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Themed Section: Recent Advances in Targeting Ion Channels to Treat Chronic Pain

REVIEW ARTICLE

Targeting nociceptive transient receptor potential channels to treat chronic pain: current state of the field

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Control of chronic pain is frequently inadequate and/or associated with intolerable adverse effects, prompting a frantic search for new therapeutics and new therapeutic targets. Nearly two decades of preclinical and clinical research supports the involvement of **transient receptor potential (TRP) channels** in temperature perception, nociception and sensitization. Although there has been considerable excitement around the therapeutic potential of this channel family since the cloning and identification of **TRPV1** cation channels as the **capsaicin** receptor more than 20 years ago, only modulators of a few channels have been tested clinically. TRPV1 channel antagonists have suffered from side effects related to the channel's role in temperature sensation; however, high dose formulations of capsaicin have reached the market and shown therapeutic utility. A number of potent, small molecule antagonists of **TRPA1** channels have recently advanced into clinical trials for the treatment of inflammatory and neuropathic pain, and **TRPM8** antagonists are following closely behind for cold allodynia. **TRPV3**, **TRPV4**, **TRPM2** and **TRPM3** channels have also been of significant interest. This review discusses the preclinical promise and status of novel analgesic agents that target TRP channels and the challenges that these compounds may face in development and clinical practice.

LINKED ARTICLES

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Abbreviations

AITC, allyl isothiocyanate; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglia; SP, substance P

Introduction

Acute pain is defined as an 'unpleasant sensory and emotional experience associated with actual or potential tissue damage' (http://www.iasp-pain.org/taxonomy). In this context, pain can be considered as a crucial alarm system that triggers protective mechanisms. Beneficial acute pain can become pathological chronic pain under certain conditions and the molecular pathways that underlie this transition are poorly understood, hampering drug development. Chronic pain is now understood to be a disease and constitutes the most common reason why patients seek medical care (Lambert, 2010). Indeed, the American Pain Society estimates that chronic pain affects more than 100 million Americans and costs the USA about $635 billion each year in health care costs and lost productivity (Stewart *et al.*, 2003). As the population ages, these numbers will only increase.

The current pain therapeutic market is dominated by agents that have been around for decades such as narcotic analgesics (opioids), non-steroidal anti-inflammatory drugs (NSAIDs) and gabapentinoids. Opioids are effective pain killers, but their use is limited by addiction, sedation, respiratory depression, constipation and itch (Benyamin *et al.*, 2008). In the USA, opioid abuse is seen as a medical crisis. In 2014, 28 000 Americans died from opioid overdose. That number rose 15.6% in 2015 (Rudd *et al.*, 2016) and is predicted to be even higher in 2016

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and 2017 (Katz, 2017), so doctors are anxious to avoid these drugs whenever possible. NSAIDs have only modest effects on moderate to severe pain, and their use is limited by a combination of gastrointestinal and cardiovascular side effects (http://www.fda.gov/downloads/drugs/drugsafety/ucm089162.pdf). Gabapentinoids, originally prescribed to control epileptic seizures, show efficacy in many patients but can cause sedation and weight gain (Schmidt *et al.,* 2013). This can be particularly problematic in a diabetic population where weight gain can exacerbate disease. Clearly, there is a critical need for new pain killers that are effective and safe.

In recognition of this need, the US Congress proclaimed the decade commencing on 1 January 2001 as the 'Decade of Pain Control and Research' (Brennan, 2015). This act gave new impetus (and funding) to preclinical pain research and helped identify an array of novel molecular mechanisms (many of which are reviewed in this Themed Issue) involved in the development and maintenance of chronic pain. A breakthrough discovery was the cloning of the capsaicin receptor and its identification as the cation channel **TRPV1** (Caterina *et al.,* 1997). This finding proved to be the catalyst for identification of the nociceptive TRP channels discussed in this review (**TRPV3** and **TRPV4**, **TRPA1**, **TRPM2**, **TRPM3** and **TRPM8**). When the TRPV1 channel was discovered, there was a flurry of research activities into new drug possibilities. Indeed, it took less than a decade for the first potent, low MW antagonists of TRPV1 channels to enter Phase 1 clinical trials (Szallasi *et al.,* 2007). However, despite the large investment put towards research into TRPV1 channels, there are no drugs in Phase 3 clinical trials. In this review, we aim to capture the progress in the nociceptive TRP channel field over the past 20 years. Despite the roadblocks and difficulties, TRP channels remain powerful tools in pain research and represent promising therapeutic targets.

A brief overview of TRP channels

Great discoveries sometimes have unexpected beginnings. In 1969, Cosens and Manning (1969) described a *Drosophila* mutant that was defective in light sensing: when exposed to continuous light, this fruit fly exhibited only a transient receptor potential (TRP) instead of the normal sustained response. This observation was explained by a defect in a non-selective cation channel, and the gene responsible for this abnormal light response was eventually cloned by Montell and Rubin in 1989. Six years later, mammalian homologues of the *Drosophila* TRP cation channel were discovered (Wes *et al.,* 1995; Zhu *et al.,* 1995). Since then, the number of mammalian proteins recognized as TRP channels has grown to form a superfamily of 28 ion channels (27 in humans) with varied physiological functions (see Wu *et al.,* 2010). The mammalian TRP superfamily has been divided into six subfamilies based on sequence homology: canonical (TRPC1 to TRPC7), vanilloid (TRPV1 to TRPV6), melastatin (TRPM1 to TRPM8), ankyrin (TRPA1), mucolipin (TRPML1 to TRPML3) and polycystin (TRPP1 to TRPP3). A crucial discovery was that TRP channel dysfunction ('TRP channelopathy') can cause a wide range of pathological conditions (see Nilius and Szallasi, 2014). Indeed, two TRP subfamilies, mucolipin and polycystin, were named after the hereditary diseases they are associated with: mucolipidosis and polycystic kidney disease respectively.

All TRP channels share some structural similarities, including six transmembrane spanning regions (S1 to S6) and a pore-forming loop between S5 and S6. Moreover, most TRP channels are believed to function as homo- or heterotetramers. Otherwise, few generalizations can be made about TRP channels. As TRP subfamilies are based on sequence homology and not function, channels can be grouped together that have little in common. For example, whereas TRPV1 is a non-selective cation channel implicated in pain and thermosensation expressed predominantly on nociceptive neurons, TRPV6 is the main channel for intestinal Ca^{2+} transport that controls bone mineralization (see Nilius and Szallasi, 2014).

Using electron cryo-microscopy, the structures of several TRP channels have been elucidated. Fittingly, TRPV1 was the first TRP channel whose structure was published (Liao *et al.,* 2013), followed by TRPA1 (Paulsen *et al.,* 2015), TRPV2 (Huynh *et al.,* 2016; Zubcevic *et al.,* 2016), TRPV6 (Saotome *et al.,* 2016) and, most recently, TRPP2 channels (Grieben *et al.,* 2017). Highlights of the structural features of the key channels discussed in this review are depicted in Figure 1. Consistent with their diverse structure, these channels serve diverse afferent (transduction of mechanical, chemical and thermal stimuli) and efferent (e.g. growth control, cellular differentiation, thermoregulation, vasoregulation and mediator release) functions (see Wu *et al.,* 2010; Nilius and Szallasi, 2014). Many TRP channels are polymodal, meaning that they can be activated by a wide range of physical (voltage, temperature, force, pressure and tension) and chemical stimuli (see Nilius and Szallasi, 2014). Some of the key activators are shown in Figure 2. This range of activators is important because the ability to respond to such divergent stimuli renders TRP channels coincidence detectors and cellular signal integrators. On the one hand, their ability to respond to multiple types of stimuli makes TRP channels attractive therapeutic targets, but on the other hand, this presents challenges for drug development in terms of on-target adverse effects. For example, TRPV1 channels participate in the detection of noxious heat (Caterina *et al.,* 1997; Bölcskei *et al.,* 2005), thus it is not entirely unexpected that pharmacological blockade of these channels can cause burn injuries as a side effect, by compromising noxious heat sensation (Eid, 2011; Manitpisitkul *et al.,* 2016).

Although TRP channels are evolutionarily highly conserved, their sensitivity to external stimuli shows intriguing species-related differences. For example, the TRPV1 channel detects noxious heat in many mammals including humans but not in camels and ground squirrels (Laursen *et al.,* 2016). This dramatic change in heat sensitivity results from a single amino acid substitution. Moreover, TRPV1 channels are activated by capsaicin in mammals but not in birds, forming the scientific basis of capsaicin-laced, 'squirrel-free' bird feed (Curtis *et al.,* 2000). A theory posits that the pepper plant uses capsaicin as a deterrent against mammalian herbivores but not against birds, which are needed for seed dispersal (Tewksbury and Nabhan, 2001).

