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Natural product-derived therapies for treating drug-resistant epilepsies: From ethnopharmacology to evidence-based medicine

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ABSTRACT

Ethnopharmacological relevance: Epilepsy is one of the most prevalent neurological human diseases, affecting 1% of the population in all age groups. Despite the availability of over 25 anti-seizure medications (ASMs), which are approved in most industrialized countries, approximately 30% of epilepsy patients still experience seizures that are resistant to these drugs. Since ASMs target only limited number of neurochemical mechanisms, drug-resistant epilepsy (DRE) is not only an unmet medical need, but also a formidable challenge in drug discovery.

Aim: In this review, we examine recently approved epilepsy drugs based on natural product (NP) such as cannabidiol (CBD) and rapamycin, as well as NP-based epilepsy drug candidates still in clinical development, such as huperzine A. We also critically evaluate the therapeutic potential of botanical drugs as polytherapy or adjunct therapy specifically for DRE.

Methods: Articles related to ethnopharmacological anti-epileptic medicines and NPs in treating all forms of epilepsy were collected from PubMed and Scopus using keywords related to epilepsy, DRE, herbal medicines, and NPs. The database clinicaltrials.gov was used to find ongoing, terminated and planned clinical trials using herbal medicines or NPs in epilepsy treatment.

Results: A comprehensive review on anti-epileptic herbal drugs and natural products from the ethnomedical literature is provided. We discuss the ethnomedical context of recently approved drugs and drug candidates derived from NPs, including CBD, rapamycin, and huperzine A. Recently published studies on natural products with preclinical efficacy in animal models of DRE are summarized. Moreover, we highlight that natural products capable of pharmacologically activating the vagus nerve (VN), such as CBD, may be therapeutically useful to treat DRE.

Conclusions: The review highlights that herbal drugs utilized in traditional medicine offer a valuable source of potential anti-epileptic drug candidates with novel mechanisms of action, and with clinical promise for the treatment of drug-resistant epilepsy (DRE). Moreover, recently developed NP-based anti-seizure medications (ASMs) indicate the translational potential of metabolites of plant, microbial, fungal and animal origin.

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Abbreviations

Δ9-THC	Δ9-tetrahydrocannabinol
ASM	anti-seizure medication
BBB	blood-brain barrier
BGF	bioactivity-guided fractionation
CBD	cannabidiol
CBDV	cannabidivarin
CNS	central nervous system
DRE	drug-resistant epilepsy
DS	Dravet syndrome
HTS	high-throughput screening
Hup A	huperzine A
ILAE	International League Against Epilepsy
GFAP	glial fibrillary acidic protein
GNX	ganaxolone
GPCR	G-protein-coupled receptor
LGS	Lennox-Gastaut syndrome
MAP kinase	mitogen-activated protein kinase
MES	maximal electroshock seizure
NMDA	N-methyl-D-aspartate
NP	natural product
PKC	protein kinase C
PTZ	pentylenetetrazol
RPM	rapamycin
SC	synthetic cannabinoid
TCM	traditional Chinese medicine
TSC	tuberous sclerosis complex

1. Introduction

1.1. Forms of epilepsy

Epilepsy is defined by the International League Against Epilepsy (ILAE) as a “common brain disorder characterized predominantly by an enduring predisposition to generate recurrent and unpredictable epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition’’(Fisher et al., 2014). Approximately 50 million people worldwide are effected by epilepsy, with up to 30% being resistant to current treatments (WHO, 2019).

Epilepsy can be categorized into six groups based on its etiology: (1) structural, related to visiblestructural abnormalities through neuroimaging e.g. MRI; (2) genetic, caused by mutations leading to seizures; (3) infectious, where seizures arise from brain infections like meningitis or encephalitis; (4) metabolic, where seizures are a symptom of metabolic disorders; (5) immune, where seizures are a core symptom of immune or autoimmune disorder and (6) unknown, when the etiology of seizures cannot be described using the aforementioned categories (Scheffer et al., 2017; Wei et al., 2021). Because of the variety of etiologies, the different forms of epilepsy are more accurately referred to as "the epilepsies".

Epilepsy is not only associated with seizures and physiological impairments, but also with various comorbidities, including neurodegenerative disorders, primary intracranial tumors, migraine, obstructive sleep apnea, anxiety, and depressive disorders. Epilepsy also carries an increased risk of premature death, with SUDEP (sudden unexpected death in epilepsy patients) causing around 600 deaths annually (Yuen et al., 2018; Guillen et al., 2019; Avalos et al., 2020; Casillas-Espinosa et al., 2020; Demarquay and Rheims, 2021).

The management of currently untreatable epilepsy remains a significant clinical challenge and unmet medical need. Despite the recent discovery and introduction of new anti-seizure medications (ASMs), more one third of patients with new onset epilepsy dontrespond to available medications and fail toachieve seizure freedom (Brodie et al., 2012; Glauser et al., 2013). These patients are referred to as having drug-resistant epilepsy (DRE) or treatment-resistant, epilepsy which is defined as “failure of adequate trials of two tolerated, appropriately chosen and used anti-epileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom” (Kwan et al., 2010). Treatment-resistant seizures are also known as drug-resistant or refractory seizures.

Among the epilepsy patients who do not respond to ASMs, only a small percetange benefit from alternative therapeutic options such as resective surgery of the epileptogenic zone, ketogenic diet or electrical stimulation (Li et al., 2020).

More than 50% of epilepsies have a genetic basis, and modern molecular diagnostics for epilepsy patients utilize gene panels with over 300 individual genes. Mutations in many of these genes are associated with childhood epilepsy syndromes, often coupled with significant comorbidities such as developmental delay, cognitive impairment and high mortality. Numerous childhood epilepsy syndromes, exhibit notably high levels of treatment-resistant seizures, sometimes with only few patients responding to currently approved ASMs, as in the case of CDKL5 deficiency disorder (CDD) (Leonard et al., 2022).

Interestingly, while classical ASMs have limited efficacy for childhood epilepsy syndromes, there are several cases (discussed in this review) where NP-based drugs have demonstrated anti-seizure activity in these refractory syndromes. Noteworthy examples of childhood epilepsy syndromes recently investigated in clinical trials for novel ASM drug candidates include Dravet syndrome (DS), Lennox-Gastaut syndrome (LGS), West syndrome and tuberous sclerosis complex (TSC). Therefore, NPs with a distinct mechanisms of action may offer a unique opportunity for childhood epilepsy syndromes and other DREs that do not respond to classical ASMs. Conversely, childhood epilepsy syndromes offer unique opportunities for anti-epileptic NPs, namely (1) genetic animal models allowing screening and preclinical validation of NPs for specific syndromes, and (2) commercial protection through orphan drug designation and exclusivity for drugs that otherwise may not have full patent protection (i.e. composition-of-matter claims) because of their origin as NPs.

1.2. Anti-seizure medications (ASMs)

The era of modern pharmacotherapy of seizure began in 1857 with the introduction of bromides. Since then, over 40 ASMs have been developed and marketed, expanding the range of treatment options for various epileptic syndromes (Shorvon, 2009a, 2009b). An overview of the most frequently prescribed ASMs, their chemical structure, indications and major side effects are given in Table S1. ASMs are classified into three generations, as commonly recognized in the literature (Shorvon, 2009a, 2009b; Bialer and White, 2010; Löscher and Schmidt, 2011).

Despite the wide range of therapeutic options provided by antiepileptic medications, including specific indications for certain drugs (e.g., rufinamide for Lennox-Gastaut syndrome or stiripentol for Dravet syndrome), the efficacy of third-generation ASMs in terms of improved seizure control compared to the first-line treatments for partial and generalized seizures, such as carbamazepine and valproate, is limited. Although significant progress has been made in terms of minimizing side effects, improving pharmacokinetics, and enhancing safety, most of these newer drugs have not demonstrated superior efficacy (Marson et al., 2007a, 2007b; Löscher and Schmidt, 2011).

