

TRPV1 Antagonists as a Potential Treatment for Hyperalgesia

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Abstract: The vanilloid receptor (TRPV1) is a member of the transient receptor potential family of ion channels that is highly expressed in nociceptive primary afferent sensory neurons. TRPV1 is a voltage-dependent cation channel, which can be activated at physiological membrane potentials by stimuli including noxious heat (>42 degrees), capsaicin, hydrogen ions and anandamide. Activation of TRPV1 results in release of neurotransmitters from peripheral and central nerve terminals, resulting in pain and inflammation. Endogenous inflammatory mediators also promote activation of TRPV1. Studies in TRPV1 null mice reveal that responses to noxious heat stimuli are normal but the development of thermal hyperalgesia is abolished. Several TRPV1 antagonists have recently been developed and reported to alleviate or reverse mechanical and thermal hyperalgesia associated with inflammatory pain. This review will examine the development of patented TRPV1 antagonists as a potential clinical treatment for the alleviation of pain associated with hyperalgesia and inflammation.

Keywords: TRPV1, antagonist, hyperalgesia, capsaicin, sensory neuron, inflammation, pain.

TRPV1 RECEPTOR ANTAGONISTS AND THEIR POTENTIAL AS ANALGESICS

Physiological Role of TRPV1 in Pain

The vanilloid receptor (TRPV1, formerly VR1) [1] is a member of the transient receptor potential (TRP) family of ion channels, a large group of proteins involved in the detection and integration of sensory stimuli and regulation of cellular calcium [2]. TRPV1 is closely related to 3 other TRP channels (TRPV2, 3 & 4), all of which are thought to be involved in temperature sensation [3]. TRPV1 is a cation channel that is highly expressed in a subset of primary afferent neurons and is directly activated by a wide range of stimuli including noxious heat (>42 degrees), protons, endogenous lipoxygenase products and fatty acid amides as well as an array of plant-derived chemicals such as capsaicin, gingerols, eugenol, resiniferatoxin, piperine and camphor [4–7]. Activation of TRPV1 is potentiated by endogenous pro-nociceptive mediators such as prostaglandins, bradykinin and ATP, and together these properties make TRPV1 the principle integrator of noxious information in many polymodal primary afferent neurons and is therefore a major potential target for novel analgesics [8–12].

TRPV1 Expression

TRPV1 is found in the cell bodies of small to medium sized primary afferents (located in dorsal root, trigeminal and nodose ganglia) and is heavily localized in the peripheral and central terminals of these cells. The central terminals of TRPV1-expressing neurons synapse in the superficial laminae (I and II) of the spinal cord and the trigeminal dorsal horn as well as in the brainstem nucleus tractus solitarius

[10, 13]. Peripheral terminals of TRPV1 expressing afferents are found in many structures including skin, muscle and viscera. TRPV1 is also expressed in several areas of the brain including regions of the limbic system such as the hippocampus and the amygdala, striatum, locus ceruleus, cerebellum and the hypothalamus [14] and has been shown to regulate glutamatergic and/or GABAergic synaptic transmission in several of these structures [15–17]. TRPV1 may function to regulate body temperature in the hypothalamus as well as reward and appetite [18].

TRPV1 receptors are also found in epithelial cells including keratinocytes and urothelial cells that line the bladder lumen. TRPV1 appears to be involved in normal bladder function and mechanically evoked purinergic signaling by the urothelium [19]. TRPV1, together with other temperature-detecting members of the TRPV family, TRPV3 and 4, are also expressed in keratinocytes [20–22], where they are thought to contribute to thermosensation [23]. TRPV1 is also found in mast cells, where it is likely to be involved in states of inflammation, and has an emerging role in the immune system [24, 25].

TRPV1 Function

The activation of TRPV1 in the primary afferent neuron results in the release of pro-inflammatory peptides from peripheral terminals and action potential-dependent vesicular release of glutamate and neuropeptides from the central nerve terminals. The release of glutamate from the primary afferent terminal in the spinal cord is the basic event in pain transmission, while central release of neuropeptides acts to amplify this signal and increase the sensitivity of the dorsal horn neurons to other incoming signals from the periphery. The release of CGRP and substance P in the periphery can contribute to a state of neurogenic inflammation, via their effects on blood vessel and immune cells. Thus, TRPV1 serves dual functions, firstly as a rapid and direct detector of potentially damaging thermal and chemical stimuli, which

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Table 1. *In vitro* Potency of Selected TRPV1 Antagonists

Compound (Figure)	Species	TRPV1 Affinity	IC ₅₀ against capsaicin activation of TRPV1	IC ₅₀ against pH activation of TRPV1	Reference
Capsazepine (1.3)	Rat	4.3 μ M	560 nM	246 nM	49, 63
	Human	580 nM	118 nM	58 nM	
Iodo-resiniferatoxin (1.4)	Rat	0.4 - 4nM	0.7 nM	0.55 nM	62, 63
	Human	7 nM	5 nM	0.55 nM	
Compound 41 (4.3)	Human	267 nM	65 nM	16 nM	81, 82
	Rat		102 nM	16 nM	
BCTC (4.4)	Rat	0.7 nM	35 nM	6 nM	85,86
A-425619 (5.4)	Human	n/a	5 nM	2 nM	92,93
	Rat		9 nM		
SB-366791 (6.1)	Human	n/a	6 nM	97% at 1 μ M	94
	Rat		18 nM	94% at 1 μ M	
AMG-9810 (6.2)	Human	n/a	25 nM	93 nM	97
	Rat		86 nM	294 nM	
6-I-PhAR (IDN5890)	Human		6 nM		39
	Rat		750 nM		

are perceived as painful, and secondly as a major contributor to the establishment of persistent peripheral inflammation and central sensitization.

TRPV1 and Pain

Apart from being involved in the immediate detection of noxious stimuli such as heat or tissue damage, TRPV1 has been shown to play a central role in the development and/or maintenance of persistent pain states, in particular those associated with inflammation. A common characteristic of persistent pain states is hyperalgesia, which is defined as an increased responsiveness to a noxious stimulus. This exaggerated pain response can be due to sensitization of the peripheral endings of nerve fibers, such that they respond to a given thermal or mechanical stimulus with many more action potentials, resulting in increased stimulation of dorsal horn neurons. Indeed, part of the mechanisms of TRPV1-mediated hyperalgesia may involve alterations in TRPV1 gene/protein expression. Hyperalgesia can occur in a variety of conditions including cancer, infection, post-operative pain and neuropathies associated with diabetes and HIV. Neuropathic pain, which occurs as a result of nerve damage, also frequently has a component of allodynia, which is pain resulting from a normally innocuous stimulus. In particular, normal tactile sensations are perceived as painful due to nociceptor-driven changes in the spinal cord, whereby signals from normally non-nociceptive nerve fibers are identified as noxious.

