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Transient Receptor Potential Ankyrin 1 (TRPA1) Channel as Emerging Target for Novel Analgesics and Anti-Inflammatory Agents

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1. Introduction

1.a. TRP Channels and TRPA1. The identification of the first transient receptor potential (TRP[a]) channel originated from the observation that, in contrast to the sustained light-induced current (LIC) of wild-type flies, a *Drosophila* mutant displayed a transient LIC in response to light. The TRP protein encoded by the *trp* gene forms all, or part, of a Ca^{2+} influx channel associated with the transient LIC. At present, the superfamily of the TRP channels in mammals encompasses 28 different proteins with pleitropic functions in the vast majority of cells and tissues. The TRP ankyrin 1 (TRPA1), first cloned from human fetal lung fibroblasts,[1] is the sole representative of the A subfamily, whereas the C (canonical), M (melastatin), V (vanilloid), P (polycystin), and ML (mucolipin) subfamilies include from three to eight members. Like the other TRP channels, the TRPA1 subunit consists of six putative transmembrane spanning segments (S1−6), a pore-forming loop between S5 and S6, and intracellularly located NH_2 and COOH termini. Channel subunits may assemble as homo- or heterotetramers, resulting in the formation of cation-selective channels (for further details, see refs 2−4). TRPs are nonselective Ca^{2+}-permeable cation channels, but the permeability ratio (Ca^{2+}/Na^{+}) varies markedly between different members of the superfamily and also among the individual members of each subfamily. Activation of some TRP members may result from the stimulation of either diacylglycerol (DAG)-dependent or -independent pathways that follows G-protein-coupled receptor and phospholipase C (PLC) activation.[5] However, TRP gating may also be caused by physicochemical stimuli, which act independently from receptor stimulation.[6] Some TRP channels, e.g., TRPC, have been initially proposed as the long-sought store operated channels. However, after detailed experimental scrutiny, this hypothesis has not been further confirmed,[2] whereas specific cell- and tissue-dependent functions for each individual TRP are emerging. TRPA1 exhibits 14 NH_2-terminal ankyrin repeats,[7] an unusual structural feature, from which the channel derives its name.[8,9] TRPA1 is abundantly expressed in dorsal root (DRG), vagal (VG), and trigeminal ganglion (TG) neurons and in hair cells.[7,9−11] TRPA1 has been considered as a suitable candidate for the mechanosensitive transduction of vertebrate hair cells and for the hearing function.[9,11]However, this role has not been confirmed by studies[12,13] conducted with TRPA1 null mice, which revealed no evident deficit in auditory function. Thus, these findings exclude that TRPA1 represents an essential component of the transduction mechanism in hearing.[12−14] In contrast, localization of TRPA1 to somatosensory neurons is offering increasing proof for a primary role of the channel in nociceptive transduction and neurogenic inflammation.

Calcium plays a major role in TRPA1 functioning because it not only acts as a permeant ion but also controls activation[15] and desensitization of TRPA1.[16] A recent study[17] provided evidence that TRPA1 has a wide pore that allows a much larger calcium flux than predicted and that regulation of calcium permeability and diameter of the TRPA1 pore is stimulus dependent. Thus, calcium largely contributes to the inward TRPA1 current in a dynamic fashion. On the other hand, TRPA1 may represent an important determinant of the calcium signal in nociceptors, thereby regulating various calcium-dependent processes, including TRPA1 activation and inactivation, TRPV1 desensitization, and neurotransmitter release.

1.b. Primary Sensory Neurons and TRPA1. The heterogeneous population of primary sensory neurons can be differentiated according to electrophysiological, morphological, neurochemical, and other criteria. According to the conduction velocity of the action potential, peripheral afferent and efferent neurons have been divided in those with the unmyelinated slowest conducting fibers, designated C, and the

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[a] Abbreviations: ALE, advanced lipoperoxidation end-products; CFA, complete Freund's adjuvant; CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary; CN, 1-chloroacetophenone; COPD, chronic obstructive pulmonary disease; CQ, clioquinol; CS, 2-chlorobenzylidene malononitrile: DAG, diacylglycerol; DRG, dorsal root ganglion; ESI-MS, electrospray ionization mass spectrometry; FAAH, fatty acid amide hydrolase; FLIPR, fluorescent imaging plate reader; GAs, general anesthetics; HαSS, α-hydroxysanshool; Hβ SS, β-hydroxysanshool; HEK cells, human embryonic kidney cells; LIC, light-induced current; NKA, neurokinin A; PAR2, proteinase activated receptor-2; PG, prostaglandin; PIP_2, phosphatidylinositol bisphosphate; PKA, protein kinase A; PLC, phospholipase C; RCS, reactive carbonyl species; RNS, reactive nitrogen species; ROS, reactive oxygen species; RR, ruthenium red; SNL, spinal nerve ligation; SP, substance P; TCEB, trichloro(sulfanyl)ethylbenzamides; TG, trigeminal ganglion; THC, Δ^9-tetrahydrocannabinol; TRP, transient receptor potential; TRPA1, transient receptor potential ankyrin 1; TRPM, transient receptor potential melastatin; TRPML, transient receptor potential mucolipin; TRPP, transient receptor potential polycystin; TRPV, transient receptor potential vanilloid; VG, vagal ganglion; VGAs, volatile general anesthetics.

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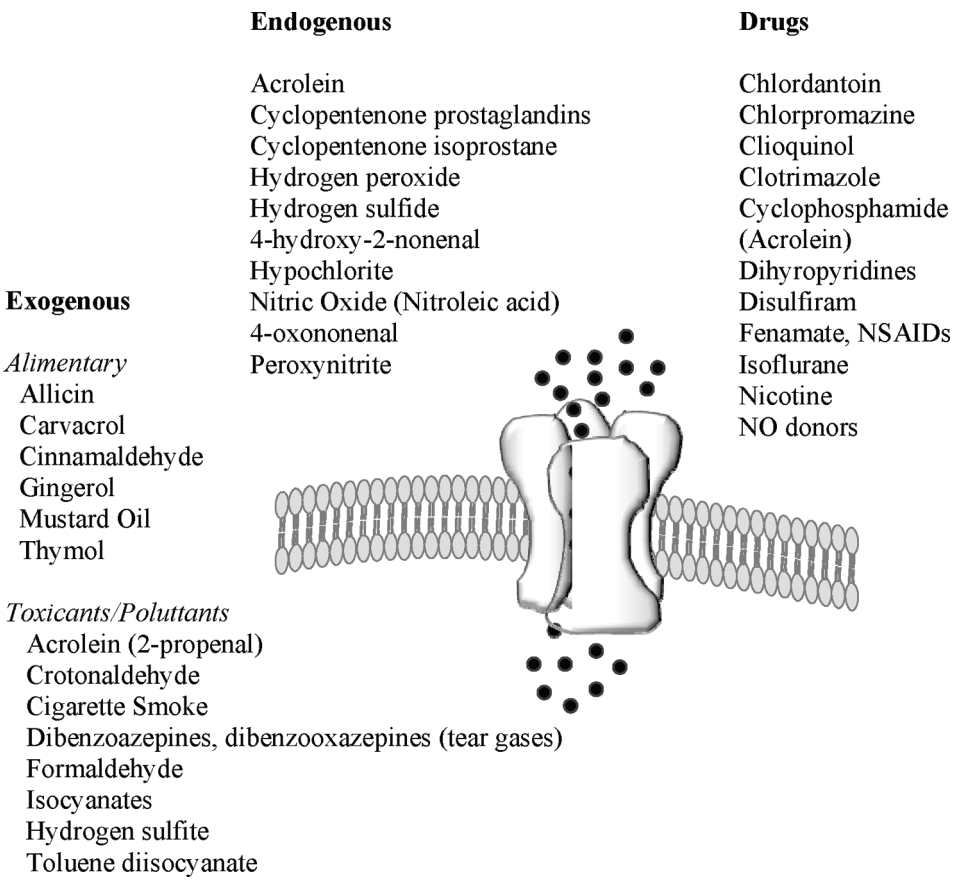


Figure 1. The TRPA1 channel is gated by a host of exogenous or endogenously produced agents, thereby evoking influx of cations, including calcium (black dots) and sensory nerve activation. A number of drugs have been also reported to activate TRPA1.

faster conducting myelinated fibers, designated Aδ, Aβ, and Aα. There is a general consensus that only the smallest diameter and slowest conducting nerve fibers, the C- and Aδ-fibers, carry from nociceptors the afferent signal, which in humans is perceived as pain. However, A-fiber nociceptors that conduct in the Aβ conduction velocity range also seem to be involved in pain transmission.[18,19] Thus, both unmyelinated C-fibers and myelinated A-fibers conducting in the A(δ,β) velocity range sense and transmit nociceptive signals. In addition they also signal for non-noxious innocuous mechanical, warm, and cold stimuli. Finally, it should be considered that not all C-fibers and A(δ,β) fibers are nociceptors. Nociceptive neurons can also be classified according to their differential expression of membrane channels. TRPV1 expressing neurons (sensitive to heat and low extracellular pH) also express the TRPA1 (sensitive to a host of chemical irritants), whereas TRPM8 (sensitive to cold) is expressed by a different neuronal subpopulation.[7] Additional criteria to conduction velocity and channel expression result in an increasing complexity and subdivision of the different neuronal categories. Nevertheless, there is emerging and robust evidence that neurons that produce and release neuropeptides are those that express both the TRPV1 and TRPA1 channels on their plasma membranes (see below[20]), and TRPA1-positive neurons are a proportion of the TRPV1-positive subpopulation.[7] Hence, activators of TRPV1 and TRPA1 are capable of eliciting the functional consequences that result from neuropeptide release.

