Full article title: **TRP CHANNEL COOPERATION FOR NOCICEPTION: THERAPEUTIC OPPORTUNITIES**

Authors:

Dorien Bamps, Clinical Pharmacology and Pharmacotherapy, Department of Pharmaceutical and Pharmacological Sciences, Center for Clinical Pharmacology, UZ Leuven, Leuven, Belgium
dorien.bamps@kuleuven.be
https://orcid.org/0000-0002-3441-4290

Joris Vriens, Laboratory of Endometrium, Endometriosis and Reproductive Medicine, Department of Development and Regeneration, G-PURE, KU Leuven, Leuven Belgium
joris.vriens@kuleuven.be
https://orcid.org/0000-0002-2502-0409

Jan de Hoon, Clinical Pharmacology and Pharmacotherapy, Department of Pharmaceutical and Pharmacological Sciences, Center for Clinical Pharmacology, UZ Leuven, Leuven, Belgium
jan.dehoon@uzleuven.be
https://orcid.org/0000-0002-5215-9836

Thomas Voets, Laboratory of Ion Channel Research, VIB-KU Leuven Centre for Brain & Disease Research & KU Leuven Department of Cellular and Molecular Medicine, Leuven Belgium
thomas.voets@kuleuven.vib.be
https://orcid.org/0000-0001-5526-5821

Corresponding author: Thomas Voets, thomas.voets@kuleuven.vib.be

Key words: TRP channel, TRPV1, TRPA1, TRPM3, nociception, drug development

ABSTRACT

Chronic pain treatment remains a sore challenge, and in our aging society, the number of patients reporting inadequate pain relief continues to grow. Current treatment options all have their drawbacks, including limited efficacy and propensity of abuse and addiction; the latter exemplified by the ongoing opioid crisis. Extensive research in the last decades has focused on mechanisms underlying chronic pain states, thereby producing attractive opportunities for novel effective and safe pharmaceutical interventions. Members of the transient receptor potential (TRP) ion channel family represent innovative targets to tackle pain sensation at the root. Three TRP channels, TRPV1, TRPM3 and TRPA1, are of particular interest, as they were identified as sensors of chemical and heat-induced pain in nociceptor neurons. This review summarizes the knowledge regarding TRP channel-based pain therapies, including the bumpy road of clinical development of TRPV1 antagonists, the current status of TRPA1 antagonists, and future perspectives of targeting TRPM3.

MANUSCRIPT

Although generally described as an unpleasant sensation, pain is vital to detect noxious stimuli in the environment and to protect our body from the harm they may cause. By contrast, chronic pain no longer fulfills a physiological goal; it represents an incapacitating pathology that burdens millions of patients worldwide (1, 2). The condition may arise from a degenerative process or an incurred lesion, but pointing out the culprit is not always obvious. Yet, the prevalence of chronic pain is only expected to increase in our aging population. Effective treatment of this healthcare problem is therefore a top priority, but unfortunately continues to present a major challenge. Currently marketed analgesics such as nonsteroidal anti-inflammatory drugs and paracetamol can be very effective to treat common aches, but often provide inadequate pain relief in chronic pain patients. On the other hand, opioids are the most potent analgesics available, yet their use is burdened by unwanted side effects, the development of tolerance and an increasing addiction problem (3–5). Other classes of analgesic drugs, such as gabapentinoids, tricyclic antidepressants or serotonin/norepinephrine reuptake inhibitors are effective in only a limited number of chronic pain patients. Consequently, there is a high demand for novel analgesics with an improved efficacy and safety profile.

Promising pain research in the last decades has shed light on the physiology of pain and the pathological events underlying chronic pain conditions, thereby creating opportunities for novel pharmaceutical interventions. One attractive approach is to target the very beginning of the pain pathway (Figure 1), focusing on nociceptive receptors. In particular, several members of the transient receptor potential (TRP) superfamily are notorious for their role in nociception, for example the burning sensation associated with chili peppers or the pungent taste of wasabi.

1. TRP ION CHANNELS, A PROMISCUOUS FAMILY

Discovery of TRP channels traces back to 1969, when a *Drosophila melanogaster* mutant was isolated that exhibited a transient receptor potential in its photoreceptors upon continuous illumination (6, 7). The *Drosophila trp* gene was cloned in 1989 (8), and further research led to the identification of a large TRP family consisting of more than 50 members. In mammals, 28 TRP

channels have been characterized, grouped into six subfamilies: TRP Ankyrin (TRPA), TRP Canonical (TRPC), TRP Melastatin (TRPM), TRP Mucolipin (TRPML), TRP Polycystin (TRPP) and TRP Vanilloid (TRPV) (9). These ion channels generally possess six transmembrane domains, but the overall structure differs a lot between subfamilies. Furthermore, there is a wide heterogeneity in gating mechanisms, including activation by various endo- or exogenous ligands as well as voltage- and temperature-gated channels. As such, the TRP family contributes to a variety of physiological functions including vision, hearing, taste perception, thermosensation and responding to different environmental stimuli (9). Nonetheless, the ion channel family is probably most (in)famous for its role in nociception. In fact, TRP channels constitute the largest group of nociceptive ion channels involved in pain sensation in mammals.

The growing interest in TRP channels as transducers of painful stimuli started about 20 years ago, following the discovery and subsequent cloning of Transient Receptor Potential Vanilloid 1 (TRPV1). Back then baptized Vanilloid Receptor Subtype 1 (VR1), the ion channel was described as a non-selective cation channel sensitive to capsaicin, responsible for the pungent taste of chili peppers, thermal stimuli within the noxious range as well as protons, corresponding to conditions of inflammation, infection or ischemia (10). Being an integrator of diverse noxious stimuli, TRPV1 is predominantly expressed in a subset of primary sensory neurons, in particular thinly myelinated Aδ fibers and unmyelinated C fibers, both peptidergic and non-peptidergic (sidebar 1). In addition, the capsaicin receptor has been identified in the spinal cord and brain as well as in non-neuronal tissues like the bladder and skin, although the function of the ion channel in these tissues remains poorly understood (11).

2. TRPV1 AND THE HEAT-ACTIVATED TRP TRIO

The generation of TRPV1-deficient mice further highlighted the channel's involvement in acute and inflammatory pain. Indeed, these animals lacked a pain response to capsaicin, and also had a significantly reduced (but still robust) avoidance response to noxious heat. More intriguingly, TRPV1-deficient mice failed to develop heat hyperalgesia following tissue inflammation induced by

the injection of carrageenan or Freund's Complete Adjuvant (12, 13). Based on these promising preclinical results, TRPV1 soon aroused interest as a novel target for inflammatory pain conditions, and, to date, TRPV1 remains the most thoroughly studied member of the TRP family. Moreover, its discovery as a heat sensor opened the door towards the identification of a subset of thermosensory TRP channels, the so-called thermoTRPs (14), which include channels that are activated by heating and/or cooling over a broad thermal range between 5 and 55 °C. Whereas the role of the menthol-sensitive channel Transient Receptor Potential Melastatin 8 (TRPM8) in conveying cool temperatures is well established and generally accepted, the exact contribution of for instance Transient Receptor Potential Ankyrin 1 (TRPA1) to the detection of noxious cold, or the roles of Transient Receptor Potential Vanilloid 3 (TRPV3), Transient Receptor Potential Vanilloid 4 (TRPV4) and Transient Receptor Potential Melastatin 2 (TRPM2) to warmth sensation remain to be fully uncovered (15).

Recent research revealed that noxious heat sensing in mice depends on a set of three TRP channels, including not only TRPV1 but also TRPA1 and Transient Receptor Potential Melastatin 3 (TRPM3). Functionality of at least one of these three TRPs was shown sufficient, yet crucial to maintain acute heat sensitivity, as triple knockout mice, deficient for all three heat-activated channels, lacked the protective withdrawal reaction when exposed to noxious heat, making them vulnerable to burn injuries (16). Notably, all three channels, which show a partly overlapping expression profile in nociceptor neurons, have been implicated in pathological pain signaling and hypersensitivity, and are therefore appealing targets for analgesic drug development. Here, we provide an overview of lessons learnt from drug development projects targeting these three TRP channels, and provide an outlook into how these may one day lead to novel analgesic drugs to treat various types of pathological pain.

2.1 TARGETTING TRPV1, THE HOT-HEADED FAMILY MEMBER

Although the molecular basis, interaction with the TRPV1 vanilloid binding site (Figure 2), took longer to decipher, chili peppers have been known to provoke pungency and pain since time immemorial. In addition to its widespread use to spice-up food, the principle of capsaicin-induced

acute pain is still utilized nowadays in for example capsaicin-containing pepper sprays for policing or self-defense. On the other hand, TRPV1 shows analgesic potential. Different approaches (Figure 3) have been tested to combat pain, starting with agonists and antagonists of TRPV1 (17).

2.1.1 INHIBITING PAIN BY OVERWHELMING TRPV1

The desensitizing and analgesic effects of capsaicin (Table 1) are nothing new. To illustrate, alcohol or gum enriched with capsaicin is an old trick to relieve toothache, where the analgesic effect arises from a lasting refractory state following excitation of sensory neurons by capsaicin. During this *"*refractory*"* or *"*desensitized*"* period, sensory neurons no longer respond to subsequent capsaicin stimulation nor to other stimuli such as noxious heat. Of course, desensitization can also be exploited therapeutically. Indeed, a wide range of capsaicin containing formulations, including creams and occlusive patches are on the market for a variety of pain syndromes, ranging from minor muscle or joint aches to chronic painful conditions such as diabetic neuropathy, post-herpetic neuralgia and arthritis (18, 19). However, the clinical value of these capsaicin-containing formulations is limited by the delicate balance between its irritating and desensitizing effects. Indeed, when the concentration acquired at the sensory nerve endings is not sufficiently high, capsaicin application will merely result in a stinging sensation, without the anticipated desensitization. On the other hand, one should be cautious not to produce neurotoxic effects at the high "therapeutic" doses. Accordingly, systemic administration of capsaicin is not attainable in clinical practice, despite its powerful therapeutic potential (18, 20).

In an attempt to optimize the "desensitization window", resiniferatoxin (RTX; Table 1) is currently under clinical investigation. RTX is another pungent plant product, derived from *Euphorbia resinifera*, of which the dried latex already has a long history of medical use (21). It is considered to be the ultrapotent analogue of capsaicin and is currently the most powerful TRPV1 agonist known. As such, stimulation of the TRP channel by RTX induces an exceedingly prolonged calcium influx, desensitizing the sensory neurons that express TRPV1 and thereby surpassing the pharmacological

mechanism of action associated with capsaicin (20, 22). At present, RTX is undergoing clinical trials to attain lasting pain relief in patients with advanced cancer, administrated via intrathecal or epidural injection. Preliminary results suggest that RTX can indeed evoke cell death of TRPV1-positive nociceptor neurons in human, causing prolonged pain relief (23). In addition, intra-articular injection of the plant derivative is in development for moderate to severe knee pain due to osteoarthritis, for which Sorrento Therapeutics recently progressed to phase III trials (NCT04044742). On the other hand, RTX was found unsuccessful to treat interstitial cystitis (24).

Noteworthy, in addition to overwhelming the ion channel, activation of TRPV1 in the antinociceptive descending pain pathway might be an alternative strategy to combat pain. In this respect, endocannabinoids including anandamide were identified as channel agonists and more recently, also AM404, a metabolite of paracetamol, was shown to be a potent activator of TRPV1 in the brain (25–29). Yet, besides activating and desensitizing TRPV1 using channel agonists, also TRPV1 antagonists possess the therapeutic potential to revolutionize pain management. To support early clinical development of these TRPV1 targeting drugs, capsaicin once again proved to be very useful. Following topical application or intradermal injection, capsaicin is well-known to provoke cutaneous neurogenic inflammation (Figure 1). The resulting flare, induced by an increase in dermal blood flow, can serve as a basis to evaluate TRPV1 target engagement in both animals and humans (Figure 4).

2.1.2 FIRST GENERATION TRPV1 ANTAGONISTS: TOO HOT TO HANDLE

Following the discovery of capsazepine (Table 1), the oldest TRPV1 antagonist, competing with capsaicin and RTX for the vanilloid binding site (Figure 2), TRPV1 research boomed (30, 31). Considering that TRPV1-deficient mice demonstrate reduced heat sensitivity, and do not develop inflammatory heat hyperalgesia, it was a straightforward step for the pharmaceutical industry to engage into the development of TRPV1 antagonists for pain relief. However, initial endeavors were far from successful, due to a lack of efficacy and unwanted side-effects.

SB-705498 (Table 1) from GlaxoSmithKline (GSK) was the first TRPV1 antagonist to enter clinical development in 2005. The compound was well tolerated at single oral doses up to 400 mg, and reduced both the flare evoked by topical application of capsaicin and the dermal inflammation induced by ultraviolet B irradiation (32). Based on the evident target engagement, GSK initiated phase II clinical studies. SB-705498 failed to demonstrate a positive effect on the intensity of dental pain after a 3rd molar tooth extraction, which is a well characterized model of acute inflammatory pain (33). In addition, the compound showed to be inferior to placebo against migraine headache, photo- and phonophobia (34). Following these unsuccessful results, GSK started aiming for respiratory and skin disorders as an alternative indication. Intranasal doses of the TRPV1 antagonist were evaluated to manage rhinitis, but also here only modest symptom attenuation could be obtained (35). Likewise, in the case of chronic cough, the compound failed to reduce cough counts, despite its confirmed engagement with TRPV1 (36). Finally, drug development attempts were terminated after a SB-705498-containing cream was found unsuccessful in atopic dermatitis (37). In contrast to other first-generation TRPV1 antagonists (see below), effects on core body temperature have not been reported for SB-705498. This may reflect the cautious developmental approach of GSK, which may not have resulted in a similar degree of TRPV1 antagonism as achieved with other compounds (36, 38).