Many animal models can be used to study hyperalgesia and the efficacy of compounds for its treatment. Hyperalgesia resulting from neuropathy can be studied by

making partial or complete lesions to the sciatic nerve in the leg, post-operative hyperalgesia can be studied by using incisional pain models and inflammatory models can be produced by administering noxious chemicals such as complete Freund's adjuvant (CFA) to peripheral structures, thereby inducing a local inflammatory response. The effects of these models on thermal nociception can be assessed by placing the animal on a hot plate or using a focused radiant heat source (the Hargreaves test [26]). Mechanical hyperalgesia can be assessed by observing withdrawal threshold responses to tail pinch or calibrated monofilament fibres known as von Frey hairs.

Capsaicin and Analogs as Analgesics

Capsaicin stimulation of C-fibre afferents results in pain and hyperalgesia; however, repeated or prolonged treatment with capsaicin can decrease chronic pain. Clinical trials have shown that topical capsaicin is better than placebo at reducing pain associated with post-herpetic neuralgia, diabetic neuropathy, osteoarthritis and musculoskeletal disorders (reviewed in [27, 28]). In none of these conditions is capsaicin's effectiveness particularly notable, and all the trials are compromised to the extent that the local stinging and burning that accompanies capsaicin application makes placebo application or blinded trials problematic. Topical capsaicin treatment has few serious side effects, but it frequently causes unpleasant sensations and erythema at its application site [29]. However, few patients suffer adverse events sufficiently serious to withdraw from trials [27]. While there is often some pain relief after 2 weeks, a maximum therapeutic effect may be delayed for up to 4-6 weeks [30]. The topical concentration of capsaicin is

usually 0.075 %, presumably higher concentrations would be effective more quickly, but may also be more likely to produce unacceptable side effects. A single application of a much higher concentration of topical capsaicin (8%) has been trialed in healthy subjects, and interestingly, the effects on markers of skin nerve function were similar to that produced by prolonged treatment with a lower concentration of drug while the unpleasant effects were relatively minimal [31]. However, when applied to patients with chronic, severe neuropathic pain, high dose capsaicin administration had to be accompanied by regional nerve block and on occasions opioids as rescue medication [32]. In patients, this dose appeared to provide relief from pain, but the study has not, to our knowledge, been successfully followed up.

The primary mechanism(s) of capsaicin-induced analgesia have not been unequivocally established and there are a number of more or less plausible possibilities. Obviously, if TRPV1 receptor activation itself is producing pain, then capsaicin application may desensitize the receptor, thus blocking the source of the signal to the primary afferent. Capsaicin application also produces a release of substance P and CGRP in the skin and it is possible that continued application of capsaicin depletes peripheral terminals of these pro-nociceptive substances. Finally, continuous or repetitive capsaicin application leads to a blunting of many cutaneous sensory modalities [33], and this is associated with a reversible loss of epidermal nerve fibres [33] coinciding with the onset of the sensory deficits. It should be noted that for the most part, the studies investigating the mechanism of action of capsaicin have been carried out in healthy volunteers, and how these possible mechanisms relate to what happens in patients with an established pain state is an open question.

Because capsaicin appears to have some therapeutic utility, attempts have been made to develop TRPV1 agonists with fewer initially unpleasant effects; i.e. a reduced pungency. Compounds such as olvanil (NE-19550 [34]) and capsaite [35] robustly activate TRPV1 *in vitro* and have anti-hyperalgesic effects *in vivo*, but are apparently not irritant to the skin (but see [36]). Neither drug has been reported to be an effective topical analgesic, probably because they get broken down or sequestered before they reach sensory nerve endings [35, 37], a finding which presumably also explains their lack of pungency. The TRPV1 agonist resiniferatoxin (RTX) has been successfully trialed for use in some types of urinary incontinence [38], and these findings have also spurred the development of potent new TRPV1 agonists such as phenylacetylvanil [39], which lack the potentially tumorigenic phorbol ester structure present in RTX.

An alternative approach to capsaicin treatment for hyperalgesia is the use of TRPV1 antagonists. Several TRPV1 antagonists have recently been developed and reported to alleviate or reverse mechanical and thermal hyperalgesia associated with inflammatory pain. This review will firstly examine the general characteristics required of a therapeutic TRPV1 antagonist using examples of characterized TRPV1 compounds. Then the different structural classes of current patented TRPV1 antagonists will be outlined and the potential of TRPV1 antagonists as

pharmacological tools for the alleviation of pain associated with hyperalgesia and inflammation will be discussed.

TRPV1 Antagonists

Antagonist Mechanisms of Action

TRPV1 channels are activated by several stimuli that have no obvious physical relationship to each other, i.e. capsaicin, protons and heat. A capsaicin binding site has been identified on an intracellular portion of TRPV1, while protons clearly affect channel activation by interactions with extracellular glutamate residues (reviewed in Tominaga, 2005 [40]). No specific residues have been identified as being involved in heat activation of the channel. There are no obvious differences in the types of TRPV1 current evoked by each of these activators, suggesting that each distinct type of stimulus promotes a similar final change in channel conformation that leads to its opening. The idea of common pathway of TRPV1, activation has been formalized by Nilius and colleagues [8, 41], who have presented a model of receptor gating in which external stimuli shift the normally very positive activation potential for TRPV1 to more hyperpolarized voltages, meaning that the channel will open at physiological membrane potentials. These findings have important implications for the design of TRPV1 antagonists.