The calcitonin gene-related peptide (CGRP) and the tachykinins, substance P (SP) and neurokinin A (NKA), are the main peptide transmitters produced and transported to both central and peripheral endings of a subpopulation of neurons with C- and A(δ)-fibers. Ca^{2+}-dependent release of neuropetides from endings located to the dorsal spinal cord has been associated with nociceptive signaling, whereas the release from peripheral endings produces a series of pro-inflammatory responses collectively referred to as neurogenic inflammation.[21] Neurogenic inflammatory responses include vascular and nonvascular (bronchoconstriction, mucous gland secretion, positive inotropic and chronotropic cardiac effects, and other effects) responses. Neurons, which have the double ability to sense irritant or painful stimuli either of exogenous origin or produced endogenously under circumstances of inflammation or tissue injury and to simultaneously orchestrate an early, local, defensive response, have been designated as "the nocifensor system".[22] Although this pioneering proposal has encountered mixed fortune, it has recently been corroborated by preclinical and clinical findings that have assigned a role in migraine mechanism to neurogenic inflammation.[23] In fact, cutaneous vasodilatation by topical application of the selective TRPV1 agonist capsaicin in rhesus monkey forearm was blocked by telcagepant,[24] a CGRP receptor antagonist. More importantly, telcagepant[25] and another CGRP antagonist, olcegepant,[26] were reported to reduce the headache of migraine attacks. These observations propose a 2-fold hypothesis. First, CGRP is the mediator of neurogenic vasodilatation, a major component of the neurogenic inflammatory repertoire brought about by activation of peptidergic (and most likely TRPA1-expressing) primary sensory neurons in primates

and presumably in man.[27] Second, neurogenic and CGRP-driven vasodilatation in intracranial and extracranial arterial vessels plays a major role in generating the headache and the associated symptoms of a migraine attack. Theoretically, all TRPA1 agonists may release neuropeptides and by this mechanism may cause vascular responses, including plasma protein extravasation in postcapillary venules and neutrophil adhesion to the venular endothelium (both mediated by SP NK1 receptors in endothelial cells) and arterial and arteriolar vasodilatation (mediated by CGRP receptor in vascular smooth muscle cells). Failure of tachykinin NK1 receptor antagonists[28,29] and success of CGRP receptor antagonists[25,26] in clinical trials have assigned to the CGRP-mediated neurogenic vasodilatation, and not to SP-mediated plasma protein extravasation, a key role in neurogenic inflammation in humans and in migraine mechanism.

Soon after its cloning, the TRPA1 channel has been reported to be abundantly expressed in somatosensory neurons and to contribute to nociceptive responses evoked by a series of pungent agents often used in traditional medicine or as part of worldwide dietary habits, including cinnamaldehyde (contained in cinnamon),[30] allyl isothiocyanate (mustard oil, mustard, wasabi), allicin (garlic),[31,32] carvacrol, thymol, gingerol, and eugenol (contained in various pungent spices or vegetables).[33] By different functional and morphological criteria a more precise functional characterization of TRPA1 expressing neurons has been very recently obtained. By using radial stretch in combination with live-cell Ca^{2+} imaging different, mechanosensitive or -insensitive sensory neuronal categories have been identified.[20] A group of small-diameter stretch-sensitive cells could be further subdivided into a cluster of small-diameter cells sensitive to hydroxy-α-sanshool, a two pore K^+ channel antagonist contained in *Szechuan* pepper, and the TRPV1 agonist capsaicin, and a second one that comprises large-diameter cells that respond to hydroxy-α-sanshool but not to capsaicin. The former neuron type likely corresponds to a high threshold nociceptor subpopulation and the latter to a low threshold proprioceptor subpopulation. Moreover, stretch insensitive neurons fall into two groups of small-diameter cells. The first group is composed of peptidergic neurons sensitive to capsaicin and to the TRPA1 selective agonist mustard oil, and the second group is composed of a small cohort of menthol-sensitive cells.[20] Thus, TRPA1 expressing neurons that obligatorily coexpress TRPV1 are those apparently insensitive to mechanical stimulation and that, because they contain neuropeptides, bring about neurogenic inflammation (Figure 1).

1.c. Thermosensation. Alongside TRPV1, TRPA1, and TRPM8, other subclasses of somatosensory neurons express additional members of the TRPV family of channels, namely, TRPV2, TRPV3, and TRPV4. TRPV channels as a whole exhibit highly temperature-sensitive gating properties. The expression of most of these channels (TRPV1, TRPV2, TRPV3, and TRPV4) in the peripheral sensory system[27] underscores their primary role in sensing thermal stimuli, from mild to hot noxious temperatures. The unique role of these channels in thermosensation is challenged by more recent and increasing evidence. For example, the participation of TRPV2 in early phagocytosis by macrophages[34] highlights an unforeseen and fundamental function in innate immunity mediated by the TRPV2 channel, expressed in non-neuronal cells. On the other hand, TRPM8, expressed by a specific subpopulation of primary

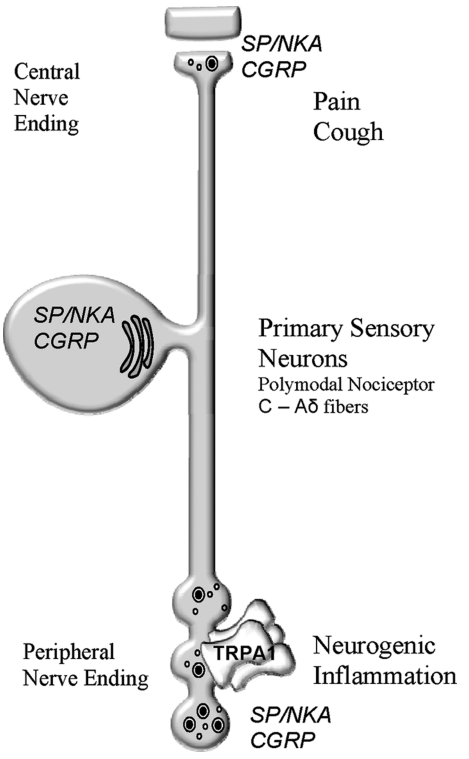


Figure 2. The TRPA1 is expressed by a subpopulation of peptidergic somatosensory neurons (that also express the TRPV1 channel). TRPA1 gating results in the release of the neuropeptides substance P (SP) and neurokinin A (NKA) and the calcitonin gene-related peptide (CGRP). Their release at the central nerve terminals is associated with pain transmission and the activation of reflex responses (cough), whereas when released from peripheral endings they produce neurogenic inflammation.

sensory neurons, is a channel activated by mild cold temperatures and the cooling sensation given by menthol is dependent on its ability to gate the TRPM8 channel.[30,35] If the role of TRPA1 as a sensor of noxious cold has been controversial, more recent findings propose that TRPA1 contributes to cold sensation. If TRPA1 expressed in heterologous systems was first activated at temperatures (~17 °C) close to the threshold of noxious cold for humans (15 °C),[7] the rat and human orthologues of TRPA1 were found to be insensitive to cold stimulation.[36] Negative results after cold activation were obtained in mouse TRPA1 channels heterologously expressed in human embryonic kidney (HEK293) cells,[9] whereas a subsequent study,[37] in a similar preparation, confirmed the original observation that TRPA1 responds to noxious cold. Conflicting results also extended to TRPA1 null mice. The effects of exposure to cold surface or to the evaporative cooling action of acetone were found similar[12] or significantly reduced[13] in TRPA1 null mice compared to wild type mice. These contradictory findings appeared to be resolved by subsequent work, where TRPA1 null mice were still able to sense cold but showed a reduced behavioral response to noxious cold.[38] Furthermore, mice in which NaV1.8-expressing sensory neurons were eliminated by diphtheria toxin A exhibited a strongly reduced expression of TRPA1 in DRG neurons and lacked TRPA1-mediated nociceptive responses to formalin and cold.[39] Thus, noxious cold sensing in vivo requires somatosensory neurons that express both NaV1.8 and TRPA1.

1.d. Mechanical Stimuli. TRPA1 has also been proposed to serve as a detector of mechanical stimuli, although this hypothesis remains highly controversial. The TRPA1 worm orthologue was found to be sensitive to mechanical pressure,[40] and heterologously expressed mammalian TRPA1 is activated by molecules causing a curvature of the membrane, which has been demonstrated to activate mechanosensitive ion channels.[41] In contrast with these encouraging data, conflicting results have been obtained in in vivo testing. In fact, TRPA1 null mice displayed only weak defects in mechanosensory behavior, and results in paradigms of mechanosensation were generally inconsistent.[12,42] However, a third study reported deficits in mechanical thresholds in TRPA1 null animals.[13] The role of TRPA1 in mechanosensation has been strengthened by a recent study that reported TRPA1 expression in epidermal and hair follicle keratinocytes as part of the mechanotransduction system.[43] In addition, C-fiber nociceptors from TRPA1 null mice exhibited action potential firing rates 50% lower than those in neurons from wild-type mice C-fibers across a wide range of force intensities. Surprisingly Aδ-fiber mechanonociceptors from TRPA1 null mice had reduced firing and Aβ and D-hair mechanoreceptive fibers were also altered.[43] Current available data do not support an established, primary role for TRPA1 in mechanosensation and in mechanical allodynia and hyperalgesia but suggest that TRPA1 modulates in a subtle way the excitability of mechanosensitive afferents.

1.e. Chemical Irritants. Much more robust and conclusive evidence has been accumulated in the past 5 years with respect to the ability of TRPA1 to respond to a host of environmental irritants (Figure 2). In fact, TRPA1 is gated by structurally diverse compounds that share the common and unique ability to form covalent adducts with thiol groups. This long list includes, in addition to allyl isothiocyanate and cinnamaldehyde, many other molecules, all highly reactive compounds that activate TRPA1 by covalently modifying cysteine residues within the amino terminal cytoplasmic domain of the channel.[44,45] Identification of the importance of thiol reactivity via Michael addition (see further sections of this review) has proposed TRPA1 as a general sensor for an array of chemically diverse environmental toxicants. These include acrolein (2-propenal), a highly reactive α,β-unsaturated aldehyde present in tear gas, vehicle exhaust, or smoke from burning vegetation,[12] and other volatile irritants such as hypochlorite, hydrogen peroxide, formalin, and isocyanates.[46−48]

Most of the TRPA1 activators are thiol-reactive electrophiles that covalently bind with cysteine and lysine residues in the cytosolic part of the channel. Of the three cysteines found to be responsible for human TRPA1 activation,[44] only one, C619, corresponds to those identified in the mouse channel (C415, C422, and C622).[49] Covalent modification of a lysine side chain also contributes to the residual response found in cysteine mutated channel.[44] Data obtained by point mutagenesis experiments strongly suggest that the pore region of TRPA1 is involved in the coupling between the covalent binding of electrophilic compounds and channel gating.[50] Other TRPA1 agonists, which are not nonelectrophilic, do not covalently bind TRPA1 cysteine residues because their chemical structures do not support such a mechanism of activation, which instead probably relies on a more traditional binding pocket.[51] Additional agonists, including menthol, have an opposing and concentration-dependent action on TRPA1.[52] A large variety of medicines have been identified as capable of targeting TRPA1. Specific adverse effects produced by some of these drugs such as isofluorane,[51,53] nicotine,[54] NO donors,[55] or the metabolic byproducts of chemotherapeutic agent, cyclophosphamide,[12,56] may be explained by the ability of TRPA1 to cause pain and neurogenic inflammation. However, for other currently used medicines, including dihyropyridines,[57] chlorpromazine,[41] clotrimazole,[58] or fenamate nonsteroidal anti-inflammatory drugs,[59] the association between their role as TRPA1 agonists and their therapeutic properties or most frequent side effects is less obvious. Of particular relevance in this context is the observation that the first and second phases of the nociceptive and inflammatory response to formalin (formaldehyde) are entirely mediated by TRPA1, as both phases are blunted in TRPA1 null mice or after pharmacological blockade of the TRPA1 channel.[47]

