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CRITICAL REVIEW – INVITED COMMENTARY

The ups and downs of alkyl-carbamates in epilepsy therapy: How does cenobamate differ?

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Abstract

Since 1955, several alkyl-carbamates have been developed for the treatment of anxiety and epilepsy, including meprobamate, flupirtine, felbamate, retigabine, carisbamate, and cenobamate. They have each enjoyed varying levels of success as antiseizure drugs; however, they have all been plagued by the emergence of serious and sometimes life-threatening adverse events. In this review, we compare and contrast their predominant molecular mechanisms of action, their antiseizure profile, and where possible, their clinical efficacy. The preclinical, clinical, and mechanistic profile of the prototypical γ-aminobutyric acidergic (GABAergic) modulator phenobarbital is included for comparison. Like phenobarbital, all of the clinically approved alkyl-carbamates share an ability to enhance inhibitory neurotransmission through modulation of the $GABA_A$ receptor, although the specific mechanism of interaction differs among the different drugs discussed. In addition, several alkyl-carbamates have been shown to interact with voltage-gated ion channels. Flupirtine and retigabine share an ability to activate K^+ currents mediated by KCNQ (Kv7) K^+ channels, and felbamate, carisbamate, and cenobamate have been shown to block Na^+ channels. In contrast to other alkyl-carbamates, cenobamate seems to be unique in its ability to preferentially attenuate the persistent rather than transient Na^+ current. Results from recent randomized controlled clinical trials with cenobamate suggest that this newest antiseizure alkyl-carbamate possesses a degree of efficacy not witnessed since felbamate was approved in 1993. Given that ceno-bamate's mechanistic profile is unique among the alkyl-carbamates, it is not clear whether this impressive efficacy reflects an as yet undescribed mechanism of action or whether it possesses a unique synergy between its actions at the $GABA_A$ receptor and on persistent Na^+ currents. The high efficacy of cenobamate is, however, tempered by the risk of serious rash and low tolerability at higher doses, meaning that further safety studies and clinical experience are needed to determine the true clinical value of cenobamate.

KEYWORDS

antiseizure drugs, carisbamate, felbamate, flupirtine, $GABA_A$ receptor, meprobamate, retigabine, voltage-gated Na^+ channels

1 | INTRODUCTION

The discovery in 1951 of the dicarbamate meprobamate as a new anxiolytic that also possessed antiseizure activity[1] triggered the search for new central nervous system (CNS)-active carbamate compounds,[2-6] finally resulting in the discovery of the dicarbamate felbamate in 1969.[6] Since then, a total of three alkyl-carbamates (i.e., felbamate, retigabine, and cenobamate) have been approved for treatment of epilepsy (Figure 1), making alkyl-carbamates one of the most successful chemical classes in the history of antiseizure drug (ASD) development. Another alkyl-carbamate, carisbamate, received provisional approval from the US Food and Drug Administration (FDA) in 2008, but this was withdrawn in 2010 because of inconsistent efficacy across different clinical trials in patients with drug-resistant focal epilepsy.[7] Nonetheless, in 2012, carisbamate received an orphan drug designation for the management of infantile spasms and is currently in clinical development for therapy of Lennox–Gastaut syndrome.[8]

Cenobamate, the most recent drug of the series of alkyl-carbamates developed for epilepsy therapy, showed remarkable antiseizure efficacy in clinical trials, suggesting that this novel ASD brings substantial promise for patients with focal seizures that have been difficult to control with other medications, and with the potential for freedom from seizures.[9] However, impressive clinical efficacy was also described for felbamate shortly after its approval in 1993 for the management of focal seizures and Lennox–Gastaut syndrome.[10] Within 1 year, ~120,000 people had been exposed to felbamate. Unfortunately, this extensive postapproval use revealed a previously unknown risk of life-threatening idiosyncratic adverse events (aplastic anemia and hepatic failure) not seen in preapproval studies,[11] which led to a dramatic reduction in its use. For experienced pharmacologists and toxicologists, these idiosyncratic adverse events of felbamate were not entirely surprising, because induction of aplastic anemia was previously reported for the structurally similar alkyl-dicarbamate meprobamate.[11,12] In a later development, a new type of adverse effect (i.e., blue skin discoloration and pigment changes in the retina) was reported for retigabine, leading to its withdrawal in 2017.[13]

The aim of this review is to critically discuss the "ups and downs" of alkyl-carbamates in epilepsy therapy. Are they all the same, or do they differ in their pharmacology, toxicology, and clinical efficacy? Is cenobamate really a "game changer,"[9] or is it too early to assess its efficacy, tolerability, and safety? Finally, with the evolving era of precision medicine for rare monogenetic epilepsies, will alkyl-carbamates such as retigabine and carisbamate attract renewed interest? We briefly review each of the alkyl-carbamates shown in Figure 1; compare their preclinical efficacy in animal seizure and epilepsy models, mechanisms of action, clinical efficacy, and safety; and finally, discuss additional alkyl-carbamates in the preclinical pipeline.

Key Points

- Alkyl-carbamate drugs have efficacy in experimental models of seizures and epilepsy and in the clinical management of seizure disorders
- Two classes of alkyl-carbamates exist; dicarbamates (e.g., felbamate) and monocarbamates (e.g., retigabine, carisbamate, cenobamate)
- Most alkyl-carbamates act as positive allosteric modulators of the $GABA_A$ receptor and enhance inhibitory neurotransmission
- Most also possess additional modes of action, including effects on voltage-gated Na^+ and K^+ channels and on ionotropic glutamate receptors
- In addition to their recognized clinical efficacy, alkyl-carbamates have often been associated with serious adverse reactions

2 | ALKYL-CARBAMATES WITH PRECLINICAL AND CLINICAL ANTISEIZURE EFFECTS

2.1 | Meprobamate

Meprobamate, a carbamylated derivative of propanediol (2-methyl-2-propyl-1,3-propanediol dicarbamate; Figure 1), was introduced as an antianxiety drug in 1955. It was the first drug to be used as an anxiolytic agent; it was also used as a sedative–hypnotic and, less frequently, as an ASD. Meprobamate was developed by Frank Berger at Wallace Laboratories, a division of Carter Products (later Carter-Wallace; Cranbury, New Jersey) in the early 1950s, based on previous experience with the sedating or "tranquilizing" effect of mephenesin in rodents.[14] The report of Berger[14] marked the beginning of investigations of modern sedatives with useful antianxiety and minor tranquilizing properties. After its approval, meprobamate rapidly became the first blockbuster psychotropic drug in American history,[15] but it was later largely replaced by the benzodiazepines (BDZs) due to their wider therapeutic index. In addition to its anxiolytic and sedative effects, meprobamate exerts antiseizure activity in animal models (see below) and epilepsy patients. Meprobamate appears to act by modulating the γ-aminobutyric acid type A ($GABA_A$) receptor via a barbiturate-like action,[16] but other mechanisms may contribute to its pharmacology (see below for more detailed discussion). Based on its GABAergic effect, meprobamate has abuse potential and may induce dependence

