Methyl-1-[3-(3-pyridinyl)phenyl]-1H-imidazo[4,5-b]pyridine (D)

Compound	GABAA $\alpha 3\beta 2\gamma 2$ (Max rel. to RF*)	Human hepatic clearance
	Efficacy (%)	QH (%)
Compound-1	49	< 25
Compound-A	36	71
Compound-B	70	75
Compound-C	18	< 25
Compound-D	13	

* relative to the potentiating effects of 0.5 μ M diazepam (= RF) on the same oocytes.

Conclusion

5 Compared to the reference compounds (A-D), Compound-1 has a unique profile modulating $\alpha 3\beta 3\gamma 2$ -containing GABA_A receptors with an efficacy of 49%, and having a metabolic stability measured as clearance in hepatocytes with a QH of less than 25%. The low %QH predicts a long half-life for Compound-1 when dosed to humans. As demonstrated with the efficacy data in oocytes and clearance data in hepatocytes, relatively small structural differences between two molecules may have a large impact

10 on the biological activity.

In contrast to Compound-1, Compound-A and Compound-B have low metabolic stability measured in hepatocytes and high QH of 71% and 75%, respectively, predicting a short half-life in humans. Despite the structural similarities with Compound-

1, Compound-C and Compound-D have low efficacy on $\alpha_3\beta_3\gamma_2$ -containing GABA_A receptors of 18% and 13%, respectively.

Methods of therapy

5 In one aspect, the present invention relates to 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, for use as a medicament.

10 In one aspect, the present invention relates to Compound-1, or a pharmaceutically acceptable salt thereof, for use in the treatment, prevention or alleviation of a disease or a disorder or a condition of a mammal, including a human, which disease, disorder or condition is responsive to modulation of the GABA_A receptor complex.

15 In Example 5, it is demonstrated that Compound-1 dose dependently protected the mice against 6Hz induced partial seizures with minimum effective dose of 1 mg/kg.

20 In one aspect, the present invention relates to Compound-1, or pharmaceutically acceptable salt thereof, for use in the treatment, prevention and/or alleviation of epilepsy. Epilepsy may include acute repetitive seizures (ARS) and other types of seizures.

Seizure types may be classified using the International League Against Epilepsy (ILAE) 2017 Classification of Seizure Types Basic Version (Fisher RS, et al. Instruction manual for the ILAE 2017 operational classification of seizure types. *Epilepsia*, 25 58(4):531–542, 2017), wherein seizures are first categorized by type of onset. A seizure is classified as having focal onset if it originates (by clinical pattern or EEG localization) in one hemisphere of the brain and generalized if appearing apparently simultaneously in both hemispheres. A third category allow for the origin focality being unknown. Confidence level for focal versus generalized onset is set at 80%. When a 30 seizure type begins with the words “focal,” “generalized,” or “absence,” then the word “onset” may be presumed.

35 The seizures can include sensory (visual, hearing, or smell), psychic, autonomic, and motor phenomena depending on which part of the brain is involved. Muscle jerks may start in a specific muscle group and spread to surrounding muscle groups in which

case it is known as a Jacksonian march. Automatisms may occur, which are non-consciously generated activities and mostly simple repetitive movements like smacking the lips or more complex activities such as attempts to pick up something.

- 5 There are six main types of generalized seizures tonic-clonic, tonic, clonic, myoclonic, absence, and atonic seizures. They all involve loss of consciousness and typically happen without warning. The seizures may be acute repetitive seizures (ARS).

10 Epilepsy includes focal onset seizures, generalized onset seizures, and seizures of unknown onset. Focal onset seizures may be with awareness or with impaired awareness. Further, focal onset seizures may be motor onset or nonmotor onset. Generalized onset seizures may be with motor onset (tonic-clonic and other motor onset) or nonmotor onset seizures (absence seizures). Seizures of unknown onset includes motor onset (tonic-clonic and other motor) and nonmotor onset seizures.

15 In one embodiment, the epilepsy is characterized by focal epilepsy. In one embodiment, the epilepsy is characterized by myoclonic generalized seizures. In one embodiment, the epilepsy is characterized by tonic clonic generalized epilepsy.

20 In one aspect, the present invention relates to a method for treatment, prevention and/or alleviation of epilepsy, said method comprising administering Compound-1, or a pharmaceutically acceptable salt thereof, to a subject in need thereof. In some embodiments, said method further comprises administering an anti-epileptic drug (AED). By the term "an anti-epileptic drug (AED)" is meant a drug different from
25 Compound 1. The anti-epileptic drug may be Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner. Preferably the anti-epileptic drug is selected from the group
30 consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels.

35 In one aspect, the present invention relates to use of Compound-1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment, prevention and/or alleviation of epilepsy.

In some embodiments, the subject is a mammal, such as a human. In some embodiments, the subject is suffering from epilepsy.

Combination treatments

5 As demonstrated in Example 5, Compound-1 has synergistic effect with levetiracetam in 6Hz model of focal epilepsy, and a synergistic effect with valproic acid in 6Hz model of focal epilepsy. It is also demonstrated in Example 6 that Compound-1 has synergistic effect with levetiracetam in the PTZ model of myoclonic generalized epilepsy. Further, it is demonstrated in Example 7 that Compound-1 has additive effect
10 with retigabine in the maximal electroshock model of tonic clonic generalized epilepsy.

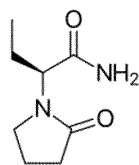
In one aspect, the present invention relates to a combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridine-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug
15 (AED) selected from the group consisting of Levetiracetam, valproic acid, and Retigabine for use as a medicament.

In one aspect, the present invention relates to a combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridine-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug
20 (AED) for use in the treatment, prevention and/or alleviation of epilepsy. The anti-epileptic drug may be Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or
25 encukalner/azetukalner. Preferably the anti-epileptic drug is selected from the group consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels.

