Medicinal Chemistry, Pharmacology and Clinical Implications of TRPV1 receptor antagonists

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ABSTRACT

Transient receptor potential vanilloid 1 (TRPV1) is an ion channel expressed on sensory neurons triggering an influx of cations into sensory cells. TRPV1 receptors function as homotetramers responsive to heat, proinflammatory substances, lipoxygenase products, resiniferatoxin, endocannabinoids, protons, and peptide toxins. Its phosphorylation increases sensitivity to both chemical and thermal stimuli while desensitization involves a calcium-dependent mechanism resulting in receptor dephosphorylation. TRPV1 functions as a sensor of noxious stimuli and may represent a target to avoid pain and injury. TRPV1 activation has been associated to chronic inflammatory pain and peripheral neuropathy. Its expression is also detected in non-neuronal areas such as bladder, lungs and cochlea where TRPV1 activation is responsible for pathology development of cystitis, asthma and hearing loss. Therefore, modulation of TRPV1 channel activity is under consideration for the therapy of chronic pain, cough, bladder disorders, diabetes, obesity, and hearing loss. This review offers a comprehensive status of the art about TRPV1 receptor in pathophysiology and highlights how drug development targeting this channel could have a clinical therapeutic potential. This review also summarizes advances of medicinal chemistry leading to the identification of highly selective TRPV1 antagonists and their analysis of structure–activity relationships (SARs) highlighting how drugs targeting this channel could be better developed.

Key words: Transient receptor potential vanilloid 1; pathophysiology; TRPV1 antagonists; medicinal chemistry; structure–activity relationships

1. INTRODUCTION

Why peppers taste hot? The heat sensation is due to capsaicin, a chemical molecule present in peppers, daily consumed on a global scale. Capsaicin interacts with specific sensory neurons and in particular binds to the vanilloid receptor (VR1), a member of the superfamily transient receptor potential (TRP) ion channel referred as TRPV1. Through this interaction the capsaicin molecule produces the same sensation, or signal to the brain, that normal heat produces when activating the TRP receptors. This is why eating peppers makes your mouth feel really hot, even though it's not.

TRPV1 is a non-selective cation channel abundantly expressed in the nociceptors (c-fibers).

In this review article, we offer an overview about the role of TRPV1 in the regulation of key physiological signaling and important pathologies associated with its activation. In recent years our research group has investigated a large series of TRPV1 derivatives with interesting properties from a chemical and pharmacological point of view, showing *in vitro* and *in vivo* affinities for the TRPV1 receptor. Considering that several pharmaceutical industries are involved in the field and many patents are reported, and that it is often difficult to obtain exact information regarding the status of molecules in early stages of clinical development, we perform a review on TRPV1 receptor ligands (since 2008 to date) from published articles and patent literature, to highlight how drugs targeting this channel could be important clinically and better developed.

Moreover, we have also tried to describe the potential of TRPV1 antagonists as therapeutic agents for treating pain, cough, bladder disorders, diabetes and obesity by reporting their clinical status of development. The medicinal chemistry section of this review summarizes the 2008-2016 advances of the new chemical entities development as TRPV1 antagonists, the structure–activity relationships (SAR) analysis, the corresponding biological activities combined with the therapeutic potential of compounds.

2. STRUCTURE, REGULATION AND DISTRIBUTION

A. Structure

The family of vanilloid TRP channels includes six members, from 1 to 6. Only the TRPV1 subtype is stimulated by vanilloids, such as capsaicin, present in chilli peppers. It was in 1960 that Jancsó found that capsaicin was able to activate sensory nerves,[1] then, 15 years later the presence of a capsaicin receptor was detected in their plasma membranes.[2] Subsequently, the TRPV1 receptor was cloned in mouse, human and guinea-pigs,[3–5] showing marked species-related differences in pharmacological profiles. For example, birds and rabbits, differently from rodents and humans, are insensitive to the pungent action of capsaicin. The human Trpv1 gene is located on chromosome 17p13 and encodes a 95 kDa protein containing 839 amino acids residues.[6] TRPV1 resembles voltage-gated potassium channels as suggested by single-particle electron cryo-microscopy studies allowing the identification of transmembrane topology and subunit organization of TRPV1.[7–9] Furthermore, research performed on TRPV1 knockout (KO) mice sanctioned a crucial role for this receptor in noxious heat perception *in vivo*.[10] Noxious stimuli open TRPV1 channels located in sensory nerve endings with consequent membrane depolarization, thereby initiating action potentials that propagate to the spinal cord and brain. TRPV1 currents evoked by a variety of agonists exhibit pronounced outward rectification, increasing current at positive compared to negative membrane potentials thus reducing TRPV1 activation when cells are near resting potential.[11–14] A series of exogenous and endogenous activators of TRPV1 have been characterized, such as Vanilloids (e.g. Olvanil, Resiniferatoxin, RTX)[15, 16], Capsinoids (e.g. Capsiate)[17], Camphor[18], heat >43°C and pH <5.9[19], Anandamide[13, 14], Lipoxygenase products [8](e.g. LTB4)[20, 21], N-acyldopamines[21, 22], Bradykinin[23], PAR-2 agonists[24], nerve growth factor (NGF)[23], ATP[23, 25] suggesting the importance of cross-talk mechanisms.

The TRPV1 receptor is a homotetramer that consists of six transmembrane domains with a pore region located between the fifth and sixth domain, and long intracellular N- and C- terminal tails.[6] Within the N-terminal tail, six ankyrin repeat domains allow binding of calmodulin and ATP to modulate TRPV1 activation.[26] The C-terminus contains a TRP domain as well as binding sites for phosphoinositide 4,5-biphosphate (PIP2) and calmodulin.

Fig. 1

At the receptor level, capsaicin binds to the region that spans transmembrane domains 3 to 4 of the TRPV1 receptor. Bound capsaicin orients in a "tail-up, head-down" configuration where the vanillyl and amide groups form specific interactions that anchor the ligand to the receptor.[27] Since ligands bind to the cytosolic side of TRPV1[28], these agonists must traverse the plasma membrane to access the intracellular ligand-binding site of TRPV1.[29] One exception is proton activation at pH of <6.0. TRPV1 receptor forms a cation channel permeable to mono- , such as sodium and potassium, and divalent cations, with 10-fold higher preference for calcium, and an exceptionally high permeability to large cations.[30] Its activation leads to the elevation of cytosolic Ca^{2+}, and the subsequent release of neuropeptides such as substance P and calcitonin-gene related peptide by exocytosis.[31, 32] Interestingly, TRPV1-mediated ion permeation changes depending on the activation state.[33] Selectivity for calcium is also modified, depending on the extracellular calcium levels. Both agonist concentration and identity affect these dynamic permeability effects. In particular, a strong increase in permeability of large cations, such as *N*-methyl-d-glucamine (NMDG) or the propidium dye YO-PRO1, is induced by high concentrations of capsaicin, in contrast to low concentrations that normally do not change it, unless when protein kinase C (PKC) phosphorylated TRPV1. Moreover, some agonists, e.g. camphor and heat, induce only few changes in large cations permeability.[34]

Mutagenesis studies revealed that Met547 and Thr551 of human and rodent TRPV1 are necessary for capsaicin sensitivity.[35] Furthermore, the highly conserved tyrosine in position 511 (Y511) of the rTRPV1 was the first residue to be identified as a necessary participant in the vanilloid-mediated response,[36] its rotation was implicated in the vanilloids bound state[8, 37–39] and, more recently, it was found to entrap vanilloids in their binding site, prolonging channel activity.[40] Moreover, the aminoacid residues Glu600 and Glu648 within the extracellular loop domain are important for TRPV1 activation by protons. A series of aminoacid residues located in the pore region and C-

terminal domains are crucial for heat sensitivity e.g. 1696A, W697A, and R701A resulted in total loss or reduction of heat sensitivity.[41]

B. Regulation

TRPV1 is regulated by phosphorylation inducing an increased sensitivity to both chemical and thermal stimuli. Indeed TRPV1 have multiple phosphorylation sites at various serine and threonine residues for protein kinase A (PKA), PKC, c-Src kinase and calcium-calmodulin kinase II (CaMKII), in addition to putative sites for capsaicin and proton binding.[12, 42–47] This is relevant because several inflammatory agents stimulate PKA and/or PKC through G-protein coupled receptor (GPCR)-depending pathways.[48, 49] Phosphorylation induced by PKA, via cyclic AMP signaling, and PKC, via IP3 signaling, produced different effects. In particular, PKA decreases TRPV1 desensitization capsaicin-mediated by phosphorylating Ser116, thus allowing an higher sensitivity to the TRPV1 agonist.[43] PKC phosphorylation instead increases sensitization of this receptor to heat, protons or agonists and is generally triggered by inflammatory agents, through IP_3 turnover.[11, 29, 50] Furthermore, PKC potentiates TRPV1 activity by shifting voltage-dependent activation to more negative potentials thus rising the possibility of channel opening at normal membrane potentials.[6]

Interestingly, TRPV1 sensitization is obtained also through soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-dependent exocytosis by a rapid enrollment of TRPV1, located in subcellular vesicules, in the plasma membrane under inflammatory conditions.[51–53]

Recently, it has been reported that TRPV1 function is strongly modulated by cyclin-dependent kinase 5 (Cdk5)-mediated phosphorylation at position threonine-407 (mouse)/T406 (rat), position critical for the function of TRPV1 by modulating ligand-sensitivity, activation, and desensitization kinetics as well as voltage-dependence.[54] These results indicate that Cdk5-mediated phosphorylation of rat TRPV1 (rTRPV1) at T406 plays an important role in the molecular process of transduction of nociceptive stimuli and pain signaling.

TRPV1 undergoes to homologous desensitization after repeated or prolonged exposure to capsaicin or resinaferatoxin in a calcium-dependent way that leads to dephosphorylation.[29, 55] The mechanism involved is through calcineurin, a protein phosphatase 3, which dephosphorylates serine and threonine residues, targets of PKA activity. Indeed, this desensitization is reduced by PKC phosphorylation.[46, 56, 57]

In addition, numerous different pathways such as interactions of molecules e.g. calmodulin,[26, 58] ATP,[26] or A-Kinase Anchoring Protein 79/150 (AKAP150)[59] or the privation of phosphoinositol 4,5-diphosphate from the plasma membrane[53, 60] take part in receptor desensitization.

Finally, it has been found that endocytosis plays a role in dose- and time-dependent capsaicin-induced desensitization through a clathrin-independent pathway targeting the channel for lysosomal degradation.[61]

C. Distribution

According to its role in pain, nociception and heat perception, TRPV1 expression has been originally detected in primary afferent nociceptors of the dorsal root ganglia (DRGs), trigeminal ganglia (TG), and vagal ganglia.[3, 19, 62] These neurons form unmyelinated or myelinated nerve fibres, C or Aδ, respectively.

Even though subsequent studies demonstrated a much wider distribution in the central nervous system (CNS) e.g. in dopaminergic neurons of the substantia nigra, hippocampus, hypothalamus, cortex, cerebellum, in the dentate gyrus and the nucleus accumbens, more recently it has been revealed through TRPV1 reporter mice that only few brain regions contain these receptors.[63–67] TRPV1 is expressed also in non-neuronal cells, e.g. epidermal keratinocytes,[68] urothelium,[69–71] liver hepatocytes,[72] polymorphonuclear granulocytes,[73] pancreatic β-cells,[74] endothelial cells,[75] mononuclear cells,[76, 77] arteriolar smooth muscle cells,[67] mesenteric arteries,[78] preadipocytes and adipose tissue.[79]

3. PATHOPHYSIOLOGICAL ROLES

A. Thermoregulation

The administration of the TRPV1 agonists capsaicin, RTX, rinvanil, and arvanil has been demonstrated to induce severe hypothermia associated with skin vasodilatation,[80–84] thus suggesting a role for this receptor in thermoregulation. Hypothermia caused by TRPV1 agonists is transient in nature because it usually lasts only for a few hours, most probably due to TRPV1 desensitization.[81] However, it has been reported that continuous infusion of the TRPV1 agonist dihydrocapsaicin, a component of chili pepper, due to its desirable hypothermic profile with regards to the duration and depth, controls body temperature in a fashion which is promising for patients survivors of out of hospital cardiac arrest.[85]

Interestingly, evaluating the effect of TRPV1 antagonists on capsaicin-mediated hypothermia it has been demonstrated that these ligands alone rise body temperature. Hypertermia was obtained by using a range of chemically different antagonists and was not observed in TRPV1 KO mice[86] suggesting that this phenomenon is due to inhibition of TRPV1 receptors. Therefore, being these antagonists highly selective it has been argued that TRPV1 channel tonically affects body temperature regulation.[81, 87] The mechanism associated with this effect is a vasoconstriction producing a reduction in heat loss through skin and increased thermogenesis. The site of action of hyperthermia induced by TRPV1 antagonists has been located in peripheral visceral TRPV1 channels, outside the blood brain barrier.[86, 87] Hypertermia was transient because it was attenuated after consecutive doses of antagonist allowing the hypothesis that clinical development of antagonists in humans could not be a problem.[88]

B. Pain

TRPV1 has been recognized to be a major contributor to pain for important reasons. Firstly, it is known that TRPV1 receptor undergoes desensitization following agonist stimulation thus alleviating pain in rodents,[89] and in humans.[90] Secondly, this channel is overexpressed in inflammation being stimulated by inflammatory mediators to induce pain behaviors in rats. [91–94] Thirdly, TRPV1 KO show decreased thermal hypersensitivity after inflammation,[10, 95] and finally TRPV1 antagonists block pain behavior in rodent models of inflammation,[96–101] osteoarthritis,[97] and

cancer.[102–104] Since TRPV1 is considered an attractive target for an analgesic agent, both agonists and antagonists are being considered for therapeutic evaluation.