As mentioned above, several hereditary diseases caused by defects in genes encoding TRP channels have been described (see Nilius and Szallasi, 2014). Given the pivotal role that TRP channels are thought to play in nociceptive transduction (Patapoutian *et al.,* 2009; Moran *et al.,* 2011a), it is somewhat unexpected that so far, only an obscure human painful pain condition, Familial Episodic Pain Syndrome, has been linked

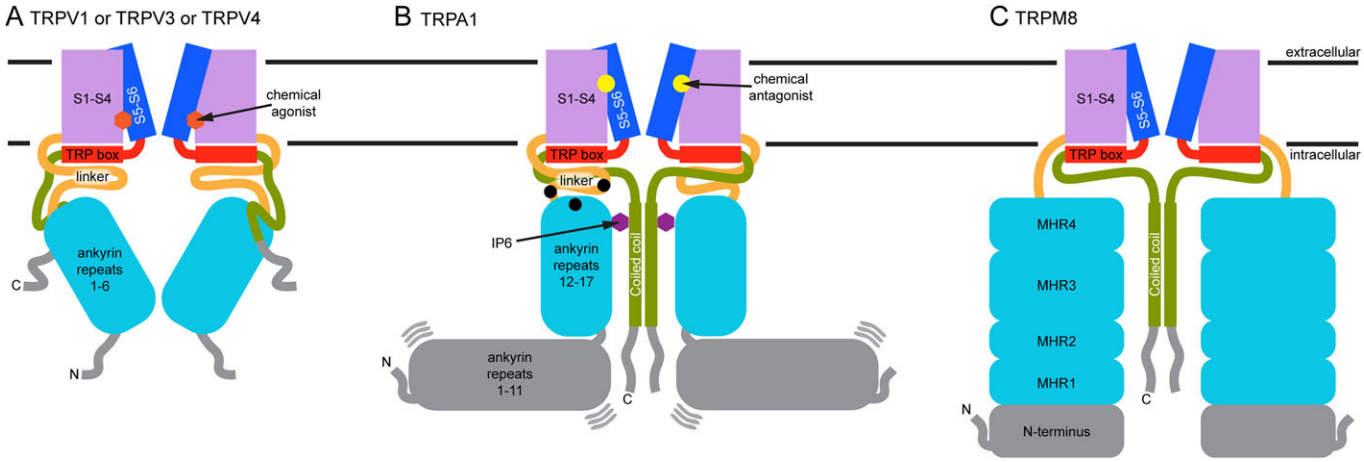

Figure 1

Schematic diagrams of TRP channels, showing two of the four subunits in the tetrameric structures. All panels follow the same colour scheme from N- to C-terminus: N-terminal cytoplasmic domain, turquoise; linker, yellow; S1–S4, lilac; S5–S6, blue; TRP-box helix, red; C-terminus, green; and flexible N- or C-terminal extensions, grey. (A) TRPV1, TRPV3 and TRPV4 proteins contain six ankyrin repeats in their N-terminus. The C-terminus interacts with the ankyrin repeats, connecting the cytoplasmic regions. A chemical agonist (e.g. capsaicin or resiniferatoxin for TRPV1) binding site was identified at the interface of the S1–S4 and S5–S6 regions. Of note, TRPV4 contains a proline-rich region in its flexible N-terminal extension. (B) TRPA1's unique structural features include a C-terminal coiled-coil region and 17 ankyrin repeats in the N-terminus; repeats 1–11 were not modelled in the cryoEM structure and are likely to connect to repeats 12–17 through a flexible link. An IP6 molecule bridges the N-terminal ankyrin repeats to the C-terminal coiled-coil. A chemical antagonist, A967079, binds within the S5–S6 pore region (yellow circle). Cysteines sensing reactive electrophiles are represented as black circles in the membrane-proximal N-terminal linker region that surrounds the TRP-box helix (red). (C) No structure is yet available for TRPM8, but its transmembrane domain is homologous to that of TRPV1 and TRPA1. The N-terminal region contains 'melastatin homology regions' (MHRs), and its C-terminus contains a predicted coiled coil.

to a TRP channelopathy, namely, a gain-of-function mutation in TRPA1 channels (Kremeyer *et al.,* 2010). That said, multiple polymorphisms in TRP channels have been linked to pain susceptibility (Nilius *et al.,* 2007; Binder *et al.,* 2011; Carreno *et al.,* 2012; Park *et al.,* 2016).

The vanilloid (capsaicin) receptor TRPV1: a 'hot' pain target

The neuroanatomy and pharmacology of TRPV1-expressing neurons

The prototypical nociceptive TRP channel is the capsaicin receptor TRPV1. Indeed, a whole subdivision of nociceptive sensory neurons (capsaicin-sensitive neurons) is named after their unique sensitivity to **capsaicin** (structure shown in Figure 3; see Szallasi and Blumberg, 1999). These neurons are bipolar cells with somata in the dorsal root ganglia (DRG), trigeminal and nodose ganglia. Generally speaking, capsaicin-sensitive neurons detect harmful stimuli both in the external and internal milieu and convey this information to the CNS. For example, central axons of DRG neurons enter the dorsal horn of the spinal cord where they form synapses with second-order neurons that subsequently relay information to the somatosensory cortex.

The existence of a capsaicin receptor was first postulated based on the strict structure–activity relations for capsaicin-like activity (Szolcsányi and Jancsó-Gábor, 1975). Specific binding of [^{3}H]**resiniferatoxin** furnished a biochemical proof for this receptor (Szallasi and Blumberg, 1989), which was eventually identified as the cation channel TRPV1 (Caterina *et al.,* 1997). The TRPV1 channel is a polymodal receptor: its activators range from innocuous warmth and noxious heat (above 43°C) to changes in pH (both acidic and alkaline) to 'endovanilloids' (e.g. anandamide) and a variety of pungent plant products (see Figure 2). The latter group includes capsaicin (responsible for the piquancy of hot chilli pepper), resiniferatoxin (from the latex of *Eucalyptus resinifera*), piperine (the pungent ingredient in black pepper), gingerol and zingerone (from ginger), camphor and eugenol (an essential oil found in cloves that exerts substantial biological effects) (see Szallasi *et al.,* 2007). Not unexpectedly, TRPV1 channels are activated by painful venoms from a wide range of species including jellyfish (Cuypers *et al.,* 2006), spiders (Siemens *et al.,* 2006) and centipedes (Yang *et al.,* 2015).

Connoisseurs of hot, spicy food are intimately familiar with the predominant pharmacological actions of capsaicin from personal experience. Capsaicin induces profuse perspiration (known as gustatory sweating) and causes a burning sensation on the tongue and oral mucosa that dissipates ('desensitizes') upon repeated challenge. It is, however, a mystery why the same pungent sensation that repels animals is found pleasurable by so many human beings (Szallasi, 2016). Capsaicin-sensitive neurons not only detect harmful stimuli (afferent function), but their peripheral endings are also sites of release for a variety of neuropeptides, most notably substance P (SP) and calcitonin gene-related peptide (CGRP), that, in turn, trigger the biochemical cascade known as neurogenic inflammation (efferent function). Neurogenic

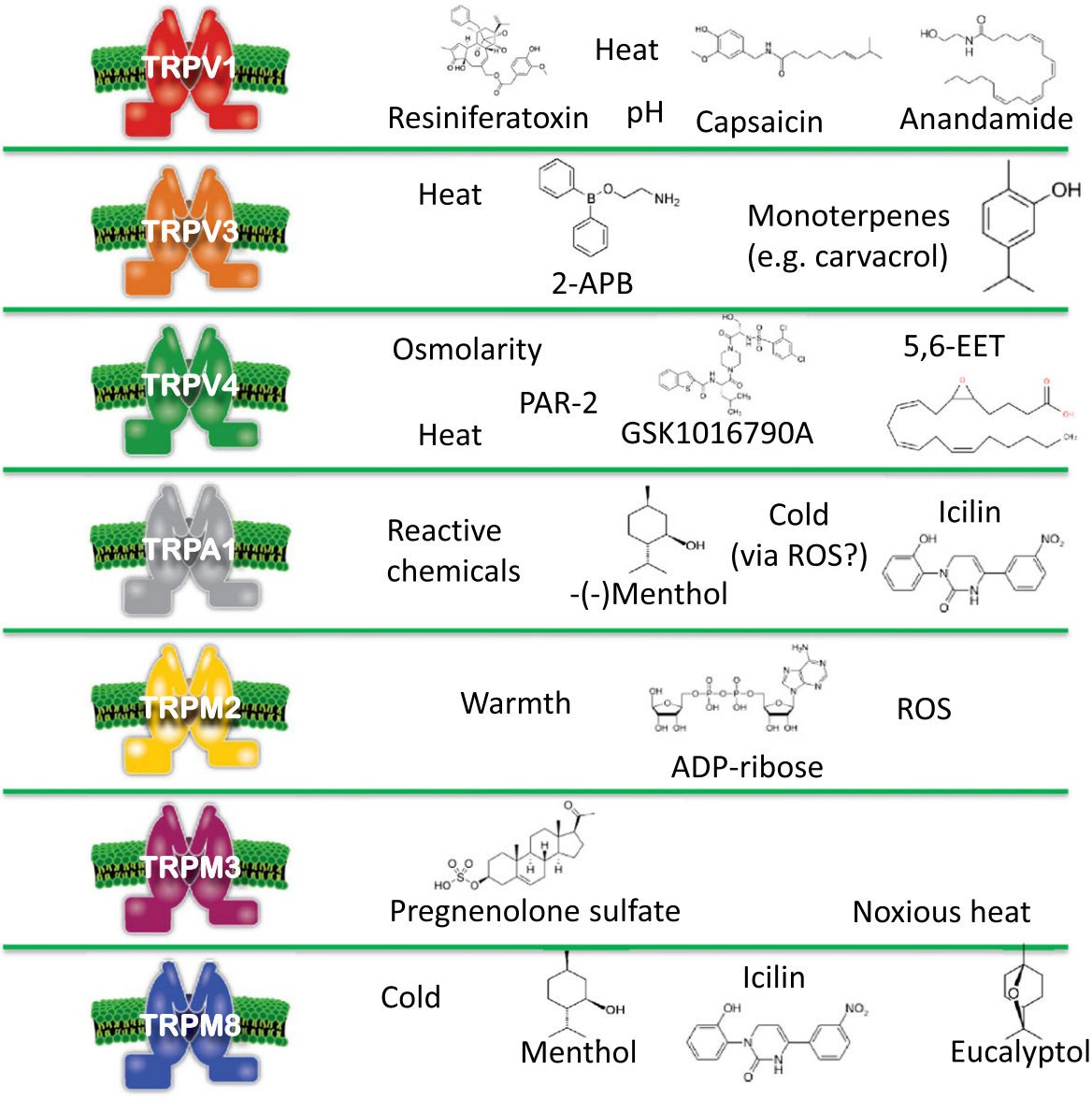

Figure 2

Common TRP channel activators. Each row contains a TRP channel discussed here and some of its commonly used activators. It should be noted that many of the activators are not selective. For example, many of the monoterpenes (menthol, etc.) and 2-APB activate many channels.

inflammation is thought to play an important role in the pathogenesis of various disease states, ranging from migraine through arthritis to diabetes (reviewed in Jancsó *et al.*, 2009).