In one aspect, the present invention relates to a method for treatment, prevention
30 and/or alleviation of epilepsy, said method comprising administering Compound-1, or a pharmaceutically acceptable salt thereof, in combination with an anti-epileptic drug (AED) to a subject in need thereof. The anti-epileptic drug may be Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin,
35 Zonisamide, Cenobamate, or encukalner/azetukalner. Preferably the anti-epileptic drug

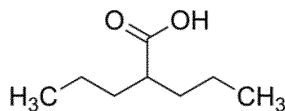
is selected from the group consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels.

- 5 In one aspect, the present invention relates to use of a combination of Compound-1, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug (AED) in the manufacture of a medicament for treatment, prevention and/or alleviation of epilepsy. The anti-epileptic drug may be Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate,
- 10 Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner. Preferably the anti-epileptic drug is selected from the group consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels.
- 15 In some embodiments, the AED is Levetiracetam. In some embodiments, the AED is valproic acid. In some embodiments, the AED is Retigabine. (S)-2-(2-Oxopyrrolidin-1-yl)butanamide, also called Levetiracetam, sold under the brand name Keppra among others, is a medication used to treat epilepsy.



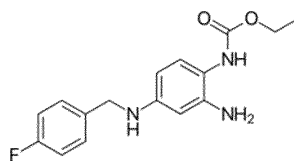
20 Levetiracetam

- Valproate (VPA) and its valproic acid (2-propylpentanoic acid), sodium valproate, and valproate semisodium forms are medications primarily used to treat epilepsy and bipolar disorder and prevent migraine headaches. They are useful for the prevention of
- 25 seizures in those with absence seizures, partial seizures, and generalized seizures.



Valproic acid

- Ethyl N-[2-amino-4-[(4-fluorophenyl)methylamino]phenyl]carbamate, also called
- 30 Retigabine, is antiseizure drug that acts through potassium channels.



Retigabine

Instead of Retigabine other drugs with similar mode of action can be used, for example
5 other K_v7 potassium channel openers, or XEN1101 also known as encukalner or
azetukalner, and other selective K_v7.2/K_v7.3 potassium channel openers.

In one aspect, the present invention relates to a kit-of-parts for simultaneous,
sequential or separate administration of at least two separate unit dosage forms (A)
10 and (B), wherein
(A) comprises of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridine-1-yl)-
4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof;
and
(B) comprises an anti-epileptic drug (AED).

15 The anti-epileptic drug may be Brivaracetam, Ethosuximide, Gabapentin, Lacosamide,
Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate,
Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or
encukalner/azetukalner. Preferably the anti-epileptic drug is selected from the group
consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure
20 medications with similar mode of action, i.e. activation of Kv7 channels.

In one aspect, the present invention relates to a kit-of-parts as defined herein, for the
treatment, prevention and/or alleviation of epilepsy such as acute repetitive seizures
(ARS).

25

In one aspect, the present invention relates to a method for the treatment, prevention
and/or alleviation of epilepsy comprising administering a kit-of-parts as defined herein,
to a subject in need thereof.

30 In one aspect, the present invention relates to use of a kit-of-parts as defined herein, in
the manufacture of a medicament for treatment, prevention and/or alleviation of
epilepsy.

Items

- 5 1. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof.
- 10 2. A pharmaceutical composition comprising 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof.
- 15 3. The pharmaceutical composition according to item 2, wherein the composition further comprises one or more adjuvants, excipients, carriers, buffers, diluents, and/or other customary pharmaceutical auxiliaries.
- 20 4. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, for use as a medicament.
- 25 5. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, for use in the treatment, prevention and/or alleviation of epilepsy.
- 30 6. A method for treatment, prevention and/or alleviation of epilepsy, said method comprising administering 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, to a subject in need thereof.
- 35 7. The method according to item 6, wherein the method further comprises administering an anti-epileptic drug (AED).
8. The method according to item 7, wherein the anti-epileptic drug is Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner, preferably, wherein the anti-epileptic drug is selected from the group consisting

of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels.

- 5 9. Use of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment, prevention and/or alleviation of epilepsy.
- 10 10. A combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug (AED) for use as a medicament.
- 15 11. The combination of item 10, wherein the anti-epileptic drug is Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner, preferably, wherein the anti-epileptic drug is selected from the group consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels
- 20 12. A combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug (AED) for use in the treatment, prevention and/or alleviation of epilepsy.
- 25 13. The combination of item 12, wherein the anti-epileptic drug is Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner, preferably, wherein the anti-epileptic drug is selected from the group consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels
- 30 14. A method for treatment, prevention and/or alleviation of epilepsy, said method comprising administering a combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-
- 35

yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug (AED) to a subject in need thereof.

- 5 15. The method of item 14, wherein the anti-epileptic drug is Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner, preferably, wherein the anti-epileptic drug is selected from the group consisting of
- 10 Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels.
- 15 16. Use of a combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, and an anti-epileptic drug (AED) in the manufacture of a medicament for treatment, prevention and/or alleviation of epilepsy.
- 20 17. The use according to item 16, wherein the anti-epileptic drug is Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner, preferably, wherein the anti-epileptic drug is selected from the group consisting of
- 25 Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels
- 30 18. A kit-of-parts for simultaneous, sequential or separate administration of at least two separate unit dosage forms (A) and (B), wherein (A) comprises of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridine-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof; and (B) comprises an anti-epileptic drug (AED) or a pharmaceutically acceptable salt thereof.
- 35 19. The kit-of-parts according to item 13 for the treatment, prevention and/or alleviation of epilepsy.