Pain can generally be identified as nociceptive or neuropathic depending on the pathogenesis.

C. Nociceptive pain

Nociceptive pain includes inflammatory pain, due to the activation of the nociceptors by a series of proinflammatory molecules e.g. cytokines, chemokines, lipids, kinases. In this environment TRPV1 channels play a crucial role in the mechanism at the basis of inflammatory pain because they can be directly activated by inflammatory mediators.[105, 106] Furthermore, sensitization occurs in inflammatory conditions that can increase TRPV1 channel expression in sensory neurons,[92] induce a rapid translocation from the cytoplasm to the plasma membrane[51, 107–109] and modify at posttranslational level TRPV1 receptors [12, 24, 110–114] with phosphorylation mechanisms responsible for an increase of functionality.[115]

TRPV1 has been implicated in osteoarthritis (OA).[116, 117] TRPV1 is present at high level in nociceptive nerve fibres that innervate the articular capsule of the joint and is increased in the sensory afferent fibres innervating the OA joint. When OA was induced in rats by intra-articular monoiodoacetate [MIA] injection, DRG neurons showed an upregulation of TRPV1.[118] These animals presented an increase in Calcitonin gene related peptide (CGRP) production triggered by TRPV1 after stimulation with capsaicin.[119] The TRPV1 antagonist A-889425 [1-(3-methylpyridin-2-yl)-N-(4-(trifluoromethylsulfonyl) phenyl)-1,2,3,6-tetrahydropyridine-4-carboxamide] was able to counteract increased spontaneous firing activity in spinal neurons, presumably linked to pain.[116] Pain behavior, measured as weight-bearing asymmetry, in mice with MIA-induced OA was blocked by intra-articular administration of the TRPV1 antagonist JNJ17203212 [4-[3-(trifluoromethyl)-2-pyridinyl]-N-[5-(trifluoromethyl)-2-pyridinyl]-1-piperazinecarboxamide].[120] Stimulation with capsaicin led to desensitization and as a consequence reduced both pain and bone damage induced by MIA. [121] In TRPV1 KO mice, a decrease in tissue damage and mechanical hyperalgesia was observed in a model of chronic arthritis. [122] Further evidences supporting the role of TRPV1 in pain

OA derive from human studies where an increased density of TRPV1 has been observed in patients with OA.[123] Accordingly, variant of TRPV1 with loss-of-function has been associated to a reduced risk of pain in OA.[124] All together, these data support an important role for TRPV1 antagonists to relieve OA pain even though two of them, ABT-116 and AZD1386, failed to give significant pain relief in this pathology.[125–128]

However, as in other preclinical pain models, e.g. diabetic neuropathy, it has been observed that the pain relief effect of TRPV1 antagonism was affected by the duration of the pathology, it has been hypothesized that TRPV1 antagonists may be useful when administered at the beginning of the disease, to avoid the occurrence of chronic pain. Furthermore, we have to consider that OA is a complex pathology where TRPV1 is only one pain target. [129, 130]

TRPV1 is thought to play a role in dental pain being expressed in most trigeminal ganglion neurons innervating tooth pulp.[131] In particular, TRPV1 are increased in trigeminal ganglion neurons in a model of pulpitis[131] and are responsible of thermal hyperalgesia. Accordingly, agonist-induced desensitization of TRPV1 present on sensory neurons inhibited inflammation and consequent bone loss in a rodent model of periodontal disease.[132] Furthermore, the TRPV1 antagonist AZD1386 exerts analgesic effect versus acute pain after molar extraction in humans.[133] Overall these data suggest that TRPV1 may be a useful therapeutic target in patients with periodontal disease.

It is well recognized that TRPV1 is involved in pain and hyperalgesia throughout the alimentary canal in response to peristaltic movements and/or distension that may be induced by inflammatory conditions.[134] TRPV1 are widely located in the digestive tract where affect taste, visceral sensation, gastrointestinal (GI) motility and functions. In particular, TRPV1 are expressed in extrinsic sensory neurons, intrinsic enteric neurons, epithelial, and endocrine cells.[135, 136] The highest presence of TRPV1 in the alimentary canal is in spinal and vagal primary afferent neurons where TRPV1 may contribute to the cough induced by gastro-esophageal reflux of gastric contents (GERD), thus producing pain.[137] Furthermore, the sensitivity of TRPV1 to low pH renders it a target for sensing heartburn provoked by GERD. Accordingly, TRPV1-KO mice present a reduced esophagitis

following acid exposure in comparison to wild type littermates.[138] Therefore, clinical trials have been conducted to evaluate the potential role of TRPV1 antagonists in patients affected by GERD, but unexpectedly the results obtained with AZD1386 indicated the lack of involvement of TRPV1 in heat-, mechanically- and electrically-evoked esophageal pain in these patients.[139] Interestingly, in the context of acute pancreatitis induced by alcohol abuse TRPV1 is activated and responsible for a reduction of the disease.[140, 141] TRPV1 is overexpressed in colonic biopsies derived from patients with inflammatory bowel disease (IBD).[142] Interestingly, the TRPV1 antagonist JYL1421 inhibited colorectal distension and ameliorated colitis, indicating TRPV1 as a contributor to the pathophysiology of visceral pain.[143]

TRPV1 has been indicated as a good therapeutic target in cancer pain.[102] Indeed, TRPV1 activation plays a critical role in the generation of bone cancer pain, and its expression is increased within distinct subpopulation of DRG neurons.[144] Both treatment with RTX and genetic deletion of TRPV1 reduced bone cancer pain.[136, 145]

D. Neuropathic pain

Neuropathic pain is generated by a damage of somatosensory nervous system and is comprehensive of diabetic neuropathy, chemotherapy-induced neuropathy, post-herpetic neuralgia, spinal cord injury, and phantom limb pain. After a primary lesion or injury affecting peripheral nerve there is a modification of TRPV1 channel expression in sensory neurons. In particular, a TRPV1 decrease has been observed in the injured neurons in response to peripheral axonal injury possibly due to the loss of trophic delivery.[146] However, their upregulation has been observed in some neurons near damaged nerve.[147, 148] Evidence from KO mice suggested that TRPV1 channels did not affect pain induced by nerve injury or diabetes.[10, 149, 150]

E. Diabetes and Obesity

The role of TRPV1 in the control of metabolism and glucose homeostasis is well established.[151–153] Indeed, TRPV1 has been associated to the occurrence of both type 1 and type 2 diabetes mellitus[154] due to its effects on appetite and weight regulation,[155] glucagon-like peptide-1 (GLP-1)

production,[156, 157] pancreatic function modulation and increase of insulin secretion,[155] adiponectin[158] and leptin signaling control,[159] modulation of neuronal activity. [152, 160–162]

Interestingly, dietary capsaicin reduced inflammation, insulin resistance and hepatic steatosis in obese mice fed with a high-fat diet.[163] This agonist induced a lower fasting glucose levels, lower insulin and leptin levels, and improved glucose tolerance versus obese mice without capsaicin treatment, thus leading to prevention of type 2 diabetes occurrence. Similar beneficial effects have been observed also with topical application of capsaicin that produced weight less and a decrease of adipose tissue related to lower expression levels of tumor necrosis factor-α and interleukin(IL)-6 and higher expression of adipokines and other genes involved in lipid metabolism.[164] Interestingly, capsinoids associated with physical exercise blocked diet-induced obesity by increasing energy consumption.[165] More recently, a synergistic antiobesity effect by a combination of capsinoids and cold temperature through promoting beige adipocyte biogenesis has been observed.[166]

The beneficial effects of capsaicin in metabolic profile have been observed in TRPV1 KO mice showing dysregulated leptin signaling and obesity increase.[167] However, in a previous study, it was found that in mice lacking TRPV1 there was a protection from diet-induced obesity.[168]

F. Bladder disorders

The expression of TRPV1 in normal urinary bladder has been well studied. Initial studies detected TRPV1 receptors in membranes from urinary bladder, then TRPV1 was found in nerve fibres,[169–171] in urothelial cells, and in myofibroblasts.[69, 172] In particular, in urothelial cells activation of TRPV1 channel induced an increase in intracellular calcium and nitric oxide production that was not observed in TRPV1 KO mice.[69] Furthermore, in *in vivo* animal studies capsaicin increased bladder contraction frequency and decreased the threshold of volume necessary to trigger voiding.[173] Under pathological conditions such as overactive bladder it has been found that TRPV1 receptors are upregulated in urothelial cells and nerve fibers and administration of TRPV1 antagonist reduced amplitudes of bladder contractions. [174] The coexistence of TRPV1 channels and tropomyosin receptor kinase A (TrKA) receptors has been shown to be essential in bladder control and

sensitivity. Indeed, it has been demonstrated that NGF increases expression of TRPV1 channels in the cell membrane of urothelial cells, increased ATP release in response to capsaicin through TrKA activation via a phosphatidylinositol 3-Kinase and PKC signalling pathway. Under inflammatory conditions a TrKA blocker was able to inhibit pain behavior. Furthermore, bladder inflammation elicits increased expression of TRPV1 channels in the membrane of urothelial cells.[175] Mechanical bladder hyperactivity, typical of cystitis, was antagonized by a TRPV1 blocker and was not revealed in TRPV1 KO mice.[176, 177] Indeed, in the rat bladder, the TRPV1 antagonist JTS-653 blocked overactivity without affecting normal micturition.[178] In particular, TRPV1 channels were upregulated in neurons innervating the bladder and the related DRGs.[179]

G. Cough

Numerous literature data report a potential involvement for TRPV1 receptor in cough with capsaicin presenting unique protussive effects.[180–182] As for the expression of TRPV1 channels they are distributed through the respiratory system, including in the nose, bronchi and vessel wall.[183–186] More recently, TRPV1 expression has been observed in primary cultures of epithelial cells isolated from human airways, where the receptor is upregulated in the airways of patients with refractory asthma mediating IL-8 release potentially relevant for long term cough reactivity.[187] Importantly, it has been demonstrated that the most common cause of cold, human rhinovirus (HRV), infecting neuronal cells, induces an overexpression of TRPV1 through NGF-, IL-6- and IL-8-dependent mechanisms even though it has to be clarified whether this upregulation is crucial for HRV-dependent cough.[188] It has been observed an high level of TRPV1 and TGFß2 in blood and adenoid body specimens of children with upper airway cough syndrome in comparison with healthy subjects.[189] Another evidence for the role of TRPV1 in cough derived from finding about tiotropium bromide, a muscarinic antagonist used as a drug for chronic obstructive pulmonary disease (COPD), that inhibited cough triggered by TRPV1 agonist.[190] Contrasting results on TRPV1 role in cough derive from genetic studies as a single point mutations (SNPs) in TRPV1 channel decreases cough threshold in subjects with a history of workplace exposure.[191] However, asthmatic patients with a

loss of function SNP of TRPV1 appear to be more sensitive to cough.[192] Furthermore, the SB705498 ligand, a TRPV1 antagonist, even though was able to reduce cough reflex sensitivity to capsaicin, did not decrease cough frequency in patients affected by refractory chronic cough[193] suggesting that the role of TRPV1 in cough deserves other investigations.[180]

H. Hearing loss

TRPV1 is expressed in the organ of Corti and in spiral ganglion cells where it is involved in the cochlear homeostasis.[194] The cochlear TRPV1 could serve as a sensor of cisplatin-induced oxidative stress and as mediator of cochlear damage. Induction of TRPV1 clearly results from ROS generation in the cochlea.[195] Interestingly, salicylate could induce tinnitus through activation of TRPV1 in the rat auditory pathway.[196]

4. MEDICINAL CHEMISTRY OF TRPV1 ANTAGONISTS

While both TRPV1 agonists and antagonists have been targeted as potential analgesics in various animal models of neuropathic pain, the major focus of the drug discovery attempt has been on the identification of TRPV1 antagonists. Over the past several years, several pharmaceutical companies focused efforts drug discovery on the TRPV1 receptor antagonists. Starting with structures of lead agonists such as the natural products, capsaicin and its ultrapotent selective analog RTX (compounds **1** and **2**, respectively, Fig. 2), passionate medicinal chemistry labors have been generated potent antagonists along with better understanding of TRPV1 pharmacology. The prototypical TRPV1 antagonist, structurally related to the capsaicin, is the N-(4-chlorophenethyl)-4,5-dihydro-7,8-dihydroxy-1*H*-benzo[*c*]azepine-2(3*H*)-carboxamide **3** (capsazepine, Fig. 2) that was widely used in the past to dissect TRPV1-mediated responses. It has been shown that capsazepine blocked capsaicin-mediated performances in rodents and has become a precious tool for studying the effects of TRPV1 antagonists in neuropathic pain models. Challenges to improve on the poor physical properties associated with the capsazepine scaffold have led to a large number of diverse structures as potent TRPV1 antagonists.