Importantly, TRPV1 channels are downstream targets for various pro-inflammatory and pain-producing agents such as prostaglandins, bradykinin, ATP, 5-HT, activators of the protease-activated receptors (PAR) 1, 2 and 4, nerve growth factor and tumor necrosis factor-α. These compounds initiate an allosteric modification of the TRPV1 channel protein, the result of which is an increase in the probability of channel opening by heat, protons and capsaicin (see Szallasi *et al.*, 2007).

As expected, TRPV1 null mice are less responsive to acute noxious heat (Caterina *et al.*, 2000; Bölcskei *et al.*, 2005), though the deficit is not absolute and is somewhat dependent on how the stimulus is applied. For example, the impact is more pronounced in a tail immersion test than it is on a hot plate at 50°C, and there is no significant difference in response to radiant heating at an intensity of 8 (Caterina *et al.*, 2000). Somewhat surprisingly, a recent study observed only a modest (~20%) decrease in the number of trigeminal ganglion neurons that respond to noxious heat after pharmacological blockade with the TRPV1 channel antagonist **JNJ17203212** (Yarmolinsky *et al.*, 2016).

Transgenic mice injected with short interfering RNAs that silence TRPV1 expression also show attenuated capsaicin-induced pain behaviour and sensitivity towards noxious heat (Kasama *et al.*, 2007), a phenotype similar to that was observed in the TRPV1 knockout (KO) mice. Two independent studies with TRPV1-deficient (KO) mice conclusively showed that the TRPV1 channel is also a critical channel for mediating thermal hyperalgesia under inflammatory pain conditions

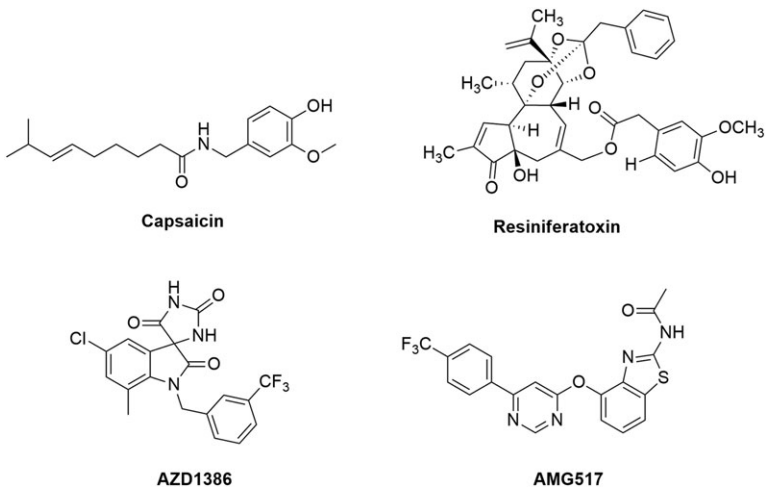

Figure 3

Chemical structures of TRPV1 channel modulators. Capsaicin and resiniferatoxin are agonists, while AMG-517 and AZD1386 are antagonists.

(Caterina *et al.,* 2000; Davis *et al.,* 2000). Combined, these findings created tremendous interest in developing low MW antagonists of TRPV1 channels with an anti-hyperalgesic profile. However, these findings also foreshadowed the issues that would shadow the development of TRPV1 channel antagonists: these compounds reduced subjects' ability to sense noxious heat and caused elevated body temperature (discussed below).

Capsaicin desensitization in clinical practice for reversible analgesia

What makes capsaicin unique among natural irritants is that the initial pain response that it causes is followed by a lasting refractory state, traditionally referred to as desensitization, in which the previously excited neurons are unresponsive not only to a repeated capsaicin challenge but also to various unrelated stimuli (see Szallasi and Blumberg, 1999). 'Capsaicin desensitization' is an unfortunate term because it has nothing to do with receptor desensitization as it is understood by most pharmacologists. While TRPV1 channels do desensitize upon repeated stimulation, it is not how capsaicin exerts its effects as a reversible analgesic agent. For historical reasons, however, the phrase 'capsaicin desensitization' remains firmly entrenched in the literature (Szolcsányi, 2004). Capsaicin desensitization remains an ill-defined, complex and poorly understood phenomenon. Clinically useful desensitization, which begins a few hours after treatment and may last for several months, most likely corresponds to 'defunctionalisation' of sensory neurons and is believed to involve reprogramming of the gene expression profile in the affected cells (Szallasi, 1996). There is good evidence that the expression of pro-algesic substances, such as SP, are down-regulated whereas endogenous analgesic peptides, such as galanin and somatostatin, are, conversely, increased after capsaicin treatment (see Szallasi and Blumberg, 1999). This phenomenon was referred to as vanilloid-induced messenger plasticity (Szallasi, 1996). Added to this, capsaicin initiates a massive Ca^{2+} influx that, in turn, leads to a breakdown of the cytoskeleton by activating Ca^{2+}-dependent proteases (Chard *et al.,* 1995). Intra-axonal transport is halted (Kawakami *et al.,* 1993), which depletes perikarya of nerve growth factor and stops the flow of neuropeptides and TRPV1 channels to the periphery (reviewed in Szallasi and Blumberg, 1999). Mitochondrial dysfunction in sensory neurons may also contribute to capsaicin desensitization (Bley, 2013). Indeed, swollen mitochondria are a well-established ultrastructural signature of capsaicin treatment.

By definition, capsaicin desensitization is reversible (Szallasi and Blumberg, 1999). Thus, capsaicin desensitization, for instance, by the use of site-specific capsaicin injections to relieve localized pain, has to be clearly distinguished from the permanent analgesia achieved by intrathecal or intra-ganglionic injections of resiniferatoxin, which are used as 'molecular scalpels' to kill sensory neurons. In experimental animals, there is a complete loss of capsaicin receptors, as detected by [^{3}H]resiniferatoxin binding, 4 h after systemic administration of capsaicin, followed by a slow but full recovery over the course of 4 weeks (Szallasi and Blumberg, 1992). The loss and full recovery of capsaicin receptors parallels the loss and reappearance of nociception (capsaicin-evoked eye-wipings) and neurogenic inflammation. Importantly, skin punch biopsies taken from volunteers exposed to high-dose capsaicin revealed a similarly reversible loss of TRPV1-like immunoreactivity in the dermis (Malmberg *et al.,* 2004; Kennedy *et al.,* 2010).

Low-concentration capsaicin creams, like Zostrix (0.075%) and Capzasin (0.1%), are popular over-the-counter analgesic agents. Despite their broad use, it is still unclear how these creams might work. It is unlikely that low-dose capsaicin can achieve clinically meaningful desensitization; instead, these creams might function as counterirritants. Not surprisingly, a recent Cochrane review on low-concentration capsaicin found insufficient evidence to make any treatment recommendations (Derry and Moore, 2012).

High concentration capsaicin creams caused significant problems both for patients and health care providers. Most troublesome was that vaporized capsaicin provoked severe cough or even bronchoconstriction in sensitive individuals that limited the dose of capsaicin that could be applied to the skin (Fuller *et al.,* 1985). To avoid this dose-limiting side

effect, occlusive patches (NGX-4010) (Noto *et al.,* 2009) and liquid formulations (NGX-1998) (Remadevi and Szallasi, 2008) containing high capsaicin concentrations of capsaicin were developed. NGX-4010 (sold under the brand name, Qutenza) is a dermal patch containing 8% capsaicin that can be used to treat localized pain. As of today, the clinical use of NGX-4010 in the European Union is approved for peripheral neuropathic pain of diverse aetiology. This decision was based on the ELEVATE trial (NCT01713426) that showed non-inferiority to pregabalin for pain reduction after 8 weeks. In the USA, the FDA approved the use of NGX-4010 only for the treatment of post-herpetic neuralgia. A Cochrane review of four clinical studies with NGX-4010 involving 1272 patients with post-herpetic neuralgia found a significant improvement in the patients' global impression of change score (Derry *et al.,* 2013). In an open-label study, the mean duration of the response (~30% reduction in Numerical Pain Rating Score compared to placebo) was 22-weeks, and a small subset of the patients maintained a response even after 40 weeks (Backonja *et al.,* 2010). Importantly, NGX-4010 was well tolerated with over 98% of study subjects completing the 30 to 60 min treatment (Peppin *et al.,* 2011).