Antagonists of capsaicin binding should block the activation of TRPV1 by other chemical stimuli such as anandamide, *N*-arachidonoyldopamine (NADA) and lipoxygenase metabolites but they will only interfere with activation of the channel by heat and protons, if they also interact in an inhibitory way with residues of the channel involved in channel activation. The observation that some capsaicin antagonists poorly inhibit activation of the channel by heat or protons [42, 43] suggests that the capsaicin pharmacophore is comprised of several functional elements i.e. one (or more) elements involved in drug binding and another one (or more) involved in promoting TRPV1 activation. This idea has recently been formalized with the proposal of different functional classes of antagonists-Group A compounds, which can block capsaicin and proton activation; and Group B compounds, which block activation of TRPV1 by capsaicin but not protons [44]. A further potential site of TRPV1 activation has been suggested by the observation that camphor activates cloned TRPV1 at site distinct from capsaicin, and in a manner insensitive to capsazepine [7]. Thus, inhibition of capsaicin binding to or activation of TRPV1 may not be predictive of a compound that is able to inhibit TRPV1 activation by natural stimuli, and other functional assays are essential to define the full effectiveness of antagonists.

Inhibition of capsaicin binding/activation of TRPV1 is, however, likely to remain an important requirement for useful antagonists. There is an ever increasing number of endovanilloid compounds related to anandamide (*N*-acyl-ethanolamines [45]) and *N*-arachidonyl amino acids such as NADA being identified [46], and it has recently been reported that TRPV1 activation leads to the production of such compounds-which in turn leads to a further potentiation of TRPV1 activity ([47]).

Apart from binding to regions involved in capsaicin modulation of the channel, TRPV1 antagonists could also

conceivably be targeted to the channel pore to directly block ion flux (similar to ruthenium red [48]), or to extracellular regions destabilized by protons. It is also possible that antagonists could block TRPV1 activation through interfering with conformational changes required for channel activation at sites completely distinct from those important for protons or capsaicin actions, such as the putative camphor activation site [7]. Thus, compounds that do not displace binding of existing ligands to TRPV1 may still effectively inhibit activation of the channel, and this possibility is considered when designing or interpreting the results of drug screens.

TRPV1 Antagonists and Structure-Activity Relationship Studies

Capsazepine is a synthetic analog of capsaicin and was the first competitive antagonist of capsaicin to be characterized [49] Fig. (1-1 and 1-2). Capsazepine also competes for binding with the potent TRPV1 agonist resiniferatoxin in rat spinal cord, sensory neurons and guinea-pig airway membranes [50]. As well as effectively abolishing capsaicin induced TRPV1 currents in sensory neurons [49], and in heterologously expressing cell lines [51], capsazepine is also an effective inhibitor of the antinociceptive properties of capsaicin in animal behavioral studies [52].

The effectiveness of capsazepine as a TRPV1 inhibitor is however, species specific. Heat and pH induced TRPV1 currents are inhibited by capsazepine in a similar manner in human and guinea pig [53], but capsazepine only incompletely inhibits rat TRPV1 responses to heat and it fails to block activation of rat, mouse or rabbit TRPV1 by protons [42, 43, 54]. These *in vitro* findings are supported by

results showing that capsazepine produces significant reversal of carrageenan-induced thermal hyperalgesia and a reduction in mechanical hyperalgesia in models of neuropathic pain in the guinea pig but is ineffective in the rat and mouse [55]. Additionally, capsazepine has been shown to have significant non-TRPV1-mediated actions including inhibitory activity at nicotinic acetylcholine receptors, voltage-gated calcium channels, hyperpolarization-activated cation channels (I_h) and the putative cold-sensing channel TRPM8, all of which are important mediators of sensory neuron pain transduction [56-60]. Capsazepine is also an activator of the delta-subunit of the human epithelial sodium channel (ENaC) [61]. Despite capsazepine exhibiting a low potency relative to all other TRPV1 antagonists, it has been a fundamental pharmacological tool in defining the effects of TRPV1 activation, and it is often used for comparative studies with the newly identified TRPV1 antagonists discussed in this review.

Initially identified as a pure antagonist, I-RTX is also a modification of a TRPV1 agonist, the plant alkaloid, resiniferatoxin Fig. (1-3). The placing of an iodine atom at the 5' carbon of the vanillyl moiety of RTX transformed the agonist into an antagonist with a 40-fold higher potency than capsazepine, whilst maintaining its selectivity at TRPV1 [62]. Whole cell patch clamping studies in human TRPV1 (hTRPV1) transfected CHO cells, demonstrated I-RTX inhibition of capsaicin-induced activation of hTRPV1 with a potency of 5.4 nM, compared with capsazepines potency of 118 nM [63]. Iodo-resiniferatoxin effectively blocks responses to capsaicin, protons, and heat in human TRPV1 receptors [62], however, studies by Seabrook *et al.* [63] show that I-RTX is relatively ineffective for capsaicin induced paw flinching studies, compared with capsazepine.

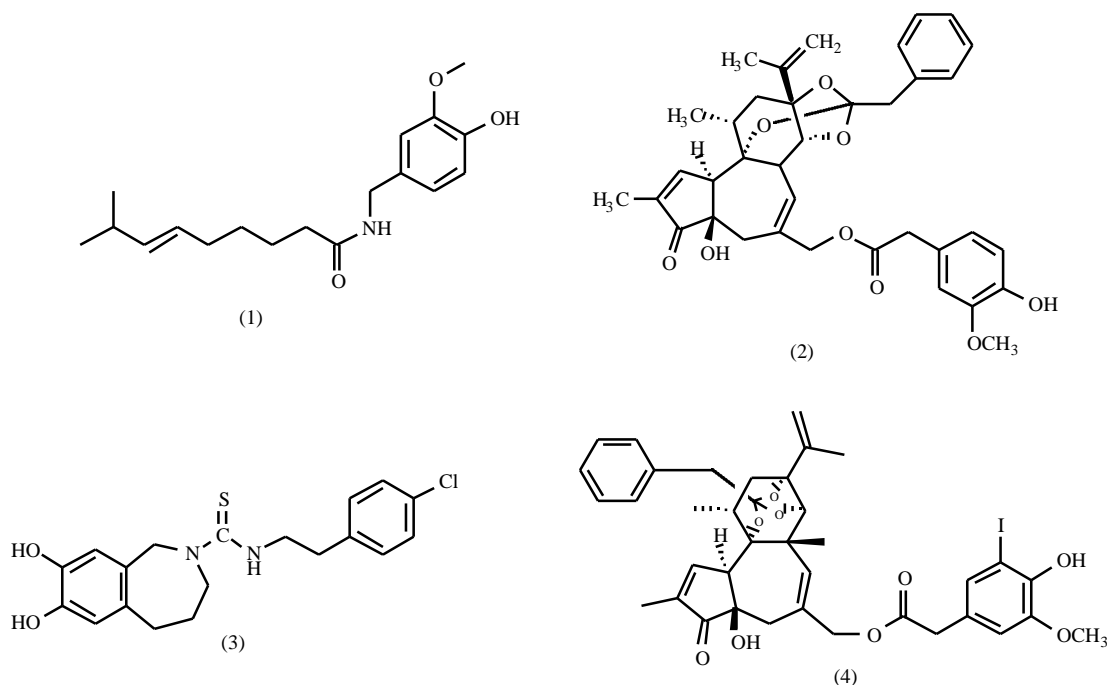


Fig. (1). (1,2) Structures of TRPV1 agonists capsaicin and resiniferatoxin (3,4) Structures of modified analogs of capsaicin and resiniferatoxin, capsazepine and iodo-resiniferatoxin.