Oxidative decomposition of polyunsaturated fatty acids (linoleic acid, arachidonic acid) initiates chain reactions that lead to the formation of a variety of reactive carbonyl species (RCS, three to nine carbons in length), the most reactive and cytotoxic being, in addition to dialdehydes or ketoaldehydes, the highly reactive α,β-unsaturated aldehydes, 4-hydroxy-*trans*-2-nonenal and acrolein. By reacting with nucleophilic sites in proteins, RCS generate advanced lipoperoxidation end-products (ALE), including Schiff base adducts or Michael adducts.[60] Most of the toxic and tissue damaging effects of RCS/protein adducts are due to their ability to disrupt protein functions.[60] Thus, the oxidative burst produced following neutrophilic and macrophagic activation at sites of inflammation generates reactive molecules that among several other actions target the TRPA1 to elicit pain and neurogenic inflammation (Figure 2). 4-Hydroxy-*trans*-2-nonenal[61] and 4-oxononenal[62] have been reported to cause nociceptive behavior via a selective action at TRPA1. Also, reactive oxygen species (ROS), as oxygen peroxide,[37,46] or reactive nitrogen species (RNS), as peroxynitrite[37] and nitrooleic acid,[63] target TRPA1. Another endogenous agent produced as part of the immediate host defense response, hypochlorite, and the exogenous toxicant hydrogen sulfide, which is also produced endogenously and has been proposed as a novel mediator in the cardiovascular system and other systems, gate the TRPA1.[46,64] Nonenzymatic dehydration within the cyclopentane rings of PGE_2, PGE_1, and PGD_2 produces the cyclopentenone prostaglandins PGA_2, PGA_1, and PGJ_2, whose biological actions are not mediated by activation of the classical G-protein-coupled prostanoid receptors but rather through interaction with other target proteins,[65] including the nuclear receptor peroxisome proliferator-activated receptor γ.[65] Cyclopentenone PGs are characterized by the presence of a reactive α,β-unsaturated carbonyl group within their cyclopentenone ring, which confers to them the ability to stimulate TRPA1, thereby producing an early nociceptive response that is absent in TRPA1 null mice[66] and that is clearly distinguished from the delayed nociception mediated by the proinflammatory action produced via the prostanoid receptors by classical PGs.[66] Thus, analgesia evoked through cyclooxygenase inhibition by nonsteroidal anti-inflammatory drugs most likely results from the blockade of two final pathways. The first is derived from inhibition of the classical proinflammatory action of PGs at the prostanoid receptors and results mainly from blunting nociceptor sensitization,[67] while the second is produced by inhibition a newly identified, proalgesic mechanism, mediated by a direct

excitatory action by cyclopentenone PGs on nociceptors via TRPA1 stimulation.[66]

1.f. TRPA1 Modulation. TRPA1 expression and activity undergo remarkable plasticity by a series of inflammatory mediators and by its own intrinsic function via activation of specific intracellular pathways or channel trafficking. TRPA1 is both activated and sensitized through inflammatory receptor pathways in a manner similar to TRPV1.[12,16,30,68] However, additional mechanisms, specific for TRPA1, seem to operate to maintain channel function and to preserve it from desensitization. Several TRPA1 agonists covalently modify the channel.[32] TRPA1-mediated nocifensive behavior can be sensitized in vivo via protein kinase A (PKA) and PLC signaling[12,16,30] and by activating TRPA1 with its ligands by increasing TRPA1 membrane levels.[69] TRPA1 translocation to the membrane seems to represent one of the mechanisms controlling TRPA1 functionality upon acute activation or inflammatory signals.[69] Phosphatidylinositol bisphosphate (PIP_2) hydrolysis by PLC and PKA activation are the two pathways that heighten the sensitivity of TRPA1 following bradykinin receptor or proteinase activated receptor-2 (PAR-2). However, it has been reported that PIP_2 hydrolysis not only can sensitize but also can slightly desensitize the channel.[70] These in vitro findings have in vivo counterparts because bradykinin-induced hyperalgesia was absent in TRPA1 null mice,[12,13] and a TRPA1 antagonist attenuated bradykinin-induced mechanical hyperalgesia.[42]

1.g. TRPA1 and Airway Diseases. Skin exposure to or accidental inhalation of a number of aggressive environmental irritants or industrial pollutants has been reported to cause asthma-like symptoms, including coughing, wheezing, chest tightness, dyspnea, and heightened sensitivity to chemical and physical stimuli. These conditions have been labeled under different names, including irritant-induced asthma,[71] reactive airways dysfunction syndrome,[72] and occupational asthma if exposure occurs at the workplace[73] and may outlast for months or years the short-lived exposure to the irritant molecule.[71] Of interest for the present discussion is the recent identification as TRPA1 stimulants, of many of the agents known to cause these conditions and including chlorine gas and reactive oxygen species,[46] acrolein,[10,12] nitric oxide, via nitrooleic acid formation,[63] isocyanates,[48] and toluene diisocyanate.[74] Although the pathway(s) that, activated by these agents, causes acute to chronic respiratory symptoms remains to be clarified, a recent paper[75] has reported that genetic deletion or pharmacological inhibition of the TRPA1 diminishes a variety of allergen-induced cellular and biochemical inflammatory responses, thereby suggesting that neurogenic and TRPA1-mediated inflammation is a major contributor in a model of asthma.

Chronic obstructive pulmonary disease (COPD) is characterized by chronic bronchitis and emphysema, which result in a progressive and fatal deterioration of the respiratory function. COPD will be the forth/fifth cause of death worldwide in 2020. The major causative agent of COPD is the cigarette smoke habit. However, the precise mechanism that leads 10−15% of smokers to develop COPD is unknown. Cigarette smoke inhalation causes an early inflammatory response in the airways that is partly neurogenic because it is abated by defunctionalization of sensory nerve terminals by capsaicin pretreatment.[76] However, because the selective TRPV1 receptor antagonist capsazepine[77] failed to prevent airway inflammation evoked by cigarette smoke,[78] this effect could not be ascribed to a response mediated by TRPV1 activation. The apparent contradiction was resolved recently[10] by showing that among the ∼5000 constituents of cigarette smoke, two α,β-unsaturated aldehydes acrolein and crotonaldehyde, by stimulation of TRPA1 on capsaicin-sensitive sensory neurons, mediate airway neurogenic inflammatory responses evoked by cigarette smoke. Thus, in the early phase of bronchial inflammation evoked by cigarette smoke the TRPA1 pathway plays a major role, and it is possible that neurogenic mechanisms due to TRPA1 channel activation also contribute to the subsequent and terminal stages of COPD.

Inhalation of ozone is a major health risk in industrialized nations. Ozone can affect lung function and has been shown to exacerbate asthma via different proinflammatory pathways, including neurogenic mechanisms.[79] The recent observation that ozone selectively activated cinnamaldehyde-sensitive C-fibers and HEK293 cells transfected with TRPA1, but failed to activate nontransfected HEK293 or HEK293 transfected with the capsaicin-sensitive TRPV1 channel, suggests that ozone is a selective activator of a subset of airway C-fibers by directly stimulating TRPA1[80] and that the previously identified neurogenic proinflammatory action of ozone is mediated by TRPA1.[81]

Inhibition of neurogenic inflammation and of its detrimental consequences evoked by repeated exposure to irritant stimuli represents an appealing perspective in the clinical development of selective and high affinity TRPA1 antagonists. On the other hand, the use of such antagonists, with the resulting TRPA1 blockade, may contribute to the alarming condition of the protective system orchestrated by TRPA1 activation no longer sensing noxious and potential tissue damaging agents. It is emphasized, however, that at present there is no evidence in favor of or against the hypothesis that TRPA1 inhibition causes predictable serious adverse reactions.

1.h. Non-Neuronal Localization of TRPA1. Following the early detection of TRPA1 in a subpopulation of nociceptive neurons with C and A fibers, which also mediate neurogenic inflammation, major attention has been paid to the functional consequences to this neuronal localization of the TRPA1 channel. However, apart from the fact that the cloning of the channels has been obtained from a human fetal lung fibroblast cell line,[1] more recently, additional non-neural cells have been identified as capable of expressing functional TRPA1 channels. In isolated, pressurized cerebral arteries, allyl isothiocyanate caused a concentration-dependent dilatation that was accompanied by a decrease in smooth muscle intracellular calcium ($[Ca^{2+}]_i$). These responses were inhibited by the TRPA1 known channel blockers, by small and intermediate conductance Ca^{2+}-activated K^+ channel blockers, and by blockers of inwardly rectifying K^+ channels. These findings suggest that activation of endothelial TRPA1 elicits vasodilatation of cerebral arteries by a mechanism that ultimately involves K^+ channels in arterial myocytes.[82]

Enterochromaffin cells of the luminal part of the gastrointestinal tract are believed to respond to the chemical composition of the gut. Release of the elevated serotonin content of enterochromaffin cells is one of the consequences of such stimulation. However, the molecular and cellular mechanisms underlying this response have not been identified. TRPA1 was found to be highly expressed in enterochromaffin cells, and its stimulation resulted in serotonin release, which ultimately causes the contraction of isolated guinea pig ileum via serotonin 5-HT_3 receptor activation.[83]