There is a persistent need for new ASMs and drug candidates with innovative mechanisms of action. Three main strategies have been employed in the development of new ASMs. Firstly, based on preclinical

drug testing in animal models of acute seizures suggested by the Anticonvulsant Screening initatives, such as the NINDS Epilepsy Therapy Screening Program (ETSP), most of the drugs were discovered in MES and PTZ animal epilepsy models. Secondly, the majority of existing drug therapies acts through similar mechanisms of action (i.e. targeting voltage-gated ion channels and the GABAergic system). The discovery of new targets will therefore be crucial for the emergence of better treatments. Lastly, while most currently available drugs focus on suppressing seizures (the consequence), there is a growing recognition of the need for drugs that address the underlying cause of the disease, with potential anti-epileptogenic or disease-modifying effects (Pitkänen, 2010). In the case of genetic epilepsies, there are already medicines, such as rapamycin, that target the specific cause of the disease by interacting with the altered mTOR pathway (Section 3.2.1 provides further details).

2. Anti-epileptic herbal drugs

In developing countries traditional medicine remains a primary source of healthcare, including the treatment of epilepsy. At present, 80% or more epilepsy patients in these areasrely on local healers, who primarily prescribe herbal preparations and decoctions due to limited access and affordability of conventional ASMs. Consequently, many studies have reported the anti-seizure activity of medicinal plants, plant extracts or enriched fractions. Most of these studies were conducted on rodent animal models, where significant effects could be monitored (Quintans Júnior et al., 2008).

Several reviews (Table 1) have already summarized the currently available ethnopharmacological information related to herbal drugs used in epilepsy. The biological and pharmacological validation of traditional knowledge, e.g. by performing *in vivo* assays, are not only a a condition for development of new therapies based on herbal preparations, but also of great interest for the safety and improved usage of potentially anti-epileptic herbal medicines in developing countries. However, to date, very few studies have led to the identification of active principles, likely because of the lack of assays compatible with high-throughput screening (HTS) and bioactivity-guided fractionations (BGF) studies.

The validity of ethnopharmacological information in these studies is influenced by how epilepsy is diagnosed and classified in herbal medicine. Seizure classification varies depending on the type of medicine. In Ayurvedic medicine, epilepsy is considered as a mental disease, and is classified based on three elements (vata, pitta, kapha) of the human body. The description of seizure types has very little resemblance with definition of seizures in Western medicine. Traditional Chinese medicine (TCM) also has its own classification, describing 4 subtypes of epilepsy that differ from the ILAE definitions (Ekstein and Schachter, 2010).

In addition to the symptomatic diagnosis of epilepsy, the preparation of the plant extract for treatment plays a vital role. Typically, decoctions, macerations, infusions or hydro-alcoholic extracts are utilized. It is important to consider these methods when searching for the active principles during phytochemical investigations. The choice of an appropriate extraction method and solvent is critical, as different compounds will be present in extracts depending on their polarity and the solvents used. Polar compounds will be extracted more effectively by polar solvents, while hydrophobic components will have a stronger affinity for lipophilic solvents. It is essential to maintain similar conditions to those employed by traditional healers to ensure consistency. Based on the extensive ethnopharmacology information gathered to date, there is a compelling interest to carry out "reverse pharmacology" approaches based on empirical knowledge of traditional healers or anecdotally observed therapeutic effects (Butterweck and Nahrstedt, 2012), which also applies to new chemical entities of natural origin suitable for the treatment of epilepsy. To facilitate the discovery process it is crucial to establish bioassays that (1) align with bioactivity-guided fractionation methods and (2) demonstrate a good predictability for human epilepsies.

Table 1 demonstrates the antiseizure activity of numerous medicinal plants, as evidenced by *in vitro* and animal studies, leading to the discovery of various NPs.

3. Drugs and drug candidates derived from natural sources

Here we review recently approved drugs and drug candidates in clinical trials that are NPs with a strong link to ethnopharmacology, including cannabidiol, rapamycin, ganaxolone and huperzine (Fig. 1).

3.1. NPs derived from plants

3.1.1. Cannabinoids

3.1.1.1. Historical background and therapeutic indication. *Cannabis sativa* L. (Cannabaceae) has been widely used for centuries as a recreational drug and botanical drug for the treatment of a wide range of diseases (Deiana, 2013; Devinsky et al., 2014). English neurologists used cannabis to treat epilepsy in the late 19th century before it fell out of favor because of psychoactive effects inducing behavior, coordination and memory impairments (Devinsky et al., 2014).

While today more than 100 cannabinoids have been described in *C. sativa* the two primary cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) were isolated from *C. sativa* and *C. indica* only in the 1940s (Devinsky et al., 2014) and structurally elucidated in the 1960s already but the major targets for Δ9-THC was only found and confirmed in the late early 1990s. Δ9-THC activates cannabinoid type-1 receptors, which are highly abundant GPCRs in the brain, and cannabinoid type-2 receptors, which are mainly found in immune cells. Cannabinoid receptors are activated by called endocannabinoids. In the CNS, endocannabinoids are identified mainly as 2-arachodinoylglycerol (2-AG) and arachidonoylethanolamine (anadamide). The endocannabinoid system has been shown to play a major role in epilepsy and is therefore a drug target (Cristino et al., 2020).

Cannabidivarin (CBDV), isolated from different species (*C. sativa, C. indica)* of cannabis in the late 1960s, is lately under investigation for its anticonvulsant activity (Hill et al., 2012; Bialer et al., 2015).

3.1.1.2. Chemistry, pharmacology, mechanism of action. Since there are numerous publications and reviews on the pharmacology and phytochemistry of cannabinoids (Soltesz et al., 2015; Golub and Reddy, 2021), here we will focus primarily on aspects relevant for its development as an anti-seizure drug.

Cannabinoids are lipophilic terpenophenolic compounds present as acids in the resin found in the trichomes of mainly female inflorescences (Pertwee, 2008; Hill et al., 2012). The decarboylation product Δ9-THC is the major psychoactive cannabinoid and a potent partial agonist of CB1 and CB2 receptors. Δ9-THC (Dronabinol) has been used to treat nausea and vomiting caused by chemotherapy and loss of appetite and weight loss in people who have acquired immunodeficiency syndrome (AIDS). Given its role in negatively modulating synaptic glutamate release, the CB1 receptor strongly inhibits glutamate-mediated seizures in animal models. Paradoxically, strong CB1 receptor activation can also induce seizures, possibly via the inhibition of synaptic GABA release and by enhancing glutamatergic transmission in the hippocampus (Funada and Takebayashi-Ohsawa, 2018). In agreement with human studies and case reports, this study found that SCs generally produced more seizures than THC (Breivogel et al., 2020). Cannabigerol, the canonical precursor of cannabinoids does not interact significantly with cannabinoid receptors but it is weak agonist of α-adrenoreceptors and an antagonist of 5-HT_{1A}, which may contribute to the potential of cannabis to affect neuronal excitability via voltage-gated and ligand-gated ion channels (Cascio et al., 2010; Pertwee, 2008).

The gain of interest in *C. sativa* for ASM research is primarily related to the anticonvulsant activity of both Δ^9-THC and CBD in several animal

Table 1
Recent reviews on medicinal plants used in traditional medicine for epilepsy.