20. The kit-of-parts according to item 18 and/or 19, wherein the anti-epileptic drug is Brivaracetam, Ethosuximide, Gabapentin, Lacosamide, Levetiracetam, Perampanel, Pregabalin, Primidone, Rufinamide, Sodium valproate, Tiagabine, Topiramate, Vigabatrin, Zonisamide, Cenobamate, or encukalner/azetukalner, preferably, wherein the anti-epileptic drug is selected from the group consisting of Levetiracetam, valproic acid, Retigabine and other antiseizure medications with similar mode of action, i.e. activation of Kv7 channels
21. A method for the treatment, prevention and/or alleviation of epilepsy, comprising administering the kit-of-parts according to any one of item 18 to 20, to a subject in need thereof.
22. Use of a kit-of-parts according to item any one of item 18 to 20 in the manufacture of a medicament for treatment, prevention and/or alleviation of epilepsy.
23. The combination for use according to item 10, the kit-of-parts for use according to item 20, the method of treatment according to item 6 or item 21, the use according to item 9 or 17, wherein the AED is Levetiracetam.
24. The combination for use according to item 10, the kit-of-parts for use according to item 20, the method of treatment according to item 6 or item 21, the use according to item 9 or 17, wherein the AED is valproic acid.
25. The combination for use according to item 5, 10, the kit-of-parts for use according to item 20, the method of treatment according to item 6 or item 21, the use according to item 9 or 17, wherein the AED is Retigabine.
26. The method of treatment, the use, the combination for use, the kit-of-parts for use according to any one of the preceding claims, wherein epilepsy includes focal onset seizures, generalized onset seizures, and seizures of unknown onset.

27. The method of treatment, the use, the combination for use, the kit-of-parts for use according to claim 26, wherein focal onset seizures is awareness or with impaired awareness or wherein focal onset seizures is motor onset or nonmotor onset, or wherein generalized onset seizures is with motor onset (tonic-clonic and other motor onset) or nonmotor onset seizures (absence seizures), or wherein seizures of unknown onset include motor onset (tonic-clonic and other motor) and nonmotor onset seizures.
28. The method of treatment, the use, the combination for use, the kit-of-parts for use according to any one of the preceding claims 26 or 27, wherein epilepsy is characterized by focal epilepsy, including epilepsy characterized by myoclonic generalized seizures or epilepsy characterized by tonic clonic generalized epilepsy.
29. A method for manufacturing 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, the method comprising reacting 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with 4-fluoro-5-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile.
30. The method according to item 29, wherein the reaction is performed in presence of a palladium catalyst and a base.
31. The method according to item 30, wherein the palladium catalyst is $\text{Pd}(\text{Dppf})\text{Cl}_2\cdot\text{CH}_2\text{Cl}_2$.
32. The method according to any one of items 29 or 31, wherein the base is an alkali metal carbonate, such as Na_2CO_3 .
33. The method according to any one of items 29 to 32, wherein the method further comprises reacting 2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with 2-bromo-4-fluoro-1-nitrobenzene to generate 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.

34. The method according to any one of items 29 to 33, wherein the method further comprises reducing 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol to 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.

5

35. The method according to any one of items 29 to 34, wherein the method further comprises reacting 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with tert-Butyl nitrite and DMSO to generate 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.

10

36. A compound selected from the group consisting of:

2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol;

2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol;

2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol; and

15

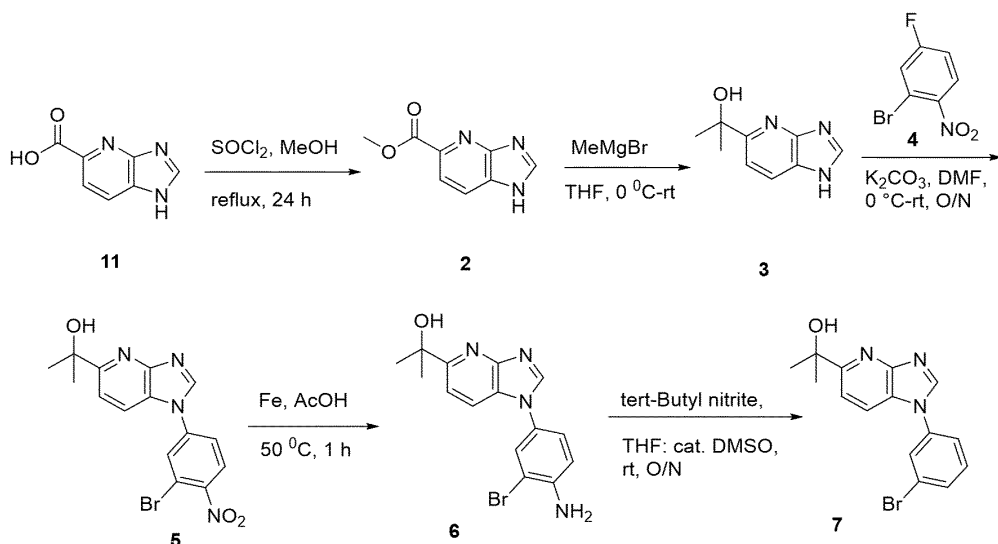
2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol,

or a salt thereof.

Examples

Example 1: Synthesis of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (11)

20



Synthesis of methyl 1H-imidazo[4,5-b]pyridine-5-carboxylate (2): To a stirred solution of compound-11 (50 g, 0.306 mol) in MeOH (500 mL) added thionyl chloride

(26.64 mL, 0.367 mol). Resulting reaction mixture refluxed for 24 h. Concentrated under *vacuo* to remove excess of thionyl chloride. Residue obtained was diluted with aq. NaHCO₃ solution to pH 7. Solids obtained filtered through Büchner funnel to get compound-2 (49 g, 90%); m/z (LC-MS): 178.10 [M+H]⁺.