The lack of activity of I-RTX after intraplantar administration was suggested to be due to limited access to the site of inflammation in the skin due to high lipophilicity and poor aqueous solubility. Interestingly, a recent study has demonstrated that I-RTX is a weak partial agonist at recombinant rat TRPV1, and this activity is sufficient to produce some capsaicin-like effects *in vivo* [64].

Other TRPV1 Antagonists Derived from TRPV1 Agonists

Halogenation of TRPV1 agonists to produce antagonists is turning out to be a useful general strategy. In contrast with resiniferatoxin, where 5' iodination of the homovanillic moiety produces a compound with less intrinsic agonist activity than 2' iodination (62, 65), 6' iodination of a series of vanillamide compounds including nordihydrocapsaicin, nonivamide, arvanil and phenacetylirinvanil produced potent antagonists of TRPV1 [39, 66, 67]. In each case the 6'-iodo compounds showed higher inhibitory potency against capsaicin than the corresponding 5-iodo analogs [39, 66, 67], and iodine was the most effective halogen substitution. The effectiveness of these compounds against activation of TRPV1 by heat or protons has not been determined, and their *in vivo* effectiveness has not been assessed. TRPV1 antagonists have also been developed on the basis of structural modification of synthetic thiourea-based agonists [68, 69]. In these cases, replacement of the 3' phenolic hydroxyl group on the terminal benzyl ring with a methylsulfonyl-amino group resulted in the reversal of agonist activity [68, 69]. KJM 429, the antagonist compound described in [68], only effectively inhibited capsaicin activation of TRPV1, but addition of F to the 2' carbon of the benzyl moiety of this compound (JYL 1421) resulted in an antagonist that also fully reversed heat and proton activation of TRPV1 [68,69]. Thus, halogenation in general, and iodination in particular, is an important concept in the further development of novel TRPV1 antagonists from existing agonists.

The above studies demonstrate that structural manipulations of natural exogenous TRPV1 agonists can result in the reversal of TRPV1 activity. Structural adaptation of other natural, exogenous TRPV1 agonists such as gingerol, piperine and camphor [5-7] in order to confer antagonism has not yet been explored and may result in interesting findings. Indeed, the array of endogenous TRPV1 activators such as anandamide and lipoxigenase metabolites [11, 12], are also possible leads for antagonist development.

PATENTED COMPOUNDS

TRPV1 antagonists are promising in their potential as therapeutic analgesics. In order to identify the most effective TRPV1 modulators, many pharmaceutical companies use large-scale high-throughput screening (HTS) of chemical libraries, performed using cells expressing recombinant TRPV1. The library paradigm has the benefit of searching through a multitude of chemically diverse compounds, thus steering away from the approach of structure-activity relationship studies that were used to design capsazepine and iodo-resiniferatoxin. Many of the recently patented compounds described in this review have been identified through HTS. The influx of calcium induced by TRPV1 activation is used to measure the functional activity of the

compounds, usually using the fluorescent Ca^{2+} chelating dye fluo-4, and a fluorescence imaging plate reader (FLIPR).

RECENTLY PATENTED TRPV1 ANTAGONISTS

Fused Azabicyclic, Heterocyclic and Amide Compounds

Abbot Laboratories have developed several novel TRPV1 antagonists consisting of fused azabicyclic, heterocyclic and amide compounds. Compounds referred to application numbers US20040157849A1 [70], US20040209884A1 [71] and US20050113576A1 [72] Fig. (2-1) were developed and assessed by Chih-Hung *et al* and WO05016890A1 [73] developed by Bayburt *et al*. *In vitro* investigation utilized hTRPV1 in 1321N1 human astrocytoma cells and functional activity was determined using a Ca^{2+} influx assay. The most potent TRPV1 antagonists examined in these patents had IC_{50} values of 0.1 nM vs. capsaicin.

Although animal models of hyperalgesia were not used to determine the *in vivo* effectiveness of these compounds, the antinociceptive effects of the azabicyclic compounds against an acute pain stimulus were examined using a modification of the abdominal constriction assay described in Collier *et al*, [74]. Each animal received an intraperitoneal (i.p.) injection of 0.3 mL of 0.6% acetic acid in normal saline to evoke writhing and abdominal constrictions were quantified from 5 to 20 mins. The most potent azabicyclic compound had antinociceptive effects with ED_{50} s of 1mg/kg, confirming the ability of the compound to be bioactive *in vivo*. Gomtsyan *et al* used the *in vitro* and *in vivo* techniques described above, to examine the TRPV1 antagonist effectiveness exhibited by fused heterocyclic compounds in patent applications, US20040254188A1 [75] and US20050043351A1 [76] Fig. (2-2). The most potent fused heterocyclic compounds described in these applications also had an *in vitro* IC_{50} of 0.1 nM and *in vivo* IC_{50} of 1 mg/kg.

'Amide' compounds have also been patented as TRPV1 receptor antagonists for use in the treatment of pain and inflammatory thermal hyperalgesia. Compounds described in patent applications WO05040121A2 [77] and US20050085512A1 [78] Fig. (2-3), exhibited the same maximal potency in *in vivo* and *in vitro* assays as the fused azabicyclic and heterocyclic compounds. However, further studies are required to examine the efficacy of these compounds to inhibit thermal and mechanical hyperalgesia as well as to determine their oral bioavailability.