The chemical section of the present review will provide an update of the progress made in SARs in the field with particular focus on the TRPV1 antagonists developed from 2008 onwards. Representative compounds and key characterization data covering multiple chemical series are highlighted.

Fig. 2

A. Phenyl- /Benzyl- Urea

The 1,3-disubstitued ureas represent the major classes of the TRPV1 antagonists. The search for TRPV1 antagonists in Abbott Laboratories was started with identification of 7-hydroxynaphthalene urea **4** (Fig. 3) that was discovered as a part of high-throughput screening (HTS) campaign. Despite its potent cellular activity, compound **4** did not exhibit in vivo activity in animal models of inflammatory pain and was not orally bioavailable. Subsequently, the replacement of hydroxy-naphthyl group with a variety of nitrogen containing bicyclic heteroaromatics produced the 5-isoquinoline urea **5** (A-425619**,** Fig. 3) with better pharmacokinetic properties and higher aqueous solubility.[197] It blocked capsaicin-evoked increases in intracellular calcium concentrations in HEK293 cells expressing recombinant human TRPV1 receptors (IC_{50} = 5 nM). A-425619 showed similar potency (IC_{50} = 3-4 nM) to block TRPV1 receptor activation by anandamide and N-arachidonoyl-dopamine. Similar to capsazepine, A-425619 demonstrated competitive antagonism of capsaicin-evoked calcium flux, showing 25- to 50-fold more potency than capsazepine in blocking TRPV1 activation.[198] Exploration of the SAR in this chemical series by replacement of the benzyl lipophilic portion with an indane moiety led to the identification of the indazolyl urea **6** (ABT-102, Fig. 3)**,** a potent antagonist (IC_{50} = 5-7 nM) of capsaicin-induced Ca^{2+} influx in human recombinant TRPV1 receptors.[199] ABT-102 was effective in blocking nociception in rodent models of inflammatory, post-operative, osteoarthritic, and bone cancer pain.[117] Moreover, it was found that repeated administration of ABT-102 for 5-12 days increased its analgesic activity in models of post-operative, osteoarthritic, and bone cancer pain while the associated hyperthermic effects were attenuated. Oral administration (100 μmol/kg) of **6** elicited a clear elevation (0.8 °C) in core body

temperature.[200] However, in a randomized controlled trial it was not able to reduce hip arthritic pain in a model of dog OA.[201]

Evaluation of the SAR on the benzyl urea series revealed that introduction of lipophilic groups at 2-position, while maintaining the 4-CF_3 substituent provided more in vivo activities when compared to the corresponding pyridinyl analogs. The lipophilic 2-(*tert*-butyl)ethyl substitution led to compound **7** (Fig. 3) which despite a positive pharmacokinetic profile and increase aqueous solubility relative to **6**, was excluded from any preclinical development showing to be a time-dependent inhibitor of CYP3A4 metabolism. The N1 metylation of indazole nucleus yielded **8** (ABT-116, Fig. 3) with favorable in vitro activity and CYP inhibition profile.[202]

In a related effort, PharmEste s.r.l. disclosed in a patent publication the urea TRPV1 antagonists characterized by a bicyclic heteroaryl portion. Among this series, the 2-oxobenzoimidaloes **9** and **10** (Fig. 2) displayed antagonist activities exhibiting a complete abolition of capsaicin at 300 nM (IC_{50} **=** 1 and 0.51 nM, respectively). Compound **9** showed a significant anti-hyperalgesic effect in chronic constriction injury-induced mechanical hyperalgesia test.[203]

Starting from a naphthol-based urea series with low oral bioavailability, Bayer Yakuhin's Research Center identified the tetrahydronaphthols as TRPV1 inhibitors with oral bioavailability in rats. Tetrahydronaphthol derivative **11** (Fig. 3) showed high activity (hTRPV1 IC_{50} = 3.3 nM), it exhibited outstanding exposure after oral administration, good oral bioavailability in rats (F = 74%, 1 mg/kg oral dose, 0.1 mg/kg iv) and adequate free fraction in rat plasma (fu = 0.28%). The area under the curve (AUC) relates to plasma levels obtained after oral administration to rats at 1 mg/kg for **11** was 650 (ng h/ml). In this group of compounds the enantiomers showed a low eudismic ratio at the receptor level.[204,205]

Fig. 3

B. Dibenzyl- Urea/Thiourea

Following the discovery of dibenzylurea and relative thiourea derivatives[206] as analogs of natural product capsaicine, in PharmEste research laboratories, a program was begun to reach

TRPV1 antagonists with improved aqueous solubility and metabolic stability. The new series of O-hydroxyalkyl urea derivatives (compounds **12** and **13**, Fig. 4) were tested against capsaicin-induced secondary allodynia in rats, prevented its pro-allodynic effect by 53.1% and 47.9% of inhibition, respectively. The *R*- and *S*-isomers of compounds **12** and **13** were synthesized in order to appreciate the difference in acting with respect to the racemic compounds. The most active isomers in calcium influx assay were the *R*-enantiomers with a values of IC_{50} = 7 and 53 nM, respectively. The O-hydroxyalkyl ureas showed improvement in terms of metabolic stability and cytotoxicity.[207]

It has been reported that isosteric replacement of the phenolic hydroxyl group in the thiourea TRPV1 agonists such as compounds **14** and **15** with the methylsulfonamido moiety provided the potent antagonists **16** and **17**, respectively (Fig. 4), which inhibited the activation by capsaicin of rat and human TRPV1 expressed in CHO cells. In particular, the N-[2-(4-*tert*butylbenzyl)-3-pivaloyloxypropyl]-N′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea **17** (Fig. 4) showed high affinity with a K_i value of 54 nM for the inhibition of rat TRPV1 [^{3}H]RTX binding and potent antagonism with a K_i value of 7.8 nM for the inhibition of Ca^{2+} uptake in response to capsaicin with a low level of residual agonism.[208,209]

In a related effort, to optimize the 4-methylsulfonamide TRPV1 antagonists, the amide surrogates of the parent thiourea antagonists was designed.[210] An extensive SAR investigation of the amide antagonists led to the identification of the α-methyl amide analogues which showed very capable activities. Among them, the two *α*-methyl amide antagonists, **18** and **19** (Fig. 4) showed high binding affinities, with K_i values of 4.12 and 1.83 nM, respectively, they also exhibited potent antagonism with K_i values of 0.58 and 5.2 nM, respectively, in rTRPV1/CHO cells.[210]

Continuing attempt to examine the effect of α-methylation on affinity for TPRV1 receptor, the α-methylated analogs of simplified RTX thiourea antagonist were synthesized. The SAR analysis indicated that the C_2-configuration was not important for activity; while the amide analogs preferred the *S*-configuration for receptor activity, the thiourea compounds privileged the *R*-configuration. In vitro binding competition assay with [^{3}H]RTX and by a functional $^{45}Ca^{2+}$ uptake assay using rat

TRPV1 heterologously expressed in CHO cells, compound **20** (Fig. 4) provided antagonism activity with a K_i of 10 nM and K_{iCAP} of 23.9 nM.[211]

Fig. 4

C. Chromane and Tetrahydroquinoline Urea

Further optimization efforts were undertaken by Abbott Laboratories to address the pharmacokinetic profile and potency of the previous series of urea class of TRPV1 antagonists. A series of structural modifications, including conformational restriction of the benzylic group of 5-isoquinoline urea **5** (A-425619**,** Fig. 3) was achieved by generating the chromane and tetrahydroquinoline bicyclic scaffolds, which usually contribute to a higher solubility of the resulting ureas compared with the original rigid indane scaffold. Compounds **21** and **22** (Fig. 4) are the most representatives of this family, showing an IC_{50} values of 5 and 7 nM for the hTRPV1 receptor, respectively, in calcium influx assay.[212] From the in vitro SAR of the chromane ureas was emerged that different substituents such as the small lipophilic trifluoromethyl, bulky *tert*butyl, and the basic piperidine ring were tolerated, while the site of the functional group assignment was the key for in vitro potency. In addition, the *R*-enantiomer showed greater potency than the S-enantiomer. The chromane urea **21** was efficacious in multiple animal pain models and possessed a brain/plasma ratio of 0.42 in rats. From the SAR analysis, the position of the aryl substituent is a key factor for in vitro activity; and the 7- and 8-positions are the favorable sites for higher potency. Better in vitro activity was obtained from the large N-methyl or N-benzyl substituted analogs. Although, the tetrahydroquinoline ureas were found to be inhibitors of the cytochrome (CYP)3A4, the best combination of potency and CYP3A4 inhibition was achieved with **22** (hTRPV1 IC_{50} = 7 nM, CYP3A4 = 47% inhibition).[212]

Subsequently, an additional series of chromane ureas characterized by substitution at the 3 position (methyl, chlorine, or amine) of isoquinoline core were included in a patent publication by the Abbott Laboratories.[213] One of this large number of family is urea **23** (Fig. 5) with superior profile

against all the three subtypes of CYP 450 enzyme CYP2C9, CYP2D6, and CYP3A4 showing limited transient temperature effect on core body temperature of rats.[213] In a related study, a patent publication claimed also the phenylcyclopentane urea as TRPV1 antagonists. Several ureas were prepared by replacing the chromane ring with phenylcyclopentyl moiety. A large number of compounds were prepared exemplified by **24** and **25** (Fig. 5), bearing the indazole and isoquinoline cores, respectively, linked by urea function to the arylcycloalkyl portion of molecule. Most compounds of the reported patent were potent TRPV1 antagonists that inhibited the increase in cellular calcium in response to the capsaicin and showed little or no impairment of the subject's ability to sense noxious temperature. Moreover, when they tested in various model of neuropathic pain, exhibited about 25% or more the calcium flux response remaining upon acid activation of TRPV1.[214]

Further SAR studies on the chromane series and continues attempts to optimize the nociceptive and thermoregulatory functions of TRPV1 receptor led to a new patent publication by the Abbvie Inc.[215] Several compounds claimed in this publication inhibited the response to capsaicin but only partially blocked receptor activation by pH 5.0 solution. Those were also tested for their effect on noxious thermosensation using the tail immersion assay, where less than a 10% increased tail withdrawal latency when administered orally. An example of these urea-based compounds is derivative **26** (Fig. 4).

More recently, the isoquinoline urea derivative including chromane moiety such as in compound **27** (Fig. 5) with *R* configuration was found to be devoid of hyperthermic effects at high dose (hTRPV1 IC_{50} = 2 nM in calcium influx assay). This data encouraged a more focused investigation of SAR to optimization of the chromanyl urea series, concluding in the discovery of *R*-1-(7-chloro-2,2-bis(fluoromethyl)chroman-4-yl)-3-(3-methylisoquinolin-5-yl)-urea **28** (A-1165442, Fig. 5), a TRPV1 antagonist with good analgesic efficacy, temperature-neutral profile, favorable pharmacokinetic profile, and good efficacy against osteoarthritis pain in rodents (hTRPV1 IC_{50} = 9 nM in calcium influx assay).[216]

Fig. 5

D. Piperazine Urea

A high-throughput screening performed by the Johnson & Johnson Pharmaceutical Research by fluorescence cell-based assay utilizing the Ca2+ permeability of TRPV1 led to the identification of several series of agonists and antagonists including the pyridinylpiperazine ureas.[217] The representative hit **29** (hTRPV1 IC_{50} = 74 nM and rTRPV1 IC_{50} = 100 nM, Fig. 6), inhibited phorbol 12-myristate-13-acetate (PMA)-, and anandamide-induced Ca^{2+} influx mediated by TRPV1 (determined using FLIPR). Further SAR studies led to piperazine carboxamide **30** (BCTC**,** Fig. 6) that inhibited capsaicin-induced activation of rat TRPV1 (IC_{50} = 35 nM) and acid-induced activation of rat TRPV1 (IC_{50} = 6 nM).[218] This compound has been extensively profiled in animal models of inflammatory and neuropathic pain.[219] Poor metabolic stability, aqueous solubility, oral bioavailability has disqualified it for further development. Following investigations focused on the piperazine urea scaffold to improve the pharmacokinetic profile of compound **30** produced *R*-4-(6-chloro-4-methylpyridazin-3-yl)-N-(6-fluorobenzo[*d*]thiazol-2-yl)-2-methylpiperazine-1 carboxamide **31** (Fig. 6) that was recognized as a second-generation of BCTC analogs. It had a methyl chain on the piperazine ring, while *tert*butylphenyl and pyridinyl nucleus were replaced with benzothiazolyl and pyridazinyl groups, respectively. Compound **31** (hTRPV1 IC_{50CAP} = 226 nM, hTRPV1 IC_{50pH} = 103 nM) showed better metabolic stability, longer half-life, aqueous solubility and decreased inhibition of HERG (human *Ether-à-go-go*-Related Gene) channel.[220]

