The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept

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Abstract | The clinical use of TRPV1 (transient receptor potential vanilloid subfamily, member 1; also known as VR1) antagonists is based on the concept that endogenous agonists acting on TRPV1 might provide a major contribution to certain pain conditions. Indeed, a number of small-molecule TRPV1 antagonists are already undergoing Phase I/II clinical trials for the indications of chronic inflammatory pain and migraine. Moreover, animal models suggest a therapeutic value for TRPV1 antagonists in the treatment of other types of pain, including pain from cancer. We argue that TRPV1 antagonists alone or in conjunction with other analgesics will improve the quality of life of people with migraine, chronic intractable pain secondary to cancer, AIDS or diabetes. Moreover, emerging data indicate that TRPV1 antagonists could also be useful in treating disorders other than pain, such as urinary urge incontinence, chronic cough and irritable bowel syndrome. The lack of effective drugs for treating many of these conditions highlights the need for further investigation into the therapeutic potential of TRPV1 antagonists.

Ten years ago in 1997, the field of somatic sensory biology and pain research witnessed the breakthrough work of David Julius and colleagues that led to the cloning of the first vanilloid (capsaicin) receptor, transient receptor potential vanilloid subfamily, member 1 (TRPV1)[1]. Capsaicin, which is responsible for the piquancy of hot-chilli peppers, is a versatile natural compound, the biological use of which is covered by more than 900 patents. Uses range from food flavouring and bird seeds (included to repel squirrels) to pepper spray for self-defence and ointments for the relief of neuropathic pain[2]. Capsaicin is unique among naturally occurring irritant compounds in that the initial neuronal excitation that it evokes is followed by a durable refractory state during which the previously excited neurons are unresponsive to a broad range of seemingly unrelated stimuli[2]. This effect, traditionally referred to as desensitization, has a clear therapeutic potential. In fact, capsaicin-containing creams have been in clinical use for decades to relieve painful conditions such as diabetic neuropathy[3].

Generally speaking, capsaicin-sensitive neurons are bipolar neurons with unmyelinated axons (C-fibres) and somata in sensory (dorsal root and trigeminal) ganglia[4]. Of note, a subset of sensory neurons with thin myelinated axons (Aδ fibres) is also capsaicin sensitive[4]. As discussed later, the expression of TRPV1 in Aδ fibres is upregulated during nerve-injury-induced thermal hyperalgesia[5] and diabetic neuropathy[6], which makes TRPV1 an important target for pain relief. The peripheral termini of capsaicin-sensitive neurons are sites of release for various pro-inflammatory neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) that, in turn, initiate the biochemical cascade collectively known as neurogenic inflammation[7,8]. Disease states that have a significant neurogenic inflammatory component include migraine, asthma, inflammatory bowel disease (IBD), interstitial cystitis and osteoarthritis (reviewed in REF. 7). The central fibres of capsaicin-sensitive neurons enter the dorsal horn of the spinal cord where they form synapses with second-order neurons[2,4]. The central role of TRPV1 in the initiation of the neurogenic inflammatory response and the transduction of pain is well established[2–4,7,8]. Of note (as reviewed in REFS 9,10), TRPV1 is also present in brain nuclei and non-neuronal tissues. As to the biological roles of TRPV1 receptors in these tissues, speculations are abundant, but conclusive evidence is still absent.

The cloning of TRPV1 represented a significant step in our understanding of the molecular mechanisms that underlie the transduction of noxious thermal and

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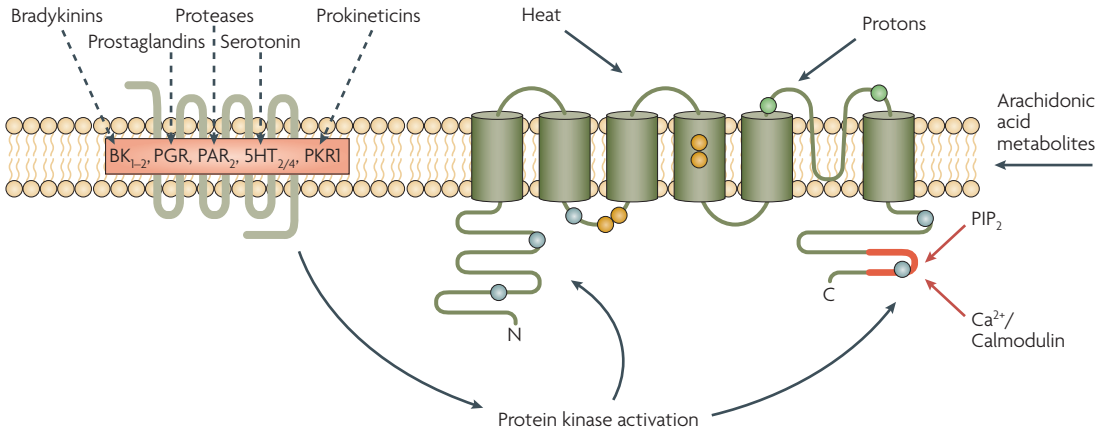


Figure 1 | **Schematic summary of TRPV1 signal integration in the peripheral nociceptor terminal.** Solid arrows indicate transient receptor potential vanilloid subfamily, member 1 (TRPV1)-sensitizing stimuli. The red arrows indicate negative regulation by phosphatidylinositol 4,5-bisphosphate (PIP_2), calcium and calmodulin. Receptors and cognate ligands known to mediate the sensitization of TRPV1 are shown on the left. These largely sensitize TRPV1 through protein-kinase activation, although increased arachidonic acid metabolite production and PIP_2 hydrolysis are also important. Coloured circles represent amino-acid residues that have been identified to be important in particular functions: orange, vanilloid binding (Y511, S512, L547, T550); blue, protein kinase phosphorylation sites (S116, T370, S502, T704, S800); and green, low-pH activation (E600, E646). The red line indicates the carboxy-terminal domain of TRPV1, which has been shown to interact with both PIP_2 and calmodulin.

chemical stimuli by sensory neurons[1]. TRPV1 is now recognized as a molecular integrator of inflammatory mediators (FIG. 1). Consistent with this hypothesis, TRPV1-homozygous-null mice (knockouts) are devoid of thermal hypersensitivity that occurs in response to an acute hind-paw injection of pro-inflammatory agents (for example, complete Freund's adjuvant; CFA), which suggests a clinical value for TRPV1 antagonists as novel analgesic drugs[11,12]. A substantial investment of resources by pharmaceutical companies has led to the discovery of an array of potent and selective small-molecule TRPV1 antagonists, some of which are already undergoing clinical trials (TABLE 1). TRPV1 antagonists are of great interest in that they represent a new strategy in pain relief, because unlike traditional analgesic agents that block the inflammatory response and the propagation and transmission of pain, TRPV1 antagonists aim to prevent pain by blocking an important sensor (transducer) of noxious stimuli on polymodal sensory neurons. The clinical value of TRPV1 antagonists might be the litmus test for the feasibility of this novel approach.

In this Review, we attempt to give a summary of the key characteristics of TRPV1, an overview of the intriguing clinical findings with TRPV1 agonists and a critical assessment of the potential therapeutic effects of the TRPV1 antagonists. We also briefly discuss the salient features of other thermo transient-receptor-potential (thermoTRP) channels[13,14] that are relevant to the pharmacology of TRPV1-expressing cells. TRPV1 is the most studied and validated TRP channel among the thermoTRP class[15], nonetheless, there is mounting evidence to suggest that channels of the TRP family might be the next generation of ion-channel targets that are involved in inflammatory pain[14–16].

The molecular pharmacology of TRPV1

TRPV1, similar to other TRP channels, is a putative six-transmembrane-spanning protein with a pore region localized between transmembrane segments 5 and 6 (REFS 1,17). Consistent with a role in nociception, TRPV1 is a non-selective cation channel with a preference for calcium that is directly activated by capsaicin and noxious temperatures — with an activation threshold *in vitro* of approximately 43°C (REF. 1). These data suggest that TRPV1 might be inactive at a normal body temperature. However, TRPV1 is an exceptional channel in that it is a polymodal nociceptor exhibiting a dynamic threshold of activation that could be significantly lowered under inflammatory conditions (FIG. 1). TRPV1 is thought to mediate the phenomenon of peripheral sensitization that involves a reduction in the threshold of activation and an increase in the responsiveness of the peripheral termini of nociceptors[16]. Indeed, agents in the 'inflammatory soup' act together to lower the activation threshold of TRPV1 (FIG. 1). The growing list of agents that can activate and/or sensitize TRPV1 include: mild acidification[18,19]; bradykinin (an endogenous inflammatory peptide that causes hyperalgesia)[20,21]; nerve-growth factor[21]; anandamide (arachidonoylethanolamide)[22]; arachidonic acid metabolites such as *N*-arachidonoyl-dopamine (NADA, structure shown in FIG. 2) and *N*-oleoyldopamine[23]; lipoxygenase products (12-hydroperoxyeicosatetraenoic acid (12-HPETE) and 15-HPETE)[24]; leukotriene B_4 (REF. 25); prostaglandins[26]; adenosine and ATP[27]; prokineticins[28]; polyamines (such as spermine, spermidine and putrescine)[29]; and venoms from jellyfish[30] and spiders[31].