Abbott Laboratories have also recently identified novel TRPV1 receptor antagonists with various bicyclic heteroaromatic pharmacophores [79]. The lead compound, 7-hydroxynaphthalene urea was identified through HTS, and exhibited good potency values in TRPV1 inhibitory calcium influx assays; however, it did not exhibit *in vivo* activity in animal models of inflammatory pain and was not orally bioavailable Fig. (2-4). Structure-relationship studies revealed the importance of the hydroxyl group for activity, thought to contribute to hydrogen-bonding interactions with the TRPV1 receptor. However, the hydroxyl group also appears to contribute to the poor pharmacokinetic properties of the compound. Replacement of the hydroxy-naphthyl group to a variety of 6,6-fused heteroaromatic moieties with one and two nitrogen atoms resulted in several compounds

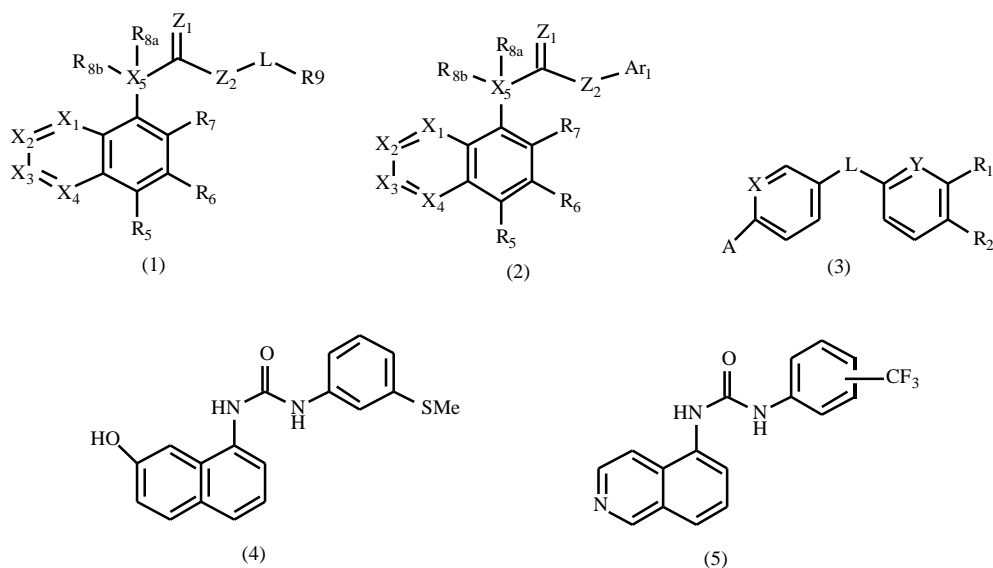


Fig. (2). Structures of TRPV1 antagonist compounds. (1) Fused azabicyclic compounds US20050113576A (2) Fused compound 20040254188A1 (3) Amide, US20050085512A1 (4) 7-hydroxynaphthalene urea (5) 5-isoquinoline containing bicyclic heterocyclic pharmacophore.

exhibiting a similar or higher potency than the 7-hydroxynaphthalene. The most effective compound was a 5-isoquinoline-containing compound, Fig. (2-5), which exhibited an IC_{50} at hTRPV1 of 4 nM and also exhibited 46% bioavailability as well as *in vivo* activity in models of inflammatory pain.

Fused Pyridine Derivatives

A Novartis research group patented a number of fused pyridine derivatives, based on the formula in Fig. (3), as novel TRPV1 antagonists in 2004 (application number, US20040138454 [80]). The activity of these compounds was measured using Ca^{2+} fluorescence assays in Chinese hamster ovary (CHO) cells expressing human TRPV1. The antagonism of both capsaicin and low pH-induced calcium were tested and the compounds was found to effectively block calcium uptake in concentration range from 1 nM to 10 μ M. The fused pyridine compounds were also found to be

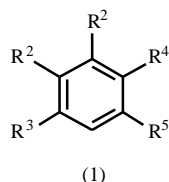


Fig. (3). Structure of fused pyridine derivative, US20040138454.

potent and efficacious anti-hyperalgesic agents following oral administration in a rat model of neuropathic pain. Peripheral neuropathy was induced by partial ligation of the left sciatic nerve and mechanical hyperalgesia was assessed from paw withdrawal thresholds measured on the ipsilateral (ligated) and contralateral (non-ligated) hind paws. Drug effects were studied 11-15 days post ligation. The mean paw withdrawal threshold \pm s.e.m for the left (ligated) paw was

compared to that of the right (non-ligated) paw. Single oral administration of the compounds produced an effective reversal of mechanical hyperalgesia in the partially denervated rat hind paw and produced mechanical hyperalgesia at 0.1mg/kg. Rapid onset of activity with a long duration of action was also reported [80].

Pyridyl Piperazinyl Ureas

Another structural class of TRPV1 antagonists is the pyridyl piperazinyl ureas [81]. TRPV1 activity of pyridyl piperazinyl urea compounds was also assessed using FLIPR assays in HEK293 cells expressing recombinant rat and human TRPV1, resulting in the identification of a potent TRPV1 antagonist, 4-(3-trifluoromethylpyridin-2-yl) piperazine-1-carboxylic acid (5-trifluoromethylpyridin-2-yl) amide, developed by Johnson & Johnson (patent application number US20050049241 [82] Fig. (4-2)). The formula described in this application, now referred to as 'compound 41' due to its recent publication by Swanson *et al* [81], was compared to the TRPV1 antagonist activity of a 'Comparative example A' Fig. (4-1). These compounds differ in the position of the piperazine-1-carboxylic acid (5-trifluoromethyl-pyridin-2-yl)-amide. The '3-chloro-pyridin-2-yl' of the comparative example is substituted for a '3-Trifluoromethyl-pyridin-2-yl' and these structural manipulations result in a significant increase in potency of TRPV1 antagonism, from an IC_{50} approximately 120 nM to 25 nM. In radioligand binding studies of human TRPV1 transfected HEK293 cells using a [3 H] resiniferatoxin, compound 41 exhibited a K_i value of 267 nM compared to 1020 nM for the comparative example [82].

