Turning a Drug Target into a Drug Candidate: A New Paradigm for Neurological Drug Discovery?

Steven D. Buckingham, Harry-Jack Mann, Olivia K. Hearnden, and David B. Sattelle*

The conventional paradigm for developing new treatments for disease mainly involves either the discovery of new drug targets, or finding new, improved drugs for old targets. However, an ion channel found only in invertebrates offers the potential of a completely new paradigm in which an established drug target can be re-engineered to serve as a new candidate therapeutic agent. The L-glutamate-gated chloride channels (GluCls) of invertebrates are absent from vertebrate genomes, offering the opportunity to introduce this exogenous, inhibitory, L-glutamate receptor into vertebrate neuronal circuits either as a tool with which to study neural networks, or a candidate therapy. Epileptic seizures can involve L-glutamate-induced hyper-excitation and toxicity. Variant GluCls, with their inhibitory responses to L-glutamate, when engineered into human neurons, might counter the excitotoxic effects of excess L-glutamate. In reviewing recent studies on model organisms, it appears that this approach might offer a new paradigm for the development of candidate therapeutics for epilepsy.

1. Introduction
1.1. L-Glutamate Receptors of Vertebrates and Invertebrates

L-glutamate is the main excitatory neurotransmitter in the brains of vertebrates, including humans. Vertebrate L-glutamate receptors consist of several subtypes, including both ligand-gated ion channels (ionotropic receptors) and G-protein coupled (metabotropic) receptors. L-glutamate can signal through 3 classes of ligand-gated cation channels, namely α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, N-methyl-D-aspartate receptors (NMDA) receptors, and kainate (KA) receptors, all of which are excitatory. There are also 3 classes of metabotropic receptors, all with modulatory roles (mGluRs I, II, or III).[11] NMDA receptors play a key role in brain synaptic transmission[2] and calcium entry via NMDA receptors plays an important role in synaptic plasticity, which in turn is critical for learning and memory and is also linked to memory deficits such as those associated with Alzheimer’s disease.[3] Intracellular calcium is a key second messenger in cells and its levels are normally very tightly regulated as prolonged intracellular calcium overload can be damaging or even toxic to cells.[4] Following its release and synaptic action, L-glutamate is removed via uptake into astrocytes via the L-glutamate transporter 1 (GLT-1)[5,6] and the L-glutamate/L-aspartate transporter (GLAST).[6] This is essential to retain normal sensitivity to the neurotransmitter.

Invertebrates, by contrast, have not only L-glutamate-gated cation channels, but also L-glutamate-gated anion channels (GluCls) (Figure 1A,B), which are selectively permeable to chloride ions. The GluCls differ from L-glutamate-gated cation channels, and are members of the Cys-loop ligand-gated ion channel (Cys-loop LGIC) superfamily of receptors with roles in controlling locomotion, feeding, and the processing of sensory input.[12] Fascinatingly, in invertebrates Cys-loop LGICs are much more diverse with respect to ligand-gated anion channels[13] (Figure 1C). Insights into GluCls began in the mid-1970s. By then, there was growing evidence that L-glutamate was very likely the excitatory neurotransmitter at insect neuromuscular junctions.[14] In 1976 Stuart Cull Candy showed there were two classes of extrasynaptic responses to L-glutamate detected on the insect muscle surface, one excitatory, one inhibitory. The receptors mediating extrasynaptic hyperpolarising responses to L-glutamate were termed H receptors.[15] Extrasynaptic GluCls were also present on identifiable insect neurons.[16] Studies on crustacean stomatogastric ganglion neurons showed that GluCls were postsynaptic...
L-glutamate-gated chloride channels (GluCls) are members of the Cys-loop ligand-gated ion channel superfamily (Cys-loop LGICs). Their stoichiometry remains poorly understood but functional homomers and heteromers have been reported. Thus component subunits are either identical or highly homologous. A) Each GluCl subunit contains an extensive N-terminal extracellular domain containing the dicysteine loop characteristic of the superfamily and the binding site loops at adjacent subunit interfaces that make up the orthosteric binding site where the neurotransmitter L-glutamate and glutamatergic agonists bind. Individual subunits possess four transmembrane (TM) regions, the second of which line the anion channel. An extended TM3-TM4 cytoplasmic loop precedes a short extracellular C-terminal region. B) Schematic illustration of a pentameric GluCl molecule. Structural studies have located the ivermectin binding site in the transmembrane region and the picrotoxin binding site at the inner (cytoplasmic end) end of the channel. C) The Table shows the rich diversity of the Cys-loop LGIC family in invertebrates compared to their vertebrate counterparts, particularly in the case of ligand-gated anion channels.