Amgen also invested substantially in small molecule TRPV1 antagonists. Of the compounds evaluated in vitro, the polymodal antagonist AMG-517 (Table 1), a potent blocker of TRPV1 when activated not only by capsaicin but also by other stimuli such as heat or low pH, was selected for clinical development (39, 40). However, the expectation to develop a selective new analgesic drug was soon tempered due to problematic hyperthermic side effects. In a single-dose phase I study, AMG-517 evoked a marked but reversible increase in body temperature. The maximum temperature recorded in human was 39.9 °C (40). A similar hyperthermic reaction was also observed in animals treated with AMG-517, where the effect was attenuated upon repeated dosing, suggesting it may be circumvented in a clinical setting as well (41). As such, Amgen proceeded to a multiple-dose phase I

trial, which demonstrated that also humans habituate to the hyperthermic effect induced by 10 mg of AMG-517. Furthermore, the temperature elevation was found to be plasma concentration-dependent with some individual susceptibility (40). Still, it was unknown whether the concentration of AMG-517 required to obtain analgesia in humans was below or above the threshold triggering hyperthermia. To investigate this hypothesis, a phase II study was initiated in patients with acute dental pain after molar extraction. These subjects also experienced marked hyperthermia, but unlike in the phase I trial, temperature elevations persisted for days and individual susceptibility was no longer a unique case. About one third of the participants experienced elevated body temperatures of 39 to 40.2 °C, with the highest temperature registered in a subject receiving the lowest dose, only 2 mg of AMG-517 (40). The different hyperthemic responses in these phase I and phase II studies was unexpected, but is most likely an on-target effect of TRPV1 blockade, since TRPV1 knockout mice do not develop this drug-induced hyperthermia (42). Consequently, clinical studies involving AMG-517 were discontinued before uncovering its analgesic potential (40).

Recently, also Abbott, since 2013 divided into Abbott and Abbvie, published clinical results on their polymodal antagonist ABT-102 (Table 1). The analgesic capacities were assessed in a phase I crossover trial involving etoricoxib, tramadol and low doses of ABT-102. Painful stimulation was evoked using a CO_2-laser, on both normal and inflamed skin. In this experimental pain study, a 6 mg dose of ABT-102 was found effective to reduce pain and even superior to the active controls (43). Unfortunately, also this compound displayed temperature-related side effects, prompting its withdrawal from clinical development. In rats, ABT-102 evoked hyperthermia, an adverse on-target effect that attenuated within two days of recurrent dosing (44). Also in humans, ABT-102 induced a dose-dependent increase in core body temperature although participants never exceeded 39 °C. By the seventh dosing day, the body temperature was no longer significantly different from placebo subjects (45, 46). Accordingly, the TRPV1 antagonist presented worthy therapeutic potential in the context of chronic pain, if it were not for another temperature-related side effect. Indeed, ABT-102 was also found to affect the vital ability to sense noxious heat in subjects. With the first dose of the

antagonist, subjects encountered elevated cutaneous and oral heat pain thresholds as well as increased withdrawal latencies from a 49 °C water bath. Unlike the hyperthermic effects, these deficits maintained after repeated dosing. Thermosensation only recovered when the antagonist was nearly washed-out. As a result, development of ABT-102 was terminated due to a substantial clinical risk of burns (46).

Similarly, also AstraZeneca attempted to develop a polymodal TRPV1 antagonist. AZD1386 (Table 1) entered clinical development in 2008 with a single-dose study to investigate its effect on capsaicin- and heat-induced pain (47). Relying on the encouraging antinociceptive effect and evident target engagement, AstraZeneca continued to invest in their antagonist. A slight hyperthermic effect was noted, but not considered clinically significant as the largest rise in body temperature concerned 1.2 °C, and the highest temperature recorded was 38 °C (48, 49). Furthermore, the hyperthermia attenuated after multiple dosing, unlike the impact on skin heat perception with the heat pain threshold increasing on average 4.8 °C compared to placebo (47, 49). Yet, based on these overall mild temperature-related side effects, AstraZeneca proceeded to evaluate the analgesic activity of their antagonist. In patients experiencing pain following wisdom-tooth extraction, the primary endpoint focused on pain intensity over an eight hours period, but did not reach statistical significance. Instead, AZD1386 was shown to induce a very rapid but temporary analgesic effect. Within 15 minutes after receiving an oral solution containing AZD1386, subjects rated their pain intensity considerably lower than placebo. Surprisingly, the reported analgesia did not last longer than one hour, unlike the elevated heat pain threshold, which persisted up to five hours after dosing. Furthermore, the analgesic effect was obtained at relatively low plasma concentrations, that only peaked after one hour, when the analgesia already started to decrease (48). As such, the potential of AZD1386 for chronic pain conditions remained undecided, and, unfortunately, the drug candidate had to be withdrawn from further development as several patients in an osteoarthritic pain study exhibited elevated hepatic enzymes. Note that also this study hinted at some analgesic effect, as a reduction in pain intensity

using the numerical rating scale could be demonstrated. However, no clinically relevant effect on the Western Ontario and McMaster osteoarthritis index (WOMAC) could be obtained (50).

In addition, Johnson & Johnson Pharmaceutical Research & Development developed a TRPV1 antagonist aimed at treating osteoarthritic pain. Mavatrep, otherwise known as JNJ-39439335 (Table 1), originated from a benzo[*d*]imidazole platform and is a potent competitive antagonist of capsaicin-, pH- and heat-evoked currents mediated by TRPV1 (51). After confirming dose-dependent target engagement (52), the analgesic potential was assessed in patients with chronic osteoarthritic pain of the knee. Mavatrep diminished pain intensity following a stair climbing effort and improved osteoarthritic pain and stiffness using the WOMAC questionnaire, although the latter could not be confirmed in a multiple-dose study (53, 54). Unfortunately, also this compound suffered from temperature-related side effects. As only the hyperthermic effect and not the thermohypoesthesia minimized upon repeated dosing, the company terminated clinical development (52).

Many other pharmaceutical companies experienced similar obstacles in the quest for TRPV1 antagonists. MK-2295/NGD8243 (Table 1), a development collaboration between Merck and Neurogen, demonstrated significantly impaired sensitivity to noxious heat, with subjects requiring more than one minute extra to pull their hand out of a 49 °C water bath. Moreover, up to 40% of subjects did not recognize 70 °C water as noxious (55, 56). On the other hand, V116517 (Table 1) from Purdue Pharma displayed a clean safety profile in human, although a dose-dependent increase in body temperature was described in rats (57, 58). Nonetheless, no additional clinical studies have been registered for this compound. Likewise, clinical development of GRC-6211 (Table 1), a potent and competitive TRPV1 antagonist by Eli Lilly and Glenmark Pharmaceuticals, was suspended without providing details (59–61). Japan Tobacco halted development of JTS-653 (Table 1), one of the most potent polymodal TRPV1 antagonists (62) and also DWP05195 (structure not disclosed) from Daewoong Pharmaceuticals did not go beyond phase II trials (63). At the moment, only Tivanisiran, former SYL1001, a small interfering RNA (siRNA) targeting TRPV1 is still under development for dry eye disease, where the nociceptive channel is thought to be involved in ocular

pain and inflammation (64). So far, topical administration of SYL1001 was shown effective without tolerability issues (65, 66).

2.1.3 SECOND GENERATION TRPV1 ANTAGONISTS: A MORE COOLED DOWN APPROACH

In an attempt to achieve TRPV1-mediated analgesia without the accompanying hyperthermic effects, modality-selective antagonists were developed. Amgen was one of the pioneers to experiment with different TRPV1 modulators. Interestingly, AMG-8562 (Table 1), an antagonist that blocks capsaicin- but not heat-induced TRPV1 stimulation and potentiates activation by noxious pH, did not elicit hyperthermia in rats. On the other hand, AMG-7905 (Table 1), a compound that antagonizes activation by capsaicin but potentiates heat and proton stimulation, produced striking hypothermia. Accordingly, AMG-8562 was considered the long-awaited holy grail in TRPV1 drug development. The thermoneutral molecule was further evaluated in different experimental pain models, where it produced analgesic effects similar to those described for first generation polymodal TRPV1 antagonists. However, AMG-8562 presented a different pharmacological profile on human TRPV1 as the compound blocked activation by protons, instead of the potentiation demonstrated in rats. Hence, it was expected that AMG-8562 would still evoke hyperthermia in humans. As a result, the compound was never pursued clinically. Nonetheless, Amgen demonstrated that it is possible to separate the hyperthermic effects associated with TRPV1 modulation from their antihyperalgesic effects, paving the way for second generation TRPV1 antagonists (67).

Other pharmaceutical companies soon jumped on board to develop TRPV1 antagonists without the undesired effects on body temperature and noxious heat threshold. NEO6860 (structure not disclosed), developed by the NEOMED institute, is such a new generation antagonist that blocks TRPV1 activation by capsaicin, but not by heat or protons. A first-in-human study in 2015 discovered no relevant effect on core body temperature, nor heat pain threshold at doses ascending from 50 to 1200 mg. The only temperature-related adverse event was a slight perception of feeling hot. Due to

the well-known involvement of TRPV1 in heat sensation, this was believed to be an on-target effect. Other evidence of target engagement was provided by a reduction of the pain and flare induced by intradermal capsaicin injection (68, 69). To evaluate the analgesic properties of their modality-selective antagonist, NEOMED set up a proof-of-concept study in patients with osteoarthritic knee pain. NEO6860 provided pain relief based on the numerical rating scale pre- and post-exercise, the patient's global impression of change and to a smaller degree, the WOMAC (70). However, further research is necessary to fully comprehend the therapeutic potential of this compound.

PharmEste also developed a TRPV1 antagonist with an apparently clean safety profile. Remarkably, PHE377, alternatively named V-377 (Table 1), was reported to antagonize both capsaicin- and proton-induced activation of TRPV1 (71). As such, there was skepticism when the first-in-human trial was initiated. According to the company website, different phase I clinical trials confirmed the favorable safety profile of PHE377. However, PharmEste did not support this claim with data and for reasons unknown, the clinical development was terminated.

2.1.4 THE FUTURE OF TRPV1 ANTAGONISTS: EXTINQUISHED?

In conclusion, despite significant investments, there is currently no TRPV1 antagonist registered for clinical use. The efforts to develop small molecules targeting TRPV1 have produced multiple potent and selective inhibitors, but none of these compounds came close to market authorization. Several reasons are at the source of this unsatisfactory return on investment. Most importantly, acute pharmacological inhibition of TRPV1 has been shown to evoke hyperthermia, independent of the chemical structure of the antagonist. This on-target side effect appears related to the blockade of constitutively active TRPV1 channels on thermal afferents in the abdomen. These neurons show basal activity under physiological circumstances, which leads to a tonic suppression of autonomic cold defense mechanisms. Acute inhibition of TRPV1 reduces the basal activity of these thermal afferents, which in turn reduces the tonic suppression, ultimately causing hyperthermia. Although concerning, the evoked temperature elevation is not permanent as compensatory mechanisms can develop over

time (72). Also in human, the hyperthermic effect of some TRPV1 antagonists attenuated upon repeated administration, while the analgesic effect remained. Still, in an attempt to overcome this side effect, TRPV1 antagonists with different pharmacological profiles were investigated. The hyperthermic effect correlated with the proton mode of TRPV1 activation: antagonists with a high potency to block stimulation by protons induce hyperthermia, whereas antagonists that potentiate the TRPV1-mediated proton response even cause hypothermia (72, 73). Interestingly, Garami et al. recently suggested that in humans, but not in rats, the heat activation mode of TRPV1 also plays a role in temperature regulation, while the capsaicin mode was shown to be irrelevant (74). As such, second generation, modality-selective TRPV1 antagonists still hold some promise, but the clinical potential of this generation has not yet been thoroughly explored.

In addition to the numerous attempts to block TRPV1 activity, interest in selective channel agonists rekindled to target local anesthetics towards sensory neurons. Most local anesthetics, including lidocaine, exert their effect by interacting with sodium channels embedded in the cell membrane, thereby reducing the neuronal excitability and consequently, generating anesthesia. However, as these drugs do not specifically target sensory neurons, common side effects include low blood pressure and local paralysis. In an attempt to stay away from sympathetic and motor nerve fibers, sodium channel blockers in combination with TRPV1 agonists were shown to produce pain-specific local anesthesia by chaperoning the anesthetic through the pore of the TRPV1 channel (Figure 3) (75). In mice, this approach was shown to induce a long-lasting anesthetic effect, without the burning sensation generally known to accompany capsaicin injection (76). However, to the best of our knowledge, the clinical potential of this combination was never investigated in human.

2.2 TRPA1, THE ONLY CHILD OF THE FAMILY

As an alternative drug target to TRPV1, Transient Receptor Potential Ankyrin 1 (TRPA1) caught the eye. This only mammalian member of the TRPA subfamily, is highly co-expressed with TRPV1 in nociceptor neurons, and was initially described as a noxious cold sensor, although its role in cold

sensing in mice and man remains a matter of debate (77–80). In contrast, the role of TRPA1 in nociceptive responses to chemical compounds is well established. TRPA1 can be activated by a broad variety of irritants. This includes numerous natural pungents like cinnamaldehyde (Table 2), responsible for the flavor and odor of cinnamon, allicin, present in garlic and allyl isothiocyanate (Table 2), the pungent ingredient in wasabi and mustard oil. Besides, also acrolein, a toxicant in cigarette smoke and tear gasses is a known channel agonist. All these compounds activate TRPA1 through the covalent modification of reactive cysteine and lysine residues in the channel's N-terminus (Figure 5). Yet, also non-electrophilic agonists have been described, including menthol, nicotine (81), and cannabidiol. Moreover, multiple endogenous compounds released under conditions of oxidative stress and inflammation have been described to activate TRPA1 (82–84), evidently pointing toward a role of the channel in nociception and pathological pain. This has been confirmed in several animal models, showing effects of genetic ablation or pharmacological inhibition of TRPA1 inflammatory pain as well as in diabetic and chemotherapy-induced painful neuropathy (85–91).

Notably, the familial episodic pain syndrome (FEPS) provides genetic evidence that variations in the TRPA1 gene are capable of altering pain sensitivity in humans. FEPS results from a point mutation in the channel gene, associated with an enhanced response to both chemical and thermal TRPA1 activation. Individuals suffering from the syndrome describe debilitating episodes of upper body pain, often elicited by fasting, cold and physical stress (92). Based on all these findings, TRPA1 is considered to be a potential analgesic and anti-inflammatory target and as such has been pursued by several pharmaceutical companies.

2.2.1 TRPA1 ANTAGONISTS: MORE THIN ON THE GROUND

In contrast to TRPV1, where agonist-induced desensitization is an established means of analgesia (see above), using TRPA1 agonists to induce pain relief has been poorly explored. Yet, a recent study related the antinociceptive effect of acetaminophen to activation of TRPA1 in the spinal cord. It was proposed that the sodium and calcium influx following stimulation of spinal TRPA1 will generate excitatory postsynaptic currents, yet the subsequent inhibition of voltage-gated sodium and calcium

channels on the central terminals in the dorsal horn will reduce neuronal excitability, providing a mechanism for the antinociceptive effect of the common analgesic, at least preclinically (93). Nonetheless, pharmaceutical companies mainly invested in channel antagonists as was the case with TRPV1.