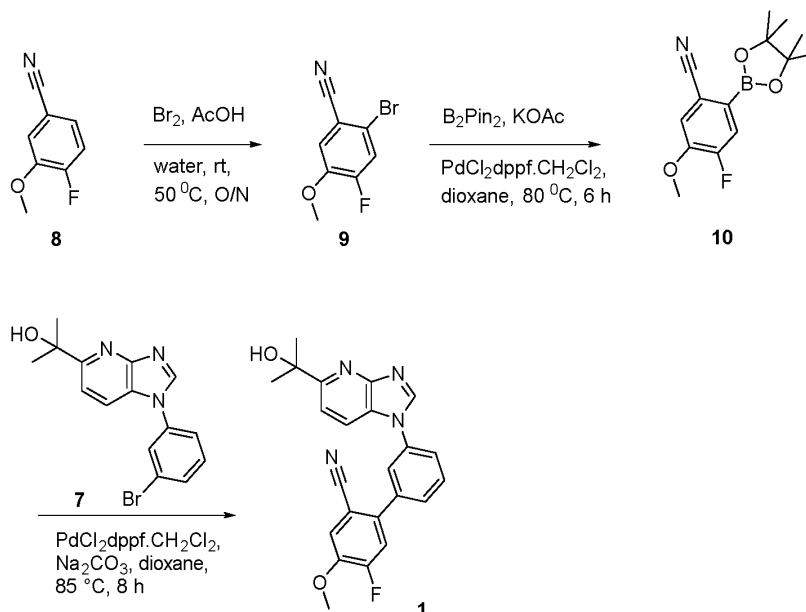
- 5 **Synthesis of 2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (3):** To a solution of compound-2 (25 g, 0.141 mol) in THF (500 mL) at 0 °C added MeMgBr (330 mL, 3 M in ether). Reaction mixture stirred at rt for 24 h. Reaction quenched using sat. solution of ammonium chloride, solids generated. Organic layer decanted. Solid obtained dissolved in 400 mL of water and extracted using IPA:CHCl₃ (3:7, 2 x 2 L). Combined
10 organic layer dried over anhydrous Na₂SO₄ and concentrated under *vacuo* to get compound-3 (42 g, 84%); m/z (LC-MS): 177.75 [M+H]⁺.

- Synthesis of 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (5):** To a solution of compound-3 (20 g, 0.113 mol) in DMF (200 mL) at 0 °C added K₂CO₃ (39 g, 0.282 mmol) followed by 2-bromo-4-fluoro-1-nitrobenzene (4) (24.74 g,
15 0.113 mol). Reaction mixture stirred at rt for 24 h. Reaction mixture diluted with water (1 L), extracted using MeOH:DCM (1:9, 3 x 1L). Combined organic layer dried over anhydrous Na₂SO₄ and concentrated under *vacuo* to get crude compound-5. Crude compound purified using combiflash chromatography (60 % EtOAc in hexane) to get pure enough compound-5 (24 g, 56%). *Note:* Polar major peak is the desired
20 regioisomer and nonpolar peak is the undesired regioisomer; m/z (LC-MS): 378.65 [M+H]⁺.

- Synthesis of 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (6):** To a solution of compound-5 (24 g, 31.91 mmol) in AcOH (200 mL) under argon at 0 °C added Fe powder (1.78 g, 31.91 mmol). Reaction mixture allowed to
25 come to rt and heated at 50 °C for 40 min to 1 h time based on TLC. After completion of the reaction, the reaction solution was filtered through celite bed, washed with 10% MeOH/DCM. The filtrate was washed with water, brine, dried over anhydrous Na₂SO₄ and concentrated under *vacuo* to give crude product. The crude product was purified by combiflash chromatography (60-70% EtOAc in hexane) to get the desired
30 compound-6 as yellowish solid (14.5 g, 64%); m/z (LC-MS): 333.95 [M+H]⁺.

- Synthesis of 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol (7):** To a solution of compound-6 (14.5 g, 41.90 mmol) in THF (145 mL) under argon at 0 °C added tert-Butyl nitrite (24.92 mL, 209.5 mmol). After stirring for 10 min added DMSO (15 mL, 209.5 mmol). Resulting reaction mixture stirred under argon for 16 h to
35 48 h based on TLC. After completion of the reaction, the reaction mixture was

quenched with ice cold water (200 mL) and extracted with 10% MeOH in DCM (3 x 500 mL). The total organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under *vacuo* to give crude product-7 as brownish gummy. The crude compound-7 was purified by combiflash chromatography (60-70% EtOAc in hexane) to give desired compound-7 (11.5 g, 83%) as yellowish solid; m/z (LC-MS): 333.80 [M+H]⁺.



Synthesis of 2-bromo-4-fluoro-5-methoxybenzonitrile (9): To a stirred solution of 4-fluoro-3-methoxybenzonitrile (8) (2 g, 13.233 mmol) in AcOH:water (1:1, 20 mL) was added bromine (0.685 mL, 13.233 mmol) at room temperature. The reaction mixture was heated at 50 °C for 16 h. Reaction mixture cooled and diluted with icecold water, solids obtained, filtered and dried under high *vacuo* to get compound-9 (2 g, 65.78%) as white solid; HNMR (CDCl₃, 300 MHz): δ 3.92 (s, 3 H), 7.20 (d, J = 8.1 Hz, 1 H), 7.39 (d, J = 9.9 Hz, 1 H).