Further structure-activity studies of compound 41 postulate that the essential template for antagonism is a 4-pyridinyl-2-ylpiperazine-1-carboxylic acid phenylamide. The most potent compounds had a 3-substituent on the pyridine and an electro-withdrawing group in the para-position of the

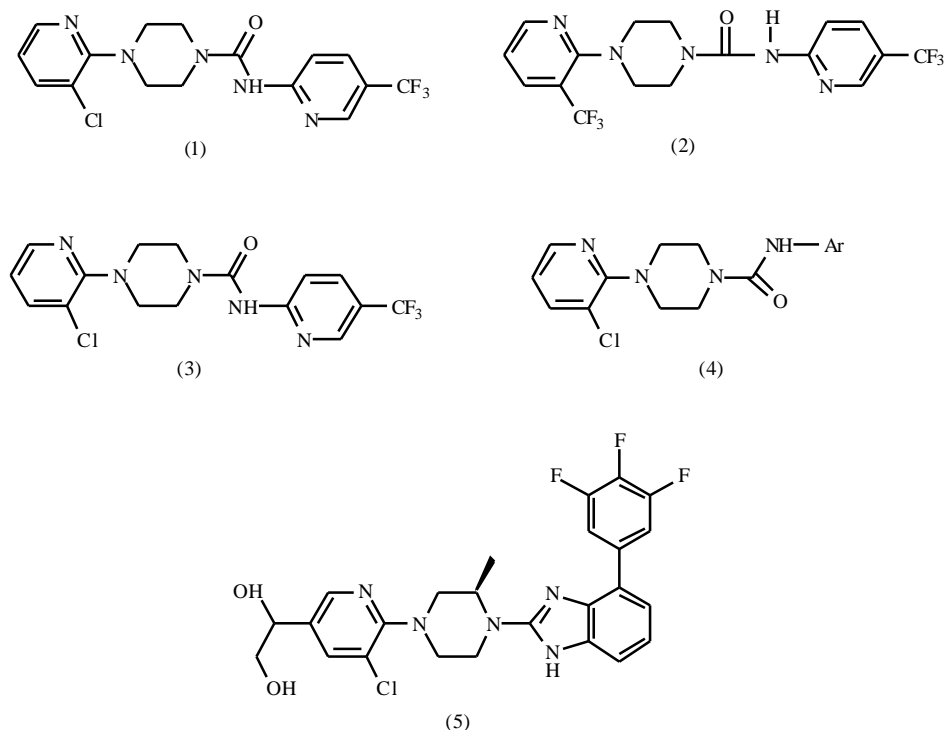


Fig. (4). Pyridyl piperazinyl urea compounds (1) Comparative example A (2) US20050049241 (Compound 41) (3) BCTC (4) Pyridazinylpiperazine analog based on BCTC (5) 2-(piperazine-1-yl)-1H-benzimidazole.

phenyl-amide fragment [81]. Swanson *et al.* also showed that compound 41 is a potent antagonist of low pH induced TRPV1 activation of both human and rat TRPV1, with an IC_{50} of around 16 nM. Pharmacokinetic assessment in the rat showed 100% oral bioavailability of compound 41, and *in vivo* studies showed that pre-treatment with 24 $\mu\text{mol/kg}$ i.p. completely prevented thermal hyperalgesia produced by intraplantar injection of 5 μg of the TRPV1 agonist NADA. The same dosage also prevented capsaicin-induced tactile allodynia. However, as has been noted with other TRPV1 antagonists, compound 41 also caused mild hyperthermia, which may be due to inhibition of a constitutively active endogenous vanilloid pathway. In addition, a study by Bannon *et al.* [83] reported that acute treatment with a novel TRPV1 antagonist AMG8163 (Amgen) produced a dose-dependent increase in body core temperature of rats, which at higher doses persisted for approximately 20 hours. The AMG8163-induced hyperthermia was not blocked by acetaminophen (paracetamol).

Another TRPV1 antagonist containing a similar structure is the 4-(2-pyridyl)piperazine-1-carboxamide analogue, N-(tert-butylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyridazine-1(2H)-carboxamide (BCTC) Fig. (4-3) [84]. BCTC is a highly potent TRPV1 antagonist that effectively inhibits proton-induced activation of rTRPV1 (but not rabbit TRPV1 [44]) and reverses the behavioral effects of inflammation and neuropathic pain in rats [85]. However, BCTC exhibits poor metabolic stability, moderate oral bioavailability and does not produce dose-proportionate pharmacokinetics when given orally to rats [85, 86]. In addition, BCTC also exhibits

non-specific inhibitory effects on the cold receptor TRPM8, with an IC_{50} of approximately 1 μM [59, 60].

2-(Piperazine-1-yl)-1H-Benzimidazole

Several piperazine-1-carboxanilides with a structure similar to that of BCTC were yielded from a search of the Amgen HTS database [87]. The structure-activity relationships of constrained analogues of the BCTC template were generated by incorporating the carboxanilide function into a 5-membered heterocyclic ring, condensed with the adjacent aromatic ring. Fig. (4-5) The resulting potent and orally available compound exhibited a markedly improved potency at rat TRPV1 (IC_{50} of 0.9 nM) compared to the initial BCTC analog (IC_{50} of 87 nM). In addition, 'compound 7' inhibited inflammation-induced thermal hyperalgesia by CFA by 46% [87].

Pyridazinylpiperazines

The synthesis and evaluation of a series of pyridazinylpiperazine compounds as TRPV1 antagonists were also reported. Fig. (4-4). Capsaicin and pH 5.5-induced calcium influx was assessed using FLIPR assays and the most potent compound exhibited an IC_{50} value of 9 nM. The bioavailability of 3 mg/kg oral dose of the pyridazinylpiperazine compounds was calculated to be 70% (with a maximum plasma concentration of 2689 ± 143 ng/mL at 3 h) compared to BCTC, which showed marginal oral bioavailability only at a high oral dose (40 mg/kg) [88].

Urea Derivatives

Several other urea derivatives have also been patented as TRPV1 antagonists. These include the heteroaromatic urea derivatives described in patent application number 20050107388 [89], developed by Merck & Co., Inc. Compounds were antagonists exhibiting IC_{50} values of around 200 nM when CHO cells expressing human TRPV1 receptors were exposed to capsaicin during calcium influx assays. Fig. (5-1). Investigators at Johnson & Johnson developed several novel α -aminotetralin-derived urea compounds (patent application number US20050187291 [90]) based on the formula in Fig. (5-2). One of the compounds, (1-(1-benzyl-6-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-3-isoquinolin-5-yl-urea), referred to as Compound 33, potently blocked the increase in intracellular calcium at hTRPV1 elicited by low pH and anandamide with an IC_{50} value of 40 nM. Blockade of activation of TRPV1 by protein kinase C (PKC) phosphorylation at low pH had an IC_{50} of 70 nM. This compound was compared to capsazepine, which had an IC_{50} of 110 nM and 370 nM for acidic and PKC phosphorylation (at low pH) activation of TRPV1 respectively. Several compounds including compound 33 were also tested in their ability to inhibit carrageenan-paw induced thermal hyperalgesia in the rat. The ED_{50} value for inhibition by compound 33 was 0.276 mg/kg p.o. The ability of the compounds to inhibit the binding of [3 H]-RTX was also assessed. Compound 33 exhibited a K_i of 3 nM at hTRPV1 when expressed in HEK293 cells.