Over a decade ago, Hydra Biosciences took the first step towards therapeutic TRPA1 antagonists with HC-030031 (Table 2), a xanthine derivative (94). The compound was shown to diminish mechanical hypersensitivity in rodent models of inflammatory and neuropathic pain (95) and was found effective to reduce inflammation and hyperactivity of the airways in wild-type mice exposed to airway allergens (96). HC-030031 soon became a stalwart friend in preclinical TRPA1 research, and put the channel in the spotlight as a drug target for diabetic neuropathy, chemotherapy-mediated neuropathic pain, inflammatory bowel disease and respiratory disorders (97). Yet, from a pharmacokinetic perspective the caffeine (Table 2) derivative showed less promise, with a half-life of around 30 minutes in rats, high clearance and only micromolar potency (98). Nevertheless, it inspired pharmaceutical companies to develop more potent TRPA1 antagonists with enhanced pharmacokinetic properties.

In 2012, Glenmark Pharmaceuticals selected GRC-17536 (Table 2) from a series of caffeine-based TRPA1 antagonists to initiate phase I development. The drug was well tolerated and demonstrated a decent pharmacokinetic profile (99). A subsequent proof-of-concept study in patients with painful diabetic peripheral neuropathy delivered a promising statistically significant and clinically relevant response in the subgroup without denervation, without affecting the central nervous system or inducing other drug-related adverse effects (100). In addition, Glenmark evaluated the compound in patients with refractory chronic cough, but no significant improvement in cough frequency could be established (101). GRC-17536 is now a candidate for out-licensing, but full clinical development is supposedly hampered by the drug's poor pharmaceutical characteristics as the structure is rather bulky, lipophilic and poorly soluble (102).

Although Glenmark was the first company to take a TRPA1 antagonist to clinical development, also Hydra Biosciences continued to work on better channel blockers. In 2012, the company announced a phase I clinical trial for CB-625 (Table 2), a joint collaboration effort with Cubist Pharmaceuticals, but pharmacokinetic concerns supposedly halted further development of this nanomolar potent TRPA1 modulator (102, 103).

In general, the first class of xanthine-based antagonists suffered from pharmaceutical and pharmacokinetic flaws, despite strong inhibitory potencies. As such, several companies shifted their focus towards other classes of small molecule TRPA1 antagonists. Over the recent years, companies such as Amgen, Abbott, AstraZeneca, Janssen, Merck, Pfizer and Roche all described channel modulators with diverse medicinal chemistry. Some of these compounds were shown to reduce nocifensive behavior in rats exposed to cinnamaldehyde or mustard oil, but for the most part not much is known about these development attempts (102). Among other concerns, inconsistent effects across species proved to be a hurdle in the further progress of these drug candidates (104, 105). For example, Amgen disclosed a series of species-specific TRPA1 modulators including AMG9090 (Table 2), an antagonist of hTRPA1 but a partial agonist at rTRPA1 (106). Interestingly, an antagonist developed by Novartis (Table 2) was shown to affect the cold perception of naïve mice at -5 °C, without altering body temperature (107). On the hand, none of the subjects included in a human TRPA1-specific pain study reported a cold sensation after intradermal injections of JT010 and A-967079 (Table 2), selective agonist and antagonist of TRPA1, respectively (108).

In 2015, the Orion corporation initiated a phase I study for ODM-108, a negative allosteric TRPA1 modulator. The chemical structure of this antagonist has not been disclosed so far, but likely resembles the alkyne-containing compounds (Table 2) patented in the same year (109). Unfortunately, the structural modifications were no golden ticket to success as Orion terminated the study early due to complex pharmacokinetic results. However, no safety concerns were reported (NCT02432664).

Also Hydra Biosciences tried a different approach. In addition to their HC-030031 analogue CB-625, the company developed HX-100 (structure not disclosed), a structurally different TRPA1 antagonist. Based on promising preclinical safety and efficacy results, Hydra Biosciences teamed up with Boehringer-Ingelheim to advance HX-100 into clinical trials for painful diabetic neuropathy and allergic asthma. The phase I clinical trial followed a standard single and multiple ascending dose design and was completed in 2016. Hydra Biosciences was expected to quickly proceed to phase II development, but dose-related skin and musculoskeletal adverse events in healthy volunteers put an end to the fairy tale. Recently, Eli Lilly acquired Hydra's preclinical program of TRPA1 antagonists, committed to develop new therapeutic options for chronic pain patients. Currently, the company is evaluating the safety, tolerability and pharmacokinetic effects of LY3526318, previously known as HX-260 (Table 2). In addition, target engagement will be assessed using cinnamaldehyde-induced dermal blood flow changes in healthy females (NCT03977974;NCT04183283) (110, 111).

2.2.2 THE FUTURE OF TRPA1 ANTAGONISTS: DESOLATE?

Taking into account that TRPA1 focused drug development started only a decade ago, it is not surprising that antagonists are largely outnumbered by TRPV1 targeted drug candidates. However, the numerous patent applications filed by different pharmaceutical companies demonstrate a general interest in selective TRPA1 channel antagonists (for review see (112)). The therapeutic potential of the ion channel is further supported by promising clinical results, yet development efforts are tormented by poor pharmaceutical and pharmacokinetic properties, and at the moment, the growing TRPA1 interest appears to have stumbled over the hurdles along the way. Besides the pharmacokinetic concerns, TRPA1 antagonists might exert an effect on body temperature and/or thermosensation. These temperature-related side effects remain a subject of discussion as several preclinical studies in mice delivered contradictory results on the importance of TRPA1 in thermoregulation (16, 113, 114). However, unlike for TRPV1, an effect on human thermosensation or thermoregulation has not been described for TRPA1 so far. Yet, the bumpy road travelled with

TRPV1 antagonists makes investors rather reticent when it comes to TRP drug development. In addition, the striking differences between human and rodent TRPA1 are another complication damping industry's enthusiasm. As the sequence homology of human and rodent TRPA1 is less than 80%, the relevance of these animal experiments may be questioned (115). In this respect, experiments in primates might be more instructive, as it is known that monkeys and humans share similar TRPA1 pharmacology (104). Finally, TRPA1 can be found in a large variety of tissues, although the channel is primarily expressed on nociceptive neurons. This opens the door to several on-target, yet undesirable side effects. Nonetheless, no major adverse events were described for the few TRPA1 antagonists that advanced to clinical development, making us wonder whether TRPA1 is undeservedly left behind. In this respect, characterizing the TRPA1 binding sites for channel antagonists would be of great service to breathe new life into the development efforts.

Still, if medicinal chemistry efforts fail to produce successful compounds, TRPA1 agonists might be able to ease the pain. In addition, also monoclonal antibodies against TRPA1 provide therapeutic possibilities. The true potential of this approach has been questioned due to the limited extracellular pore loop of TRP channels. Yet, in 2014 Amgen described two monoclonal antibodies, 2B10 and 2D1, acting as antagonists of multiple modes of TRPA1 activation (116). Unfortunately, the feasibility of these antagonist antibodies as therapeutics remains an important, underexplored opportunity in TRP targeted drug development.

To conclude, the last has not been said when it comes to TRPA1 targeted drug development, and as our knowledge on TRPA1 modulation increases, this only child might pave the road to effective TRP-based analgesics.

2.3 TRPM3, THE NEW KID ON THE BLOCK

Recently, the spotlight moved to Transient Receptor Potential Melastatin 3 (TRPM3). Initially described as a volume-regulated calcium permeable channel in the human kidney, this member of the melastatin subfamily is now recognized as a polymodal nociceptor, sensitive to a variety of physical and chemical stimuli, most notably the endogenous neurosteroid pregnenolone sulfate (Table 3) and

the 1,4-dihydropyridine nifedipine (Table 3) (117, 118). TRPM3 mRNA was detected at levels similar to TRPV1 and TRPA1 in mouse sensory neurons, and the three channels show a largely overlapping expression profile (119, 120). Likewise, activation of TRPM3 can provoke pain, as exemplified by the pain evoked by injection of pregnenolone sulfate into the hindpaw of wild-type but not TRPM3 knockout mice (120). Later, the synthetic small molecule CIM0216 (Table 3) was described as a more potent chemical ligand (121). Remarkably, CIM0216 was shown to open an alternative permeation pathway in addition to the central calcium permeable pore. Also combined stimulation with pregnenolone sulfate and the antifungal drug clotrimazole (Table 3), but not nifedipine and clotrimazole, causes the alternative permeation pathway to open (122). Activation of this additional permeation pathway is thus strongly stimulus dependent, but can greatly enhance action potential firing and thereby intensify TRPM3-mediated pain (122). In addition to its role as a nociceptive chemosensor, TRPM3 is activated by a heat. Like TRPV1 knockout animals, elimination of TRPM3 abolishes inflammatory heat hyperalgesia. Preclinical studies in rodents are highly promising and indeed suggest that pharmacological inhibition of TRPM3 can alleviate pain and hyperalgesia (123). Interestingly, recent evidence also provided a role for G-protein-coupled receptors, including μ opioid receptors, in the modulation of TRPM3, but not TRPV1 and TRPA1. Upon activation of peripheral μ opioid receptors, the $G_{\beta\gamma}$ subunit can dissociate and directly bind to TRPM3, thereby inhibiting the channel's activity (124–129). On top of that, the non-steroidal anti-inflammatory drug diclofenac (Table 3) was shown to antagonize the Melastatin 3 family member, in addition to its effect on other TRPs (130, 131). In human, a de novo mutation in TRPM3's S4–S5 linker region was recently described in subjects with developmental and epileptic encephalopathies. In addition to epilepsy and intellectual disability, some probands reported altered heat and pain thresholds (132). Altogether, inhibition of TRPM3 has drawn attention as a viable strategy to combat pain. Of course, if we have learned anything from the past TRP drug development attempts, it is the importance of a favorable safety profile in addition to the analgesic potential. So far, TRPM3 is presumed not to contribute to body temperature regulation, as neither channel agonists nor antagonists

were shown to disturb core temperature in mice (133, 134). Nonetheless, potentially dangerous impairments in the acute response to noxious heat remain a concern, as TRPM3 antagonists were shown to attenuate heat sensation (133, 134). Yet, only minor deficits are expected as long as the capsaicin receptor remains functional. In line with the clinical results obtained with TRPA1, functionality of TRPV1 is thought to be sufficient to maintain the initial response to acute heat as 43 °C is hypothesized to be the turning point between innocuous warmth and noxious heat (135). Besides these possible temperature-related issues, the broad expression pattern of TRPM3 raises a reasonable concern for other on-target side effects. Still, the generation of knockout mice revealed no major deficits and also the administration of TRPM3 antagonists like primidone (Table 3) and the flavone isosakuranetin (Table 3) was reassuringly devoid of serious side effects (133, 134). Nonetheless, the biggest impediment in the development of TRPM3 targeted drugs is the availability of suitable drug candidates.

3. CONCLUSION AND FUTURE PERSPECTIVES

Pain management continues to present an area of substantial unmet medical need. With the discovery of nociceptive TRP channels, one hoped to develop a new class of potent, safe analgesics. Yet, for the time being, the ugly TRP side has been most prominent. Activation or inhibition of a TRP channel may be beneficial for pain relief while at the same time inducing unacceptable adverse effects. Indeed, clinical development of TRPV1 antagonists was largely halted because drugs caused hyperthermia and put patients at risk for scalding injuries by elevating the heat pain threshold. On the other hand, TRPA1-targeted drug development suffered from pharmaceutical and pharmacokinetic complications. Nonetheless, companies that find a way to successfully exploit the fair face of TRP channels might hold a golden ticket towards pain relief. In this respect, TRPM3 is hitting the headlines as the latest TRP member to tackle. Bearing the lessons learned from the past, this ion channel might indeed be just the cannon we need to combat persistent pain.

ACKNOWLEDGEMENTS

Scientific illustration toolkits from Motifolio were used to generate some of the figures shown in this manuscript

REFERENCES

1. Nahin RL. 2015. Estimates of Pain Prevalence and Severity in Adults: United States, 2012. *J. Pain*. 16(8):769–80

2. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. 2006. Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *Eur. J. Pain*. 10(4):287–287

3. Lynch ME, Watson CPN. 2006. The pharmacotherapy of chronic pain: a review. *Pain Res. Manag.* 11(1):11–38

4. Massimo Calabresi. 2015. Why America Can't Kick Its Painkiller Problem. *Time*

5. Skolnick P. 2018. The Opioid Epidemic: Crisis and Solutions. *Annu. Rev. Pharmacol. Toxicol. Annu. Rev. Pharmacol. Toxicol*. 58:143–59

6. Cosens DJ, Manning A. 1969. Abnormal electroretinogram from a Drosophila mutant

7. Minke B, Wu CF, Pak WL. 1975. Induction of photoreceptor voltage noise in the dark in Drosophila mutant. *Nature*. 258(5530):84–87

8. Montell C, Rubin GM. 1989. Molecular characterization of the drosophila trp locus: A putative integral membrane protein required for phototransduction. *Neuron*. 2(4):1313–23

9. Gees M, Owsianik G, Nilius B, Voets T. 2012. TRP channels

10. Julius D, Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 389(6653):816–24

11. Bevan S, Quallo T, Andersson DA. 2014. TRPV1. In *Handbook of experimental*

pharmacology. 222:207–45

12. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, et al. 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 288(5464):306–13

13. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, et al. 2000. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature*. 405(6783):183–87

14. Vriens J, Nilius B, Voets T. 2014. Peripheral thermosensation in mammals

15. Dhaka A, Viswanath V, Patapoutian A. 2006. TRP ion channels and temperature sensation. *Annu. Rev. Neurosci.* 29(1):135–61

16. Vandewauw I, De Clercq K, Mulier M, Held K, Pinto S, et al. 2018. A TRP channel trio mediates acute noxious heat sensing. *Nature*. 555(7698):662–66

17. Nilius B, Appendino G. 2013. Spices: The savory and beneficial science of pungency. *Rev. Physiol. Biochem. Pharmacol.* 164:1–76