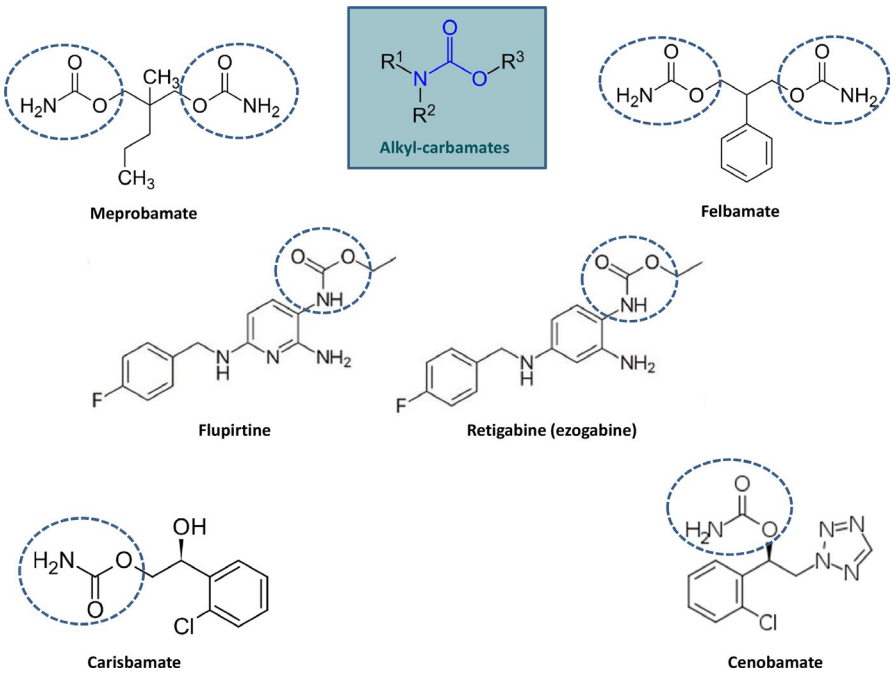

FIGURE 1 Alkyl-carbamates with antiseizure activity. Note that meprobamate and felbamate are dicarbamates, whereas the other drugs are monocarbamates

with continued use; it is a Drug Enforcement Administration (DEA) Schedule IV controlled drug in the United States (as is the anticonvulsant and sedative/hypnotic drug phenobarbital, which also mainly acts via the $GABA_A$ receptor). The major unwanted effects of the usual sedative doses of meprobamate are drowsiness and ataxia. However, soon after its approval, meprobamate was reported to induce potentially fatal aplastic anemia as a rare idiosyncratic adverse effect.[11,12] That aplastic anemia was later also associated with felbamate (see below) may indicate that this severe adverse event is a class effect of dicarbamate esters of propanediol.

2.2 | Felbamate

Felbamate (2-phenyl-1,3-propanediol dicarbamate) was synthesized and developed by Wallace Laboratories in the 1950s as part of their efforts to explore structure–activity relationships with meprobamate.[6,17] As shown in Figure 1, felbamate is a close structural analogue of meprobamate, both being dicarbamate esters of propanediol. In contrast to meprobamate, however, felbamate lacked pronounced tranquilizing or sedative activity, so it did not receive much attention until approximately 30 years later, when Wallace Laboratories submitted felbamate to the Epilepsy Branch of the National Institute of Neurological Disorders and Stroke (NINDS) for anticonvulsant screening.[18] Felbamate's antiseizure profile was similar to that of phenobarbital and valproate. Later studies indicated that felbamate, similarly to meprobamate (and phenobarbital), enhances GABAergic neurotransmission at the $GABA_A$ receptor, but it also blocks the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors and voltage-dependent Na^+ channels.[19] In contrast to meprobamate (and phenobarbital), felbamate is not listed in the DEA Schedules, possibly as a result of its lower potency in modulating the $GABA_A$ receptor (see more detailed discussion below). Based on its promising preclinical profile and positive findings in regulatory trials, felbamate was approved in 1993 for the treatment of focal seizures (with or without generalization) in adults and for focal and generalized seizures associated with Lennox–Gastaut syndrome in children.[20] It was the first drug shown to be effective in treating Lennox–Gastaut syndrome in controlled trials.[21] In its first year on the market, felbamate reached an estimated patient population of 120,000, but soon after its introduction, it was associated with multiple cases of aplastic anemia and acute liver failure, and its use has been restricted ever since.[10,22] A total of 31 cases of aplastic anemia and 18 cases of hepatic failure were reported following approval of the drug in the United States.[23] Risk of aplastic anemia in patients who take felbamate is believed to be between 27 and 209 cases per million, with the most probable risk estimate being 127 cases per million.[22] The mechanism of these rare idiosyncratic adverse effects is unknown, but they are likely to be due to bioactivation of felbamate to a highly reactive electrophilic toxic metabolite.[24] Although felbamate is not indicated as first-line antiseizure therapy, its utility in treating patients with severe epilepsy who have inadequately responded to alternative drugs is undisputed.[22,23] Furthermore, there are certain precautions that can minimize the risk of serious adverse reactions associated with felbamate (see the section Risk of Severe Idiosyncratic Adverse Events With Different Alkyl-Carbamates), thereby providing an option in refractory cases where no other drug works.[22,25]

2.3 | Flupirtine

The centrally acting analgesic and muscle relaxant flupirtine (N-[2-amino-6-[(4-fluorophenyl)methylamino]-3-pyridinyl] carbamic acid ethyl ester; Figure 1) was developed in the 1970s and 1980s by Chemiewerk Homburg (later ASTA Medica, a branch of the Degussa group) in Germany in close cooperation with its US partner Carter-Wallace Laboratories.[26-29] It has been marketed since 1984 in European countries as an effective, nonopioid, non-nonsteroidal anti-inflammatory drug (non-NSAID) analgesic for the management of acute, moderate-to-severe cases of pain.[30] The antiseizure effects of flupirtine were also evaluated within the NINDS-funded Anticonvulsant Screening Program at the University of Utah by the group of Ewart A. Swinyard.[26] Flupirtine was reported to exert antiseizure effects in animal models of epilepsy and in a pilot study in patients with refractory seizures.[26] It was shown to act as an opener of voltage-dependent KCNQ (K_V7) potassium channels and as a $GABA_A$ receptor modulator,[29,31] and was the first KCNQ opener in therapeutic use, introduced long before retigabine.[29] However, following its widespread use as an analgesic, flupirtine was reported to cause rare but occasionally fatal liver injury, leading to restrictions on its use.[13] Clinical trials with flupirtine in patients with epilepsy were discontinued due to the development of a more potent (and reportedly less toxic) analogue, retigabine (ezogabine). Recently, however, interest in the antiseizure activity of flupirtine has been reignited because of studies indicating that it is more effective than either of two commonly used ASDs, phenobarbital and diazepam, in suppressing neonatal seizures and status epilepticus in rats.[32-35] Furthermore, a combination of flupirtine and diazepam was shown to be effective in terminating established (BDZ resistant) status epilepticus in three adult rat models.[36]

2.4 | Retigabine (ezogabine)

As shown in Figure 1, retigabine (known as ezogabine in the United States) is a close structural analogue of flupirtine. Unlike the branched dicarbamates felbamate and meprobamate, retigabine (*N*-[2-amino-4-(4-fluorobenzylamino)phenyl] carbamic acid ethyl ester) is a monocarbamate compound. In the early 1990s, molecular modeling studies at ASTA Medica (Frankfurt, Germany), including quantitative structure–activity studies and pharmacophore modeling, resulted in the development of a number of desazaflupirtine derivatives with anticonvulsant activity superior to that obtained with flupirtine.[37] The most potent of these derivatives was D-20443, the dihydrochloride of retigabine, which was subsequently developed as the free base (D-23129 or retigabine) due to technological reasons and a superior impurity profile.[38] Retigabine exerted a broad spectrum of antiseizure activities in animal models.[38] One of the first studies to investigate a potential mechanism of action for retigabine reported that de novo synthesis of GABA within the hippocampus was increased by retigabine,[39] although this was later called into question.[40] Subsequent studies showed that retigabine, similar to other alkyl-carbamates, potentiates GABA-induced currents in rat cortical neurons through a non-BDZ binding site mechanism.[41] Most likely as a result of its effects on $GABA_A$ receptors, retigabine is a DEA Schedule V controlled substance (substances in the DEA Schedule V have a low potential for abuse relative to substances listed in Schedule IV). Interestingly, in the prescribing information of retigabine (Potiga) provided by GSK, drug effects such as euphoria-type and drunken-like subjective responses were described for patients treated with retigabine, which could be a consequence of the GABAergic component of this drug. Furthermore, withdrawal symptoms are observed upon discontinuation of retigabine in animal studies. In rats, abrupt discontinuation of chronic oral administration of retigabine (at either 3, 10, or 30 mg/kg/day over 28 days) induced behavioral alterations, such as piloerection, increases in high step gait, and tremors, that were mild and also distinct from the more prominent withdrawal signs induced by chronic administration of a BDZ.[42] Nevertheless, these data suggest that retigabine produces a withdrawal syndrome indicative of physical dependence, which is important because such a withdrawal syndrome is a typical class effect of drugs that potentiate GABAergic transmission.