Urea derivatives from Bayer pharmaceuticals, patent application US20050154230A1 [81], were also reported to exhibit TRPV1 antagonist activity in Ca^{2+} mobilization studies from DRG neurons and show good selectivity, although in common with most of the patents discussed, few details of how selectivity was established were provided.

The selective TRPV1 receptor antagonist A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea], Fig. (5-

3), an isoquinoline compound demonstrates a high degree of specificity at TRPV1 and exhibits IC_{50} values of 5 nM in hTRPV1 expressing HEK cells against activation by capsaicin [92]. A-425619 showed similar potency in native TRPV1 channels of rat dorsal root ganglion neurons. Honore *et al* [93] reported that A-425619 dose-dependently reduced capsaicin induced mechanical hyperalgesia (ED_{50} 45 μ mol/kg p.o.) and was also effective in models of inflammatory and postoperative pain. A-425619 reduced complete Freund's adjuvant-induced chronic inflammatory pain after oral administration (ED_{50} 40 μ mol/kg p.o.) and maintained its efficacy against post-operative pain after twice daily dosing p.o. for 5 days [93].

Cinnamides

A group of TRPV1 antagonists based on cinnamides include AMG 9810 and SB-366791 (N-(3-methoxyphenyl)-4-chlorocinnamide. Fig. (6-1,2). SB-366791 is a compound isolated *via* high-throughput screening of a chemical library and exhibits an IC_{50} of approximately 6 nM in hTRPV1-HEK293 cells [94]. SB366791 also inhibited capsaicin-induced hypothermia, eye wiping movements and vasodilatation of the knee joint at 500 μ g/kg i.p. dose 2mg/kg, whilst CZP was found to be ineffective [95]. The potency of cinnamides depends on the optimum groups being in the para position (R_{para}) of the phenyl ring of the cinnamide, these groups were tert-butyl, isopropyl, and trifluoromethyl [96].

The TRPV1 antagonist AMG 9810, studied in CHO cells stably transfected with hTRPV1 and rTRPV1 showed lower potencies with IC_{50} values of 24 nM and 86 nM respectively, and this compound exhibited high first-pass metabolism and poor oral availability in rats [97]. The pharmacological selectivity of AMG 9810 was tested against a wide range of receptors, enzymes, and ion channels. No significant interactions were observed up to concentrations of 4 μ M, where assays revealed inhibitory activity at additional TRP channels including TRPV3, TRPV4, TRPM8 and TRPA1

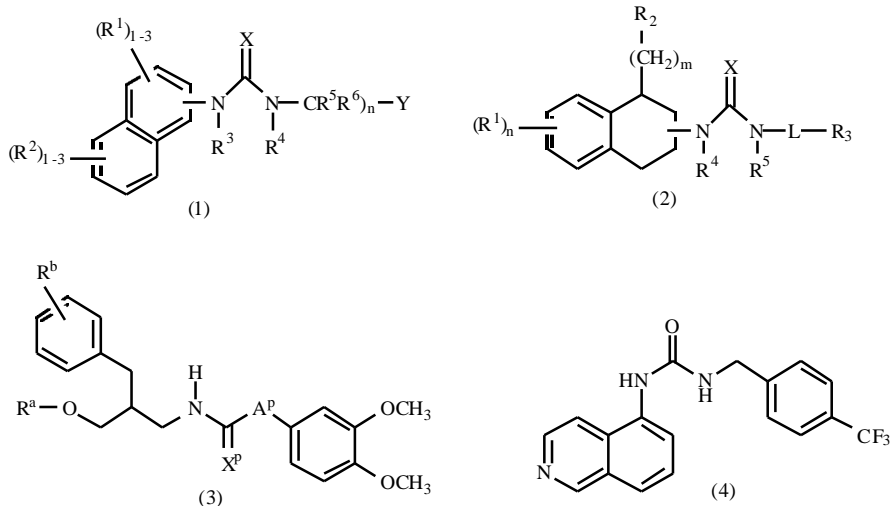


Fig. (5). Structures of urea derivatives, (1) US20050107388A1 (2) α -aminotetralin-derived urea, US20050187291 (3) Urea derivative, US20050154230A1 (4) Isoquinolin urea derivative, A-425619.

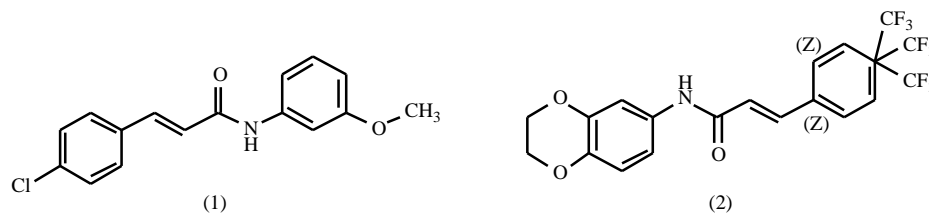


Fig. (6). Structures of cinnamide derivatives (1) SB-366791 and (2) AMG 9810.

[97]. An improved selectivity can be observed with TRPV1 isoquinoline urea derivative antagonist A-425619 Fig (5-3). A-425619 was profiled in a large panel of *in vitro* binding assays where it was found to be largely inactive, and only exerted inhibitory activity at TRPM8 with an IC_{50} of $8\mu\text{M}$. All other tested targets were not affected up to $10\mu\text{M}$. Detailed information regarding the selectivity for targets other than TRPV1 is not usually described.

