Blockers of Voltage-Gated Sodium Channels for the Treatment of Central Nervous System Diseases

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Abstract: Voltage gated sodium channels play important roles both in vital physiological functions and several pathological processes of the central nervous system. Epilepsy, chronic pain, neurodegenerative diseases, and spasticity are all characterized by an over-excited state of specific groups of central neurons that is accompanied by an abnormally increased activity of sodium channels. An efficient strategy of controlling such diseases is to use blockers that preferentially act on these over-excited cells. State dependently acting agents, such as phenytoin, or lamotrigine, leave normal physiological functions relatively intact, resulting in a favorable therapeutic window. Nine isoforms of the channel forming alpha subunit are known, which show distinct expression patterns in different tissues. Another possible way to decrease the chance of adverse effects is to develop agents selectively inhibiting the channel subtype involved in the pathomechanism of the disease to be treated.

Many recent patents claim sodium channel blockers with improved characteristics regarding state dependency or subtype selectivity. Such agents may offer a breakthrough in the treatment of a variety of central nervous system diseases. This review focuses on the current trends in sodium channel research, surveying the traditional and newly emerging therapeutic fields, and the diverse medicinal chemistry of sodium channel blockers.

Keywords: Voltage-gated sodium channel, channel blockers, state-dependent action, central nervous system diseases, pharmacotherapy, hyperexcitability disorders, side-effect, epilepsy, chronic pain, spasticity, excitotoxicity.

INTRODUCTION

Several central nervous system (CNS) diseases, which can be collectively termed neuronal hyperexcitability disorders, are characterized by an altered balance between excitatory and inhibitory neuronal functions, so that increased excitability can be demonstrated at both behavioral and cellular levels. The shift in balance may disturb normal neuronal functioning, while severe and sustained over-excitation can result in neuronal death. Such diseases include epilepsies, chronic neuropathic or inflammatory pain states, migraine and neurodegeneration related to ischemia or chronic diseases, etc. Neurons involved in hyperexcitability CNS disorders frequently display high-frequency bursts of action potentials, paroxysmal depolarization shifts, plateau potentials, or sustained depolarization [1-4]; Altered functioning can be restored by either enhancing inhibitory, or decreasing excitatory synaptic transmission at the level of neurotransmitter receptors by using appropriate modulator compounds. Voltage-gated sodium channels (VGSCs) are responsible for action potential generation and propagation, and an increased activity of these channels is characteristically accompanied with the above disease states. Furthermore, non-inactivating (persistent) sodium currents may contribute to abnormal activity, and sustained depolarization [1,5-7]. Thus, attenuation of abnormally increased sodium channel activity represents another efficient way of alleviating neuronal over-excitation [8-10].

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Indeed, VGSC blocking drugs have long been used in the therapy of various CNS diseases. The antiepileptic drugs phenytoin and carbamazepine were introduced 7 and 5 decades ago, respectively, but the fact that their antiepileptic effect is based on VGSC inhibition was recognized only twenty years ago.

Recognition of the significance of VGSCs as a molecular target of CNS drugs prompted most drug companies to conduct intensive research for therapeutically useful agents. Since VGSCs also play important roles in a wide variety of vital physiological functions; the therapeutic window of VGSC blockers is a critical issue.

The present review discusses the possibilities for developing VGSC blocking drugs with acceptable side effect profile for the treatment of CNS diseases. Multiple isoforms of the sodium channel protein exist [9,11-13], which possess different functional characteristics, and display distinct expression patterns in various parts of the CNS. Thus, theoretically, it is possible to design subtype-selective therapeutic agents, which block only those neurons which are involved in a particular disease. An important feature of VGSC blocking drugs is that they more efficaciously inhibit the over-active sodium channels characteristic of neuronal hyperexcitability diseases, than those participating in normal physiological functions [8]. Therefore, another way to widen the therapeutic window of VGSC blocking drugs is to enhance the state-dependence of their action [14].

These new trends of sodium channel research will be surveyed in the light of the recently published scientific and patent literature. In addition, traditional and newly emerging therapeutic fields, and the diverse medicinal chemistry of

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sodium channel blockers with CNS indication, will be reviewed.

STRUCTURE AND DIVERSITY OF VGSCs

VGSCs are heteromeric assemblies of one alpha and one or more beta subunits, the former being responsible for most channel functions such as voltage sensing, gating, ion permeation, and inactivation [11]. Beta subunits can modulate the function of the pore forming alpha subunits and help their integration into the plasma membrane. Alpha subunits are huge (about 260 kDa) proteins containing four homologous domains (DI-DIV) each with six transmembrane segments (S1-S6) and a re-entrant loop between S5 and S6, which contributes to the formation of the inner lining of the channel (Fig. **1**). Transmembrane segments are interconnected with extra- or intracellular loops, and both N- and C terminals of the protein molecule are located intracellularly. According to earlier hypotheses homologous domains embrace one pore permeable to sodium ions. A recent model, based on cryo-electron microscopy and single-particle image analysis of the solubilized sodium channel, the so-called "twist-sprinkler" model [15,16], proposes a more complex 3-D structure, containing four channel pores. The functions of the different parts of the channel are reasonably well-understood; e.g. molecular determinants of voltage sensing, ion selectivity, inactivation, etc. have been identified [11]. Inactivation of the channel occurs via multiple independent mechanisms; of which fast inactivation is the best understood [17]. The intracellular loop between DIII and DIV contains a lipophilic sequence, the isoleucine, phenylalanine, and methionine (IFM) motif, which binds to a region located on DIVS6 shortly after channel opening, thus causing a blockade of ion flux (Fig. **1**). Furthermore, regulatory sites have been described through which channel function can be modified via phosphorylation or drug binding [11,17,18].

Different isoforms of the alpha subunit have been cloned and characterized in detail. Na_V 1.1-1.9 [12] show distinct expression patterns in muscle cells and various parts of the peripheral and central nervous system. Na_V 1.5, and Na_V 1.4 are mainly expressed in the heart and skeletal muscle, respectively, while Na_V 1.7-1.9 are characteristic of sensory neurons. Brain-type sodium channels include Na_V 1.1-1.3, and 1.6 [8,13]. However, these expression patterns are not absolute, and, with the possible exception of Na_V 1.8, practically all subunits can be detected in the CNS [19]. The physiological properties (e.g. kinetics or voltage dependence of activation and inactivation) of each channel subtype are characteristically different. Antibodies and a variety of pharmacological tools can help to discriminate between the subtypes. Various Conus and spider toxins specifically block different VGSC subtypes, while tetrodotoxin (TTX) is traditionally used for differentiating TTX-sensitive (TTX-s) and TTX-resistant (TTX-r) subsets of VGSCs [9] (Table **1**).

As shown in Fig. (**2**), there is a substantial sequence identity among the various alpha subunit proteins of VGSCs [8, 9, 13, 15]. According to the Entrez protein database the homology/identity is more than 70/80 % among TTX-s sodium channels, but it is above 60 % even between Na_V 1.7 and the TTX-r Na_V 1.8.

Nax represents another, less-characterized subfamily of sodium channel proteins, with about 50 % sequence homology to the Na_V 1 family [12]. Nax proteins are unlikely to act as VGSCs. According to recent data they may be involved in osmoregulation and salt intake behavior [20].

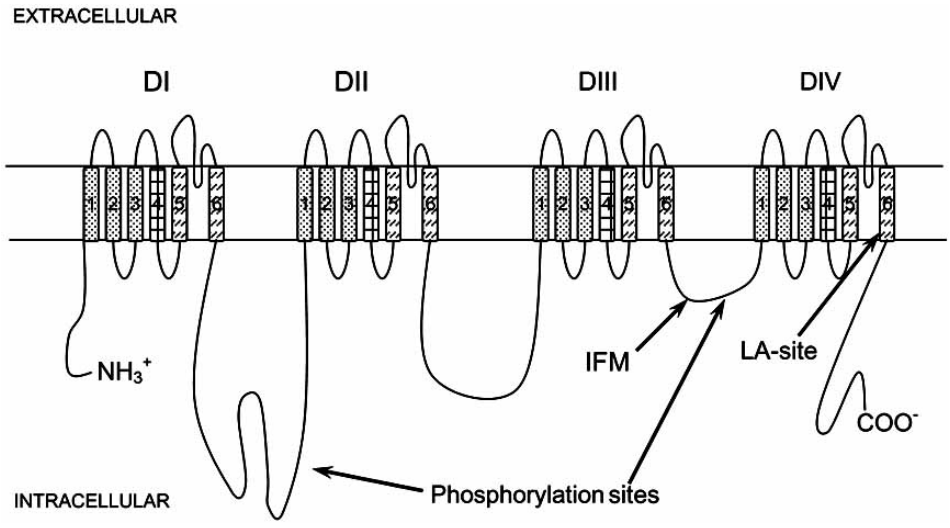


Fig. (1). Schematic structure of the alpha subunit of voltage gated sodium channels. The protein (260 kDa) contains four homologue domains (DI-DIV), each consisting of six transmembrane helices (S1-S6) and a hairpin like P loop between helices S5 and S6. The pore of the channel is formed by helices S5 and S6 and the P loops, while the voltage sensor of the channel builds up from the positively charged S4 helices. Arrows mark the most important sites on the molecule regarding function or pharmacology of the channel. The so called IFM motif has an important role in the fast inactivation process. Activation-related conformational changes cause binding of the IFM motif to a site formed by amino acid residues located mainly on DIVS6. The IFM binding site largely overlaps with the LA site, which binds all therapeutically used, state dependently acting sodium channel blockers. Phosphorylation sites for PKA and PKC, involved in the regulation of channel functions, are also marked by arrows.

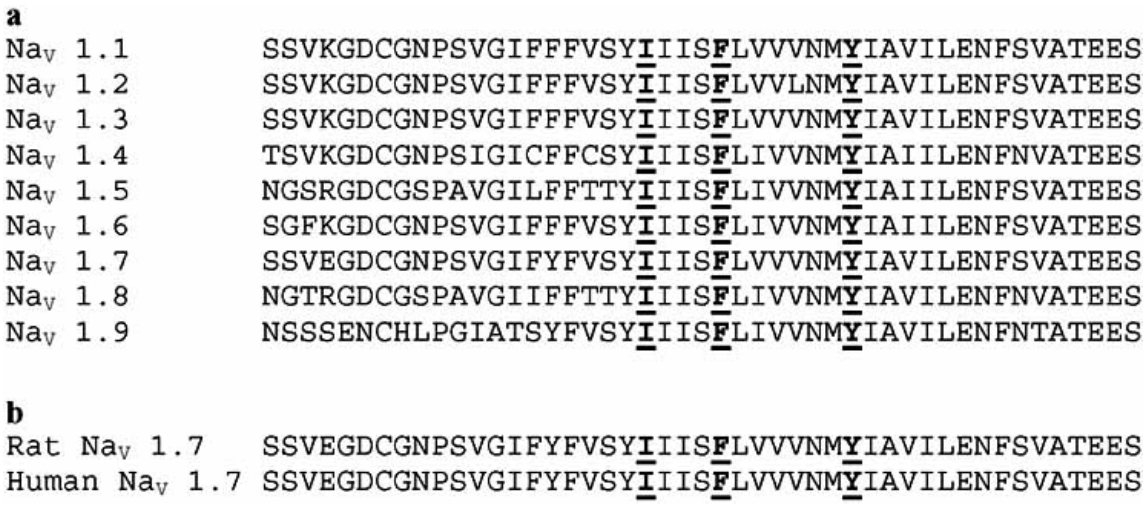


Fig. (2). Multiple sequence alignment among alpha subunits at the region of the local anesthetic binding site of various human VGSC alpha subunits (**a**), and between rat and human Na_V 1.7 alpha subunits (**b**). Bold and underlined letters show residues that are part of the ligand binding site according to protein mutation data.

Table 1.

compound	selectivity	ref
GIIIA/B	for rat Na_V 1.4 against human Na_V 1.4	[49]
μCTX	for Na_V 1.4 against brain type sodium channels	[50]
PIIIA	for Na_V 1.4 against brain type sodium channels	[51]
δCTX	for brain type sodium channels against Na_V 1.4	[9]
PaurTx3	for Na_V 1.2	[52]
CcoTx3	for Na_V 1.5	[52]
ambroxol	about 3 times for TTX-R over TTX-S sodium channels	[53]
ralfinamide	about 2 times for TTX-R over TTX-S sodium channels	[54]
VX-409	not known	dailydrugnews.com (Daily Essentials) February 7, 2006
A-803467	300-1000 selectivity for Na_V 1.8 over Na_V 1.2; Na_V 1.5; Na_V 1.7	[55]

The diversity of sodium channels is further increased by alternative splicing, although the functional significance of different splice variants is not yet understood [9].

PHARMACOLOGICAL MODULATION OF VGSCs

Several toxin binding sites on the alpha subunit of VGSCs have been identified [21, 22], and on binding to the different sites, toxins cause well-characterized changes in the channel function. Notably, TTX binds to site-1, and prevents ion flow by occluding the outer opening of the pore. Batrachotoxin (BTX), veratridine, or aconitine, binding to toxin site-2, prevents inactivation and causes permanent channel activation.

The local anesthetic (LA) site, where all clinically applied VGSC blockers are thought to bind, partially overlaps with the BTX site [23]. Since this binding area also overlaps with a site involved in inactivation (the binding site for the IFM motif), all VGSC blockers have an effect on the process of fast inactivation in that they shift the membrane potential dependence of inactivation so that the channels become inactivated at more hyperpolarized voltages. This site and its interaction with various pharmacological agents will be discussed later in greater details. It is to be noted, that some agents also affect the process(es) underlying slow inactivation [24,25], but the molecular details of this interaction are less understood.

Point mutation studies identified several amino acid residues located on DIVS6 of VGSCs that are involved in the binding [26] of therapeutic agents. As shown in Fig. (**2a**) isoleucine, phenylalanine and valine residues (at positions 1760, 1764, and 1771 respectively in Na_V 1.2), are identical

in all channel subtypes. The sequence environment of the local anesthetic binding site is also highly similar as illustrated by a multiple sequence alignment among human alpha subunit sequences (according to the Entrez protein database). Na_V 1.9 shows somewhat greater differences.

The homology among VGSCs of various mammalian species is also very high. For example, in the case of Na_V 1.7 the homologies are above 95 % between human and dog, human and rat as well as between human and mouse. The amino acid sequences of human and rat Na_V 1.7 channels in the neighborhood of the LA site are identical (Fig. **2b**).

The relatively favorable side effect profiles of practically all therapeutic agents acting on the LA site is generally attributed to their state-dependent action. For example, lamotrigine has a much higher affinity to inactivated than to resting channels, therefore its IC_{50} is several hundred times higher on hyperpolarized VGSCs than on VGSCs in depolarized membranes [27]. Furthermore, its blockade is frequency dependent, i.e. the activity of neurons firing with high-frequency is blocked by lamotrigine to a greater extent than normal, low-frequency, activity. This preferential action on over-active functional states of VGSCs [14, 28] results in a relatively high therapeutic index. State-dependency is a broad term covering (a) higher binding affinity to one of the inactivated (fast, medium-slow, slow, ultra-slow) channel states [17], (b) better access to the binding site when the channel is activated (activity dependency, or open channel blockade), or (c) an ability to promote the transition between different channel states (e.g. from open to inactive) [25].

We are just beginning to understand which features are important for an ideal VGSC blocker in terms of state dependency. The required features may be different with agents belonging to different therapeutic classes, since various neuronal hyperexcitability diseases of the CNS are characterized by distinct patterns of firing and membrane potential fluctuations of particular subsets of CNS neurons.