For localized pain, site-specific capsaicin injections represent an attractive therapeutic approach. ALGRX-4975 is an injectable capsaicin (0.25 mg·mL^{-1}) preparation now being developed by Centrexion (Boston, MA) as CNTX-4975. In early clinical studies, ALGRX-4975 (Adlea) showed some promise for indications like Morton's neuroma or management of post-operative pain in bunionectomy or knee-replacement surgery patients (reviewed in Remadevi and Szallasi, 2008). In the ACTIVE-1 and -2 Phase 3 clinical trials, Adlea, however, missed its primary endpoint of reducing post-operative pain. In the later TRIUMPH study, two-third of patients with chronic pain due to degenerative joint disease (osteoarthritis) achieved a 50% or greater reduction of pain after receiving a single injection of CNTX-4975 at a dose of 1 mg (http://www.biosciencetechnology.com/news/2017/01/non-opioid-pain-treatment-shows-promise-phase-2-study). Nearly one in four patients achieved an almost complete pain relief. The maximum effect was seen at week 5, and a measurable difference between the treatment group and placebo persisted to 12 weeks after treatment. Of note, Centrexion is also evaluating CNTX-4975 in companion animals with osteoarthritis.

Topical or systemic resiniferatoxin for reversible analgesia

Resiniferatoxin (see Figure 2 for structure) is an ultrapotent capsaicin analogue with a unique spectrum of pharmacological actions (Szallasi and Blumberg, 1999). In rodents, systemic resiniferatoxin is a remarkable analgesic agent (reviewed in Kissin and Szallasi, 2011). In rats, a complete, long-lasting (up to a month) and fully reversible desensitization of the neurogenic inflammatory pathway was achieved by means of a single (300 μg) s.c. injection of resiniferatoxin (Szallasi and Blumberg, 1999). A similar loss of thermal hyperalgesia was noted in rats with neuropathic pain due to sciatic nerve injury. In these animals, resiniferatoxin also abolished the guarding behaviour of the injured hind paw, indicative of spontaneous pain. Although resiniferatoxin-treated rats showed a normal lifespan, some long-term effects were noted, including the development of hair loss, skin ulcers and incontinence (spotty voiding behaviour) (reviewed in Szallasi and Blumberg, 1999). These adverse effects clearly preclude the use of systemic resiniferatoxin in patients. By contrast, topical resiniferatoxin can be safely used in patients. Indeed, intravesical resiniferatoxin reduced the incidence and severity of catheter-related bladder discomfort in patients after transurethral resection of prostate without any significant side effect (Zhang *et al.,* 2012).

'Molecular neurosurgery': resiniferatoxin as a 'molecular scalpel' to achieve permanent analgesia

Neurons in sensory (DRG and trigeminal) ganglia collect noxious information from well-defined anatomical areas, the so-called dermatotomes. Therefore, it is an attractive idea to target these ganglia by resiniferatoxin for the relief of highly localized pain (Brown *et al.,* 2015a; Cimino Brown, 2016). To ablate sensory neurons, resiniferatoxin may be injected either directly into the sensory ganglia or given intrathecally to target the ganglionic nerve roots (Iadarola and Gonnella, 2013). In a proof-of-concept study, resiniferatoxin was injected into the trigeminal ganglia of rats (0.02 and 0.2 μg) and monkeys (2 μg) (Karai *et al.,* 2004; Neubert *et al.,* 2005). In both species, intratrigeminal resiniferatoxin injection produced a unilateral block of the eye-wiping response evoked by intraocular capsaicin drops. This effect was rapid in onset (detected 24 h after the microinjection) and most likely permanent as it did not return by the termination of the experiments (in the rat, 350 days, and 4 months in the monkey). Intratrigeminal resiniferatoxin administration also blocked neurogenic inflammation over the entire trigeminal distribution area. In pigs, resiniferatoxin injected into lumbar DRG under computerized tomography guidance abolished withdrawal responses to infrared laser (Brown *et al.,* 2015b). Inspired by these promising preclinical studies, a clinical trial with patients suffering from chronic, intractable bone pain due to cancer metastatic was initiated (NCT0252261).

For pain affecting multiple dermatomes, resiniferatoxin may be administered intrathecally (Cimino Brown, 2016). Canine osteosarcoma is a naturally occurring bone cancer pain that usually affects the long bones in one limb. In dogs, resiniferatoxin injections can be made into either the lumbar cistern (for hind limb tumours) or the cisterna magna (for forelimb tumours). In the initial dose-finding study, resiniferatoxin was delivered into the cisterna magna and the fore paws were tested with a radiant thermal stimulus. Intracisternal administration of resiniferatoxin (1, 2 or 3 μg·kg^{-1}) resulted in a nearly complete loss of noxious heat sensitivity when tested 2 days after treatment (Brown *et al.,* 2015a). Pet dogs with intractable osteosarcoma pain were enrolled into the intrathecal resiniferatoxin administration studies. Most of the animals were candidates for euthanasia because of poor pain control. As intrathecal resiniferatoxin causes an acute pain reaction, the injection was performed under general anaesthesia. The treatment was well tolerated, and the animals were discharged to home the day after treatment. Intrathecal resiniferatoxin administration caused a

profound analgesic action: the animals became ambulatory, walking on four legs (Brown *et al.,* 2015a; Cimino Brown, 2016). Resiniferatoxin administration, however, had no influence on disease progression: while 18 dogs were alive 2 weeks after treatment, 12 weeks later only four dogs survived (Iadarola and Mannes, 2011).

The first human patient with intractable cancer pain was enrolled into a Phase 1 resiniferatoxin trial at the National Cancer Institute in October, 2009 (NCT0252261). A total of 10 patients have received intrathecal resiniferatoxin treatment: all showed lasting analgesia with no significant adverse effects (Heiss *et al.,* 2014). At present, this study is on hold while new drug product is being manufactured (Michael Iadarola, personal communication). Although not reported in preclinical studies, it is a reasonable assumption that resiniferatoxin injected into the trigeminal ganglia would relieve migraine and trigeminal neuralgia pain.

Antagonists of TRPV1 channels as a new generation of analgesic drugs: expectations, disappointments, new hopes

The phenotype of the TRPV1 null mouse (attenuated thermal hyperalgesia under inflammatory conditions) triggered tremendous interest in developing small molecule antagonists with an anti-hyperalgesic profile (Caterina *et al.,* 2000; Davis *et al.,* 2000). It took less than a decade for the first antagonists to enter clinical trials after cloning of the TRPV1 protein (Szallasi *et al.,* 2007). The medicinal chemistry and pharmacology of TRPV1 channel antagonists have been extensively reviewed (see Gharat and Szallasi, 2008; Kort and Kym, 2012). Representative antagonists are depicted in Figure 3. Here, we focus on adverse effects hampering drug development and potential strategies to avoid these complications.

The two major adverse effects of TRPV1 channel antagonists are: (i) a transient increase in body temperature (febrile reaction) and (ii) a long-lasting compromise of noxious heat sensation leading to burn injuries. Neither side effect was completely unexpected. It has been known for over a century that TRPV1 channel agonists induce a transient drop in body temperature. Indeed, this hypothermic response is so characteristic that it was used by pharmacologists for decades to detect capsaicin-like bioactivity (Szolcsányi, 1982). If TRPV1 agonists induce hypothermia, TRPV1 antagonists may be expected to cause hyperthermia. Indeed, most antagonists of TRPV1 channels elevated body temperature in clinical studies, though the duration and magnitude of the febrile reaction varied significantly among the compounds tested. For example, AstraZeneca reported a modest increase in body temperature (~0.4°C in average) in most patients after an oral dose of **AZD-1386** (55 mg), exceeding 38°C only in one individual (Krarup *et al.,* 2011). By contrast, in 2008, Amgen terminated its Phase 1b dental pain (molar extraction) study with its clinical candidate molecule, **AMG517**, prematurely, because of the lasting (1–4 days) and marked febrile response (up to 40.2°C) that it caused in human volunteers (Gavva *et al.,* 2008).

The mechanism by which antagonists of TRPV1 channels elevate body temperature is still hotly debated. Capsaicin reduces rectal temperature in experimental animals (Jancsó-Gábor *et al.,* 1970). To explain the popularity of hot, spicy food in tropical climates, it was speculated that capsaicin also provides a cooling effect in humans ('capsaicin as air-conditioner') (Szallasi, 2016). Capsaicin-treated rats have red ears (due to dilated blood vessels) and they seek cool surfaces (Jancsó-Gábor *et al.,* 1970). Capsaicin-treated dogs pant (Schertel *et al.,* 1986) as do goats. Thus, one may argue that capsaicin tricks animals (and maybe also humans) into feeling hot and thereby activates counter-regulatory mechanisms to lose heat. If this holds true, TRPV1 channel antagonists may cause the opposite effect. Indeed, a common side effect of these antagonists is feeling cold (Manitpisitkul *et al.,* 2016). To reconcile these findings, it was argued that the TRPV1 channel functions as a body heat-sensor with endogenous tone (Gavva, 2008). Yet TRPV1 null mice have normal body temperature though they show perturbations in circadian rhythm (Szelényi *et al.,* 2004). Thus, the exact role of TRPV1 channels in body temperature regulation under physiological conditions remains to be elucidated (Romanovsky *et al.,* 2009).