In addition to its effects on GABAergic inhibitory transmission, retigabine acts as an opener of K^+ channels in neuronal cells,[43,44] a mechanism that is thought to be mainly responsible for its antiseizure activity (but see more detailed discussion below). The effect of retigabine on K^+ channels is characterized by activation of the K_V7 class of voltage-gated K^+ channels. It is specific for channels containing $K_V7.2$ to $K_V7.5$ subunits, and has particular affinity for channel assemblies containing dimers of $K_V7.2/K_V7.3$ and $K_V7.3/K_V7.5$ subunits.[45]

Retigabine was originally licensed in the United States and Europe in 2011 for the treatment of focal seizures in adults.[46] Its use was later restricted due to the emergence of idiosyncratic adverse effects characterized by blue tissue discoloration, and although subsequently withdrawn by the manufacturer (GSK), there remains interest in the use of retigabine as a precision therapy in severe epileptic encephalopathies due to mutations in the *KCNQ* genes.[19,47] In 2018, Xenon Pharmaceuticals received orphan drug designation for treatment of patients with *KCNQ2* mutations with retigabine, and the company added retigabine to its epilepsy pipeline.

2.5 | Carisbamate

The monocarbamate carisbamate (*S*-2-*O*-carbamoyl-1-*o*-chlorophenyl-ethanol; Figure 1) was initially discovered and developed by the South Korean company

SK-Biopharmaceuticals and, in 1998, in-licensed by Johnson & Johnson Pharmaceutical Research & Development in the United States, who evaluated carisbamate as a novel ASD. The compound demonstrated potent antiseizure activity in a variety of in vivo seizure models including hippocampal and corneal kindling.[48] Several potential mechanisms of action have been proposed, including use-dependent block of Na^+ channels, activation of presynaptic Cl^- conduction leading to depression of excitatory neurotransmission, and inhibition of voltage-gated Ca^{2+} channels, but apparently there is no effect on GABAergic transmission.[49-52] Following several controlled clinical trials with carisbamate in patients with refractory focal epilepsy, in 2008 Johnson & Johnson received provisional approval from the FDA to market carisbamate.[53] However, carisbamate failed to demonstrate consistent efficacy across regulatory trials; thus, its new drug application and medical authorization application submissions were withdrawn in January 2010, and the clinical program in epilepsy was discontinued by Johnson & Johnson. In 2012, SK-Biopharmaceuticals, the company that initially developed the drug, received an orphan designation for the management of infantile spasms (West syndrome). Furthermore, SK-Biopharmaceuticals is currently performing trials with carisbamate in Lennox–Gastaut syndrome.[8]

2.6 | Cenobamate

As with carisbamate, the monocarbamate cenobamate ([(1R)-1-(2-chlorophenyl)-2-(tetrazol-2-yl)ethyl] carbamate; Figure 1) was discovered and developed by SK-Biopharmaceuticals.[54] Similarly to other alkyl-carbamates reviewed here, cenobamate exerted broad-spectrum antiseizure activity in preclinical models.[54,55] Cenobamate seems to act primarily by two mechanisms (see below for more detailed discussion); it blocks persistent Na^+ currents (I_{NaP}s) and enhances both phasic and tonic GABA inhibition .[56,57] Based on drug discrimination studies, cenobamate was designated as a Schedule V controlled substance by the DEA, indicating limited physical or psychological dependence relative to the drugs in Schedule IV.

On the basis of its marked efficacy in clinical trials in patients with therapy-resistant focal epilepsy,[9] cenobamate was approved in 2019 for this indication in the United States. In the first published Phase 2b efficacy study of cenobamate for treatment-resistant focal seizures,[58] high doses produced high seizure-freedom rates, suggesting that cenobamate can outperform existing treatment options.[9] However, such high doses of cenobamate were also associated with a risk of drug rash with eosinophilia and systemic symptoms (DRESS) and low tolerability. In a subsequent Phase 3, multicenter, open-label safety study in 1339 cenobamate-treated patients, using a start-low (12.5 mg/d), go-slow titration approach, no cases of severe idiosyncratic adverse effects (including DRESS) were observed,[59] but many more exposures are needed to determine the true safety profile of the drug.[9]

3 | COMPARISON OF PRECLINICAL ANTISEIZURE EFFICACY OF ALKYL-CARBAMATES IN ANIMAL MODELS

For preclinical development of novel ASDs for pharmacoresistant focal epilepsy, a battery of mouse and rat models is used, including the 6-Hz mouse and rat models of drug-resistant focal seizures and kindling models, such as amygdala, hippocampal, or corneal kindling in mice and rats.[60] Such a battery of models is also used in the current version of the Epilepsy Therapy Screening Program of the NINDS.[61] Furthermore, simple rodent models such as the maximal electroshock seizure (MES) test and the subcutaneous pentylenetetrazol (PTZ) seizure test, which have been used over decades in ASD screening, are still included in most of the current screening programs.[60] In addition, simple tests of "minimal neurological deficit" such as the rotarod test are used to estimate the safety margin (or "protective index") between the median effective dose (ED_{50}) and the median neurotoxic dose (TD_{50}).

As illustrated in Tables 1 and 2, the monocarbamates retigabine, carisbamate, and cenobamate show comparable efficacies and potencies in rodent seizure models. Interestingly, their potency does not appear to decrease when increasing the stimulation strength in the 6-Hz mouse model from 22 to 32 to 44 mA (Table 1), whereas most other ASDs lose antiseizure potency and/or efficacy when the current is increased in this test.[55,60,62] Furthermore, all three drugs were effective in kindled rats, including the lamotrigine-resistant amygdala kindling rat model (Table 2). With respect to safety margins, carisbamate and cenobamate exert antiseizure activity in all models at doses well below their TD_{50}, whereas this is not always the case with retigabine (Tables 1 and 2).

Only limited seizure model data could be found for meprobamate and flupirtine, but the ED_{50} s of the dicarbamate felbamate in the different models clearly differ from respective ED_{50} s of the three monocarbamates (Tables 1 and 2). The most important difference is that felbamate loses antiseizure potency with increasing stimulation strength in the 6-Hz test, whereas this was not observed with retigabine, carisbamate, and cenobamate. Similarly, felbamate is less potent in kindled rats compared to the monocarbamate drugs (Table 2).

The broad antiseizure efficacies of the monocarbamates retigabine, carisbamate, and cenobamate differ

TABLE 1 Antiseizure potencies of alkyl-carbamates in mouse models

Compound	Route	Time of test, min	ED_{50}, mg/kg						Rotarod test, TD_{50}, mg/kg	References
			MES	sc PTZ	6 Hz 22 mA	6 Hz 32 mA	6 Hz 44 mA	Kindled seizures		
Meprobamate	ip	60	127	66	?	?	?	?	85	Frey and Bartels, 1997[149]
Felbamate	ip	60	35.5	126	13.1	69.5	241	?	220	Guignet et al., 2020[55]
Flupirtine	po	?	51	39	?	?	?	?	174	Seaman et al., 1986[26]
Retigabine	ip	15	9.3	13.5	?	26	33	24.1 (corneal kindled)	20.5	Rostock et al., 1996[38]; Bialer et al., 2009[150]; Rowley and White, 2010[151]; Bankstahl et al., 2013[152]
Carisbamate	ip	15	7.9	20.4	20.7	21.4	27.6	?	46	Bialer et al., 2009[150]
Cenobamate	ip	15	9.8	28.5	11	17.9	16.5	?	58	Bialer et al., 2013[54]
Phenobarbital	ip	30	21.8	13.2	?	14.8	18.3	9.4 (corneal kindling)	69	Barton et al., 2001[62]; Bankstahl et al., 2013[152]; Koneval et al., 2020[153]

Note:: Phenobarbital is shown for comparison. "?" indicates that no data were found in the public domain.

Abbreviations: ED_{50}, median effective dose; ip, intraperitoneal; MES, maximal electroshock seizure; po, by mouth; PTZ, pentylenetetrazol; sc, subcutaneous; TD_{50}, median neurotoxic dose.