Employment of a tetrahydro-pyrimidoazepine core as a bioisosteric alternate for the piperazine urea resulted in the discovery of a novel series of TRPV1 antagonists. Utilizing the disclosed SAR from the piperazine urea series reported previously by the same research group, the 3-trifluoromethyl-pyridine was chosen as the favorite substituent on the piperidine ring nitrogen. The best compound of this study, the pyrimido-[4,5-*d*]azepine **32** (hTRPV1 IC_{50} = 6 nM, Fig. 6) was examined in an in vivo model of inflammatory pain and carrageenan-induced thermal hyperalgesia. Despite its low

oral bioavailability, compound **32** significantly attenuated thermal hyperalgesia, expressed as % maximal possible effect (% MPE), when dosed orally at 30 mg/kg.[221] Subsequently, the structural modifications to improve aqueous solubility produced the piperazine analoge **33** (Fig. 5) which bears an *iso*butyl substituent (hTRPV1 IC_{50} = 11 nM).[222] This compound provided improved rat pharmacokinetics (CL = 0.7 L/h/kg) compared to **32** (CL = 3.1 L/h/kg), was orally bioavailable, and gave a significant reversal of carrageenan-induced thermal hyperalgesia at 5 and 30 mg/kg in rats.[222]

More recently, a published report by the Discovery Research, Purdue Pharma LP, described the SAR exploration effort around the 3-chloropyridin derivative BCTC to identify novel potent TRPV1 antagonists with improved aqueous solubility and pharmacokinetic properties. The bioisosteric replacement of the central piperazine core of BCTC with tetrahydropyridine nucleus led to 2-pyridyl-3,6-dihydropyridine carboxamide derivatives as TRPV1 antagonists. An extended SAR of the new tetrahydropyridine template was concluded in the discovery of analogue **34** (V116517, Fig. 6), which was evaluated in acute inflammatory complete Freund's adjuvant (CFA) model for its ability to reverse thermal hyperalgesia. In this model, V116517 showed dose-dependent reversal of thermal hyperalgesia with an ED_{50} of 2 mg/kg. It also exhibited high potency for blocking proton activation of TRPV1 in inflamed tissue.[223]

Fig. 6

E. Pyrazole- Carboxamide/Urea

TRPV1 antagonists based on pyrazole nucleus have been reported in several patent publications by Grüenenthal GmbH, in where an extensive SAR investigation around the pyrazole based urea and carboxamide derivatives was descried.[224] The chemical structure of this new series was based on substitution of the benzyl ring of the previously N-benzylurea compounds with the heterocyclic pyrazole ring. The first series of compounds bearing an O-containing phenyl acetamide (**35**), phenyl propionamide (**36**) or phenyl urea (**37**) functions is depicted in Fig. 7, which showed high affinities for TPRV1 receptor (hK_{iCAP} values of 1, 2, and 2 nM, respectively).

Subsequently substituted pyrazolyl carboxamide/urea derivatives bearing a phenyl moiety substituted with an N-cyclic group were designed [225]. In this group of compounds the phenyl nucleus was substituted with various heterocycloalkyls, such as azetidinyl, thiazolidinyl, pyrrolidinyl, piperidinyl, and morpholinyl rings. The best compounds of this series, the azetidine derivative **38** and thiazolidine **39** (Fig. 7) displayed antagonist activities at 10 μM on the hTRPV1 receptor with K_i affinity values of 15 and 29 nM, respectively. Grüenenthal GmbH research group has also investigated the pyrazolyl carboxamide and urea derivatives bearing a phenyl moiety substituted with a sulfonyl or sulfonamide groups as vanilloid receptor ligands**.** An example of the sulfonyl derivatives is showed in Fig. 6 (Compound **40**) that exhibited a K_i value of 9 nM in binding experiments.[226] The best results were obtained with the propionamide compounds bearing a sulfonamide chain such as in **41**, **42**, and **43**, (Fig. 7) which showed excellent affinity for hTRPV1 receptor with K_i values of < 1 nM (hK_{iCAP} = 0.9, 0.7, and 0.3 nM).[227]

Replacing of the phenyl ring of **42** with a pyridine moiety resulted in a new family of pyridinyl-propanamide/urea compounds **44** and **45**, respectively depicted in Fig. 7 as hTRPV1 antagonists (**44,** K_{iCAP} = 1 nM, **45,** K_{iCAP} = 3 nM) that have been enclosed in a patent publication.[228]

Fig. 7

F. Pyridine- Propanamide/Urea

Lee. J. and coworkers from Seoul National University published the 2-substituted-pyridine propanamide derivatives as TRPV1 receptor antagonists through replacement of the phenyl ring of previous *N*-4-*tert*-butylbenzyl-2-(4-methylsulfonylaminophenyl) propanamide (**46,** hTRPV1 K_i = 46.2 nM, Fig. 8) with a pyridine ring.[229] The SARs investigations of the 2-substituent in the pyridine moiety by various groups including amino, oxy, thio and alkyl functions designed compound **47** (Fig. 8), showing high antagonism with hTRPV1 K_{iCAP} = 0.3 nM and IC_{50pH} = 8.4 nM. The effect of chiral center on activity was studied and the *R*-enantiomer of **47** exhibited greater affinity for the TRPV1 receptor than the *S*-enantiomer. It showed analgesic activity in a neuropathic

pain model with modest TRPV1-related hyperthermia in mice.[230] Within the 2-arylsubstituted pyridine propanamide analoges, compounds such as **48** (Fig. 8) showed anti-allodynia in a mouse neuropathic pain model and blocked capsaicin-induced hypothermia in a dose-dependent manner.[231] In this study, the SARs of 2-alkyl/alkenyl pyridine derivatives as hTRPV1 antagonists were also investigated. Several compounds in the series showed outstanding and stereospecific TRPV1 antagonism with high potencies. The 2-cyclohexyl derivative **49** (K_{iCAP} = 0.6 nM, IC_{50pH} = 43.4 nM, $IC_{50heat45°C}$ = 14 nM, Fig. 8) was preferred for further study and was shown to antagonize capsaicin-induced hypothermia in a dose-dependent manner, consistent with its action in vivo being through TRPV1, and it showed analgesic activity in a rat neuropathic pain model.[232]

Subsequently, an additional series of more lipofile analogues, which were found to possess high TRPV1 antagonist activity were described by the same research group. The SAR analysis indicated that the lipophilicity of the 2-oxy substituents was a key determinant of antagonism. The 2-*iso*butyloxy with low lipophilicity and 2-benzyloxy analogues **50** and **51,** respectively (Fig. 8) showed analgesic activity in the formalin test in mice with full efficacy.[233] In a related effort to optimize the properties of the antagonistic 2-(3-fluoro-4-methylsulfonamidophenyl) propanamide template by incorporation of various alkyl, dialkyl and aryl groups at the α-position in the acetamide central chain was investigated.[234] From this study was emerged that the steric repulsion of the α-substituent was determinant for antagonistic potency. Within this series, compound **52** (Fig. 8) showed excellent antagonism with a value of hTRPV1 K_i = 0.1 nM was more potent than the parent **47** while it showed weaker potency for the other activators. In addition, the docking study of **47** indicated that its high potency could be attributed to a specific hydrophobic interaction of the *m*-tolyl group with the receptor.

In a related effort, further pyridine-urea linked to different aromatic nucleus such as isoquinoline and indazole moieties were claimed by Grünenthal GmbH in a patent publication.[235] In particular, the N-[{2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)-pyridin-3-yl}methyl]-N'-(6,6-fused heterocyclic) urea (for example compound **53,** Fig. 8) showed highly potent TRPV1 antagonism to

capsaicin, antagonized against stimulation by heat and was efficacious in the formalin pain model.[236] Molecular modeling analysis with hTRPV1 homology model supplies the binding mode of compound **53** with the receptor in which hydrogen bonding between the pyridine nitrogen and Ser512 being important for high potency.

Fig. 8

G. Isoxazole Carboxamide

The optimization effort to improve both TRPV1 potency and solubility led to the discovery of isoxazole-3-carboxamide derivatives as TRPV1 antagonists by the N.V. Organon and Pharmacopeia LLC companies.[237,238] The synthesis and SARs of 5-phenylisoxazole derivatives showed that the 1*S*, 3*R*-3-aminocyclohexanol entity was a fundamental stereochemically to confer both TRPV1 potency and solubility. The trifluoromethyl in 4-positione of the 5-phenyl group was shown to impart strong potency to the molecules at TRPV1 whilst, introduction of fluoro substituents were found to make important contributions to the compounds exhibiting the required balance of solubility and potency. Compounds **54** (hTRPV1 IC_{50} = 3 nM) and **55** (IC_{50} = 79 nM) (Fig. 9) were identified as the most promising compounds from this series in calcium assay and were progressed into animal studies. Both compounds were able to attenuate the acute inflammatory thermal response in the rat CFA assay but suffered from poor solubility and high plasma protein binding.[239] Subsequently, Merck Research Laboratories described the structural modifications by substitution of the 4-position of **54** with specific polar functionality to improve solubility and physicochemical properties.[240] In particular, the 4-*iso*propylmethylamino derivative **56** (hTRPV1 pIC_{50} = 7.2 in calcium influx assay, Fig. 9) showed clear attenuation of the acute inflammatory thermal response in the rat Capsaicin Hargreaves assay, although its lower in vitro potency as compared to compound **54**. In this series, the 4-*iso*butylmethylamino derivative **57** (pIC_{50} = 9, Fig. 9) demonstrated improved oral bioavailability (33%) as compared to compound **54** demonstrating significant

attenuation of the acute inflammatory thermal response in the rat capsaicin Hargreaves assay (p <0.01).[241]

Fig. 9

H. Piperidine Carboxamide

Piperidine carboxamides were developed by the Johnson & Johnson Pharmaceutical Research and Development as potent antagonists of the TRPV1 receptor.[242] The initial compound **58** (Fig. 10) was a weak antagonist with a value of IC_{50} =600 nM for human TRPV1 receptors. Further optimization efforts were undertaken by structural modifications in the polar portion of this molecule leading to the identification of benzo[1,4]oxazin-3-one analog **59** (Fig. 10), that offered improved stability with significant value of activity (IC_{50} = 5 nM). Compound **59** was evaluated for in vivo efficacy in a rodent model of thermal hyperalgesia, which at an oral dose of 30 mg/kg produced a small but non-significant decrease in radiant heat latency at 30 min post-dose.[242]

Purdue Pharma L.P. has also disclosed in a patent publication the phenylpiperidine-1-carboxamides (example **60**, Fig. 10) and structurally similar compounds such as **61** (Fig. 9, IC_{50CAP} = 33.8 nM, IC_{50pH} = 1.1 nM) as human TRPV1 receptor antagonists. Within these series, a large number of compounds reduced FCA-induced thermal hyperalgesia with 50% to 100% reversal of mechanical hyperalgesia.[243]

Fig. 10

I. Pyrazolopyridine-3-/Imidazopyridine-3- Carboxamide

Glaxo Group Limited has claimed in several patent publications the pyrazolo[1,5-*a*]pyridine and imidazo[1,2-*a*]pyridine carboxamides as TPRV1 receptor antagonists. These bicyclic chemotypes are characterized by presence of a phenoxyethyl chain linked to the carboxamide function such as in compounds **62** and **63** (Fig. 11). Most of the synthesized derivatives showed a pIC_{50} greater than 7.8 in the capsaicin assay and greater than 4.6 in the acid stimulus assay.[244] Structural modifications on this series by introduction of a cyclobutyl ring in the central portion and the hydroxymethyl group

as a side chain on the heterocyclic core produced the new family of N-cyclobutyl-imidazopyridine or N-cyclobutyl--pyrazolopyridine carboxamides as TRPV1 antagonist in recombinant HEK-293 cells expressing TRPV1 assay (for example **64**, Fig. 11).[245] It showed a pIC_{50} of 9.1 and 9.2 in the capsaicine and in the pH assays, respectively.