The mechanism by which TRPV1 integrates such diverse inputs as protons, heat and capsaicin (structure shown in FIG. 2) has important implications for drug

Table 1 | **Current clinical status of TRPV1-targeted therapies**

Therapy name	Compound	Company	Action	Route(s)	Indication(s)	Clinical stage
Transacin	Capsaicin	NeurogesX	Agonist	Transdermal patch	HIV neuropathy-associated pain	Phase III
WL-1001 WL-1002	Civamide (cis-capsaicin)	Winston Laboratories	Agonist	Intranasal, topical	Cluster headache, migraine, osteoarthritis pain	Phase III (headache, osteoarthritis) Phase II (migraine)
ALGRX4975	Capsaicin	Anesiva	Agonist	Injection	Pain	Phase II
SB-705498	SB-705498	GlaxoSmith-Kline	Antagonist	Oral	Migraine, dental pain	Phase II* (migraine) Phase I (dental pain)
NGD 8243	NGD 8243	Neurogen/ Merck	Antagonist	Oral	Pain	Phase II
AMG 517	AMG 517	Amgen	Antagonist	Oral	Pain	Phase I
GRC 6211	GRC 6211	Glenmark	Antagonist	Oral	Osteoarthritis pain, dental pain, incontinence, neuropathic pain	Phase I

*This compound is no longer listed in the GlaxoSmithKline pipeline for migraine. TRPV1, transient receptor potential vanilloid subfamily, member 1.

development. Generally speaking, protein-kinase-dependent phosphorylation of TRPV1 causes sensitization (for example, protein kinase A (PKA)[32] and C (PKC)[33,34]), whereas dephosphorylation by protein phosphatases promotes desensitization[35,36]. Well-described analgesic agents can affect these pathways. For example, morphine blocks TRPV1 sensitization by preventing its phosphorylation by PKA and the synthetic cannabinoid WIN 55,212-2 inhibits TRPV1 through calcineurin-mediated receptor protein dephosphorylation[37,38]. These findings are physiologically relevant as cannabinoid receptor CB1 and δ-opioid receptors are co-expressed with TRPV1 on sensory fibres[39,40]. Furthermore, there is preliminary evidence that suggests that the phosphorylation state of TRPV1 might be disease specific[41]. If so, it might have important implications for drug development as the pharmacological activity of some compounds are affected by the phosphorylation state of TRPV1 (REF. 42).

The capsaicin-binding domain in TRPV1 was first reported by Julius and co-workers in 2002 (REF. 43). Defining the key residues in TRPV1 that are responsible for mediating agonist versus antagonist activity is expected to aid the development of clinically useful ligands. This notion is emphasized by the finding that a single amino-acid mutation (S512Y, located in the intracellular loop between transmembrane domains 2 and 3) converts the potent TRPV1 antagonist iodoresiniferatoxin (I-RTX) into an intrinsic agonist[44]. Resiniferatoxin (RTX, structure shown in FIG. 2) is an ultrapotent capsaicin analogue isolated from the latex of the perennial *Euphorbia resinifera Berg*[2]. High affinity [^{3}H] RTX binding has been linked to S512Y and to other critical residues (in particular, residue 547) in transmembrane domains 3 and 4 (REF. 44). A third domain that is involved in capsaicin gating, but not heat or proton activation, was localized to the pore region of TRPV1 (REFS 44,45). Based on these findings a new model of TRPV1 proposes a 'paddle structure' in which the transmembrane 3 and 4 regions form a gating paddle and residues such as 547 constitute an intracellular vanilloid-binding site[44].

Extracellular amino acids located near the pore domain (FIG. 1) have been implicated in the pH sensitivity of TRPV1 (REF. 46). Recent studies demonstrate that the rabbit anti-rat TRPV1 polyclonal antibody 156H (raised with a synthetic peptide corresponding to the pre-pore loop, E600 to P623) acts as a full antagonist of proton activation but only as a partial antagonist of capsaicin, anandamide and heat activation[47]. It was proposed that antibody 156H locks the channel conformation in the closed (non-conducting) state. Recently, it has been shown that it is the C-terminal domain of TRPV1 that confers heat sensitivity to the channel[48].

It has been postulated that TRPV1 antagonists fall into two categories: class A antagonists, which block the effects of both capsaicin and protons, such as SB-705498, and class B compounds, which are more selective for capsaicin, such as capsazepine[49] (FIG. 2). In addition to sensitization, TRPV1 exhibits agonist-induced channel desensitization that should be distinguished from defunctionalization of the whole neuron by TRPV1 agonists[50]. This is of importance when trying to extrapolate findings in animals 'desensitized to capsaicin' to studies that used antagonists for blockade of TRPV1.

TRPV1: splice variants and related channels

Scant but provocative evidence suggests the existence of TRPV1 splice variants in various tissues. The first rat TRPV1 splice variant to be cloned[51], named stretch-inactivated channel, is also the most controversial in that it appears to be derived from two related but independent genes, raising speculations about its authenticity[52]. The first bona fide TRPV1 splice variant, TRPV1 5′ (originally described as VR1 5′sv), lacks the first approximately 0.5 kb of canonical rat TRPV1

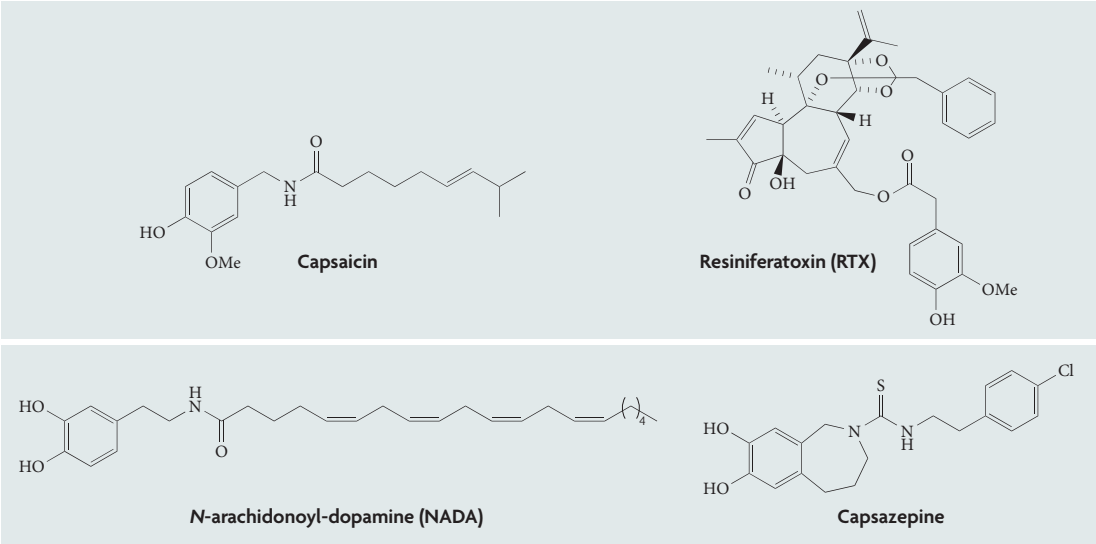


Figure 2 | **Chemical structures of selected TRPV1 ligands.** Capsaicin, the pungent ingredient in hot-chilli peppers; resiniferatoxin, an ultrapotent capsaicin analogue isolated from the cactus-like plant *Euphorbia resinifera Berg*; *N*-arachidonoyl-dopamine, an endogenous lipid mediator in brain nuclei; and the first generation transient receptor potential vanilloid subfamily, member 1 (TRPV1) antagonist, capsazepine.

sequence and is detectable in dorsal root ganglia (where its expression is 12-fold lower than that of TRPV1) and the CNS (at levels comparable to those of TRPV1)[53]. When heterogeneously expressed, TRPV1 5′ is not responsive to vanilloid agonists and the biological role of endogenous TRPV1 5′ is unknown[54]. The recently identified $TRPV1_{VAR}$ is believed to represent a truncated form of the canonical TRPV1 that is present at high levels in renal papillary, but not medullary, lysates[55]. Similar to TRPV1 5′, $TRPV1_{VAR}$ is non-functional[55]. However, when co-expressed with TRPV1, $TRPV1_{VAR}$ acts as a dominant negative modulator[55]. Interestingly, the non-functional murine TRPV1 splice variant TRPV1β can also act in a dominant negative manner[56]. The human TRPV1 variant TRPV1B is closely related to the murine TRPV1β; it is, however, activated by both capsaicin and protons[57]. The number of TRPV1 splice variants is expected to grow in the foreseeable future. For instance, in the kidney at least three distinct TRPV1-related transcripts were detected, which were not present in other tissues[55]. Antagonists designed to inhibit TRPV1 may also act on the splice variants but it is too early to speculate about the possible outcome of such an interaction, as the biological roles of TRPV1 splice variants have yet to be delineated.