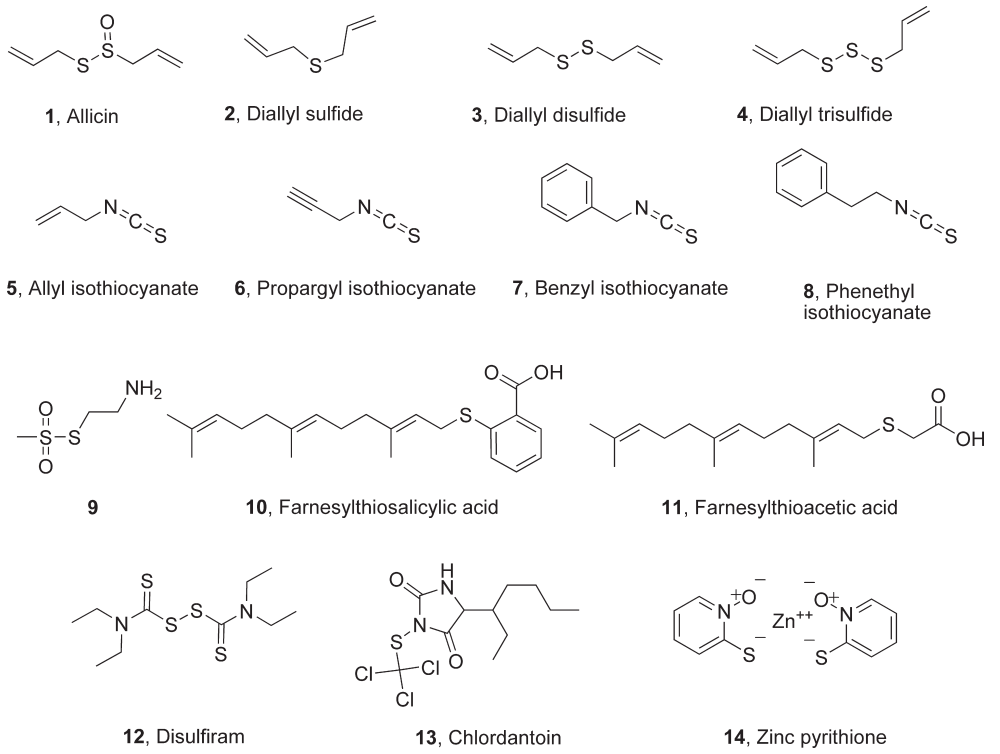


Figure 3. Organosulfur compounds as TRPA1 agonists.

A human pancreatic endocrine cell line (QGP-1 cells) was also found to express, along with entrochromaffin cell marker genes, the TRPA1 channel, and TRPA1 agonists evoked serotonin release from these cells, a response that was inhibited by TRPA1 antagonists.[84] The functional consequences for gastrointestinal motility of the presence of TRPA1 in endocrine cells of the gut may be of relevance. In fact, TRPA1 agonists have been shown to delay gastric emptying in rats, an effect abolished by serotonin depletion or 5-HT_3 receptor antagonism.[85] Numerous heat-sensitive TRPs have been identified in non-neuronal cell populations of the skin. In mice, TRPA1 expression was not solely confined to intraepidermal endings of somatosensory thin-caliber nerve fibres but also to many large-caliber axons and lanceolate and Meissner endings. More importantly, epidermal and hair follicle keratinocytes have been found to express TRPA1 message and protein.[43] The hypothesis that a modulatory role of TRPA1 in keratinocytes,[86] interacting with sensory terminals to modify their mechanical firing properties TRPA1, is involved in thermo- and mechanosensation is of interest but clearly requires further investigation.

In rat urinary bladder, TRPA1 immunoreactivity has been localized to unmyelinated sensory nerve terminals within the urothelium, suburothelial space, and muscle layer as well as around blood vessels.[87] Additional localization has been detected in urothelial cells at both transcriptional and protein levels.[87] Functional responses produced by TRPA1 agonists suggest that the channel may activate the micturition reflex[87] and, in association with other channels (TRPV1, TRPV4), expressed in neurons and/or urothelial cells,[88] may contribute to detrusor overactivity or other inflammatory urinary bladder conditions.[56]

2. TRPA1 Channel Activators

As already mentioned, both exogenous (mainly pungent ingredients from plants, irritant chemicals from air pollution or cigarette smoke and some marketed drugs) and endogenous (originated under inflammatory conditions) agents have been identified as TRPA1 agonists.[89] Several TRPA1 activators are derived from the vegetal realm where evolution promoted the development of ingenious defensive systems against herbivorous predators. For example, plants of the genus *Brassica* (mustard), *Allium* (onion), and *Cinnamomum* (cinnamon), widely known because of their alimentary use, produce pungent isothiocyanate, thiosulfinate, and α,β-unsaturated aldehyde compounds, which are now known to produce acute pain by activating TRPA1. In contrast with other chemoceptors, which are usually stimulated by ligands with rather conserved structures, a unique feature of TRPA1 is that the channel appears to be activated by a variety of structurally unrelated compounds. In this section we report a detailed description of the specific chemical properties and structures of TRPA1 agonists. The list of compounds also includes those that exhibit low potency or poor selectivity because in an early phase of TRPA1 research this kind of information may be still valuable.

2.a. Organosulfur Compounds. *Allium sativum*, commonly known as garlic, belongs with onions, leeks, chives, and shallots to the plant of the genus *Allium*. Some sulfur compounds have been identified as responsible for the distinctive pungency, lachrymatory effects, and spicy aroma of garlic.[31,32] Fresh garlic extract activates mTRPA1 and hTRPA1 and to a lesser extent rTRPV1, as well as excites a subset of cultured sensory neurons from trigeminal ganglia,[31,32] while baked cloves were shown to lack any activity to these thermo-TRPs. Allicin[31] (**1**, Figure 3, Table 1) is an unstable organosulfur component of fresh garlic, and its pungent activity is due to its activity to both TRPA1 and TRPV1.[90] This chemical is generated from alliin by a reaction catalyzed by the vacuolar enzyme alliinase after the clove has been bruised, cut, or cracked. Like other high-reactive thiosulfinates, allicin has a short life in aqueous

solution, yielding more stable organosulfur byproducts such as diallyl sulfide (**2**), diallyl disulfide (**3**), and diallyl trisulfide (**4,** Figure 3, Table 1). While alliin is odorless, **1** and the related derivatives **2**−**4** are volatile compounds responsible for the typical garlic aroma and flavor that have made it one of the most widely diffused culinary ingredients.[31] EC_{50} values for purified **1** for mTRPA1, hTRPA1, and rTRPV1 were 1.32, 1.91, and 51.22 μM, respectively (Table 1).[31] Compounds **2**, **3**, and **4** increased $[Ca^{2+}]_i$ in hTRPA1-expressing CHO cells in a concentration-dependent manner with EC_{50} values of 254, 7.55, and 0.49 μM, respectively.[91] Ca^{2+} response by these molecules significantly decreased in the presence of specific TRPA1 antagonists. Many TRPA1 agonists have been recognized to activate the channel by covalent binding to cysteine residues at the inner cellular domain (see "TRPA1 Ion Channel Activation/Inactivation Mechanism").[44,45,49] TRPA1 activation by derivatives **2**−**4** is probably based on modification of critical cysteine residues. Compound **4** is, indeed, known to exert a cancer inhibiting effect by blocking tubulin polymerization through the modification of cysteine residues in β-tubulin of microtubule.[91]

Table 1. Organosulfur Compounds as TPRA1 Activators[a]

compd	EC_{50} (μM)
1	1.32[31]
	1.91[31]
2	254[91]
3	7.55[91]
	125[31]
4	0.49[91]
5	33.5[49]
	1.9[115]
	5[96]
6	18.4[49]
7	0.319[129]
8	10[94]
9	1580[49]
10	5[96]
	7[96]
	7.3[112]
11	90[96]
	100[96]
12	3[96]
	3[96]
13	3[96]
	3[96]
14	2.4[98]
	0.46[98]
	0.38[98]

[a] Structures in Figure 3.

The above-mentioned organosulfur compounds share structural similarities with allyl isothiocyanate (**5**, Figure 3, Table 1), the pungent ingredient of wasabi, horseradish, and mustard oil.[36,92,93] This chemical induces inflammatory edema and hyperalgesia by activating TRPA1 on primary sensory neurons. Other isothiocyanates behaved as TRPA1 activators (propargyl isothiocyanate **6**, benzyl isothiocyanate **7**, and phenethyl isothiocyanate **8**; Figure 3),[44,49,94] but the high reactivity of these molecules has severely limited their use as pharmacological tools. Allyl isothiocyanate was

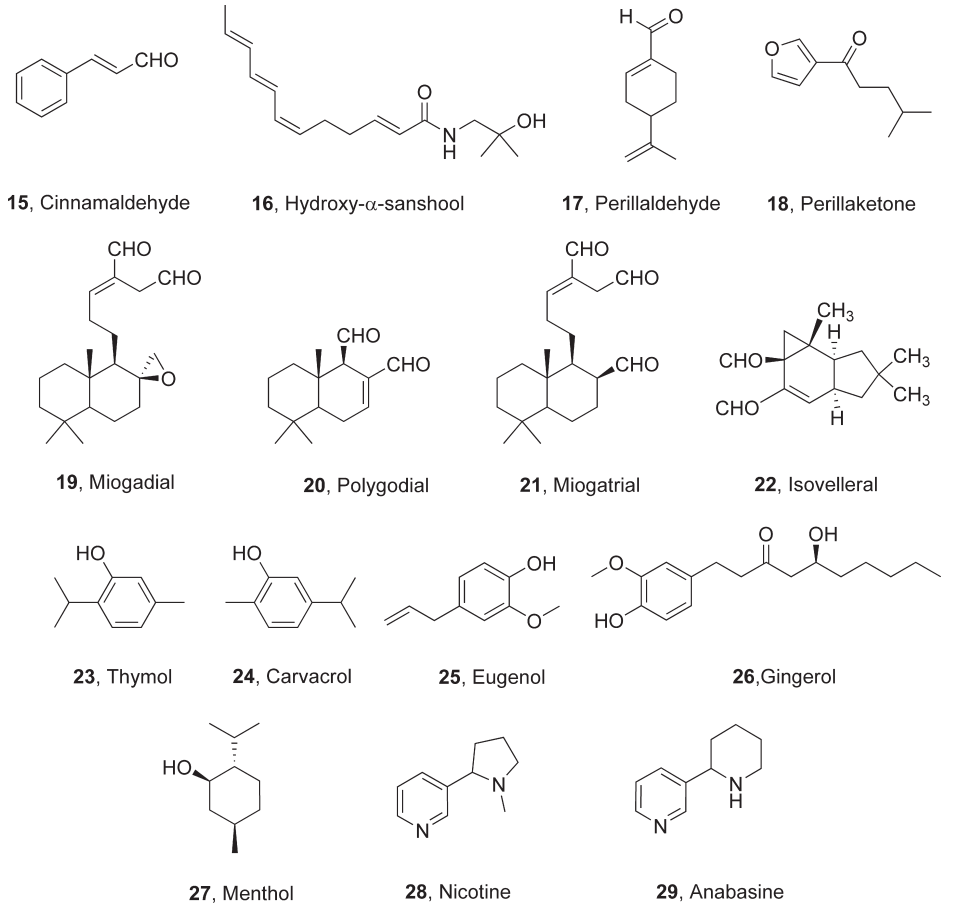


Figure 4. Phytochemicals as TRPA1 agonists.