TABLE 2 Antiseizure potencies of alkyl-carbamates in rat models

Compound	Route	Time of test, min	ED_{50}, mg/kg		Rotarod test, TD_{50}, mg/kg	References
			MES	Kindled seizures		
Meprobamate	ip	60	?	68 (fully amygdala kindled rats)	?	Frey and Bartels, 1997[149]
Felbamate	ip	30	35	296 (fully hippocampal kindled rats)	>500	Guignet et al., 2020[55]
Flupirtine	po	?	47	?	116	Seaman et al., 1986[26]
Retigabine	ip	10/30	5.1	3.2 (lamotrigine-resistant fully amygdala kindled rats)	10	Rostock et al., 1996[38]; Metcalf et al., 2019[154]
Carisbamate	ip/po	15/?	4.4 (po)	22.5 (ip; fully hippocampal kindled rats)	39.5 (ip)	Novak et al., 2007[48]; Bialer et al., 2009[150]
Cenobamate	ip	15	2.9	16.4 (fully hippocampal kindled rats)	38.9	Guignet et al., 2020[55]
Phenobarbital	ip	60	12	16 (fully amygdala kindled rats)	41	Löscher et al., 1986[155]; Löscher and Nolting, 1991[156]

Note:: Phenobarbital is shown for comparison. "?" indicates that no data were found in the public domain.

Abbreviations: ED_{50}, median effective dose; ip, intraperitoneal; MES, maximal electroshock seizure; po, by mouth; TD_{50}, median neurotoxic dose.

from most other clinically used ASDs, except for the barbiturate phenobarbital, which is shown for comparison in Tables 1 and 2. This may indicate that these drugs share mechanistic aspects of phenobarbital, which mainly acts by potentiating the inhibitory neurotransmitter GABA via a barbiturate binding site on the $GABA_A$ receptor complex.[63]

4 | OTHER PHARMACOLOGICAL EFFECTS OF ALKYL-CARBAMATES

In addition to antiseizure activity, several alkyl-carbamates exert significant disease-modifying or antiepileptogenic effects in rodent models of epilepsy. For instance, felbamate counteracted the development of PTZ kindling in rats,[64]

and retigabine was the most effective drug in a series of compounds at blocking rapid kindling in developing rats.[65] Similarly, carisbamate has been shown to delay amygdala kindling in rats[66] and to exert powerful disease-modifying effects in the lithium-pilocarpine mode,[67] but failed to affect epileptogenesis in a rat model of epilepsy precipitated by traumatic brain injury.[68] At this time, it is not clear whether cenobamate possesses any disease-modifying activity.

Apart from effects on seizures, meprobamate exerts anxiolytic and sedative–hypnotic activities, which formed the basis for its clinical use (see above). In contrast, felbamate has only minimal anxiolytic and sedative–hypnotic effects at therapeutic doses, most likely as a result of its less potent effects on the $GABA_A$ receptor (see below). Retigabine was reported to exert anxiolytic activity in two mouse models of anxiety, and its effect in one of these models was blocked by the K_v7 channel inhibitor XE-991,[69] again suggesting this as an important mechanism of action of the drug. Similarly, cenobamate has been proposed to possess efficacy in mouse and rat models of anxiety.[54]

Another pharmacological effect of alkyl-carbamates is their analgesic activity. Analgesia is a class effect of GABA-potentiating drugs[70-72] but also of KCNQ activators such as flupirtine and retigabine.[28,29] Meprobamate is a constituent of various combination analgesics and may exert its beneficial activity in such combinations at least in part because of its sedative and muscle relaxant properties. Felbamate is effective in rodent models of acute and chronic pain and against neuropathic pain in patients.[73-75] Likewise, flupirtine exerts relatively strong analgesic effects in acute and chronic pain states in animal models as well as in humans.[28,29] Retigabine is arguably the most widely studied alkyl-carbamate derivative in models of nociceptive and neuropathic pain and, together with its analogue flupirtine (the only alkyl-carbamate approved for pain treatment), provided a template for developing more selective Kv7 openers as analgesic drugs.[27,29,76] In apparent contrast, carisbamate, which had been reported to exert an antiallodynic effect in rats,[77] did not demonstrate efficacy in controlled trials in neuropathic pain.[78] Limited data exist for cenobamate in this regard, although it has been reported to be more efficacious than gabapentin in the spinal nerve ligation (Bennett and Chung) model of neuropathic pain.[53]

As discussed above, most alkyl-carbamates have some abuse potential, most likely as a result of their GABAergic activity, and are thus DEA Schedule IV (meprobamate) or V (retigabine, cenobamate) regulated substances. The exceptions are felbamate and carisbamate, possibly because of lower potency at the $GABA_A$ receptor. For comparison, phenobarbital and BDZs are Schedule IV regulated drugs because of their higher abuse liability.

5 | COMPARISON OF MECHANISMS OF ACTION OF ALKYL-CARBAMATES

Figure 2 summarizes the main mechanisms of action of alkyl-carbamates that may explain their antiseizure effects. A more detailed comparison is shown in Table 3, in which phenobarbital is included for comparison. Most alkyl-carbamates seem to share a phenobarbital-like action on the $GABA_A$ receptor, but other effects may also contribute to their antiseizure efficacy.

$GABA_A$ receptors are ligand-gated chloride channels composed of five subunits that can belong to different subunit classes.[79,80] Most synaptic $GABA_A$ receptors are composed of two α, two β, and one γ2 subunit and mediate phasic inhibition, whereas receptors composed of two α, two β, and one δ subunit are predominantly or exclusively located extrasynaptically, where they respond to ambient GABA in the extracellular milieu and confer tonic (long-term) inhibition, thereby controlling network excitability. ASDs can be differentiated into drugs that potentiate synaptic inhibition or extrasynaptic inhibition or both.[81] ASDs that potentiate both types of GABAergic inhibition (including barbiturates, neurosteroids such as allopregnanolone, and several alkyl-carbamates) may be more effective in the treatment of acute and spontaneous recurrent seizures than drugs that act only on synaptic inhibition.[81,82]

$GABA_A$ receptors are the site of action of a variety of pharmacologically and clinically important drugs, such as BDZs, barbiturates, neuroactive steroids, general anesthetics, ethanol, and several convulsant compounds.[79,80,83,84] GABA acts at orthosteric sites located within the two extracellular $\beta^+\alpha^-$ interfaces of $GABA_A$ receptors, whereas the positive allosteric modulation exerted by BDZs involves interaction with the extracellular $\alpha^+\gamma2^-$ interface and that of barbiturates, neuroactive steroids, ethanol, and general anesthetics seemingly involves interaction with solvent accessible pockets in the transmembrane domain of the receptor. In contrast, a variety of convulsant drugs such as picrotoxinin and other "cage convulsants" block the chloride channel of $GABA_A$ receptors by binding to a site within the channel.[79,80] This picrotoxinin binding site on the $GABA_A$ receptor is typically characterized by the high-affinity ligand [^{35}S]*t*-butylbicyclophosphorothionate (TBPS), a bicyclophosphate derivative with potent picrotoxin-like convulsant activity.[16,80] A structurally related compound, [^{3}H]*t*-butylbicycloorthobenzoate (TBOB), can also be used for this purpose.[85] TBPS binding to $GABA_A$ receptors can be allosterically inhibited by barbiturates, including phenobarbital, whereas BDZs, in the absence of GABA, allosterically increase TBPS binding in in vitro preparations of rodent and human brain.[80,86,87] Thus, TBPS (or TBOB) binding is often