18. Kaneko Y, Szallasi A. 2014. Transient receptor potential (TRP) channels: a clinical perspective. *Br. J. Pharmacol.* 171(10):2474–2507

19. Szallasi A, Blumberg PM. 1999. Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol. Rev.* 51(2):159–212

20. Kissin I, Szallasi A. 2011. Therapeutic targeting of TRPV1 by resiniferatoxin, from preclinical studies to clinical trials. *Curr. Top. Med. Chem.* 11(17):2159–70

21. Appendino G, Szallasi A. 1997. Euphorbium: Modern research on its active principle, resiniferatoxin, revives an ancient medicine. *Life Sci.* 60(10):681–96

22. Brown DC. 2016. Resiniferatoxin: The Evolution of the “Molecular Scalpel” for Chronic Pain Relief. *Pharmaceuticals (Basel).* 9(3):

23. Heiss J, Iadarola M, Cantor F, Oughourli A, Smith R, Mannes A. 2014. A Phase I study of the intrathecal administration of resiniferatoxin for treating severe refractory pain associated with advanced cancer. *J. Pain*. 15(4):S67

24. Wong GY, Gavva NR. 2009. Therapeutic potential of vanilloid receptor TRPV1 agonists and antagonists as analgesics: Recent advances and setbacks. *Brain Res. Rev.* 60(1):267–77

25. Ross RA. 2003. Anandamide and vanilloid TRPV1 receptors

26. Zygmunt PM, Petersson J, Andersson DA, Chuang HH, Sørgård M, et al. 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*. 400(6743):452–57

27. Smart D, Gunthorpe MJ, Jerman JC, Nasir S, Gray J, et al. 2000. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br. J. Pharmacol.* 129(2):227–30

28. Zygmunt PM, Chuang H, Movahed P, Julius D, Högestätt ED. 2000. The anandamide transport inhibitor AM404 activates vanilloid receptors. *Eur. J. Pharmacol.* 396(1):39–42

29. Mallet C, Barrière DA, Ermund A, Jönsson BAG, Eschalier A, et al. 2010. TRPV1 in brain is involved in acetaminophen-induced antinociception. *PLoS One*. 5(9):

30. Bevan S, Hothi S, Hughes G, James IF, Rang HP, et al. 1992. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *Br. J. Pharmacol.* 107(2):544–52

31. Walpole CSJ, Bevan S, Bovermann G, Boelsterli JJ, Breckenridge R, et al. 1994. The Discovery of Capsazepine, the First Competitive Antagonist of the Sensory Neuron Excitants Capsaicin and Resiniferatoxin. *J. Med. Chem.* 37(13):1942–54

32. Chizh BA, O'Donnell MB, Napolitano A, Wang J, Brooke AC, et al. 2007. The effects of the TRPV1 antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. *Pain*. 132(1):132–41

33. Palmer J, Lai R, Thomas N, Bullman J, Ali Z, et al. 2009. Randomised, placebo-controlled, parallel-group study of the effect of SB-705498, a TRPV1 receptor antagonist, on pain following third molar tooth extraction. *Eur. J. Pain*. 13(S1):S202

34. Chizh B, Palmer J, Lai R, Guillard F, Bullman J, et al. 2009. A randomised, two-period cross-over study to investigate the efficacy of the trpv1 antagonist sb-705498 in acute migraine. *Eur. J. Pain*. 13(S1):S202

35. Bareille P, Murdoch RD, Denyer J, Bentley J, Smart K, et al. 2013. The effects of a TRPV1 antagonist, SB- 705498, in the treatment of seasonal allergic rhinitis. *Int. J. Clin. Pharmacol. Ther*. 51(7):576–84

36. Khalid S, Murdoch R, Newlands A, Smart K, Kelsall A, et al. 2014. Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: A double-blind randomized controlled trial. *J. Allergy Clin. Immunol.* 134(1):56-62.e4

37. Gibson RA, Robertson J, Mistry H, McCallum S, Fernando D, et al. 2014. A randomised trial evaluating the effects of the TRPV1 antagonist SB705498 on pruritus induced by histamine, and cowhage challenge in healthy volunteers. *PLoS One*. 9(7):

38. Allerton, C, Fox D. 2014. *Pain Therapeutics- Current and Future Treatment Paradigms*. 135–137 pp.

39. Broad LM, Keding SJ, Blanco M-J. 2008. Recent progress in the development of selective TRPV1 antagonists for pain. *Curr. Top. Med. Chem.* 8(16):1431–41

40. Gavva NR, Treanor JJS, Garami A, Fang L, Surapaneni S, et al. 2008. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain*. 136(1):202–10

41. Gavva NR, Bannon AW, Hovland DN, Lehto SG, Klionsky L, et al. 2007. Repeated administration of vanilloid receptor TRPV1 antagonists attenuates hyperthermia elicited by

TRPV1 blockade. *J. Pharmacol. Exp. Ther.* 323(1):128–37

42. Garami A, Pakai E, Oliveira DL, Steiner AA, Wanner SP, et al. 2011. Thermoregulatory phenotype of the Trpv1 knockout mouse: Thermoeffector dysbalance with hyperkinesis. *J. Neurosci.* 31(5):1721–33

43. Schaffler K, Reeh P, Duan WR, Best AE, Othman AA, et al. 2013. An oral TRPV1 antagonist attenuates laser radiant-heat-evoked potentials and pain ratings from UV_B-inflamed and normal skin. *Br. J. Clin. Pharmacol.* 75(2):404–14

44. Honore P, Chandran P, Hernandez G, Gauvin DM, Mikusa JP, et al. 2009. Repeated dosing of ABT-102, a potent and selective TRPV1 antagonist, enhances TRPV1-mediated analgesic activity in rodents, but attenuates antagonist-induced hyperthermia. *Pain.* 142(1–2):27–35

45. Othman AA, Nothaft W, Awni WM, Dutta S. 2013. Effects of the TRPV1 antagonist ABT-102 on body temperature in healthy volunteers: pharmacokinetic/ pharmacodynamic analysis of three phase 1 trials. *Br. J. Clin. Pharmacol.* 75(4):1029–40

46. Rowbotham MC, Nothaft W, Duan WR, Wang Y, Faltynek C, et al. 2011. Oral and cutaneous thermosensory profile of selective TRPV1 inhibition by ABT-102 in a randomized healthy volunteer trial. *Pain.* 152(5):1192–1200

47. Karlsten R, Jonzon B, Quiding H, Carlsson MA, Segerdahl M, Malamut R, Björnsson M MF. TRPV1 antagonist AZD1386 inhibits capsaicin and heat evoked pain in healthy volunteers. *Abstr. 13th World Congr. Pain. Seattle IASP, 2010.*

48. Quiding H, Jonzon B, Svensson O, Webster L, Reimfelt A, et al. 2013. TRPV1 antagonistic analgesic effect: A randomized study of AZD1386 in pain after third molar extraction. *Pain.* 154(6):808–12

49. Gomtsyan A, Brederson JD. 2015. Clinical and Preclinical Experience with TRPV1 Antagonists as Potential Analgesic Agents. In *TRP Channels as Therapeutic Targets: From*

Basic Science to Clinical Use, pp. 129–44. Elsevier Inc.

50. Miller F, Björnsson M, Svensson O, Karlsten R. 2014. Experiences with an adaptive design for a dose-finding study in osteoarthritis. *Scand. J. Pain*. 5(3):209–209

51. Parsons WH, Calvo RR, Cheung W, Lee Y-K, Patel S, et al. 2015. Benzo[*d*]imidazole Transient Receptor Potential Vanilloid 1 Antagonists for the Treatment of Pain: Discovery of *trans* -2-(2-{2-[2-(4-Trifluoromethyl-phenyl)-vinyl]-1 *H* -benzimidazol-5-yl}-phenyl)-propan-2-ol (Mavatrep). *J. Med. Chem.* 58(9):3859–74

52. Manitpisitkul P, Brandt M, Flores CM, Kenigs V, Moyer JA, et al. 2016. TRPV1 antagonist JNJ-39439335 (mavatrep) demonstrates proof of pharmacology in healthy men: a first-in-human, double-blind, placebo-controlled, randomized, sequential group study. *Pain reports*. 1(4):e576

53. Mayorga AJ, Flores CM, Trudeau JJ, Moyer JA, Shalayda K, et al. 2017. A randomized study to evaluate the analgesic efficacy of a single dose of the TRPV1 antagonist mavatrep in patients with osteoarthritis. *Scand. J. Pain*. 17(1):134–43

54. Manitpisitkul P, Flores CM, Moyer JA, Romano G, Shalayda K, et al. 2018. A multiple-dose double-blind randomized study to evaluate the safety, pharmacokinetics, pharmacodynamics and analgesic efficacy of the TRPV1 antagonist JNJ-39439335 (mavatrep). *Scand. J. Pain*. 18(2):151–64

55. Crutchlow M, Dong Y, Schulz V, Van Hoydonck P, Laethem T, et al. 2009. Pharmacologic inhibition of TRPV1 impairs sensation of potentially injurious heat in healthy subjects. *110th Annu. Meet. Am. Soc. Clin. Pharmacol. Ther.*

56. R. Eid S. 2011. Therapeutic Targeting of TRP Channels - The TR(i)P to Pain Relief. *Curr. Top. Med. Chem.* 11(17):2118–30

57. Arendt-Nielsen L, Harris S, Whiteside GT, Hummel M, Knappenberger T, et al. 2016. A

randomized, double-blind, positive-controlled, 3-way cross-over human experimental pain study of a TRPV1 antagonist (V116517) in healthy volunteers and comparison with preclinical profile. *Pain*. 157(9):2057–67

58. Tafesse L, Kanemasa T, Kurose N, Yu J, Asaki T, et al. 2014. Structure-activity relationship studies and discovery of a potent transient receptor potential vanilloid (TRPV1) antagonist 4-[3-chloro-5-[(1 S)-1,2-dihydroxyethyl]-2-pyridyl]-N-[5-(trifluoromethyl)-2-pyridyl]-3, 6-dihydro-2 H-pyridine-1-carboxamide (V116517) as a clinical candidate for pain management. *J. Med. Chem.* 57(15):6781–94

59. Glenmark Pharmaceuticals, Eli Lilly. 2007. *Glenmark's lead candidate for pain, GRC 6211, to enter Phase II*. Press Release. https://pipelinereview.com/index.php/2007052912039/Small-Molecules/Glenmarks-lead-candidate-for-pain-GRC-6211-to-enter-Phase-II.html

60. Glenmark Pharmaceuticals, Eli Lilly. 2008. *Further Clinical Trials in Osteoarthritis Pain Suspended for GRC 6211*. Press Release. https://www.fiercebiotech.com/biotech/further-clinical-trials-osteoarthritis-pain-suspended-for-grc-6211

61. Xia R, Dekermendjian K, Lullau E, Dekker N. 2011. TRPV1: A Therapy Target That Attracts the Pharmaceutical Interests. In *Transient Receptor Potential Channels*, pp. 637–65. Springer, Dordrecht

62. Kitagawa Y, Miyai A, Usui K, Hamada Y, Deai K, et al. 2012. Pharmacological characterization of (3S)-3-(hydroxymethyl)-4-(5-methylpyridin-2-yl)-N-[6-(2,2,2-trifluoroethoxy)pyridin-3-yl]-3,4-dihydro-2H-benzo[b][1,4]oxazine-8-carboxamide (JTS-653), a novel transient receptor potential vanilloid 1 antagonist. *J. Pharmacol. Exp. Ther.* 342(2):520–28

63. Lee J, Kim BH, Yu KS, Kim HS, Kim JD, et al. 2017. A first-in-human, double-blind, placebo-controlled, randomized, dose escalation study of DWP05195, a novel TRPV1

antagonist, in healthy volunteers. *Drug Des. Devel. Ther.* 11:1301–13

64. Pan Z, Wang Z, Yang H, Zhang F, Reinach PS. 2011. TRPV1 activation is required for hypertonicity-stimulated inflammatory cytokine release in human corneal epithelial cells. *Investig. Ophthalmol. Vis. Sci.* 52(1):485–93

65. Benitez-Del-Castillo JM, Moreno-Montañés J, Jiménez-Alfaro I, Muñoz-Negrete FJ, Turman K, et al. 2016. Safety and efficacy clinical trials for SYL1001, a novel short interfering RNA for the treatment of dry eye disease. *Investig. Ophthalmol. Vis. Sci.* 57(14):6447–54

66. Bleau A-M, Ruz V, González V, Martínez T, Vargas B, Jimenez AI. 2018. Development of tivanisiran, a topical siRNA designed to treat dry eye disease. *Integr. Clin. Med.* 2(2):

67. Lehto SG, Tamir R, Deng H, Klionsky L, Kuang R, et al. 2008. Antihyperalgesic Effects of (R,E)-N-(2-Hydroxy-2,3-dihydro-1H-inden-4-yl)-3-(2-(piperidin-1-yl)-4-(trifluoromethyl)phenyl)-acrylamide (AMG8562), a Novel Transient Receptor Potential Vanilloid Type 1 Modulator That Does Not Cause Hyperthermia in Rats. *J. Pharmacol. Exp. Ther.* 326(1):218–29

68. Brown W, Leff RL, Griffin A, Hossack S, Aubray R, et al. 2017. Safety, Pharmacokinetics, and Pharmacodynamics Study in Healthy Subjects of Oral NEO6860, a Modality Selective Transient Receptor Potential Vanilloid Subtype 1 Antagonist. *J. Pain*. 18(6):726–38

69. Chiche D, Brown W, Walker P. 2016. NEO6860, a novel modality selective TRPV1 antagonist: results from a phase I, double-blind, placebo-controlled study in healthy subjects. *J. Pain*. 17(4):S79

70. Arsenault P, Leff R, Katz N, Walker P, Chiche D. 2017. Analgesic Potential of NEO6860, a Modality Selective TRPV1 Antagonist, in Osteoarthritis Knee Pain: Results of a Randomized, Controlled, Proof-of-Concept Trial. *ACR Meet. Abstr.*

71. Nilius B, Szallasi A. 2014. Transient Receptor Potential Channels as Drug Targets: From the

Science of Basic Research to the Art of Medicine. *Pharmacol. Rev.* 66(3):676–814

72. Romanovsky AA, Almeida MC, Garami A, Steiner AA, Norman MH, et al. 2009. The transient receptor potential vanilloid-1 channel in thermoregulation: a thermosensor it is not. *Pharmacol. Rev.* 61(3):228–61