Unexpectedly, the concept of a TRPV1-related stretch-inhibited channel was rekindled by the recent finding of pronounced serum hyperosmolarity in *TRPV1*$^{-/-}$ mice[58]. Osmosensory supraoptic nucleus neurons apparently express an N-terminal splice variant, but not the full length, TRPV1. Based on these findings it was surmised that *Trpv1* might encode a central component of the putative hypothalamic osmoreceptor[58].

TRPV1 is the founding member of the thermoTRP subfamily of sensory transducers[13,14] (FIG. 3, TABLE 2) that belong to the larger TRP superfamily of cation channels, which were first described in *Drosophila melanogaster*. Ironically, in this populous receptor subfamily, TRPV1 remains the only 'vanilloid receptor' that is a target for capsaicin and other vanilloids. So far, nine thermoTRP channels have been reported to be activated by changes in temperature. Six of them (TRPV1 to TRPV4, TRP subfamily melastatin, member 8 (TRPM8) and TRP subfamily ankyrin, member 1 (TRPA1)) are expressed in the sensory system and/or skin keratinocytes, and their function in thermosensation is well characterized. The thermoTRP subfamily has two major subdivisions, namely heat- and cold-sensitive channels[13]. TRPV1 is the archetypal, noxious heat-sensitive TRP channel — the group also includes TRPV2 (REF. 59), TRPV3 (REF. 60) and TRPV4 (REF. 61). Cold-responsive TRP channels include TRPM8 and TRPA1 (REFS 62–64). TRPM2 (REF. 65), TRPM4 (REF. 66) and TRPM5 (REF. 67) have recently been shown to be modulated by warm temperatures, but their role in thermosensation remains unclear.

Broadly speaking, thermoTRP channels are expressed on specific subsets of sensory neurons (some also in keratinocytes and other non-neuronal tissues) where they respond to chemicals and to a wide range of temperatures, from innocuous cold and warm to painfully hot or cold (BOXES 1,2). The initial identification of TRPV1 as a noxious stimuli transducer and its validation as a promising target against pain have brought tremendous interest in identifying additional TRPV1-related channels. To date, the TRPV subfamily has a total of six members, TRPV1 to TRPV6, of which TRPV1 to TRPV4 are the focus of interest as potential targets for novel analgesic drugs (BOX 1; FIG. 3). The other two channels, TRPV5 and TRPV6, appear to be constitutively active and are thought to have a role in vitamin-D-dependent calcium uptake in the kidney and intestine, respectively[68].

Expression of TRPV1: implications for therapy

TRPV1 RNA and/or protein expression has been described in various discrete cells, but it is most prevalent in sensory neurons[53]. As reviewed elsewhere[15,69], there is mounting evidence that TRPV1 expression is regulated in sensory neurons at the transcriptional and post-transcriptional levels: for instance, elevated TRPV1-protein levels have been observed in animal models of inflammatory hyperalgesia[70]. These findings are in agreement with the increase in TRPV1-like immunoreactivity that is detected in painful human disease conditions such as IBD[71], faecal urgency, irritable bowel syndrome[72], vulvodynia[73] and mastalgia[74]. In other words, TRPV1-protein upregulation may work together with mechanisms of channel sensitization to drive increased nociceptor afferent activity (mediating pain) as well as neurogenic efferent activity effects through SP and CGRP release (which mediate local vascular and inflammatory effects). Surprisingly, a diffuse loss of TRPV1-positive axons was reported in patients with painful peripheral neuropathies[75]. This finding might provide a rationale to explain the disappointing results obtained in some of the clinical trials that used topical capsaicin for the indication of diabetic neuropathy (reviewed in REF. 2).

Until recently, TRPV1-expressing thin myelinated Aδ fibres received little attention as they comprise a minor subpopulation of Aδ fibres[76]. TRPV1 is, however, not only upregulated on thin myelinated primary afferent neurons in mice with diabetic neuropathy[6] but is also ectopically expressed on Aδ fibres during nerve-injury-induced thermal hyperalgesia[5,77]. Consequently, it was suggested that an increased TRPV1 expression on myelinated fibres might contribute to the anti-hyperalgesic effect of topical capsaicin in diabetic neuropathic pain[75]. Of course, this consideration would also apply to TRPV1 antagonists.

Although neuropeptide release from sensory nerve fibre endings is generally viewed as pro-inflammatory[4,7], some data indicate positive effects on sensory fibre activation and/or SP and CGRP release under certain physiological conditions. For example, acute intragastric capsaicin administration was reported to protect against gastric ulcer formation in animal experiments and in healthy volunteers[78,79]. Hence, although it is theoretically possible that desensitizing doses of TRPV1 agonists or acute pharmacological blockade by TRPV1 antagonists might ameliorate pain while exacerbating pathology, little data exist that addresses this possibility directly. Of particular relevance in this regard is the gastrointestinal (GI) tract, which is rich in TRPV1-positive sensory nerves[80] that will be directly exposed to high concentrations of TRPV1 antagonists if they are given orally as analgesic drugs.

The distribution and possible function of TRPV1 in the CNS will be detailed below. It should be noted, however, that TRPV1 is expressed in higher brain structures[81] that are thought to be involved in pain processing, such as the cingulated cortex (reviewed in REF. 82). As elegantly demonstrated by workers at Abbott, who compared the analgesic activity of TRPV1 antagonists with poor versus good CNS penetration, central TRPV1 exposure might be important for broad-spectrum analgesias[83].

TRPV1 antagonists: for pain relief and more

Overall, the role of TRPV1 as a molecular integrator of noxious stimuli and as an initiator of the neurogenic inflammatory response is universally accepted[7,8]. As a result, numerous companies have initiated programmes to identify TRPV1 modulators. Literature and patent searches, and web-site visits identified the following companies as having active preclinical TRPV1 research activities or programmes: GlaxoSmithKline, Neurogen/Merck, Amgen, Novartis, Abbott, AstraZeneca, Johnson & Johnson, Janssen, Bayer, Takeda, Vertex, Pacific Corp., Euro-Celtique, Digital-Biotech, Schwartz, Renovis/Pfizer, Grünenthal, Glenmark, and Purdue. The result of these efforts has been the identification of many novel and potent TRPV1 antagonists. A select group of these compounds is shown in TABLE 3a,b.

In general, significant *in vitro* and/or *in vivo* data exist for these examples in peer-reviewed publications such that a picture of TRPV1-antagonist effects is beginning to emerge. These compounds and their analogues also provide the basis for a developing structure–activity relationship (SAR) for small-molecule TRPV1 antagonists. For entries 1–9 in TABLE 3a,b, this can be generalized as a central hydrogen-bond acceptor/donor motif flanked by a lipophilic side chain on one side and an aromatic group that incorporates a hydrogen-bond acceptor on the other side (FIG. 4). In TABLE 3a,b, entries 2–7 incorporate structural features that can be traced back to the prototypical TRPV1 antagonist capsazepine (entry 1 in TABLE 3a). Further refinements of the urea/amide motif, such as restricting the number of accessible conformations, have provided examples such as the quinazoline and benzimidazole analogues shown in TABLE 3b (entries 8 and 9, respectively), which are further removed structurally from capsazepine but still retain the key binding elements outlined in FIG. 4. Other TRPV1 antagonists have emerged that do not fit as readily into this model (for example, entry 10 in TABLE 3b). Selectivity data is available for some of the compounds in TABLE 3a,b; in specific cases, broad receptor screening by radioligand binding has been reported (see TABLE 3a,b for references). It should be noted, however, that there is little data available on selectivity as assessed in functional assays. So, for example, although we know that BCTC (*N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide) is a functional inhibitor of TRPM8 (TABLE 3a) its effects on other ion channels are unclear[84].