The target that mediates the effect of capsaicin on body temperature regulation was localized to the brain by microinjection studies (reviewed in Szolcsányi, 2015). Therefore, it was unexpected that TRPV1 channel antagonists that do and those that do not cross the blood–brain barrier, both caused a hyperthermic response (Cui *et al.,* 2006). To explain this surprising finding, it was postulated that TRPV1 channels involved in body temperature regulation are present in the viscera, probably in the gastrointestinal tract (Gavva, 2008). This theory is, however, in conflict with the apparent inability of intra-abdominal resiniferatoxin administration (that desensitizes TRPV1 receptors in the gastrointestinal tract) to abolish the febrile reaction to TRPV1 channel antagonists (Szolcsányi, 2015).

To rid the TRPV1 channel antagonists of the undesirable effects on body temperature, many groups pursued modality specific inhibitors (http://www.painresearchforum.org/forums/discussion/50422-modality-specific-trpv1-antagonist-analgesia-what-are-prospects). The TRPV1 channel has three major means of activation: heat, protons and capsaicin. Interestingly, TRPV1 channel antagonists that do not interfere with proton activation of the receptor do not induce hyperthermia in rats (even if heat activation is blocked) (Lehto *et al.,* 2008). Using one such antagonist, **JYL1421**, in the rat, it was possible to eliminate hyperthermia while preserving the analgesic activity. Sadly, this effect was highly species-dependent. This compound that did not cause hyperthermia in the rat, but still elevated body temperature both in dogs and monkeys (Gavva *et al.,* 2007). This unexpected result suggests that TRPV1 channel antagonists must be tested against several species both *in vitro* and *in vivo* for the potential to cause hyperthermia, before advancing to the clinic.

Several other companies have reported second-generation TRPV1 channel antagonists that did not raise body temperature. It is unclear if these compounds were developed rationally or found by serendipity. PHE377 (PharmEste) is interesting in that it was reported to block all the three major means of activating TRPV1 channels, although it did not cause any detectable hyperthermia in rats or dogs (Trevisani and Gatti, 2012). PHE377 completed a

Phase 1 clinical trial but was not advanced into Phase 2 studies for undisclosed reasons. NeoMed is developing NEO6860 for osteoarthritic pain. According to a recent presentation, this compound does not affect body temperature in humans (Chiche *et al.,* 2016). Key structures that have been published are shown in Figure 3.

The observation that TRPV1 channel antagonists can elevate noxious heat sensation threshold and thereby cause burn injuries was not unforeseeable either. TRPV1 null mice are deficient in acute heat pain sensation, and resiniferatoxin desensitization markedly increases the temperature required to evoke a response in treated animals (Bölcskei *et al.,* 2010). Indeed, noxious heat is a major means of activating TRPV1 channels (Caterina *et al.,* 1997). Merck/Neurogen was developing MK-2295 for the potential treatment of pain and cough. This compound markedly increased the noxious heat pain threshold in humans (quantitative thermal sensory tests like pain evoked by hand immersion into or sipping hot water), placing the study participants at the risk of scalding injury (see Eid, 2011). Some subjects even perceived scalding water as cold and wanted to increase the temperature! Unlike the hyperthermia response that attenuated upon repeated application, the increase in heat pain threshold showed no sign of tachyphylaxis. This adverse effect was not specific to MK-2295. Increased heat pain threshold was found in the non-sensitized skin of healthy volunteers following oral administration of 400 mg of **SB-705498** (Chizh *et al.,* 2007), and similar results were observed by AstraZeneca with the TRPV1 channel antagonist, **AZD1386** (Krarup *et al.,* 2011). Mild oral (gum) and skin burn injuries were also noted in patients taking the Janssen TRPV1 channel antagonist, mavatrep (JNJ-39439335) (Manitpisitkul *et al.,* 2016). Unfortunately, because of the observed safety issues, efficacy of only a few TRPV1 antagonists have been reported, so it is difficult to assess how well a compound that thoroughly engaged the target would relieve pain.

Recently, the generally accepted pivotal role of TRPV1 channels in noxious heat sensation has been challenged (Yarmolinsky *et al.,* 2016). In mice, the TRPV1 channel antagonist **JNJ-17203212** had only a modest effect on noxious heat (48°C) stimulation with >75% of trigeminal ganglion neurons still showing a robust response. By contrast, JNJ-17203212 completely eliminated the neuronal response to innocuous warmth (35 to 43°C). Furthermore, in this study, TRPV1 KO mice lost their ability to discriminate between warm and cold liquids but still showed a strong aversion to hot drinks. Based on these results, authors propose a central role for TRPV1 channels in sensing innocuous warmth, but not noxious heat. Though interesting, this study is difficult to reconcile with the burn (scalding) injuries reported consistently by several groups as an adverse effect in human subjects taking TRPV1 channel antagonists (Chizh *et al.,* 2007; Eid, 2011; Krarup *et al.,* 2011; Manitpisitkul *et al.,* 2016). Where all studies agree is the indispensable role of TRPV1 channels in the development and maintenance of thermal hyperalgesia that develops after burn injury or inflammation (Caterina *et al.,* 2000; Davis *et al.,* 2000; Yarmolinsky *et al.,* 2016). These studies suggest a therapeutic potential for antagonists of TRPV1 channels in both thermal injury patients and victims of sun-burn.

Targeting TRPM8 channels to relieve cold allodynia

The TRPM8 channel is a cold-sensitive receptor (McKemy *et al.,* 2002; Peier *et al.,* 2002a). Indeed, mice with ablated TRPM8 channels are unable to distinguish warm temperatures from cold (Knowlton *et al.,* 2013). These animals do not avoid noxious cold either. The cold-sensitivity of TRPM8 channels is regulated by phosphoinositide interacting regulator of TRP channels (PIRT), a phosphoinositide binding protein (Tang *et al.,* 2013), and the cold-sensing phenotype of PIRT null mice is very similar to that of the TRPM8 null animals. Importantly, cold allodynia is markedly attenuated in the TRPM8 KO mice (Bautista *et al.,* 2007; Colburn *et al.,* 2007; Dhaka *et al.,* 2007). Combined, these findings identify TRPM8 channels as therapeutic targets for the cold hypersensitivity that is commonly associated with neuropathic pain.

The medicinal chemistry and pharmacology of TRPM8 channels are detailed elsewhere (DeFalco *et al.,* 2011; Fernández-Pena and Viana, 2012; Weyer and Lehto, 2017). Here, it suffices to mention that, as illustrated in Figure 2, TRPM8 channels are activated by cooling compounds such as **menthol** (in peppermint) and the synthetic menthol congeners, **icilin** (McKemy *et al.,* 2002; Peier *et al.,* 2002a; Bödding *et al.,* 2007) and WS-12 (Sherkheli *et al.,* 2012). As a matter of fact, TRPM8 channels were often referred to as 'menthol receptors'. Menthol is not, however, selective for TRPM8 channels, as it modulates both TRPV3 and TRPA1 channels (Macpherson *et al.,* 2006). Furthermore, menthol was shown to block both voltage-gated Na^+ and Ca^{2+} channels (Swandulla *et al.,* 1987; Haeseler *et al.,* 2002). Of note, menthol sensitivity was reduced, but not lost, in the TRPM8 null mice (Colburn *et al.,* 2007). Cold hypersensitivity to menthol was lost in the TRPA1 KO, but not in the TRPM8 null, animals (Gentry *et al.,* 2010). These findings suggest caution in ascribing results obtained with menthol, solely to activity on TRPM8 channels. For example, one study reported that menthol relieves pain in certain neuropathic models (Proudfoot *et al.,* 2006), but a second study using the TRPM8 'super agonist' icilin was unable to replicate this finding (Hagenacker *et al.,* 2014). Thus, the analgesic potential of TRPM8 channel agonists remains controversial. That being said, menthol has long been used as a topical analgesic.

TRPM8 channels are expressed in a subpopulation of primary afferent sensory neurons originating from both dorsal root and trigeminal ganglia (Dhaka *et al.,* 2008). Most studies agree that TRPM8-positive neurons are distinct from those expressing TRPV1 and/or TRPA1 channels (Takashima *et al.,* 2007). A number of studies have investigated the expression of TRPM8 channels in experimental models of chronic pain with conflicting findings. For example, TRPM8 levels (measured by *in situ* hybridization) decreased in the injured L5 DRG and remained unchanged in the neighbouring (uninjured) L4 DRG in the spinal nerve ligation model of neuropathic pain (Obata *et al.,* 2005). By contrast, in the chronic constriction injury (CCI) model, TRPM8 mRNA levels showed a delayed increase (reaching statistical significance only at 14 days post-CCI) in affected

DRG neurons (Frederick *et al.,* 2007). In the same model, immunostaining revealed a rapid (already noticeable 4 days after surgery) increase in the percentage of TRPM8-immunoreactive neurons when compared with the sham-operated group (Xing *et al.,* 2007; Su *et al.,* 2011). Importantly, the development of cold allodynia paralleled the increase in TRPM8 staining after CCI (Rossi *et al.,* 2012).