Table 2. Phytochemicals as TPRA1 activators[a]

compd	EC_{50} (μM)	source
15	19[49]	*Cinnamomun cassia* (bark), *Languas galanga* (rhizome)
	15[115]	
	6.5[57]	
16	66.2[103]	*Zanthoxylum piperitum* (Szechuan pepper)
17	41[107]	*Perilla frutescens*
18	19.7[107]	*Perilla frutescens*
19	0.2[108]	*Zingiber mioga* (fresh flower buds)
	0.4[108]	
20	0.059[108]	*Polygonum hydropiper* (leaves)
	0.67[108]	
	0.4[109]	
21	0.13[108]	*Zingiber mioga* (fresh flower buds)
	0.63[108]	
22	130[109]	fungus *Lactarius vellereus*
	0.5[109]	
	2.58[109]	
23	6[110]	*Thymus vulgari*, *Origanum vulgare*
	64[110]	
	127[110]	
	62.5[112]	
24	7[110]	*Origanum vulgare*
27	95[52]	*Mentha piperita*
	28.4[112]	
	IC_{50} = 56[52]	
	IC_{50} = 73.4[112]	
	IC_{50} = 68[113]	
28	17[54]	*Nicotina tabacum* (leaves)

[a] Structures in Figure 4.

shown to activate other TRPs (including TRPV1).[95] Similar to garlic organosulfur derivatives, allyl isothiocyanate could be conjugated with cysteines via an addition to form dithiocarbamates. TRPA1 activation has been detected after application of millimolar concentration of an organosulfur reagent known for its ability to form a disulfide bond with cysteines (2-aminoethyl methanethiosulfonate, **9**).[49]

By use of a combination of Ca^{2+} fluorescent assays and whole-cell electrophysiology, some sulfur-containing compounds that possess TRPA1-stimulating properties have been recently discovered (Figure 3, Table 1).[96] These include several lipid compounds (farnesylthiosalicylic acid, **10**, farnesylthioacetic acid, **11**) and two commercially available drugs: disulfiram (**12**, Antabuse) and chlordantoin (**13**, Sporostacin). The activity of **12** and **13** was significantly reduced at the quadruple cysteine/lysine mutant of the channel (TRPA1-M4), while **10** and **11** retained to some extent the ability to excite TRPA1-M4-transfected cells. These findings would suggest that the action mechanism of **10** differs from that of reactive agents such as **5**; nonetheless, the precise mechanism by which the compound activates TRPA1 needs further elucidation. Inhibition of aldehyde dehydrogenase by **12** results in elevated levels of acetaldehyde following alcohol intake, leading to a syndrome characterized by a series of unpleasant symptoms including nausea, vomiting, and headache. The drug inhibits the enzyme by inducing the formation of an intramolecular disulfide bridge involving key cysteine residues in the active site.[97] The inability of **12** to activate the TRPA1-M4 mutant channel suggests that modification of critical cysteine residues underlies the TRPA1 agonist activity of this compound. Whether the TRPA1 agonist activity contributes to the alcohol-sensitizing effects of this molecule in any way is unclear.

Another antifungal and amoebicidal drug, clioquinol (5-chloro-7-iodoquinolin-8-ol, CQ), has been found to exert some agonist activity toward TRPA1.[98] CQ was withdrawn from the market when it was linked to severe side effects, and moreover, almost 50% of patients exposed to CQ complained of pain and 40% exhibited cold sensitivity. Behavioral experiments from TRPA1 −/− mice demonstrated that the channel is required for the nociceptive effects of CQ in vivo. Given that CQ exerts its antiparasitic actions by acting as a Zn^{2+} ionophore, another known Zn^{2+} ionophore, zinc pyrithione (**14**), has also been evaluated and found to be active in whole-cell voltage clamp recordings as TRPA1 agonist (EC_{50} = 2.4 μM). No currents evoked by **14** have been recorded in the presence of Zn^{2+} chelators. Moreover, Zn^{2+} was shown to stimulate TRPA1 by itself (EC_{50} = 7.5 ± 1 nM). In conclusion, **14** activated TRPA1 by an atypical and indirect mechanism. Other divalent cations such as Mn^{2+}, Cu^{2+}, Cd^{2+}, and the same Ca^{2+} have been shown to directly activate TRPA1 channel.[96,99]

2.b. Phytochemicals and Related Compounds as TRPA1 Agonists. Cinnamaldehyde (**15**, Figure 4, Table 2) is the main constituent of cinnamon oil (70%) and is extensively used for its flavoring properties in the food industry or as an ingredient of toothpastes. When orally administered to human subjects, the compound is perceived as a burning and tingling sensation.[100] This molecule activates TRPA1-expressing CHO cells in micromolar concentrations as demonstrated in various assays (see EC_{50} values from different experiments in Table 2) with selectivity for TRPA1 against TRPM8 and TRPV1, indicating that its pungent properties are mainly, if not exclusively, due to TRPA1 stimulation.

Szechuan pepper (*Zanthoxylum piperitum*, Japanese pepper tree) is commonly used in East Asia as a food spice because it produces a pungent, tingling sensation with slight lemony overtones.[101,102] Sanshools has been recognized as the component responsible for the spicy taste of Szechuan pepper (~3%). The main sanshools found are α-, β-, γ, and δ-sanshools and their analogues possessing one hydroxyl group on the amide moiety. Hydroxysanshools have four double bonds in their alkyl chains (Figure 4). They differ from the configuration of one double bond and the length of the polyenic system (12 carbons for α- and β-sanshools versus 14 carbons for γ- and δ-sanshools). Hydroxysanshool with four double bonds in the all-trans-configuration is called β-hydroxysanshool (HβSS), whereas α-hydroxysanshool (HαSS) contains a double bond in the cis-configuration (see Figure 4, **16**). Interestingly, distinctive sensations can be induced by each purified sanshool.[102] The molecular mechanisms by which **16** induces these sensations have been a matter of debate. This chemical has been particularly analyzed for its capability to interact with TRPA1, but it was shown to activate the channel only at high micromolar concentration (EC_{50} = 66.2 μM),[103,104] while TRPV1 activation occurred at much lower concentrations (EC_{50} = 1.1 μM).[105] For this reason, new synthetic molecules structurally related to **16** have been synthesized with the aim to improve TRPA1 vs TRPV1 selectivity.[105] Sanshool derivatives with variations in their alkyl chains have been prepared. In addition, the amide function has been modified by replacing the 2-methylpropan-2-ol moiety by amino acids having different chemical properties. This study led to more selective TRPA1 agonists, easier to elaborate and with improved potency when compared to **16**. In particular, the alkylamides derived from linolenic acid and palmitoleic acid condensed with

alanine showed increased potency (EC_{50} from Ca^{2+} assay of 20.3 and 25 μM, respectively) in activating TRPA1 channel when compared to **16** along with significant increased selectivity vs TRPV1 (EC_{50} of palmitoleic acid derived amide of 115 μM). It should be remembered, however, that in a recent study three members (KCNK3, KCNK9, and KCNK18) of the pH-sensitive two-pore K^+ family of channels were molecular targets for sanshool action.[106]

Perilla frutescens is a food plant commonly used in Asian cuisine, especially in Korea (kaennip) and Japan (shiso).[107] Traditional Chinese medicine proposes extracts of this plant as remedies for atopic dermatitis because of possible anti-inflammatory and antiallergic properties. Perillaldehyde (**17**, Figure 4, Table 2) and perillaketone (**18**) have been extracted from the leaves of *P. frutescens*, where they are accumulated as secondary metabolites. After their isolation, the two bioactive components were submitted to in vitro assays in which they exhibited agonist properties at TRPA1 (EC_{50} values of 41.0 and 19.7 μM for **17** and **18**, respectively; see Table 2). Because of their relatively simple chemical structures, **17** and **18** might be considered as new interesting hit compounds for structure−activity relationship studies.

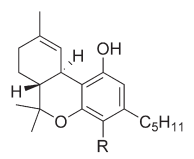
Terpenes represent the largest group of natural products synthesized by plants, fungi, microorganisms, and animals as chemical deterrents against herbivores, fungivores, and predators.[108,109] Indeed, some terpenes produce a painful, pungent sensation in humans, probably related to their deterrent function. Recent research on terpene irritants has focused on sesquiterpenes, a class of terpenes consisting of three isoprene units. More than 80 terpenoids with an α,β-unsaturated 1,4-dialdehyde moiety have been identified so far. For example, miogadial (**19**) and polygodial (**20**, Figure 4), which have an α,β-unsaturated 1,4-dialdehyde moiety, are pungent compounds found in the fresh flower buds of *Zingiber mioga* Roscoe and the leaves of water pepper *Polygonum hydropiper*, respectively. Leaves and bark from these plants are used for analgesic and anti-inflammatory preparations to treat dental and stomach pain and other painful conditions.[108] Although initially painful, **20** acts as an analgesic through desensitization of sensory neurons. Miogatrial (**21**) is a novel terpenoid isolated from *Z. mioga* Roscoe that does not show stimulatory activity in the human tongue despite possessing the α,β-unsaturated 1,4-dialdehyde moiety. Compounds **19**, **20**, and **21** increased $[Ca^{2+}]_i$ in hTRPA1-CHO cells with EC_{50} values of 0.20, 0.059, and 0.13 μM, respectively.[108] Moreover, in patch-clamp experiments performed with mTRPA1-HEK293 cells, large membrane currents were elicited by **19** (1 μM), **20** (3 μM), and **21** (3 μM). These responses were inhibited by selective TRPA1 blockers. The potencies of these terpenoids are among the highest of plant-derived TRPA1 agonists, suggesting that the α,β-unsaturated 1,4-dialdehyde moiety plays an important role for channel activition.

Another intensively studied sesquiterpene is isovelleral (**22**, Figure 4, Table 2), the pungent product of the fungus *Lactarius vellereus*.[109] It has been found to display TRPV1 antagonist activity with IC_{50} values similar to those of capsazepine and ruthenium red (RR). The terpene **22** induced robust influx of Ca^{2+} into HEK-293T cells transiently transfected with human ($EC_{50} = 0.50 \pm 0.13$ μM) or mouse ($EC_{50} = 2.58 \pm 1.09$ μM) TRPA1 cDNA. Agonist activity of **20** and **22** on a TRPA1 mutant, with substitutions of four potential acceptor sites, has been determined. Surprisingly, despite the fact that **20** and **22** contain the α,β-unsaturated dicarbonyl moiety potentially capable of forming Michael adducts, the ability of the compounds to activate the mutant receptor was basically unaltered. These data suggest that dialdehyde sesquiterpenes activate TRPA1 through a mechanism different from that of small reactive unsaturated α,β-aldehydes.