The progress of small-molecule drug discovery is also evident in the initiation of clinical trials of TRPV1 antagonists. Amgen, GlaxoSmithKline and Neurogen/Merck have advanced TRPV1 antagonists into clinical testing (TABLE 1). Amgen reported initiation of Phase I trials of AMG517 in September 2004. GlaxoSmithKline's SB-705498 (TABLE 3b) is in Phase I trials for dental pain (SB-705498 Dental Pain Study) and reached Phase II for acute migraine headache (Use Of SB-705498 In The Acute Treatment Of Migraine). In May 2006, GlaxoSmithKline presented Phase I clinical data of SB-705498 in healthy volunteers[85]. SB-705498 significantly reduced capsaicin-evoked flare and acute heat-evoked pain on non-sensitized skin. Furthermore,

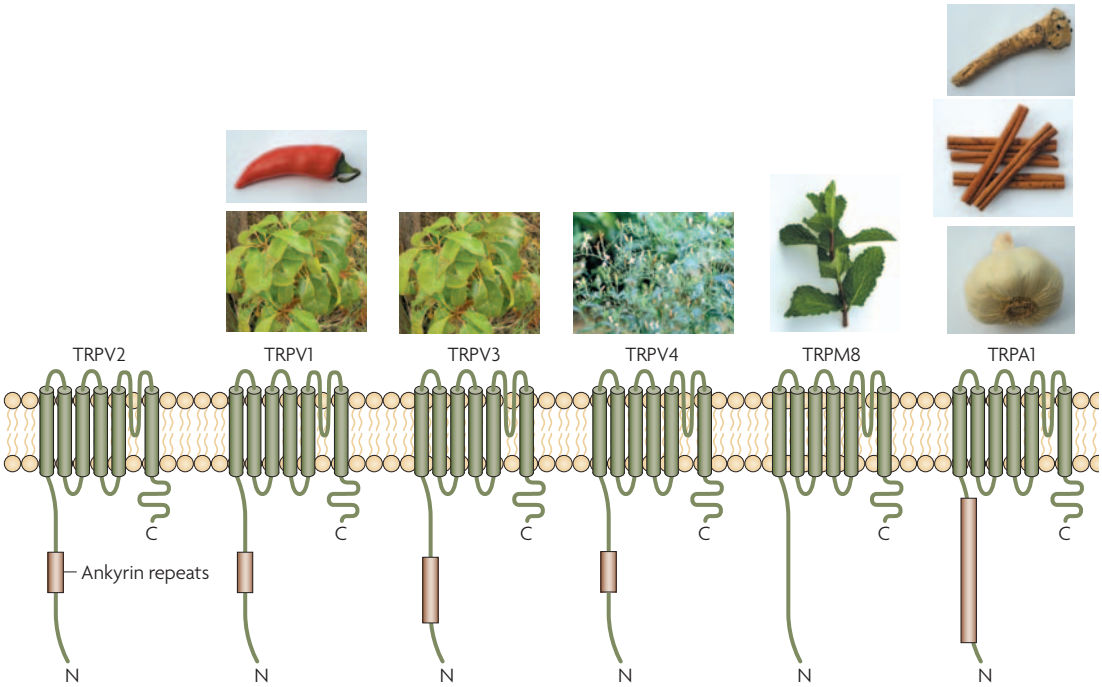


Figure 3 | **Activation of thermoTRPs by naturally occurring compounds.** Schematic depiction of the predicted membrane topology of the thermoTRPs and their activation by natural ligands. These channels are thought to have six transmembrane domains with a proposed pore region between segment 5 and 6. The amino and carboxy termini are cytoplasmic. Channels with ankyrin repeats in their amino termini are indicated. In addition to their thermal sensitivity, thermo transient-receptor-potential (thermoTRP) channels are activated by natural compounds. TRP vanilloid subfamily, member 1 (TRPV1) is activated by capsaicin, which is responsible for the piquancy of hot-chili peppers; TRP melastatin subfamily, member 8 (TRPM8) by menthol, the active ingredient in green mint; TRP ankyrin subfamily, member 1 (TRPA1) by pungent compounds such as cinnamaldehyde, isothiocyanates and allicin, active ingredients in cinnamon, horseradish and garlic, accordingly; TRPV1 and TRPV3 by camphor, isolated from the wood of the camphor laurel tree (*Cinnamomum camphora*); and TRPV4 by bisandrographolide, present in the Chinese herbal plant *Andrographis paniculata*. Photograph of *Adrographis paniculta* © Kazuo Yamasaki, Teikyo Heisei University, Japan.

SB-705498 reduced heat-evoked pain after ultraviolet B (UVB)-evoked inflammation. In November 2006, Neurogen/Merck announced the initiation of Phase II trials with NGD-8243/MK-2295 in acute pain.

The advance of compounds into Phase II clinical trials suggests that TRPV1 antagonists exhibit a benign profile in laboratory animals that are used for preclinical toxicology studies. This speculation is consistent with the unremarkable phenotype of *TRPV1*$^{-/-}$ mice under 'normal' physiological conditions[11,12] — although they do exhibit increased urine voiding or 'spotting' behaviour[86]. There is evidence for an endogenous TRPV1 'tone' in the regulation of core body temperature[87]. Acute systemic capsaicin administration results in a rapid drop in body temperature[88], whereas acute systemic TRPV1 antagonist treatment manifests in a significant increase in body temperature[87]. However, no difference in circadian body temperature fluctuation is observed in *TRPV1*$^{-/-}$ mice versus wild-type mice[89], so the effect of chronic treatment with TRPV1 antagonists in this regard is unclear. Nonetheless, *TRPV1*$^{-/-}$ mice show an attenuated fever in response to lipopolysaccharide (LPS)[89].

One might speculate that TRPV1 antagonists will affect certain physiological processes because of the distribution of TRPV1-containing sensory nerve fibres in a multitude of tissues, the expression of TRPV1 in non-sensory neuron cells and the regulation of TRPV1 expression by ongoing pathophysiology. The potential impact of TRPV1 antagonists in disease states, including pain, is considered below.

Effects of TRPV1 antagonists on the skin and musculoskeletal systems. In the skin, the neurogenic inflammatory response includes vasodilation and edema (flare response), which are mediated by CGRP and SP, respectively[7]. However, in the knee joint of the mouse, capsaicin evokes an unexpected vasoconstrictor effect, the mechanism of which is unclear[90]. Consistent with the hypothesis that TRPV1 activation is a crucial event in C-fibre nociceptor activation under inflammatory conditions, *TRPV1*$^{-/-}$ do not exhibit thermal hypersensitivity in response to an acute hind-paw injection of pro-inflammatory agents, such as CFA or carrageenan[11,12]. These observations have been recapitulated in rats and guinea pigs, which were administered with the TRPV1 antagonists, capsazepine, BCTC, A-425619, AMG9810, compound 46ad and compound 26 (entries 9 and 10 in TABLE 3b). In a CFA model of experimental arthritis, joint swelling is attenuated, but not absent, in *TRPV1*$^{-/-}$ mice, which indicates that mechanisms other than TRPV1 are

Table 2 | **Function of thermoTRP channels and their relevance to pain**

Channel	Thermal threshold	Function and phenotype	Gene-targeted deletion	References
TRPV1	≥43°C	Involved in noxious heat detection and mediates thermal hyperalgesia under inflammatory conditions	Two independent knockout studies	1, 11, 12
TRPA1	≤17°C	Involved in noxious cold detection, mechanical and mustard oil- and bradykinin-induced hyperalgesia	Two independent knockout studies	63, 172, 177
TRPV2	≥53°C	Responds to noxious heat in heterologous systems; upregulated during inflammation	Not reported so far	58, 152
TRPV3	≥33°C	Involved in warm and noxious heat detection	One study	59, 154, 157
TRPV4	≥25°C	Involved in warm temperature sensation; controversial reports about its involvement in mediating noxious heat pain and thermal hyperalgesia	Two independent knockout studies	60, 158–164
TRPM8	≤23°C	Role in the detection of innocuous and noxious cold sensations remains to be determined	Not reported so far	61, 62

TRPA1, transient receptor potential subfamily ankyrin, member 1; TRPM8, TRP subfamily melastatin, member 8; TRPV1,2,3,4, TRP subfamily vanilloid, member 1,2,3 or 4.

also involved in the development of joint inflammation[91]. Importantly, histology showed no major differences between TRPV1 wild-type and knockout mice, which suggests that TRPV1 is not involved in the development of joint abnormalities[91]. In a mouse model of cancer pain, systemic administration of the TRPV1 antagonist JNJ-17203212 (TABLE 3a) results in efficacy according to several behavioural measures of pain. *TRPV1*$^{-/-}$ mice show a similar extent of efficacy as *TRPV1*$^{+/+}$ controls that were treated with the antagonist[92].

Interestingly, *TRPV1*$^{-/-}$ mice develop mechanical hypersensitivity to the same extent as wild-type mice[11,12]. By contrast, TRPV1-antagonist treatment alters mechanical hypersensitivity induced by inflammation or nerve injury in rats[91,93–96]. This incongruous result might be explained by 'off-target' activities of the agents used in these studies. In fact, capsazepine inhibits voltage calcium channels[97] and nicotinic acetylcholine receptors[98], and BCTC inhibits TRPM8 activity with high affinity (with an IC_{50} of 143 nM)[84]. But poor selectivity seems an unlikely explanation given the structural diversity of the reported antagonists. For instance, stretch sensitivity in colonic afferents is reduced in *TRPV1*$^{-/-}$ mice, which provides experimental support for the involvement of TRPV1 in aspects of mechanical-force-sensitive signalling[99]. Moreover, TRPV1 sensitization might underlie an increase in generator potential in nociceptors, therefore driving an increased spontaneous action-potential firing in these cells post-injury[15]. If so, traditional preclinical pain models, which rely on evoked withdrawal thresholds, might underestimate the true analgesic potential of TRPV1 antagonists. Indeed, A-425619 gives statistically significant reversal of the hind-limb weight-bearing differential that is induced by the unilateral injection of sodium monoiodoacetate into the knee joint of rats[94]. Furthermore, JNJ-17203212 was judged efficacious in mouse cancer pain on the basis of, in part, improvements in spontaneous flinching and guarding of the affected hind-limb[94]. These data suggest that constant spontaneous pain, sometimes referred to as burning pain, might be positively affected by TRPV1 antagonist administration.