MODULATION OF VGSCs VIA G PROTEIN-COUPLED RECEPTORS

The activity of VGSCs can be modulated by drugs acting on G protein-coupled receptors via activation of protein kinases. Specific sites (Fig. **1**) on the alpha subunit can be phosphorylated by either PKA or PKC [29]. Phosphorylation of an identified serine residue in the intracellular loop between DIII and DIV by PKC can alter the inactivation characteristics of the channel. For example, activation of 5-HT_{2C} receptors in prefrontal cortex neurons results in a negative shift in the voltage-dependence of fast inactivation accompanied by a reduction of the peak current due to a PKC mediated phosphorylation process [18]. Such mechanisms can also be activated by other neurotransmitters, like dopamine, or acetylcholine [30, 31]. D1 receptor stimulation also results in phosphorylation of VGSC serine residues in the cytoplasmic linkers between DI and DII (S554 and S573) and DIII and DIV (S1506) by PKC. Concurrent phosphorylation by PKA seems necessary for a maximal current reduction [32]. Chen *et al*. (2006) [33] suggested an enhanced slow inactivation after phosphorylation of the sodium channel protein via either PKA or PKC. Though the effect of PKC-mediated phosphorylation is inhibitory in all TTX-s neuronal VGSCs, an increase of TTX-r currents was reported in DRG cells after stimulation of PKC [34].

The above data suggest that abnormal functioning of VGSCs in CNS diseases can be restored by drugs modulating G protein-coupled receptors. Since VGSC blockers prefer the inactivated state of the channel, an alteration of the inactivation of VGSCs via G protein-coupled receptor-mediated phosphorylation may result in an increased efficacy of these state-dependently acting agents. This raises the possibility that combination of a mono-aminergic agonist with a VGSC blocker or a compound with dual activity on both targets may be more efficacious, than pure VGSC blockers. This possibility will be discussed in a later section of this review.

SIDE EFFECTS OF VGSC BLOCKING DRUGS

A complete blockade of sodium channels is incompatible with life, and even moderate inhibition of VGSCs can seriously impair vital (e.g. cardiac or CNS) functions.

Some adverse effects of VGSC blocking CNS drugs may, however, be the consequence of off-target effects. Since the presently available agents have not been optimized for high affinity for VGSCs, high doses are needed to reach the required therapeutic effect. Such dosing increases the chances of actions through targets unrelated to sodium channels. Important examples are hepatotoxicity caused by carbamazepine [35], gingival hyperplasia by phenytoin [36] and rush by lamotrigine [37].

VGSCs are genetically related to other voltage gated channel proteins, and the structure of the LA site is homologous to certain drug binding sites on calcium and potassium channels [38]. This implies there is an additional side effect risk when using VGSC blocking drugs. Importantly, many of them inhibit hERG potassium channels, which can cause serious cardiac arrhythmias. Therefore, early testing of newly synthesized compounds for hERG activity is obligatory in VGSC blocker projects [39].

Unintended blockade of peripheral VGSCs by CNS active compounds can also cause adverse effects, such as arrhythmia, or blood pressure decrease. Obviously, these potential side effects can be decreased by improving the brain penetration of the compounds. However, in the case of VGSC blockers used in the treatment of chronic pain a peripheral component of action (i.e. blockade of the activity of afferent fibers and peripheral sensitization) may be desirable [40, 41]. The recently recognized role of VGSCs in the immune system [42] suggests that blockers may cause adverse effects by inhibiting immune functions. However, no such side effects have been reported.

Due to the ubiquitous expression and widespread physiological role of VGSCs in the CNS, therapeutic doses may be responsible for CNS side effects also. The most important among them include ataxia, impaired motor coordination, muscle weakness, cognition impairment, and psychiatric disorders, etc [21]. Interestingly, many VGSC blockers induce seizures at high doses [43]. This effect may be mediated by potassium channels [44].

Side effect risks can be potentially decreased by designing agents that selectively act on the particular subtype(s) of VGSC involved in individual disease processes, but subtype selective agents may still have serious side effects due to the multiple physiological roles of most sodium channel subtypes [19]. Knocking out the widely expressed Na_V 1.2 [45] as well as Na_V 1.7 [46] results in lethality. VGSC type 1.8 has the most restricted localization, but its full inhibition can still cause side effects. Thus, Matthews *et al*. [47] reported deficits in the mechanical perception in Na_V 1.8 knock out mice. On the other hand, eliminating affinity for heart-type sodium channels (Na_V 1.5) is obviously a favorable feature of a CNS agent.

We suggest that developing VGSC blockers with subtype selectivity and enhanced state-dependent action could give the best results in terms of a low side effect profile.

SUBTYPE SELECTIVE VGSC BLOCKERS

The heterogeneity of VGSCs apparently offers the possibility of developing subtype selective pharmaceutical agents that only block the VGSCs specifically involved in the disease to be treated. The fact that various natural toxins can block only a subset of sodium channels [9, 48] indicates that it is, in principle, possible to design such blockers (Table **1**) [49-55].

Examples where this rationale may work exist in the area of chronic pain. Thus, Na_V 1.7, 1.8 and 1.9, characteristically expressed in small dorsal root ganglion (DRG) cells, have been shown to be involved in pain sensation and chronic pain states [56].

Several lines of experimental evidence indicate the possible therapeutic utility of Na_V 1.7 VGSC blockers in inflammatory pain. Increased expression of this TTX-s channel have been reported in experimental pain states evoked by injection of carrageenan [57] or complete Freund's adjuvant [58]. Furthermore, the COX-2 inhibitor ibuprofen, which effectively blocked inflammatory pain, also reversed this up-regulation. By knocking out Na_V 1.7 genes in DRG cells, Nassar found reduced or abolished inflammatory hyperalgesia [46]. Thus specific blockade of Na_V 1.7 seems to be a promising approach for the treatment of inflammatory pain. Unfortunately, no specific blockers have been available up to now, so the real proof of this concept is missing. Furthermore, Na_V 1.7 is also expressed in the intestines and sympathetic ganglion cells [19,59], which may raise some safety concerns.

Other VGSC subtypes that can serve as drug targets for the treatment of pain states are the TTX-r sodium channels Na_V 1.8 and 1.9, which is exclusively and predominantly located on DRG cells respectively. Results show that Na_V 1.8 is a necessary mediator of NGF-induced thermal hyperalgesia [60], and also has an important role in PGE2 induced hyperalgesia [61]. Intrathecal administration of antisense oligodeoxynucleotides against this channel partially prevented hyperalgesia associated with neuropathic pain [62]. Nevertheless a decreased expression of Na_V 1.8 (as well as 1.6) was observed in DRG cell bodies of rats with diabetic neuropathy [63]. This latter observation may be explained by a re-distribution of the channel protein from the cell body to the neuronal processes in pain states [64]. On the contrary, an up-regulation of Na_V 1.9 was found [64] in the same model. Other investigators suggest a major role of Na_V 1.9 in inflammatory pain states [65]. Furthermore, Na_V 1.3, which is expressed at high level in developing neurons, has been also shown to participate in the maintenance of various chronic pain syndromes at the levels of DRG and spinal cord as well as at higher brain centers [66-68].

These changes in expression indicate specific roles for a subset of sodium channels in various chronic pain states, which may serve as the basis for developing subtype-specific therapies. New data, however, suggest that practically all these channels are expressed also in neurons of higher brain areas, at least at the mRNA level [19]. Thus the concept needs further verification.

Mutation of specific sodium channel genes has been implicated in several other CNS disorders, such as erythermalgia, where mutation of Na_V 1.7 causes a severe burning pain in the extremities, or familial generalized epilepsy with febrile seizures, where mutant brain-type sodium channels (Na_V 1.1 and 1.2) are involved in the development of epileptic seizures [69,70]. Application of VGSC blockers selective for the mutant channel may be useful tools to control the symptoms of these channelopathies.

Finally, subunit-specific changes in VGSC expression have been observed in multiple sclerosis, ataxia and other cerebellar symptoms [71], and also in certain forms of epilepsies [72]. Subtype-specific channel blockade may be therapeutically effective as a symptomatic treatment in these conditions.

Due to the high degree of amino acid sequence homology among the alpha subunits of various subtypes of VGSC, especially around the LA site (see above), it is not an easy task to find subtype selective blockers. Thus, only a few such agents have been reported to be in active development. Ambroxol [53] and ralfinamide (NW-1029) [54] have higher affinity for TTX-r VGSCs, than to TTX-s channels. The selectivity of these drugs (Table **1**), however, is not really high, and a marginal subtype selectivity demonstrated under *in vitro* conditions does not necessarily have therapeutic significance. The enigmatic compound, VX-409, which is being developed by Vertex, in collaboration with Glaxo for pain indications, has been reported as a "subtype selective sodium channel modulator" in a company report (Vertex Pharmaceuticals Press Release 2006, February 07; www.integrity.prous.com), but no information is available about which subtype is targeted is available. A-803467, being developed by Abbott and Icagen (pain indication), has high selectivity for Na_V 1.8 [55] over other subtypes of VGSC (Table **1**), however, and may thus be a useful tool in preclinical proof of concept studies.

The development of agents selectively acting on specific subtypes of VGSCs remains a promising possibility and several drug companies have been conducting such programs, as illustrated by the high number of patents submitted over the last few years (see later). Some compounds selectively inhibiting Na_V 1.2 are under development by Gruenenthal (for analgesia; Drug Data Rep 2001, 23(3): 221; www.integrity.prous.com) and CoCensys (for epilepsy and

analgesia) [73]. Other subtype-selective blockers of Vertex, being developed against pain, act on Na_V 1.3, 1.8 and Ca_V 2.2. Merck has several Na_V 1.7 blockers in the preclinical phase, again with the analgesia indication [39, 74], just like Glaxo's selective Na_V 1.8 blocker, GW-286103 [8]. Patents of Icagen also claim Na_V 1.8 blockers as analgesic drugs, while Ionix is developing selective Na_V 1.8 blockers against inflammatory bowel disease and multiple sclerosis. Pfizer, Vernalis as well as Vertex also have subtype (Na_V 1.8) selective VGSC blocking compounds targeted as analgesics.

OPTIMIZATION OF COMPOUNDS FOR STATE-DEPENDENT BLOCKADE

In a review paper Clare *et al*. [8] provided a comprehensive structure-activity relationship study on a great number of structural relatives of the anticonvulsant drug lamotrigine. They identified compounds that displayed therapeutic selectivity. Compounds were found that had strong antiepileptic effect, but negligible analgesic effect (such as GW273293), or selective analgesics, such as GW286103, lacking anticonvulsant activity. Since these differential effects were not associated with specific VGSC subtypes, their molecular basis is not known.

Some authors emphasize that it may be possible to improve therapeutic efficacy, and in particular therapeutic index by designing broad-spectrum Na^+ channel blockers with higher potency and a greater level of state-dependence, i.e "optimizing the compounds for biophysical parameters, as opposed to subtype selectivity" [14]. Ilyin *et al.* (2006) reported the synthesis and pharmacological actions of PPPA (2-[4-(4-chloro-2-fluorophenoxy)phenyl]-pyrimidine-4-carboxamide), a novel analogue of the older Pardue Pharma's compound, V102862. Compared with carbamazepine and lamotrigine, PPPA showed much higher efficacy, faster onset kinetics and higher dependence on channel state, i.e. higher preference to the inactivated state. It was equally effective in recombinant Na_V 1.2 and native, TTX-r sodium channels of DRG cells. At low oral doses, it was effective in neuropathic-, inflammatory and post-operative pain models, but did not cause motor impairment up to 30 mg/kg, p.o., showing that enhancing the state dependency of VGSC blocking action is a feasible way to widen the therapeutic window.

According to the "modulated receptor theory" [75], preferential binding to one (or more) of the inactivated channel states is regarded as the basis of state-dependent action (therefore low side effect profile) of VGSC blockers. On the contrary, Mike *et al*. (2004) emphasize that state dependency can be achieved by other mechanisms [25]. They showed that GBR 12909 has a pronounced state/ frequency dependent action on sodium channels in spite of similar binding affinities to the resting and inactivated states. These authors also emphasize the significance of differences between the actions on various (slow, medium, or fast) inactivation states [24,25,76].

Advanced voltage clamp techniques, using carefully designed voltage protocols, allow a detailed analysis of VGSC blockers on different parameters, such as affinities to the inactivated and resting states, frequency-dependency, binding and unbinding kinetics, effect on the voltage- and time dependence of inactivation, effect on the recovery from inactivation, etc. [14,76]. Although abnormally increased activity of VGSCs is a common feature of all neuronal hyperexcitability-associated CNS disorders, the fine characteristics of abnormal functioning of VGSCs can be different in various diseases.

Thus, it is probable that VGSC blockers belonging to different therapeutic categories need to meet different requirements concerning the above mentioned electrophysiological parameters, or "biophysical properties". It is important to understand these subtle differences in order to design drugs optimized for the treatment of specific CNS diseases. One way to develop the necessary knowledge is to study the differences between the VGSC blocking mechanisms of representative members of each therapeutic category.

COMPOUNDS ACTING ON SITES OTHER THAN THE LA SITE

The potent sodium channel blocker TTX, which acts on neurotoxin site-1, is under active development (tectin; Phase II/III) for cancer pain by Wex Pharmaceuticals. Wex and Esteve also have tectin in Phase II trials for opiate dependence. Preclinical data suggest that it has analgesic effects in models of inflammatory and neuropathic pain at low systemic doses that caused no adverse events [77]. These findings indicate that a considerable part of pain-related VGSC functions involve TTX-s channels.

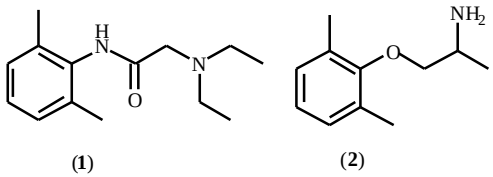
Certain mu, as well as delta conotoxins (Table **1**) can block VGSCs in a subtype-selective manner. Some preclinical data suggest the usefulness of mu conotoxins in chronic pain syndromes [78]. Among these toxins there are specific blockers both for TTX sensitive and resistant VGSCs [9, 79, 80]. The scorpion toxin OD1, for example, is a selective modulator of Na_V 1.7 [48]. These compounds could serve as starting points for programs to develop subtype selective VGSC blocking agents for different clinical conditions.

The possibility of modulating sodium channel functions by drugs acting on one of the beta subunits of sodium channels has not been studied extensively so far. Beta subunits of VGSC also show distinct patterns of expression, and may be responsible for specific physiological and pathological functions in the CNS [81,82]. They can affect channel activation and inactivation through modulation of the alpha subunits' function [83]. Furthermore, beta 2 has a regulatory role in the expression of alpha subunits. Thus beta subunits can be regarded as potential drug targets for similar indications as classical VGSC blocking drugs. Some experimental data call attention to the up-regulation of beta 2 and 3 subunits in chronic pain, and suggest their importance in the development of chronic pain syndromes [84,85]. Furthermore, recent results demonstrate a role of beta 4 subunits in the pathophysiology of Huntington's disease [86]. Nevertheless, no VGSC blocker targeting a beta subunit is under development at present.

THE USE OF VGSC BLOCKERS IN VARIOUS CNS THERAPEUTIC FIELDS

The local anesthetic lidocaine (**1**) is the prototype of VGSC blockers. Several derivatives of lidocaine, such as

mexiletine (**2**) are used mostly in non-CNS therapeutic fields (Fig. **3**).



(**1**) (**2**)

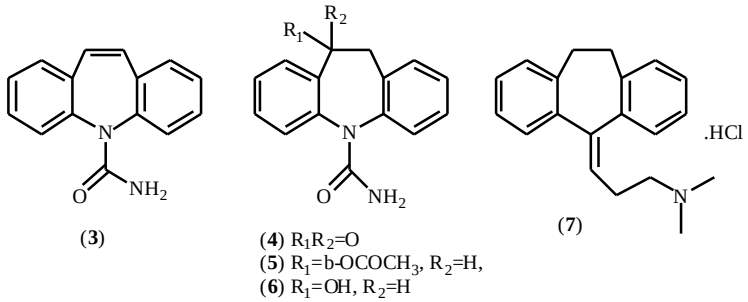
Fig. (3). Lidocaine (**1**) and mexiletine (**2**).