Synthesis of 4-fluoro-5-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (10): To a sealed tube containing stirred solution of compound-9 (2 g, 8.694 mmol) in dioxane (20 mL) added B₂Pin₂ (3.31 g, 13.041 mmol) and KOAc (1.28 g, 13.041 mmol,) at room temperature under argon atmosphere. The resulting mixture was degassed using N₂ for 5 min and added PdCl₂dppf.CH₂Cl₂ (0.355 g, 0.435 mmol) and heated to 80 °C for 6 h. The progress of the reaction monitored by TLC. After completion of the reaction water was added and extracted with EtOAc (3 x 100 mL). Combined organic layers washed with brine and concentrated under reduced pressure.

Crude compound purified by combiflash chromatography using (5% EtOAc in hexane) to get compound-10 (1.7 g, 70.83%) as off white solid, HNMR (DMSO-d₆, 300 MHz): δ 1.31 (s, 12 H), 3.93 (s, 3 H), 7.49 (d, J = 12 Hz, 1 H), 7.71 (d, J = 7.8 Hz, 1 H).

Synthesis of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-

5 **4-methoxy-[1,1'-biphenyl]-2-carbonitrile (1):** To a stirred solution of compound-7 (600 mg, 1.806 mmol) in dioxane (15 mL) was added compound-10 (1.5 g, 2.709 mmol) followed by Na₂CO₃ (0.383 g, 3.612 mmol). The reaction mixture was purged with argon for 10 min, added PdCl₂dppf. CH₂Cl₂ (0.074 g, 0.090 mmol) and again purged with argon for 5 min. The reaction mass was heated at 85 °C for 8 h. Reaction
10 mixture was diluted with water (20 mL) and extracted with 10% MeOH in CH₂Cl₂ (3 x 50 mL). The total organic layer was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under *vacuo* to give crude product as brown gummy. The crude product was purified using combiflash chromatography (60-70% EtOAc in hexane) to get compound-1 (170 mg, 23%, HPLC: 99.5%) as off white solid. HNMR
15 (DMSO-d₆, 400 MHz): δ 1.53 (s, 6 H), 3.98 (s, 3 H), 5.30 (s, 1 H), 7.69-7.80 (m, 2 H), 7.80-7.96 (m, 2 H), 7.80-7.85 (m, 2 H), 7.94 (s, 1 H), 8.23 (d, J = 8.7 Hz, 1 H), 8.88 (s, 1 H). HRMS calculated for: [C₂₃H₁₉FN₄O₂+H]⁺ 403.1565; found: 403.1532.

Example 2: *In vitro* ³H-flumazenil binding

20

Tissue preparation

Cerebral cortices from male Wistar rats (150-200 g) were homogenized for 5-10 s in 20 mL Tris-HCl (30 mM, pH 7.4) using an Ultra-Turrax homogenizer. All procedures were performed at 0-4°C unless otherwise indicated. The homogenate was centrifuged at
25 27,000 x g for 15 min and the pellet was washed three times with buffer (centrifuged at 27,000 x g for 10 min). The washed pellet was homogenized in 20 mL of buffer and incubated on a water bath (37°C) for 30 min and then centrifuged for 10 min at 27,000 x g. The pellet was then homogenized in buffer and centrifuged for 10 min at 27,000 x g. The final pellet was re-suspended in 30 mL buffer and the preparation was frozen and
30 stored at -20°C.

Binding assay

The membrane preparation was thawed and centrifuged at 2°C for 10 min at 27,000 x g. The pellet was washed twice with 20 mL Tris-citrate (50 mM, pH 7.1) using an Ultra-
35 Turrax homogenizer and centrifuged for 10 min at 27,000 x g. The final pellet was re-

suspended in Tris-citrate (500 mL (for 550 µL assay) or 200 mL (for 220 µL assay) buffer per g of original tissue), and then used for binding experiments. Aliquots of 500 µL tissue suspension were added to 25 µL of test solution and 25 µL of ³H-flumazenil or ³H-flunitrazepam (1 nM, final concentration), mixed and incubated for 40 min at 2°C. Non-specific binding was determined using clonazepam (1 µM, final concentration). After incubation the 550 µL samples were added 5 mL of ice-cold buffer and poured directly onto Whatman GF/C glass fibre filters under suction and immediately washed with 5 mL ice-cold buffer. The 220 µL samples were filtered over UniFilter GF/C glass fiber filters using a Tomtec cell harvester. The filters were washed with approximately 5 mL ice-cold buffer. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting. Specific binding was calculated as the difference between total binding and non-specific binding.

Data analysis

The concentration of the test substance which inhibits the specific binding of ³H-flumazenil or ³H-flunitrazepam by 50% is identified as an IC₅₀ value.

Four concentrations of test compound were used to determine the inhibition curves from which the IC₅₀ values were determined. If a full curve was not available a 25-75% inhibition of specific binding must be obtained, before calculation of an IC₅₀:

$$IC_{50} = (\text{applied test substance concentration, mg/kg}) * \frac{1}{\left(\frac{C_o}{C_x} - 1\right)}$$

where C_o is specific binding in control assays and C_x is the specific binding in test assays. (The calculations assume normal mass-action kinetics).

Since the determined IC₅₀ values depend on the concentration of the radioligand, these values are converted to K_i values using the following equation:

$$K_i = \frac{IC_{50}}{\left(\frac{L}{K_d} + 1\right)}$$

where L is the concentration and K_d is the dissociation constant of the radioligand.

Results

IC₅₀ for Compound-1 was 0.0023 µM.

Conclusion

This example demonstrates that Compound 1 potentially displaces flumazenil.

Example 3: *Xenopus laevis* oocyte experiments

5 Preparation

Xenopus laevis oocytes were obtained from Ecocyte Bioscience (Dortmund, Germany). Here, lobes of ovaries were removed surgically from adult female *Xenopus* frogs. Lobes were then cut into small pieces with surgical knives and oocytes were dispersed as well as defoliated using 0.2% collagenase and gentle agitation. After dispersal for 1-2 hr, 10 oocytes were washed with modified Barth's solution and stage V and VI oocytes were selected and shipped. Upon receipt, oocytes were maintained at 18 °C in modified Barth's solution.