It is now clear that GluCls are only found in invertebrates. The closest vertebrate ligand-gated ion channels are glycine receptors, which also gate chloride channels. The novelty of GluCls resides in the fact that their permeability to chloride ions makes them inhibitory; all responses to L-glutamate described hitherto had been excitatory.

1.2. GluCl: The Target of Ivermectin, a Major Animal Health Drug

The importance of GluCls as drug targets was recognized when they were shown to be targeted by the macrocyclic lactone worming agent, ivermectin (Figure 2). The origins of this discovery stemmed from Satoshi Omura’s quest for novel substances with biological activity isolated from a newly described bacterium (Streptomyces avermitilis). William Campbell, working at Merck, identified antiparasitic activity in the *S. avermitilis* culture and isolated the active constituent, named avermectin. Further studies led to the generation of ivermectin, which is active against several parasites of domestic animals and farm livestock. Ivermectin went on to become the best known worming agent for livestock but was also found to be very effective in removing ticks. It is the first agent known to remove both internal and external parasites, a property earning it the classification of “endectocide”. It was the first endectocide discovered and is still in use today, due at least in part to its ease of use, broad spectrum of activity, relatively slow onset of resistance and, with the sole exception of collie dogs, an excellent safety profile. The effectiveness of ivermectin is due to its opening the GluCl channel, causing membrane hyperpolarization and hence inhibiting neuronal firing. At higher concentrations than those active on GluCls, ivermectin also acts upon GABA and glycine receptors.

The effectiveness of ivermectin, which still remains a mainstay for the control of animal parasites, has inspired the development of a number of successful ivermectin-like compounds (Figure 2). For example, doramectin was developed by mutating *S. avermitilis*; based on this work, selamectin was subsequently derived. Abamectin is a widely-used acaricide and insecticidal seed protectant and is used in the control of fire ants imported to the USA. Eprinomectin was developed in the mid-1990s and is used as a topical endectocide. Moxidectin belongs to a related class of macrocyclic lactones, the milbemycins. Unlike ivermectin, which was isolated from *S. avermitilis*, the milbemycins were isolated from *Streptomyces hygroscopus* and from *Streptomyces cyaneogriseus*. Although the milbemycins and avermectins form distinct subfamilies of the macrocyclic lactones (Figure 2), they are chemically very similar. Milbemycins also act on similar GluCl binding sites, based on their strong cross-resistance.

2. GluCl Ligand (Ivermectin) Transitions from an Animal Health Drug to a Human Drug

2.1. A Treatment for River Blindness

Onchocerciasis (River Blindness), which affects 25 million people, is the result of infection by a parasitic nematode worm...
Onchocerca volvulus. The common name for the disease stems from its transmission following bites by blackflies of the genus Simulium, whose early life stages are aquatic. In those infected by the parasite, worms migrate to the eye causing visual impairment or blindness. Globally, onchocerciasis is a major infectious cause of blindness, second only to trachoma, and is a neglected tropical disease. Lymphatic filariasis, another neglected tropical disease, causes chronic tissue swelling and leads to the disabling clinical symptoms, referred to as elephantiasis (lymphedema) resulting from infection by parasitic worms, notably Wuchereria bancrofti, Brugia malayi, and Brugia timori. Elephantiasis afflicts over 120 million people, 40 million of whom are severely affected.\(^{[36]}\)

The recommended treatment for both onchocerciasis and lymphatic filariasis is ivermectin. In the case of onchocerciasis, ivermectin is given every 6 months for the lifespan of the adult worms, or for as long as the infected person has evidence of skin or eye infection. Ivermectin kills worm larval stages, thereby preventing tissue damage but it does not kill the adult worms. The antibiotic doxycycline does kill the adult worms by eliminating the Wolbachia bacteria on which adult worms depend. Clinicians may recommend treatment with both ivermectin and the antibiotic. Before any treatment commences, it is vital to establish that patients are not also infected with Loa loa, another filarial parasite often found in the same regions of Africa where O. volvulus is prevalent. Co-infection with L. loa can lead to severe side effects to the medications used to treat onchocerciasis. A strategy recommended by the global filariasis elimination programme is a single-dose ivermectin with or without albendazole. Thus ivermectin, while retaining utility for animal health applications, has fully transitioned into a drug for treating human infections. Campbell and Omura were recipients of the Nobel Prize in Physiology or Medicine in 2015\(^{[37]}\) for their roles in the discovery of ivermectin.