EPILEPSY

Abnormally enhanced excitation of cortical neuronal circuits during epileptic seizures, characterized by paroxysmal depolarization shifts and high frequency bursts of neurons, is (among others) a consequence of over-activation of sodium channels. Persistent sodium currents can be recorded from the neurons affected, like during hypoxic conditions [1,3]. VGSC blockers are highly effective under these conditions, and epilepsy is a traditional therapeutic indication for state-dependently acting VGSC blocking drugs (see [8] for review) (Figs. **4** and **5**). One of the most widely used antiepileptic agents, lamotrigine (**8**), is useful against partial and secondarily generalized seizures as well as generalized seizures. Notably, it is suitable for controlling absence seizures of childhood absence epilepsy and generalized seizures of juvenile myoclonic epilepsy [87]. Carbamazepine (**3**) and phenytoin, as well as their novel derivatives, oxcarbazepine (**4**) and fosphenytoin (3-(hydroxymethyl)-5,5-diphenylhydantoin phosphate ester) proved to be useful in the therapy of epilepsies [88]. Novel analogues of oxcarbazepine (**4**), eslicarbazepine (**6**) and eslicarbazepine acetate (BIA 2-093; **5**) are under clinical trial (Phase III) in epilepsy patients [89, 90].

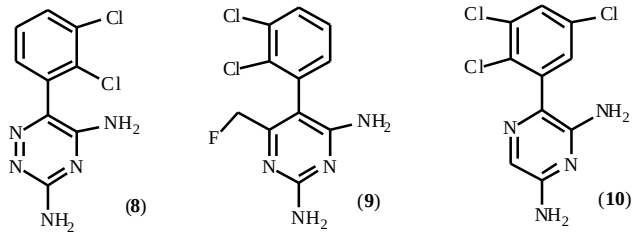
Topiramate (**12**), is especially suitable for the treatment of complex partial seizures [91], as it has combined sodium channel blocking, AMPA receptor antagonist and GABA receptor agonist actions [92]. Zonisamide (**11**) (Fig. **6**) is a novel sodium channel blocking and T-type calcium channel blocking agent launched for the oral treatment of epilepsy. Rufinamide (**13**) is an antiepileptic drug candidate of Eisai awaiting registration in the USA and the EU. It is recommended as an adjunctive therapy in the oral treatment of seizures associated with Lennox-Gastaut syndrome in children and with partial-onset seizures in adult patients.

In spite of the availability of a great number of antiepileptic drugs, a high proportion of epilepsy patients do not respond satisfactorily to current therapies [90]. Thus



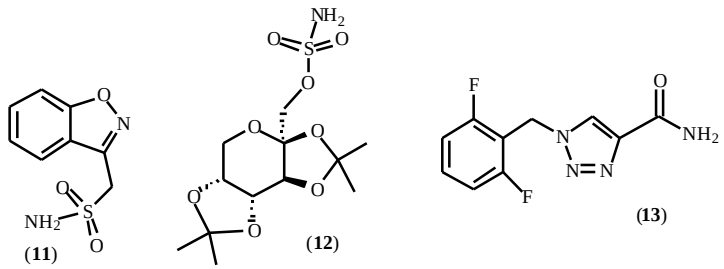
(**3**) (**4**) R_1R_2=O
(**5**) R_1=b-$OCOCH_3$, R_2=H,
(**6**) R_1=OH, R_2=H (**7**)

Fig. (4). Carbamazepine (**3**), and related structures; oxcarbazepine (**4**); eslicarbazepine acetate (**5**), licarbazepine (**6**), and amitriptyline (**7**).



(**8**) (**9**) (**10**)

Fig. (5). Lamotrigine (**8**) and derivatives; BW-4030W92 (**9**) and GW-273293 (**10**).



(**11**) (**12**) (**13**)

Fig. (6). Zonisamide (**11**), topiramate (**12**) and rufinamide (**13**).

there is a need for improved antiepileptic agents. Several VGSC blocker drug candidates are under active clinical development for the improved treatment of epileptic disorders. Among others, safinamide mesylate, a mixed sodium and calcium channel blocker, dopamine transporter and MAO B inhibitor, is in Phase III, while BW-534U87 (**18**) and the lamotrigine derivative GW-273293 (**10**) are in Phase II clinical investigations for epilepsy. A VGSC blocking action (among others) plays a role in the antiepileptic action of felbamate (2-phenyl-1,3-propanediol dicarbamate). Novel analogues of felbamate (fluoro-felbamate and RWJ-333369) are also being tested as drug candidates [89]. It is not clear to what extent the VGSC blocking action of these non-specific agents contribute to their clinical actions.

PAIN

Several lines of evidence indicate the crucial involvement of VGSCs in the development of pathological pain [9,93]. Over-expression of distinct subtypes of VGSCs contribute to the hyperexcitability of sensory primary afferents and higher-order sensory neurons in chronic inflammatory, and neuropathic pain states [9,93-96]. Several old (lidocaine (**1**), mexiletine (**2**), carbamazepine (**3**), phenytoin, lamotrigine (**8**)) and newly developed (oxcarbazepine (**4**), crobenetine (**21**), ralfinamide (**24**)) sodium channel blockers have proved to be effective in the treatment of various types of chronic pain in animal models [97, 98], and some of them are used clinically also [8, 95]. All of the clinical agents, however, have other diseases as their primary indication. Some clinical studies with well-established antiepileptic drugs are in progress. For example, lamotrigine is in Phase III for the treatment of postherpetic neuralgia and neuropathic pain, and Phase II/III studies are under way to evaluate TTX's use for the treatment of chronic cancer pain. Zonisamide (**11**) (Fig. **6**) and TTX have also been studied for the treatment of neuropathic pain.

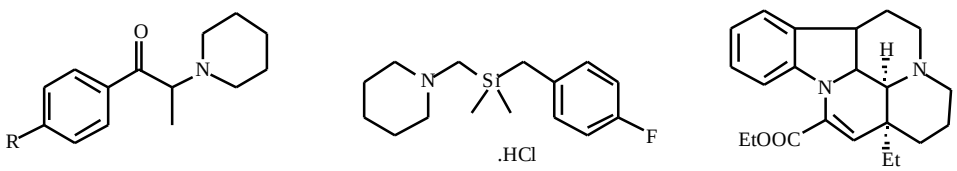
Significant efforts have been made by drug companies to identify new VGSC blocking agents with improved analgesic activity and fewer dose-limiting side effects (see [9] for review). Presently, available agents are those that are also used to treat epilepsy, and sometimes other diseases, and have not been optimized for chronic pain indications. Such an optimization can be based on the specific involvement of certain VGSC subtypes in pathological pain, or on the subtle details of the biophysical interaction between the drug and the channel (see the corresponding paragraphs). Pardue Pharma used the second approach to develop V102862 (4-(4-fluorophenoxy)benzaldehyde semicarbazone), and its novel derivatives, such as PPPA [14,99,100], which possess binding affinities in the order of ten-nanomolar, improved state-dependence of action, and good *in vivo* analgesic effects, accompanied by a low side effect profile. PPPA is a promising drug candidate for neuropathic, inflammatory and post-surgical pain.

Migraine, which is frequently associated with severe headache, is also a target for VGSC blocker drugs [101, 102]. Topiramate (**12**) (Fig. **6**) is being commercialized for the prophylaxis of migraine headache, and lamotrigine has been reported effective in the treatment of migraine auras, isolated auras and to some extent migraine headaches [4]. Sodium channels have been implicated in the phenomenon of cortical spreading depression, which is thought to be responsible for the visual symptoms associated with migraine attacks [4]. Available evidence also suggests the efficacy of VGSC blocking drugs in the prophylaxis of primary headache [103]. Thus, lamotrigine (**8**) was effective in some cases of short-lasting unilateral neuralgiform headache attacks [104]. Eisai is conducting Phase II trials with zonisamide (**11**) for the treatment of migraine and fibromyalgia, a chronic, widespread, musculoskeletal pain, combined with muscle stiffness, soft tissue tenderness and additional neuropsychological symptoms [105]. Amitriptyline (**7**), a sodium channel blocker and reuptake inhibitor of norepinephrine and serotonin, is already in Phase III trials for the treatment of fibromyalgia. Two further compounds, ralfinamide (**24**) (Fig. **6**) and BW-4030W92 (**9**) (Fig. **5**), are in Pase II trials in patients with neuropathic pain.

SPASTICITY AND TREMOR

Spasticity disorders are characterized by hyperexcitability of spinal motoneurons, which may be due to a weakened central inhibitory, or an enhanced excitatory control over segmental neuronal circuits in the spinal cord [2,106]. Tolperisone (**14**) (Fig. **7**) and eperisone (**15**) have long been used in the treatment of muscle spasticities of neurological origin, and painful muscle spasms due to rheumatologic conditions.

Like other centrally acting muscle relaxants, these agents inhibit segmental spinal reflex transmission [107, 108]. Ono and co-workers [107] implicated a lidocaine-like membrane stabilizing action in their pharmacological effects. This assumption was further supported by the molecular modeling studies of Fels [109], who concluded that the structural features of tolperisone and lidocaine allow action through a common binding site. Since then several lines of indirect- and direct evidence have been published supporting the view that tolperisone has a state-dependent blocking action on VGSCs [108, 110]. In fact, several sodium channel blocking agents, used in a variety of therapeutic fields, have been shown to inhibit spinal reflexes in a simple *in vitro* model



R=Me (**14**)
R=Et (**15**)

(**16**)

(**17**)

Fig. (7). Tolperisone (**14**), eperisone (**15**) silperisone (**16**) and vinpocetine (**17**).

[111]. Thus spasticity disorders represent a potential therapeutic target for new VGSC blocking drugs, in addition to other neuromuscular syndromes, such as certain types of dyskinesias [102].

In a rat model, where spasticity is evoked by chronic injury of the sacral spinal cord, Li *et al*. (2004) [106] demonstrated that persistent sodium and calcium currents underlie abnormal plateau potentials, which may be responsible for motoneuronal hyperexcitability. VGSC blockers may alleviate this state by either a postsynaptic action, or by a presynaptic inhibition of transmitter (glutamate) release from nerve terminals. Silperisone (**16**) (Fig. **7**), a silicon-containing analogue of tolperisone, which has improved muscle relaxant action in *in vitro* and *in vivo* models, is equipotent with tolperisone on sodium channels, but has a more pronounced action on calcium channels [108], emphasizing the importance of additional effects on calcium channels for muscle relaxant agents.

Tolperisone-like VGSC blockers and other centrally acting muscle relaxants also inhibit drug-induced tremor in animal experiments [112]. Phase II trials with zonisamide (**11**) are under way for the treatment of essential tremor.

ISCHEMIC NEURONAL DAMAGE AND NEURODEGENERATIVE DISEASES

Sustained over-excitation of neurons occurs during brain hypoxia, resulting in cell death in hippocampal and neocortical areas, and abnormal functioning of VGSCs play a significant role in ischemic neuronal damage [1,10]. In spite of huge research efforts and the promising neuroprotective action of several drug candidates in animal experiments, there is no suitable pharmacotherapy of stroke at present. Many putative anti-stroke agents, with different modes of action, have failed in recent clinical trials [113]. Among VGSC blockers sipatrigine, a novel derivative of lamotrigine, was tested in stroke patients (Phase II) a few years ago, but no development has been reported during the last couple of years. Enecadin-HCl (4-(4-fluorophenyl)-2-methyl-6-[5-(1-piperidinyl)pentyloxy]pyrimidine.HCl) is a mixed calcium and sodium channel blocker, and nitric oxide synthase inhibitor, which is currently undergoing Phase I clinical trials for the treatment of acute ischemic stroke.

The neuroprotective agent vinpocetine (**17**) (Fig. **7**) has long been used for the treatment of vascular dementias, and an action on sodium channels may contribute to its pharmacological effects [114].

Sodium channel blocking drugs may be suitable for treating some chronic neurodegenerative diseases. Antiepileptics are efficient in animal models of such disorders [115, 116], and riluzole (**19**) (Fig. **8**) can be safely used in patients with amyotrophic lateral sclerosis (ALS) [117]. Lamotrigine (**8**), while ineffective in ALS [118], is in Phase II trials for the treatment of secondary progressive multiple sclerosis. Safinamide (**23**) improved motor functions in patients with Parkinson's disease [119], and zonisamide (**11**) is before registration for this indication in Japan. Riluzole (**19**) is also reported to slow down the progression of the abnormalities in an animal model of Parkinson's disease [120], and is in Phase III trials for the treatment of Huntington's disease.

Phenytoin was found neuroprotective in an animal model of glaucoma [121]. Nerispirdine HCl (**20**) (Fig. **8**), an acetylcholine release enhancer and dual ion channel blocker (sodium, potassium), is currently in Phase II clinical trials for the treatment of chronic spinal cord injury.

PSYCHIATRIC INDICATIONS

VGSC modulating effect may play a role in the antidepressant effects of tricyclic antidepressants drugs, such as amitriptyline [122] (**7**) and desipramine, and of the selective serotonine reuptake inhibitor (SSRI) fluoxetine [76]. Moreover, several lines of evidence indicate the utility of antiepileptic agents in bipolar depression [123,124]. Lamotrigine (**8**) has recently been approved for the maintenance treatment of bipolar patients, and has a well-documented "mood stabilizing effect" [125]; i.e. it reduces the rate of cycling between manic and depressive states. It has proved to be safe and well tolerated [126]. Lamotrigine (**8**) was also effective as an adjunctive therapy to SSRI in the treatment resistant unipolar depression [127].

Eslicarbazepine (**5**), and licarbazepine (**6**) are in Phase II and Phase III trials respectively for the treatment of bipolar disorder. Riluzole (**19**) is also in Phase II for the same indication. Furthermore, the latter drug is also being tested in pediatric patients with obsessive-compulsive disorder.

VGSC blocker drugs are also reported to have anxiolytic effects in an animal model of anxiety [128].

In addition to several other pharmacological activities, a common feature of topiramate (**12**) and carbamazepine (**3**) is their sodium channel blocking action [129]. The former drug is under Phase III trials for obsessive-compulsive disorder as adjunctive therapy. Carbamazepine (**3**) was launched for the treatment of psychosis, including the prophylaxis of manic-depressive psychosis unresponsive to lithium and as an adjunct to antipsychosis therapy for the symptomatic treatment of acute phase schizophrenia, as well as for the

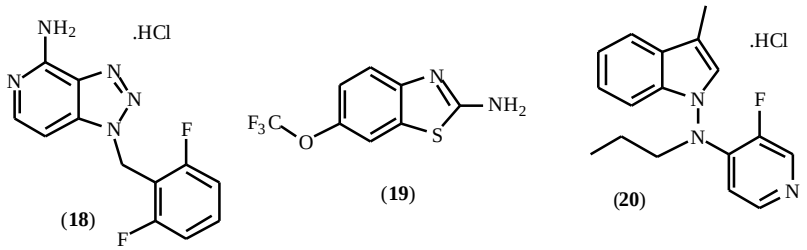


Fig. (8). BW-534U87 (**18**), riluzole (**19**), and nerispirdine (**20**).

treatment of acute manic and mixed episodes associated with bipolar disorder. The significance of the VGSC blocking action of topiramate (**12**) and carbamazepine (**3**) in these actions remains to be determined.