Injection of cRNA into oocytes

15 For injection, the oocytes were placed in a custom designed chamber in modified Barth's solution and injected with 25-50 nl of cRNA mixture using a Pico Pump (WPI). The cRNA mixture contained GABA_AR subunits α_x , β_2 , and γ_{2s} in the ratio of 3:1:3 and in a total concentration of 0.5 µg/µl. Following injection, oocytes were maintained at 18°C in modified Barth's solution for 1-5 days.

20

Two-electrode voltage clamp

Electrophysiological responses from *X. laevis* oocytes were measured using the two-electrode voltage clamp technique. Single oocytes were placed in custom designed recording chambers that were continuously perfused with 2 mL/min OR2 (90 mM NaCl, 25 2.5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES pH 7.4). Recording electrodes were fabricated from borosilicate glass tubings with filament (Sutter BF150-110-10) using a DMZ-Universal puller (Zeitz Instrument), backfilled with 2 M KCl and when submerged into OR2 solution the electrode resistances were in the range of 0.5-1 MΩ. The oocyte was impaled using manual micro manipulators and allowed to 30 equilibrate at a holding potential of -50 mV to -80 mV for at least 1 min to ensure a maximal leak current of 100 nA before the experiment was initiated. Currents were amplified by a Geneclamp 500B amplifier (Axon), low-pass filtered at 20Hz, digitized at 200 Hz by a Digidata 1322A (Axon) and then recorded as well as analyzed by a PC (Compaq Evo) using the pClamp9 suite (Axon).

35

Drug application system

Compound solutions were applied through a capillary tube, with an inner diameter of 1.5 mm (Modulohm 214813), placed approximately 2 mm from the oocyte and connected through Teflon tubing to a Gilson 233XL autosampler. Gilson 735 software suite was used to control all the Gilson equipment (233XL autosampler, 402 diluter and Minipuls 3 pumps) and to trigger recording by pCLAMP9. A flow rate of 2.5 mL/min through the capillary tube during applications ensured a rapid exchange of liquid surrounding the oocyte (in the order of few sec). The application length was sufficient to obtain peak currents. The time interval between recordings was 5 min, during which the oocyte was perfused with OR2 through the capillary tube as well.

Experimental data set

For each experimental set, GABA was freshly dissolved in OR2 in a concentration known to give rise to EC_5 - EC_{20} elicited currents for a given $GABA_A$ subtype combination (0.5-5 μ M) and this solution was then used for controls as well as a stock solution for dissolving the compounds to test in the experiment. A complete experimental set contained 4 control traces of GABA, a reference 0.5 μ M diazepam ($>EC_{90}$) trace, 10 GABA control traces and finally 5 test traces of a compound in increasing concentrations. Each oocyte was discarded after one experimental set.

Reference diazepam potentiation was calculated in percent by comparing the diazepam trace to the control trace immediately before. Likewise, modulatory effects of the compound were obtained by comparing to the control trace immediately before the test traces. To enable comparison of effects of a compound between individual oocytes, all compound potentiations were normalized to the reference diazepam potentiation on the same oocyte.

Results

Results are shown in Figure 7. The results demonstrate that Compound 1 is a $GABA_A$ $\alpha 2/\alpha 3/\alpha 5$ positive allosteric modulator. Notably, it has no modulation of $\alpha 1$. It is well accepted that the enhanced effect of $GABA_A$ $\alpha 1$ receptors cause the dose-limiting adverse effects of the benzodiazepines, such as sedation and motoric instability in addition to tolerance development, cognitive impairment, abuse liability and physical

dependence.(Shinotoch, H et al. (1989), Engin, e. (2023). These results suggest that Compound 1 will have limited adverse effects.

Compound 1 was compared to prior art compounds A, B, C, and D with respect to activity on GABAA $\alpha 3\beta 2\gamma 2$. The results are shown in the table below.

Compound	GABAA $\alpha 3\beta 2\gamma 2$ (Max rel. to RF*)
	Efficacy (%)
Compound-1	49
Compound-A	36
Compound-B	70
Compound-C	18
Compound-D	13

** relative to the potentiating effects of 0.5 μ M diazepam (= RF) on the same oocytes.*

Example 4: Human hepatocyte clearance, %QH

In this example, the test compound was incubated with cryopreserved hepatocytes for different time points and disappearance of the test compound was monitored by LC-MS/MS.

Preparation of test solutions

Solutions of test compounds were prepared from 10 mM stock solutions in DMSO by dilution to 50 μ M with acetonitrile/water (1:1) and further dilution with InvitroGRO KHB medium to 2 μ M test solutions.

Incubation

The cryopreserved human hepatocytes (5*10⁶ cell vial from BioIVT) were thawed and suspended in invitroGRO HT medium (45 ml, 37 °C). The cell suspension was centrifuged at 50 g for 5 min at 20 °C. The medium was removed, and the pellet resuspended in InvitroGRO KHB medium (2 ml, 37 °C). The cell count was determined using the Trypan Blue exclusion method and the cell suspension diluted with InvitroGRO KHB medium to 1*10⁶ cells/ml. Aliquots of cell suspension were added to each well and preincubated for 15 min on a shaker table in a CO₂ incubator at 37 °C before 1:1 dilution with test solution (2 μ M, 37 °C). The mixtures were incubated in the CO₂ incubator at 37

°C for a total of 120 min. Aliquots were transferred at six timepoints (t=5, 10, 30, 60, 90, 120 min) to a "STOP" plate kept on ice containing acetonitrile (5% AcOH and internal standard) in each well, resulting in 1:3 dilution of incubation mixture with acetonitrile (5% AcOH and internal standard). The "STOP" plate was centrifuged at 3000 g for 5 min at 4 °C. The supernatant was diluted 5:95 with MilliQ water before LCMS analysis.