73. Garami A, Pakai E, McDonald HA, Reilly RM, Gomtsyan A, et al. 2018. TRPV1 antagonists that cause hypothermia, instead of hyperthermia, in rodents: Compounds' pharmacological profiles, in vivo targets, thermoeffectors recruited and implications for drug development. *Acta Physiol.* 223(3):e13038

74. Garami A, Shimansky YP, Rumbus Z, Vizin RCL, Farkas N, et al. 2020. Hyperthermia induced by transient receptor potential vanilloid-1 (TRPV1) antagonists in human clinical trials: Insights from mathematical modeling and meta-analysis. *Pharmacol. Ther.*, p. 107474

75. Binshtok AM, Bean BP, Woolf CJ. 2007. Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. *Nature*. 449(7162):607–10

76. Gerner P, Binshtok AM, Wang C-F, Hevelone ND, Bean BP, et al. 2008. Capsaicin Combined with Local Anesthetics Preferentially Prolongs Sensory/Nociceptive Block in Rat Sciatic Nerve. *Anesthesiology*. 109(5):872–78

77. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, et al. 2003. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell*. 112(6):819–29

78. Zygmunt PM, Högestätt ED. 2014. TRPA1. , pp. 583–630. Springer, Berlin, Heidelberg

79. Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, et al. 2009. TRPA1 acts as a cold sensor in vitro and in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 106(4):1273–78

80. Chen J, Kym PR. 2009. TRPA1: The species difference. *J. Gen. Physiol.* 133(6):623–25

81. Talavera K, Gees M, Karashima Y, Meseguer VM, Vanoirbeek JAJ, et al. 2009. Nicotine activates the chemosensory cation channel TRPA1. *Nat. Neurosci.* 12(10):1293–99

82. Andersson DA, Gentry C, Moss S, Bevan S. 2008. Transient Receptor Potential A1 Is a Sensory Receptor for Multiple Products of Oxidative Stress. *J. Neurosci.* 28(10):2485–94

83. Materazzi S, Nassini R, Andre E, Campi B, Amadesi S, et al. 2008. Cox-dependent fatty acid metabolites cause pain through activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci.* 105(33):12045–50

84. Sawada Y, Hosokawa H, Matsumura K, Kobayashi S. 2008. Activation of transient receptor potential ankyrin 1 by hydrogen peroxide. *Eur. J. Neurosci.* 27(5):1131–42

85. Nazıroğlu M, Braidy N. 2017. Thermo-Sensitive TRP Channels: Novel Targets for Treating Chemotherapy-Induced Peripheral Pain. *Front. Physiol.* 8:1040

86. Trevisan G, Materazzi S, Fusi C, Altomare A, Aldini G, et al. 2013. Novel Therapeutic Strategy to Prevent Chemotherapy-Induced Persistent Sensory Neuropathy By TRPA1 Blockade. *Cancer Res.* 73(10):3120–31

87. Zhao M, Isami K, Nakamura S, Shirakawa H, Nakagawa T, Kaneko S. 2012. Acute Cold Hypersensitivity Characteristically Induced by Oxaliplatin is Caused by the Enhanced Responsiveness of TRPA1 in Mice. *Mol. Pain*. 8:1744-8069-8–55

88. Materazzi S, Fusi C, Benemei S, Pedretti P, Patacchini R, et al. 2012. TRPA1 and TRPV4 mediate paclitaxel-induced peripheral neuropathy in mice via a glutathione-sensitive mechanism. *Pflügers Arch. - Eur. J. Physiol.* 463(4):561–69

89. Wei H, Hämäläinen MM, Saarnilehto M, Koivisto A, Pertovaara A. 2009. Attenuation of Mechanical Hypersensitivity by an Antagonist of the TRPA1 Ion Channel in Diabetic Animals. *Anesthesiology*. 111(1):147–54

90. Eberhardt MJ, Filipovic MR, Leffler A, de la Roche J, Kistner K, et al. 2012. Methylglyoxal

activates nociceptors through transient receptor potential channel A1 (TRPA1): a possible mechanism of metabolic neuropathies. *J. Biol. Chem.* 287(34):28291–306

91. Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, et al. 2006. TRPA1 Mediates the Inflammatory Actions of Environmental Irritants and Proalgesic Agents. *Cell*. 124(6):1269–82

92. Kremeyer B, Lopera F, Cox JJ, Momin A, Rugiero F, et al. 2010. A Gain-of-Function Mutation in TRPA1 Causes Familial Episodic Pain Syndrome. *Neuron*. 66(5):671–80

93. Andersson DA, Gentry C, Alenmyr L, Killander D, Lewis SE, et al. 2011. TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Δ9-tetrahydrocannabiorcol. *Nat. Commun.* 2(1):551

94. McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, et al. 2007. TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. U. S. A.* 104(33):13525–30

95. Eid SR, Crown ED, Moore EL, Liang HA, Choong K-C, et al. 2008. HC-030031, a TRPA1 Selective Antagonist, Attenuates Inflammatory- and Neuropathy-Induced Mechanical Hypersensitivity. *Mol. Pain*. 4:1744-8069-4–48

96. Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, et al. 2009. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc. Natl. Acad. Sci.* 106(22):9099–9104

97. Chen J, Hackos DH. 2015. TRPA1 as a drug target—promise and challenges. *Naunyn. Schmiedebergs. Arch. Pharmacol.* 388(4):451–63

98. Rech JC, Eckert WA, Maher MP, Banke T, Bhattacharya A, Wickenden AD. 2010. Recent advances in the biology and medicinal chemistry of TRPA1. *Future Med. Chem.* 2(5):843–58

99. Glenmark Pharmaceuticals Ltd. 2012. *Glenmark's novel molecule "GRC 17536" for pain and respiratory conditions successfully completes Phase I trials in Europe.*

https://www.glenmarkpharma.com/sites/default/files/Glenmark_GRC_17536_Phase_I_completion_0.pdf

100. Glenmark Pharmaceuticals. 2017. *Glenmark's TRPA1 Antagonist 'GRC 17536' Shows Positive Data in a Proof of Concept Study*. https://www.prnewswire.com/news-releases/glenmarks-trpa1-antagonist-grc-17536-shows-positive-data-in-a-proof-of-concept-study-275445961.html

101. EudraCT Number 2013-002728-17. 2016. Clinical trial results: A Phase 2a, Multi-Centre, Randomised, Double-Blind, Parallel Group, Placebo-Controlled Study to Evaluate Efficacy, Safety and Tolerability of Inhaled GRC 17536, Administered for 4 Weeks, in Patients with Refractory Chronic Cough

102. Skerratt S. 2017. Recent Progress in the Discovery and Development of TRPA1 Modulators. *Prog. Med. Chem.* 56:81–115

103. Cubist Pharmaceuticals, Hydra Biosciences. 2012. *Cubist Pharmaceuticals and Hydra Biosciences Announce Plans to Begin Phase 1 Clinical Trial for Novel TRPA1 Modulator to Treat Acute Pain*. http://hydrabiosciences.com/pdf/press_releases/2012_01_10.pdf

104. Bianchi BR, Zhang X-F, Reilly RM, Kym PR, Yao BB, Chen J. 2012. Species comparison and pharmacological characterization of human, monkey, rat, and mouse TRPA1 channels. *J. Pharmacol. Exp. Ther.* 341(2):360–68

105. Reese RM, Dourado M, Anderson K, Warming S, Stark KL, et al. 2020. Behavioral characterization of a CRISPR-generated TRPA1 knockout rat in models of pain, itch, and asthma. *Sci. Rep.* 10(1):1–11

106. Klionsky L, Tamir R, Gao B, Wang W, Immke DC, et al. 2007. Species-specific pharmacology of Trichloro(sulfanyl)ethyl benzamides as transient receptor potential ankyrin 1 (TRPA1) antagonists. *Mol. Pain.* 3:39

107. Rooney L, Vidal A, D'Souza A-M, Devereux N, Masick B, et al. 2014. Discovery, Optimization, and Biological Evaluation of 5-(2-(Trifluoromethyl)phenyl)indazoles as a Novel Class of Transient Receptor Potential A1 (TRPA1) Antagonists. *J. Med. Chem.* 57(12):5129–40

108. Heber S, Gold-Binder M, Ciotu CI, Witek M, Ninidze N, et al. 2019. A human trpa1-specific pain model. *J. Neurosci.* 39(20):3845–55

109. Orion Corporation, Arvela R, Holm P, Vesalainen A. 2015. TRPA1 modulators. *WO2015144976*

110. Buntinx L, Chang L, Amin A, Morlion B, de Hoon J. 2017. Development of an *in vivo* target-engagement biomarker for TRPA1 antagonists in humans. *Br. J. Clin. Pharmacol.* 83(3):603–11

111. Van der Schueren BJ, de Hoon JN, Vanmolkot FH, Van Hecken A, Depre M, et al. 2007. Reproducibility of the capsaicin-induced dermal blood flow response as assessed by laser Doppler perfusion imaging. *Br. J. Clin. Pharmacol.* 64(5):580–90

112. Preti D, Saponaro G, Szallasi A. 2015. Transient receptor potential ankyrin 1 (TRPA1) antagonists. *Pharm. Pat. Anal.* 4(2):75–94

113. Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang D-S, et al. 2006. TRPA1 Contributes to Cold, Mechanical, and Chemical Nociception but Is Not Essential for Hair-Cell Transduction. *Neuron*. 50(2):277–89

114. de Oliveira C, Garami A, Lehto SG, Pakai E, Tekus V, et al. 2014. Transient receptor potential channel ankyrin-1 is not a cold sensor for autonomic thermoregulation in rodents. *J. Neurosci.* 34(13):4445–52

115. Chen J, Kang D, Xu J, Lake M, Hogan JO, et al. 2013. Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat. Commun.* 4(1):2501

116. Lee KJ, Wang W, Padaki R, Bi V, Plewa CA, Gavva NR. 2014. Mouse Monoclonal Antibodies to Transient Receptor Potential Ankyrin 1 Act as Antagonists of Multiple Modes of Channel Activation. *J. Pharmacol. Exp. Ther.* 350(2):223–31

117. Grimm C, Kraft R, Sauerbruch S, Schultz G, Harteneck C. 2003. Molecular and Functional Characterization of the Melastatin-related Cation Channel TRPM3. *J. Biol. Chem.* 278(24):21493–501

118. Wagner TFJ, Loch S, Lambert S, Straub I, Mannebach S, et al. 2008. Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic β cells. *Nat. Cell Biol.* 10(12):1421–30

119. Vriens J, Held K, Voets T. 2017. The nociceptive TRPM3 channel as potential therapeutic target for chronic pain. *Intern. Med. Rev.* 3(8):

120. Vriens J, Owsianik G, Hofmann T, Philipp SE, Stab J, et al. 2011. TRPM3 Is a Nociceptor Channel Involved in the Detection of Noxious Heat. *Neuron*. 70(3):482–94

121. Held K, Kichko T, De Clercq K, Klaassen H, Van Bree R, et al. 2015. Activation of TRPM3 by a potent synthetic ligand reveals a role in peptide release. *Proc. Natl. Acad. Sci.* 112(11):E1363–72

122. Vriens J, Held K, Janssens A, Tóth BI, Kerselaers S, et al. 2014. Opening of an alternative ion permeation pathway in a nociceptor TRP channel. *Nat. Chem. Biol.* 10(3):188–95

123. Vriens J, Voets T. 2018. Sensing the heat with TRPM3. *Pflügers Arch. - Eur. J. Physiol.*

124. Dembla S, Behrendt M, Mohr F, Goecke C, Sondermann J, et al. 2017. Anti-nociceptive action of peripheral mu-opioid receptors by G-beta-gamma protein-mediated inhibition of TRPM3 channels. *Elife*. 6:

125. Alkhatib O, da Costa R, Gentry C, Quallo T, Bevan S, Andersson DA. 2019. Promiscuous G-Protein-Coupled Receptor Inhibition of Transient Receptor Potential Melastatin 3 Ion

Channels by Gβγ Subunits. *J. Neurosci.* 39(40):7840–52

126. Yudin Y, Rohacs T. 2019. The G-protein-biased agents PZM21 and TRV130 are partial agonists of μ-opioid receptor-mediated signalling to ion channels. *Br. J. Pharmacol.* 176(17):bph.14702

127. Badheka D, Yudin Y, Borbiro I, Hartle CM, Yazici A, et al. 2017. Inhibition of transient receptor potential Melastatin 3 ion channels by G-protein βγ subunits. *Elife*. 6:

128. Quallo T, Alkhatib O, Gentry C, Andersson DA, Bevan S. 2017. G protein βγ subunits inhibit TRPM3 ion channels in sensory neurons. *Elife*. 6:

129. Vangeel L, Benoit M, Miron Y, Miller PE, De Clercq K, et al. 2020. Functional expression and pharmacological modulation of TRPM3 in human sensory neurons. *Br. J. Pharmacol.* bph.14994

130. Suzuki H, Sasaki E, Nakagawa A, Muraki Y, Hatano N, Muraki K. 2016. Diclofenac, a nonsteroidal anti-inflammatory drug, is an antagonist of human TRPM3 isoforms. *Pharmacol. Res. Perspect.* 4(3):1–14

131. Hu H, Tian J, Zhu Y, Wang C, Xiao R, et al. 2010. Activation of TRPA1 channels by fenamate nonsteroidal anti-inflammatory drugs. *Pflügers Arch. - Eur. J. Physiol.* 459(4):579–92

132. Dyment DA, Terhal PA, Rustad CF, Tveten K, Griffith C, et al. 2019. De novo substitutions of TRPM3 cause intellectual disability and epilepsy. *Eur. J. Hum. Genet.* 27(10):1611–18

133. Krügel U, Straub I, Beckmann H, Schaefer M. 2017. Primidone inhibits TRPM3 and attenuates thermal nociception in vivo. *Pain*. 158(5):856–67

134. Straub I, Krugel U, Mohr F, Teichert J, Rizun O, et al. 2013. Flavanones That Selectively Inhibit TRPM3 Attenuate Thermal Nociception In Vivo. *Mol. Pharmacol.* 84(5):736–50

135. Patapoutian A, Peier AM, Story GM, Viswanath V. 2003. ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nat. Rev. Neurosci.* 4(7):529–39

136. Fein A. 2014. *Nociceptors and the perception of pain*

137. Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, et al. 2006. Glial Cell Line-Derived Neurotrophic Factor Family Members Sensitize Nociceptors In Vitro and Produce Thermal Hyperalgesia In Vivo. *J. Neurosci.* 26(33):8588–99