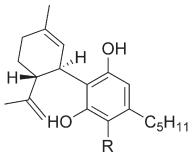
Thymol (**23**, Figure 4), a phenolic monoterpene compound, is one of the predominant components of oil derived from thyme (*Thymus vulgari*) and oregano (*Origanum vulgare*).[33] Although the molecular requirements for its pungency are not yet known, **23** activates various receptors such as TRPV3 and TRPM8. It has been also observed that the molecule stimulates hTRPA1-HEK293 cells in a concentration-dependent manner, with an EC_{50} value of 6 μM.[110] The ability of eight commercially available alkyl-substituted phenols to activate hTRPA1 was evaluated in an analogous membrane potential assay.[110] Many of these compounds (i.e., 2-*tert*-butyl-5-methylphenol ($EC_{50} = 3$ μM), carvacrol (**24**, Figure 4, Table 2, $EC_{50} = 7$ μM), 2,6-dimethylphenol, and 2,5-dimethylphenol) showed low potency at the examined target. SAR analysis revealed that the potency of the screened alkyl-substituted phenols generally diminished with a decrease of the log *P* value. It has also been observed that the presence of sterically demanding and branched alkyl groups, such as isopropyl or *tert*-butyl, at the ortho-position of the phenol ring increased potency. The 2,6-diisopropylphenol (EC_{50} = 4 μM) was indeed more active than 2,6-dimethlyphenol ($EC_{50} = 31$ μM), 2,5-dimethylphenol ($EC_{50} = 57$ μM), and 3,4-dimethylphenol ($EC_{50} = 67$ μM). Phenol had no activity up to 1 mM. Taken together, the log *P* values and alkyl substituent pattern suggest that alkylphenols might bind in a hydrophobic pocket to activate TRPA1. The mechanism of action of **23** on hTRPA1 is still unclear. However, differences in the respective kinetics of channel activation would indicate that **23** and cinnamaldehyde act on different binding sites and through distinctive mechanisms. This is in agreement with the nonelectrophilic nature of this molecule and related simple alkylphenols, which are unlikely to act on TRPA1 via a covalent mechanism. Eugenol (**25**) and gingerol (**26**),[30] para-substituted guaiacol derivatives extracted from the essential oils of clove and from fresh ginger, respectively, along with trinitrophenol,[41] methyl *p*-hydroxybenzoate (methylparaben),[111] and methyl salicylate,[30] are additional examples of phenol-derived compounds exerting some activity at the TRPA1 channel.

Menthol (**27**) is a naturally occurring monoterpene alcohol obtained from peppermint (*Mentha piperita*) or other mint oils. It elicits a cooling sensation when topically applied to the skin or mucous membranes of the airways,[52] a popular effect that is extensively exploited in oral health care products (mouthwashes, toothpastes) and confectionery products. Menthol sensation by mammalian primary neurons has been primarily ascribed to TRPM8,[112] a member of the transient receptor potential (TRP) superfamily of cation channels. Karashima et al. discovered a bimodal activation of TRPA1 by **27** showing that submicromolar to low-micromolar concentrations (1−30 μM) cause robust channel activation in mTRPA1-CHO cells, whereas higher concentrations (1 mM) lead to reversible channel block. A potency (EC_{50} value) of 95 μM has been reported for a racemic mixture of DL-menthol, whereas for the mTRPA1-mediated block of the whole-cell conductance, the calculated IC_{50} was 56 μM.[52] Macpherson et al. furnished an IC_{50} value of 68 μM for DL-menthol inhibition of cinnamaldehyde activated TRPA1 currents.[113] While mouse TRPA1 showed bimodal

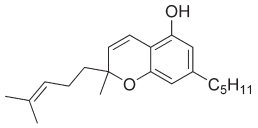
Table 3. Phytocannabinoids as TPRA1 Activators



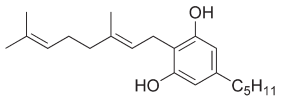
30, Δ^9-Tetrahydrocannabinol (R = H)
31, Δ^9-Tetrahydrocannabinol acid (R = COOH)



32, Cannabidiol (R = H)
33, Cannabidiol acid (R = COOH)



34, Cannabichromene



35, Cannabigerol

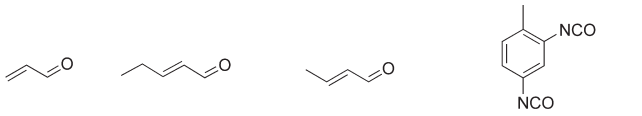
compd	EC_{50} (μM)	assay	cell type
30	0.23	$[Ca^{2+}]_i$	rTRPA1-HEK293
31	0.24	$[Ca^{2+}]_i$	rTRPA1-HEK293
32	0.096	$[Ca^{2+}]_i$	rTRPA1-HEK293
33	12	$[Ca^{2+}]_i$	rTRPA1-HEK293
34	0.06	$[Ca^{2+}]_i$	rTRPA1-HEK293
	34.3	$[Ca^{2+}]_i$	DRG neurons
35	3.4	$[Ca^{2+}]_i$	rTRPA1-HEK293

behavior against menthol, the human isoform of the channel seems to be activated only by menthol, whereas TRPA1 from nonmammalian species appears to be insensitive to this compound and the difference might refer to a different calcium dependent inactivation of hTRPA1 and mTRPA1. It is therefore possible that the molecule modulates TRPA1 in a species-specific manner. The mechanism of the bimodal behavior of menthol (which has been suggested to be mimicked by thymol) is uncertain. Lee et al. supposed that channel activation and desensitization may overlap in the presence of elevated concentrations of ligand.[110]

Nicotine (**28**, Figure 4) is an alkaloid obtained from the leaves of the tobacco plant (*Nicotina tabacum*) belonging to the family of *Solanaceae*, with typical taste and smell. If contacting mucous membranes, it arouses a burning pain sensation that is supposed to hamper both compliance and efficacy of all the smoking cessation therapies based on nicotine replacement (especially nicotine nasal sprays) because of local irritation occurring during the treatment.[54] The alkaloid was found to inhibit hTRPV1, indicating that TRPV1 is not directly involved in the irritation caused by nicotine, whereas nicotine was shown to be equipotent to mustard oil in activating inward TRPA1-mediated currents (EC_{50} = 17 μM).[54] Given its nonelectrophilic nature, the alkaloid does not seem to act via covalent interactions. A behavioral experiment demonstrated that nasal instillation of nicotine, mimicking nasal sprays, provoked airway constriction in wild-type, but not in TRPA1null, mice. These studies suggest that TRPA1 behaves as a ionotropic nicotine receptor that is distinct from the classical nicotine receptor and that inhibition of TRPA1 might represent an interesting approach for developing smoking cessation therapies with less adverse effects. Anabasine (**29**), a nicotine congener found in the tree tobacco plant (*Nicotiana glauca*), has been reported to induce TRPA1 activation similar to nicotine.

Bisogno et al. demonstrated for the first time that Δ^9-tetrahydrocannabinol (THC, **30**, Table 3) and cannabidiol (**32**), bioactive components of *Cannabis sativa*, could exert some of their pharmacological effects through interaction with TRPV1 channels.[114] Some recent findings have disclosed that cannabis extracts and **32** might modulate TRPA1 channel opening,[115] as rat TRPA1-HEK293 cells responded to **30** (EC_{50} of 0.23 $\pm$ 0.03 μM) with an increase in $[Ca^{2+}]_i$. Other phytocannabinoids have been tested in the same conditions (EC_{50} values are reported in Table 3). Cannabidiol acid (**33**) and Δ^9-tetrahydrocannabinol acid (**31**) behaved as partial agonists in this assay compared with mustard oil, and this information might be of some value for the development of TRPA1 antagonists. Selected phytocannabinoids were also tested in DRG neurons using a Ca^{2+} imaging approach, and it is remarked that the potency of cannabichromene (**34**) in neonatal DRG neurons (EC_{50} of 34.3 μM) was slightly lower than that in HEK-293 cells (61 nM). The rank of potency of phytocannabinoids (**34** > **32** > **30** > **31** > **35** > **33**) appears to reflect the expected electrophilic nature of the molecules. The proposed role TRP channels as ionotropic cannabinoid receptors, however, requires endogenous cannabiod levels compatible with the potency of these agonists for the different TRP channels.

Table 4. Environmental Irritants as TPRA1 Activators



36, Acrolein **37**, 2-Pentenal **38**, Crotonaldehyde **39**, Toluene diisocyanate

compd	EC_{50} (μM)
36	5^{12}
	0.8^{10}
	$>1^{129}$
37	5^{12}
38	16^{10}
	23^{10}
39	10^{81}

2.c. Environmental Irritants. TRPA1 is a recognized target for several environmental irritants, particularly formaldehyde[47] and unsaturated aldehydes.[12] Biological responses evoked by these agents are summarized in previous sections of the review.[46,116−118] Acrolein (2-propenal, **36**, Table 4), a noxious and reactive α,β-unsaturated aldehyde,[12] and 2-pentenal (**37**) produce significant muscle $[Ca^{2+}]_i$ increases in TRPA1-transfected cells but not in those expressing TRPV1, TRPV2, or TRPM8 with EC_{50} of 5 μM for both compounds.[12] The mechanism of TRPA1 activation by acrolein seems clearly related to the electrophilic nature of β-carbon of the α,β-unsaturated aldehyde.