As with TRPV1 channels, significant interest exists in both agonists and antagonists of TRPM8 channels. The medicinal chemistry of TRPM8 channel blockers is at a much earlier stage, however. Notable TRPM8 channel antagonists include JNJ41876666, AMG9678, **AMTB**, **PBMC** and, most recently, PF-05105679 (see Figure 4 for structures). In rats, these compounds were reported to inhibit cold hypersensitivity in the CCI model of chronic neuropathic pain. Disappointingly, PBMC did not attenuate cold hypersensitivity in a preclinical model of chemotherapy (oxaliplatin)-induced neuropathy (Knowlton *et al.,* 2011) despite the postulated role of TRPM8 channels in this condition (Gauchan *et al.,* 2009). Little is known about the activity of TRPM8 channel antagonists in humans. PF-05105679 (900 mg dosed orally) was reported to provide efficacy in the cold pressor test (Andrews *et al.,* 2015). There is good evidence that, as observed with TRPV1 channels, the TRPM8 channels are involved in thermoregulation. Thus, menthol increases, and pharmacological TRPM8 channel blockade decreases, body temperature (Knowlton *et al.,* 2011; Gavva *et al.,* 2012). The hypothermic response to TRPM8 channel antagonists is mild (less than 1°C) and disappears upon repeated dosing. Therefore, unlike the febrile response to TRPV1 antagonists, it may not pose a problem for developing TRPM8 antagonists as therapeutics (Gavva *et al.,* 2012). Indeed, PF-05105679 did not have any measurable effect on the body temperature of study subjects; however, it did induce a poorly tolerated hot sensation localized to the head (mouth and face), torso and upper extremities (Andrews *et al.,* 2015). It is unclear if this adverse effect was off-target or represents a general problem for TRPM8 channel antagonists.

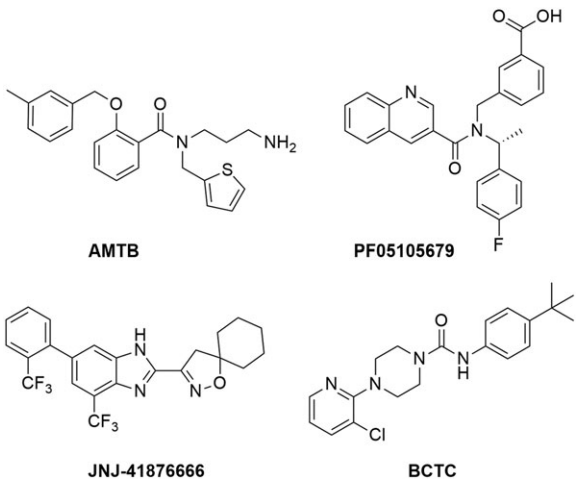

Figure 4
Chemical structures of TRPM8 antagonists.

TRPA1 channels as analgesic targets

The pungency of mustard, the burn of wasabi and the sting of cinnamon are all due to activation of TRPA1 channels (Bandell *et al.,* 2004; Jordt *et al.,* 2004). Based on our own personal experiences, we all know that inducing TRPA1 channel activity is sufficient to cause pain. Experiments with rodents have shown that functional TRPA1 channels are required for chemical nociception, and genetic deletion or pharmacological inhibition lead to vastly attenuated responses to a wide variety of noxious chemicals including tear gases (Brone *et al.,* 2008; Bessac *et al.,* 2009), formaldehyde (low concentrations) (McNamara *et al.,* 2007; Braz and Basbaum, 2010) and acrolein (a reactive aldehyde present in smoke and burning fat) (Bautista *et al.,* 2006; Birrell *et al.,* 2009). In addition to responding to these exogenously produced reactive chemicals, a variety of by-products of tissue damage and inflammation also drive TRPA1 channel activity. These include cyclopentenone prostaglandins (Andersson *et al.,* 2008; Cruz-Orengo *et al.,* 2008; Taylor-Clark *et al.,* 2008), products of lipid peroxidation such as 4-hydroxynonenal (Trevisani *et al.,* 2007), hypochlorite, a product of the myeloperoxidase pathway in neutrophils (Bessac *et al.,* 2008), and methylglyoxal (Eberhardt *et al.,* 2012; Andersson *et al.,* 2013), a product of aberrant glucose metabolism.

Particularly relevant activators of TRPA1 channels are ROS and nitrogen species (Bessac *et al.,* 2008; Sawada *et al.,* 2008). Multiple pathways converge on the production of these reactive species and thus link TRPA1 channels to an even broader array of biological function. Stimuli that can activate these channels can extend as far as light. UV light can activate TRPA1 channels through ROS generation (Babes *et al.,* 2016) with contributions from GPCR signalling pathways in melanocytes (Bellono *et al.,* 2013; Bellono *et al.,* 2014). Metabolites of acetaminophen (paracetamol) are another group of reactive chemicals that have been shown to activate TRPA1 channels (Andersson *et al.,* 2011). In mice, the anti-nociceptive effects of acetaminophen require functional TRPA1 channels, suggesting that modulation of these channels may underlie the therapeutic utility of this widely used analgesic compound. In addition, the non-electrophilic Δ^9-tetrahydrocannabiorcol also activates TRPA1 channels and requires functional channels to be anti-nociceptive (Andersson *et al.,* 2011). These findings may be akin to the analgesic capacity of the TRPV1 channel agonists, capsaicin and resiniferatoxin, which lead to functional desensitization. Indeed, recent work with systemic activators of TRPA1 channels suggest that activating the channel may be a viable means of reducing inflammation and generating hypoalgesia (Kistner *et al.,* 2016).

What allows TRPA1 to detect and respond to such a pleiotropic group of chemicals? The N-terminus of the channel is characterized by a series of cysteine residues that are subject to reversible or irreversible covalent modification (Hinman *et al.,* 2006; Macpherson *et al.,* 2007). This modification is sufficient to open the channel, causing ion flux. Instead of requiring a very specific shape to interact in a lock and key type mechanism, TRPA1 channels seem to respond to virtually any molecule capable of modifying cysteine residues. In addition to being activated by cysteine modification, TRPA1 currents are also potentiated by elevations in intracellular

calcium concentrations (Zurborg *et al.,* 2007; Wang *et al.,* 2008). This feature is important because it links TRPA1 channel activity to a plethora of other cell signalling mechanisms, including activation of the often co-expressed TRPV1 channels and Gq activation.

An area of controversy in the TRPA1 field has been the role of cold in activating TRPA1 channels. *In vivo,* TRPA1 channels do not act as a sensor for environmental cold. There is no difference in a thermal preference test between TRPA1 KO and wild-type mice (Bautista *et al.,* 2007; Knowlton *et al.,* 2010). In addition, extensive characterization of the effects of blocking TRPA1 channels has revealed no influence on body temperature (Chen *et al.,* 2011; de Oliveira *et al.,* 2014). While a role in environmental cold sensing is unlikely, TRPA1 channels may participate in the detection of noxious cold. Although some groups see no difference in cold sensing between TRPA1 wild-type and KO mice (del Camino *et al.,* 2010; Knowlton *et al.,* 2010; Chen *et al.,* 2011), there are also reports relying on KO mice that show deficiencies in noxious cold responses (Karashima *et al.,* 2009; Miyake *et al.,* 2016). It is likely that these discrepancies are due to methodological differences and that there is an effect on the detection of noxious cold under certain conditions. However, it is unclear if this is a direct effect on the channel or is mediated by the release of other signalling molecules that potentiate TRPA1 channels such as intracellular calcium (Wang *et al.,* 2008), proinflammatory mediators or ROS (Miyake *et al.,* 2016).

In vitro, activity of recombinantly expressed TRPA1 channels increases with noxious cold (Story *et al.,* 2003). However, the level of activation is minimal compared to what is seen with chemical agonists such as **allyl isothiocyanate (AITC)** (del Camino *et al.,* 2010). The effects of cold become significant though when other activators are present in both recombinant systems and acutely isolated DRG neurons. In the presence of an activator such as AITC, even moderate cooling (to 20°C) potentiates channel activity of heterologously expressed rat and human TRPA1 channels (del Camino *et al.,* 2010). Again, it is unclear whether cold acts directly on the channel or releases known TRPA1 channel activators such as ROS (Miyake *et al.,* 2016) that then work in concert with other chemical activators. The channel behaviour observed *in vitro* may explain why TRPA1 channels play such significant roles in cold allodynia, induced by a variety of insults including chemotherapeutic agents, nerve injury and inflammation. TRPA1 null mice have substantially attenuated cold allodynia evoked by oxaliplatin, cisplatin and bortezomib (Nassini *et al.,* 2011; Trevisan *et al.,* 2013). These findings have been reproduced by pharmacological inhibition with **HC-030031** (Nassini *et al.,* 2011; Trevisan *et al.,* 2013), a peptide blocker of TRPA1 channels (Tonello *et al.,* 2017), or the more advanced TRPA1 channel antagonist, HC-068559 (Moran *et al.,* 2011b). Although studies have suggested that oxaliplatin and cisplatin cause cold hypersensitivity in part by up-regulating expression of TRPA1 channels in sensory neurons (Ta *et al.,* 2010; Yamamoto *et al.,* 2015), increased sensitivity of the TRPA1 channel may also play a role. Bortezomib, for example, increases the sensitivity of neurons to AITC and ROS, two TRPA1 channel activators (Trevisan *et al.,* 2013). In addition, Abbott observed significant effects of **A-967079** on cold allodynia in a CCI model (Chen *et al.,* 2011), and similar

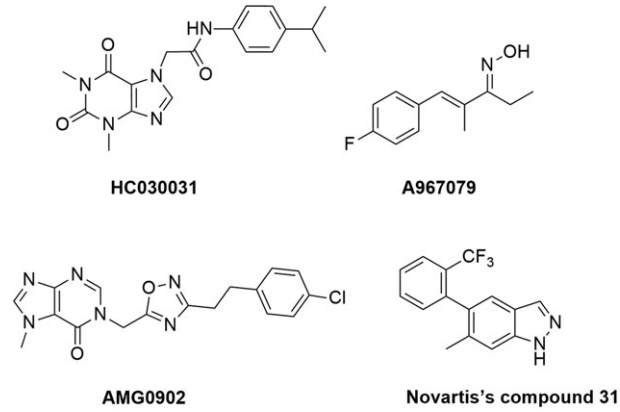

Figure 5

Chemical structures of TRPA1 antagonists.

findings were reported for HC-030031 after spared nerve injury (del Camino *et al.,* 2010). Structures for key antagonists may be found in Figure 5. Blocking TRPA1 channel function also reduces cold hypersensitivity associated with inflammation. In a Complete Freund's Adjuvant (CFA) cold model, blocking TRPA1 channels with either HC-030031 (Da Costa *et al.,* 2010; del Camino *et al.,* 2010) or **AP-18** (Petrus *et al.,* 2007) attenuated cold responses.