A related series of N-cyclobutyl-imidazopyridine methylamine analogues is also reported by the same company, wherein 8-*trans*-3-(2,3-dichlorophenoxy)cyclobutylamino)methyl)H-imidazo[1,5-a]pyridin-6-yl)methanol **65** (Fig. 11) was assayed in vivo, showing clear dose response for the inhibition of capsaicin-induced bronchoconstriction.[246, 247]

J. Pyrrolopyridazine

AstraZeneca R&D with a HTS campaign where the Ca^{2+} influx assay was replaced by the Rb^{+} atomic absorption spectroscopy assay discovered a novel indolizine class of compounds as TRPV1 antagonist. This new chemotype proved to be unstable in the presence of light and oxygen and the metabolic stability was poor. Consequently, the addition of a heteroatom onto the bicyclic core structure to improve the light stability and the metabolic stability by reducing the lipophilic character led to the pyrrolopyridazine scaffold. This class of compounds exhibited the same level of potency, no light stability issue and a similar drug metabolism and pharmacokinetic (DMPK) profile, while the metabolic stability problem inherent to the indolizine class remained. A significant metabolic stability improvement was achieved by combining eLogD kept below 3 and a less flexible hydroxylamide moiety such as in compound **66** (Fig. 11). The inhibitory effect of **66** against the human TRPV1 ligand-gated ion channel expressed in CHO cells was $pIC_{50} = 5.5$.[248]

Fig. 11

K. Benzothiazol Carboxamide

A HTS campaign using the fluorescence cell-based assay that measures Ca^{2+} influx performed by the AstraZeneca R&D led to the identification of the benzothiazole hit **67** (Fig. 12). This compound was a potent and competitive antagonist with capsaicin at the hTRPV1 receptor, with an IC_{50} of 27

nM, but displayed poor metabolic stability in human microsomes and had low aqueous solubility (2.2 μM at pH 7.4).[249] Further efforts to improve both aqueous solubility and metabolic stability profile, through the attachment of polar groups to the benzothiazole core and by blocking metabolic sites, resulted to the recognition of 4-bromo-N-(2-(hydroxymethyl)benzothiazol-5-yl)-2-methylbenzamide **68** (IC_{50} = 52.3 nM, Fig. 12). It was active in a rat carrageenan model of inflammatory pain with no observed body temperature effects seen at an efficacious dose.[249]

A parallel research activity performed by Purdue Pharma L.P. has claimed the benzothiazol-2-carboxamides bearing 2,3-dihydroxypropyl pyridine-benzamide chain (compound **69**, IC_{50} = 32 nM, Fig. 12) as TRPV1 ligand, possessing moderate receptor affinity.[250] Extensive SAR studies by Shionogi & Co. Ltd aimed at improving the metabolic profile led to the 4-(4-fluoro-6-(2,3-dihydroxypropyl)pyridin-3-yl)-N-(6-fluorobenzo[*d*]thiazol-2-yl)piperazine-1-carboxamide **70** (Fig. 12).[251]

Fig. 12

L. Oxazole, Triazine

A series of non urea TRPV1 antagonists were synthesized and evaluated by the Abbott Laboratories, to address CNS penetration and pharmacokinetic properties of their previous urea molecule ABT-102. The bioisosteric replacement of the urea functionality with 2-arylaminooxazoles led to the 5-monosubstituted and 4,5-disubstituted 2-arylaminooxazoles as novel antagonists of the transient receptor potential vanilloid.[252]

Exploration of SAR in this chemical series revealed that the key to the high potency was the hydroxyl group at the 7-position of the tetrahydronaphthalene ring on the amino group of the oxazole nucleus and a *para*-substitution on the phenyl group at the 5-position of the oxazole was preferred. Within this series, compound **71** (hTRPV1 IC_{50} = 3.2 nM, Fig. 13), was resolved and the *R*-enantiomer with the better pharmacokinetic profile was orally active in animal models of pain, exhibiting a statistically significant ($p < 0.01$) 52% increase in the paw withdrawal latency in the rat

carrageenan hotbox model of thermal hyperalgesia at 10 μmol/kg po, and an ED_{50} of 14 μmol/kg (95% CI, 11–20 μmolkg po) in the rat osteoarthritis model of chronic pain.

Non urea TRPV1 antagonists based on the atypical triazine scaffold were identified by Messeguer and coworkers.[253] This series that behaved as uncompetitive antagonists acting as open channel blockers are therapeutically attractive because of their detection of over-activated TRPV1 channels, which could reduce the potential of unwanted side effects.[254] Of this class, 2,4,6-trisubstitued-1,3,5-triazine **72** (Fig. 13) inhibited whole-cell currents from rat TRPV1 injected *Xenopus* oocytes with an IC_{50} of 50 nM, showing strong voltage dependency (IC_{50} = 50 nM).[253]

Fig. 13

M. Biarylcarboxamide

PharmEste srl disclosed in a patent publication the biarylcarboxyarylamide series as a novel chemotype of TRPV1 receptor antagonist. The SAR of new derivatives designated the N-(4-chlorophenyl)-6-(isoquinolin-5-yl)pyridine-3-carboxamide **73** (V394, Fig. 14) as the most preferred compound. In radioligand binding assay, the saturation curve of [^{3}H]-RTX to TRPV1 expressed in rat spinal cord showed a K_D value of 0.21 nM and a B_{max} value of 57 fmol/mg protein. The IC_{50} value of V394 that inhibited capsaicin-evoked Ca^{2+} mobilization was 0.83 nM. In the capsaicin-induced secondary allodynia in rat, V394 produced an important preventive effect (55%).[255]

O. Acrylamide

PharmEste srl published the isoquinolineacrylamide bearing an ionoinc substructure as TRPV1 antagonists.[256] Structural modifications on the 3 position of cyclohexen moiety produced the (2*E*)-3-(3-(4-chlorophenoxy)-2,6,6-trimethylcyclohex-1-enyl)-N-(isoquinolin-5-yl)acrylamide **74** (Fig. 14)**,** exhibiting a significant preventive effect (54%) against the pro-allodinic effect of capsaicine (K_i = 4.6 nM).

AmorePacific Corporation, through the synthesis of over 2,000 new compounds has discovered

a novel class of non-vanilloid TRPV1 antagonists. Among them, the pyridine-3-yl-acrylamide **75** (PAC-14028**,** Fig. 14) showed important efficacies against varied disease models that include visceral pain, inflammatory bowel disease, and inflammatory pain. In particular, PAC-14028 has been reported as a new drug for atopic dermatitis and pruritus.[257] It has completed a Phase II clinical study for Skin Pruritus (ClinicalTrials.gov Identifier: NCT02052531).

N. Dienamide

Fuji Research Park has developed a chemical series of 5,5-diphenylpentadienamides for targeting TRPV1 in vitro and in vivo.[258] In this study, they investigated a variety of replacements for the 5-position of dienamides with the goal of improving the related pharmacokinetics. The SAR analysis suggested that substitution with alkoxy groups on the phenyl ring at the 5-position increased the ability to penetrate the blood−brain barrier. This investigation concluded in the discovery of compound **76** (Fig. 14), which showed a good pharmacokinetic profile. The *R*-/*S*-enantiomers of **76** were prepared by introduction of the optically active 5-amino-3-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline that was obtained from a diastereomer salt resolution using optically active tartaric acid as the resolving reagent.

The functional antagonist activity IC_{50} values for **76**, based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells, were 0.14 nM and 0.35 nM, respectively. This compound was found to be effective at reversing mechanical allodynia in rats in a dose-dependent manner, and it reversed thermal hyperalgesia in a model of neuropathic pain induced by sciatic nerve injury.

Fig. 14

P. Benzoimidazole

Very recently, Researchers in the Janssen R&D designed a series of benzo[*d*]imidazole TRPV1 antagonists from the biarylamide scaffold **77** (Fig. 15). Evaluation of the SAR and optimization of the new TRPV1 pharmacophore led to the identification of benzoimidazole **78** (named

Mavatrep/JNJ-39439335, Fig. 15), bearing a trifluoromethylphenylvinyl chain in 5 poistion.[259] Mavatrep showed high affinity against TRPV1 receptor and exhibited potent in vitro functional activity and robust oral efficacy in multiple models of inflammatory pain at relatively low plasma levels. Mavatrep is an orally bioavailable hTRPV1 antagonist (K_i = 6.5 nM) that exhibited minimal effect on the enzymatic activity (IC_{50} > 25 μM) of CYP isoforms 3A4, 1A2, and 2D6.

Fig. 15

5. CLINICAL TRIALS

The therapeutic value of TRPV1 antagonists has been investigated and numerous compounds are now in clinical trial for different pathologies.

The TRPV1 antagonist ABT-102 (Fig. 3) entered phase I clinical studies for pain-associated with inflammation, tissue injury and ischemia. The antagonist was well tolerated with repeated dosing and increased the mean core body temperature by 0.6°C in healthy volunteers after short-term administration.[260] By day 7, temperature increases were no longer significant for any dose tested. ABT-102 increased cutaneous and oral heat pain thresholds and the deficit in noxious heat perception did not attenuate with the 7-day.[260] However, the last update for ABT-102 in the clinicaltrials.gov website was in 2010 and no study results have been posted.

AZD1386 (Fig 16) entered phase II for potential oral treatment of OA, dental and GERD pain. Even if in phase II trial in subjects with dental pain AZD1386 was effective in reducing pain after third molar extraction,[139] the drug did not significantly reduce pain in patients with OA or GERD.[139] Therefore, the phase II clinical trial with AZD1386 was terminated for lack of analgesic efficacy.[127]

SB705498 was studied in different clinical studies for dermatitis atopic (phase I), rhinitis, migraine, cough, dental pain after tooth extraction, and irritable colon (phase II). SB705498 did not induce any serious adverse effects in preliminary human studies.[261] Results have not been revealed until now.

AMG517 (Fig 16) entered a phase Ib dental pain (molar extraction) study but was discontinued because it induced a strong hyperthermia (up to 40.2°C) in human volunteers.[81]

A phase II trial with GRC-6211 (Fig 16) for OA pain was suspended due to undisclosed reasons.[136]

MK-2295 (Fig 16) has been developed for the potential treatment of pain and cough. In Phase II this drug significantly raised the threshold to heat pain in humans but induced hyperthermia.[262]

The TRPV1 antagonist PHE377 (Fig 16) has completed a phase I clinical trial aimed to treat diabetic neuropathic pain and post herpetic neuralgia but the results have not been disclosed and now it has been withdrawn.

JTS-653(Fig 16) has been studied for the potential treatment of pain and overactive bladder in a phase II study.

XEN-D0501 (Fig 16) entered phase II development for the indications of overactive bladder and chronic cough. The TRPV1antagonist was well tolerated and induced only mild hyperthermia (0.74°C) at the highest dose tested.[263]

PAC-14028 (Fig 14) entered in phase I clinical trials for cumulative skin irritation, and in phase II for patients with skin pruritus, rosacea, atopic dermatitis after a successful completion of preclinical studies.[264]

DWP-05195 is now in Phase II for neuropathic pain and post-herpetic neuralgia treatment as oral administration. No study results have been disclosed.

JNJ-39439335 entered in phase I clinical trials to evaluate the relief of paradoxical pain induced by a thermal grill experimental model and the efficacy to treat patients with chronic osteoarthritis pain of the knee. No data results have been posted.

SYL-1001 has been studied in clinical trials of phase II for ocular pain associated with dry eye syndrome. No study results have been disclosed.

NEO 6860 is currently under study in phase II for the treatment of osteoarthritic pain.

Fig. 16

6. Conclusions

The first recorded report describing evidence for a heat-activated ion channel in the pain pathway originates from 1997. Now, almost twenty years later, many ligands that selectively target these receptors have been developed and have enabled researchers to identify potential therapeutic areas for drug development. In particular, the transient receptor potential vanilloid subtype ion channel TRPV1 has been functionally linked to many pathophysiological states in preclinical studies.

First of all, TRPV1 is considered as a highly validated pain target because *i)* its agonists such as capsaicin cause desensitization of TRPV1 channels that reduce pain in preclinical species, and *ii)* its antagonists relieve pain behaviors in rodent models of inflammation, osteoarthritis, and cancer. Hence, modulators of TRPV1 channels are potentially useful in the treatment of inflammatory and neuropathic pain in clinical trials. However, despite the widespread clinical use and success of the TRPV1 agonist capsaicin, for instance, in the local treatment of postherpetic neuralgia, any TRPV1 antagonist is now available in therapy. TRPV1 antagonism caused hyperthermia as a dose-limiting adverse effect that hampers therapeutic utility. Not least, the blockade of physiological functions mediated by these important proteins may account for the difficulty in the validation of an antagonist as a drug. Nevertheless, pharmaceutical companies have invested millions of dollars for drug screening and lead optimization programs that have identified selective and potent TRPV1 antagonists, many of which are undergoing clinical trials as analgesic drugs.

While a primary area of interest is the role of this channel in mediating pain, a number of researchers are studying the ability of agonists or antagonists of TRPV1to relieve symptoms of other important diseases. In particular, TRPV1 role in metabolism, diabetes, insulin resistance and obesity, urinary incontinence, cough, arthritis and hearing loss has to be elucidated.