Another α,β-unsaturated aldehyde, crotonaldehyde (**38**, Table 4), has been recently identified as a TRPA1 stimulant.[10] This compound is contained in cigarette smoke and air pollution or produced endogenously by oxidative stress. It seems to cause a significant tussive effect that is selectively inhibited by TRPA1 antagonists.[116] Cigarette smoke aqueous extract, **36** and **38**, mobilizes Ca^{2+} in cultured guinea pig jugular ganglia neurons and provokes the contraction of isolated guinea pig bronchi at micromolar concentrations.[10] These responses were abolished by a TRPA1-selective antagonist but not by the TRPV1 antagonist capsazepine or by ROS scavengers. A recent study of the physiological effects mediated by **36**, **38**, and other TRPA1 agonists identified the TRPA1 channel as a novel initiator of the cough reflex in

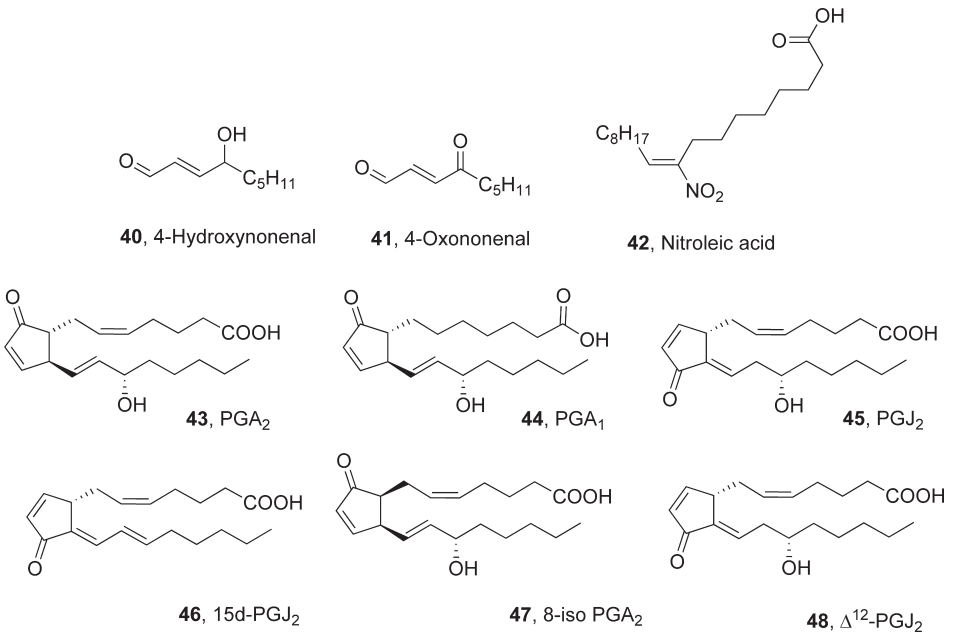


Figure 5. Endogenous TRPA1 ligands.

Table 5. Endogenous TRPA1 Activators[a]

compd	EC_{50} (μM)
40	27[61]
	77[61]
	19.9[119]
41	1.9[119]
42	1[63]
43	24[66]
44	15.1[66]
46	8.9[66]
	18[96]
	22 μM[96]
	5.6[119]
47	22.4[66]

[a] Structures in Figure 5.

guinea pigs.[116] Toluene diisocyanate (**39**),[74] a reactive compound extensively used in the manufacture of polymeric derivatives, can cause respiratory symptoms in exposed workers. The molecule causes Ca^{2+} influx in hTRPA1-HEK cells (EC_{50} of 10 μM). Additional isocyanates, such as methyl isocyanate and hexamethylene diisocyanate, environmental irritant and ingredients of tear gases, activated the hTRPA1 with a potency comparable to that of mustard oil (EC_{50} of 25 and 2.6 μM, respectively).[48] All the above-reported findings would clearly suggest that TRPA1 may be hopefully regarded as a novel target for cough studies in experimental animals and in man. More interestingly, TRPA1 antagonists may be developed with the aim to identify novel antitussive approaches. These results would also suggest the TRPA1 channel as a possible target for pharmacological treatment of symptoms resulting from environmental irritants exposure.[48]

2.d. Endogenous TRPA1 Activators. Asthma and COPD are examples of chronic diseases characterized by oxidative stress, a condition in which an abnormal production of reactive oxygen species (ROS) produces tissue damage and inflammation. ROS-mediated oxidative decomposition of polyunsaturated fatty acids of cell membranes results in increased amounts of lipid peroxidation products, including some α,β-unsaturated aldehydes that have been detected in the airspaces, breath, sputum, lungs, and blood from patients with both asthma and COPD.

4-Hydroxy-2-nonenal (**40**, Figure 5, Table 5)[61] is the most abundant and reactive carbonyl species, generated through peroxidation of ω6-polyunsaturated fatty acids such as linoleic acid and arachidonic acid in response to tissue injury, inflammation, and oxidative stress. It has been demonstrated that **40** robustly activates native or recombinant TRPA1 channels and that its nocifensive and neurogenic inflammatory actions are eliminated by pharmacologic or genetic inhibition of TRPA1 function. This endogenous α,β-unsaturated aldehyde elicited rapid, sustained, and concentration-dependent increase of $[Ca^{2+}]_i$ both in rTRPA1-HEK cells ($EC_{50} = 27\,\mu$M) and in a subset of rat/mouse DRG neurons ($EC_{50} = 77\,\mu$M). 4-Oxononenal (**41**)[62] is a highly reactive electrophilic oxoalkenal produced during oxidative stress. Like **40**, **41** is formed downstream of arachidonic acid and linoleic acid and share the same immediate precursor (4-hydroperoxy-2-nonenal). Compounds **40** and **41** differ at the C4 position where **41** possesses a ketone group in place of the hydroxyl group. This dramatically increases the electrophilic reactivity of **41**, thus increasing its potential to form Michael adducts with cysteine residues. The bis-carbonyl derivative **41** proved indeed to be 10-fold more potent than **40** at activating hTRPA1 ($EC_{50} = 2\,\mu$M). Moreover, **41** evokes a contraction of isolated guinea pig bronchial smooth muscle. Another hydroxyalkenal, 4-hydroxyhexenal,[62] formed downstream of docosahexaenoic acid, eicosapentaenoic acid, and linolenic acid, also activates TRPA1, albeit with lower potency (EC_{50} = 38.9 μM).[119]

Nitration of phospholipids, a consequence of excessive nitric oxide production during inflammation (nitrative stress), results in the formation of nitrated fatty acids (e.g., nitrooleic acid, **42**). Taylor-Clark et al.[63] found that **42** activates TRPA1 channels with an approximate EC_{50} of 1 μM in a Ca^{2+} imaging assay; thus, this nitro compound can be considered the most potent endogenous TRPA1 agonist thus far described. Oleic acid failed to activate

hTRPA1, suggesting that the presence of the NO_2 group would be crucial to TRPA1 channel activation. Data obtained with mutant receptors clearly suggest that **42** activates TRPA1 via covalent modification. It is likely that the electrophilic C=C−NO_2 moiety is responsible for the activity. It has been intriguingly observed that the rank order of potencies of the above-mentioned endogenous TRPA1 electrophilic agonists (pEC_{50} = 6 (**42**) > 5.8 (**41**) > 5 (**40**)) seems to reflect the rank order of their kinetics (second-order rate constants) of the reaction with glutathione.[63] As already mentioned, a novel mechanism through which reactive prostanoids may activate nociceptive neurons independent of prostaglandin receptors has been proposed.[65] Cyclopentenone prostaglandins, including PGA_2 (**43**), PGA_1 (**44**), PGJ_2 (**45**), 15-d-PGJ_2 (**46**), 8-iso-PGA_2 (**47**), and Δ^{12}-PGJ_2 (**48**) because of the presence of a highly reactive α,β-unsaturated bond in their structures, are TRPA1 agonists[66,120] (structures and EC_{50} values for selected compounds are reported in Figure 5 and Table 5, respectively).

2.e. Marketed Drugs. Several marketed drugs have been discovered to activate TRPA1 channel as an additional target, for instance, the above-mentioned disulfiram (**12**),[96] chlordantoin (**13**),[96] and CQ[96] (see Organosulfur Compounds). Cyclophosphamide has been proposed to induce nociception through the indirect interaction of its metabolite, acrolein, with the TRPA1 channel.[56] In addition, some nonsteroidal anti-inflammatory drugs were recently proved to interact with this target.[59]

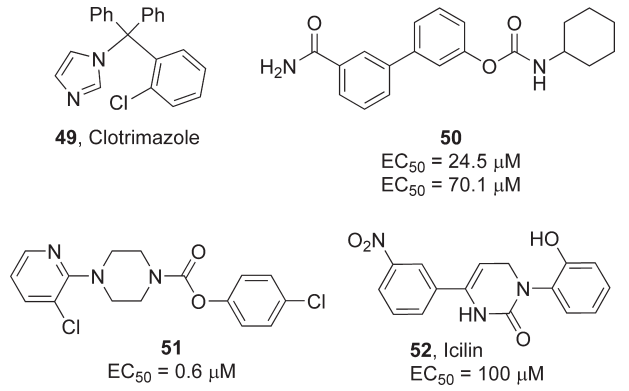
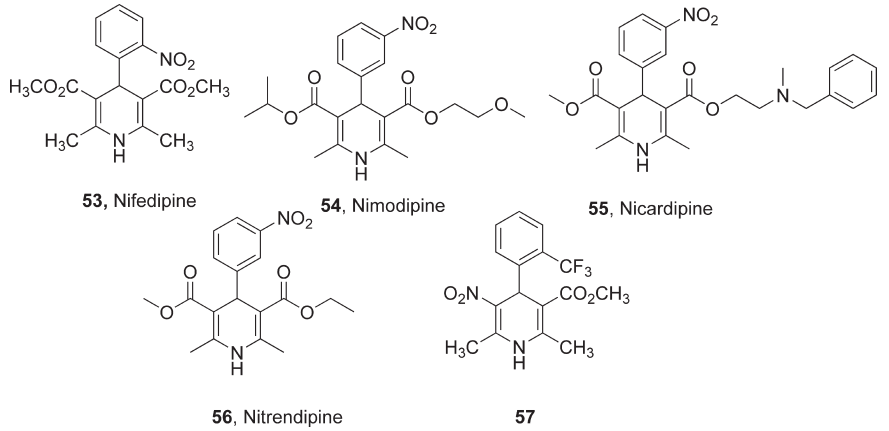


Figure 6. Structural unrelated TRPA1 agonists.

2.e.i. Clotrimazole. Clotrimazole (**49**, Figure 6) is an antifungal agent commonly used for the topical treatment of fungal infections of the skin, vagina, and mouth. Although mostly well tolerated, topical application of clotrimazole-based medicaments occasionally causes local skin and mucous membrane irritation, and a proportion of patients complained of a burning sensation. Intraplantar injection of **49** evoked nocifensive behavior and thermal hyperalgesia in mice.[58] The side effects could be partially explained by interaction of the drug with TRP channels. This molecule has been in fact identified as an agonist of TRPA1 because it activated HEK293 cells expressing TRPA1 at 10 μM. The drug has also been recognized as an agonist of TRPV1 and a potent antagonist of TRPM8. In contrast, **49** did not affect Ca^{2+} levels in cells expressing TRPV2, TRPV3, and TRPV4 channels up to 10 μM. The nonelectrophilic nature of **49** structure would not suggest a covalent binding mechanism of TRPA1 channel activation.