Effect of TRPV1 antagonists on the GI system. TRPV1-immunoreactive fibres constitute over half of the sensory afferents that project into the viscera (reviewed in REF. 80). Most of the TRPV1-containing innervation of the GI system is of spinal origin. Elevated TRPV1 immunoreactivity has been observed in colonic sensory nerve fibres in patients with IBD[71], in rectal sensory nerve fibres in patients with rectal hypersensitivity and faecal urgency[72], and in oesophageal mucosa sensory fibres in patients with gastroesophageal reflux disease[100]. Therefore, a clear hypothesis for the use of TRPV1 antagonists in treating the pain that results from GI disease exists and, as reviewed elsewhere[80], numerous preclinical studies have explored the role of TRPV1 in the gut. Although it is unclear whether TRPV1 antagonists administered therapeutically can reverse ileitis-induced pain based on preclinical models, systemic capsazepine decreases nociceptor signalling as assessed by c-fos staining in an L-arginine-induced pancreatitis model[101], and decreases markers of inflammation in multiple GI-inflammation models including caerulein-induced pancreatitis in rats[102]. Capsazepine also decreases physiological responses to colorectal or jejunal distension in mice[103], an effect comparable to observations in *TRPV1*$^{-/-}$ mice[99]. A limitation in our understanding of TRPV1 antagonist utility in treating disorders of the GI system is that capsazepine is the only agent studied in these preclinical models so far. However, the extent of TRPV1 expression in sensory neurons and its potential to contribute to spontaneous firing strongly suggest that TRPV1 antagonists will be useful agents to treat GI-disease pain.

Effect of TRPV1 antagonists on the urinary system. The few published reports that describe the effects of TRPV1 antagonists in preclinical models of bladder hyperreflexia give tantalizing pieces of data that suggest a TRPV1-antagonist use in bladder hyperreflexia states. As reviewed recently, instillation of capsaicin or RTX results in decreased micturition frequency presumably because of a desensitization of the sensory neuron fibres that innervate the urinary bladder[104]. Indeed, TRPV1

Box 1 | **Warm and hot-temperature-activated thermoTRPs**

TRPV2. Transient receptor potential vanilloid subfamily, member 2 (TRPV2) responds to noxious heat at temperatures that exceed 53°C (REF. 58) and is upregulated under inflammatory conditions[153]. So far, no null-mutant mice of TRPV2 have been reported and its therapeutic potential or role in noxious stimuli detection remains to be evaluated.

TRPV3. TRPV3 is expressed in dorsal root ganglia and keratinocytes where it may act as a heat sensor and responds to warm temperatures (33–39 °C)[59,154,155]. Interestingly, TRPV3 strongly sensitizes to multiple applications of heat and/or sensitizers such as camphor, 2-APB (2-aminoethyl diphenylborate), carvacrol, thymol and eugenol, indicating a potential role in nociception[156–158]. Indeed, *TRPV3*-mutant mice demonstrate a deficit to noxious acute thermal stimulation at temperatures ≥ 50°C (REF. 158). In contrast to TRPV1 (REFS 11,12), *TRPV3*-mutant mice showed normal behaviour in models of inflammatory pain suggesting that TRPV3 may not be of therapeutic value for such indications[158].

TRPV4. TRPV4 is expressed in the skin and dorsal root ganglion neurons and responds to warm temperatures (25–34°C)[60,159]. Temperature and other TRPV4 modulators, such as anandamide, arachidonic acid, epoxyeicosatienoic acid and α-phorbol didecanoate (PDD) work together to activate the channel[160]. Recently, it was shown that bisandrographolide A from the Chinese herbal plant *Andrographis paniculata* activates TRPV4 (REF. 161). In addition to a deficit in osmoregulation, *TRPV4*-mutant mice exhibited a higher threshold to intense mechanical stimulation[162,163]. Surprisingly, *TRPV4*-mutant and wild-type mice behaved normally in the hot-plate assay (up to 50°C) or when exposed to radiant heat, suggesting no function in acute thermal sensation[164]. By contrast, *TRPV4*-mutant mice exhibit higher withdrawal latency in response to heat applied to their tail (45–46°C)[165]. *TRPV4*-mutant mice behaved normally in temperature gradient assays after intraplantar complete Freund's adjuvant injection; other studies, however, suggested that TRPV4 has an essential role in models of carrageenan-induced thermal and inflammatory mediator-induced mechanical hyperalgesia[164,166]. Furthermore, spinal administration of antisense oligodeoxynucleotides to TRPV4 abolished mechanical hyperalgesia in a model of taxol-induced neuropathic pain[167]. Given the inconsistencies in these studies, the role of TRPV4 in inflammatory pain remains unclear.

TRPM2, TRPM4 and TRPM5. Transient receptor potential melastatin subfamily, member 2 (TRPM2) was recently shown to be activated by warm temperatures (≥ 35 °C) apparently by direct gating of the channel[168]. Although β-NAD^+ or ADP-ribose are known agonists of TRPM2, the activity of the channel is enhanced by co-application of heat[168]. TRPM4 (REF. 66) and TRPM5 (REF. 67) are non-selective cation channels that are impermeable to calcium and are activated by elevated levels of intracellular calcium. Both channels can be activated by bringing the temperature to between 15 and 35°C. TRPM5 is indirectly involved in the transduction of taste in which it is thought to be activated downstream of taste receptor type 1, member 1 (T1R), T2R and phospholipase C-β2 (PLCβ2) by elevated calcium in the cytosol[169]. Although TRPM2, TRPM4 and TRPM5 are sensitive to heat, no expression in sensory neurons was reported for these channels. Therefore, their function in thermosensation or pain remains unknown.

immunoreactivity has been detected in afferents in human bladder biopsies and increased immunoreactivity has been correlated with neurogenic detrusor overactivity[105]. Intriguingly, instillation of RTX or botulinum neurotoxin type A, both of which are effective treatments for detrusor overactivity, decrease the extent of TRPV1 immunoreactivity in the bladder[105,106]. Direct application of capsazepine to the bladder decreases micturition frequency in cyclophosphamide-treated, but not control, rats under anesthaesia[107]. By contrast, *TRPV1*$^{-/-}$ mice demonstrated reduced voiding reflex in response to controlled filling under anesthaesia, but also demonstrate increased spotting or voiding frequency while conscious, relative to wild-type mice[86]. The apparent differences between the capsazepine and knockout studies might be related to species differences in bladder physiology. Nonetheless, these studies raise the possibility that TRPV1 antagonists can effect an alteration in voiding frequency.

Effect of TRPV1 antagonists on the respiratory system. In human airways, TRPV1 is believed to represent an important target for toxicants[108]. As reviewed elsewhere[109], SP released from capsaicin-sensitive neurons mediates robust bronchoconstriction through neurokinin 2 (NK2; also known as TACR2) receptors and stimulates seromucous secretion from bronchial glands by interacting with NK1 (also known as TACR1) receptors. These changes attract inflammatory cells that, in turn, release proteases such as trypsin and tryptase. These enzymes activate protease-activated receptor 2 (PAR2)[110], which further stimulates TRPV1 through a PKCε-dependent mechanism[111], causing a positive-feedback loop that results in increased vanilloid and citric acid-induced cough in guinea pigs[112].

Collier and Fuller first described the cough-inducing effects of capsaicin in a clinical setting[113]. This work clearly shows that the activation of sensory fibres is necessary for the cough response. Capsaicin also reduces airway conductance in humans, and the dose of capsaicin required to cause a cough is reduced in patients with asthma and chronic obstructive pulmonary disease (reviewed in REF. 109). Similarly, patients with a chronic cough show increased tussive sensitivity to capsaicin and have higher levels of TRPV1 immunoreactivity in airway sensory fibres[114]. As elevated bradykinin levels are mechanistically involved in angiotensin-converting enzyme (ACE)-inhibitor-induced chronic cough, further mechanistic support for the role of TRPV1 in coughing comes from the observation that bradykinin stimulates airway afferents in a TRPV1-dependent manner[115]. Not surprisingly, capsazepine and I-RTX have been shown to block capsaicin and citric acid-induced cough responses in guinea pigs[116,117], and BCTC attenuates cough responses to ovalbumin antigen in ovalbumin-sensitized guinea pigs[118]. As capsaicin and citric acid are widely used for clinical experimental medicine purposes[119], and as the guinea pig is a well-established preclinical species for the study of coughing[120], these results demonstrate the potential for TRPV1 antagonists to be clinical anti-tussives[121].