2.2. Resistance Threatens Usefulness of the GluCl-Targeting Drug Ivermectin

As with so many antiparasitic drugs and pest control agents, the use of ivermectin has been limited by the rise of resistance.\(^{[38]}\)
Resistance to ivermectin was first reported in sheep in 1979[39] whereas moxidectin resistance was first reported in 1995.[41] In 2000, one group found that resistance to ivermectin in the genetic model organism, *Caenorhabditis elegans*, could arise when three GluCl genes were mutated[45] while deletions in another GluCl (glc-1) were found to be associated with resistance in natural populations of *C. elegans*.[46] A list of all GluCl genes present in the free-living nematode (*C. elegans*) and a parasite (*Haemonchus contortus*) are shown in Table 1. There are other candidate targets of ivermectin resistance. For example, mutations of other neuronal proteins such as DYF-7 also confer resistance.[42] More work is needed to understand better the molecular basis of ivermectin resistance in nematodes. There are also reports of resistance in insects,[43–47] in which only one GluCl gene is present.[12] Whatever the causes of resistance, the loss of the protective power offered by ivermectin and related macrocyclic lactones will have a significant impact on animal health and hence agricultural productivity. Thus, new chemistries are needed to ensure a pipeline of new compounds as candidate replacements.

### 2.3. New Natural Product Drug Leads that Target GluCls

To combat resistance problems, new synthetic and natural products are being pursued. Okaramines (Figure 2) are prenylated indole alkaloids of fungal origin, which may serve as candidate leads for a new generation of GluCl targeting drugs. Okaramines were purified from the fermentation products of *Penicillium simplicissimum* growing on the pulp (Japanese, okara) left over from the production of soya cake and tofu. The first okaramines isolated (A and B) (Figure 2) were shown to be highly toxic to larvae of the silkworm (*Bombyx mori*).[48] The okaramine-treated silkworm larvae were rapidly killed. This alerted Kazuhiro Matsuda and colleagues to the possibility that they might act on ion channels, which are known targets of several classes of fast acting insecticides.[49] Like ivermectin, okaramine B produced inhibitory responses on silkworm (*B. mori*) larval neurons that reversed close to the chloride equilibrium potential.[26] Studies on cloned receptors (*B. mori* GluCls, *B. mori* GABA-gated chloride channels) functionally expressed in *Xenopus laevis* oocytes were pursued. Okaramine B activated silkworm GluCls but not silkworm GABA-gated chloride channels.[26] In the same study it was shown that okaramine B, the most active okaramine on insect GluCls, was not active on human glycine receptors or human GABA-gated chloride channels. Subsequent studies showed that the GluCl target site actions of 4 okaramines (A, B, Q, and B-H2, see Figure 2) agreed well with their insecticidal potency.[50] In addition to targeting insect GluCls, okaramine B also targets GluCls of the tick *Ixodes scapularis*.[51] When compared to ivermectin, okaramines have an even more attractive specificity profile, being inactive against insect GABA receptors and against human GABA or glycine receptors.[26] Okaramines N, J, and G have been chemically synthesized and okaramine I (Figure 2) has been generated by a biosynthetic route.[55]

The insect order Lepidoptera is second only to the Coleoptera (beetles) in the number of species it contains and many cultivated crop plants that make up the world’s food supply as well as trees that make up the world’s forests are targeted by larvae of caterpillars of lepidopteran pests. However, it is important to note that many lepidoptera are nectar feeders and therefore pollinators. It will be of interest to explore okaramine selectivity further in the search for new natural product-based leads for novel, safer, pest control chemicals.