Data analysis

The half-life ($t_{1/2}$) of the test compound was calculated from the first-order rate constant k , obtained as the slope of the time vs. $\ln(\text{area})$:

$$t_{1/2} = \frac{\ln(2)}{k}$$

The intrinsic clearance was calculated from k considering the cell concentration, human hepatocellularity and human liver weight:

$$CL_{int} = \frac{k * V * \text{hepatocellularity} * \text{liver weight}}{M * 1000}$$

where V is the volume of the incubation and M is the number of cells in the incubation.

Human hepatic clearance was calculated using the well-stirred model:

$$CL_h = \frac{Q * f_{u,b} * CL_{int}/f_{u,i}}{Q + f_{u,b} * CL_{int}/f_{u,i}} * 0.06$$

where Q is the human liver blood flow. No corrections related to unbound fraction in the blood ($f_{u,b}$) and unbound fraction in the incubation ($f_{u,i}$) was applied and $f_{u,b}/f_{u,i}$ assumed to be 1. %QH describes the percentage of the drug cleared by first passage through the liver and was calculated as hepatic clearance relative to liver blood flow:

$$QH\% = \frac{CL_h}{Q} * 100\%$$

Results

Compound	Human hepatic clearance
	QH (%)
5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (1) (Compound 1)	< 25
2-[3-[5-(1-hydroxy-1-methyl-ethyl)benzimidazol-1-yl]phenyl]benzonitrile (Compound A)	71
2-fluoro-6-[2-fluoro-5-[5-(1-hydroxy-1-methyl-ethyl)benzimidazol-1-yl]phenyl]benzonitrile (Compound B)	75
2-fluoro-6-[6-[5-(1-hydroxy-1-methyl-ethyl)benzimidazol-1-yl]-2-pyridyl]benzonitrile (Compound C)	< 25

Conclusion

- 5
- Compound-1 has a metabolic stability measured in hepatocytes with QH of less than 50%, indicating that Compound 1 has a long half-life when dosed in humans.

Example 5: Anti-convulsant effect of Compound-1 in 6Hz model of partial epilepsy

- 10
- Most currently available anti-epileptic drugs (AED) are discovered through chemical or electrical induced seizure models in animals especially rodents. Preclinical animal models are used to demonstrate efficacy and safety of an investigational new AED before its introduction to healthy subjects as part of Phase I trials. Animal seizure models can also be predictive of the epilepsy types observed in the patients, e.g., the
- 15
- low frequency (6Hz) and long duration (3s) corneal stimulation produces psychic / psychomotor seizures in animals which are believed to reflect the focal seizure phenotype of patients. The AED effective in this model can be used to treat focal and treatment resistant epilepsy in patients.

Method

- 20
- Female NMRI mice (BomTac:NMRI, 20-30 gr) from Charlers River Germany are used. The animals were housed in a controlled environment (constant temperature 22 ± 1 °C, humidity 40-60%, light on 0600-1800 h) and free access to food (Altromin 1324) and water. All animal procedures were conducted in accordance with the guidelines set by

the European Community Council Directives 86/609/EEC and approved by the Danish ministry of justice ("Dyreforsøgstilsynet").

Psychomotor seizures were induced via corneal stimulation (6 Hz, 0.2 ms rectangular pulses at 20 mA for 3 s) using a ECT unit 57800 from Ugo Basile (Italy, Europe). At the time of drug administration, a drop of 0.5% tetracaine was applied to the eyes of all animals. The electrodes of the corneal stimulator are soaked in 0.9% saline. The mice were restrained during corneal stimulation and released immediately after and observed for the presence or absence of seizure activity. Seizure activity included one of the following behavioral components: stunned, posture awkward but upright, forelimbs often crossed and hindlimb wide spread, tail is frequently held practically vertically (Straub-tail), stunning may occasionally be preceded by a few seconds of running with a rolling gait, face and forelimb movements resemble "purposeful" automatisms, not infrequently the mouse will stand on almost erect on its hindlimbs while exhibiting automatic behavior, or catatonia is often present. The duration of any of these seizure variables is 10-75 seconds (Toman 1951; Brown, Schiffman et al. 1953; Barton, Klein et al. 2001).

Compound preparation and administration

5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile (Compound-1) was dissolved in 5% dimethyl sulfoxide (DMSO) and 30% hydroxypropyl beta-cyclodextrin (HPbCD) in MilliQ water. The compound was dosed to the free base weight and is given peroral, 60 minutes before electrical stimulation in a volume of 10 ml/kg.

Data analysis

If any of the seizure symptoms described above were present in the mouse, 0% protection was noted and if none of the above symptoms were present, the mouse was labeled 100% protection. This was plotted as a function of logarithmic dose and fitted to a sigmoid-dose response (with variable slope (GraphPad Prism) and used to estimate the dose that would give a 50% protection from seizures (N=10 / group). The statistically significant is calculated using non-parametric One-Way ANOVA, and Kruskal-Wallis post hoc test, with significance at $p < 0.05$. The result is presented as mean $\pm$ SEM for each experiment.

Results

The results are presented in Figures 1, 2 and 3. It is demonstrated that Compound-1 dose dependently protected the mice against 6Hz induced partial seizures with minimum effective dose of 1 mg/kg. Further, it is demonstrated that Compound-1 has synergistic effect with levetiracetam in 6Hz model of focal epilepsy, and that Compound-1 has synergistic effect with valproic acid in 6Hz model of focal epilepsy.