7. ABBREVIATIONS

AKAP150 = A-Kinase Anchoring Protein 79/150
CaMKII = calmodulin kinase II
Cdk5 = cyclin-dependent kinase 5
CFA = complete Freund's adjuvant
CGRP = Calcitonin gene related peptide
CNS = central nervous system
COPD = chronic obstructive pulmonary disease
DRGs = dorsal root ganglia
GERD = gastro-esophageal reflux of gastric contents
GI = gastrointestinal
GLP-1= glucagon-like peptide-1
GPCR = G-protein coupled receptor
HRV = human rhinovirus
hTRPV1= human TRPV1
HTS high-throughput screening
IBD = inflammatory bowel disease
IL = interleukin
KO = knockout
MIA = intra-articular monoiodoacetate
NGF = nerve growth factor
NMDG = *N*-methyl-d-glucamine
OA = osteoarthritis
PIP2 = phosphoinositide 4,5-biphosphate
PKA = protein kinase A
PKC = protein kinase C
rTRPV1= rat TRPV1
RTX = Resiniferatoxin
SARs = structure–activity relationships
SNARE = N-ethylmaleimide-sensitive factor attachment protein receptor
SNPs = single point mutations
TG = trigeminal ganglia
TrKA = tropomyosin receptor kinase A
TRPV1= Transient receptor potential vanilloid 1
VR1= vanilloid receptor

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Figure Captions

Figure 1. *Schematic diagram that shows key structural features of TRPV1 receptors. Six transmembrane domains TM constitute TRPV1 receptors with a pore region located between TM5 and TM6 domains and N- and C- long and intracellular terminals. Six ankirin repeat domains are contained in the N-terminal tail allowing binding of calmodulin (CaM) and ATP. A TRP domain is present in the C-terminal tail in addition to the binding sites for PIP2 and CaM. Putative sites for capsaicin and protons are shown.*

Figure 2. *TRPV1 Agonists (**1, 2**), TRPV1 Antagonist (**3**).*

Figure 3. *Phenyl-/Benzyl- Urea TRPV1 antagonists.*

Figure 4. *TRPV1 antagonists (Dibenzyl Ureas **12**, **13**), TRPV1 agonists (Dibenzyl Thioureas **14**, **15**), TRPV1 antgonists (Dibenzyl Thioureas **16**, **17**), TRPV1 antgonists (α-Methyl Amides **18-20**).*

Figure 5. *SAR optimization of Chroman and Tetrahydroquinoline Urea TRPV1 antagonists.**Figure 6.** Piperazine Ureas (**29-30**), related Pyrimido[4,5-d]azepines (**32**, **33**) and, Tetrahydropyridine (**34**).*

Figure 6. *Piperazine Ureas (**29-30**), related Pyrimido[4,5-d]azepines (**32**, **33**) and, Tetrahydropyridine (**34**).*

Figure 7. *SAR optimization of Pyrazole Carboxamide and Pyrazole Ureas (**35-43**) leading to Pyridinyl- Propanamide/Ureas (**44**, **45**) as TRPV1 antagonists.*

Figure 8. *Pyridine Propanamide and Pyridine Urea TRPV1 antagonists.*

Figure 9. *Isoxazole Carboxamide TRPV1 antagonists.*

Figure 10. *Piperidine Carboxamides TRPV1 antagonists.*

Figure 11. *Pyrazolopyridine-3-carboxamide (**62**), Imidazopyridine-3-carboxamides (**63-65**), and Pyrrolopyridazine (**66**).*

Figure 12. *Benzothiazol-5-carboxamides (**67**, **68**), Benzothiazol-2-carboxamides (**69**, **70**).*

Figure 13. *Oxazole (**71**) and Triazine (**72**) TRPV1 antagonists.*

Figure 14. *Biarylcarboxyarylamide (**73**), Acrylamides (**74**, **75**), and Dienamide (**76**) TRPV1 antagonists.*

Figure 15. *SAR optimization of biarylamide (**77**) leading to benzoimidazole (**78**).*

Figure 16. *Selected TRPV1 antagonists in clinical development.*

Figure 1. *Key structural features of TRPV1 receptors.*

Schematic diagram that shows key structural features of TRPV1 receptors. Six transmembrane domains TM constitute TRPV1 receptors with a pore region located between TM5 and TM6 domains and N- and C- long and intracellular terminals. Six ankirin repeat domains are contained in the N-terminal tail allowing binding of calmodulin (CaM) and ATP. A TRP domain is present in the C-terminal tail in addition to the binding sites for PIP2 and CaM. Putative sites for capsaicin and protons are shown.

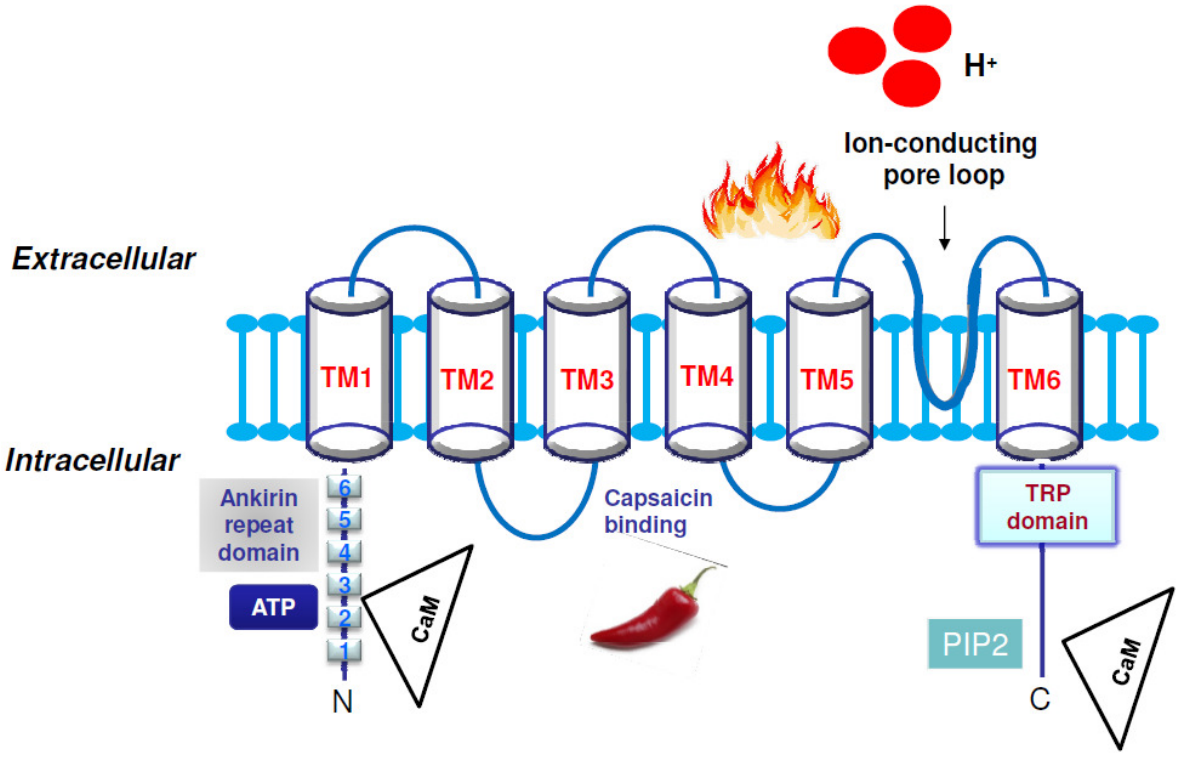
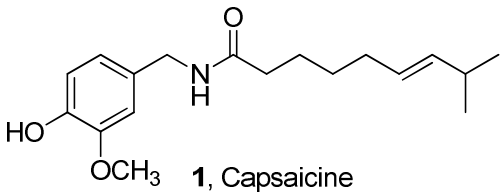
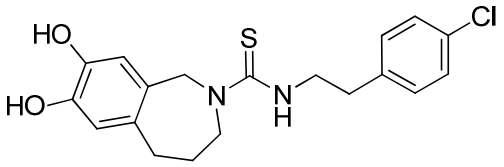


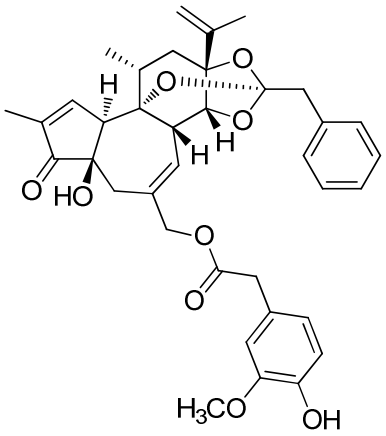
Figure 2. *TRPV1 Agonists (**1, 2**), TRPV1 Antagonist (**3**).*



1, Capsaicine

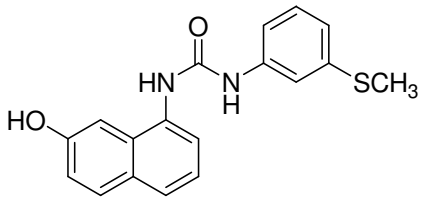


3, Capsazepine



2, Resiniferatoxin

Figure 3. *Phenyl-/Benzyl- Urea TRPV1 antagonists.*



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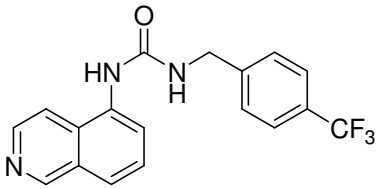

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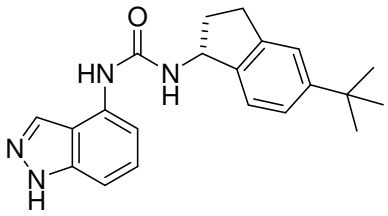
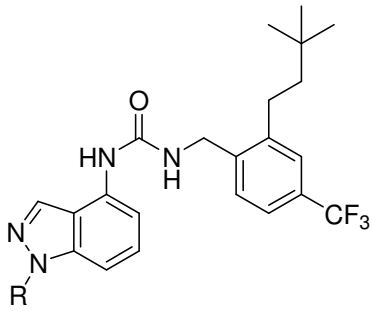
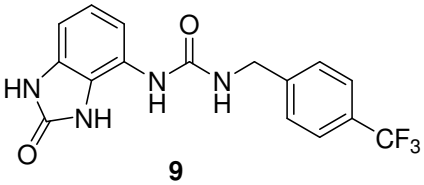


6 (R)**,** ABT-102



7, R = H
8, (ABT-116), R = CH_3



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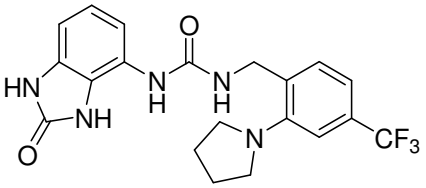

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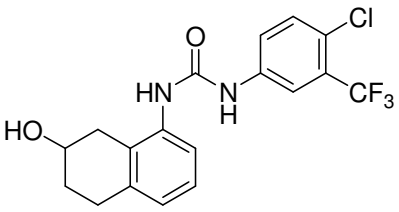

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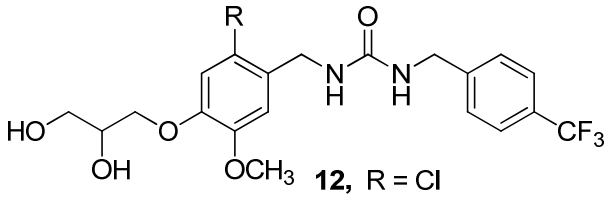
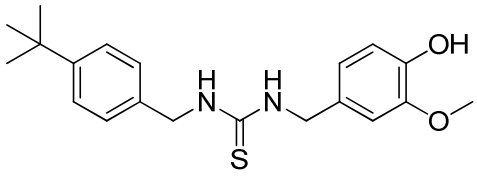
Figure 4. *TRPV1 antagonists (Dibenzyl Ureas* ***12***, ***13****), TRPV1 agonists (Dibenzyl Thioureas* ***14***, ***15****), TRPV1 antgonists (Dibenzyl Thioureas* ***16***, ***17****), TRPV1 antgonists (α-Methyl Amides* ***18-20****).*



12, R = Cl
13, R = Br



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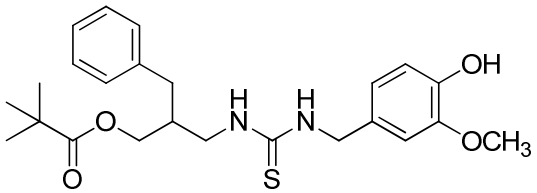

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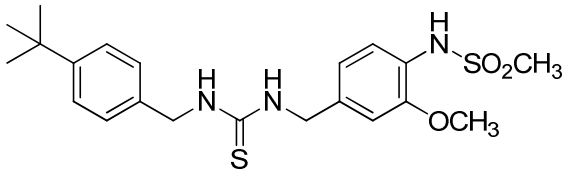

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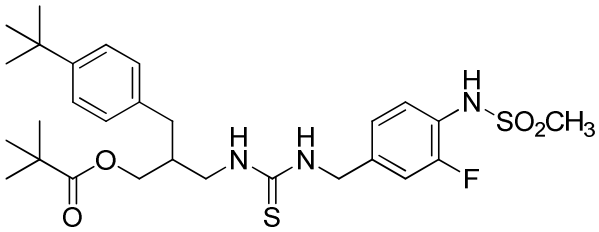
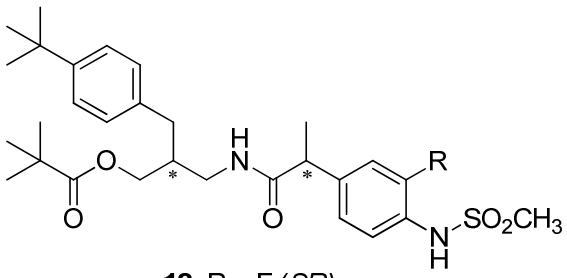
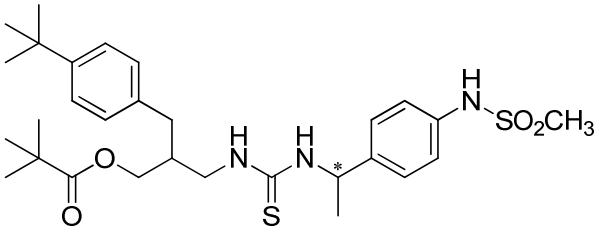