Effects of TRPV1 antagonists on the vascular system. BIBN-4096BS, also known as olcegepant, is a potent CGRP-receptor antagonist that blocks the vasodilator effects of CGRP and exhibits efficacy in patients with migraine and cluster headache[122]. As CGRP is strongly co-expressed in many TRPV1-expressing nerve fibres, including sensory fibres that innervate the dural vasculature[123], it is plausible to consider the possibility that activation of TRPV1 could partially underlie a neurogenic-mediated component of headache. Indeed, TRPV1 might be activated by mediators that are released from the vasculature during a headache[124]. For example, anandamide that is released from the endothelium can activate TRPV1 that is expressed on trigeminal afferents in the cerebrovasculature[125]. Unfortunately, little information is available on the efficacy of TRPV1 antagonists in preclinical models of headache.

The co-expression of CGRP and TRPV1 also implies other vascular effects of TRPV1 modulation. CGRP that is administered intravenously has a hypotensive

Box 2 | **Cool and noxious cold-temperature-activated thermoTRPs**

TRPM8. Transient receptor potential melastatin subfamily, member 8 (TRPM8) responds to cool temperatures with an activation threshold of 23–27°C (REFS 62,63). Interestingly, compared with TRPV1 (TRP vanilloid subfamily, member 1), TRPM8 shows the opposite mechanisms of activation. For instance, TRPV1 is sensitized by heat, acidification and protein kinase C (PKC), whereas TRPM8 exhibits a decrease in its activity in response to these agents. TRPM8 is believed to function as an innocuous cool receptor. In agreement with this, TRPM8 expression is restricted to a subset of dorsal root ganglia and trigeminal neurons that do not express known markers of nociception[62,63]. Although TRPM8 and TRPV1 clearly define distinct subpopulations of sensory neurons *in vivo*[170], there is an overlap in their functional expression in cultured and acutely dissociated dorsal root ganglion neurons using capsaicin and menthol as agonists[171]. Mutant mice lacking in TRPM8 have yet to be reported. The generation of such mice should ultimately define the role of TRPM8 in the detection of innocuous and noxious cold sensations.

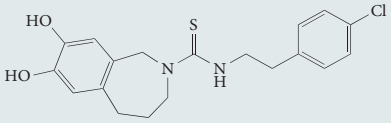
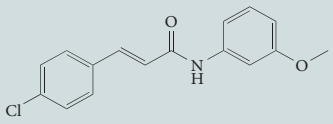
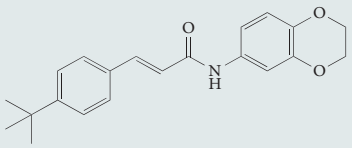
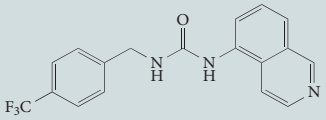
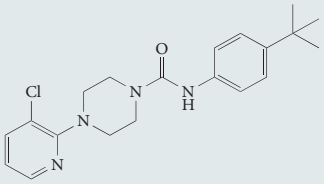
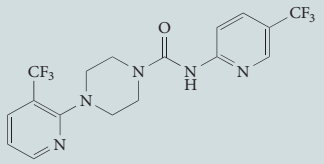
TRPA1. Transient receptor potential ankyrin subfamily, member 1 (TRPA1) is activated by noxious cold temperatures (≤ 17 °C), and is co-expressed with TRPV1 on sensory neurons[64]. TRPA1 is also activated by pungent compounds and irritants such as cinnamaldehyde, isothiocyanate, allicin and acrolein (a metabolized byproduct of chemotherapeutic agents that is also present in tear gas and vehicle exhaust)[172–176]. Recent studies have suggested that many of the structurally diverse TRPA1 agonists (most of which are electrophilic in nature) activate the channel through covalent modification of reactive cysteine residues within the receptor[177]. Two independent knockout studies showed that *TRPA1*-mutant mice did not develop thermal and mechanical hyperalgesia after intraplantar injection of bradykinin and mustard oil[172,178]. *TRPA1*-mutant mice also showed reduced sensitivity to intense cold stimulation and a higher threshold of activation in response to painful punctuate mechanical stimulation[178]. In a spinal nerve-ligation model (SNL), TRPA1 mRNA expression was increased in the nearby uninjured L4 dorsal root ganglia. Furthermore, antisense knockdown of TRPA1 suppressed cold hyperalgesia in SNL rats[179]. TRPA1 antagonism might prevent acrolein side effects resulting from cyclophosphamide- or ifosfamide-based chemotherapy. Finally, TRPA1 may also serve as a pharmacological target for pulmonary oedema and respiratory irritation caused by environmental irritants related to the acrolein class[172].

effect[126], and has been suggested to confer a beneficial counterbalance to the development of hypertension[127]. Intravenous injection of capsazepine increased systolic blood pressure in Dahl salt-resistent rats that were maintained on a high-salt diet for 3 weeks[128]. By contrast, Dahl salt-sensitive rats on a high-salt diet, which exhibit significantly increased systolic blood pressure, are unaffected by capsazepine treatment[128]. Intriguingly, cations can sensitize TRPV1 currents and activate nociceptive signalling by elevated ionic strength, which suggests a role for TRPV1 in mediating physiological responses to high salt. Moreover, *TRPV1*$^{-/-}$ mice exhibited a decreased serum arginine-vasopressin response to increased serum osmolarity, which was induced by high-salt intake. It should be noted, however, that no alteration in baseline cardiovascular parameters is observed in *TRPV1*$^{-/-}$ mice, although the Bezold–Jarisch reflex (increased vagal, parasympathetic, efferent discharge to the heart elicited by stimulation of chemoreceptors), which is initiated by high plasma concentrations of anandamide (20 mg per kg, intravenously) is decreased in *TRPV1*$^{-/-}$ mice versus *TRPV1*$^{+/+}$ controls[129]. Taken together, these results suggest that TRPV1 might be involved in the pressor response to high serum-sodium concentrations, although the related channel TRPV4, which is known to be sensitive to osmolarity changes and is expressed in the hypothalamus and kidney, has also been implicated in high salt-induced responses[130].

TRPV1 might be involved in mediating a sensory nerve fibre response to ischaemia, which could include neurogenic release of CGRP. However, the literature that describes the physiological effect of CGRP and TRPV1 on the cardiovascular system response to ischaemia is filled with contradictions. Several studies demonstrate no effect of the CGRP antagonist CGRP(8–37) on acute myocardial ischaemia-induced changes in coronary arteriole microvessel diameter in dogs or on myocardial ischaemia-induced infarct size in pigs[131,132]. These studies observed decreased mean aortic and arterial pressure, respectively, in animals that were administered with high doses of exogenous CGRP. The studies also observed CGRP(8–37)-mediated inhibition of the exogenous CGRP effect, which suggests that endogenous CGRP has little effect on coronary vascular tone during ischaemia. Moreover, studies in rats with the CGRP antagonist BIBN-4096BS demonstrate that blockade of CGRP has no detrimental effect after a 60 min myocardial ischaemia-induced infarct size in rats, even though plasma concentrations of CGRP were increased by 50% during ischaemia[133]. However, one study indicates that ischaemic preconditioning — a brief coronary artery occlusion, which has been shown to provide some protection against damage caused by a subsequent prolonged occlusion — is mediated by endogenous CGRP[134]. This is based on the observation that pretreatment of rats with BIBN-4096BS before ischaemic preconditioning (15 min coronary artery occlusion) results in greater myocardial-infarct size and creatine-kinase release than controls in response to prolonged ischaemia (45 min coronary artery occlusion)[134]. A study using the Langendorff apparatus reported that hearts from C57BL/6J mice that were treated with capsazepine before 40 min of no-flow global ischaemia exhibited decreased cardiovascular performance after reperfusion (increased left-ventricular developed pressure and coronary flow) in comparison with hearts from control mice[135]. Identical experiments in *TRPV1*$^{-/-}$ mice generated results similar to those of capsazepine-treated *TRPV1*$^{+/+}$ mice[135]. However, these investigators observed no effect of CGRP(8–37) on post-ischaemic recovery in similar experiments. Because the preclinical data set contains contradictions on many levels (for example, the lack of significant effects of CGRP(8–37) in pigs, dogs and mice versus the effects of capsazepine and *TRPV1*$^{-/-}$ genotype in mice versus the contradictory findings with BIBN-4096BS), an understanding of TRPV1 antagonist effect in ischaemia is challenging.