Example 6: Anti-convulsant effect of Compound 1 in pentylenetetrazol (PTZ) model of myoclonic generalized seizures

Pentylenetetrazol (PTZ) is a GABA_A receptor antagonist and is used as a chemo convulsant in rodents. The administration of PTZ can cause generalized convulsive and non-convulsive seizures depending on the dose used. It is one of the widely used models for screening of novel test compounds for their anti-convulsant effect.

Compound preparation and administration

Compound-1 was dissolved in 5% dimethyl sulfoxide (DMSO) and 30% hydroxypropyl beta-cyclodextrin (HPβCD) in MilliQ water. Pentylenetetrazol was dissolved in physiological saline solution. Female NMRI mice were pretreated with vehicle or test compound 60 minutes in a volume of 10 ml/kg before intravenously infusion of the PTZ-solution (5 mg/ml) at a speed of 0.7 ml/min through a cannula placed in the tail vein. The duration from initiation of the infusion to appearance of clonic convulsions was noted.

Data analysis

The dose of PTZ required for inducing convulsion in each mouse was calculated in mg/kg body weight (N=10 / group). The minimal effective dose (MED) dose that induce significant increase in PTZ threshold is identified using ANOVA and post hoc Fisher's LSD ($\alpha < 0.05$, graph pad prism). The efficacy is calculated as the % increase in PTZ threshold from vehicle. The result is presented as mean $\pm$ SEM for each experiment.

Results

The results are presented in Figures 4 and 5. It is demonstrated that Compound-1 dose dependently protected the mice against PTZ induced myoclonic generalized seizures with minimum effective dose of 0.3 mg/kg. Further, it is demonstrated that Compound-1

has synergistic effect with levetiracetam in the PTZ model of myoclonic generalized epilepsy.

Example 7: Anti-convulsant effect of Compound-1 in maximal electroshock threshold (MEST) model of tonic clonic generalized seizures

Method

Apparatus: Rodent Shocker type 221 (high current), HUGO SACHS ELEKTRONIK, Stimulus settings: 50Hz, 0,2 s – variable current (mA), Stimulus current intensity was based on 'up and down' method. Each mouse was stimulated only once at any given current intensity and the current intensity of the stimulation in the next animal depended on the outcome of the previous stimulation. The current in the Kimballs method changes by the log scale (0.06). The seizure threshold was expressed as convulsive current value (CC₅₀) which represents the current threshold (mA) predicted to produce tonic convulsions in the 50% of mice. Each mouse receives current stimulus once independent of the outcome.

The anticonvulsant compound should increase the CC₅₀ value.

Data analysis

The convulsive current threshold (CC₅₀) for the treatment group was calculated in mA (N=10 / group). The minimal effective dose (MED) dose that induced significant increase in CC₅₀ was identified using ANOVA and post hoc Fisher's LSD ($\alpha < 0.05$, graph pad prism). The efficacy was calculated as the % increase in CC₅₀ threshold from vehicle. The result is presented as mean $\pm$ SEM for each experiment, $\alpha < 0.05$.

Results

The results are presented in Figure 6. It is demonstrated that Compound-1 has additive effect with retigabine in the maximal electroshock model of tonic clonic generalized epilepsy.

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Claims

1. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-
[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof.
5
2. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-
[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, for
use as a medicament.
- 10 3. 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-
[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, for
use in the treatment, prevention and/or alleviation of epilepsy.
- 15 4. A combination of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-
1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable
salt thereof, and an anti-epileptic drug (AED) for use in the treatment,
prevention and/or alleviation of epilepsy.
- 20 5. A kit-of-parts comprising at least two separate unit dosage forms (A) and (B),
wherein
(A) comprises of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-
b]pyridine-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically
acceptable salt thereof; and
(B) comprises an anti-epileptic drug (AED) or a pharmaceutically acceptable
25 salt thereof; and optionally
(C) instructions for the simultaneous, sequential or separate administration of
(A) and (B), to a subject in need thereof.
- 30 6. The combination for use according to claim 4 or the kit-of-parts for use
according to claim 5, wherein the AED is Levetiracetam.
7. The combination for use according to claim 4 or the kit-of-parts for use
according to claim 5, wherein the AED is valproic acid.

8. The combination for use according to claim 4 or the kit-of-parts for use according to claim 5, wherein the AED is Retigabine.
9. The kit-of-parts according to any one of claims 5 to 8 for the treatment,
5 prevention and/or alleviation of epilepsy.
10. A method for manufacturing 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, the method comprising reacting 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-
10 2-ol with 4-fluoro-5-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile.
11. The method according to claim 10, wherein the reaction is performed in presence of a palladium catalyst, such as Pd(Dppf)Cl₂-CH₂Cl₂, and a base,
15 such as an alkali metal carbonate, for example Na₂CO₃.
12. The method according to any one of claims 10 to 11, wherein the method further comprises reacting 2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with 2-bromo-4-fluoro-1-nitrobenzene to generate 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.
20
13. The method according to any one of claims 10 to 12, wherein the method further comprises reducing 2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol to 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.
25
14. The method according to any one of claims 10 to 13, wherein the method further comprises reacting 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol with tert-Butyl nitrite and DMSO to generate 2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol.
30
15. A compound selected from the group consisting of:
2-(1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol;
2-(1-(3-bromo-4-nitrophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol;
35 2-(1-(4-amino-3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol; and

2-(1-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-5-yl)propan-2-ol,
or a salt thereof.

- 5 16. A method for treatment, prevention and/or alleviation of epilepsy, said method comprising administering 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, to a subject in need thereof.
- 10 17. Use of 5-fluoro-3'-(5-(2-hydroxypropan-2-yl)-1H-imidazo[4,5-b]pyridin-1-yl)-4-methoxy-[1,1'-biphenyl]-2-carbonitrile, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treatment, prevention and/or alleviation of epilepsy.