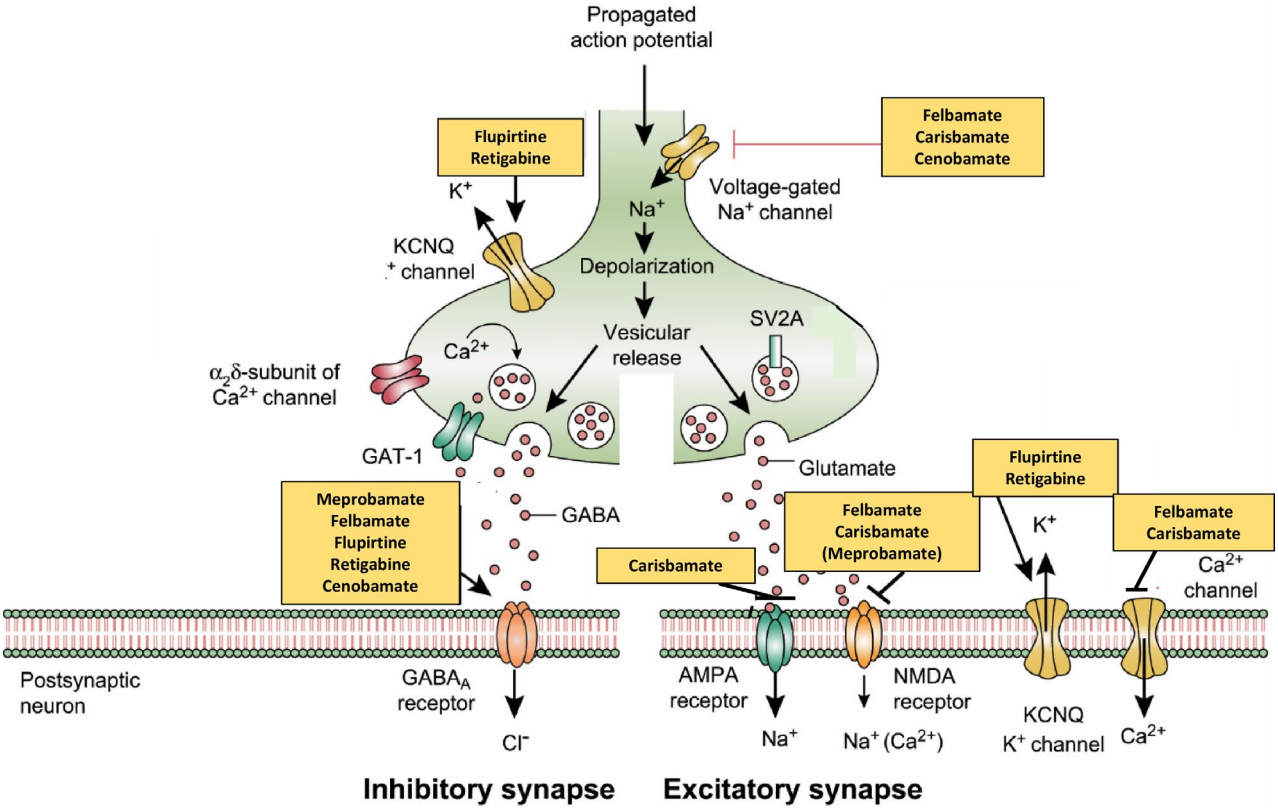

FIGURE 2 Presumed mechanisms of action of alkyl-carbamates. The figure has been modified from Löscher and Schmidt[147] and Löscher et al.[148] AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ; GABA, γ-aminobutyric acid; GAT-1, GABA transporter 1; KCNQ, voltage gated Kv7 potassium channels; NMDA, N-methyl-D-aspartate; SV2A, synaptic vesicle protein 2A

used as a probe to discriminate drugs acting via BDZ and non-BDZ recognition sites at the $GABA_A$ receptor.

Meprobamate was the first carbamate for which interactions with GABAergic neurotransmission were reported.[16,88-93] Some early studies suggested that meprobamate may bind to the BDZ recognition site of the $GABA_A$ receptor,[90,91] but this was disputed in other studies.[88,89,92] Instead, in line with a barbiturate-like action, meprobamate was reported to inhibit TBPS binding at the $GABA_A$ receptor–chloride channel complex[16] and to allosterically enhance BDZ binding.[93] Meprobamate was found to have behavioral actions distinct from BDZs and more characteristic of barbiturates,[94] although there are also differences between meprobamate and barbiturates, both pharmacologically and clinically.[95] Rho et al.[96] reported that meprobamate acts as a barbiturate-like positive allosteric modulator (PAM) of $GABA_A$ receptors, enhancing GABA-evoked responses in rat hippocampal neurons and, in the absence of GABA, directly activating Cl^- currents that could be attenuated by the $GABA_A$ receptor antagonists bicuculline and picrotoxin. Furthermore, the barbiturate antagonist bemegride inhibited meprobamate-mediated direct gating of the $GABA_A$ receptor in a concentration-dependent manner, consistent with a competitive nature of inhibition.[97] Meprobamate acts on both synaptic and extrasynaptic $GABA_A$ receptors.[97] In the absence of a selective antagonist for the barbiturate recognition site of the $GABA_A$ receptor, it is difficult to pharmacologically evaluate the possibility that meprobamate (and other alkyl-carbamates) act at the same site on the $GABA_A$ receptor as barbiturates. However, at high concentrations (3 $mmol \cdot L^{-1}$), meprobamate did reduce the potentiation of GABA currents by 1 $mmol \cdot L^{-1}$ pentobarbital, which is compatible with the possibility that meprobamate competes for binding with pentobarbital (and has lower intrinsic efficacy), but may also reflect greater channel-blocking activity of meprobamate in comparison with pentobarbital.[96] The effects of meprobamate on GABA-evoked responses, which have also been studied in recombinant human $GABA_A$ receptors,[97] are likely to explain both its antiseizure and anxiolytic/sedative effects. The channel block of the $GABA_A$ receptor at high concentrations of meprobamate limits the extent of GABA potentiation, making it a less potent CNS depressant than pentobarbital, and may explain the differences between meprobamate (and other alkyl-carbamates) and barbiturates. In addition to its effects on GABAergic inhibition, meprobamate also blocks NMDA-activated currents at high (presumably supratherapeutic) concentrations, indicating an effect on excitatory glutamatergic activity.[96]

TABLE 3 Mechanisms of action of alkyl-carbamates with antiseizure activity

Compound	GABA-evoked responses		Specific interaction with $GABA_A$ receptors		
	Synaptic (phasic currents)	Extrasynaptic (tonic currents)	Picrotoxinin recognition site (TBPS or TBOB binding)	BDZ recognition site	Receptor activation in the absence of GABA
Meprobamate	Enhanced	Enhanced	Inhibited	Allosteric enhancement of BDZ binding	Yes
Felbamate	Enhanced	?	Inhibited	No inhibition of BDZ binding	No
Flupirtine	Enhanced	Enhanced	?	?	?
Retigabine	Enhanced	Enhanced	Inhibited	?	?
Carisbamate	No effect (but see text)	?	? (but effect on picrotoxin-sensitive Cl^- channels)	?	?
Cenobamate	Enhanced	Enhanced	Inhibited	No inhibition of BDZ binding	?
Phenobarbital	Enhanced	Enhanced	Inhibited	Allosteric enhancement of BDZ binding	Yes

Note:: Phenobarbital is shown for comparison. "?" indicates that no data were found in the public domain. See text for references.

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDZ, benzodiazepine; GABA, γ-aminobutyric acid; IC_{50}, median effective dose; KCNQ, voltage gated Kv7 potassium channels; NMDA, N-methyl-D-aspartate; TBOB, [^{3}H]*t*-butylbicycloorthobenzoate; TBPS, [^{35}S]*t*-butylbicyclophosphorothionate

Felbamate resembles meprobamate in its effects on $GABA_A$ and NMDA receptors (Table 3) but is a less effective potentiator of $GABA_A$ receptors and does not directly activate $GABA_A$ receptors in the absence of GABA,[96,98-100] likely explaining why felbamate is less sedative than meprobamate. The enhancement of GABA-evoked currents by felbamate was unaffected by flumazenil, a specific BDZ recognition site antagonist, indicating that felbamate does not act at the BDZ site.[98] A 2019 study with competition photolabeling of recombinant human $GABA_A$ receptors indicated that the effects of felbamate on GABA responses do not occur by binding at barbiturate interaction sites either.[101] In contrast to meprobamate, felbamate inhibits NMDA receptors at therapeutically relevant concentrations; however, it is uncertain whether the NMDA receptor-blocking activity of felbamate is relevant to its clinical antiseizure activity.[102] Single-channel recordings have indicated that the effect of felbamate on NMDA responses occurs via a channel-blocking mechanism.[98] In addition, felbamate has been reported to exert use-dependent block of voltage-gated Na^+ and Ca^{2+} channels.[19] Overall, the barbiturate-like potentiation of GABA by felbamate is the most likely mechanism underlying its antiseizure activity, but effects on voltage-gated Na^+ channels may also contribute, particularly to its effects on seizure generalization.