### 3. The Crystal Structure of a *C. elegans* GluCl Provides Insights into Ivermectin Action

GluCls attracted considerable interest when in 2011 GLC-1 (GluCla) became the first member of the Cys-loop LGIC family to be crystallized by Ryan Hibbs and Eric Gouaux.[56] Prior to this, strong attention had been placed on structural studies of nicotinic acetylcholine receptors. The superb electron diffraction images obtained by Nigel Unwin and colleagues from native nAChRs from *Torpedo marmorata* electric organ (modified muscle) membranes with their high density arrays of receptors yielded important insights first at 4.6 Å resolution[57] and then at a resolution of 4.0 Å.[58] From this work, the 5 component subunits, the position of the ACh binding site and the cation channel in the open and closed states could be discerned. In addition, partial crystal structures of the extracellular region of the muscle nAChR with the snake toxin α-bungarotoxin bound added further insights,[59] as did the co-crystalization of the soluble acetylcholine binding protein, an excellent surrogate for the extracellular nAChR domain, with various ligands bound.[60–62]

Thus it was exciting to see the 3.3 Angstrom resolution X-ray structure of the *C. elegans* GLC-1-Fab complex. Moreover, structures were determined with L-glutamate occupying the orthosteric binding site, the binding site of the neurotransmitter L-glutamate and glutamatergic agonists.[56] L-glutamate binds in the extracellular region at the interface of adjacent subunits. Structures with the allosteric agonist ivermectin bound and structures with the open-channel blocker picrotoxin bound were also reported.[56] Picrotoxin occludes the channel at its inner, cytoplasmic end. Ivermectin binds in the transmembrane region of the receptor, stabilizing an open-pore conformation. Thus the GluCl X-ray structure has offered a framework for understanding mechanisms of fast inhibitory neurotransmission and allosteric modulation of Cys-loop LGICs.
Interestingly GLC-1 is only weakly activated by L-glutamate when expressed in X. laevis oocytes as a homomer although L-glutamate activates the β homomer (GLC-2) and the αβ (GLC-1/GLC-2) heteromer. Henry Lester and colleagues deployed mutagenesis on the closely related C. elegans GluCl β subunit (GLC-2) to demonstrate cation-π interactions between Y206 and the protonated amine of L-glutamate. They also identified other important ionic and hydrogen bond interactions between the ligand and the binding site consistent with the crystal structure. [63]
4. GluCls: Repurposing a Drug Receptor as a Neurobiology Tool and a Candidate Therapeutic

4.1. Engineered GluCls as Switches to Help Understand Neuronal Circuitry

The identification and cloning of drug receptors that cause the cell to respond to the presence of a particular compound gave rise to the prospect of exploiting those receptors as tools to manipulate populations of neurons, turning the receptor into a probe with which to study neural circuits. Optogenetics, for example, takes the approach of exogenously expressing an engineered photoreceptor in a target population of neurons and using the channel activator (light) to control this switch. Another engineering approach known as chemogenetics takes this one step further by introducing a “designer receptor” into the target organism and activating it with a ligand that otherwise exerts no effect on the organism.

This chemogenetic approach was adopted in a body of work in Henry Lester’s lab, taking advantage of the fact that there are no GluCls in vertebrates. Lester’s group adapted the C. elegans GluCl α (GLC-1) and β (GLC-2) encoding genes and heterologously expressed them in cultured rat hippocampal cells (Figure 3). The expression of this construct did not affect the background activity of the neurons but when ivermectin was applied, the cells were strongly hyperpolarized and spiking was blocked. The effect of ivermectin was reversible, but it was not possible to determine whether that was due to dissociation of the ligand from its binding site or to receptor turnover (Figure 3).

There were concerns that endogenous L-glutamate could also inactivate neurons and further mutations of the GluClβ construct were undertaken including the removal of its L-glutamate sensitivity. The Y182F mutation reduced the L-glutamate response to about 15% of that observed for the wild type subunit, with no impact on the response to ivermectin. Interestingly the tyrosine at position Tyr-182 aligns with nicotinic receptor residue Trp-149 which is crucial to cation-γ interactions with ACh (65) and so it was concluded that tyrosine 182 is important for L-glutamate interactions in GluCls. In a further development, fluorescent proteins were introduced into the M3-M4 intracellular loop of the GluCl without impact on receptor function facilitating the detection of expression levels of the new tool.