138. Ji R-R, Samad TA, Jin S-X, Schmoll R, Woolf CJ. 2002. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron*. 36(1):57–68

139. Julius D. 2013. TRP Channels and Pain. *Annu. Rev. Cell Dev. Biol.* 29(1):355–84

140. Richardson JD, Vasko MR. 2002. Cellular Mechanisms of Neurogenic Inflammation. *J. Pharmacol. Exp. Ther.* 302(3):839–45

141. Gao Y, Cao E, Julius D, Cheng Y. 2016. TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature*. 534(7607):347–51

142. Buntinx L, Vermeersch S, de Hoon J. 2015. Development of anti-migraine therapeutics using the capsaicin-induced dermal blood flow model. *Br. J. Clin. Pharmacol.* 80(5):992–1000

143. Grant AD, Gerard NP, Brain SD. 2002. Evidence of a role for NK_1 and CGRP receptors in mediating neurogenic vasodilatation in the mouse ear. *Br. J. Pharmacol.* 135(2):356–62

144. Aubdool AA, Kodji X, Abdul-Kader N, Heads R, Fernandes ES, et al. 2016. TRPA1 activation leads to neurogenic vasodilatation: involvement of reactive oxygen nitrogen species in addition to CGRP and NO. *Br. J. Pharmacol.* 173(15):2419–33

145. Suo Y, Wang Z, Zubcevic L, Hsu AL, He Q, et al. 2020. Structural Insights into Electrophile Irritant Sensing by the Human TRPA1 Channel. *Neuron*. 105(5):882-894.e5

146. Hayes P, Meadows HJ, Gunthorpe MJ, Harries MH, Duckworth DM, et al. 2000. Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. *Pain*. 88(2):205–15

147. Shin JS, Wang M-H, Hwang SW, Cho H, Cho SY, et al. 2001. Differences in sensitivity of vanilloid receptor 1 transfected to human embryonic kidney cells and capsaicin-activated channels in cultured rat dorsal root ganglion neurons to capsaicin receptor agonists. *Neurosci. Lett.* 299(1–2):135–39

148. Correll CC, Phelps PT, Anthes JC, Umland S, Greenfeder S. 2004. Cloning and pharmacological characterization of mouse TRPV1. *Neurosci. Lett.* 370(1):55–60

149. Gunthorpe MJ, Hannan SL, Smart D, Jerman JC, Arpino S, et al. 2007. Characterization of SB-705498, a potent and selective vanilloid receptor-1 (VR1/TRPV1) antagonist that inhibits the capsaicin-, acid-, and heat-mediated activation of the receptor. *J. Pharmacol. Exp. Ther.* 321(3):1183–92

150. Rami HK, Thompson M, Stemp G, Fell S, Jerman JC, et al. 2006. Discovery of SB-705498: A potent, selective and orally bioavailable TRPV1 antagonist suitable for clinical development. *Bioorganic Med. Chem. Lett.* 16(12):3287–91

151. Holland C, van Drunen C, Denyer J, Smart K, Segboer C, et al. 2014. Inhibition of capsaicin-driven nasal hyper-reactivity by SB-705498, a TRPV1 antagonist. *Br. J. Clin. Pharmacol.* 77(5):777–88

152. Surowy CS, Neelands TR, Bianchi BR, McGaraughty S, El Kouhen R, et al. 2008. (R)-(5-tert-butyl-2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)-urea (ABT-102) blocks polymodal activation of transient receptor potential vanilloid 1 receptors in vitro and heat-evoked firing of spinal dorsal horn neurons in vivo. *J. Pharmacol. Exp. Ther.* 326(3):879–88

153. Rowbotham MC, Nothaft W, Duan RW, Wang Y, Faltynek C, et al. 2011. Oral and

cutaneous thermosensory profile of selective TRPV1 inhibition by ABT-102 in a randomized healthy volunteer trial. *Pain.* 152(5):1192–1200

154. Othman AA, Nothaft W, Awni WM, Dutta S. 2012. Pharmacokinetics of the TRPV1 Antagonist ABT-102 in Healthy Human Volunteers: Population Analysis of Data From 3 Phase 1 Trials. *J. Clin. Pharmacol.* 52(7):1028–41

155. Othman AA, Cheskin H, Locke C, Nothaft W, Dutta S. 2012. A Phase 1 Study to Evaluate the Bioavailability and Food Effect of 2 Solid-Dispersion Formulations of the TRPV1 Antagonist ABT-102, Relative to the Oral Solution Formulation, in Healthy Human Volunteers. *Clin. Pharmacol. Drug Dev.* 1(1):24–31

156. Krarup AL, Ny L, Åstrand M, Bajor A, Hvid-Jensen F, et al. 2011. Randomised clinical trial: the efficacy of a transient receptor potential vanilloid 1 antagonist AZD1386 in human oesophageal pain. *Aliment. Pharmacol. Ther.* 33(10):1113–22

157. Manitpisitkul P, Shalayda K, Russell L, Sanga P, Solanki B, et al. 2017. Pharmacokinetics and Safety of Mavatrep (JNJ-39439335), a TRPV1 Antagonist in Healthy Japanese and Caucasian Men: A Double-Blind, Randomized, Placebo-Controlled, Sequential-Group Phase 1 Study. *Clin. Pharmacol. Drug Dev.*

158. Manitpisitkul P, Shalayda K, Russell L, Sanga P, Williams Y, et al. 2017. Bioavailability and Pharmacokinetics of TRPV1 Antagonist Mavatrep (JNJ-39439335) Tablet and Capsule Formulations in Healthy Men: Two Open-Label, Crossover, Single-Dose Phase 1 Studies. *Clin. Pharmacol. Drug Dev.*

159. Rajagopal Bakthavatchalam, Charles A. Blum, Brielmann HL, Caldwell TM, Lombaert S De, et al. 2003. Substituted quinazolin-4-ylamine analogues as modulators of capsaicin. *WO2003062209*

160. Tafesse L, Kanemasa T, Kurose N, Yu J, Asaki T, et al. 2014. Structure−Activity

Relationship Studies and Discovery of a Potent Transient Receptor Potential Vanilloid (TRPV1) Antagonist 4-[3-Chloro-5-[(1S)-1,2-dihydroxyethyl]-2-pyridyl]-N-[5-(trifluoromethyl)-2-pyridyl]-3,6-dihydro-2H-pyridine-1-carboxamide (V116517) as a Clinical Candidate for Pain Management

161. Glenmark Pharmaceuticals, Gharat L, Joshi U, Joshi Neelima. 2006. Substituted benzofused derivatives and their use as vanilloid receptor ligands. *WO2007042906*

162. Kitagawa Y, Miyai A, Usui K, Hamada Y, Deai K, et al. 2012. Pharmacological characterization of (3S)-3-(hydroxymethyl)-4-(5- methylpyridin-2-yl)-N-[6-(2,2,2-trifluoroethoxy)pyridin-3-yl]-3, 4-dihydro-2H-benzo[b][1,4]oxazine-8-carboxamide (JTS-653), a novel transient receptor potential vanilloid 1 antagonist. *J. Pharmacol. Exp. Ther.* 342(2):520–28

163. Arsenault P, Chiche D, Brown W, Miller J, Treister R, et al. 2018. NEO6860, modality-selective TRPV1 antagonist: A randomized, controlled, proof-of-concept trial in patients with osteoarthritis knee pain. *Pain Reports*. 3(6):

164. PharmEste SRL, Baraldi G, Borea A, Pierangelo G. 2006. Sulfonamido compounds that antagonise the vanilloid TRPV1 receptor. *WO2006045498*

165. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, et al. 2004. Noxious Cold Ion Channel TRPA1 Is Activated by Pungent Compounds and Bradykinin. *Neuron*. 41(6):849–57

166. Hu H, Tian J, Zhu Y, Wang C, Xiao R, et al. 2010. Activation of TRPA1 channels by fenamate nonsteroidal anti-inflammatory drugs. *Pflugers Arch. Eur. J. Physiol.* 459(4):579–92

167. Hinman A, Chuang HH, Bautista DM, Julius D. 2006. TRP channel activation by reversible covalent modification. *Proc. Natl. Acad. Sci. U. S. A.* 103(51):19564–68

168. Nagatomo K, Kubo Y. 2008. Caffeine activates mouse TRPA1 channels but suppresses human TRPA1 channels. *Proc. Natl. Acad. Sci. U. S. A.* 105(45):17373–78

169. Bianchi BR, Zhang XF, Reilly RM, Kym PR, Yao BB, Chen J. 2012. Species comparison and pharmacological characterization of human, monkey, rat, and mouse TRPA1 channels. *J. Pharmacol. Exp. Ther.* 341(2):360–68

170. Anupindi R, Mukhopadhyay I, Thomas A, Kumar S, Chaudhari S., et al. 2010. GRC 17536, a novel, selective TRPA1 antagonist for potential treatment of respiratory disorders. *Eur. Respir. Soc. Annu. Congr.*

171. Mukhopadhyay I, Gomes P, Aranake S, Shetty M, Karnik P, et al. 2011. Expression of functional TRPA1 receptor on human lung fibroblast and epithelial cells. *J. Recept. Signal Transduct.* 31(5):350–58

172. Metcalf C, Gu Y, Kimball D, Li Q, Lippa B, et al. 2013. Inhibiting transient receptor potential ion channel TRPA1. *WO2013023102*

173. Perner RJ, Kort ME, Didomenico S, Jun C, Vasudevan A. 2009. Trpa1 antagonists. *WO2009089082*

174. Chen J, Joshi SK, DiDomenico S, Perner RJ, Mikusa JP, et al. 2011. Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. *Pain.* 152(5):1165–72

175. Krügel U, Straub I, Beckmann H, Schaefer M. 2017. Primidone inhibits TRPM3 and attenuates thermal nociception in vivo. *Pain.* 158(5):856–67

176. Straub I, Mohr F, Stab J, Konrad M, Philipp S, et al. 2013. Citrus fruit and fabacea secondary metabolites potently and selectively block TRPM3. *Br. J. Pharmacol.* 168(8):1835–50

SIDEBAR 1: PEPTIDERGIC VERSUS NON-PEPTIDERGIC AFFERENTS

Interestingly, TRPV1 expression has been reported in two distinct populations of C fibers. One specific class, termed peptidergic afferents, can induce the release of neuropeptides including calcitonin-gene related peptide (CGRP) and substance P (SP). However, not all nociceptors express SP and CGRP. As they develop, some nociceptive C fibers switch off the tropomyosin receptor kinase A (TrkA) with high affinity for the neurothrophin nerve growth factor (NGF). Instead, they begin to express the glial cell line derived neurotrophic factor (GDNF) receptor Ret. These neurons are known as non-peptidergic nociceptors. Peptidergic nociceptors preserve TrkA expression, and only these neurons express SP and CGRP (11, 136). Interestingly, elevated levels of both NGF and GDNF have been described in circumstances of inflammation, in which these factors are known to upregulate the expression of TRPV1 and as such facilitate thermal hyperalgesia (137, 138). On the other hand, the neuropeptides released from peptidergic afferents following TRP activation are known to evoke neurogenic inflammation (Figure 1) and as such contribute to the typical hypersensitivity and pain symptoms following tissue injury (139).

FIGURE CAPTIONS

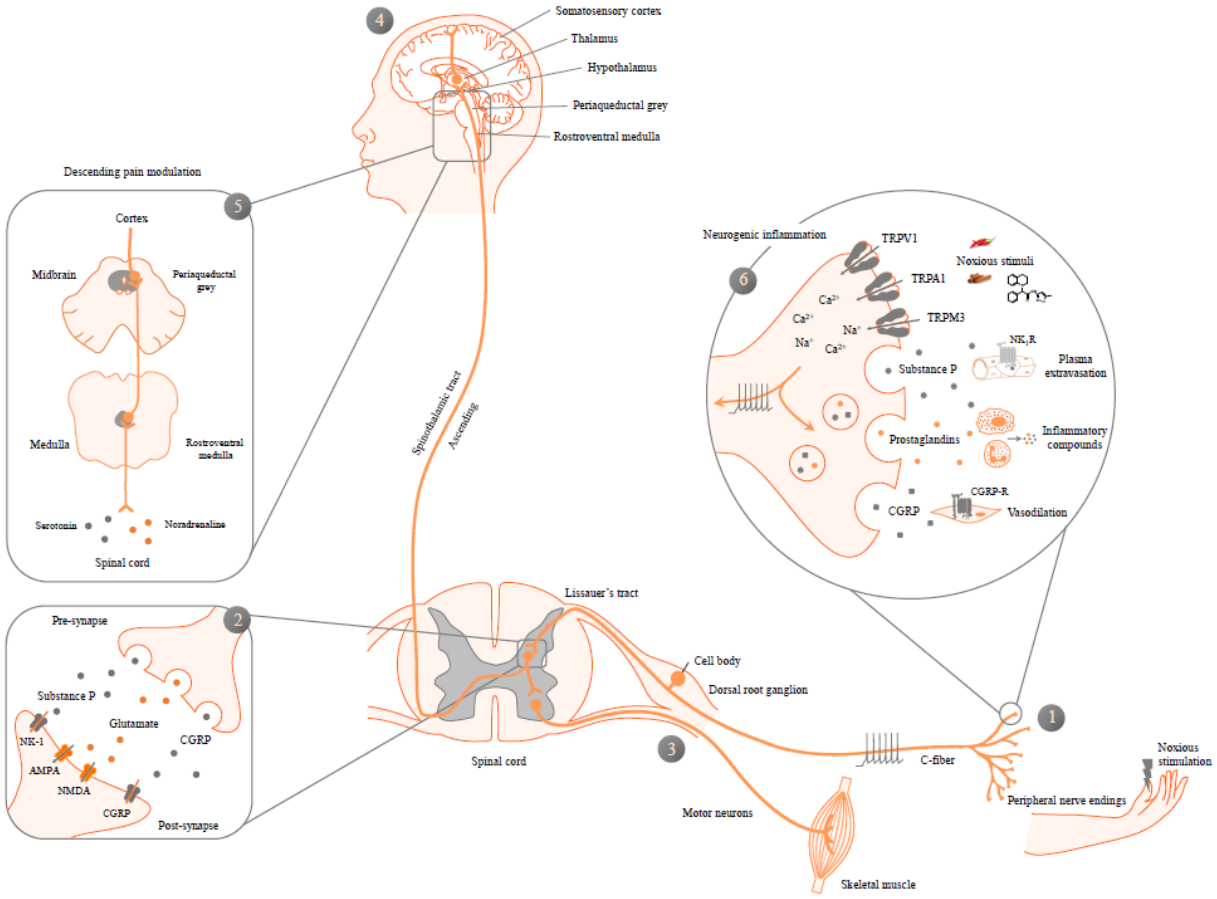


Figure 1: Pain and neurogenic inflammation following the activation of nociceptive TRP channels. (1) In the event of painful stimulation, nociceptive ion channels including TRPV1, TRPA1 and TRPM3 can be activated. (2) The nociceptive signal then travels up to the spinal cord via myelinated Aδ-fibers or unmyelinated C-fibers, terminating in the upper laminae of the dorsal horn. Within the spinal cord, second-order neurons are activated through the release of neurotransmitters, including glutamate, substance P (SP) and calcitonin gene-related peptide (CGRP). (3) To ensure a rapid withdrawal reflex, the nociceptive information is communicated with motorneurons. (4) A sensation of pain arises when the spinothalamic tract passes the information to the brain, where the thalamus relays the nociceptive signal to the cortical areas. (5) In these higher brain regions, also descending systems originate that can attenuate or facilitate the nociceptive input in the dorsal horn. Both the periaqueductal grey and rostroventral medulla are key structures for the descending modulation of pain. Activation of TRP channels within the antinociceptive pathway is one of the strategies to obtain analgesia. (6) Besides currents conducted towards the central nervous system, the