Flupirtine at therapeutic concentrations (≤10 μmol·L^{-1}) does not affect voltage-gated Na^+ or Ca^{2+} channels, inward rectifier K^+ channels, nicotinic acetylcholine receptors, glycine receptors, or ionotropic glutamate receptors in neuronal preparations.[31] Instead, flupirtine shifts the gating of K_V7 K^+ channels to more negative potentials and the gating of $GABA_A$ receptors to lower GABA concentrations, indicating concomitant facilitation of K_V7 channels and $GABA_A$ receptors.[31] In a subsequent study on native and recombinant $GABA_A$ receptors, Klinger et al.[103] reported that flupirtine prefers extrasynaptic δ-containing $GABA_A$ receptors over synaptic receptors containing the γ-subunit, and also over K_V7 channels.

Retigabine is thought to act primarily as an opener of KCNQ (K_V7) K^+ channels, and several experimental studies support the role of Kv7 K^+ channels in the antiseizure activity of this drug.[102] Mice with a genetic defect in these channels show reduced sensitivity to the antiseizure effect of retigabine,[104] and the KCNQ inhibitor XE-991 partially blocks the antiseizure effect of retigabine in the mouse MES test.[104,105] Retigabine exerts no effects on NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors[41,106] and is only a weak inhibitor (half-maximal inhibitory concentration [IC_{50}] >100 μmol·L^{-1}) of voltage-gated Na^+ and Ca^{2+} channels.[104] However, as with other alkyl-carbamates, retigabine has been reported to interact with the GABA system. At relatively high concentrations (>10 μmol·L^{-1}), retigabine has been shown to potentiate GABA-mediated inhibitory transmission by acting as a PAM of $GABA_A$ receptors via a non-BDZ site.[41,106] Similar to barbiturates (and other alkyl-carbamates), retigabine inhibited TBOB binding to the picrotoxinin site of the $GABA_A$ receptor in the absence of GABA, indicating that retigabine

Glutamatergic transmission		Voltage-gated Na^+ channels			
NMDA-activated currents	**AMPA-activated currents**	**Transient Na^+ currents**	**Persistent Na^+ currents**	**Voltage-gated $Ca2^+$ channels**	**Voltage-gated K^+ (KCNQ) channels**
Inhibited (only at high concentrations)	?	?	?	?	?
Inhibited	?	Use-dependent block	?	Use-dependent block	?
No relevant effect	?	No effect	?	No effect	Activated
No effect	No effect	Weak inhibition	?	Weak inhibition	Activated
Inhibited	Inhibited	Use-dependent block	?	Inhibition	?
?	?	Little effect (IC_{50} > 500 µmol·L^{-1})	Block	?	?
No effect	Inhibited	No relevant effect	?	Use-dependent block	?

interacts with a site on the $GABA_A$ receptor complex that is positively allosterically coupled with the orthosteric GABA binding site.[107] Recent evidence that inhibitory effects of retigabine on seizure-like activity in hippocampal neurons persist in the presence of a blockade of Kv7 channels has bolstered the view that positive modulation of $GABA_A$ receptors likely makes a significant contribution to its antiseizure activity.[108] This view is reinforced by the observation that, at lower concentrations than those required for effects on synaptic $GABA_A$ receptors, retigabine selectively enhances extrasynaptic $GABA_A$ receptors that contain the δ-subunit.[108] Thus, various lines of evidence raise the possibility that effects of retigabine on GABA mechanisms could be of importance in its antiseizure activity.[102]

Compared to other alkyl-carbamates that are used clinically, the potential mechanisms of action of carisbamate have been less extensively studied (Table 3). Carisbamate has been reported to exert a use-dependent blocking action on voltage-gated Na^+ channels in cultured hippocampal neurons,[49] which is consistent with its inhibitory effect on repetitive action potential firing in rat piriform cortical neurons.[50] Furthermore, carisbamate appears to increase Cl^- conductance presynaptically and, under certain conditions, postsynaptically to selectively depress excitatory neurotransmission.[50] These effects were blocked by picrotoxin, indicating an effect of carisbamate on a picrotoxin-sensitive presynaptic $GABA_A$-like Cl^- conductance, but there is no evidence that carisbamate has any effect on spontaneous $GABA_A$ miniature inhibitory postsynaptic currents.[50] Similarly, in dentate gyrus granule cells, carisbamate did not affect GABAergic transmission but depressed AMPA- and NMDA-receptor-mediated excitatory neurotransmission.[51] More recently, carisbamate was reported to inhibit T-type calcium channels.[52] To our knowledge, detailed studies of carisbamate on synaptic versus extrasynaptic GABA responses are missing, as are studies on potential interaction with TBPS or TBOB binding to the $GABA_A$ receptor and experiments with recombinant $GABA_A$ receptors. As such, it is not possible, at this time, to conclude whether carisbamate has a similar or distinct mechanistic profile from other alkyl-carbamates.

Even fewer mechanistic studies are available for cenobamate (Table 3), most likely because this novel ASD was only introduced in 2019. Two mechanisms of action emerge from the few published studies on cenobamate: (1) phenobarbital-like potentiation of synaptic and extrasynaptic GABAergic inhibition by a non-BDZ mode of action, and (2) an inhibitory effect at the I_{NaP} (Table 3). With respect to GABA, cenobamate acts as a PAM of $GABA_A$ receptors in hippocampal neurons, with effects on both phasic and tonic inhibitory currents and on recombinant synaptic and extrasynaptic $GABA_A$ receptor isoforms, effects that were not antagonized by the BDZ antagonist flumazenil.[57] In line with a barbiturate-like effect, cenobamate has been reported to displace the binding of TBPS to the GABA-gated Cl^- channel, whereas the binding of GABA, muscimol, flunitrazepam, and Ro-15-1788 (flumazenil) to $GABA_A$ receptors was not inhibited.[47,109] Thus, as shown in Table 3, the effect of cenobamate on $GABA_A$ receptors resembles that of other

alkyl-carbamates and phenobarbital. With respect to voltage-gated Na^+ channels, cenobamate inhibits the noninactivating I_{NaP} more potently than the transient Na^+ current,[56] which likely contributes to the ability of this drug to suppress sustained depolarizations while sparing single action potentials and low-frequency firing. Furthermore, cenobamate was shown to enhance the inactivated state of voltage-gated Na^+ channels.[56] Notably, these reported effects of cenobamate on both $GABA_A$ receptors and on the I_{NaP} appear to occur at very similar concentrations.

Under normal physiological conditions, depolarization of the neuronal cell membrane leads to a transient inward Na^+ current (I_{NaT}) that rapidly inactivates. However, a small proportion of Na^+ channels appear to undergo rare, late openings in response to depolarization and give rise to a Na^+ current (I_{NaP}) that fails to inactivate, and is thereby termed "persistent."[110] I_{NaP}s, despite their small amplitude in comparison to transient currents, play a disproportionately large role in the regulation of neuronal excitability and repetitive firing capabilities,[110] and their existence is relevant to the pharmacology of several ASDs.[19] Substantial block of the I_{NaP} is observed with phenytoin at therapeutically relevant concentrations (conductance reduced to 20%–22% of control), which exceeds the effect of the drug on the transient current (reduced to 40%–41% of control) that underlies normal action potential generation.[111,112] Partial block of the I_{NaP} is also observed with carbamazepine (~55% of control) and topiramate (~70% of control), with potencies that can approximate (carbamazepine) or even exceed (topiramate) their effects on I_{NaT}.[110,113] Valproate has also been shown to block I_{NaP} at concentrations within the therapeutic range,[114] whereas ethosuximide does so only at supratherapeutic levels.[115] Thus, cenobamate is not the only ASD that inhibits the I_{NaP}, but, to our knowledge, it is the only alkyl-carbamate for which an effect on I_{NaP} has been reported to date. Its ability to produce a substantial block of I_{NaP} (31.5% of control at 100 $\mu mol \cdot L^{-1}$), together with the clear separation in concentrations at which it blocks persistent (IC_{50} = 53.1 $\mu mol \cdot L^{-1}$) versus transient (IC_{50} > 500 $\mu mol \cdot L^{-1}$) Na^+ currents,[56] may set it apart from most other ASDs that share this mechanism.