TRPA1 channels also appear to play a role in mechanical pain. Pressure sensing in the viscera requires TRPA1 channels. Visceromotor responses to colorectal distension are inhibited by either gene deletion or HC-030031 (Mueller-Tribbensee *et al.,* 2015). Likewise, in a skin-nerve preparation, sustained responses to noxious mechanical stimuli required functional TRPA1 channels. Nerve firing was reduced by genetic deletion or pharmacological inhibition with HC-030031. In contrast, HC-030031 had no effect on nerve firing following capsaicin stimulation, indicating these effects were not due to non-specific inhibition of action potential generation (Kerstein *et al.,* 2009). Similar effects were noted with the chemically distinct AMG0902 (Lehto *et al.,* 2016). Consistent with this, A-967079, a potent analogue of AP-18, also reduced firing of wide-dynamic range neurons in naïve rats after noxious pinch (McGaraughty *et al.,* 2010). In addition, TRPA1 KO mice showed attenuated mechanical nociception and reduced firing in response to force in a skin-nerve preparation (Kerstein *et al.,* 2009; Lehto *et al.,* 2016), though the *in vivo* effect is subtle and varies somewhat among reports (Kwan *et al.,* 2006; Petrus *et al.,* 2007). A large data set collected from many studies also supports the role of TRPA1 channels in mechanical hyperalgesia induced by inflammation. In a CFA model, pharmacological inhibition with AP-18, but not genetic deletion, ameliorates the severity of CFA-induced mechanical hyperalgesia. This observation suggests some compensatory changes in the null mouse, as AP-18 only exerts this effect in the presence of functional TRPA1 channels, indicating that the effect is indeed on target (Petrus *et al.,* 2007). Treating rats with the chemically distinct HC-030031 also reduced CFA-induced mechanical pain (Eid *et al.,* 2008) as did 'Compound 31' from Novartis (Rooney *et al.,* 2014). However, not all compounds seem to significantly reduce mechanical hypersensitivity in this model; for example,

AMG0902 exerted only a mild effect in a CFA mechanical model at a dose that showed robust inhibition of AITC-induced pain behaviours. The reason for this discrepancy remains unclear, though it may be related to the reduced ability of AMG0902 to inhibit activity induced by endogenously produced activators such as 4-ONE and methylglyoxal, compared to AITC (Lehto *et al.,* 2016).

Deletion of TRPA1 channels has also been shown to affect chronic inflammatory pain in CFA and osteoarthritis induced by mono-iodoacetate models (Garrison and Stucky, 2014; Moilanen *et al.,* 2015; Horvath *et al.,* 2016), though results between groups are not entirely consistent. This may be in part due to the age of the mice used. Studies examining pain behaviours in older mice may show more dependence on TRPA1 channels than those performed in younger ones (Garrison and Stucky, 2014). Interestingly, multiple groups report that in addition to impacting the pain associated with inflammation, deletion of TRPA1 channels also decreases the inflammation *per se* (Moilanen *et al.,* 2015; Horvath *et al.,* 2016). Similar observations have been reported in models of ulcerative colitis (Engel *et al.,* 2011) and asthma (Caceres *et al.,* 2009). These findings suggest a role for TRPA1 channels in neurogenic inflammation.

The effects of TRPA1 channels in nerve injury-induced mechanical hypersensitivity are varied. Consistent results have been observed in models of peripheral neuropathic pain and more ambiguous data with central neuropathic pain. Amgen reports that AMG0902 does not show efficacy in a Chung model of neuropathic pain due to nerve ligation (Lehto *et al.,* 2016) whereas Merck reported that the Hydra compound HC-030031 reduced mechanical hypersensitivity (Eid *et al.,* 2008). These compounds may exert different effects due to intrinsic properties, or the discrepant results may be due to the divergent protocols employed: Merck tested 6 weeks after L5/L6 ligation (Eid *et al.,* 2008) and Amgen tested 2 weeks post-surgery (Lehto *et al.,* 2016).

A wide variety of reports testing both pharmacological agents and gene deletions have suggested that TRPA1 channels play a critical role in mechanical hypersensitivity after chemotherapy-induced peripheral neuropathy. These include oxaliplatin (Nassini *et al.,* 2011; Materazzi *et al.,* 2012; Zhou *et al.,* 2016), bortezomib (Trevisan *et al.,* 2013; Tonello *et al.,* 2017) and paclitaxel (Materazzi *et al.,* 2012; Moran *et al.,* 2011a). Given the similarities in mechanisms between chemotherapy-induced peripheral neuropathy and painful diabetic neuropathy, it is not surprising that there has also been significant data implicating TRPA1 channels in painful diabetic neuropathy. Methylglyoxal, a product of aberrant glucose metabolism that is found at elevated levels in diabetics, directly activates this channel (Eberhardt *et al.,* 2012; Koivisto *et al.,* 2012; Ohkawara *et al.,* 2012; Andersson *et al.,* 2013). Indeed, methylglyoxal-induced spontaneous pain behaviour is absent in TRPA1 KO mice (Andersson *et al.,* 2013). Like diabetic patients, rats with peripheral neuropathy induced by streptozotocin also have impaired pancreatic function and increased levels of methylglyoxal (Huang *et al.,* 2016). Consistent with this, TRPA1 channel inhibitors show significantly reduced mechanical hypersensitivity in the streptozotocin model of neuropathic pain (Wei *et al.,* 2009, 2010). Although administering the TRPA1 channel antagonist directly to the paw was sufficient to reduce the mechanical hypersensitivity, intrathecal delivery exerted a more substantial effect, suggesting a role for spinal TRPA1 channels (Wei *et al.,* 2010).

One curious observation that Orion Pharma made was that the pharmacodynamic effects outlasted the anticipated plasma exposure of the TRPA1 antagonist being tested (Wei *et al.,* 2009). This led them to look at whether antagonism of TRPA1 channels altered the nerve damage in the streptozotocin model. When they co-treated animals with streptozotocin and a TRPA1 antagonist, CHEM-5861528, the usual loss of intra-epidermal nerve fibres was greatly reduced (Koivisto *et al.,* 2012). This suggests that TRPA1 channel antagonists have the potential for disease modification along with analgesia in diabetic peripheral neuropathy. How much of this is due to the diabetic condition and how much is due to direct effects of streptozotocin on TRPA1 channels (Andersson *et al.,* 2015) remains to be determined. But this fibre sparing, combined with the observation that acute treatment with a TRPA1 channel antagonist prevents the development of bortezomib- or oxaliplatin-induced chemotherapy-induced peripheral neuropathy (Trevisan *et al.,* 2013), gives reason for optimism that blocking TRPA1 channels could reduce neuropathy. Only one TRPA1 channel antagonist has reported to have gone through human Phase 2 clinical trials, Glenmark's GRC 17536. Though the full results have not been published, Glenmark reported that in a pre-specified subset of patients with painful diabetic neuropathy and intact sensory responses GRC 17536, significantly reduced pain scores. No notable side effects were reported (http://www.prnewswire.com/news-releases/glenmarks-trpa1-antagonist-grc-17536-shows-positive-data-in-a-proof-of-concept-study-275445961.html). Because of physical chemical properties of the compound, GRC 17536 was administered as an oral suspension of granules (https://www.clinicaltrialsregister.eu/ctr-search/trial/2011-005879-16/DE), so it is unlikely that this compound will proceed further without a formulation breakthrough, but these reports certainly support the possibility that inhibiting TRPA1 channels may be analgesic in painful diabetic neuropathy in patients.

TRPV4 channels and pain

The mammalian orthologue of the *Caenorhabditis elegans* osmosensor *osm-9*, TRPV4, responds to hypotonicity like its nematode counterpart (Liedtke *et al.,* 2000; Strotmann *et al.,* 2000). It is a broadly expressed, non-selective cation channel most closely related to TRPV1, TRPV2 and TRPV3 channels. Not surprisingly based on its amino acid structure, it is potentiated by heating (Guler *et al.,* 2002; Watanabe *et al.,* 2002a, b), though genetic deletion suggests that it plays little role in thermal sensation (Huang *et al.,* 2011). The phorbol ester 4α-phorbol 12,13-didecanoate (4-αPDD) (Watanabe *et al.,* 2002a, b), the arachidonic acid metabolite **5′,6′-epoxy-8Z,11Z,14Z-eicosatrienoic acid** (Watanabe *et al.,* 2003), and PAR-2 receptor stimulation (Grant *et al.,* 2007) all increase channel activity as illustrated in Figure 2.