Another role for TRPV1 in the sensory nerve fibre response to ischaemia might be the transduction of pain signals that originate from mediators that are released by damaged tissue. In one study, all cardiac afferents in ferrets, which exhibited increased spontaneous firing activity in response to 5 min myocardial ischaemia (coronary artery occlusion), were all judged to be C-fibres[136]. Following a 15–20 min recovery period, the receptive fields of these fibres on the epicardial surface were treated with I-RTX. A second 5 min myocardial ischaemia challenge revealed a significant decrease in spontaneous firing[137]. This observation suggests an

Table 3a | **Summary of important TRPV1 antagonists**

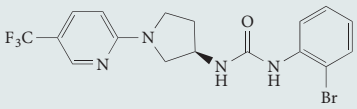
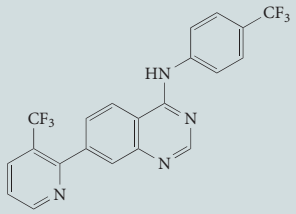
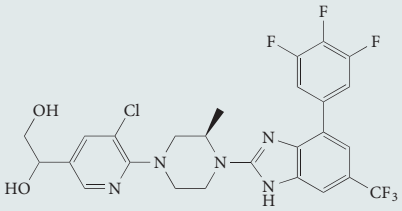
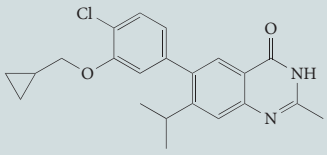
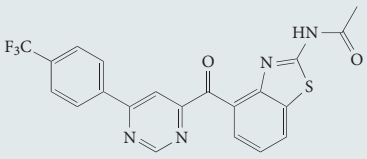
Name	Structure	Comments	References
Capsazepine (thiourea)		• rIC_{50} = 420 nM ($^{45}Ca^{2+}$ uptake) • Inhibits voltage-activated calcium channels and nicotinic acetylcholine receptors • Significantly reversed CFA-induced mechanical hyperalgesia in guinea pigs	180 97, 98 96
SB-366791 (cinnamide analogue)		• hK_i = 18 nM (FLIPR) • Selective versus TRPV4 and other TRP channels • Inhibits capsaicin and heat-mediated activation of TRPV1	182
AMG-9810 (cinnamide analogue)		• hIC_{50} = 25 nM ($^{45}Ca^{2+}$ uptake) • hIC_{50} of > 4 μM at TRPV3, TRPV4, TRPA1 and TRPM8 • Inhibits CFA-induced thermal (30 mg per kg) and mechanical hyperalgesia (100 mg per kg)	183
A-425619 (urea analogue)		• hIC_{50} = 5 nM (FLIPR) • TRPM8 IC_{50} = 8 μM; TRPA1 IC_{50} > 10 μM • Inhibits CFA-induced thermal hyperalgesia (ED_{50} = 10 mg per kg)	183 94
BCTC (urea analogue)		• hIC_{50} = 35 nM (FLIPR) • TRPM8 IC_{50} = 143 nM • Inhibits CFA-induced thermal and mechanical hyperalgesia (3–30 mg per kg, orally) • Reduces tactile allodynia and thermal hyperalgesia in a partial nerve-ligation model	184 84 95
JNJ-17203212 (urea analogue)		• hIC_{50} = 65 nM (FLIPR) • Elicits ~1°C increase in core body temperature in rats (30 mg per kg, orally) • Attenuates nocifensive behaviours in an *in vivo* model of bone-cancer pain	87 92

BCTC, *N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide; CFA, complete Freund's adjuvant; ED_{50}, half-maximal effective dose; FLIPR, fluorescence imaging plate reader; hIC_{50}, half maximal inhibitory concentration in humans; hK_i, inhibiton constant in humans; rIC_{50}, half maximal inhibitory concentration in rats; TRP, transient receptor potential receptor; TRPA1, TRP subfamily ankyrin, member 1; TRPM8, TRP receptor subfamily melastatin, member 8; TRPV1,3,4, TRP receptor subfamily vanilloid, member 1,3 or 4.

important role for TRPV1 in sensory afferent response to transient ischaemia. However, earlier work in rats indicated that the bradykinin-induced cardiac-sympathetic reflex (increased renal-sinusoidal nerve activity and increased mean arterial pressure), although mediated in part by TRPV1-expressing sensory fibres, was not dependent on TRPV1 (REF. 137). So, although cardiac sensory fibres have a role in mediating chest pain and associated autonomic reflexes, TRPV1 is not the only channel or receptor suggested to have a role in mediating sensory neuron responses to ischaemia; these channel and receptors include acid-sensing ion channel 3 (ASIC3; also known as ACCN3), TREK1 (also known as KCNK2) and bradykinin receptors.

Effects of TRPV1 antagonists on the CNS. Histochemical studies have confirmed the expression of TRPV1 in the CNS, including the hypothalamus and substantia nigra[81]. As mentioned previously, the hypothalamic supraoptic nucleus expresses an N-terminal variant of TRPV1, which is important in the arginine-vasopressin responses to high serum osmolarity[58]. Hypothalamic expression also helps to explain the hypothermia that is induced by injection of capsaicin into the hypothalamus[138]. Conversely, systemic administration of a TRPV1 antagonist (JNJ-17203212) to rats results in transient hyperthermia of about 1°C (REF. 87). This unanticipated result suggests that a TRPV1 tone exists, possibly in the anterior hypothalamic area, which is involved in the setting and sensing of core body temperature. *TRPV1*$^{-/-}$ mice exhibit core body temperatures that are identical to the wild-type mice[89], observations that are corroborated by experiments in which rats have been given a TRPV1 antagonist chronically (R. K. Conley, S. Boyce and D. N. Cortright, unpublished observations). Intriguingly, *TRPV1*$^{-/-}$ mice demonstrate a significantly smaller

Table 3b | **Summary of important TRPV1 antagonists**

Name	Structure	Comments	References
SB-705498 (urea analogue)		• rIC_{50} = 32 nM (FLIPR) • Phase I: reduced capsaicin-evoked flare and acute heat-evoked pain on non-sensitized skin	185 85
Quinazoline analogue		• hIC_{50} = 1 nM (FLIPR) • Achieved 80% block of carrageenan-induced thermal hyperalgesia at 3 mg per kg (MED 0.1 mg per kg)	186
Compound 46ad (benzimidazol analogue)		• hIC_{50} = 1 nM ($^{45}Ca^{2+}$ uptake) • Achieved significant reversal of CFA-induced thermal hyperalgesia (30 mg per kg, orally)	187
Compound 26 (quinazolinon analogue)		• hIC_{50} = 50 nM (low pH activation) • Achieved 60% reversal of CFA-induced mechanical hyperalgesia (30 mg per kg, orally) • Achieved 57% reversal of mechanical hyperalgesia in a partial nerve-ligation model	93
AMG 517		• Initiation of Phase I clinical trials reported in September 2004 • hIC_{50} = 0.9 nM ($^{45}Ca^{2+}$ uptake) • Achieved ~40% block of CFA-induced thermal hyperalgesia at 10 mg per kg (MED 1 mg per kg)	M. Norman, personal communication
NGD 8243	Undisclosed	• Initiation of Phase II trials announced in November 2006	Neurogen, press release

CFA, complete Freund's adjuvant; FLIPR, fluorescence imaging plate reader; hIC_{50}, half maximal inhibitory concentration in humans; hK_i, inhibiton constant in humans; MED, minimum effective dose; rIC_{50}, half maximal inhibitory concentration in rats.

febrile response to injection of bacterial LPS than wild-type mice[88], although LPS-induced fever in rats is not affected by capsazepine[139].

The observation that I-RTX significantly reduces the frequency of spontaneous excitatory post-synaptic currents in substantia nigra pars compacta dopaminergic neurons *in vitro* provides additional evidence for an endogenous TRPV1 tone in the CNS[140]. Additionally, TRPV1 activation of dopaminergic mesencephalic neurons *in vitro* (by high concentrations of capsaicin or anandamide) and *in vivo* (by intranigral injection of capsaicin or anandamide) results in cell death[141]. These data might imply a neuroprotective role for TRPV1 antagonists on these neurons although this result has not yet been corroborated. The functional effect of TRPV1 on thermoregulation and, possibly, on dopaminergic neurons, supports the hypothesis that TRPV1 is functionally expressed in the brain[9,142], but additional experiments are necessary to understand the full effect of TRPV1 antagonists on CNS processes.

An emerging role for TRPV1 in glucose regulation

Several lines of evidence imply a role for TRPV1 in the regulation of plasma glucose levels. A dense meshwork of sensory nerve fibres is found in the pancreas[143], and TRPV1 is also expressed on pancreatic islet cells where it is thought to have a role in insulin release[144]. Interestingly, insulin sensitizes TRPV1 on sensory nerve endings[145], thereby creating a local feedback interaction between islet cells and primary sensory neurons that innervate the Langerhans islets. The afferent arm of this feedback loop is composed of neuropeptides, in particular CGRP, that were shown to reduce insulin release from β cells[146,147]. SP that is released from TRPV1-expressing nerves promotes neurogenic inflammation in the pancreas[102].