OTHER INDICATIONS

Lamotrigine (**8**) is marginally effective in tinnitus [130], and shows some effect in obesity [131]. TTX (tectin) is in Phase II for the reduction of withdrawal symptoms in opiate-dependent patients who are receiving methadone. Phase II trials are under way for the treatment of obesity by zonisamide (**11**). Safinamide (**23**) is in Phase II for the treatment of restless legs syndrome, while topiramate (**12**) is being tried (Phase III) against pathological gambling, and to improve the symptoms of Tourette syndrome.

METHODS FOR TESTING VGSC BLOCKERS

Until recently the main method used for establishing structure-activity relationships among newly synthesized VGSC blocking compounds was the [^{3}H]-BTX binding assay, in which [^{3}H]-batrachotoxinin A 20-α-benzoate is applied as radioligand [132]. Unfortunately, this assay has limited predictive value concerning *in vivo* efficacy, supplies little information about the state dependence of action, and is unable to identify VGSC blockers that do not displace BTX. Thus functional assays, such as $^{22}Na^+$ uptake [133] or [^{14}C]-guanidine uptake [134] were introduced to replace or complement [^{3}H]-BTX binding. These methods use veratridine to activate sodium channels and to prevent their inactivation. Veratridine toxicity measurement [135] is another traditional functional method, which, in contrast with those above, does not produce radioactive waste. However, a long incubation is needed, and veratridine can alter the physiological and pharmacological properties of the channel [136], leading to false conclusions.

Electrophysiology (whole-cell current measurement) can supply reliable and relevant information regarding the fine characteristics of the interaction of compounds with VGSCs, such as binding and unbinding kinetics, membrane potential- or use-dependence of blocking potency, effect on the time- and voltage-dependence of activation and inactivation, on recovery from inactivation, etc. It applies voltage (i.e. natural) stimulation, and drug effects can be tested, under well-defined conditions, either on native channels, or on recombinant human channels expressed in heterologous systems. However, traditional whole-cell voltage clamping is extremely time and labor consuming and is unsuitable for testing large numbers of compounds. It is more adequate for detailed characterization of selected compounds.

Regarding throughput, fluorometric measurement of membrane potential, or intracellular ion concentrations offer a much better solution for primary drug testing. Advanced fluorescent dyes and computerized plate-reader systems are now affordable even for smaller laboratories, and make these assays ideal for the rapid evaluation of subtype selectivity on recombinant channels. With fluorescence resonance energy transfer (FRET) dyes the time course of membrane changes can be monitored with reasonable time resolution. Again, the drawback of these fluorescent methods is that they usually do not supply information about the state-dependence of drug action and (usually) use veratridine to open channels. A recent paper [137] reports that the potency of state-dependent blockers is considerably higher if they are applied when the cells are already depolarized by veratridine compared with the situation when the activator is added following pre-incubation with the drug. No similar difference between the potencies of state-independently acting compounds (such as GBR 12909; 1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-(3-phenylpropyl)piperazine) was found. Thus minor changes in the assay protocol can make the assay suitable for supplying information about the state-dependence of action of compounds. Using electrical field stimulation to activate channels in a fluorescent plate reader assay, combined with computerized measurements using high-speed FRET dyes, may offer an ideal solution for studying state-dependent VGSC blocking action. Introduction of such a system was reported very recently [138].

Another approach to address the above issue is the development of automated patch clamp methods, suitable for testing compounds with reasonable throughput (even though they cannot compete with fluorescent plate reader assays in this regard). Using planar electrodes, i.e. small holes (with a diameter of approximately 1 μm) on a smooth surface, the complicated procedure of obtaining whole-cell recordings can be fully automated. A suspension of cells is pipetted above the holes, and negative pressure is applied to grab the cells and make the seal. Patches can be ruptured by pressure pulses, or perforated by applying ionophores, such as amphotericin, or nystatin. The IonWorks system from Molecular Devices [139] allows the collection of 3000 data points a day, which is a great advance over conventional systems. Another system of Molecular Devices is PatchXpress, which offers higher-quality data at the expense of lower speed. Flyion, Nanion, and Sophion also offer automated patch clamp systems with somewhat lower throughput compared with IonWorks.

The reliability of dose-response data can be greatly improved by the application of advanced drug delivery systems such as that offered by Cellectricon's Dynaflow, which (like the automated systems mentioned above) uses dissociated cells. The Dynaflow recording chamber contains parallel channels (8, 16 or 48) from which the test drugs at different concentrations are flowing out continuously. Normal patch electrodes and conventional amplifiers are used for recording. After establishing the whole-cell configuration manually, under microscopic control, the cell is lifted up from the bottom of the chamber, and moved by a computer program-controlled motorized system before the orifices of parallely aligned drug delivery channels, with preset sequence and speed.

Until recently, cellular studies related to VGSCs were performed mostly on dissociated neuronal cells, striated muscle cells or heart muscle cells. These cells generally express only a limited subset of sodium channel subunits, and can be used for studying the channel subtype-selectivity of drugs. The selectivity of the cellular test systems can be further improved by applying specific blockers (e.g. TTX), or applying appropriately chosen voltage protocols to isolate currents. E.g. currents mediated by Na_V 1.8 can be reasonably isolated from those mediated by Na_V 1.9 on the basis of their different inactivation and activation properties

[140]. Being large molecules (Mw around 260 kDa) VGSC alpha subunits are relatively difficult to clone and express stably in heterologous systems. The brain-type sodium channel Na_V 1.2a (recombinant rat brain type IIA, expressed in CHO cells) was the first to be used characterizing the pharmacological effects of CNS active drugs [141], but currently several human recombinant channels expressed in heterologous cells are available commercially. Selectivity screening of the VGSC blockers is thus now possible in cellular assays. Most of the channel subtypes can be expressed functionally in heterologous systems, with the notable exception of Na_V 1.9 [140], which could not be activated by voltage drops when expressed in HEK-293 cells. Using cellular systems expressing only the alpha subunit it is important to keep in mind that compounds may potentially act on the beta subunits as well, and these systems are unsuitable for identifying such compounds.

Electrophysiology on more complex *in vitro* preparations is also used sometimes to assess the sodium channel blocking effect of compounds. Lidocaine-like local anesthetic effect can be characterized by measuring nerve-conduction in isolated nerves [142]. Sodium channel blockers also decrease the release of synaptic transmitters [143]. Such an effect can be easily studied by recording synaptic potentials from *in vitro* preparations, like the hemisected rat spinal cord [108].

The easiest way to test the *in vivo* action of VGSC blockers is to measure their anticonvulsant effect. The maximal electroshock seizure model in mice [144] is ideal for this purpose, and is frequently used for characterizing new compounds.

PHARMACOPHORE REQUIREMENTS OF VGSC BLOCKERS ACTING ON THE LA SITE

The structures of several VGSC blockers, such as lidocaine (**1**), suggest that binding to the LA site requires a lipophilic aromatic ring and a hydrophilic amine group (a hydrogen bond donor) separated by a spacer of about 3-angstrom length [75]. The binding pocket for the aromatic moiety of VGSC blockers is well-structured, and seems to be responsible for the voltage-dependence of binding [21]. 2,6-Dimethylphenol, a structural analogue of lidocaine's aromatic tail, blocks Na_V 1.4 in a voltage dependent manner, with an efficacy similar to that of lidocaine [145]. Several other compounds lacking the amine function are known to act via the same site, and these compounds may have less cardiovascular side effects, since this basic nitrogen is critical for hERG channel inhibitory activity [39]. The tertiary amine head of lidocaine binds to another part of the LA site, where diethylamide also binds [146]. Blockers with more complex structure may interact with additional motifs of the channel protein. Lamotrigine (**8**), phenytoin, and carbamazepine (**3**) use a common binding site [147]. The pharmacophore incorporates an aromatic ring with a hydrogen bond donor, plus a second hydrogen bond acceptor/donor unit [21]. Binding to the LA site by compounds belonging to other structural families may involve further motifs [148].

The loose definition of the LA site and the complexity of the structure of the VGSC protein make it difficult to use *in silico* approaches for designing new blockers. Using structural information from roentgen crystallographic studies on the corresponding molecular parts of potassium channels combined with molecular biology results, quite complex models of the VGSC have been constructed [149], which may greatly help drug design.

Establishing a clear structure-activity relationship for drug binding to VGSCs is hindered by the fact that the accessibility of the LA site implies special structural requirements. Different VGSC blockers may use alternative routes (hydrophilic and lipophilic pathways) to reach the LA site [75]. Some compounds contain neutral amine functions, which are protonated at the site of binding, making the situation even more complicated. Optimization of VGSC blocking compounds is further hindered by the lack of bioassays that supply relevant information and have a sufficiently high throughput. In fact, the selection of the presently available therapeutic agents and that of most drug candidates has not been preceded by proper structural optimization. Consequently, their affinity to the receptor is usually low, implying high risk of off-target adverse effects. Due to the fact that the structural requirements for binding to the LA site is not very strict, many CNS drugs, with a primary action at quite different targets, also show some VGSC blocking action [147]. The contribution of this latter action to the drug's therapeutic effect is sometimes difficult to assess.

NEWLY EMERGING STRUCTURES FROM THE AREA OF VGSC RESEARCH

In this section, we review the most important compounds recently published in the patent literature. We group the compounds according to their chemical structure. According to drug databases and reports the most important novel voltage gated sodium channel blockers under development seem to be crobenetine and ralfinamide. Derivatives of these two compounds will be discussed first.

CROBENETINE DERIVATIVES

Based on crobenetine (**21**) (Fig. **9**), researchers at Boehringer Ingelheim have synthesized a series of 6,7-benzomorphan derivatives to study their binding to VGSCs [150], serving as lead compound in the synthetic program. The pendant aromatic ring of crobenetine is connected through a spacer to a complex aromatic alkaloid moiety. The hydroxyl group on the latter seems to be important for sodium channel blocking activity. As a continuation of the program, several patents were published on derivatives of crobenetine in the late nineties. The last patent of this series was filed in 2002 [151] (see e.g. (**22**), Fig. **9**) where compounds contain a simple double bond instead of the usual aromatic head. The clinical indications of crobenetine and its derivatives include (among others) neurodegenerative diseases, stroke (crobenetine is in Phase II), epilepsy, and pain [152].

BENZYLOXY-BENZYLAMINE DERIVATIVES

As shown in Fig. (**10**) many benzyloxy-benzylamines with various amine functions (**23-27**) were studied and patented as sodium channel blockers by Newron [153,154, 155].

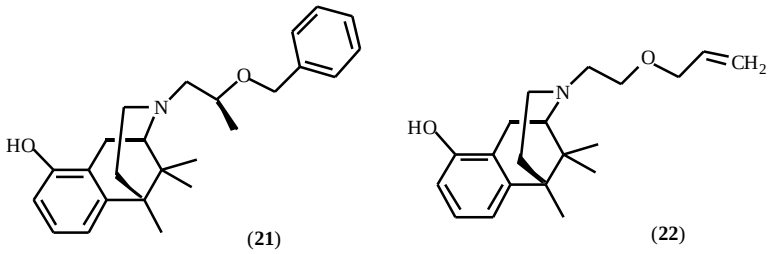


Fig. (9). Crobenetine (**21**) and a novel derivative (**22**) patented by Boehringer Ingelheim.

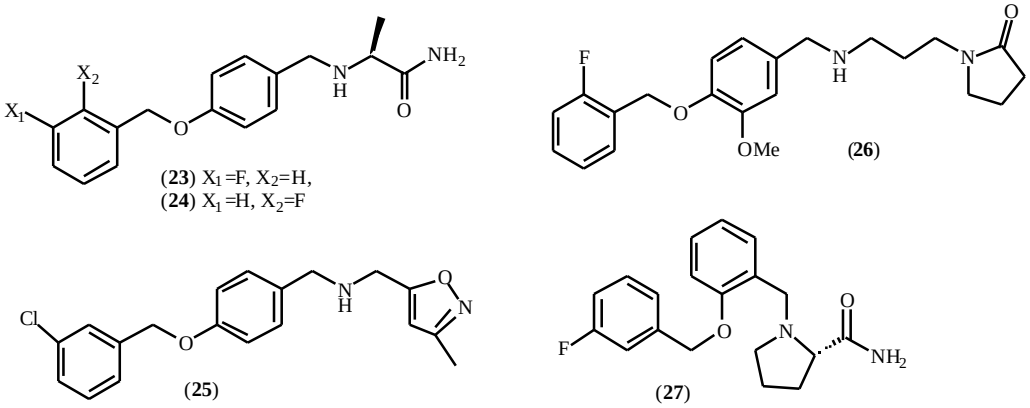


Fig. (10). Benzyloxy-benzylamine derivatives patented by Newron; safinamide (**23**) and ralfinamide (**24**).

The parent compound, safinamide (NW-1015; **23**) [156] is an antiepileptic in Phase II, which is also being tested against Parkinson's disease (Phase III) and restless legs syndrome (Phase II). Besides its sodium channel blocking activity, safinamide has a variety of molecular actions e.g. inhibition of monoamine oxidase (MAO-B), and inhibition of dopamine reuptake [157]. Ralfinamide (NW-1029; **24**) is in Phase II clinical trials for the treatment of neuropathic pain [98]. Safinamide and ralfinamide were also patented as antimigraine [158] and antiinflammatory agents [159].

AMINE DERIVATIVES CONNECTED TO AN AROMATIC MOIETY BY A SPACER

Euroceltique Sa. published several patents in 2003 and 2004 in the field of VGSC blockers. (Figs (**11a**) and (**11b**); **28-35**). Most of the compounds had a large aromatic moiety with two or three aromatic or heteroaromatic rings, e.g. pyridine (**28**) and (**30**) [160,161], pyrazine (**29**) [162], benzimidazole (**31**) [163], and an amine (mainly piperidine) function. The spacer between them could be an alkyl chain e.g. in (**31-34**) [163-166], or an ethoxy group (**30**) [161], or a carboxamido-ethyl group, (**28**) [160]. In the compounds covered by WO03076414 only aromatic and heteroaromatic rings were combined (**29**) [162]. Interes-tingly, combination of three aromatic moieties without any amine function (**35**) also showed VGSC blocking activity [167], which was confirmed also by electrophysiology. The Ki values of these compounds were between 0.18 and 1 μM. The main clinical indications include ischemia, neurode-generative conditions, such as Alzheimer's disease, ALS, Parkinson's disease, pain, and manic depression, etc.

In the case of the SCB blockers of Kotobuki Pharm Co. (**36-39**) [168-171] an aromatic moiety and an amine (piperidine or morpholine) ring were interconnected by an alkyl spacer where interestingly the piperidine ring is part of the spacer (Fig. **12**). The specific compound of WO06025471 (**38**) [170] had a piperidine ring in a carboxamide function. Disease targets include neuropathic pain (associated with diabetic neuropathy), or pain due to cancer and migraine.

Scientists at the University of Virginia have published a dimethylamine derivative (**40**) [172] for treating acute and chronic pain (Fig. **13**). This compound is similar to the above described sodium channel blockers, having an aromatic moiety-spacer-amine structure.

LAMOTRIGINE DERIVATIVES

Some years ago a great number of analogues of the phenyltriazine lamotrigine were synthesized and patented for various therapeutic fields, mainly by the Glaxo group. These include the triazine (**41**), phenylpyrimidine (**9**), and phenylpyrazine compounds (**42**). Although the structure-activity relationship among these structures have not been published in full detail, some aspects of the medicinal chemistry of lamotrigine derivatives were reviewed in two articles [8, 21]. Recently, only a few lamotrigine derivatives have been patented, e.g. the specific compound of Pfizer's patent (**43**) [173] contains a pyridine ring instead of the triazine ring of lamotrigine (Fig. **14**). Compound (**43**) is a subtype selective (Na_V 1.8) sodium channel modulator for the treatment of acute or chronic pain.