Review title	Described species	Type of assay	Additional traditional application(s) described	Remarks	References
Plants with anticonvulsant properties - a review	355 species	*in vivo* and *in vitro* models	Anticonvulsant, anxiolytic, analgesic, antidepressant		Quintans Júnior et al. (2008)
Plants used to treat epilepsy by Tanzanian traditional healers	60 species belonging to 55 genera and 45 families	*in vivo* and *in vitro* models	Anticonvulsant, anti-malaric	14 plants with possible anticonvulsant activity	Moshi et al. (2005)
Pharmacological screening of Malian medicinal plants used against epilepsy and convulsions	11 tested plants	*in vivo* and *in vitro* models	Anticonvulsant	7 active plants	Pedersen et al. (2009)
Evaluation of the sedative and anticonvulsant properties of three Cameroonian plants	3 described plants - *Millettia thonningii, Ocinum sanctum* and *Securitaca longepeduncula*	*in vivo* models: MES, NMDA, PTZ, INH (isonicotinic hydrazide acid), PIC (picrotoxin), STR (strychnine)	Epilepsy, insomnia and headaches Pain, fevers, heartaches, pneumonia, cold, stomachaches, allergy, worms, dysentery, rheumatism, jaundice, bronchitis, itch, intestinal obstruction	All 3 plants shows anticonvulsant activity	Okomolo et al. (2011)
Efficacy of Iranian Traditional Medicine in the Treatment of Epilepsy	13 described plants with antiseizure activity	*In vivo* models (MES, PTZ, PIC) and clinical trials	Anticonvulsant	Clinical trials for several plants in childhood refactory epilepsy or uncontrolled epilepsy	Abdollahi Fard and Shojaii (2013)
Review on plants with CNS-effects used in traditional South African medicine agains mental diseases	150 plant species from 63 familie	*in vitro, in vivo* models (PTZ, PIC, BIC, NMDA)	Mental illnesses, epilepsy, depression, age-related dementia, debilitative mental disorders		Stafford et al. (2008)
Medicinal plants used in Iranian traditional medicine to treat epilepsy	25 described plants	*in vivo* tests (PTZ, MES)	Anticonvulsant	11 plants shows antiepileptic activity	Sahranavard et al. (2014)
Validation of anticonvulsant and sedative activity of six medicinal plants	6 described plants	*in vivo* tests (MES, NMDA, PTZ, isonicotinichydrazide acid (INH), PIC, STR)	Epilepsy, pains, anxiety, insomnia, dizziness, headaches, migraine Antimicrobial, immunological, anti-inflammatory, analgesic, antioxidant, insecticidal, antimalaria, anti-ulcer, myorelaxant	All plants shows antiepileptic and sedative activity	Bum et al. (2009)
Review of the use of botanicals for epilepsy in complementary medical systems - Traditional Chinese Medicine	23 described plants	*in vivo* tests i.p. (PTZ, kainic acid (KA), pilocarpine, NMDA)	Anticonvulsant	Described tests for all the plants and their main active constituents	Xiao et al. (2015)
Ayurveda and botanical drugs for epilepsy: Current evidence and future prospects	55 different single herbs and formulations	*in vivo* tests (PTZ, NMDA, MES, strychnine)	Anticonvulsant	Some studies in human populations described	Sriranjini et al. (2015)
The importance of botanical treatments in traditional societies and challenges in developing countries	Described plants with anticonvulsant activity		Anticonvulsant	Botanicals used for epilepsy in developing countries described	Kakooza-Mwesige (2015)
Epilepsy in the Renaissance: A survey of remedies from 16th and 17th century German herbals	221 plants from 53 families with potent anticonvulsant activity	*in vivo* tests p.o., i.p. (PTZ, strychnine, kainic acid, isoniazid, PTX, g-butyrolacton, baclofen, bicucullin, pilocarpine, MES)	Anticonvulsant	For 49 of them confirmation from phytochemistry and *in vitro* and *in vivo* studies were found	Adams et al. (2012)
Review on phytotherapy in epilepsy	Mentioned 148 plants	*in vitro* and *in vivo* tests p.o., i.p., i.v., s.c.,	Anticonvulsant	For many of plants active constituents were find	Nsour et al. (2000)
Medicinal compounds with antiepileptic/ anticonvulsant activities	21 alkaloids, 7 flavonoids, 20 terpenoids	*in vivo* tests i.p. (PTZ, MES, kainic acid, bicuculline, pilocarpine, NMDA)	Anticonvulsant		Zhu et al. (2014)
Anticonvulsant effects of medicinal plants with emphasis on mechanisms of action	31 described plants and 7 constituents	*in vivo* tests i.p. (PTZ, MES)	Anticonvulsant	Possible mechanism of action explained	Rabiei (2017)
Herbal medicine for epilepsy seizures in Asia, Africa and Latin America: A systematic review	351 different plant species	*in vitro* and *in vivo* tests	Anticonvulsant	107 plants possess anticonvulsant activity on different models. For some possible mechanism of action explained.	Auditeau et al. (2019)
Excavating Anticonvulsant Compounds from Prescriptions of Traditional Chinese	10 classical prescription, from which 66 pure	*in vitro* and *in vivo* tests	Anticonvulsant		Zhao et al. (2018)

(*continued on next page*)

Table 1 (*continued*)

Review title	Described species	Type of assay	Additional traditional application(s) described	Remarks	References
Medicine in the Treatment of Epilepsy	compounds were tested				
Chinese Herbal Medicine for Treating Epilepsy	12 plants, different parts	*in vivo* tests	Anticonvulsant Anti-inflammatory Neuroprotective	Possible mechanism of action explained	Lin and Hsieh (2021)

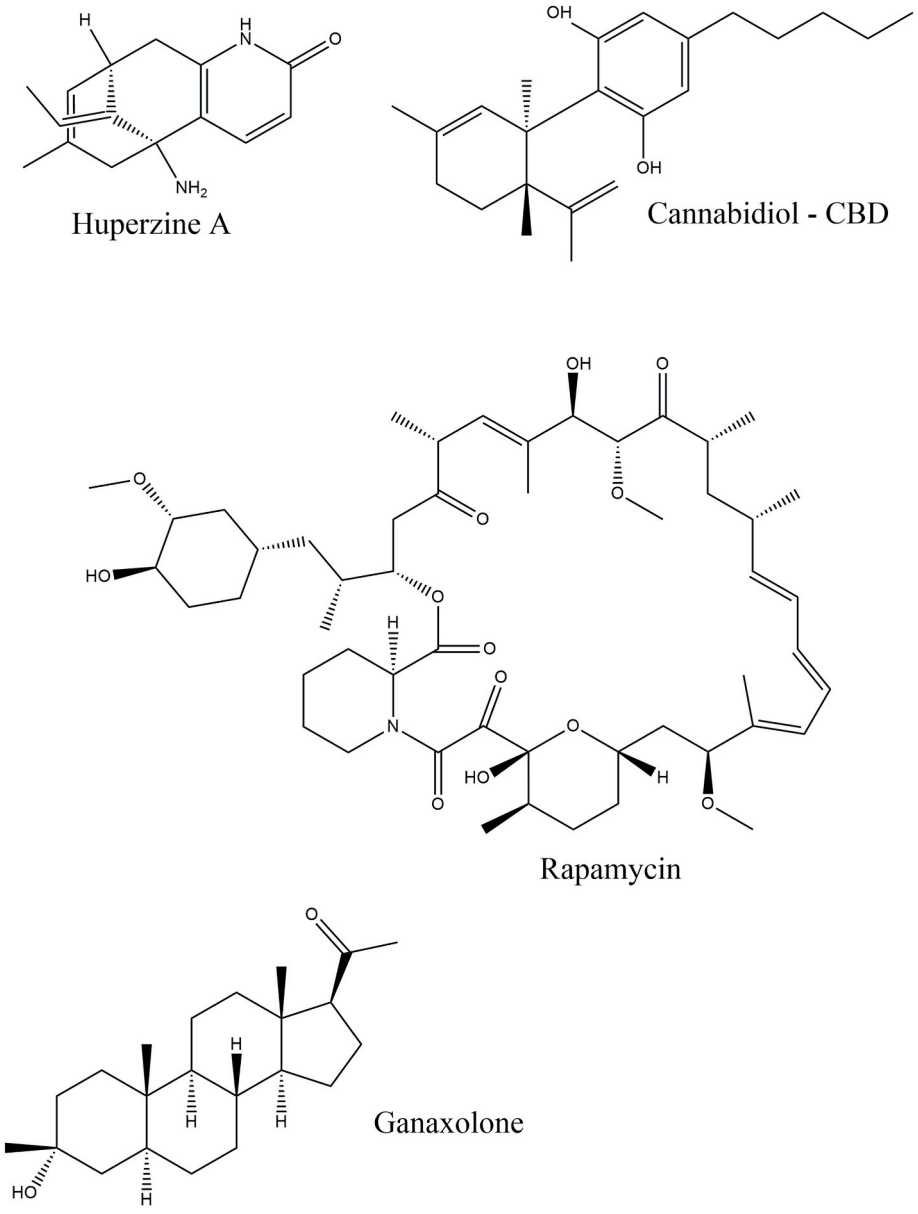

Fig. 1. Structures of NP-based drugs in clinical trials for drug-resistant epilepsies.