It is well documented that ablation of TRPV1-positive neurons by agonist (capsaicin or RTX) administration in rat neonates improves glucose tolerance in those made diabetic by streptozotocin treatment[148]. This observation is not unexpected as neonatal capsaicin

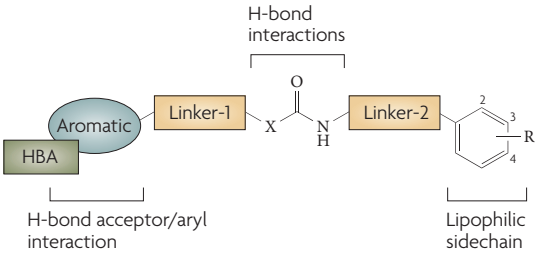


Figure 4 | **Key binding interactions of TRPV1 antagonists.** These are based on entries 1–9 in TABLE 3a,b and related structures. The indicated hydrogen-bonding motif is present in most known transient receptor potential vanilloid subfamily, member 1 (TRPV1) antagonist structures. Both H-bond donor and acceptor tend to be important for optimal potency. These are readily provided by urea (X=N), thiourea, amide (X=C) or reverse-amide functionalities, among others. Mono- or bicyclic-aryl and heteroaryl rings with a properly positioned hydrogen-bond acceptor (HBA) in this part of the molecule improve both potency and drug-like properties. Interactions (π–π) between this aryl ring and another on TRPV1 are possible, as has been suggested for the agonist capsaicin. The lipophilic side-chain interacts with a hypothetical hydrophobic binding site on TRPV1. Proper placement of lipophilic substituents (often 4-CF_3 or 4-t-Bu) is crucial for optimal TRPV1 potency. The linkers serve as scaffolding for the proper positioning/spacing of the three interactions above and, therefore, can take many forms, such as direct bonds, single-atom or double-atom spacers or ring systems.

treatment depletes CGRP (reviewed in REFS 2,4), a neuropeptide that would antagonize insulin release[147].

Treatment of obese Zucker rats, a genetic model of type 2 diabetes, with desensitizing doses of capsaicin or RTX was shown to result in significantly decreased fasting-plasma insulin levels, improved glucose tolerance through enhancement of insulin secretion and increased glucose infusion rate during euglycemic hyperinsulinaemic clamp versus vehicle controls[149]. That is, RTX treatment results both in increased insulin secretion and sensitivity, which suggests that TRPV1-expressing cells might be involved in glucose regulation. Whether the effects of desensitizing vanilloid agonist treatment can be recapitulated with TRPV1 antagonist treatment has yet to be demonstrated.

New findings obtained in non-obese diabetic (NOD) mice, which are genetically prone to develop type 1 (insulin-dependent) diabetes, imply a more important role for TRPV1 in the development of diabetes than previously thought[150]. In these animals, ablation by neonatal capsaicin treatment of TRPV1-positive neurons that innervate the pancreas (NOD^{CAPS} mice) prevents insulitis and resulting β-cell destruction that would ultimately result in type 1 diabetes, despite the systemic persistence of pathogenic T lymphocytes. Apparently, these mice carry a hypofunctional TRPV1 mutant ($TRPV1^{NOD}$) that is localized to the *Idd4.1* diabetes-risk locus. As expected, nociceptive behaviour in response to capsaicin is markedly depressed in NOD mice compared with non-obese diabetic-resistant (NOR) mice with wild-type TRPV1. Capsaicin treatment causes a dramatic reduction in insulitis without having a noticeable effect on autoimmune infiltrations elsewhere in the NOD mice. It is puzzling why the protective capsaicin effects are pancreas-specific in the NOD animals. Another unanswered question is the nature of the substance that mediates the protective action. Intra-arterial injection of SP into the NOD pancreas reverses insulitis and insulin resistance for weeks. This is an unexpected finding as neonatal capsaicin depletes, and not elevates, SP levels (reviewed in REFS 2,4), and endogenous SP that is released from TRPV1-positive fibres promotes neurogenic inflammation[102]. It is also unclear why the congenic NODxB6*Idd4* mice, that have wild-type TRPV1, are diabetes-resistant, similar to the NOD^{CAPS} animals in which TRPV1 has been chemically ablated[150]. One should, however, keep in mind that NOD mice are born with a hypofunctional TRPV1 and we cannot exclude a developmental compensatory mechanism that would create a permissive environment (an abnormal, pro-diabetogenic TRPV1) for insulitis and destruction of the β cells. This might explain why both the ablation by capsaicin of the abnormal TRPV1 neurons and/or the restoration of wild-type TRPV1 in the congenic animals rescues the phenotype[150].

TRPV1-agonist-based therapies

The lack of thermal hyperalgesia in TRPV1-null mice in models of inflammatory pain[11,12] stimulated the biotechnology and pharmaceutical companies to develop small-molecule antagonists that target TRPV1 as potential analgesic drugs. There is no doubt that such antagonists have completely overshadowed TRPV1 agonists. One should, however, keep in mind that although TRPV1 antagonists are just making their debut in the clinic TRPV1-agonist-based therapies have been used for centuries[2,15,151,152]. As already alluded to above, TRPV1 agonists and antagonists are not equivalent therapeutic approaches. Capsaicin-sensitive nerves express a myriad of receptors that are relevant to pain and inflammation of which TRPV1 is just one. TRPV1 agonists silence the whole nerve terminal, whereas antagonists selectively impair TRPV1 (REFS 2,8,15). Those who favour TRPV1 agonists argue that these compounds should be more powerful analgesic drugs than antagonists as they simultaneously block all receptors on capsaicin-sensitive nerves. By contrast, those who favour TRPV1 antagonists emphasize the initial excitation (pain) by agonist application and the potential for irreversible toxicity. In our opinion, TRPV1 agonists and antagonists are not mutually exclusive: quite the contrary, both therapeutic approaches could find their own niche in clinical practice (TABLE 1).

TRPV1 agonists aim to achieve desensitization of the sensory neurons[2,7]. As reviewed elsewhere, capsaicin-based products are associated with an intolerable burning sensation and the need for multiple applications for weeks to mediate their analgesic effect[2]. The ultrapotent capsaicin analogue RTX seems to be devoid of the above mentioned shortcomings of capsaicin, but its clinical use is riddled with problems of its own. Most importantly, RTX is a complex, highly hydrophobic molecule, expensive

to manufacture and difficult to keep in solution[2]. This might explain the strikingly dissimilar clinical outcomes in various studies that use similar patient populations (reviewed in REF. 104). Clearly, in animal experiments RTX is a highly effective and well-tolerated agent to achieve lasting and fully reversible desensitization (defunctionalization) of capsaicin-sensitive neuronal pathways[2].

Reduced-pungency capsaicin analogues and high-concentration injectable or topically applied capsaicin are being actively pursued in the clinic by companies such as NeurogesX, Winston Laboratories and Anesiva (formerly known as Corgentech). In May 2005, NeurogesX initiated Phase III trials for NGX-4010 (Transacin), a clinically administered trans-capsaicin patch to treat post-herpetic neuropathy. Furthermore, NeurogesX announced in February 2006 the positive Phase III trial results in painful HIV-associated sensory neuropathy. Winston Laboratories is developing WL-1001, an intranasal civamide (cis-capsaicin), which is currently in Phase III trials for cluster headache and Phase II for migraine prophylaxis (H. Fezatte and S. Phillips, personal communication). The company initiated Phase III trials for WL-1002, a topical civamide for treatment of osteoarthritis pain. Anesiva is developing compound 4975, a high-concentration injectable formulation of capsaicin, which is in Phase II clinical trials for the treatment of post-surgical, neuropathic and musculoskeletal-pain syndromes.

TRPV1 antagonists: concluding comments

TRPV1, perhaps the most important signal integrator in sensory nociceptors, is well established as an intriguing novel target for the treatment of pain[2,8,15,151,152]. Extensive preclinical profiling of small-molecule inhibitors of TRPV1 provides intriguing evidence that TRPV1 blockade can be a useful therapeutic approach for inflammatory, cancer and possibly neuropathic pain (reviewed in REFS 15,152). Additional preclinical data indicate that TRPV1 antagonists might provide a useful therapeutic option for urinary incontinence, pancreatitis, cough and migraine headache (recently reviewed in REF. 151). Marketed therapies for these disorders and pain have limitations in safety and/or efficacy that drive the need for novel treatment options. For example, NSAIDs (non-steroidal anti-inflammatory drugs), opiates and other analgesics are useful drugs for many patients, but exhibit dose-limiting side effects, inadequate tolerability profiles and diminished efficacy over time. Consequently, pain is often under treated. Although the extensive distribution of TRPV1-expressing sensory fibres creates potential opportunities for TRPV1 antagonists to affect many physiologies, it is in the treatment of pain that these agents may have the most promise. TRPV1 antagonism represents one of several novel mechanistic approaches to pain relief that might qualify as the next-generation analgesic. But although most of the drugs in development target the inflammatory system and the propagation and transmission of signals to the spinal cord, TRPV1 antagonists target the key mediator of nociceptive transduction. Because of TRPV1's integrative signalling properties in response to inflammatory stimuli, TRPV1 antagonists are predicted to inhibit the sensation of ongoing or burning pain (spontaneous pain) that is reported by patients suffering from chronic pain, therefore, offering an unprecedented advantage in selectively inhibiting painful signalling without mechanistic limitations.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

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CORRIGENDUM

The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept

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In Table 3b on page 367, there is an error in the structure of the compound AMG 517. The correct structure is shown below.