The authors injected the modified construct into the striatum of mice and found that by applying ivermectin they could reversibly modulate the behavior of freely moving animals (66). A widely used assay of unilateral striatal damage involves applying amphetamine. This causes increased activity in striatal neurons on the side of the brain where the amphetamine was applied, which (since the striatum plays a key role in the regulation of movement) results in turning towards the lesioned side. Lester’s group applied amphetamine systemically to mice which were unilaterally expressing the GluCl construct. As is the case for striatal lesions, application of ivermectin resulted in rotational behavior. Strikingly, this effect was readily reversible, to the extent that several ivermectin/wash cycles were feasible on a single animal. Further optimization of the construct (which they dubbed “GluClv1.0”) resulted in a yet more robust version (“GluClv2.0”) that had enhanced sensitivity to ivermectin, which meant that much lower concentrations of ivermectin were needed to produce an effect (67).

The important significance of Lester’s work is the demonstration that a “headless” (i.e., lacking L-glutamate-sensitivity) invertebrate GluCl can be used as a switch to “turn off” populations of neurons. The technique offers several distinct advantages. Unlike optogenetics, for example, no special apparatus is needed to activate the “switch”. Ivermectin is known not to produce changes in behavior and is considered a safe product. The specificity and versatility of targeting through judicious choice of promoter that has made optogenetics so successful is also available in principle to GluClv2.0. There is perhaps also future potential for an even greater margin of safety using ligands more selective than ivermectin. More selective ligands are now known (26) but at present we know little regarding their suitability for use in such studies.

4.2. GluCl Provides a Candidate Therapy for Epilepsy

The work of Lester’s lab illustrates the unique opportunities afforded by chemogenetics using the invertebrate GluCl. A more recent development has shown how GluCl might be adapted in a therapeutic application of the principles underlying chemogenetics. Dimitri Kullmann and colleagues mutated a GluCl to impart an increased L-glutamate sensitivity and expressed it in a rat model of epilepsy (68). Although the precise physiological basis of epilepsy remains elusive, it is associated with excessive neuronal firing over wide areas of the cortex. Current pharmacological treatments (such as phenytoin and valproate) ameliorate seizures largely by binding to the inactivated state of the sodium channel, and so preferentially inhibiting neurons from firing at high rates. However, this treatment only poorly distinguishes between neurons firing at abnormally highly rates (presumed to underlie seizures) and those spiking at lower rates, resulting in a very narrow therapeutic window. The rationale behind Kullmann’s exciting approach was that engineered GluCls under the appropriate promoter express outside synapses, and so will only be activated by L-glutamate that has spilled out of synapses—a situation which does not usually arise at normal firing rates but is a feature of epileptic seizures (Figure 4).

Kullmann and colleagues tested the effect of the engineered GluCl (delivered tonically through a cannula) on two models of epilepsy: generalized seizures induced with pilocarpine and a model of spontaneous focal seizures caused by application of tetanus toxin. In both models, measures of convulsant behavior were significantly reduced, and no adverse effect was seen over a large range of tests of normal behavior. This approach provides a self-correcting mechanism whereby spread of excess L-glutamate causes a hyperpolarization of the affected cells, which would balance the otherwise excitatory effect of L-glutamate acting through native receptors.

5. Conclusions, Outlook, and Challenges

L-glutamate-gated chloride channels (GluCls) are established targets for anti-parasitic drugs impacting first animal and then human health. A timeline showing this progression and their
Figure 4. GluCl engineered as a candidate therapy for epilepsy. A) An L-glutamatergic synapse showing L-glutamate released from a presynaptic nerve terminal acting on postsynaptic receptors which activate cation channels and lead to excitatory postsynaptic responses in the postsynaptic neuron. In the condition of epilepsy release of excess L-glutamate can overload the clearance machinery and lead to the prolonged presence of the neurotransmitter L-glutamate in synaptic and extrasynaptic regions. B) The introduction of an engineered L-glutamate gated chloride channels (eGluCls) into extrasynaptic regions will mean the excess L-glutamate activates chloride channels, thereby resulting in a hyperpolarising counter to L-glutamate excitation, resulting in reduction or elimination of seizures.