CURRENT AND FUTURE DEVELOPMENTS

While a high degree of selectivity for any drug is important, there is, however, another substantial unknown factor with regard to the use of TRPV1 antagonists as therapeutic analgesics. TRPV1 is expressed in many peripheral tissues and the CNS, and physiological roles outside of sensory neuron function are poorly understood. Therefore, it is likely that TRPV1 antagonists will possess actions unrelated to the inhibition of pain. The block of TRPV1 activation may prove beneficial in inflammatory states, particularly when there is increased TRPV1 expression, but TRPV1 is also involved in physiological processes that are not associated with inflammatory states. In addition to the proposed roles for TRPV1 in regulation of body temperature and synaptic transmission in the brain, TRPV1 is suggested to play a cellular protective role against noxious stimuli in gastric mucosal epithelial cells [98,99]. Another role for TRPV1 has been described in the rat pancreas, where in addition to expression in innervating neurons, TRPV1 is also expressed in islet beta cells where it may function as a calcium channel involved in the modulation of insulin secretion [100].

The investigations described above provide strong pre-clinical validation for the use of TRPV1 antagonists as antihyperalgesics. TRPV1 antagonists are also patented for use as a potential treatment of several other clinical disorders including irritable bowel syndrome, urinary tract disorders and tracheobronchial and diaphragmatic dysfunctions. Increasing use of high throughput screening in the identification of novel TRPV1 antagonists has limited the need for activity-structure relationship studies based on agonist compounds and most of the recently patented TRPV1 antagonist compounds are not structurally related to TRPV1 agonists, unlike earliest antagonists capsaizepine and iodo-resiniferatoxin. However, what is required of a clinically applicable compound is the ability to inhibit ligand, proton and heat activation of TRPV1, as well as good potency, bioavailability, and a high degree of selectivity.

Several of the recently described TRPV1 antagonists exhibit good oral bioavailability, which is a favorable property of a clinical analgesic. Of the described patent

compounds, the fused pyridine derivatives described in patent application US20040138454 [80], and later studies of pyridyl piperazine (patent application number, US20050049241 [82] demonstrated oral activity. However, depending on the required site of action, topical, as opposed to oral administration of the TRPV1 antagonist would less likely be associated with unwanted effects.

The development of TRPV1 antagonists has improved enormously since the first descriptions of capsazepine. Unlike capsazepine, most of the described patent TRPV1 antagonist compounds are structurally unrelated to TRPV1 agonists, and are therefore less likely to exert partial agonist activity, as reported for I-RTX [64]. The emerging importance of endogenous agonist activation and potentiation of TRPV1 function suggest that useful TRPV1 antagonists will need to block capsaicin activation of the channel, although the higher doses that may be required for a competitive antagonist to block TRPV1 activation by endogenous ligands could increase the likelihood of unspecific effects.

The importance of ligand efficacy and the best ways in which it is detected this in another theme to emerge from recent studies of TRPV1. Capsaicin was the prototypic TRPV1 agonist and was considered *ipso facto* a full agonist at the receptor, but piperine has been reported to have a higher efficacy than capsaicin, leading to the redefinition of capsaicin as a partial agonist [6]. Other partial agonists of TRPV1 have been identified, but whether partial agonist activity is detected can depend on the type of bioassay used. For example, anandamide is clearly a partial agonist of TRPV1 in electrophysiological studies [11, 101, 102], yet when examined in assays measuring intracellular calcium increases it has been frequently described as a full agonist (103-105). This suggests that measurements of intracellular calcium may be a more sensitive detector of TRPV1 activation, but the partial agonist activity of I-RTX has not been apparent in such assays (e.g. [39]). The partial agonist activity of I-RTX was eventually detected using standard electrophysiological techniques [64], despite many investigators previously reporting purely inhibitory activity with this compound in electrophysiological assays e.g. [15, 62]. These somewhat confusing results can probably be explained on the basis of receptor expression levels, the species from which the TRPV1 was derived, the cells in which the receptor was expressed and the phosphorylation state of the channel. Nevertheless, the results highlight the need for bioassays that can detect a wide range of efficacies at TRPV1, and not be insensitive to weak agonists or easily saturated by partial agonists. The most direct possible measure of TRPV1 activation is using patch clamp

electrophysiology to measure single TRPV1 channels in cell membranes. In such an experiment, agonists increase the open probability of the channel, while partial agonists would be expected to produce a smaller increase open probability [11]. It is possible to construct concentration/open probability curves for agonists with such assays [11], but they are technically demanding and unsuited to drug screening. The recent insights into the interaction between membrane voltage and agonist activation of TRPV1 [8, 41] suggest the possibility that recording single TRPV1 channel activity at various membrane potentials could provide a sensitive measurement of TRPV1 activity for ligands with a wide range of efficacies, as even very weak agonists would be expected to increase channel open probability at depolarized membrane potentials.

Whether partial agonists would have any additional therapeutic benefit over full agonists or pure antagonists remains to be seen, but it has been suggested that both anandamide [11] and camphor [7] produce exaggerated desensitization of TRPV1 when compared with the initial receptor activation. If these observations can be translated into structural features, agonists which produce prolonged desensitization of the TRPV1 without much initial pain may be a possibility. More speculatively, if ligands can be developed which selectively inhibit proton and ligand activation of TRPV1 without affecting temperature-dependent gating of the channel, it may be possible to preserve normal sensory function but limit the ability of the channel to integrate noxious stimuli.

TRPV1 antagonists are reported to be effective in the reduction of mechanical hyperalgesia, and in models of inflammatory and post operative pain, but the mechanisms underlying hyperalgesia are still to be fully understood. Many other neuronal receptors and ion channels are also implicated in the development and maintenance of hyperalgesia. For example, alterations in the expression of voltage-gated sodium, calcium and potassium channels have been associated with neuropathic pain conditions resulting in hyperalgesia. There is also a large amount of evidence implicating the activation of prostaglandin, purinergic and bradykinin receptors in inflammatory responses [106-108]. NMDA-type glutamate receptors appear to be necessary for mediating heat hyperalgesia in a variety of animal models of chronic pain [109, 110], but they appear not to be important for mediating enhanced responses to heat after incision [111]. Thus, the use of TRPV1 antagonists may be sufficient for the relief of some hyperalgesic states, but as hyperalgesia is a term that describes a complex series of conditions with different causes, TRPV1 antagonists are likely to be used in conjunction with other analgesics. Nonetheless, the development of TRPV1 antagonists would seem to be a more patient friendly manipulation of TRPV1 when contrasted with the pain-inducing TRPV1 desensitization or denervation required to achieve analgesia with the use of TRPV1 agonists.

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