17



18, R = F (*SR)*
19, R = Cl (*SR*)



20 (*R*)

Figure 5. *SAR optimization of Chroman and Tetrahydroquinoline Urea TRPV1 antagonists.*

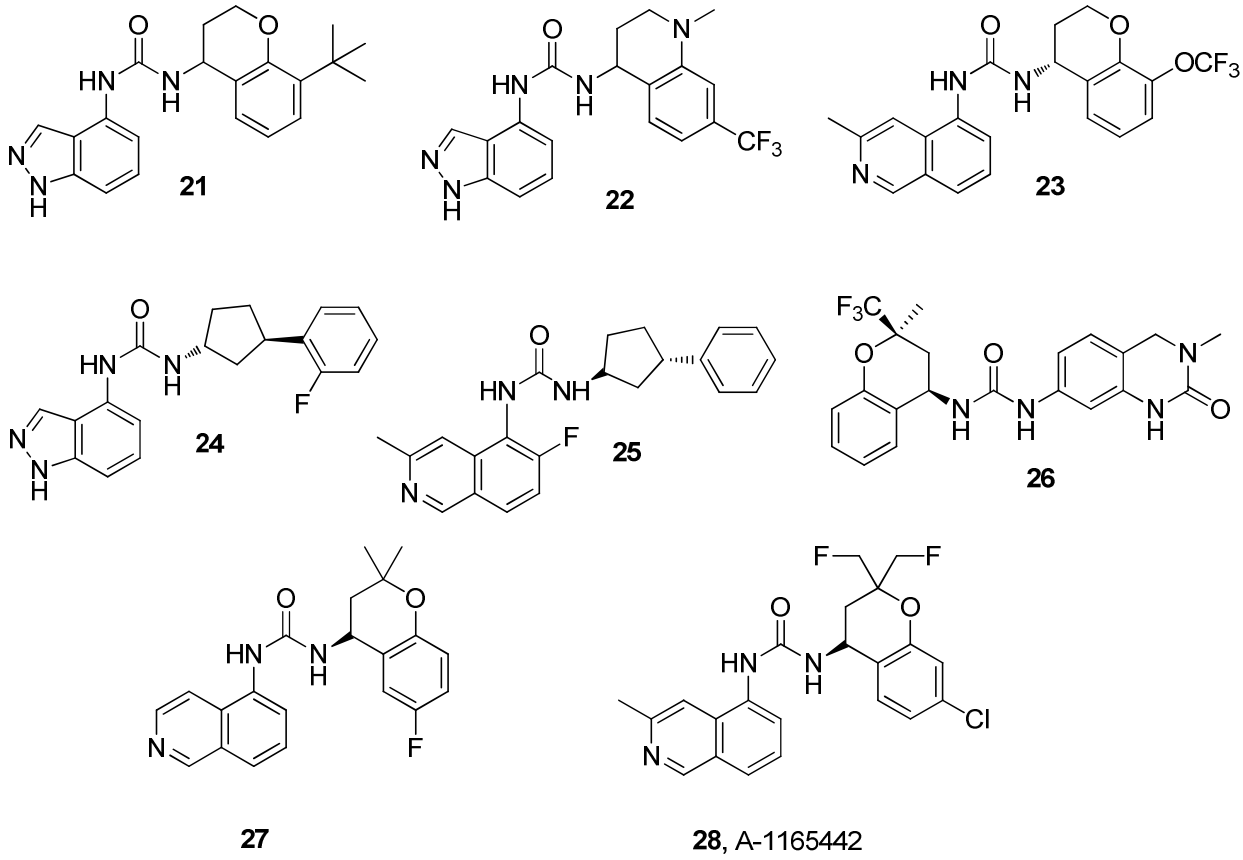


Figure 6. *Piperazine Ureas (**29-30**), related Pyrimido[4,5-d]azepines (**32**, **33**) and, Tetrahydropyridine (**34**).*

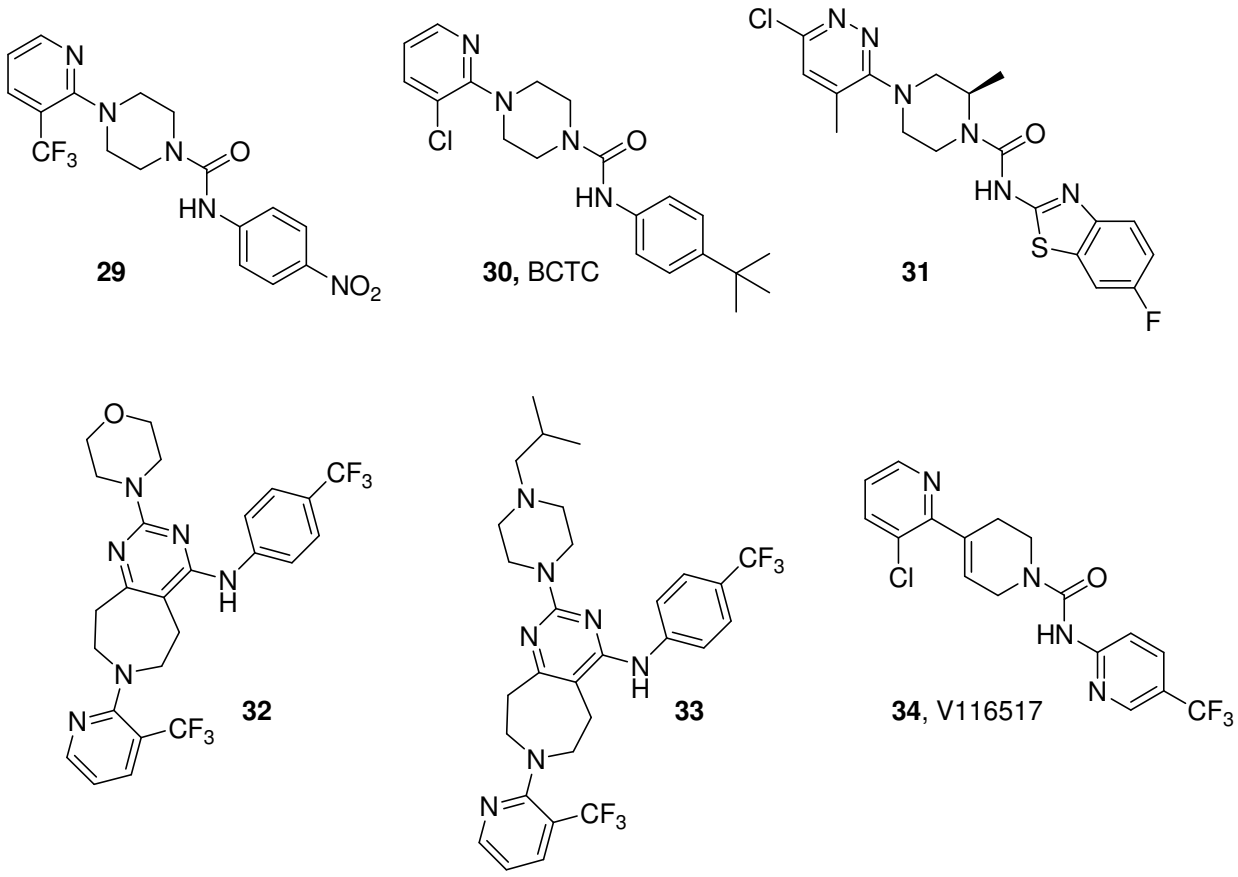
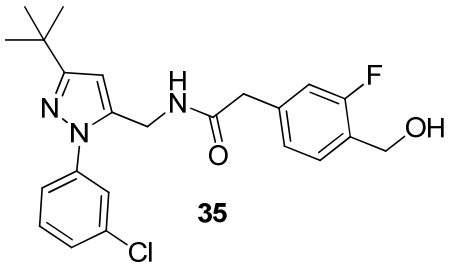
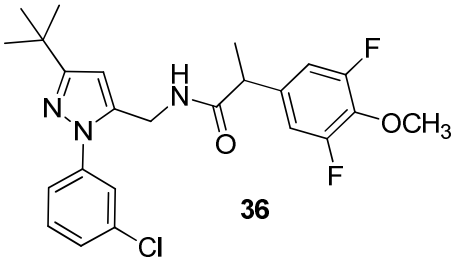


Figure 7. *SAR optimization of Pyrazole Carboxamide and Pyrazole Ureas (**35-43**) leading to Pyridinyl- Propanamide/Ureas (**44**, **45**) as TRPV1 antagonists.*



35



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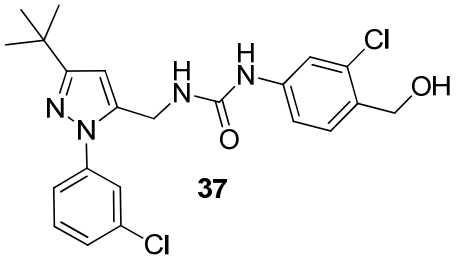

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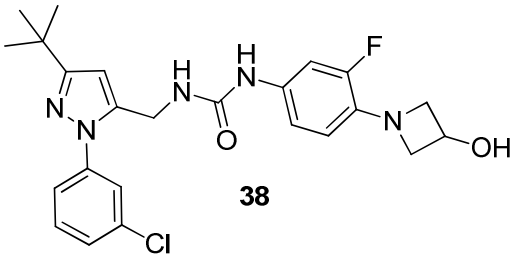

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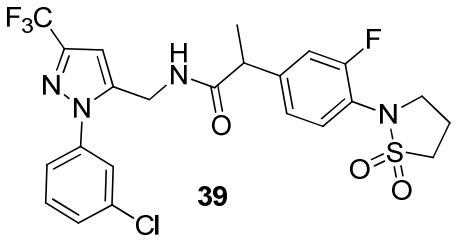

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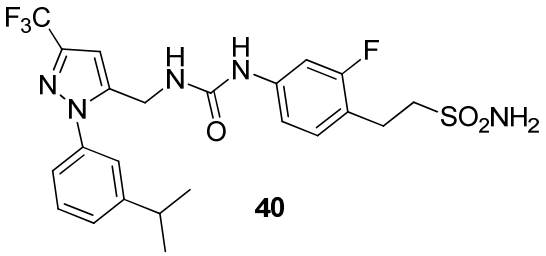

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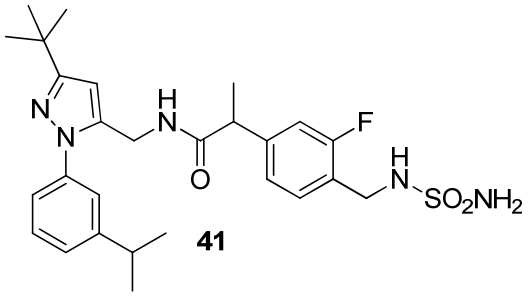

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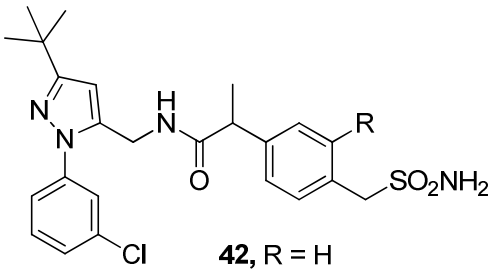
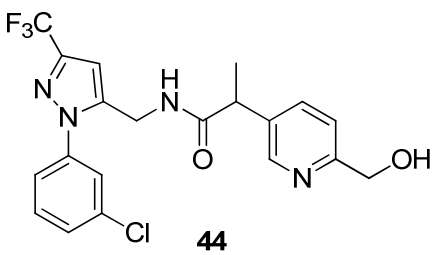


42, R = H
43, R = CH_3



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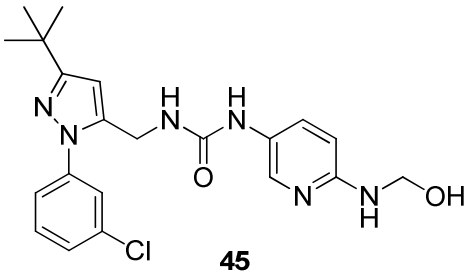
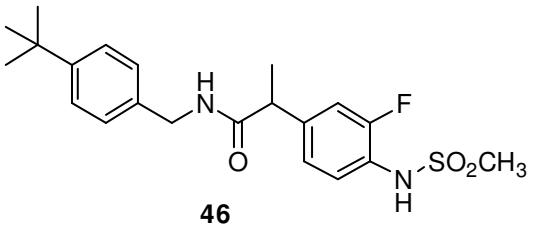