models, but both compounds have also been shown to exhibit contra-indicatory proconvulsant effects in healthy animals (Devinsky et al., 2014). Δ^9-THC is the major psychoactive compound of cannabis showing a range of aversive effects like impaired learning and memory, thereby limiting its potential use as an ASM, whereas CBD is the major nonpsychoactive compound, and has shown promising data as an anti-convulsant (Devinsky et al., 2014). For that reason, GW Pharma has developed Epidiolex (100 mg CBD per 1 ml solution) which is approved in different countries to treat seizures associated with Lennox-Gastaut syndrome (LGS), Dravet syndrome, or Tuberous Sclerosis Complex (TSC). As adjugant therapy, Epidiolex significantly reduced seizures in patients living with LGS, Dravet syndrome, or TSC for whom multiple previous seizure medicines did not work well.

Overall, CBD has an attractive safety profile with very few side effects, like induction of transaminases in liver (Hill et al., 2012; Campos et al., 2016). Despite its rather poor oral bioavailabilty, CBD has a therapeutic potential in other neuropsychiatric disorders such as anxiety, schizophrenia and addiction (Fusar-Poli et al., 2009), as well as to its antipsychotic and antinausea effects (Mechoulam et al., 2002; Campos et al., 2016). It is generally accepted that CBD is a potent negative

allosteric modualtor of CB1 receptors *in vitro* and *in vivo* (Straiker et al., 2018), which may also explain why it limits the aversive effects of THC. At high concentrations, CBD acts as allosteric modulator of the μ receptor and as an inverse agonist of CBD2, and the latest interaction might be partly responsible of its anti-inflammatory activity (Thomas et al., 2007; Scuderi et al., 2009).

Promising preclinical data on several animal models of epilepsy and anecdotal human data are encouraging the ongoing exploration of cannabinoids as anticonvulsants.

3.1.1.3. Preclinical data. CBD and its derivative CBD propyl homolog (cannabidivarin) (Devinsky et al., 2014) are both anticonvulsants in animal models.

CBD has shown activity in MES (Consroe and Wolkin, 1977), magnesium-free, 4-aminopyridine, audiogenic and all GABA inhibition-based models at doses from 50 to 400 mg/kg, while most effects occur at dose higher than 100 mg/kg. Based on rodent data reported to date, CBD currently appears to be the cannabinoid with the greatest therapeutic potential for epilepsy. Investigation of the potential of Sativex® for epilepsy, which is commercially available compound containing a mixture of CBD:Δ^9-THC, has revealed its efficacy in treatment of focal seizures, including temporal lobe epilepsy, as well as for generalized convulsion induced by electroshock and GABA receptors blockers (Scuderi et al., 2009). Epidiolex (≥98% pure CBD) was approved by FDA in 2018, and by the EMA in 2019, for the treatment of Lennox-Gastaud syndrome, Dravet syndrome and Tuberous Sclerosis Complex.

In recent studies CBD showed dose-dependent protection in the acute seizure models – mouse 6 Hz 44 mA (ED_{50} 164 mg/kg), mouse MES (ED50 83.5 mg/kg) and rat MES (ED_{50} 88.9 mg/kg). In chronic models, CBD produced dose-dependent protection in corneal kindled mice (ED_{50} 119 mg/kg). No protection was noticed in the lamotrigine resistant amygdala kindled rat (even up to 300 mg/kg)(Klein et al., 2017).

Recently CBD and CBDa studies on Male Sprague-Dawley CD albino rats (100−120 g) in acute seizure model MES 0.2 s, 60 Hz, 150 mA stimulus showed dose-dependent activity for both constituents. CBD (ED_{50} 68.78 mg/kg) and CBDa (ED50 of 76.61 mg/kg) were administered i. p. 1 h and 2 h prior to stimulation(Goerl et al., 2021).

3.1.1.4. Clinical data. In 2018, the U.S. Food and Drug Administration approved Epidiolex (CBD) as an oral solution for the treatment of seizures associated with two rare and severe forms of epilepsy, Lennox-Gastaut syndrome and Dravet syndrome, in patients two years of age and older. This was a milestone in cannabinoid-based therapies and the first FDA approval of a drug for the treatment of patients with Dravet syndrome. There are multiple ongoing clinical trials with cannabinoids (clinicaltrials.gov). Different routes of administration have been used to deliver CBD but the most common one is smoked *C. sativa*. When CBD is administrated through a specialized device in vaporization or solization, the peak plasma concentration of CBD is reached after around 10 min, with a bioavailability of 31%. Unfortunately, the *per os* route via an oil-based capsule containing CBD achieves only poor bioavailability (around 6%) because of a first-pass liver metabolism but 20–50 higher doses are needed p. o. in comparison to i. v. and induce severe intoxication (Hill et al., 2012; Jones et al., 2012).

Cannabinoids have been approved for other indications including pain, nausea, and spasticity. A comprehensive metaanalysis on the efficacy of CBD in different forms of epilepsy was published by Lattanzi et al. (2018). Several clinical trials for CBD in DRE forms have been performed or are ongoing (Davis et al., 2021; Gaston et al., 2019). The Phase III clinical trials for the treatment of Dravet syndrome and Lennox-Gastaut have been finished (Devinsky et al., 2017; Laux et al., 2019). Here the frequency of convulsive seizures was decreased by a median of 38.9% in the CDB group, whereas the placebo group showed a median decrease of 13.3%. The study assigned 120 childeren and young adults with the Dravet syndrome and drug-resistant seizures. CBD is currently in phase IV clinical trials for the treatment of diopathic generalized epilepsy (NCT04899050) (Privitera et al., 2021).

Because of regulatory issues (cannabis-derived compounds are e.g. classified in the US as Class 1 substances, indicative of a high potential for abuse), epidemiologic data are limited and the therapeutic use of marihuana, as compared to CBD as a monosubsance, in epilepsy remains controversial (Detyniecki and Hirsch, 2015; Markle and Nativio, 2019). All available clinical data involve the administration of cannabis over short periods only. There is limited data regarding the long-term exposure of epilepsy patient to cannabinoids, and these data come mainly from CBD (Arnold et al., 2023; Devinsky et al., 2019). Because of poor clinical data and the psychoactive dimension of THC, there is an ongoing debate regarding the use of marihuana in epileptic patients (Russo et al., 2005; Cortesi and Fusar-Poli, 2007; Sirven, 2014; Szaflarski and Bebin, 2014).

Nevertheless, there is still an acute need for unbiased data on safety and efficacy gathered from correctly done double-blind, placebo controlled, randomized clinical trials (Cilio et al., 2014; Friedman and Devinsky, 2015).