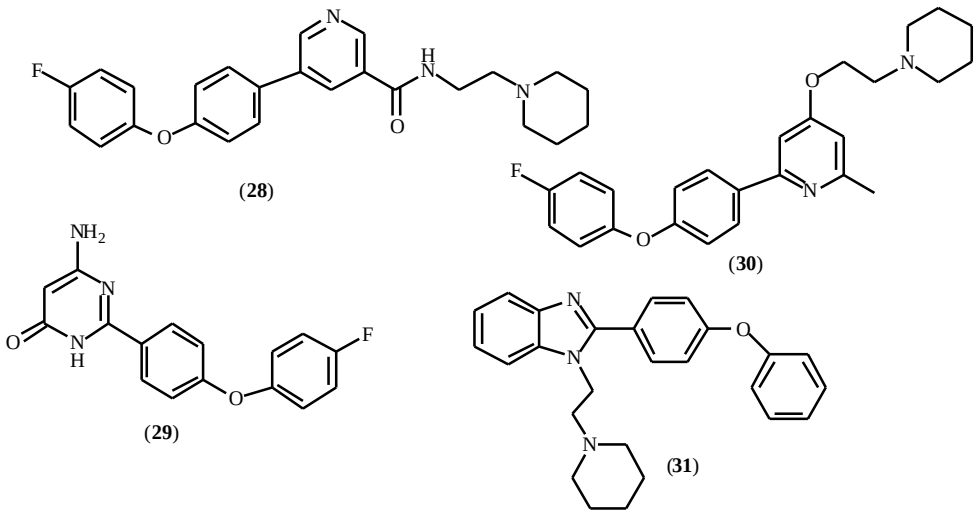


Fig. (11a). Compounds with an amine function interconnected with an aromatic moiety by a spacer, patented by Euroceltique.

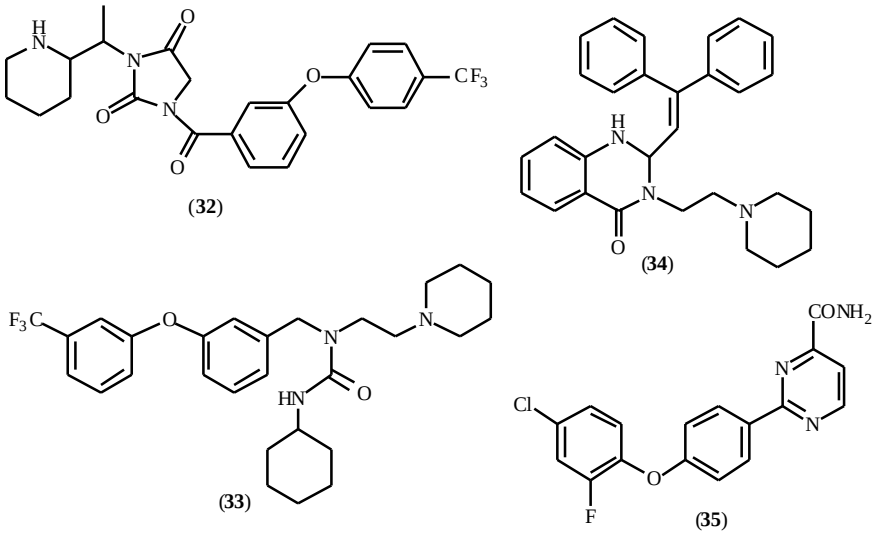


Fig. (11b). Compounds with an amine function interconnected with an aromatic moiety by a spacer, patented by Euroceltique.

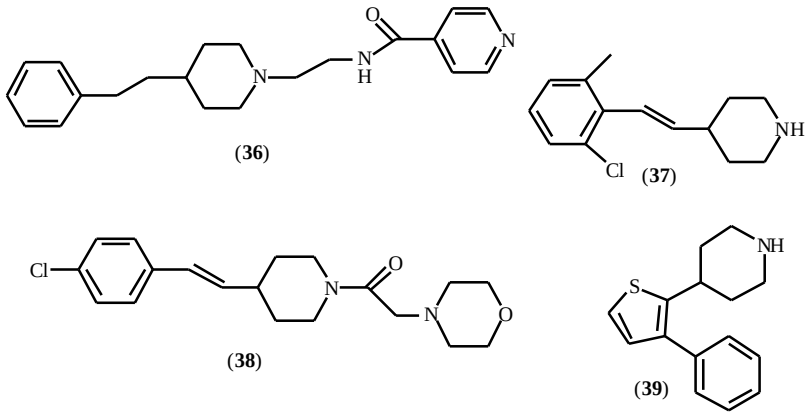
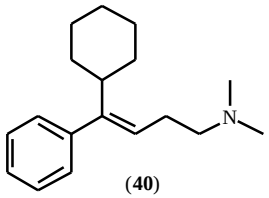
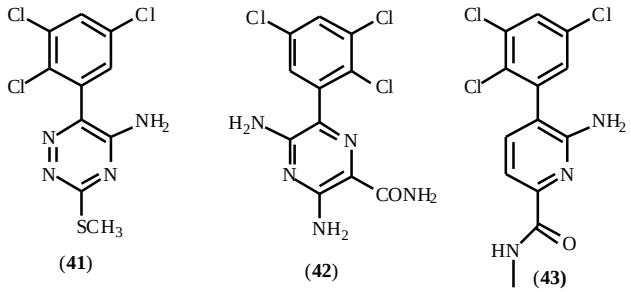


Fig. (12). Compounds with an amine function interconnected with an aromatic moiety by a spacer, patented by Kotobuki.



(**40**)

Fig. (13). Compound with an amine function interconnected with an aromatic moiety by a spacer, patented by University of Virginia.



(**41**) (**42**) (**43**)

Fig. (14). Lamotrigine derivatives.

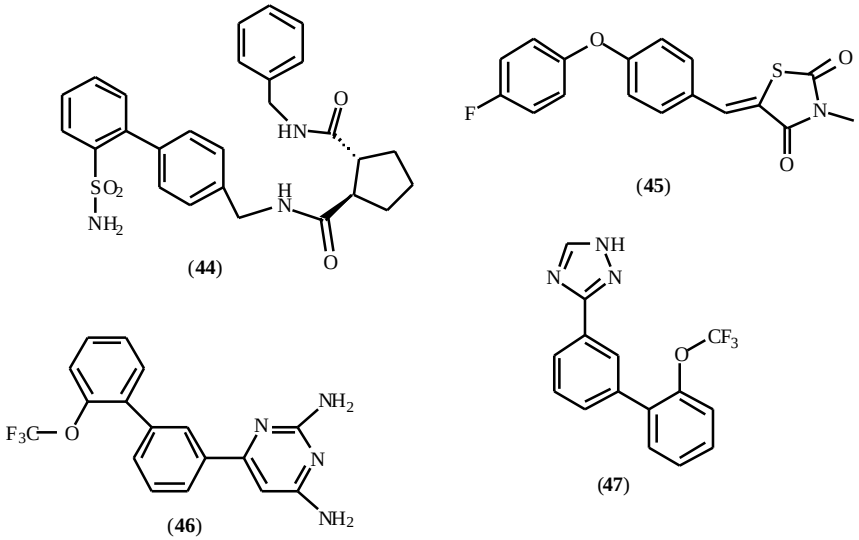
MISCELLANEOUS COMBINATIONS OF AROMATIC AND HETEROAROMATIC RINGS

Recently, published VGSC blockers of the Merck company (Figs. **15a** and **15b**) usually combine either three (**44**) [174], or two aromatic rings with one heteroaromatic ring. The latter group include phenoxyphenyl derivatives (**45**) [175], biphenylpyrimidines (**46**) [176], biphenyltriazoles (**47**), (**48**) and (**51**) [177-179], biphenylpyrazoles (**49**) [180], and biphenyl-thiazoles (**50**) [181]. These Merck compounds may contain an amide group (**44**) [174], but never contain an amine function. Compound (**44**) is a subtype selective Na_V 1.7 blocker, which does not affect cardiac hERG channels [182]. The indications of these Merck's compounds (**44-50**) include various pain syndromes (both inflammatory and neuropathic), irritable bowel synd-rome, epilepsy, and multiple sclerosis. Their VGSC blocking potencies range from 0.05-50 μM in an electrophysiological assay.

Piperazine derivatives of phenylpyrazole-carboxylic acid (**52**) (Fig. **16**) were patented by Atkinson for the treatment of neuropathic pain [183]. Interestingly there were phenyl-pyrazole-carboxamide derivatives among the above. Similarly to the above mentioned Merck compounds the patented SCB blockers of Icagen Inc. [184-186] contains three, sometimes four aromatic or heteroaromatic rings, connected with carboxamide groups instead of an amine function. CNS disorders and various types of pain (inflammatory and/or neuropathic pain**)** were targeted by these compounds.

VGSC blockers with a preferential action on persistent sodium currents were patented by Ehring (Fig. **17**). In the case of compound (**53**) [187] a benzene and a thiophene ring was connected by a carboxamide-containing alkyl spacer. The therapeutic targets included neurodegenerative, inflammatory and immune diseases. The compounds of US2005049287 [188], e.g. (**54**), were developed for treating chronic pain.

The pharmaceutical company Vertex has become very active in the field of voltage gated sodium and/or calcium channel inhibitor research in the last few years. Vertex has patented various pyrimidine derivatives (**55-59**) [189-193] and other nitrogen containing heterocycles, e.g. quinolines (**60**) [194] or benzimidazoles (**61**) [195] as sodium channel blockers (Fig. **18a** and **18b**). Claimed clinical indications are neurodegenerative disorders, chronic pain (both neuropathic and inflammatory), headache, irritable bowel syndrome, and epilepsy. According to company news, Phase I clinical trials with VX-409, a leading sodium channel modulator of Vertex (co-developed with GlaxoSmithKline) are expected in early 2007. This compound is stated to possess a subtype selective VGSC blocking action, and displays analgesic activity in animal models of both inflammatory and neuropathic pain.



(**44**) (**45**) (**46**) (**47**)

Fig. (15a). Compounds with a combination of aromatic and heteroaromatic rings, patented by Merck.

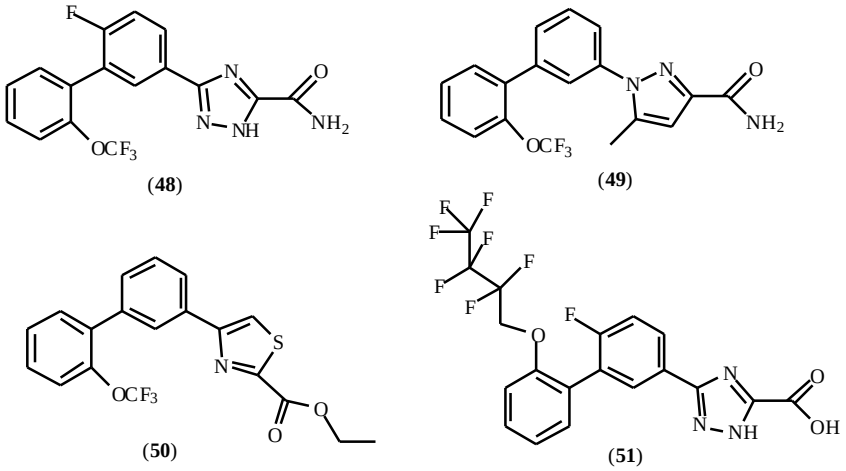


Fig. (15b). Compounds with a combination of aromatic and heteroaromatic rings, patented by Merck.

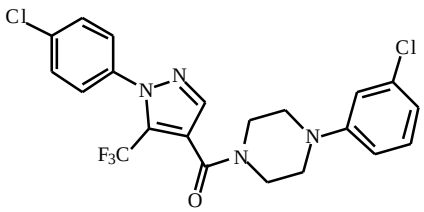


Fig. (16). Biarylcarboxamide compounds patented by Atkinson.

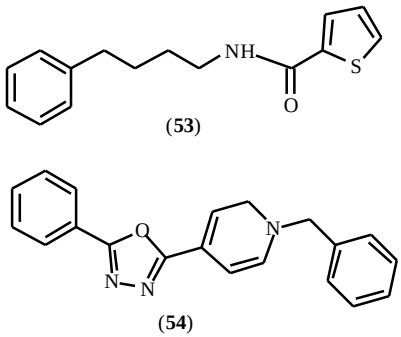


Fig. (17). Compounds with a combination of aromatic and heteroaromatic rings, patented by Ehring.

However, it is not disclosed which VGSC subtype is affected. The chemical structure of this enigmatic compound has not been published either, thus it is not clear if it is covered by any of the above patents.

Ionix Pharm. Ltd. patented sensory neurone specific sodium channel inhibitors (**62-63**) [196,197] for treating bowel disorders, bladder dysfunction, tinnitus, pain, and demyelinating diseases, etc (Fig. **19**).

POSSIBLE USE OF DRUG COMBINATIONS AND DRUGS WITH COMBINED ACTIVITY ON MULTIPLE MOLECULAR TARGETS

It is increasingly recognized that treatment with drug combinations, or drugs acting on multiple targets may offer better control than simple monotherapy in some diseases characterized by multi-factorial pathomechanisms [198]. This seems to hold especially true in relation to VGSC blockers. Several reports and patents published recently state that various neurological or psychiatric diseases can be better cured when a VGSC blocker is combined with other drugs. These combinations will be overviewed in this section.

As discussed earlier, modulation of VGSCs through G protein-coupled receptors may result in an increased state dependent action of VGSC blockers. A patent application by Glaxo scientists [199] claims the use of a composition containing an inhibitor of both norepinephrine and dopamine reuptake, such as bupropion, and a sodium channel blocker, such as lamotrigine, as an improved therapy of neuropathic pain.

According to a recently published Richter patent [111] co-administration of various selective 5-HT reuptake inhibitors invariably resulted in a substantial potentiation of the action of a number of VGSC blocking compounds on spinal neurotransmission. The combination of lamotrigine and sertraline was selected to exemplify this enhanced effect, which may result in improved antispastic and analgesic actions. Notably, the motor impairing effect of lamotrigine was not enhanced by sertraline, so that the therapeutic index of the combination was higher than that of lamotrigine alone. The results of spinal reflex studies and whole-cell patch clamp experiments suggested that SSRIs acted via increasing serotonin tone, which promoted phosphorylation of the VGSC protein via a PKC mediated mechanism.

Another patent of Richter claims that dextromethorphan significantly increases the effectiveness of the VGSC blocker tolperisone in animal models of various CNS diseases. The mechanism of action of the potentiation is not discussed, but it is conceivable that the 5-HT uptake blocking effect of dextromethorphan plays a role [200].

A patent by TEVA claims that co-administration of the VGSC blocker riluzole and the MAO-B inhibitor rasagiline results in a favorable neuroprotective effect in the treatment of amyotrophic lateral sclerosis [201]. The same drug

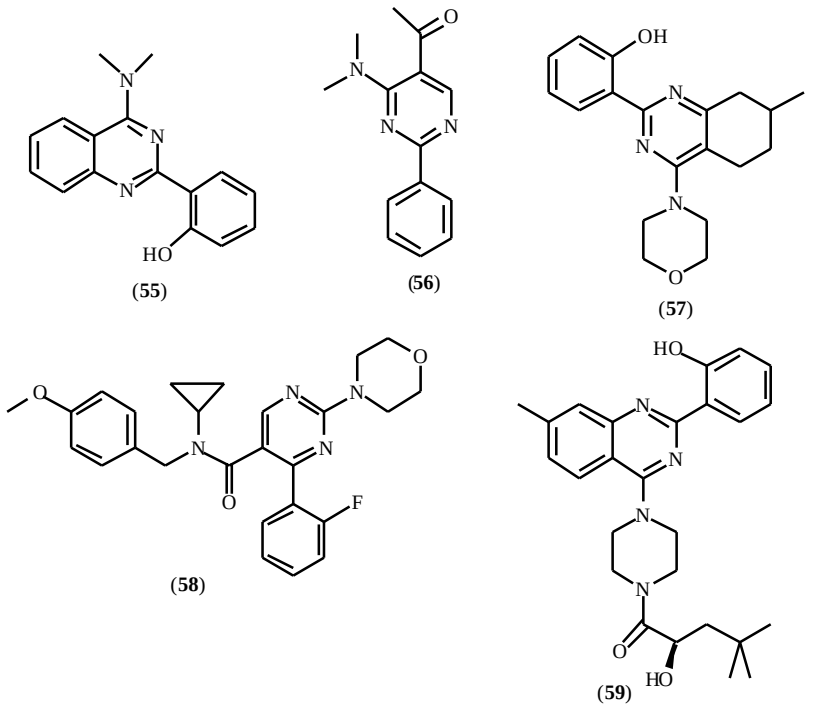


Fig. (18a). Compounds with combined aromatic and heteroaromatic rings patented by Vertex.