45

Figure 8. *Pyridine Propanamide and Pyridine Urea TRPV1 antagonists.*



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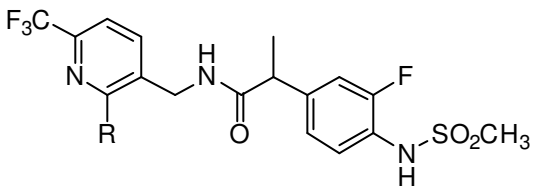
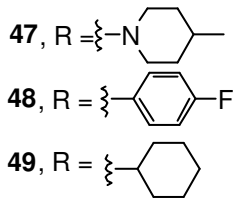
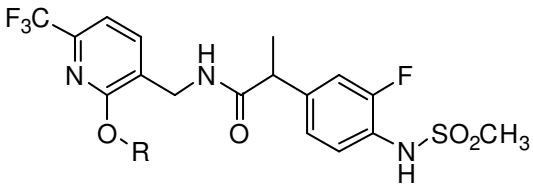


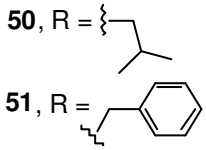


47, R =

48, R =

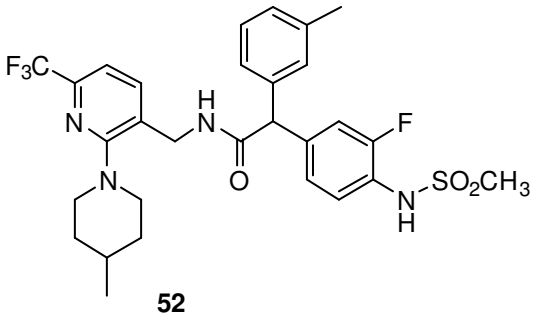
49, R =



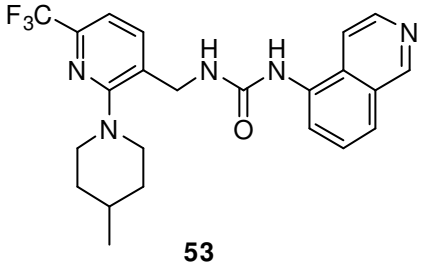


50, R =

51, R =

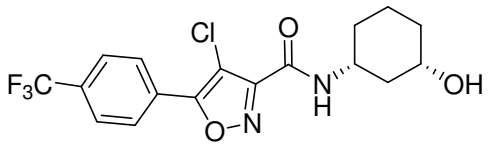


52



53

Figure 9. *Isoxazole Carboxamide TRPV1 antagonists.*



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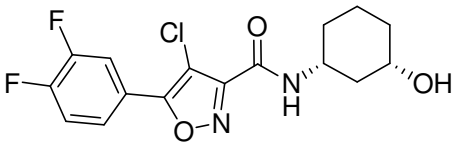

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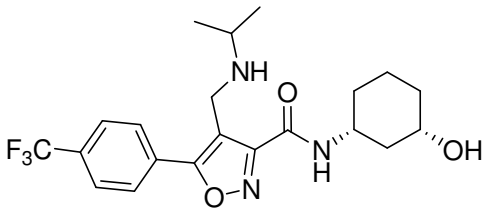
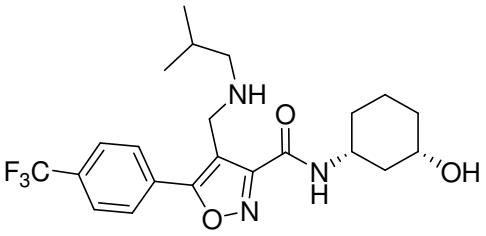
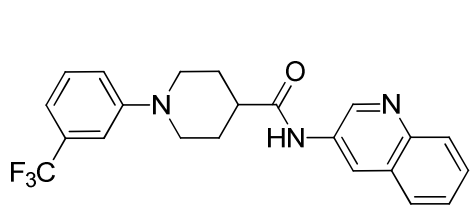


56



57

Figure 10. *Piperidine Carboxamides TRPV1 antagonists.*



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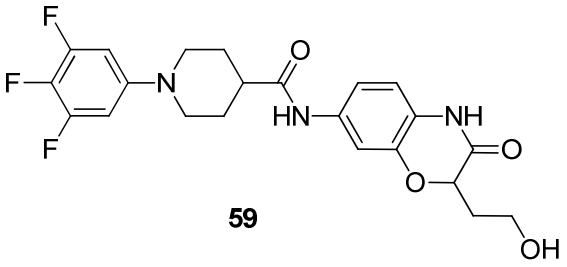

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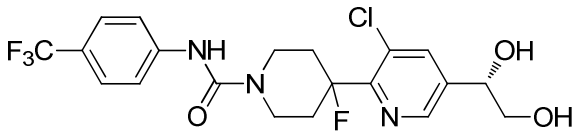

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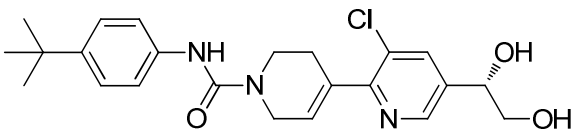


61

Figure 11. *Pyrazolopyridine-3-carboxamide (**62**), Imidazopyridine-3-carboxamides (**63-65**), and Pyrrolopyridazine (**66**).*

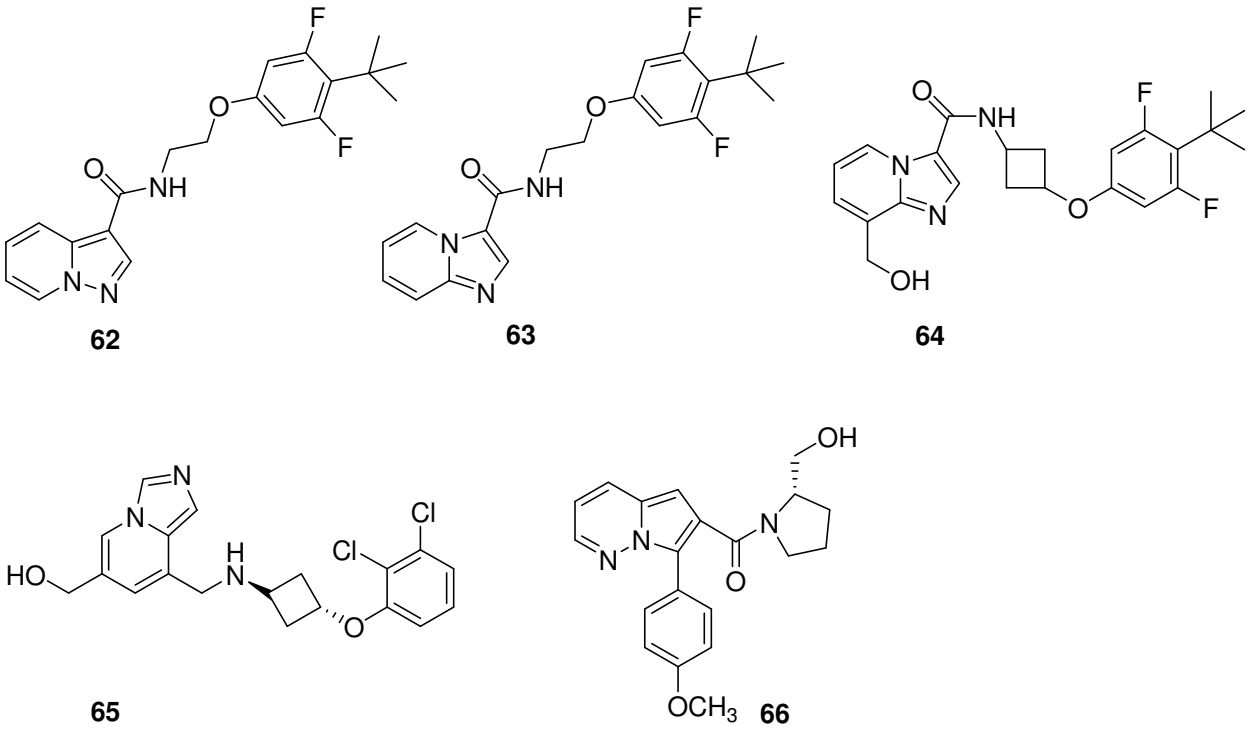
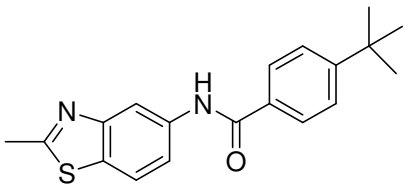
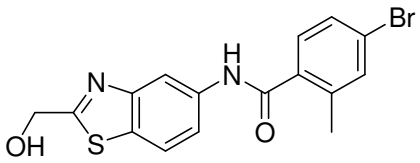


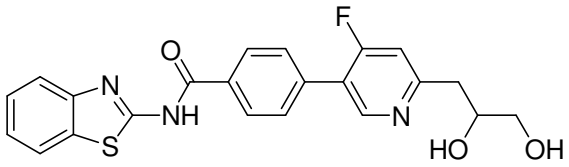
Figure 12. *Benzothiazol-5-carboxamides (**67**, **68**), Benzothiazol-2-carboxamides (**69**, **70**).*



67



68



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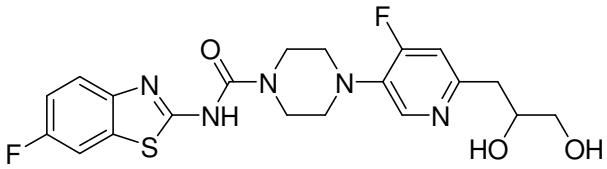
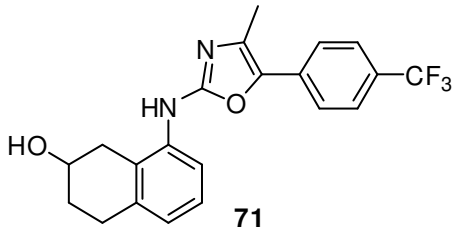
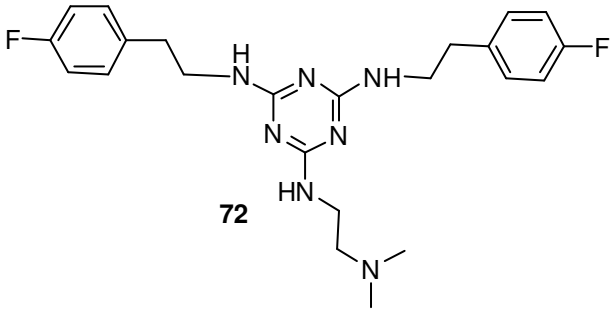


70

Figure 13. *Oxazole (**71**) and Triazine (**72**) TRPV1 antagonists.*

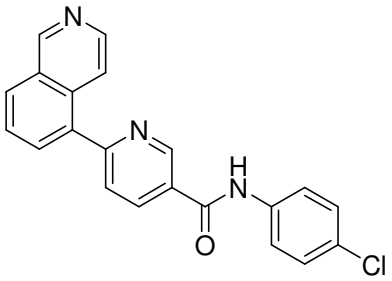


71

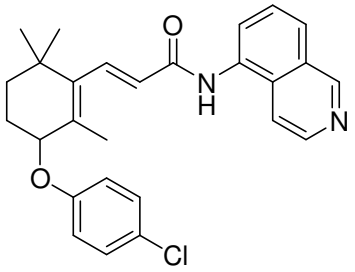


72

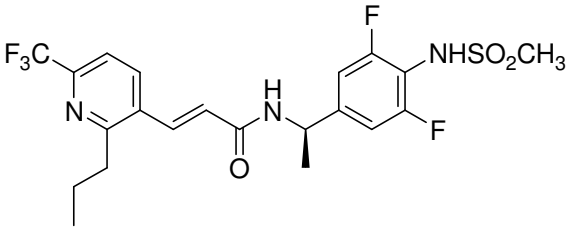
Figure 14. *Biarylcarboxyarylamide (**73**), Acrylamides (**74**, **75**), and Dienamide (**76**) TRPV1 antagonists.*



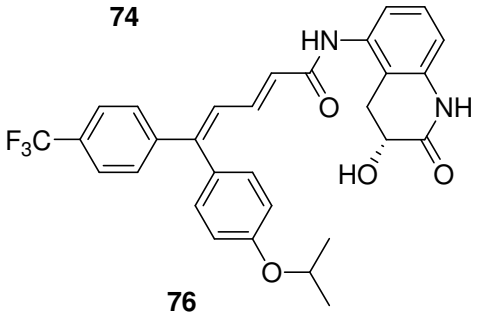
73, V394



74

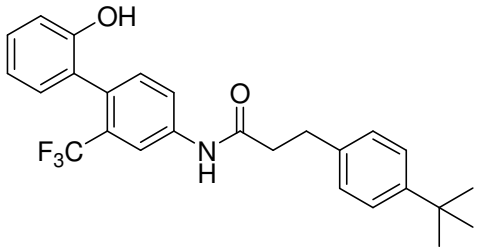


75, PAC-14028



76

Figure 15. *SAR optimization of biarylamide (**77**) leading to benzoimidazole (**78**).*



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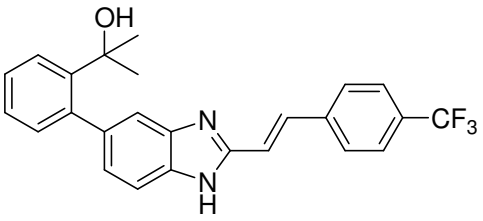

78, Mavatrep, JNJ39439335

Figure 16. *Selected TRPV1 antagonists in clinical development.*