Several mutations in the TRPV4 channel are associated with variable disease phenotypes in humans. These include a range of skeletal dysplasias, arthropathies, congenital-distal spinal motor atrophy and hereditary motor and sensory

neuropathy type 2c (Charcot–Marie–Tooth Disease type 2C) (Nilius and Voets, 2013). The sensory neuropathy is far less common than the motor neuropathy and manifests itself as a typically mild sensory loss, but pain has also been reported (Fleming and Quan, 2016). Although most of the pathogenic mutations are reported to be gain-of-function, it has also been suggested that some of the mutations are loss-of-function. Other factors are also likely involved as the same mutation can result in different phenotypes in different patients (Dai *et al.,* 2010). Distinguishing between gain- and loss-of-function mutations can be more difficult than it might seem, especially with calcium permeant ion channels: over-activity can lead to toxicity and subsequent down-regulation or internalization, so caution should be used when distinguishing between gain- and loss-of-function.

Activation *in vitro* of TRPV4 channels by 4α-PDD (Watanabe *et al.,* 2002a) or PAR-2 activation sensitizes sensory afferents to mechanical stimulation (Grant *et al.,* 2007). Genetic deletion or knock-down of TRPV4 in mice leads to attenuated pain behaviours in a range of models including those induced by inflammatory soup (Chen *et al.,* 2007), formalin (Chen *et al.,* 2014), prostaglandin E_2 (Chen *et al.,* 2007) and taxol (Alessandri-Haber *et al.,* 2004). TRPV4 channels seem to play a particularly important role in visceral pain (Brierley *et al.,* 2008; Cenac *et al.,* 2008) and these channels have also been implicated in pancreatitis pain (Ceppa *et al.,* 2010; Kanju *et al.,* 2016) and cyclophosphamide-induced bladder cystitis (Everaerts *et al.,* 2010). Murine pancreatic nerve fibres express TRPV4 channels and functionally respond to 4α-PDD. Cerulein-induced pain behaviours are reduced when functional TRPV4 channels are absent (Ceppa *et al.,* 2010).

TRPV4 channels are robustly expressed by colonic serosal and mesenteric afferents where they co-localize with CGRP and are required for normal mechanosensory function (Brierley *et al.,* 2008; Mueller-Tribbensee *et al.,* 2015). Genetic deletion of TRPV4 channels reduced spiking frequency in response to a variety of von Frey filaments. TRPV4 KO mice also showed smaller electromyographic responses to colonic distension. In contrast, the conduction velocity and electrical activation threshold in the TRPV4-expressing fibres was unaffected, indicating that the mechanosensory defect was not due an obvious disruption of nerve fibre function (Mueller-Tribbensee *et al.,* 2015). Recent data relying on an *ex vivo* preparation of human serosal fibres also showed robust expression of TRPV4 channels. Mechanosensitivity in these fibres was reduced by treatment with the TRPV4 channel antagonist HC-067047 (McGuire *et al.,* 2016).

The most advanced TRPV4 antagonists are currently in clinical trials evaluating pulmonary function and oedema associated with heart failure (NCT02497937). Hopefully, it will be possible to test these or newer antagonists in pain indications as well.

Dual inhibition of TRPA1 and TRPV4 channels for pain relief

Reducing functional TRPV4 or TRPA1 channels has been shown to reduce pain due to formalin (McNamara *et al.,* 2007; Chen *et al.,* 2014), chemotherapy-induced peripheral neuropathy (Alessandri-Haber *et al.,* 2004; Materazzi *et al.,* 2012), colorectal distension (Mueller-Tribbensee *et al.,* 2015) and pancreatitis (Ceppa *et al.,* 2010). Some recent work has evaluated dual KO or dual combination inhibitor of these channels with promising results. Inhibiting both channels produces robust efficacy in paclitaxel-induced peripheral neuropathic pain (Materazzi *et al.,* 2012), the formalin model (Chen *et al.,* 2014) and the cerulein model of pancreatitis (Kanju *et al.,* 2016). A recent report demonstrated that the invention of dual antagonists is feasible, opening the way for a new type of TRP channel drug discovery (Kanju *et al.,* 2016).

TRPV3 channels and pain

Like other TRP channels, the TRPV3 channel is a non-selective, Ca^{2+}-permeable cation channel. Elevations in temperature activate the channel (Smith *et al.,* 2002; Xu *et al.,* 2002; Peier *et al.,* 2002a, b), which has a Q10 value for the steep phase of activation around 20 (Xu *et al.,* 2002). Unlike many other TRP channels, TRPV3 channels are sensitized upon repeated stimulation (Xu *et al.,* 2002; Peier *et al.,* 2002b) instead of desensitizing. These channels are highly expressed in skin (Xu *et al.,* 2002) but are also found in sensory neurons (Smith *et al.,* 2002; Xu *et al.,* 2002). Of particular interest from a pain perspective is that nerve injury or tissue damage seems to up-regulate TRPV3 protein in human DRG or skin (Smith *et al.,* 2002; Gopinath *et al.,* 2005). TRPV3 channels play a minimal role in thermosensation. Genetic deletion exerts only a small effect, which seems to be largely strain-specific (Huang *et al.,* 2011; Moqrich *et al.,* 2005). Human and mouse genetic data have shown a clear role for TRPV3 in keratinocyte proliferation (Yoshioka *et al.,* 2009; Lin *et al.,* 2012) but also suggest possible involvement in pain. Single nucleotide polymorphism variants in TRPV3 were linked to migraine with aura in the Spanish population (Carreno *et al.,* 2012) and fibromyalgia in the Korean population (Park *et al.,* 2016). In addition, a patient with Olmsted disease and erythromelalgia was recently shown to have a mutation in TRPV3 protein (Duchatelet *et al.,* 2014).

Several companies have pursued selective antagonists of TRPV3 channels including Abbvie (Gomtsyan *et al.,* 2016), Hydra Biosciences and Glenmark/Sanofi-Aventis (Grubisha *et al.,* 2014). The Glenmark/Sanofi-Aventis compound GRC15300 failed a Phase 2 trial in chronic peripheral neuropathy patients (a 4 week trial), and the collaboration between the two companies was terminated in 2014 (Broad *et al.,* 2016). However, recent reports suggest that these TRPV3 channel antagonists are not full blockers of the channel (Grubisha *et al.,* 2014), so it is difficult to know how to interpret these results with regards to a potential role of TRPV3 channels in pain.

TRPM2 and TRPM3 channels

Even when TRPV1 channels have been genetically deleted, animals retain a reduced ability to sense heat. As discussed above, deletion of TRPV3 and TRPV4 seems to have little effect on heat sensing, so many posit the existence of additional temperature sensitive channels. One channel that

has been proposed to sense noxious heat is the TRPM3 channel (Vriens *et al.,* 2011). The channel is expressed by a subset of heat responsive afferent neurons, and genetic deletion results in attenuated responses to noxious heat as well as reduced inflammatory thermal hyperalgesia (Vriens *et al.,* 2011). It will be interesting to learn more about this channel and to determine how it interacts with other channels that respond to changes in temperature.

Another TRPM family member, TRPM2, functions as a receptor for warm temperatures in a variety of cells including sensory, autonomic and central neurons (Song *et al.,* 2016; Tan and McNaughton, 2016). Currents are potentiated by hydrogen peroxide and attenuated by 2-APB. Genetic deletion shows that functional TRPM2 channels are key for detection of non-painful warmth and that TRPM2-deficient animals show reduced sensitivity to mild heat (Song *et al.,* 2016; Tan and McNaughton, 2016). In addition, TRPM2 channels are likely to tune body temperature *via* their role as a hypothalamic heat sensor and may limit fever responses (Song *et al.,* 2016). Whether neuronally expressed TRPM2 channels play a significant role in pain remains to be determined.

Outside the nervous system, TRPM2 channels are highly expressed by a variety of immune cells (Knowles *et al.,* 2011). Mice deficient in TRPM2 channels show reduced mechanical allodynia after spared-nerve ligation, intra-articular injection of monoiodacetate and 2,4,6-trinitobenzenesulfonic acid-induced colitis (Haraguchi *et al.,* 2012). However, these effects seem to be largely due to reduced inflammation and infiltration of circulating immune cells (Haraguchi *et al.,* 2012). Whether TRPM2 channels could be a viable target for pharmaceutical development remains to be seen given the important roles it seems to play in the immune system in innate immunity (Knowles *et al.,* 2011) and in thermoregulation (Song *et al.,* 2016; Tan and McNaughton, 2016).

Conclusions

Despite the disappointments associated with the early TRPV1 antagonist programmes, the TRP family remains an exciting group of targets for the treatment of a variety of pain disorders. TRPV1 agonists are being used in a number of pain disorders, and continued development and refinement of delivery systems may provide important improvements. Although additional preclinical work with earlier stage targets will be needed to determine if other family members deserve testing, the next few years should see continued clinical trials with TRPM8, TRPV4 and TRPA1 channel antagonists. This work will provide important insights about the role of TRP channels in human pain conditions and has the potential to deliver some badly needed new therapeutic agents to patients.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.,* 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.,* 2015).

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Conflict of interest

M.M.M. is a full-time employee of Hydra Biosciences, a company that focuses on the discovery and development of TRP channel modulators. She is part of the employee stock plan. A.S. has nothing to declare.

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