An inherent problem when comparing mechanisms of action of alkyl-carbamates and phenobarbital (Table 3) is the lack of "head-to-head" studies in the same preparation. One of the few exceptions is the comparison of meprobamate and felbamate on GABA-evoked responses in rat hippocampal neurons reported by Rho et al.[96] Several of the mechanisms illustrated in Table 3 have not been examined for all alkyl-carbamates, at least in part because the respective techniques were not available at the time at which some of these drugs were first developed and characterized. Thus, for instance, we simply do not know whether cenobamate differs in its effects on Na^+ channels from other alkyl-carbamates. For cenobamate, it has been suggested that the I_{NaP} may be a critical target for enhanced seizure control, especially when combined with a PAM effect that can enhance $GABA_A$ currents and subsequent inhibitory neurotransmission.[55] However, as shown in Tables 1 and 2, the preclinical antiseizure profile of cenobamate is very similar to that of other alkyl-carbamates, particularly the monocarbamates retigabine and carisbamate, for which there is no information about effects on I_{NaP}s. As shown in Table 3, the only common mechanistic effect of most, if not all, alkyl-carbamates is a phenobarbital-like enhancement of GABA responses.

It is possible that the combined (and perhaps synergistic) effect of cenobamate on GABA-mediated inhibition and on I_{NaP}, which occur at similar concentrations, contributes to its broad antiseizure profile and impressive clinical efficacy,[55] but this mechanistic profile is not unique. It is also shared, at least in part, by topiramate,[19] a drug that has a different efficacy profile in preclinical models. These differences may be explained by the additional cellular effects of topiramate (i.e., on Ca^{2+} channels and ionotropic glutamate receptors) that are not, to our knowledge, observed with cenobamate[55] but perhaps also by differences in the extent to which each drug can block I_{NaP}. Then again, given the current dearth of mechanistic information, we cannot rule out the possibility of other, as yet unknown mechanisms that additionally contribute to the antiseizure activity of cenobamate.

6 | CLINICAL EFFICACY OF ALKYL-CARBAMATES IN PATIENTS WITH DRUG-RESISTANT FOCAL EPILEPSY

Achieving seizure freedom is the primary goal in the treatment of epilepsy; however, at least 30% of patients do not become seizure-free on existing ASDs, with even higher figures in patients with focal epilepsy.[116] Thus, all new ASDs are initially evaluated as add-on treatment for patients with seizure disorders that are not adequately controlled with one or more standard agents.[117,118]

One of the most common primary outcome measures in randomized controlled trials (RCTs) aimed at demonstrating efficacy and safety of a new ASD is the percentage of subjects who experience a 50% or greater reduction in seizure frequency from baseline (i.e., the so-called "responder rate"). Until recently, seizure freedom was not generally quoted as a primary outcome measure in RCTs, largely because of the refractoriness of epilepsy in this population and limited expectation of achieving complete remission over the short trial period.[118,119] Thus, prior to the year 2000, few clinical trials adopted this indicator, and although more recent RCTs have explored seizure freedom as an outcome, they have typically shown very modest remission rates, varying from 1% to 5%, despite the chemical and pharmacological diversity of the

drugs studied.[120-122] A recent meta-analysis[122] of seizure freedom across 40 RCTs of ASDs in a total of 9136 patients with drug-refractory focal epilepsy found that eight ASDs (including the alkyl-carbamates carisbamate and retigabine) were superior to placebo, the most effective being brivaracetam, with a reported remission rate of 4%–5%.[123] This observation supports an earlier study of 63 pivotal RCTs of lamotrigine, gabapentin, topiramate, tiagabine, levetiracetam, zonisamide, pregabalin, lacosamide, and eslicarbazepine, and also a pooled analysis of the three pivotal trials conducted for both perampanel and brivaracetam, in which seizure-free rates ranged from 0% to a maximum of 6.5%.[124,125] By contrast, a recent Phase 2b trial of cenobamate in patients with drug-refractory focal epilepsy reported seizure-free rates over the 12-week maintenance period ranging from 4% at 100 mg/day to 21% at 400 mg/day, compared to just 1% in the placebo arm.[58] A further Phase 2 study reported 28% remission with cenobamate at 200 mg/day compared to 9% for placebo, albeit over a shorter, 6-week maintenance period.[126] Thus, the degree of seizure freedom associated with higher doses of cenobamate was well beyond that seen for any new ASD brought to the market in the past 25 years.[125] By way of comparison, felbamate, which was considered highly effective, was associated with a remission rate of 10% in 246 patients with drug-refractory focal epilepsy.[127]

If cenobamate maintains its high seizure-free rate in long-term use, then it may represent an important new ASD. However, regulatory trials involve a carefully selected population of patients and are conducted under rigorously standardized conditions, so data from such studies often cannot be translated into clinical practice.[119] For cenobamate, results from open-label extension (OLE) studies suggest that its efficacy is sustained for at least the first 3 years of treatment, remaining consistent with the level of effect observed in the double-blind, placebo-controlled trials.[128] Importantly, the remission rate during the last 6 months of follow-up (Months 25–30) was 20.2% of all evaluable patients. Among the patients who were seizure-free at Months 25–30, the median duration of seizure freedom during the entire OLE phase was 33.2 months.

This impressive efficacy emerging from recent RCTs with cenobamate is, however, tempered by the risk of serious rash (i.e., DRESS) and low tolerability at higher doses,[58] meaning that further safety studies and clinical experience are needed to determine the true clinical value of cenobamate.[9]

7 | RISK OF SEVERE IDIOSYNCRATIC ADVERSE EVENTS WITH DIFFERENT ALKYL-CARBAMATES

As described above, both meprobamate and felbamate have been reported to induce rare but fatal idiosyncratic adverse effects, namely, aplastic anemia and liver toxicity in patients. For felbamate, it has been proposed that an alternative metabolic pathway, which involves the formation of 3-carbamoyl-2-phenylpropionaldehyde as a potentially reactive intermediate, contributes to its bioinactivation.[10,24,129] In vitro, the aldehyde undergoes rapid facile elimination to form 2-phenylpropenal (atropaldehyde) and can also be cyclized to form the more stable 4-hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one, which may act as a reservoir of the reactive aldehyde.[130] The atropaldehyde metabolite is electrophilic and cytotoxic, and its formation has been confirmed in vivo in rodents and humans, suggesting that it may be responsible for felbamate-induced liver and bone marrow toxicity.[10,129] Under normal conditions, atropaldehyde is rapidly conjugated with glutathione, which acts as a protective mechanism by inactivating atropaldehyde before it can cause damage.[24] Most patients will maintain sufficient levels of glutathione to detoxify the reactive metabolite of felbamate.[25] Those with a history of felbamate-associated aplastic anemia had significantly lower erythrocyte glutathione peroxidase levels,[10] and clinical studies with felbamate have demonstrated an association between reactive metabolite formation and clinically relevant toxicity.[24] Possible risk factors for felbamate-associated aplastic anemia include history of cytopenia, prior history of autoimmune disorder, and a positive antinuclear antibody titer.[10] Although the use of felbamate has been restricted, this drug remains an effective and safe treatment for patients with seizures refractory to other ASDs when used in accordance with existing recommendations and with close clinical monitoring.[10,23,25] Evidence for a key role of reactive metabolites in felbamate toxicity provided a rationale for the development of fluorofelbamate (MedPointe Pharmaceuticals), a felbamate analogue that is not converted to atropaldehyde.[131]

Flupirtine is another example of an alkyl-carbamate that induces rare but potentially fatal idiosyncratic liver toxicity, a characteristic it does not seemingly share with the structurally similar drug retigabine.[13] Even after more than 30 years of use, the hepatotoxicity of flupirtine is still poorly understood. As with felbamate, a probable toxification mechanism of flupirtine has been described, caused by extensive oxidation by peroxidase enzymes and leading to the generation of unstable intermediates with the potential to interact with nucleophilic moieties, such as thiol-containing cysteine residues, and thereby covalently modify liver proteins.[13] Presumably, in healthy subjects, the reactive quinone diimines are intercepted again by glutathione, but under conditions of glutathione depletion, as are often found in the elderly, these reactive metabolites might react with liver proteins leading to hepatotoxicity. Unlike flupirtine, retigabine is not substantially metabolized via oxidation but rather undergoes Phase II reactions.[13] This difference in metabolic profile might explain the differences in hepatotoxic potential between these structurally similar drugs.