Figure 5. Timeline showing some important milestones in the development of our understanding of GluCls and their roles. The various transitions from animal health drug target to human health drug target[24] are highlighted, together with the development of a useful neurobiological tool for neural circuit modulation[64,66,67] and finally ongoing work towards a therapeutic candidate for the control of epileptic seizures.[68]

Further development as neurobiological tools and deployment as exotargets (heterologously expressed targets) to combat epilepsy[68] is illustrated (Figure 5). The attraction of using an exotarget to automatically counterbalance a disorder arising from an excess of transmitter stems from pharmacodynamics that are dependent on the disease state; the exotarget does nothing until there is an excess of neurotransmitter. The approach could perhaps be adapted to other disorders. In Huntington’s disease, the accumulation of huntingtin protein results in an increased sensitivity of NMDA receptors.[69] A therapy could be envisaged in
which expression of an engineered GluCl-based exotarget could be directed to cells expressing high levels of huntingtin, providing an inhibitory counterbalance to L-glutamate excitotoxicity. For many neurodegenerative diseases, excitotoxicity is a final common pathway suggesting that a similar approach could be applied to other conditions involving neuronal cell loss, including stroke and traumatic brain injury.

There are other ion channels found in invertebrates but not in vertebrates that could perhaps supply new seams for the exploitation of exotargets. For example, an ACh-gated chloride channel is present in nematodes and flatworms, which could perhaps be used to build an exotarget for the relief of Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFL). This disorder involves a hypersensitivity of acetylcholine receptors to their natural ligand. While the technical challenges to such approaches appear to be surmountable, a barrier to their development will be the necessary caution surrounding the heterologous expression of engineered receptors into human tissue, especially brain tissue. However, in the case of diseases which are both devastating, incurable and lack effective alternative treatments, as is the case for certain types of epilepsy and neurodegenerative diseases, the careful exploration of this novel therapeutic paradigm merits attention.

Acknowledgements
S.B. and H.-J.M. contributed equally to this work.

Conflict of Interest
The authors declare no conflict of interest.

Keywords
chemogenetic tool, epilepsy, L-glutamate-gated chloride channel, ivermectin, novel therapy

Received: January 23, 2020
Revised: May 26, 2020
Published online:

[1] S. P. H. Alexander, A. Mathie, J. A. Peters, Br. J. Pharmacol. 2011, 164, S1.
[2] K. B. Hansen, F. Yi, R. E. Perszyk, H. Furukawa, L. P. Wollmuth, A. J. Gibb, S. F. Traynelis, J. Gen. Physiol. 2018, 150, 1081.
[3] J. Liu, L. Chang, Y. Song, H. Li, Y. Wu, Front. Neurosci. 2019, 13, 43.
[4] M. J. Berridge, J. Physiol. 2014, 592, 281.
[5] K. Takahashi, J. B. Foster, C.-L. G. Lin, Cell. Mol. Life Sci. 2015, 72, 3489.
[6] O. Šerý, N. Sultana, M. A. Kashem, D. V. Pow, V. J. Blcar, Neurochem. Res. 2015, 40, 2461.
[7] J. Wang, Z.-J. Lin, L. Liu, H.-Q. Xu, Y.-W. Shi, N. He, W.-P. Liao, Seizure 2017, 44, 11.
[8] J. A. Hubbard, J. I. Suz, J. M. Yonan, D. K. Binder, Exp. Neurol. 2016, 283, 85.
[9] D. A. Sun, S. Sombati, R. E. Blair, R. J. DeLorenzo, Epilepsia 2002, 43, 1296.
[10] M. P. Mattson, in Stress: Physiology, Biochemistry, and Pathology (Ed: G. Fink), Academic Press, Massachusetts, USA 2019, Ch. 11.
[11] S. Zhang, Y. Zhu, J. Cheng, J. Tao, in Epilepsia: Advances in Diagnosis and Therapy (Ed: I. J. Al-Zwaini), IntechOpen, London 2019.
[12] A. J. Wolstenholme, J. Biol. Chem. 2012, 287, 40232.
[13] V. Raymond, D. B. Sattelle, Nat. Rev. Drug Discovery 2002, 1, 427.
[14] S. G. Cull-Candy, P. N. Usherwood, Nature, New Biol. 1973, 246, 62.
[15] S. G. Cull-Candy, J. Physiol. 1976, 255, 449.
[16] K. A. Wafford, D. B. Sattelle, Neurosci. Lett. 1986, 63, 135.
[17] M. Bidaut, J. Neurophysiol. 1980, 44, 1089.
[18] E. Marder, J. S. Eisen, J. Neurophysiol. 1984, 51, 1362.
[19] E. Marder, D. Paupardin-Tritsch, J. Physiol. 1978, 280, 213.
[20] S. Dutertre, M. Drwal, B. Laube, H. Betz, J. Neurochem. 2012, 122, 38.
[21] D. Colquhoun, L. G. Sivilotti, Trends Neurosci. 2004, 27, 337.
[22] J. Vercruysse, R. Rew, in Macrocyclic Lactones in Antiparasitic Therapy (Eds: J. Vercruysse, R. Rew), CAB Publishing, Wallingford, UK 2002, pp. 1–464.
[23] W. C. Campbell, G. W. Benz, J. Vet. Pharmacol. Ther. 1984, 7, 1.
[24] A. Crump, S. Omura, Proc. Jpn. Acad., Ser. B 2011, 87, 13.
[25] W. C. Campbell, Parasitol. Today 1985, 1, 10.
[26] S. Furutani, Y. Nakatani, Y. Miura, M. Iharra, K. Kai, H. Hayashki, K. Matsuda, Sci. Rep. 2014, 26, 4, 6190.
[27] X.-P. Feng, J. Hayashi, R. N. Beech, R. K. Prichard, J. Neurochem 2002, 83, 870.
[28] Q. Shan, J. L. Haddrell, J. W. Lynch, J. Biol. Chem. 2001, 276, 12556.
[29] A. C. Goudie, N. A. Evans, K. A. F. Graton, B. F. Bishop, S. P. Gibson, K. S. Holdom, B. Kaye, S. R. Wicks, D. Lewis, A. J. Weatherley, C. I. Bruce, A. Herbert, D. J. Seymour, Parastology 1993, 49, 5.
[30] B. J. Banks, B. F. Bishop, N. A. Evans, S. P. Gibson, A. C. Goudie, K. A. Graton, M. S. Pacey, D. A. Perry, M. J. Witty, Bioorg. Med. Chem. 2000, 8, 2017.
[31] The Merck Index Online—chemicals, drugs and biologicals. Available at: https://www.rsc.org/merck-index, retrieved on January 10th 2020.
[32] R. T. Meister, C. Sine, Crop protection Handbook, Meister Media Worldwide, Willoughby, Ohio 2014.
[33] W. L. Shoop, J. R. Egerton, C. H. Eary, H. W. Haines, B. F. Michael, H. Mrozik, P. Eskola, M. H. Fisher, L. Stlayton, D. A. Ostlind, B. J. Skelly, R. K. Fulton, D. Barth, S. Costa, L. M. Gregory, W. C. Campbell, R. L. Seward, M. J. Turner, Int. J. Parasitol. 1996, 26, 1237.
[34] C. Paraud, T. Marcotti, A. Lespine, J. F. Sutra, I. Pors, I. Devos, Vet. Parasitol. 2016, 226, 88.
[35] N. V. S. V. Melchers, S. Mollenkopf, R. Colebunders, M. Edlinger, L. E. Coffeng, J. Irani, T. Zola, J. N. Siewe, S. J. de Vlas, A. S. Winkler, W. A. Stolk, Infect. Dis. Poverty 2018, 7, 101.
[36] E. A. Ottesen, in Advances in Parasitology (Ed: D. H. Molyneux), Academic Press, Massachusetts, USA 2006, pp. 395–441.
[37] The Nobel Prize in Physiology or Medicine 2015. NobelPrize.org (accessed: January 2020).
[38] R. K. Prichard, Expert Opin. Drug Discovery 2007, 2, 541.
[39] J. V. Wyk, F. S. Malan, Vet. Rec. 1988, 123, 226.
[40] J. A. Dent, M. M. Smith, D. K. Vassilatis, L. Avery, Proc. Natl. Acad. Sci. USA 2000, 97, 2674.
[41] R. Ghosh, E. C. Andersen, J. A. Shapiro, J. P. Gerke, L. Kruglyak, Science 2012, 335, 574.
[42] L. Urdaneta-Marquez, S. H. Bae, P. Janukavicius, R. Beech, J. Dent, R. Prichard, Int. J. Parasitol. 2014, 44, 1063.
[43] A. George, C. N. Rao, S. Chike, V. Dhengre, J. Econ. Entomol. 2017, 110, 525.
[44] J. S. Ferguson, J. Econ. Entomol. 2004, 97, 112.
[45] O.-B. Wei, Z.-R. Lei, R. Nauen, D.-C. Cai, Y.-L. Gao, Insect Sci. 2015, 22, 243.