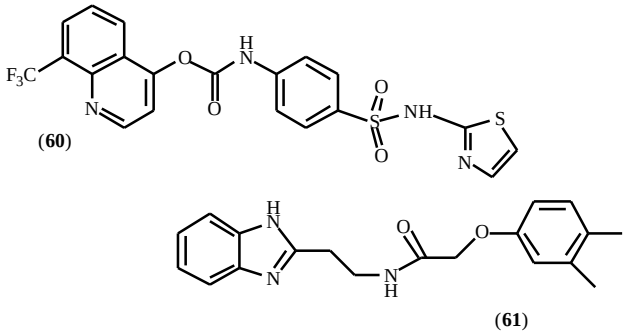


Fig. (18b). Compounds with combined aromatic and heteroaromatic rings patented by Vertex.

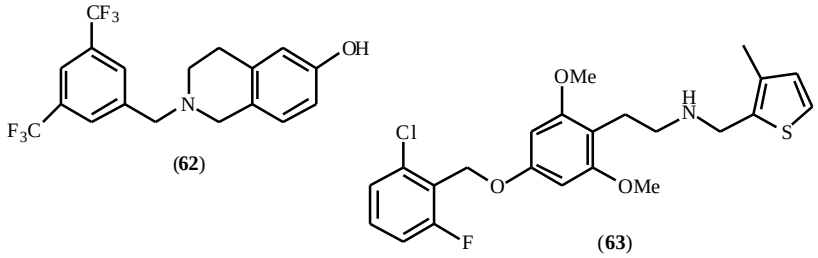


Fig. (19). Compounds with combined aromatic and heteroaromatic rings patented by Ionix.

combination was found to be effective in a mouse model of ALS [202].

Another possibility of improving therapeutic efficacy using the above principle is to develop compounds with dual or multiple actions, i.e. single molecules acting on more than one drug targets [198]. In fact, most clinically used VGSC blocking agents (phenytoin, carbamazepine, lamotrigine), do have multiple pharmacological actions. This holds especially true for tricyclic antidepressants, which are regarded as first-line treatment of neuropathic pain. Amitriptyline and desipramine clearly combine monoamine uptake inhibitory and VGSC blocking actions in one molecule. Following this

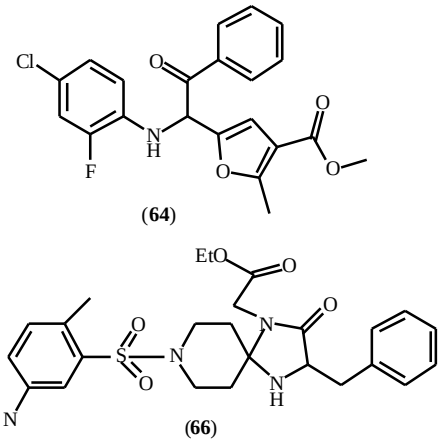


Fig. (20). Dual-activity compounds patented by Gruenenthal.

logic, a VGSC blocking compound with an additional activity on a G protein-coupled receptor (i.e. agonist of certain 5-HT, dopamine, or noradrenaline receptors) could be more efficacious, than pure VGSC blockers. Although VGSC blockade is regarded as primary mode of action of safinamide, this drug also inhibits monoamine oxidase [203], thus increasing the monoaminergic tone, which may result in an increased phosphorylation of VGSCs through PKC activation.

Gruenenthal Gmbh have published a few patents on sodium channel blockers (Fig. **20**) having additional activities, such as NMDA antagonist (**64**) [204] noradrenaline reuptake inhibitory (**65**) [205], 5-HT reuptake inhibitory (**66**) [206], or nonselective monoamine reuptake inhibitory effect (**67**) [207]. A Yoshitomi compound (**68**) (Fig. **21**), patented for the treatment of ischemic cerebrovascular diseases and neuropathic pain, combines serotonin antagonist and VGSC blocking activities [208].

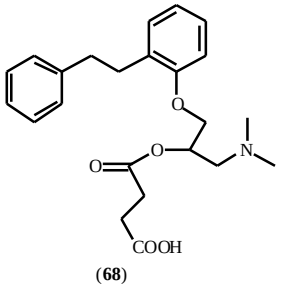
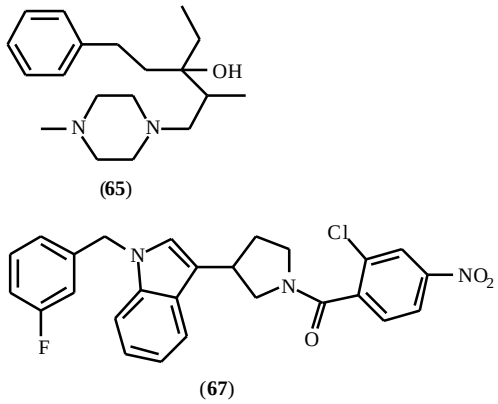


Fig. (21). Dual-activity compounds patented by Yoshitomi.

Other patents claim biaryl-piperidine derivatives (**69**) [209], and biaryl urethane compounds (**70**) [210], in which VGSC blocking activity is combined with monoamine oxidase and/or lipid peroxidation modulator activity (Fig. **22**). They are proposed for treating neurodegeneration, pain, and epilepsy.

CURRENT & FUTURE DEVELOPMENTS

Considering the huge research effort of the last few decades aimed at identifying and developing high-affinity,



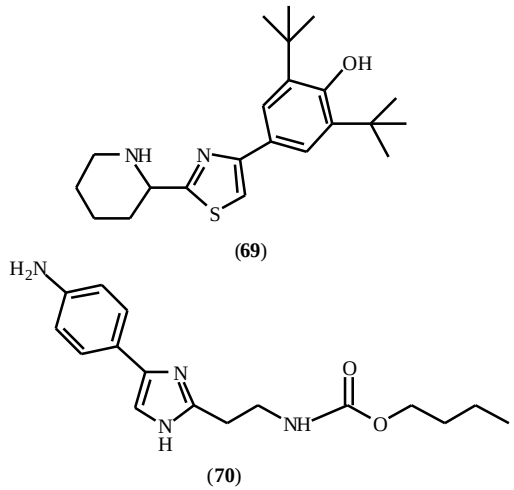


Fig. (22). Dual-activity compounds patented by Soc. Conceils Rech. Appl . Sci.

specific blockers of VGSCs for the treatment of CNS diseases, the achievements may seem somewhat disappointing. Only a few new agents have been introduced, and these are not profoundly better than older compounds that had been selected without optimizing for their VGSC blocking action. They also suffer from problems of low efficacy, low specificity regarding other targets, and a consequently high risk of adverse effects. Most of the synthetic programs used old agents, such as lamotrigine, as lead generators, so it is not surprising that only a modest advancement has been achieved. Many of these projects have been aborted before identifying suitable therapeutic agents.

Further, sodium channels represent a difficult target for structure optimization programs. Several complicating factors, such as state-dependent changes in the binding site, and the lack of really suitable test systems hinder the identification of novel drug candidates.

Nevertheless, drug companies have continued their intense research activity in the VGSC field, and several interesting families of compounds with VGSC blocking action have been identified during the last couple of years.

The increased effort is indicated by the huge number of patents filed recently, and the pertinacious interest of the research community is clearly shown by the number of scientific papers related to VGSCs. A great number of promising drug candidates is being tested in preclinical and clinical phases.

The great body of knowledge collected about the molecular structure and function among several families of compounds of VGSCs, in line with the availability of novel, high-throughput and reliable methods to characterize the VGSC blocking activity of compounds, may hold promise that the next period of research and development will be more successful.

Molecular cloning of the different VGSC subtypes and their stable expression in heterologous expression systems represent important achievements. It is notable that the exact role of beta subunits in VGSC functioning is not fully understood, therefore there has been no attempt to utilize beta subunits as possible drug targets. Similarly, understanding the role of different splice variants will certainly add new details to our knowledge on the heterogeneity of VGSCs, which can be converted to practically useful information. Further chance for a break-through is offered by a deeper understanding of the molecular mode of state-dependently acting VGSC blockers, since this knowledge can be used for searching drugs that have a more favorable interaction with channels involved in the targeted pathological process.

Besides the traditional therapeutic application fields, the utility of VGSC blockers has been shown in several additional diseases. Usually, the same old agents that were originally introduced as antiepileptics, are now used in some new fields too, first of all in chronic pain and bipolar disorder.

Finally, the recognition that drug combinations containing VGSC blocking agents, or drugs with dual/multiple actions may also help to find suitable therapeutic agents in the treatment of several CNS diseases.

REFERENCES

[1] Hammarstrom AK, Gage PW. Hypoxia and persistent sodium current. Eur Biophys J 2002; 31: 323-330.

[2] Li Y, Bennett DJ. Persistent sodium and calcium currents cause plateau potentials in motoneurons of chronic spinal rats. J Neurophysiol 2003; 90: 857-869.

[3] Avoli M, Louvel J, Pumain R, Kohling R. Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol 2005; 77: 166-200.

[4] Welch KM. Brain hyperexcitability: the basis for antiepileptic drugs in migraine prevention. Headache 2005; 45: 25-32.

[5] Lampert A, Hains BC, Waxman SG. Upregulation of persistent and ramp sodium current in dorsal horn neurons after spinal cord injury. Exp Brain Res 2006; 174: 660-666.

[6] Kuo JJ, Siddique T, Fu R, Heckman CJ. Increased persistent Na^+ current and its effect on excitability in motoneurones cultured from mutant SOD1 mice. J Physiol 2005; 563: 843-854.

[7] Rhodes TH, Lossin C, Vanoye CG, Wang DW, George AL, Jr. Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. Proc Natl Acad Sci USA 2004; 101: 11147-11152.

[8] Clare JJ, Tate SN, Nobbs M, Romanos MA. Voltage-gated sodium channels as therapeutic targets. Drug Discov Today 2000; 5: 506-520.

[9] Wood JN, Boorman J. Voltage-gated sodium channel blockers; target validation and therapeutic potential. Curr Top Med Chem 2005; 5: 529-537.

[10] Taylor CP, Meldrum BS. Na^+ channels as targets for neuroprotective drugs. Trends Pharmacol Sci 1995; 16: 309-316.

[11] Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 2000; 26: 13-25.

[12] Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol Rev 2005; 57: 397-409.

[13] Goldin AL, Barchi RL, Caldwell JH, *et al.* Nomenclature of voltage-gated sodium channels. Neuron 2000; 28: 365-368.

[14] Ilyin VI, Pomonis JD, Whiteside GT, *et al*. Pharmacology of 2-[4-(4-chloro-2-fluorophenoxy)phenyl]-pyrimidine-4-carboxamide (PPPA): a potent, broad-spectrum state-dependent sodium channel blocker for treating pain states. J Pharmacol Exp Ther 2006; 318: 1083-1093.

[15] Ogata N, Yoshida S. New perspectives on the structure and function of the Na^+ channel multigene family. Curr Med Chem - Central Nervous System Agents, 2002; 2: 59-81.

[16] Sato C, Ueno Y, Asai K, *et al*. The voltage-sensitive sodium channel is a bell-shaped molecule with several cavities. Nature 2001; 409: 1047-1051.

[17] Goldin AL. Mechanisms of sodium channel inactivation. Curr Opin Neurobiol 2003; 13: 284-290.

[18] Carr DB, Cooper DC, Ulrich SL, Spruston N, Surmeier DJ. Serotonin receptor activation inhibits sodium current and dendritic excitability in prefrontal cortex via a protein kinase C-dependent mechanism. J Neurosci 2002; 22: 6846-6855.

[19] Candenas L, Seda M, Noheda P, *et al*. Molecular diversity of voltage-gated sodium channel alpha and beta subunit mRNAs in human tissues. Eur J Pharmacol 2006; 541: 9-16.

[20] Watanabe E, Fujikawa A, Matsunaga H, *et al.* Nav2/NaG channel is involved in control of salt-intake behavior in the CNS. J Neurosci 2000; 20: 7743-7751.

[21] Anger T, Madge DJ, Mulla M, Riddall D. Medicinal chemistry of neuronal voltage-gated sodium channel blockers. J Med Chem 2001; 44: 115-137.

[22] Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. Physiol Rev 1992; 72: 15-48.

[23] Willow M, Catterall WA. Inhibition of binding of [3H]batrachotoxinin A 20-alpha-benzoate to sodium channels by the anticonvulsant drugs diphenylhydantoin and carbamazepine. Mol Pharmacol 1982; 22: 627-635.

[24] Mike A, Karoly R, Vizi ES, Kiss JP. Inhibitory effect of the DA uptake blocker GBR 12909 on sodium channels of hippocampal neurons. Neuroreport 2003; 14: 1945-1949.

[25] Mike A, Karoly R, Vizi ES, Kiss JP. A novel modulatory mechanism of sodium currents: frequency-dependence without state-dependent binding. Neuroscience 2004; 125: 1019-1028.

[26] Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. Science 1994; 265: 1724-1728.

[27] Kuo CC, Lu L. Characterization of lamotrigine inhibition of Na^+ channels in rat hippocampal neurones. Br J Pharmacol 1997; 121: 1231-1238.

[28] Kalso E. Sodium channel blockers in neuropathic pain. Curr Pharm Des 2005; 11: 3005-3011.

[29] Numann R, Catterall WA, Scheuer T. Functional modulation of brain sodium channels by protein kinase C phosphorylation. Science 1991; 254: 115-118.

[30] Carr DB, Day M, Cantrell AR, *et al*. Transmitter modulation of slow, activity-dependent alterations in sodium channel availability endows neurons with a novel form of cellular plasticity. Neuron 2003; 39: 793-806.

[31] Cantrell AR, Catterall WA. Neuromodulation of Na^+ channels: an unexpected form of cellular plasticity. Nat Rev Neurosci 2001; 2: 397-407.

[32] Cantrell AR, Tibbs VC, Yu FH, *et al*. Molecular mechanism of convergent regulation of brain Na^+ channels by protein kinase C and protein kinase A anchored to AKAP-15. Mol Cell Neurosci 2002; 21: 63-80.

[33] Chen Y, Yu FH, Surmeier DJ, Scheuer T, Catterall WA. Neuromodulation of Na^+ channel slow inactivation via cAMP-

dependent protein kinase and protein kinase C. Neuron 2006; 49: 409-420.

[34] Chahine M, Ziane R, Vijayaragavan K, Okamura Y. Regulation of Na_v channels in sensory neurons. Trends Pharmacol Sci 2005; 26: 496-502.

[35] Grieco A, Forgione A, Miele L, *et al*. Fatty liver and drugs. Eur Rev Med Pharmacol Sci 2005; 9: 261-263.

[36] Zhou LX, Pihlstrom B, Hardwick JP, *et al*. Metabolism of phenytoin by the gingiva of normal humans: the possible role of reactive metabolites of phenytoin in the initiation of gingival hyperplasia. Clin Pharmacol Ther 1996; 60: 191-198.