Although retigabine is seemingly devoid of the potential to cause idiosyncratic liver toxicity, within 2 years of retigabine's approval for clinical use, multiple cases of blue discoloration of the tissues began to emerge.[132] The hard palate, nails, lips, and the conjunctiva were particularly affected, often years after initiation of retigabine therapy, and retinal pigmentations raised concerns about possible vision loss.[13] The FDA subsequently recommended eye examinations every 6 months and discontinuation of the drug when eye discolorations were observed. The incidence rate of tissue discoloration is reported to be 3.6% per patient-year, with a median onset of approximately 4 years.[13] In biopsy samples of discolored tissue, dimers of retigabine and dimers of its main metabolite were found, partly in conjunction with melanin.[13] These dimers might be responsible for the abnormal pigmentation under retigabine treatment, which is considered an idiosyncratic adverse event.

Available evidence to date suggests that neither carisbamate nor cenobamate undergo biotransformation into reactive metabolites. The major metabolic pathways of carisbamate in humans are direct *O*-glucuronidation (44% of the dose), and hydrolysis of the carbamate ester followed by oxidation to 2-chloromandelic acid, which is subsequently metabolized in parallel to 2-chlorophenyl glycine and 2-chlorobenzoic acid.[133] Only traces of aromatic (pre)mercapturic acid conjugates have been detected in the urine (each <.3% of the dose), suggesting a low potential for reactive metabolite formation. Cenobamate is metabolized by both cytochrome P450 (CYP) and uridine 5'-diphosphoglucuron osyltransferase (UGT) enzymes.[134] Multiple CYP enzymes have appeared to be involved in cenobamate metabolism, with CYP2E1, CYP2A6, and CYP2B6 as the major enzymes contributing to oxidative metabolism in humans and, to a lesser extent, CYP2C19 and CYP3A4/5.[134] Glucuronidation of cenobamate is predominantly catalyzed by UGT2B7 and, to a lesser extent, by UGT2B4.

Despite an apparently benign metabolic profile, the early clinical development of cenobamate, during which the drug was titrated quickly, witnessed three cases of idiosyncratic DRESS among the first 953 individuals exposed to the drug, including one fatality.[9] In the RCT by Krauss et al.,[58] which also employed a rapid titration rate, three of 437 participants experienced hypersensitivity reactions, one of which was DRESS. As a result, DRESS is among the warnings and precautions for cenobamate in the US prescribing information. Interestingly, a further large-scale safety study that employed a slower titration rate than previous studies, with a start-low (12.5 mg/day), go-slow approach, reported no cases of DRESS among 1339 participants.[59] As is the case for several other ASDs, it would appear that safety concerns with cenobamate can be at least partly mitigated by cautious titration. Nevertheless, the number of cenobamate-exposed patients worldwide remains too low to assess the true risk of rare but serious idiosyncratic events with this drug.

With respect to the idiosyncratic adverse reactions described above for cenobamate, it has to be considered that cutaneous manifestations of hypersensitivity are the most common idiosyncratic reactions associated with ASD use and are independent of their antiseizure mechanisms of action.[129,135,136] Skin rashes in the vast majority of people are mild in severity, but serious and potentially life-threatening reactions, such as DRESS, Stevens–Johnson syndrome, and toxic epidermal necrosis can occur on occasion. Aromatic ASDs such as carbamazepine, oxcarbazepine, eslicarbazepine acetate, phenytoin, lamotrigine, phenobarbital, primidone, and zonisamide are all associated with idiosyncratic skin reactions, whereas alkyl-carbamates are less frequently implicated.[136]

8 | DEVELOPMENT OF NEW ALKYL-CARBAMATES WITH ANTISEIZURE EFFICACY

The success of alkyl-carbamates as ASDs has prompted intensive research with the goal of developing novel compounds with lowered risk of severe idiosyncratic adverse effects. One example, already noted above, is fluorofelbamate, which was designed to retain the broad spectrum multimechanistic activity of felbamate, with a modified metabolism that has been shown to avoid the production of the reactive atropaldehyde metabolite believed to cause its idiosyncratic toxicity.[131] Similarly, flupirtine and retigabine are being used as templates for ligand-based drug design of $K_V7.2/3$ activators,[13,137,138] although such agents are predominantly being developed for the treatment of neuropathic pain, which represents a potentially bigger and less-crowded market. Nevertheless, modulation of the $K_V7.2/3$ heterotetramer has potential for efficacy in numerous indications related to hyperexcitability,[13] and, as such, these novel compounds are also interesting candidates for epilepsy.[28] Apart from $K_V7.2/3$ selective activators, several other series of alkyl-carbamates, comprising hundreds of compounds, have been synthesized and tested for antiseizure activity.[2,139-146] Among the numerous alkyl-carbamates tested, chiral CNS-active carbamate derivatives of valproic acid were particularly interesting.[143]

9 | CONCLUSIONS

As a class, alkyl-carbamate drugs have long attracted interest for their potential efficacy in the treatment of epilepsy, from the first report of the anticonvulsant effects of meprobamate[14] to the recent development and FDA approval in 2019

of cenobamate.[9] Two distinct groups of alkyl-carbamates exist: the dicarbamates, which include meprobamate and felbamate; and the monocarbamates, which include flupirtine, retigabine, carisbamate, and cenobamate. All alkyl-carbamates have efficacy in a range of preclinical models, and most have demonstrated clinical utility in either RCTs or in the routine management of seizure disorders, with only modest differences in their spectra of activity. Some have additionally shown promise in the epileptic encephalopathies and in other, nonepilepsy indications. Most alkyl-carbamates (with the possible exception of carisbamate) appear to share a common mechanism, involving positive allosteric modulation of the $GABA_A$ receptor in a barbiturate-like manner, which often includes effects at both synaptic and extrasynaptic receptor sites that mediate phasic and tonic Cl^- currents, respectively. They appear to diverge in terms of additional mechanisms of action, which include potentiation of Kv7-mediated voltage-gated K^+ currents (flupirtine, retigabine), inhibitory effects on ionotropic glutamate receptors (felbamate, carisbamate), and blockade of both transient Na+ currents (felbamate, carisbamate) and I_{NaP}s (cenobamate).

The latest alkyl-carbamate to be approved for epilepsy—cenobamate—appears to possess an unprecedented capacity to elicit seizure freedom under the admittedly artificial conditions of regulatory RCTs. Remission rates in cenobamate trials have exceeded those of all other ASDs approved in the past 25 years by a factor of three- to fourfold and appear to be sustained in longer term follow-up. One possible explanation is cenobamate's unique combination of mechanisms of action, comprising both positive allosteric modulation of the $GABA_A$ receptor at a non-BDZ site and preferential blockade of the persistent current carried by voltage-gated Na^+ channels, and potential synergy between these cellular effects. Both actions occur at similar concentrations, and although this mechanistic profile is shared, in part, with the sulfamate-substituted monosaccharide topiramate, they differ markedly in the extent of I_{NaP} block and in their preclinical antiseizure profile. It remains to be seen whether the apparent efficacy of cenobamate is sustained during routine clinical use and also whether the idiosyncratic reactions that have often blighted this class of ASD and that were evident in some cenobamate trials, have an impact on its effectiveness in the real world.

Irrespective of the longer term outlook for cenobamate, the alkyl-carbamates will continue to represent an interesting class of compounds, with powerful antiseizure actions in experimental models and common forms of human epilepsy and with the potential to offer hope in otherwise drug-resistant epileptic encephalopathies and as precision therapies. That expectation potentially comes at the price of rare adverse reactions that can be severe and occasionally fatal but that may be surmountable with careful dosing and patient monitoring.

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CONFLICT OF INTEREST

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