3.1.2. Huperzine A

3.1.2.1. Historical background and therapeutic indications. Huperzine A (Hup A) is an alkaloid isolated in 1986 from fir mosses mainly found in China, America and Europe, *Huperzia serrata* (Thunb.) Trevis is known also under the synonym *Lycopodium serratum* Thunb. (Lycopodiaceae) (Tun and Herzon, 2012; Ferreira et al., 2016). *H. serrata* has been traditionally used in Chinese medicine for treating sprains, swelling, contusion and schizophrenia (Ferreira et al., 2016). Lycopodium alkaloids are liposoluble and are classified in four subtypes: fawcettimine-type, lycopodine-type, miscellaneous-type and lycodine-type, the latter being the subtype to which Hup A belongs. Hup A shows promising potential as a CNS drug candidate and has demonstratedactivity in both acute and chronic disorders (Gordon et al., 2001). These therapeutic applications include acetylcholine-deficit dementia, Alzheimer's disease, cerebrovascular dementia and other neurodegenerative disorders that cause ischemic or cognitive impairements. Hup A is also utilized in the treatment of myasthenia gravis, organophosphate poisoning, and schizophrenia (Ferreira et al., 2016).

With regard to epilepsy, Hup A was found to possess anti-inflammatory, antinocioceptive and anticonvulsant properties. Due to its ability to modulate the activity of the NMDA receptor, Hup A has been investigated in epilepsy models. However, achieving NMDA inhibition in humans requires very high doses, so the clinical efficacy of Hup A is preliminary attributed to its cholinergic activity. Fortunately, Hup A exhibits favorable pharmacokinetic, good tolerance, and no apparent toxicities at doses higher than the effective dose (Ferreira et al., 2016).

3.1.2.2. Chemistry, pharmacology, mechanism of action. Hup A is an unsaturated sesquiterpene alkaloid that occurs as a racemic mixture, (−)-Hup A and (+)-Hup A. Considering the low levels of these compounds in plant extracts (0.011%), the synthesis of each eutomer has been described (White et al., 2013). The (−) form of Hup A exerts 38-fold more inhibition of AChE than the (+) form (Li et al., 2007).

Hup A has the ability to penetrate the blood-brain barrier (BBB), and acts as specific AChE inhibitor. It shows minimal cholinergic toxicity, and is a cerebral NMDA antagonist. The potency of AChE inhibition by Hup A is greater than that of donepezil but less potent than tacrine. In terms of selectivity, (−)-Hup A demonstrates a 900-fold greater selectivity for AChE compared to BuChE (butyrylcholinesterase), which contributes to its safety profile (Wang and Tang, 1998; Tun and Herzon, 2012). As results, Hup A is used in Alzheimer's disease patients to enhance memory, cognitive and behavioral functions. Although it is currently not approved as a medication, it is available orally as dietary

supplement. It is also AE and antinocioceptive when administered *i. p.* in mice (Park et al., 2010). By virtue of its neuroprotective activity, Hup A improves short and long-term memory, particulary in patients with arteriosclerosis. Furthermore, it exhibits analgesic effect through muscarinic cholinergic receptors, as observed in acute thermal escape and the formalin evoked flinching response, revealing the potential of Hup A as a spinal analgesic (Zhu, 1991; Park et al., 2010).

3.1.2.3. Preclinical data. Alkaloids of the lycopodine-type include one NP, 12-deoxyhuperzine (Jiang et al., 2019), which ihas been identified as antagonist of the NMDA (N-methyl-D-aspartate) receptor (IC_{50} = 0.92 μM) (Ferreira et al., 2016). The NMDA receptor is implicated in the pathogenesis of neurodegenerative diseases such as epilepsy and Alzheimer's disease (SanMartin and Churruca, 2011). As subtype of glutamate receptors, NMDA receptors, play major roles in the modulation of synaptic plasticity, making them important for memory and learning processes.

The activation of the NMDA receptor by glutamate release leads to a significant influx of calcium ions (Ca^{2+}), which can result in oxidative stress, disruption of cellular homeostasis, and activation of downstream neurotoxic pathways. These pathways contribute to neuroinflammation by up-regulating and releasing neurotoxic cytokines (Pazdernik et al., 2001; Collombet et al., 2008; Angoa-Pérez et al., 2010; Johnson and Kan, 2010; Wang et al., 2013). The excessive excitation mediated by NMDA receptors is associated with the development of epilepsy, thus highlighting the potential of Hup A as an anti-epileptic agent. Both eutomers of Hup A are active on NMDA receptors, and (+)-Hup A at 3 mg/kg protects against SE seizures induced via NMDA activation in rodents. The inhibition of NMDA receptors in humans requires high doses of Hup A, which are are typically administered in animals.

Hup A has also been found to protect against seizures induced by beta-amyloid, glutamate, free radicals and organophosphorous-related neurotoxicities (Lallement et al., 1991, 2002). Hup A is indeed efficient in treating pre- or post-organophosphorius poisoning exposure in comparison to standard treatment which consists of administrating oximes, atropine and anticonvulsant benzodiazepines (Wang and Tang, 2005; Coleman et al., 2008). Excessive accumulation of ACh occurs upon irreversible inhibition of AChE by e.g. the binding of organophosphorous nerve agents, leading to the release of excitatory amino acids. These amino acids induce an overexcitation of NMDA receptors leading to epileptic seizures.

Hup A was efficient against PTZ-induced seizures in mice after an oral injection of 1 mg/kg while it expressed no activity in the MES model. The peak concentration was reached after 1 h. Hup A is efficient in the 6 Hz model from 0.28 to 0.78 mg/kg with a stimulation current ranging from 22 to 44 mA. The toxic dose is around 0.83 mg/kg (Bialer et al., 2007, 2009, 2010; Schachter, 2009). Hup A has also been used in dogs (at a dose of 1 g/kg twice a day) to treat complex partial seizures (Licht et al., 2002; Schneider et al., 2009). Hup A (0.6 mg/kg) suppressed seizures in the rat PTZ acute seizure model and epileptic spikes on EEG. Additionally, gamma frequency band power was enhanced. Anticonvulsant activity was found to be mediated by activation of cortical GABA transmission (Gersner et al., 2015). Hup A (0.56 or 1 mg/kg) showed protection against 6 Hz, pentylenetetrazole (PTZ)-, and maximal electroshock (MES)-induced seizures in mice with Dravet syndrome and genetic epilepsy with febrile seizures. This protection was maintained during chronic administration and muscarinic and GABA A receptors play here an important role (Wong et al., 2016).

Hup A (0.1 mg/kg) shows protection against seizure and memory impairment induced by kainic acid in mice temporal lobe epilepsy model. This activity may be due to anti-inflammatory activity in microglia and hippocampus (Mohseni-Moghaddam et al., 2019).

3.1.2.4. Clinical data. The clinical profile of Hup A has been extensively studied compared to the other NPs. It is known to be metabolized by CYP 1A2 (Ma et al., 2003; Ferreira et al., 2016). The pharmacokinetic analysis of Hup A reveals a biphasic profile combining a rapid distribution phase then a slower elimination phase. It has been suggested that to reduce cholinergic periperic side effects and improve bioavailability of Hup A in brain, the administration route should ideally be intranasal rather than oral. By using this route of administration first-pass metabolism is avoided and this enables a rapid absorption to the systemic circulation (Zhao et al., 2007).

Hup A is currently being investigated in clinical trials for its therapeutic potential (Ma et al., 2007; Rafii et al., 2011). Hup A is in a Phase II trial for focal impaired awareness seizures. The study is following 16 participants that receive Hup A 0.25 mg twice a day to reach maximum tolerated dose. The seizures are monitored by EEG recordings (NCT03474770).