[37] Ketter TA, Greist JH, Graham JA, *et al*. The effect of dermatologic precautions on the incidence of rash with addition of lamotrigine in the treatment of bipolar I disorder: a randomized trial. J Clin Psychiatry 2006; 67: 400-406.

[38] Zhorov BS, Tikhonov DB. Potassium, sodium, calcium and glutamate-gated channels: pore architecture and ligand action. J Neurochem 2004; 88: 782-799.

[39] Liang J, Brochu RM, Cohen CJ, *et al*. Discovery of potent and use-dependent sodium channel blockers for treatment of chronic pain. Bioorg Med Chem Lett 2005; 15: 2943-2947.

[40] Gribkoff VK, Wisden W. Modulators of periferal voltage-gated sodium channels for the treatment of neuropathic pain. Expert Opin Ther Pat 2005; 15: 1751-1762.

[41] Zieglgansberger W, Berthele A, Tolle TR. Understanding neuropathic pain. CNS Spectr 2005; 10: 298-308.

[42] Roselli F, Livrea P, Jirillo E. Voltage-gated sodium channel blockers as immunomodulators. Recent Patents on CNS Drug Discovery 2006; 1: 83-91.

[43] Brown DL, Ransom DM, Hall JA, *et al*. Regional anesthesia and local anesthetic-induced systemic toxicity: seizure frequency and accompanying cardiovascular changes. Anesth Analg 1995; 81: 321-328.

[44] Friederich P, Benzenberg D, Urban BW. Bupivacaine inhibits human neuronal Kv3 channels in SH-SY5Y human neuroblastoma cells. Br J Anaesth 2002; 88: 864-866.

[45] Planells-Cases R, Caprini M, Zhang J, *et al*. Neuronal death and perinatal lethality in voltage-gated sodium channel alpha(II)-deficient mice. Biophys J 2000; 78: 2878-2891.

[46] Nassar MA, Stirling LC, Forlani G, *et al*. Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. Proc Natl Acad Sci USA 2004; 101: 12706-12711.

[47] Matthews EA, Wood JN, Dickenson AH. Nav 1.8-null mice show stimulus-dependent deficits in spinal neuronal activity. Mol Pain 2006; 2: 5-10.

[48] Maertens C, Cuypers E, Amininasab M, *et al*. Potent modulation of the voltage-gated sodium channel Nav1.7 by OD1, a toxin from the scorpion *Odonthobuthus doriae*. Mol Pharmacol 2006; 70: 405-414.

[49] Cummins TR, Aglieco F, Dib-Hajj SD. Critical molecular determinants of voltage-gated sodium channel sensitivity to mu-conotoxins GIIIA/B. Mol Pharmacol 2002; 61: 1192-1201.

[50] Li RA, Ennis IL, Xue T, *et al*. Molecular basis of isoform-specific micro-conotoxin block of cardiac, skeletal muscle, and brain Na^+ channels. J Biol Chem 2003; 278: 8717-8724.

[51] Shon KJ, Olivera BM, Watkins M, *et al*. mu-Conotoxin PIIIA, a new peptide for discriminating among tetrodotoxin-sensitive Na channel subtypes. J Neurosci 1998; 18: 4473-4481.

[52] Bosmans F, Rash L, Zhu S, *et al*. Four novel tarantula toxins as selective modulators of voltage-gated sodium channel subtypes. Mol Pharmacol 2006; 69: 419-429.

[53] Weiser T, Wilson N. Inhibition of tetrodotoxin (TTX)-resistant and TTX-sensitive neuronal Na^+ channels by the secretolytic ambroxol. Mol Pharmacol 2002; 62: 433-438.

[54] Stummann TC, Salvati P, Fariello RG, Faravelli L. The anti-nociceptive agent ralfinamide inhibits tetrodotoxin-resistant and tetrodotoxin-sensitive Na^+ currents in dorsal root ganglion neurons. Eur J Pharmacol 2005; 510: 197-208.

[55] Joshi, S., Honore, P., Shieh, C., and Zhang, X. A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. 36th Annu Meet Soc Neurosci Atlanta, USA **(2006)**. Abst 245.10

[56] Djouhri L, Newton R, Levinson SR, *et al.* Sensory and electrophysiological properties of guinea-pig sensory neurones expressing Nav 1.7 (PN1) Na^+ channel alpha subunit protein. J Physiol 2003; 546: 565-576.

[57] Black JA, Liu S, Tanaka M, Cummins TR, Waxman SG. Changes in the expression of tetrodotoxin-sensitive sodium channels within dorsal root ganglia neurons in inflammatory pain. Pain 2004; 108: 237-247.

[58] Gould HJ, III, England JD, Soignier RD, *et al*. Ibuprofen blocks changes in Na v 1.7 and 1.8 sodium channels associated with complete Freund's adjuvant-induced inflammation in rat. J Pain 2004; 5: 270-280.

[59] Bartoo AC, Sprunger LK, Schneider DA. Expression and distribution of TTX-sensitive sodium channel alpha subunits in the enteric nervous system. J Comp Neurol 2005; 486: 117-131.

[60] Kerr BJ, Souslova V, McMahon SB, Wood JN. A role for the TTX-resistant sodium channel Nav 1.8 in NGF-induced hyperalgesia, but not neuropathic pain. Neuroreport 2001; 12: 3077-3080.

[61] Villarreal CF, Sachs D, de Queiroz CF, Parada CA, Ferreira SH. The role of Na(V)1.8 sodium channel in the maintenance of chronic inflammatory hypernociception. Neurosci Lett 2005; 386: 72-77.

[62] Porreca F, Lai J, Bian D, *et al*. A comparison of the potential role of the tetrodotoxin-insensitive sodium channels, PN3/SNS and NaN/SNS2, in rat models of chronic pain. Proc Natl Acad Sci U S A 1999; 96: 7640-7644.

[63] Hong S, Morrow TJ, Paulson PE, Isom LL, Wiley JW. Early painful diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat. J Biol Chem 2004; 279: 29341-29350.

[64] Hong S, Wiley JW. Altered expression and function of sodium channels in large DRG neurons and myelinated A-fibers in early diabetic neuropathy in the rat. Biochem Biophys Res Commun 2006; 339: 652-660.

[65] Priest BT, Murphy BA, Lindia JA, *et al*. Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. Proc Natl Acad Sci U S A 2005; 102: 9382-9387.

[66] Lindia JA, Kohler MG, Martin WJ, Abbadie C. Relationship between sodium channel Na(V)1.3 expression and neuropathic pain behavior in rats. Pain 2005; 117: 145-153.

[67] Zhao P, Waxman SG, Hains BC. Sodium channel expression in the ventral posterolateral nucleus of the thalamus after peripheral nerve injury. Mol Pain 2006; 2: 27-36.

[68] Hains BC, Saab CY, Waxman SG. Alterations in burst firing of thalamic VPL neurons and reversal by Na(v)1.3 antisense after spinal cord injury. J Neurophysiol 2006; 95: 3343-3352.

[69] Han C, Rush AM, Dib-Hajj SD, *et al*. Sporadic onset of erythermalgia: A gain-of-function mutation in Na(v)1.7. Ann Neurol 2006; 59: 553-558.

[70] Michiels JJ, te Morsche RH, Jansen JB, Drenth JP. Autosomal dominant erythermalgia associated with a novel mutation in the voltage-gated sodium channel alpha subunit Nav1.7. Arch Neurol 2005; 62: 1587-1590.

[71] Waxman SG. Sodium channels as molecular targets in multiple sclerosis. J Rehabil Res Dev 2002; 39: 233-242.

[72] Whitaker WR, Faull RL, Dragunow M, *et al*. Changes in the mRNAs encoding voltage-gated sodium channel types II and III in human epileptic hippocampus. Neuroscience 2001; 106: 275-285.

[73] Yang J, Gharagozloo P, Yao J, *et al*. 3-(4-Phenoxyphenyl) pyrazoles: a novel class of sodium channel blockers. J Med Chem 2004; 47: 1547-1552.

[74] Ok D, Li C, Abbadie C, *et al*. Synthesis and SAR of 1,2-*trans*-(1-hydroxy-3-phenylprop-1-yl)cyclopentane carboxamide derivatives, a new class of sodium channel blockers. Bioorg Med Chem Lett 2005; 16: 1358-1361.

[75] Hille B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. J Gen Physiol 1977; 69: 497-515.

[76] Lenkey N, Karoly R, Kiss JP, *et al*. The mechanism of activity-dependent sodium channel inhibition by the antidepressants fluoxetine and desipramine. Mol Pharmacol 2006; 70: 2052-2063.

[77] Marcil J, Walczak JS, Guindon J, *et al*. Antinociceptive effects of tetrodotoxin (TTX) in rodents. Br J Anaesth 2006; 96: 761-768.

[78] Bulaj G, Zhang MM, Green BR, *et al*. Synthetic muO-conotoxin MrVIB blocks TTX-resistant sodium channel NaV1.8 and has a long-lasting analgesic activity. Biochemistry 2006; 45: 7404-7414.

[79] Zhang MM, Fiedler B, Green BR, *et al*. Structural and functional diversities among mu-conotoxins targeting TTX-resistant sodium channels. Biochemistry 2006; 45: 3723-3732.

[80] Wang CZ, Zhang H, Jiang H, *et al*. A novel conotoxin from Conus striatus, mu-SIIIA, selectively blocking rat tetrodotoxin-resistant sodium channels. Toxicon 2006; 47: 122-132.

[81] Qu Y, Curtis R, Lawson D, *et al*. Differential modulation of sodium channel gating and persistent sodium currents by the beta1, beta2, and beta3 subunits. Mol Cell Neurosci 2001; 18: 570-580.

[82] Yu FH, Westenbroek RE, Silos-Santiago I, *et al*. Sodium channel beta4, a new disulfide-linked auxiliary subunit with similarity to beta2. J Neurosci 2003; 23: 7577-7585.

[83] Vijayaragavan K, Powell AJ, Kinghorn IJ, Chahine M. Role of auxiliary beta1-, beta2-, and beta3-subunits and their interaction with Na(v)1.8 voltage-gated sodium channel. Biochem Biophys Res Commun 2004; 319: 531-540.

[84] Coward K, Jowett A, Plumpton C, *et al*. Sodium channel beta1 and beta2 subunits parallel SNS/PN3 alpha-subunit changes in injured human sensory neurons. Neuroreport 2001; 12: 483-488.

[85] Shah BS, Stevens EB, Gonzalez MI, *et al*. beta3, a novel auxiliary subunit for the voltage-gated sodium channel, is expressed preferentially in sensory neurons and is upregulated in the chronic constriction injury model of neuropathic pain. Eur J Neurosci 2000; 12: 3985-3990.

[86] Oyama F, Miyazaki H, Sakamoto N, *et al*. Sodium channel beta4 subunit: down-regulation and possible involvement in neuritic degeneration in Huntington's disease transgenic mice. J Neurochem 2006; 98: 518-529.

[87] Choi H, Morrell MJ. Review of lamotrigine and its clinical applications in epilepsy. Expert Opin Pharmacother 2003; 4: 243-251.

[88] Ambrosio AF, Soares-Da-Silva P, Carvalho CM, Carvalho AP. Mechanisms of action of carbamazepine and its derivatives, oxcarbazepine, BIA 2-093, and BIA 2-024. Neurochem Res 2002; 27: 121-130.

[89] Rogawski MA. Diverse mechanisms of antiepileptic drugs in the development pipeline. Epilepsy Res 2006; 69: 273-294.

[90] Bialer M, Johannessen SI, Kupferberg HJ, *et al*. Progress report on new antiepileptic drugs: a summary of the Seventh Eilat Conference (EILAT VII). Epilepsy Res 2004; 61: 1-48.

[91] Nakamura J, Kuwana Y, Yukitoshi N. [Target pharmacology of topiramate, a new antiepileptic drug]. Nippon Yakurigaku Zasshi 2000; 115: 53-57.

[92] Maryanoff BE, Nortey SO, Gardocki JF, Shank RP, Dodgson SP. Anticonvulsant O-alkyl sulfamates. 2,3:4,5-Bis-*O*-(1-methyl-ethylidene)-beta-D-fructopyranose sulfamate and related compounds. J Med Chem 1987; 30: 880-887.

[93] Baker MD, Wood JN. Involvement of Na^+ channels in pain pathways. Trends Pharmacol Sci 2001; 22: 27-31.

[94] Lai J, Hunter JC, Porreca F. The role of voltage-gated sodium channels in neuropathic pain. Curr Opin Neurobiol 2003; 13: 291-297.

[95] Amir R, Argoff CE, Bennett GJ, *et al*. The role of sodium channels in chronic inflammatory and neuropathic pain. J Pain 2006; 7: 1-29.

[96] Gaida W, Klinder K, Arndt K, Weiser T. Ambroxol, a Nav1.8-preferring Na^+ channel blocker, effectively suppresses pain symptoms in animal models of chronic, neuropathic and inflammatory pain. Neuropharmacology 2005; 49: 1220-1227.

[97] Laird JM, Carter AJ, Grauert M, Cervero F. Analgesic activity of a novel use-dependent sodium channel blocker, crobenetine, in mono-arthritic rats. Br J Pharmacol 2001; 134: 1742-1748.

[98] Veneroni O, Maj R, Calabresi M, *et al*. Anti-allodynic effect of NW-1029, a novel Na^+ channel blocker, in experimental animal models of inflammatory and neuropathic pain. Pain 2003; 102: 17-25.

[99] Ilyin VI, Hodges DD, Whittemore ER, *et al*. V102862 (Co 102862): a potent, broad-spectrum state-dependent blocker of mammalian voltage-gated sodium channels. Br J Pharmacol 2005; 144: 801-812.

[100] Shao B, Victory S, Ilyin VI, *et al*. Phenoxyphenyl pyridines as novel state-dependent, high-potency sodium channel inhibitors. J Med Chem 2004; 47: 4277-4285.

[101] Cohen GL. Migraine prophylactic drugs work via ion channels. Med Hypotheses 2005; 65: 114-122.

[102] Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. Nat Med 2004; 10: 685-692.

[103] Frediani F. Anticonvulsant drugs in primary headaches prophylaxis. Neurol Sci 2004; 25: 161-166.

[104] Malik K, Rizvi S, Vaillancourt PD. The SUNCT syndrome: successfully treated with lamotrigine. Pain Med 2002; 3: 167-168.

[105] Crofford LJ. Pharmaceutical treatment options for fibromyalgia. Curr Rheumatol Rep 2004; 6: 274-280.

[106] Li Y, Harvey PJ, Li X, Bennett DJ. Spastic long-lasting reflexes of the chronic spinal rat studied *in vitro*. J Neurophysiol 2004; 91: 2236-2246.

[107] Ono H, Fukuda H, Kudo Y. Mechanisms of depressant action of muscle relaxants on spinal reflexes: participation of membrane stabilizing action. J Pharmacobiodyn 1984; 7: 171-176.

[108] Kocsis P, Farkas S, Fodor L, *et al*. Tolperisone-type drugs inhibit spinal reflexes via blockade of voltage-gated sodium and calcium channels. J Pharmacol Exp Ther 2005; 315: 1237-1246.

[109] Fels G. Tolperisone: evaluation of the lidocaine-like activity by molecular modeling. Arch Pharm (Weinheim) 1996; 329: 171-178.

[110] Hofer D, Lohberger B, Steinecker B, *et al*. A comparative study of the action of tolperisone on seven different voltage dependent sodium channel isoforms. Eur J Pharmacol 2006; 538: 5-14.

*[111] Kocsis, P., Tihanyi, K., Tarnawa, I., Thán, M., Németh, Gy.: WO05058363A2 **(2005)**.