activation of calcium-permeable TRP channels in the terminals of peptidergic C fibers can promote the local release of inflammatory mediators, including SP, CGRP and prostaglandins. Via the neurokinin-1 (NK-1) receptor expressed on endothelial cells, SP increases vascular permeability leading to plasma extravasation and thereby to swelling of the area. CGRP is a potent vasodilator, exerting its effect by interaction with the CGRP receptor on vascular smooth muscle cells. This vasodilatation causes the typical symptoms of warmth and redness. Besides, SP, CGRP as well as prostaglandins act on neighboring mast cells and immune cells, resulting in the release of inflammatory and pro-algesic mediators. This inflammatory soup in turn activates other sensory nerve endings, enlarging the response. Together these symptoms characterize neurogenic inflammation, a vicious cycle of ongoing nociceptor activation and inflammation, manifesting itself as hypersensitivity (139, 140).

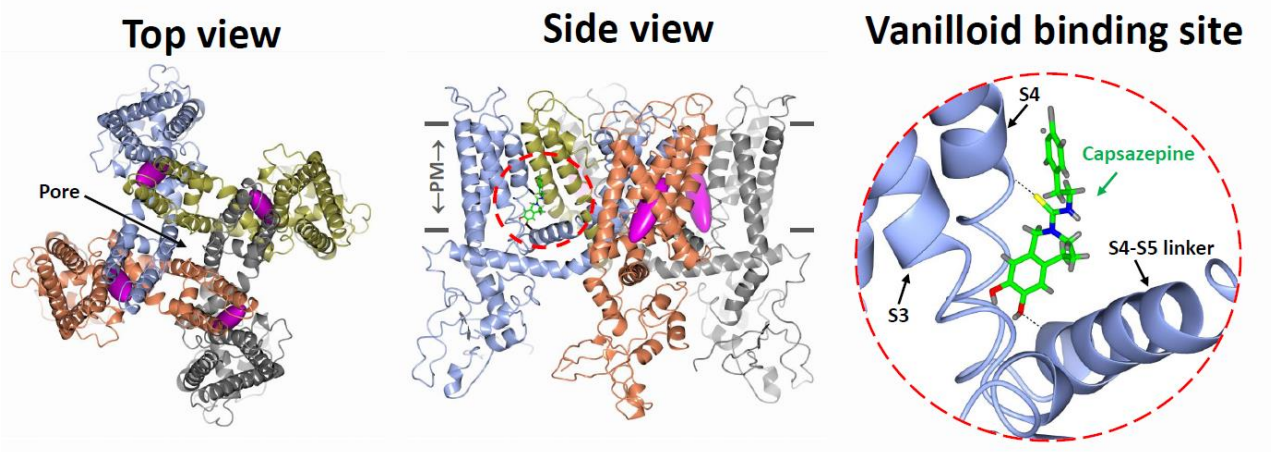


Figure 2: Structure of TRPV1. Ribbon diagram of the structure of TRPV1, as seen from the extracellular side (left) with indication of the central pore, and from the side (middle), with indication of the plasma membrane (PM). Differently colored protein regions indicate the four identical subunits of the tetrameric channel. The location of the vanilloid binding site is shown by the magenta ellopsoids. The right panel shows the details of one vanilloid binding site indicated in the middle panel. This binding site is occupied by a molecule of capsazepine, a competitive vanilloid antagonist. Structures were rendered using CCP4MG version 2.10.11 (http://www.ccp4.ac.uk/MG/), based on the atomic coordinates deposited in the Protein Data Bank under accession number 5IS0 (141).

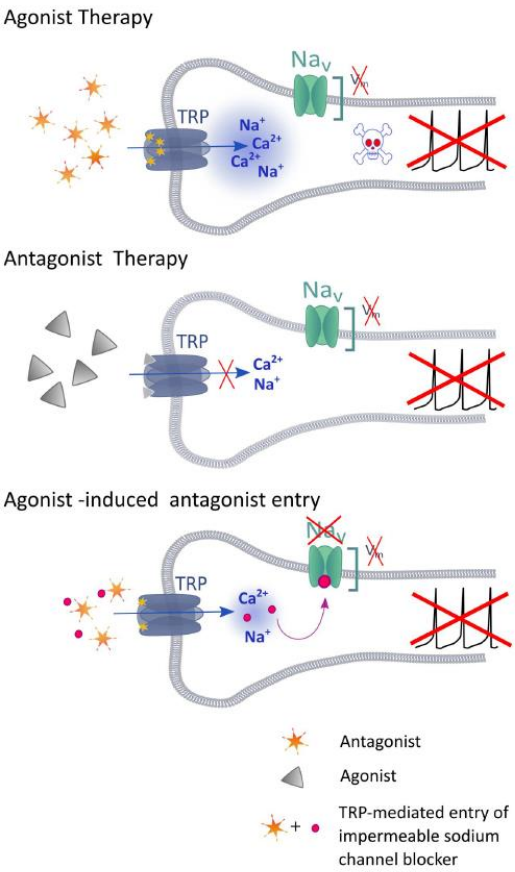


Figure 3: Therapeutic TRP strategies. (1) Agonist therapy. Opening of the TRP channel (TRPV1, TRPA1 or TRPM3) by application of an ultrapotent agonist will induce massive influx of sodium and calcium ions in the peripheral nerve endings. The extracellular calcium entering through TRP channels will overwhelm the intracellular calcium buffering capacity and thereby activate calcium-dependent proteases and cytoskeleton breakdown. As a result, local nociceptor function will be impaired for an extended period. For example, pain management with a capsaicin 8% patch to rapidly deliver the TRPV1 agonist into the skin. (2) Antagonist therapy. Selective TRP inhibitors will induce a full block of the TRP channel and prevent depolarization of the membrane potential by blocking the influx of cations like sodium and calcium. As a consequence, voltage dependent sodium channels (Na_V) will not be activated. (3) Agonist-induced antagonist entry. When combining a TRP channel agonist with local anesthetics like inhibitors of Na_V, the membrane-impermeable sodium channel blocker will be able to enter the nerve endings via the TRP pore. For example, anesthetic drugs will be chaperoned through the pore of the TRPV1 channel and block Na_V channels in all nerve endings expressing TRPV1.

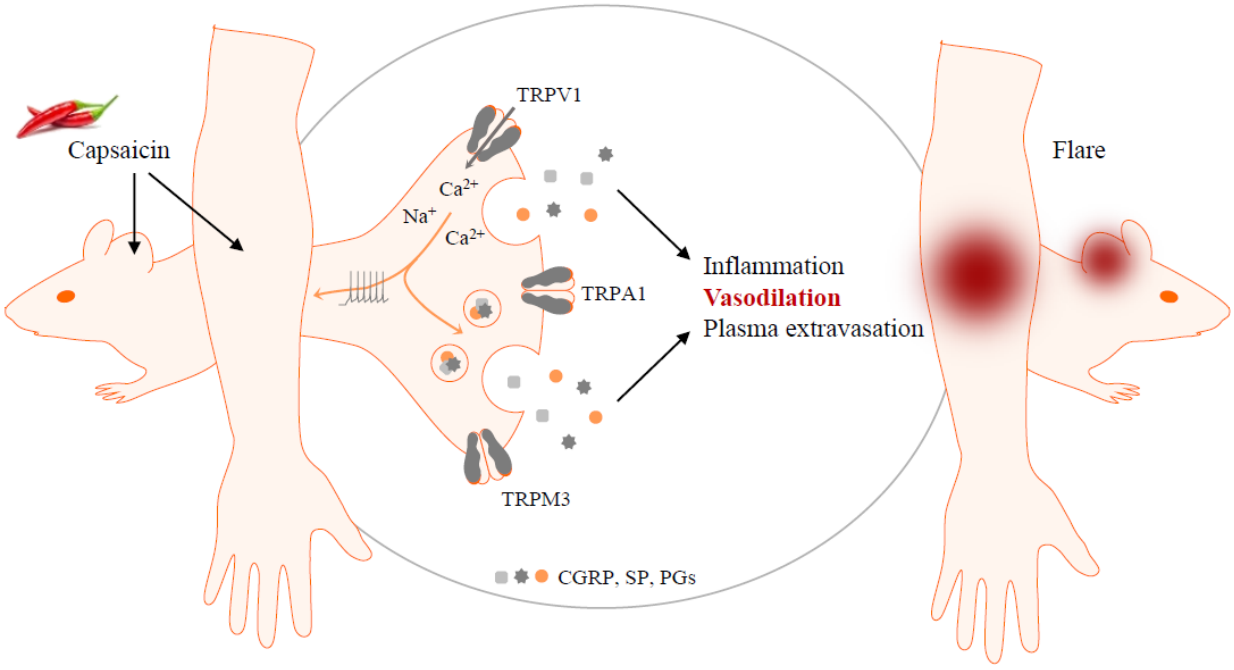


Figure 4: Capsaicin target engagement biomarker. Following administration on the ear of anesthetized mice or on the volar surface of subjects' forearm, capsaicin will activate calcium-permeable TRPV1 channels expressed on the peripheral nerve endings of sensory neurons innervating the skin. As a result, several mediators including calcitonin gene-related peptide (CGRP), substance P (SP) and prostaglandins (PGs) will be released from peptidergic dermal afferents, in turn evoking inflammation, vasodilatation and plasma extravasation, generally known as neurogenic inflammation. The vasodilatory component can be observed as a flare response with the naked eye, but also measured quantitatively as an increase in dermal blood flow. This offers a non-invasive method to evaluate TRPV1 target engagement both preclinically and in early clinical drug development (111, 142, 143). In addition to TRPV1, comparable models can be developed for other TRP channels, for example the cinnamaldehyde target engagement biomarker for TRPA1 (110, 144)

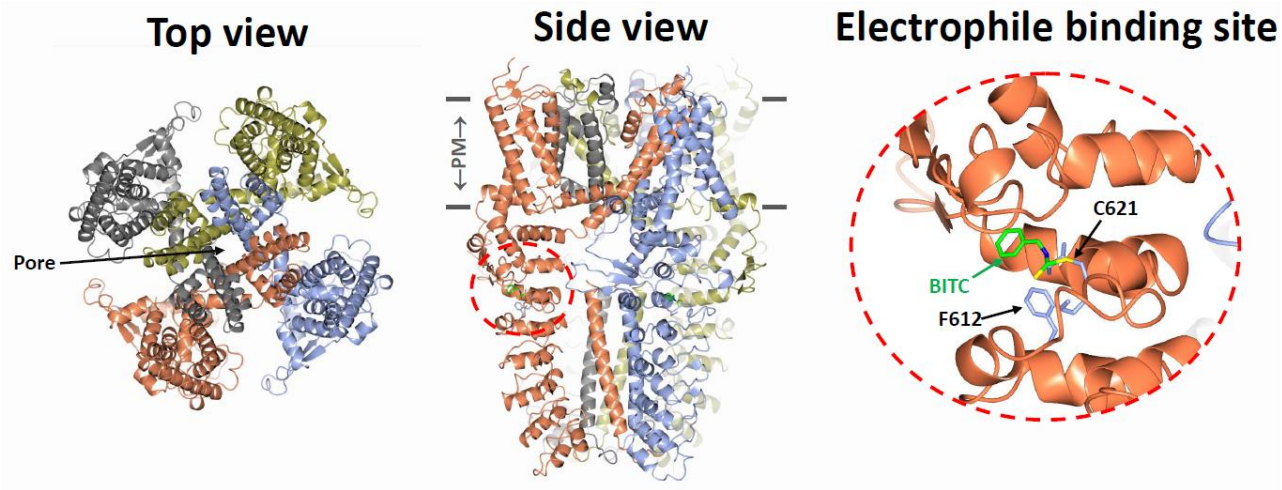
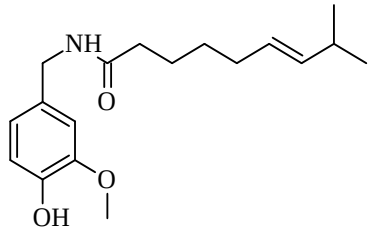
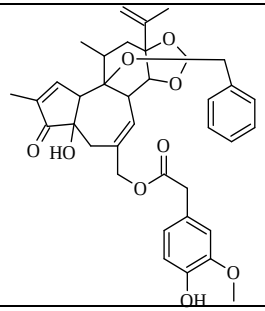
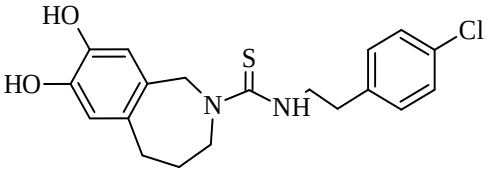
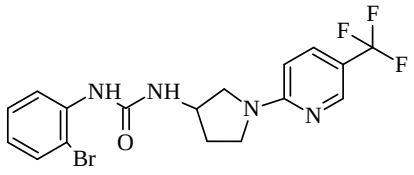
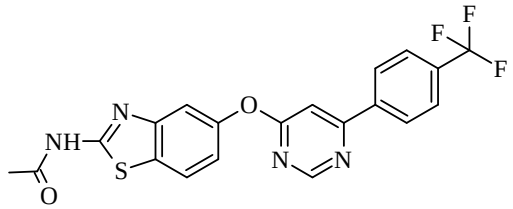
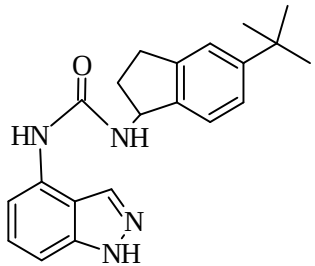
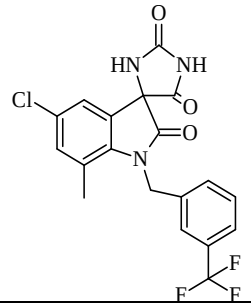
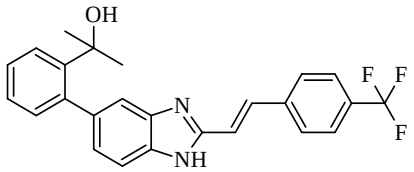
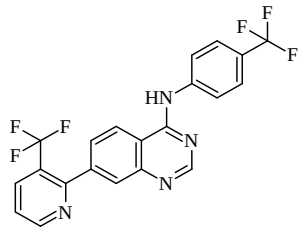
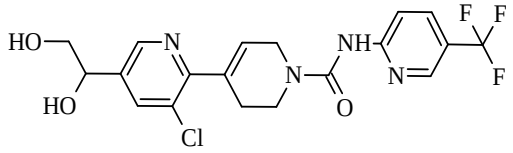
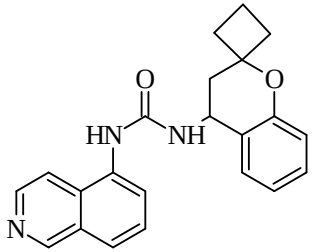