3.2. NPs derived from microorganisms and animals

3.2.1. Rapamycin

3.2.1.1. Historical background and therapeutic indication. Rapamycin (RPM) (also known as sirolimus) is a macrocyclic polyketide originally isolated from a soil microorganism of the Polynesian island of Rapa Nui (Easter Island). Originally developed as an antifungal agent, it later gained approval as an immunosuppressive drug. RPM derivative has been investigated for their potential as both an anti-neoplastic agent and for the treatment of brain lesions caused by the TSC (Newman et al., 2000; Ryther and Wong, 2012).

Rapamycin inhibits IL-2 and other cytokine receptor-dependent signal transduction mechanisms, via action on mTOR, thereby blocking the activation of T and B cells. The mammalian target of RPM (mTOR) participates in several intra- and extra-cellular pathways involved in cell growth and proliferation; and is also involved in neuronal development and synaptic plasticity (Ryther and Wong, 2012). mTOR is a serine-threonine protein kinase of 290 kDa which belongs to the phosphoinositide 3-kinase-related kinase family and has a highly conserved sequence among mammals. Analogs of mTOR can also be found in lower eukaryotes including *Drosophila* and yeast (Russo et al., 2012).

The potential of RPM for epilepsy was found through a target-oriented approach. TSC is a genetic disorder resulting from mutations in at least 5 genetic dysregulations of the mTOR pathway. The main mutations are present in the Tsc1 (hamartin) or Tsc 2 (tuberin) genes that normally act to suppress mTOR (for details see the review of Galanopoulou et al., 2012). Several symptoms are associated with TSC, including seizures, tubers, giant cell tumors, autism, mental retardation and behavior impairment and systemic complications. TSC is the most common genetic cause of epilepsy; a severe form occurs in 60–90% of TSC patients and is refractory to all ASMs and non-pharmacological therapies. Seizures in such patients can be controlled by the mTOR inhibitors such as RPM and with a RPM analogue called everolimus (Sunnen et al., 2011; Hartman et al., 2012).

The potential of RPM and everolimus are currently being investigated for various purposes. These include prevention of epilepsy in tuberous sclerosis, or cation-chloride co-transporters neonatal induced seizures. Furthermore they are also evaluated as anti-inflammatory drugs (Trinka and Brigo, 2014).

3.2.1.2. Preclinical data. RPM has shown promising antiepileptogenic effects, but its acute anticonvulsant effect in normal mice is limited. Indeed, short-term treatment of RPM protected against tonic hindlimb extension in the MES threshold test, while long-term RPM treatment protected against kainic acid-induced seizures. RPM did not exhibited activity in the 6 Hz or PTZ models, neither with short-term treatment nor with long-term treatment (Kumar et al., 2005; Tsang et al., 2007; Hartman et al., 2012).

Cortical dysplasia, as well as hemi megalencephaly are developed abnormalities with unknown etiology and are highly associated with intractable childhood epilepsy. Cortical dysplasia might result from an abnormal signaling pathway involving PI3K (phosphoinositide 3-kinase)-Akt-mTOR). Because there is no existing model to mimetize cortical dysplasia, TSC which induced abnormal brain structures (cortical tubers) is used for the research on cortical dysplasia treatment. RPM exhibited anti-seizure efficacy against cortical dysplasia after 10 mg/kg daily *i. p.* injection over 2 weeks in animals (Vinters et al., 1999; Ljungberg et al., 2009).

In a model of temporal lobe epilepsy simulating a status epilepticus through a kainate administration, RPM showed a potential against neuronal cell death, neurogenesis, and development of spontaneous epilepsy by blocking both acute and chronic seizure-induced mTOR activation. Both temporal lobe epilepsy, as well as infantile spasms, are representative of acquired forms of epilepsy and RPM showed disease-modifying behavior in infantile spasms. Infantile spasms in many cases result from genetic mutations (Zeng et al., 2008, 2009; Pellock et al., 2010; Galanopoulou et al., 2012; Devinsky et al., 2014).

Pre- or post-treatment in mice with RPM (6 mg/kg) mediates seizure-induced injury by astrocytes, a group of specialized glial cells in the CNS, implicated in epileptogenesis and seizure-induced brain injury. The involvement of the mTOR pathway in mediating this process has been suggested previously (Guo et al., 2017).

RPM was tested in mice with a conditional inactivation of the *Tsc1* gene primarily in glia (*Tsc1*^{GFAP}CKO mice, which develop in progressive epilepsy, encephalopathy, and premature death) to evaluate its potential against seizures in both early and late treatment, and indeed it suppressed seizures in addition to prolonging the survival of animals (Zeng et al., 2008; Galanopoulou et al., 2012). In another study in *Tsc1* knock-out mice, RPM (daily i. p. injection with 5 or 10 mg/kg) in early and late treatment prevented or reversed hippocampal hyperexcitability and early treatment prevented transcriptome changes (Koene et al., 2021).

3.2.1.3. Clinical data. mTOR inhibitors are currently being clinically investigated for tuberous sclerosis, and as anti-inflammatory drugs for potential anti-epileptogenic purposes (Galanopoulou et al., 2012).

RPM caused a decrease in seizures in a 10-year-old girl receiving 0.15 mg/kg/day of this compound over 10 months. The girl was suffering from TSC and was refractory to at least 9 ASM treatments and resecting surgery. Consequently, she displayed 5 to 10 seizures episodes per day, including right arm paresis. Increasing RPM doses up to 0.2 mg/kg/day resulted in side effects such as skin breakdown, mouth ulcers, and frequent viral infections without improving the anticonvulsant activity (Muncy et al., 2009).

Based on Phase III clinical trials (NCT01713946) on 366 patients that suffered from TSC in 2018, everolimus, an analogue of RPM was approved by The European Commision as an adjunctive treatment in patients from 2 years of age with seizures associated with TSC that do not respond to other treatments (Curatolo et al., 2018). Now the same drug underwent Phase II of clinical trials as adjunctive drug for patients with focal cortical dysplasia type II (NCT03198949).

The nanoparticle albumin-bound rapamycin (nab-rapamycin, ABI-009) is under evaluation in an ongoing Phase I clinical trial for refractory epilepsy patients who failed epilepsy surgery. The drug is administered *i. v.* at doses ranging from 5 mg/m^2 to determine the maximum tolerated dose (NCT03646240). The limited oral bioavailability and poor solububility of RPM pose challenges for its use. However, by combining albumin with RPM the availability and solubility of the drug can be enhanced, which offers potential advantages for further research (O'Donnell et al., 2008; Lei et al., 2021).

Despite its side effects, RPM holds promise as a potential treatment for epilepsy due to its involvement in the mTOR pathway, which plays a significant role in various epilepsy-related pathways (Galanopoulou et al., 2012). To explore it further, it is crutial to evaluate RPM in other epilepsy models beyond status epilepticus, such as the symptomatic temporal lobe epilepsy, post-traumatic epilepsy and TSC (Inoki et al., 2005; Zeng et al., 2009). These models can provide valuable insights into the efficacy of RPM as an anti-epileptogenic agent in a broader range of epileptic conditions.

3.2.2. Ganaxolone

3.2.2.1. Historical background and therapeutic indication. Ganaxolone (GNX) is a synthetic analogue of allopregnanolone, an endogenous neurosteroid, belonging to the neurosteroid class of compounds called epalons, which have sedative, anxiolytic and anticonvulsant effects (Monaghan et al., 1997). Theuse of water-soluble steroid derivatives of pregnanes for the treatment of human epilepsy was already discussed over 60 years ago, highlighting their potential. These steroid derivatives share structural similarities with GNX (Figdor et al., 1957).

3.2.2.2. Chemistry, pharmacology, mechanism of action. GNX (3α–OH–3β-methyl-5α-pregnan-20-one) is a synthetic analogue (3β-methylated) of an endogenous neurosteroid called allopregnanolone that is itself a progesterone metabolite. GNX is a derivative of natural product, but many steroids with closely related structures are known to occur in plants consumed by humans, therefore motivating the inclusion of this compound and related steroids in this review. However in this case only the most important clinical trials are presented.