[112] Farkas S, Berzsenyi P, Karpati E, Kocsis P, Tarnawa I. Simple pharmacological test battery to assess efficacy and side effect profile of centrally acting muscle relaxant drugs. J Pharmacol Toxicol Methods 2005; 52: 264-273.

[113] Green AR, Shuaib A. Therapeutic strategies for the treatment of stroke. Drug Discov Today 2006; 11: 681-693.

[114] Molnar P, Erdo SL. Vinpocetine is as potent as phenytoin to block voltage-gated Na^+ channels in rat cortical neurons. Eur J Pharmacol 1995; 273: 303-306.

[115] Willmore LJ. Antiepileptic drugs and neuroprotection: current status and future roles. Epilepsy Behav 2005; 7: 25-28.

[116] Trojnar MK, Malek R, Chroscinska M, *et al*. Neuroprotective effects of antiepileptic drugs. Pol J Pharmacol 2002; 54: 557-566.

[117] Bensimon G, Doble A. The tolerability of riluzole in the treatment of patients with amyotrophic lateral sclerosis. Expert Opin Drug Saf 2004; 3: 525-534.

[118] Ryberg H, Askmark H, Persson LI. A double-blind randomized clinical trial in amyotrophic lateral sclerosis using lamotrigine: effects on CSF glutamate, aspartate, branched-chain amino acid levels and clinical parameters. Acta Neurol Scand 2003; 108: 1-8.

[119] Stocchi F, Arnold G, Onofrj M, *et al*. Improvement of motor function in early Parkinson disease by safinamide. Neurology 2004; 63: 746-748.

[120] Bezard E, Stutzmann JM, Imbert C, *et al*. Riluzole delayed appearance of parkinsonian motor abnormalities in a chronic MPTP monkey model. Eur J Pharmacol 1998; 356: 101-104.

[121] Hains BC, Waxman SG. Neuroprotection by sodium channel blockade with phenytoin in an experimental model of glaucoma. Invest Ophthalmol Vis Sci 2005; 46: 4164-4169.

[122] Wang GK, Russell C, Wang SY. State-dependent block of voltage-gated Na^+ channels by amitriptyline via the local anesthetic receptor and its implication for neuropathic pain. Pain 2004; 110: 166-174.

[123] Bersudsky Y. Phenytoin: an anti-bipolar anticonvulsant? Int J Neuropsychopharmacol 2006; 9: 479-484.

[124] Bowden CL, Karren NU. Anticonvulsants in bipolar disorder. Aust N Z J Psychiatry 2006; 40: 386-393.

[125] Stahl SM. Anticonvulsants as mood stabilizers and adjuncts to antipsychotics: valproate, lamotrigine, carbamazepine, and

oxcarbazepine and actions at voltage-gated sodium channels. J Clin Psychiatry 2004; 65: 738-739.
[126] Bowden CL, Asnis GM, Ginsberg LD, *et al*. Safety and tolerability of lamotrigine for bipolar disorder. Drug Saf 2004; 27: 173-184.
[127] Gabriel A. Lamotrigine adjunctive treatment in resistant unipolar depression: An open, descriptive study. Depress Anxiety 2006; 23: 485-488.
[128] Mirza NR, Bright JL, Stanhope KJ, Wyatt A, Harrington NR. Lamotrigine has an anxiolytic-like profile in the rat conditioned emotional response test of anxiety: a potential role for sodium channels? Psychopharmacology (Berl) 2005; 180: 159-168.
[129] Haeseler G, Foadi N, Ahrens J, *et al*. Tramadol, fentanyl and sufentanil but not morphine block voltage-operated sodium channels. Pain 2006; 126: 234-244.
[130] Simpson JJ, Gilbert AM, Weiner GM, Davies WE. The assessment of lamotrigine, an antiepileptic drug, in the treatment of tinnitus. Am J Otol 1999; 20: 627-631.
[131] Merideth CH. A single-center, double-blind, placebo-controlled evaluation of lamotrigine in the treatment of obesity in adults. J Clin Psychiatry 2006; 67: 258-262.
[132] Catterall WA, Morrow CS, Daly JW, Brown GB. Binding of batrachotoxinin A 20-alpha-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. J Biol Chem 1981; 256: 8922-8927.
[133] Catterall WA. Membrane potential-dependent binding of scorpion toxin to the action potential Na^+ ionophore. Studies with a toxin derivative prepared by lactoperoxidase-catalyzed iodination. J Biol Chem 1977; 252: 8660-8668.
[134] Reith ME. [^{14}C]Guanidinium ion influx into Na^+ channel preparations from mouse cerebral cortex. Eur J Pharmacol 1990; 188: 33-41.
[135] Weiser T. A novel toxicity-based assay for the identification of modulators of voltage-gated Na^+ channels. J Neurosci Methods 2004; 137: 79-85.
[136] Ulbricht W. Effects of veratridine on sodium currents and fluxes. Rev Physiol Biochem Pharmacol 1998; 133: 1-54.
[137] Kolok S, Nagy J, Szombathelyi Z, Tarnawa I. Functional characterization of sodium channel blockers by membrane potential measurements in cerebellar neurons: Prediction of compound preference for the open/inactivated state. Neurochem Int 2006; 49: 593-604.
[138] Huang CJ, Harootunian A, Maher MP, *et al*. Characterization of voltage-gated sodium-channel blockers by electrical stimulation and fluorescence detection of membrane potential. Nat Biotechnol 2006; 24: 439-446.
[139] Kiss L, Bennett PB, Uebele VN, *et al*. High throughput ion-channel pharmacology: planar-array-based voltage clamp. Assay And Drug Development Technologies 2003; 1: 127-135.
[140] Delmas P, Coste B. Na^+ channel Nav1.9: in search of a gating mechanism. Trends Neurosci 2003; 26: 55-57.
[141] Ragsdale DS, Scheuer T, Catterall WA. Frequency and voltage-dependent inhibition of type IIA Na^+ channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs. Mol Pharmacol 1991; 40: 756-765.
[142] Buyukakilli B, Comelekoglu U, Tataroglu C, Kanik A. Reversible conduction block in isolated frog sciatic nerve by high concentration of bupivacaine. Pharmacol Res 2003; 47: 235-241.
[143] Salvati P, Maj R, Caccia C, *et al*. Biochemical and electro-physiological studies on the mechanism of action of PNU-151774E, a novel antiepileptic compound. J Pharmacol Exp Ther 1999; 288: 1151-1159.
[144] Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther 1952; 106: 319-330.
[145] Haeseler G, Bufler J, Merken S, *et al*. Block of voltage-operated sodium channels by 2,6-dimethylphenol, a structural analogue of lidocaine's aromatic tail. Br J Pharmacol 2002; 137: 285-293.
[146] Zamponi GW, French RJ. Dissecting lidocaine action: diethylamide and phenol mimic separate modes of lidocaine block of sodium channels from heart and skeletal muscle. Biophys J 1993; 65: 2335-2347.
[147] Kuo CC. A common anticonvulsant binding site for phenytoin, carbamazepine, and lamotrigine in neuronal Na^+ channels. Mol Pharmacol 1998; 54: 712-721.
[148] Liu G, Yarov-Yarovoy V, Nobbs M, *et al*. Differential interactions of lamotrigine and related drugs with transmembrane segment IVS6 of voltage-gated sodium channels. Neuropharmacology 2003; 44: 413-422.
[149] Lipkind GM, Fozzard HA. Molecular modeling of local anesthetic drug binding by voltage-gated Na channels. Mol Pharmacol 2005; 68: 1611-1622.
[150] Grauert M, Bechtel WD, Weiser T, *et al*. Synthesis and structure-activity relationships of 6,7-benzomorphan derivatives as use-dependent sodium channel blockers for the treatment of stroke. J Med Chem 2002; 45: 3755-3764.
*[151] Carter, A., Ensinger, H., Grauert, M., Stiller, D.A., Weiser, T.: DE 10204276 **(2002)**.
[152] Carter AJ, Grauert M, Pschorn U, *et al*. Potent blockade of sodium channels and protection of brain tissue from ischemia by BIII 890 CL. Proc Natl Acad Sci USA 2000; 97: 4944-4949.
[153] Salvati, P., Caccia, C., Melloni, P., Restivo, A., Sabido, DC., Vallese, S.: EP1524267 **(2005)**.
[154] Caccia, C., La Porta, E., Maestroni, S., Melloni, P., Sabido, DC., Salvati, P.: EP1534265 **(2005)**.
[155] Thaler, F., Sabido, DC., Farvelli, L., Gagliardi, S., Colombo, E., Salvati, P.: EP1535908 **(2005)**.
[156] Pevarello P, Bonsignori A, Dostert P, *et al*. Synthesis and anticonvulsant activity of a new class of 2-[(arylalky)amino]alkanamide derivatives. J Med Chem 1998; 41: 579-590.
[157] Chazot PL. Safinamide (Newron Pharmaceuticals). Curr Opin Investig Drugs 2001; 2: 809-813.
[158] Salvati, P., Calabresi, M., Dho, L., Veneroni, O., Melloni, P.: WO04062655 **(2004)**.
*[159] Salvati, P., Veneroni, O., Barbanti, E., Ruggero, F., Benatti, L.: WO05018627 **(2005)**.
[160] Shao, B., Goehring, R.R., Victory, S.F., Sun, Q.: WO03022276 **(2003)**.
[161] Shao, B., Goehring, R.R.: WO03022285 **(2003)**.
[162] Goehring, R.R., Victory, S.F., Kyle, D.J.: WO03076414 **(2003)**.
[163] Zhou, X., Kyle, D.J., Sun, Q.: WO04011439 **(2004)**.
[164] Sun, Q., Kyle, D.J.: WO04010950 **(2004)**.
[165] Sun, Q., Kyle, D.J., Victory, S.F.: WO04013111 **(2004)**.
[166] Sun, Q., Kyle, D.J.: WO04013114 **(2004)**.
[167] Marra, J.M., Goehring, R.R., Perez, J., Stasaitis, L.R., Liu, Y.: WO04111011 **(2004)**.
[168] Kikuchi, K., Oku, M., Hondo, T.: WO04011430 **(2004)**.
[169] Kikuchi, K., Oku, M., Fujiyasu, J.: WO04078715 **(2004)**.
[170] Kikuchi, K., Fujiyasu, J., Watanabe, T.: WO06025471 **(2006)**.
[171] Kikuchi, K., Fujiyasu, J., Watanabe, T.: WO06025517 **(2006)**.
[172] Brown, M.L.: WO06023757 **(2006)**.
[173] Lane, C.A.L., Maw, G.N., Rawson, D.J., Thompson, L.R.: WO06011050 **(2006)**.
*[174] Fisher, F.H., Li, C., Liang, J. and others: WO03101381 **(2003)**.
[175] Kuo, H.C.H., Ayer, M.B., Chakravarty, P.K., Meinke, P.T., Parsons, W.H., Tyagarajan, S.: WO04024061 **(2004)**.
[176] Chakravarty, P.K., Fisher, M.H., Parsons, W.H., Liang, J., Zhou, B.: WO04084824 **(2004)**.
[177] Chakravarty, P.K., Fisher, M.H., Palucki, B.: WO04083189 **(2004)**.
[178] Chakravarty, P.K., Fisher, M.H.: WO04083190 **(2004)**.
[179] Park, M.K., Chakravarty, P.K., Zhou, B.: WO05047270 **(2005)**.
[180] Chakravarty, P.K., Fisher, M.H., Parsons, W.H., Tyagarajan, S., Zhou, B.: WO04092140 **(2004)**.
[181] Chakravarty, P.K., Fisher, M.H., Parsons, W.H., Tyagarajan, S., Zhou, B.: WO04094395 **(2004)**.
[182] Ok D, Li C, Abbadie C, *et al*. Synthesis and SAR of 1,2-*trans*-(1-hydroxy-3-phenylprop-1-yl)cyclopentane carboxamide derivatives, a new class of sodium channel blockers. Bioorg Med Chem Lett 2006; 16: 1358-1361.
[183] Atkinson, R.N., Drizin, I., Gregg, R.J., Gross, M.F., Kort, M.E., Shi, L.: US2005020564 **(2005)**.
[184] Atkinson, R.N., Gross, M.F.: WO03037274 **(2003)**.
[185] Gross, M.F., Atkinson, R.N., Johnson, M.S.: WO03037890 **(2003)**.
[186] Atkinson, R.N., Gross, M.F., Van Rhee, M.A.: WO03037900 **(2003)**.
[187] Ehring, G.R., Adorante, J.S., Wheeler, L.A., Malone, T., Whitcup, S.M.: US2005054695 **(2005)**.

[188] Ehring, G.R., Adorante, J.S., Donello, J.E., Wheeler, L.A., Malone, T.: US2005049287 **(2005)**.
[189] Wilson, D.M., Termin, A.P., Grootenhuis, P.D.J.: WO04078733 **(2004)**.
[190] Wilson, D.M., Martinborough, E., Neubert, T.D., Termin, A.P., Gonzalez, J.E., Zimmermann, N.: WO05003099 **(2005)**.
*[191] Wilson, D.M., Termin, A.P., Gonzalez, J.E.: WO06028904 **(2006)**.
*[192] Martinborough, E., Zimmermann, N., Perni, R., Arnost, M., Bandarage, U., Maltais, F., Bemis, G.: WO06050476 **(2006)**.
*[193] Wilson, D. M., Termin, A.P., Neubert, T.D.: WO05014558 **(2006)**.
[194] Gonzalez, J.E., Termin, A.P., Martinborough, E., Zimmermann, N.: WO05013914 **(2005)**.
[195] Wilson, D.M., Termin, A.P., Gonzalez, J.E., Zimmermann, N., Zhang, Y., Fanning, L.T.D.: WO05042497 **(2005)**.
[196] Jennings, N.S., Stokes, S., Hamlyn, R.J., Tickle, D.C., Huckstep, M.R., Lynch, R., Knuttsen, L.J.S.: WO05000309 **(2005)**.
[197] Hamlyn, R.J., Lynch, R., Stokes, S., Tickle, D.: WO05005392 **(2005)**.
[198] Morphy R, Kay C, Rankovic Z. From magic bullets to designed multiple ligands. Drug Discov Today 2004; 9: 641-651.
[199] Quessy, S.T., Rudd, G.D., Winnem, M.F.: US2002147196A1 **(2002)**.
[200] Tihanyi, K., Kocsis, P., Németh, Gy., Tarnawa, I., Dalmadi, B.: WO04089352 A2 **(2004)**.
[201] Blaugrund, E., Levy, R.: WO04045515 **(2004)**.
[202] Waibel S, Reuter A, Malessa S, Blaugrund E, Ludolph AC. Rasagiline alone and in combination with riluzole prolongs survival in an ALS mouse model. J Neurol 2004; 251: 1080-1084.
[203] Marzo A, Dal Bo L, Monti NC, *et al*. Pharmacokinetics and pharmacodynamics of safinamide, a neuroprotectant with antiparkinsonian and anticonvulsant activity. Pharmacol Res 2004; 50: 77-85.
[204] Maul, C., Przewosny, M.: DE10125145 **(2002)**.
[205] Uragg, H., Maul, C., Buschmann, H., Sundermann, B., Hennies, H.-H.: DE 10161644 **(2003)**.
*[206] Sundermann, C., Sundermann, B., Oberboersch, S., Englberger, W., Hennies, H.: DE 1004014304 **(2004)**.
*[207] Sundermann, C., Sundermann, B., Bijsterveld, E.: WO05019208 **(2005)**.
[208] Suzuki, Y., Inomata, Y.: JP05206590 **(2005)**.
[209] Harnett, J., Dolo, C., Chabrier De Lassauniere, P.: WO03062232 **(2003)**.
[210] Chabrier De Lassauniere, P., Harnett, J., Bigg, D., Liberatore, A., Thurieau, C., Dong, Z.X.: WO05035510 **(2005)**.