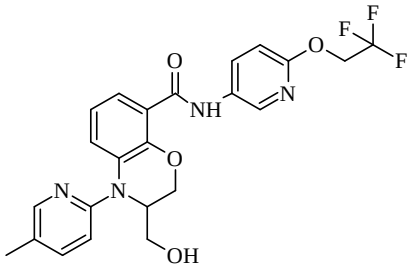
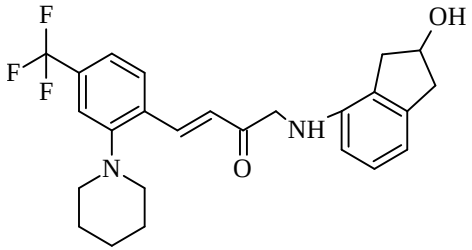
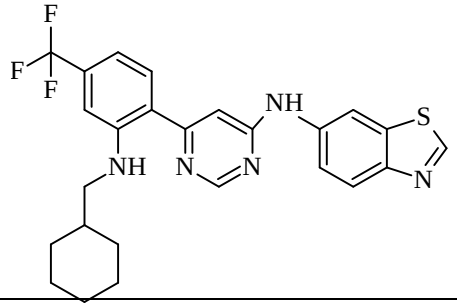
Figure 5: Structure of TRPA1. Ribbon diagram of the structure of TRPA1, as seen from the extracellular side (left) with indication of the central pore, and from the side (middle), with indication of the plasma membrane (PM). Differently colored protein regions indicate the four identical subunits of the tetrameric channel. The right panel shows the details of one electrophile binding site indicated in the middle panel. This covalent binding site is occupied by a molecule of benzyl isothiocyanate (BITC), one of the many electrophilic agonists of TRPA1. BITC is covalently bound to cysteine residue C621; the reactivity of this cysteine is strongly enhanced by the neighboring phenylalanine residue F612 through a thiol-π interaction. Structures were rendered using CCP4MG version 2.10.11 (http://www.ccp4.ac.uk/MG/), based on the atomic coordinates deposited in the Protein Data Bank under accession number 6PQP (145).

Table 1: TRPV1 agonists and antagonists

Name	Mode of action	Chemical structure	In vitro IC_{50} at hTRPV1			Effect on body temperature	Clinical status	Reference
			Capsaicin	pH	Heat			
Capsaicin	Agonist		640 nM (EC_{50} at hTRPV1)				On the market	(146)
Resiniferatoxin	Agonist		11 nM (EC_{50} at hTRPV1)				Phase III, In development	(147)
Capsazepine	Antagonist						Not pursued clinically	(148)

SB-705498 (GlaxoSmithKline)	Polymodal Antagonist		3 nM	30 nM	6 nM	No effect reported	Phase II, terminated	(32–37, 149–151)
AMG-517 (Amgen)	Polymodal Antagonist		0.76 nM	0.62 nM	1.3 nM	Hyperthermia	Phase II, terminated	(40, 41)
ABT-102 (Abbott)	Polymodal Antagonist		7 nM	Not reported	6 nM	Hyperthermia	Phase I, terminated	(43, 45, 152–155)
AZD1386 (AstraZeneca)	Polymodal Antagonist		Not reported	Not reported	Not reported	Hyperthermia	POC phase II, terminated	(47, 48, 50, 156)

JNJ-39439335 Mavatrep (Johnson & Johnson)	Polymodal Antagonist		4.6 nM	Not reported	Not reported	Hyperthermia	Phase II, terminated	(51–54, 157, 158)
MK-2295 NGD8243 (Merck and Neurogen)	Polymodal Antagonist	 Chemical structure has not been disclosed, but may represent the structure published in patent WO03062209 (159)	Not reported	Not reported	Not reported	No effect reported	Phase II, terminated	(49, 55, 56)
V116517 (Purdue Pharma)	Polymodal Antagonist		35.1 nM	39.5 nM	Not reported	No effect reported	Phase II, terminated	(57, 160)
GRC-6211 (Eli Lilly and Glenmark)	Polymodal Antagonist	 Chemical structure has not been disclosed, but may represent the structure published in patent WO2007042906 (161)	Not reported	Not reported	Not reported	No effect reported	Phase II, terminated	(59, 60)

JTS-653 (Japan Tobacco)	Polymodal Antagonist		0.24 nM	0.32 nM	Not reported	No effect reported	Phase II, terminated	(162)
AMG-8562 (Amgen)	Modality-selective Antagonist		1.75 nM (rat)	Potentiation (rat)	> 4000 (rat)	Hypothermia (rat)	Not pursued clinically	(67)
AMG-7905 (Amgen)	Modality-selective Antagonist		39 nM (rat)	Potentiation (rat)	Potentiation (rat)	Hypothermia (rat)	Not pursued clinically	(67)
NEO6880 (Neomed institute)	Modality-selective Antagonist	Structure not disclosed	41.5 nM	211 nM	No effect up to 10 μM	No effect reported	Phase II	(68–70, 163)

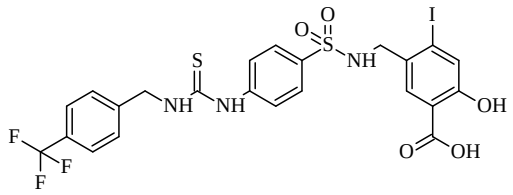
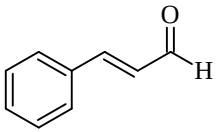
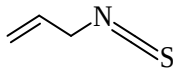
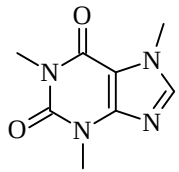
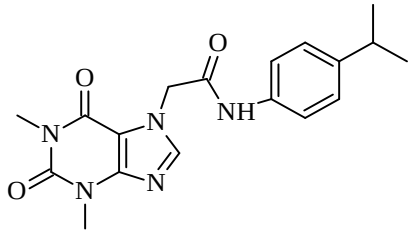
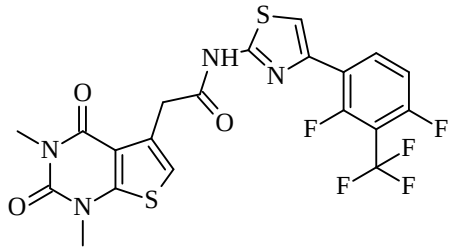
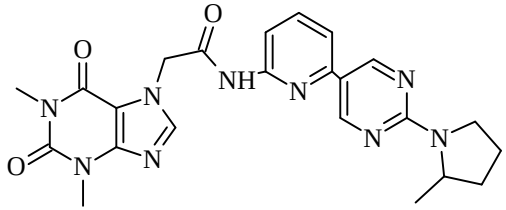
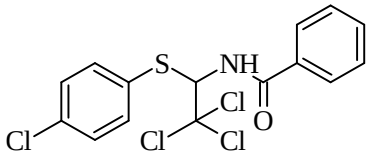
PHE377 V-377 (PharmEste)	Modality-selective Antagonist	 Chemical structure has not been disclosed, but may represent the structure published in patent WO2006045498 (164)	Not reported	Not reported	Not reported	No effect reported	Phase I, terminated	(71)

Table 2: TRPA1 agonists and antagonists

Name	Mode of action	Chemical structure	In vitro EC_{50}/IC_{50} at mTRPA1	In vitro EC_{50}/IC_{50} at hTRPA1	Clinical status	Reference
Cinnamaldehyde	Agonist		61 µM	400 µM		(165, 166)
Allyl isothiocyanate	Agonist		22 µM	64 µM		(165, 167)
Caffeine	Agonist in mouse Antagonist in human		61.8 µM Agonist	988 µM Antagonist		(168, 169)

HC-030031 (Hydra Biosciences)	Antagonist		6.8μM	6.2 μM	Not pursued clinically	(94, 169)
GRC-17536 (Glenmark Pharmaceuticals)	Antagonist	 Chemical structure has not been disclosed, but may represent the structure published by Skerratt et al. (102)	Not reported	1-6 nM	Phase II, On hold	(99–101, 170, 171)
CB-625 (Hydra Biosciences and Cubist Pharmaceuticals)	Antagonist	 Chemical structure has not been disclosed, but may represent the structure published in patent WO2013023102 (172)	101 nM (rat)	93 nM	Phase I, terminated due to pharmacokinetic issues	(172)
AMG9090 (Amgen)	Agonist in mouse Antagonist in human		66 nM Agonist (rat)	21 nM Antagonist	Not pursued clinically	(103, 106)

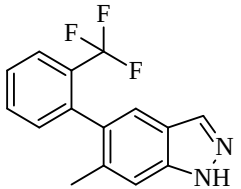
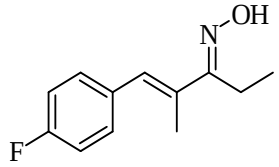
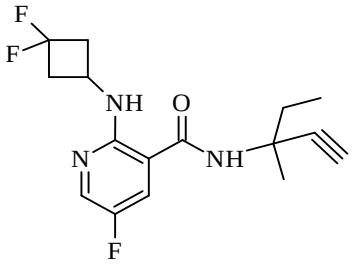
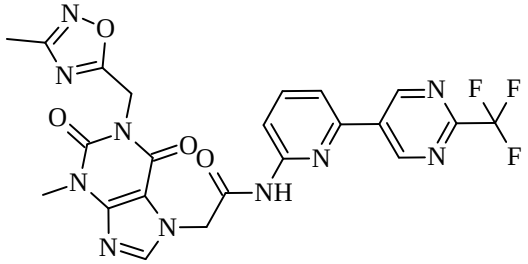
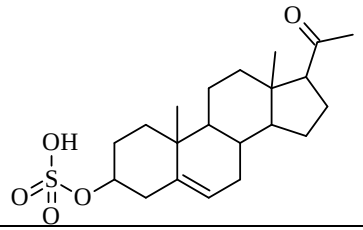
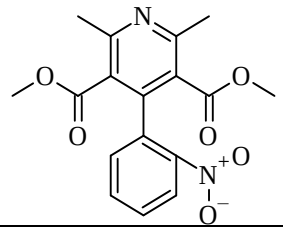
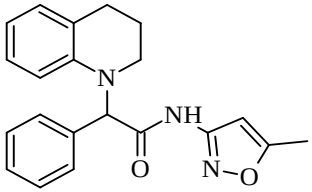
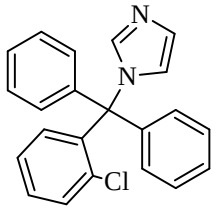
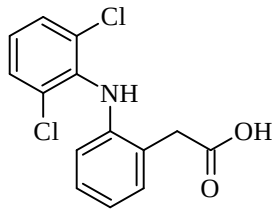
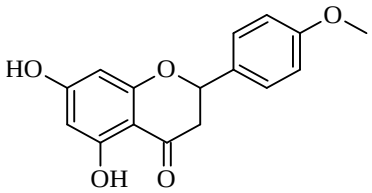
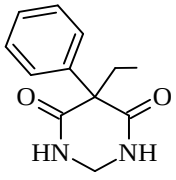
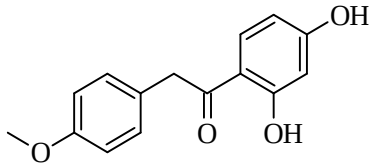
‘Compound 31’ (Novartis)	Antagonist		53 nM	15 nM	Not pursued clinically	(107)
A-967079 (Abbott)	Antagonist		296 nM	67 nM	Not pursued clinically	(173, 174)
ODM-108 (Orion Corporation)	Antagonist	 Chemical structure has not been disclosed, but may represent the structure published in patent WO2015144976 (109)	Not reported	37-100 nM	Phase I, terminated due to pharmacokinetic issues	(109)
LY3526318 HX-260 (Eli Lilly)	Antagonist		Not reported	Not reported	Phase I, In development	NCT03977974 NCT04183283

Table 3: TRPM3 agonists and antagonists

Name	Mode of action	Chemical structure	In vitro EC_{50}	In vitro IC_{50}	Reference
Pregnenolone sulfate	Agonist		23 µM		(118)
Nifedipine	Agonist		32 µM		(118)
CIM0216	Agonist		0.77 µM		(121)
Clotrimazole	Modulator				(122)
Diclofenac	Antagonist			6.2 µM	(175)

Isosakuranetin	Antagonist			50 nM	(134)
Priminidone	Antagonist			0.6 µM	(175)
Ononetin	Antagonist			0.3 µM	(176)