3.2.2.3. Clinical data (metabolism PK). GNX is used currently in several clinical trials. GNX is developed in three different formulations – intravenous injection, capsule and liquid suspension (Lattanzi et al., 2021).

A multicenter, double blind, randomized, placebo-controlled Phase III trial on partial seizure frequency in adults with DRE was conducted on 405 participants. The treatment consisted of 200 mg and 225 mg capsules GNX and the target dose was 1800 mg/day. (NTC01963208). However, the results of this study did not show a significant reduction of seizures. Reduction of partial onset seizures was 24.28% for GNX and 10.25% for placebo.

In 2020 the Phase III clinical trial in treatment of seizures in children and young adults with CDKL5 deficiency were finished. The trial on group of 101 patients, who in double-blind, randomized, placebo-controlled trials received suspension of GNX 50 mg/ml 3 times daily for 17 weeks. The results indaicate that major motor seizures frequency were reduced in patients receiving GNX (−32.2%) compared to those receiving placebo (4.0%). In the same trials 7 patients were co-diagnosed with Lennox-Gastaud Syndrome, and 4 of them demonstrated improvement in seizure frequency (mean 31.5% reduction in major motor seizure frequency) (NCT03572933) (Pestana-knight et al., 2020). In 2022 the Ztalmy (ganaxolone in form of oral suspension) was approved by FDA and now it is commercialy available for treatment of CDKL5 deficiency disorder (Knight et al., 2022).

Intravenous formulation of GNX is being evaluated in ongoing Phase III trial on subjects with status epilepticus. In a double-blind, randomized and placebo-controlled trial, 124 participants were receiving a continouos infusion of GNX for 36 h, followed by 12 h taper (NCT04391569) (Vaitkevicius et al., 2020).

The Phase II of clinical trilas for TSC was recently finished (NTC04285346). The dosage of GNX was up to 63 mg/kg/day or 1800 mg daily. After 12 weeks treatment period median in reduction in seizures was 16.6% and 1/3 of patients experienced ≥50% seizure reduction. Based on this data the Phase III is planned (Sullivan et al., 2021).

GNX underwent Phase II clinical trials for the treatment of protocadherin-19 associated female epilepsy (PCDH19). In 17 weeks treatment, double blind, randomized, placebo-controlled study, 21 females received 50 mg/ml GNX suspension. Results shows 61.5%

reduction in seizures compared to 24.0% in placebo group (NCT03865732) (Samanta, 2020). It is well-tolerated and effective against seizures in humans (Carter et al., 1997; Laxer et al., 2000; Reddy et al., 2004) and shows a comparable spectrum of activity to valproate and ethosuximide, suggesting possible efficacy against generalized seizures and therefore potential to be a new promising treatment approach in epilepsy (Nohria and Giller, 2007; Striano and Striano, 2009).

GNX is active in women with catamenial epilepsy since during premenstrual phase, the decrease in endogenous progesterone concentrations was correlated to seizure exacerbations (Monaghan et al., 1999). Other anticonvulsant activities have been reported in children with infantile spasms, complex partial seizures and refractory partial-onset seizures (Monaghan et al., 1999; Nohria and Giller, 2007). Refractory pediatric and adolescent patients have been effectively treated with GNX and only one patient expressed somnolence as side effect (Pieribone et al., 2007). The oral suspension peak concentration is obtained after 1.5–2 h after administration. GNX distribution is ubiquitous to all tissues except for plasma that contains a 5-fold higher concentration and shows a high rate of binding to plasma proteins. After *i. v.* or oral administration, GNX activity is obtained post-metabolism, for example with the major metabolite 16-OH GNX obtained after CYP 3A4 metabolism. Elimination of GNX occurs via mainly by the fecal route and around 20% in urine (Nohria and Giller, 2007).

One of the main challenges with neurosteroids is their limited bioavailability after oral administration. When complexed with the 2-hydroxypropyl or cyclodextrin excipients, doses of GNX ranging from 50 to 1500 mg were dispensed via the oral route (Monaghan et al., 1997). Each formulation should be administered after a high-fat meal to enhance the absorption (Nohria and Giller, 2007; Bialer et al., 2020).

3.3. NPs with anti-seizure efficacy in animal models of DRE

Many NPs were tested on different seizure models - mostly acute seizures induced in non-epileptic animals (i.e. the MES and *s. c.* PTZ assays) and chronic models of epilepsy with spontaneous and recurrent seizures (with pilocarpine or kainic acid). Most results are summarized in review papers presented in Table 1.

In this review the authors focused on NPs that revealed anti-seizure activity in animal models of DRE. The increasing need for *in vivo* models capable of predicting the efficacy in difficult-to-treat patients along with the lack of efficacy of some ASMs in traditional screening tests have demonstrated the importance of utilizing models that more closely resemble the human condition.

For this purpose, the 6 Hz model for partial seizures was re-evaluated and included in the ASM discovery program ("The NIH/NINDS Anticonvulsant Screening Program (ASP): recommendations from the working group's 2012 review of the Program.," 2012). Partial seizures are induced by a low frequency corneal stimulation, characterized by a brief forelimb clonus followed by stereotyped, automatistic behaviors thought to be reminiscent of auras experienced by patients with partial seizures, including twitching of the vibrissae, and Straub-tail (Barton et al., 2001). This model has a unique and interesting pharmacologic profile as it is resistant to phenytoin and numerous other "old generation" ASMs. Interestingly, levetiracetam was shown to be active in this model, whereas it failed the standard screening tests MES and *s. c.* PTZ, justifying the systematic use of this high-throughput model for early development phases.

Another model of DRE is intrahippcocampal kainate model of temporal lobe epilepsy. After injection of kainate the damage in brain is observed, particulary in limbic structures, which led to spontaneous seizures (Ben-Ari et al., 1979; Nadler et al., 1978; Löscher, 2017). Other animal models of DRE used to date for the validation of NP-derived ASM drug leads are allyglycine-induced (Leclercq et al., 2015), lamotrigine-resistant kindled model (Singh et al., 2014), model of cortical dysplasia (Ljungberg et al., 2009) and genetic models of DRE (Scheffer and Nabbout, 2019; Miljanovic et al., 2021).

Natural products with traditional therapeutic imdications and with anti-seizure efficacy in animal models of DRE are listed in Table 2. Developing novel treatments and management strategies for drug resistance represents one of the major challenges in epilepsy. Despite many years of research, the mechanisms underlying drug resistance remain largely unknown, though recent work has begun to shape our understanding more clearly (Löscher et al., 2020). NPs with anti-seizure activity in animal models of DRE, as well as with novel mechanisms of action, are potential sources of novel drug candidates for DRE.

4. Conclusion and outlook

Anti-epileptic drug discovery remains a challenging field. Despite the existence of over 25 approved medications for epilepsy, a significant number of patients, around 15–20 million epilepsy patients, do not respond to these drugs. The figure is twice the estimated number of Parkinson's disease patients worldwide. Yet, because of the difficulties to date of modeling DRE, current drug discovery approaches are unlikely to yield novel ASM candidates which adequately address this urgent medical need. Target-based approaches for epilepsy are fraught with challenges and risks, given the complexity of the pathophysiology of this disease, and the gap in our understanding of the fundamental mechanisms of epileptogenesis and pharmacoresistance. Therefore, the development of novel bioassays that adequately address these mechanisms holds great value for future advancements in the field.

Another approach that has enabled the identification of at least some of the promising anti-epileptic drug candidates and leads from traditional medicine described above, takes advantage of millennia of drug discovery efforts using the ultimate bioassay – humans. Anti-epileptic medicinal plants, which were identified by generations of traditional healers using human epilepsy patients as their model, represent a vast but still underutilized resource for drug discovery, with at least dozens if not hundreds of novel active NPs waiting to be isolated. New and improved approaches for bioassay-guided isolation, including but not limited to microfractionation and sensitive microNMR (Bohni et al., 2013), and coupled with increasingly sophisticated and disease-relevant bioassays, are likely to yield at least some novel compounds directly with the potential to address the large population of pharmcoresistant epilepsy patients.

Such approaches significantly accelerate the identification of bioactive lead molecules and facilitate the initial evaluation of their bioactivities. However, in microfractions, the true potency of specific NPs cannot be directly determined since sample quantities are often immeasurable or can only be indirectly quantified using micro-NMR methods (Dalisay and Molinski, 2009). For instance, the combination of HPLC microfractionation with *in vivo* zebrafish assays has proven to be an effective strategy for quickly localizing the active principles. Nonetheless, assessing the bioactivity of these NPs in rodent *in vivo* assays to evaluate efficacy, toxicity, pharmacokinetics, and for the *de novo* structure elucidation of unknown NPs through extensive 1D and 2D NMR experiments still necessitates milligrams of pure, well-characterized NPs (Balunas and Kinghorn, 2005).

In the ongoing search for new drugs for the treatment of DRE, and taking advantage of the wide range of novel mechanisms of action of NPs, bioactivities other than direct seizure inhibition should be included in NP-based drug discovery efforts. In this context, three classes of bioactivity hold particular interest for DRE drug discovery efforts: (1) NPs that enhance the brain bioavailability of other drugs, (2) NPs with anti-inflammatory activity capable of acting on neuroinflammation, and (3) NPs with stimulatory activity on the vagus nerve (VN).

(1) One hypothesis regarding anti-seizure medication resistance suggests that inadequate drug penetration across the blood-brain barrier (BBB) may occur due to increased expression of multidrug efflux transporters (Löscher et al., 2020). Several NPs derived from medicinal plants, such as quercetin, genistein, naringin,

Table 2
Natural products with anti-seizure efficacy in animal models of DRE.

Compound	Biological source	Traditional therapeutic indications	Proposed mechanism of action	Preclinical activity
Curcumin	*Curcuma longa* L. (Zingiberaceae)	Popular spice treat anorexia, cough, biliary disorder, abdominal pain, icterus and sinusitis (Ammon and Wahl, 1991). antioxidant activities and neuroprotective effects against oxidative stress-induced brain insults (Ammon and Wahl, 1991; Canales-Aguirre et al., 2012). anti-inflammatory, cardioprotective, immunomodulatory, cancer chemoprevention, renoprotective, antioxidant and neuroprotective properties against ischemic brain injury and neurotoxicity (J. Zhang et al., 2019)(Fan et al., 2021)(Wu et al., 2021)(Alhusaini et al., 2021)(Gholami et al., 2021)(Viegas et al., 2021).	Adenosine receptor A1 and A2; modulation of the activity of: kainate receptors, brain-derived neurotropic factor, glutamate receptors, cyclooxygenase 2, tumor suppressor p53, mitogen-activated protein kinase, sodium voltage-ion channels and kappa β-mediated transcription (Kumar et al., 2019)(Agarwal et al., 2013)(Matteucci et al., 2005)(Scapagnini et al., 2006).	several models of acute seizures in rodent, such as kainic acid-induced seizures, lithium pilocarpine-induced status epilepticus, and kindled seizures (Akula and Kulkarni, 2014)
Turmerone	*Curcuma longa* L. (Zingiberaceae)	prevention of dementia biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis (Ammon and Wahl, 1991).	against beta-secretase	mouse 6-Hz model from 0.1 to 50 mg/kg (Orellana-Paucar et al., 2013)
Piperine	*Piper nigrum* L. (Piperaceae)	Common spice hypnotic, sedative, antidepressant, myorelaxant and anticonvulsant activities (Fu et al., 2010).	presynaptic glutamate release and Ca^{2+} overloading effects.(Fu et al., 2010) GABAergic pathway (Bukhari et al., 2013; Zurowski et al., 2012).	Inhibit traumatic brain injury-induced seizure, 20 mg/kg injury in mice model (Song et al., 2020)
Berberine	*Berberis vulgaris* L. (Berberidaceae)	antimicrobial, antidiabetic, anticancer activities (Wang et al., 2017)	NMDA receptor and K + -channels	Intrahippocampal kainate mice model, 25–100 mg/kg (Mojarad and Roghani, 2014; Sedaghat et al., 2017)
Rutin	*Fagopyrum esculentum* Moench (Polygonaceae)	cytoprotective, gastroprotective, hepatoprotective, and anti-diabetic effects (Hosseinzadeh and Nassiri-Asl, 2014)	Interaction with GABA, glycine, acetylcholine, serotonin and adenosine receptors	Intrahippocampal kainite mice model; 100–200 mg/kg (Nassiri-Asl et al., 2013) 6Hz mice model 400 mg/kg (Nieoczym et al., 2014)
Quercetin	*Crataegus laevigata (Poir.) DC.* (Rosaceae)	Metabolic disorders, memory impairments, anti-viral, antineurodegenerative (Babaei et al., 2018; Heinz et al., 2010)	Antioxidant properties, Antagonism of GABAA receptor modulation of phosphatidylinositol 578 3-kinase (PI3K)/ Akt, tyrosine kinase, protein kinase C (PKC) and 579 mitogen-activated protein kinase (MAP kinase) signaling pathways (Suganthy et al., 2016)	6Hz mice model 400 mg/kg (Nieoczym et al., 2014)

piperine, borneol, and glycyrrhizin, have demonstrated the ability to enhance the bioavailability (including brain bioavailability) of various drugs (Kesarwani et al., 2013; Kowalczyk et al., 2022).

(2) Experimental evidence suggests that neuroinflammation can contribute to both a dysfunctional blood-brain barrier (BBB) and an upregulation of P-glycoprotein (P-gp) in drug-resistant epilepsy (DRE) (Löscher et al., 2020), therefore NPs with potent anti-inflammatory effects in the brain, such as terpenoids, piperine, and quercetin (Rinwa and Kumar, 2017; Pina et al., 2021), could have beneficial actions with regard to DRE by reducing inflammatory mediators such as NF-κB, IL-1β, IL-18, TNF-α and IFN-γ. The ability of these NPs to mitigate neuroinflammation highlights their potential therapeutic benefits in DRE. By targeting inflammatory pathways and modulating the production of pro-inflammatory cytokines, these NPs could help alleviate the underlying inflammatory processes associated with DRE. Further investigation is warranted to elucidate the precise mechanisms by which these NPs exert their anti-inflammatory effects in the brain and to evaluate their potential as therapeutic agents for DRE.

(3) In conclusion, we propose a hypothesis that natural products (NPs) with the ability to pharmacologically activate the vagus nerve (VN) could serve as promising neuromodulatory therapeutic options for drug-resistant epilepsy (DRE). This hypothesis is supported by the observation that certain NPs, including CBD, can stimulate the VN through the activation of TRP channels (Kowalski et al., 2020). VN activation has been associated with various beneficial effects on the CNS, including the modulation of seizure activity and the regulation of inflammatory processes. Therefore, NPs that possess the ability to activate the VN may offer a novel approach for the treatment of DRE by targeting the underlying neurophysiological mechanisms. Further research is needed to explore the potential of specific NPs in modulating VN activity and their therapeutic efficacy in the context of DRE. Understanding the precise mechanisms of action and optimizing the delivery methods of these NPs will be crucial for their successful translation into clinical applications.

CRediT author statement

S.C., A.S. and M.L. carried out the literature search and wrote the draft. All authors contributed to the study design. C.V.E., J.-L.W., A.D.C. and K.S.-W. revised the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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