2.e.ii. Dihydropyridines. 1,4-Dihydropyridines are widely marketed as antihypertensive and antianginal drugs because of their known activity as L-type Ca^{2+} channel antagonists. Some 1,4-dihydropyridines also display potent (submicromolar/nanomolar) stimulatory effects on both recombinant and native TRPA1 channels, emerging as novel potential molecular targets for these drugs.[57] Nifedipine (**53**, Table 6) showed an increase of $[Ca^{2+}]_i$ levels in mTRPA1-CHO cells with an EC_{50} of 0.4 μM. This effect was reduced by known TRPA1 antagonists. Other clinically used dihydropiridines, including nimodipine (**54**, EC_{50} = 0.8 μM), nicardipine (**55**, EC_{50} = 0.5 μM), and nitrendipine (**56**, EC_{50} = 3.8 μM)

Table 6. 1,4-Dihydropyridines as TRPA1 Activators[57]



compd	EC_{50}, μM (TRPA1)
53	0.4 ± 0.02
54	0.8 ± 1.3
55	0.5 ± 0.07
56	3.8 ± 0.3
57	32.7 ± 0.2
R-**57**	20.8 ± 1.5
S-**57**	41.5 ± 1.1

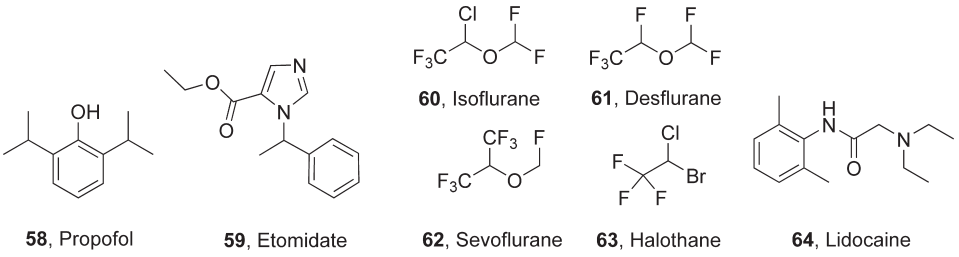


Figure 7. General and local anesthetics able to activate TRPA1.

produced a dose-dependent $[Ca^{2+}]_i$ rise, whereas diltiazem, a Ca^{2+} channel antagonist structurally unrelated to dihydropiridines, evoked nonspecific $[Ca^{2+}]_i$ increase only at very high concentrations ($EC_{50} = 1.6$ mM). The dihydropyridine derivative **57** (BayK8644[57]), behaving as a Ca^{2+} channel agonist rather than an antagonist, maintained the ability to excite TRPA1 ($EC_{50} = 32.7 \pm 0.2$ μM), further suggesting that TRPA1 and L-type Ca^{2+} channels activities are unrelated. The *R*-(+) and *S*-(−) enantiomers of **57** exerted similar potency (EC_{50} of 20.8 and 41.5 μM, respectively). The nonelectrophilic nature of 1,4-dihydropyridines would exclude the possibility that these molecules may act by covalent binding to the channel.

2.e.iii. General Anesthetics and Lidocaine as TRPA1 Channel Modulators. Some general anesthetics (GAs), whose clinical development is strictly related to their inhibitory effects in the CNS, which make them essential for the prevention of surgery-related pain, have been surprisingly found to stimulate peripheral nociceptors.[51,121] For example, the administration of the intravenous anesthetics propofol (**58**, Figure 7) or etomidate (**59**) is known to induce marked pain and burning sensation in the region of the injection for 80−90% of treated patients. Furthermore, volatile GAs (VGAs) are known to stimulate nociceptors, and their inhalation has been associated with neurogenic respiratory irritation. In the search for a possible explanation for such pain related events, both iv GAs and VGAs have been tested for their ability to activate the TRPA1 channel.[51,53] This series of experiments highlighted that **58** and **59** produced a robust activation of TRPA1 in transfected cells but were without effect on TRPV1 or TRPM8 channels. VGAs, displaying different degrees of pungency, have been compared for activity at TRPA1, and isoflurane (**60**) and desflurane (**61**) showed vigorous activity, whereas the nonpungent agents sevoflurane (**62**) and halothane (**63**) were without effect. TRPA1 mechanism of activation by GAs is not yet clear, but the nonelectrophilic nature of GAs would not support the covalent modification of N-terminal cysteines of the channel. Currently, lidocaine (**64**, Figure 7) pretreatment is the most popular method for reducing propofol/etomidate pain, but its coadministration did not demonstrate complete effectiveness. Moreover, **64** itself has been reported to activate TRPA1 expressed in the central terminals of DRG neurons.[122] Selective TRPA1 antagonists may successfully treat the pronociceptive effects of GAs.

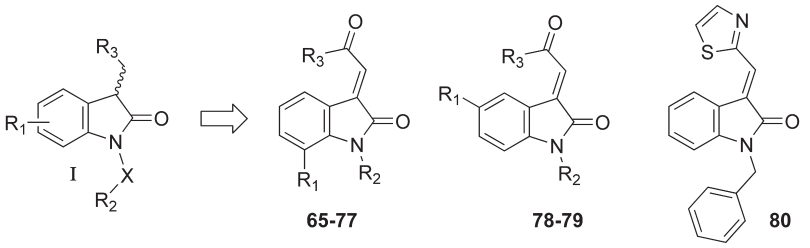
2.f. Miscellaneous Compounds. 2.f.i. FAAH Inhibitors as TRPA1 Agonists. Compound **50** (URB597,[123] Figure 6) has been described as an inhibitor of fatty acid amide hydrolase (FAAH), the enzyme responsible for degradation of the endogenous cannabinoid anandamide. FAAH inhibitors are currently under evaluation for their potential employment as non-opioid analgesic drugs. Interestingly, **50** has been found to activate TRPA1 channels. The concentration required to induce 50% increase of $[Ca^{2+}]_i$ in h-TRPA1-HEK293 cells was 24.5 μM. Activation of h-TRPA1 by this compound was further confirmed using whole-cell, patch-clamp recordings. Large inward currents were indeed induced by application of the compound, while the effect decayed upon its removal. In contrast, **50** did not induce Ca^{2+} influx through TRPV1 or TRPV4. The evaluation of a series of piperazinyl carbamates and ureas, designed on the basis of previously reported TRPV1 antagonists and FAAH inhibitors, led to the identification of compounds targeting both FAAH and TRPV1 or TRPA1 receptors.[124] 4-(3-Chloropyridin-2-yl)piperazine-1-carboxylic acid 4-chlorophenyl ester (**51**, Figure 6) is one of the most active of the series, exerting an EC_{50} value of 0.6 μM in activating TRPA1 receptor.

2.f.ii. Icilin. Icilin (**52**, Figure 6) is a cold-inducing agent used in preclinical phase investigation as an oncolytic drug (Integrity source). The molecule has been originally recognized as a potent activator of TRPM8 ($EC_{50} = 60$ nM),[125,126] while more recent findings have also highlighted its activity as an TRPA1 agonist.[127] Electrospray ionization mass spectrometry (ESI-MS) based studies would exclude reactivity of **52** to cysteines,[49] consistent with the failure of the molecule to react with glutathione in vitro. Moreover, a number of electrophysiological experiments revealed that the application of **52** (100 μM) enhanced channel activity in a rapidly reversible manner, again demonstrating the qualitative differences in mechanism of action of this chemical compared with electrophilic TRPA1 agonists. A noncovalent activation mechanism was also confirmed by using cysteine mutant TRPA1 whose responsiveness to **52** appeared unaltered.

2.f.iii. Isatin Derivatives. Some isatin derived compounds (Table 7) recently exhibited significant potency in activating TRPA1 channels.[49] Consistent with their high reactivity as Michael acceptor, these compounds can be considered among the most potent TRPA1 agonists identified so far. The *N*-methylisatin derivative **65** (Table 7), defined as supercynnamaldehyde, and its *N*-propargyl congener (**66**) exhibited submicromolar potency at hTRPA1-HEK293 in membrane currents activation. The saturation of the exocyclic α,β-double bond resulted in a complete loss of activity. Astellas Pharma confirmed activity at the TRPA1 channel of indolinone compounds represented by formula **I** (Table 7),[128] claiming these molecules are active ingredients for the prevention and/or treatment of constipation-type irritable bowel syndrome, atonic constipation, and/or functional gastrointestinal disorder. Current available data reported are, however, limited, thereby indicating the need for more detailed manipulation of the indolinone nucleus for any well-founded SAR discussion.

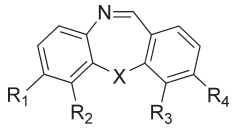
2.f.iv. Dibenzoazepines, Dibenzooxazepines, and Tear Gases. Commonly used as active components of tear gases, 1-chloroacetophenone (CN), dibenzo[*b*,*f*][1,4]oxazepine (**81**, Table 8),

Table 7. Indolinone Derivatives as TRPA1 Channel Activators[49,128]



compd	R_1	R_2	R_3	EC_{50} (μM)
65	H	CH_3	CH_3	0.8
66	H	CH_2CCH	CH_3	0.1
67	F	$CH_2CH(CH_3)_2$	OH	4.27
68	H	3-(CH_3OCO)-benzyl	NH_2	1.2
69	H	cyclohexylmethyl	OH	4.4
70	H	cyclopentylmethyl	OH	3
71	F	cyclopentylmethyl	OH	0.51
72	F	cyclopentylmethyl	NH_2	0.66
73	Cl	$CH_2CH(CH_3)_2$	OH	0.61
74	F	cyclobutylmethyl	OH	1.16
75	F	cyclopentylmethyl	$N(CH_3)_2$	3.76
76	F	$CH_2CH(CH_2CH_3)_2$	morpholin	1.29
77	F	$(CH_2)CH(CH_3)_2$	OH	0.83
78	F	benzyl	OH	4.45
79	F	$CH_2CH(CH_3)_2$	OH	4.63
80				5.65

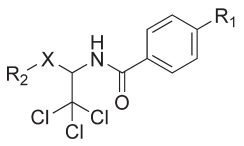
Table 8. Dibenzoazepines and dibenzooxazepines as TRPA1 agonists[129,130]



compd	X	R_1	R_2	R_3	R_4	pEC_{50}
81	O	H	H	H	H	9.52
82	CH_2	H	H	H	H	8.52
83	CH_2	H	H	CO_2CH_3	H	10.00
84	CH_2	CO_2CH_3	H	H	H	9.24
85	O	H	H	H	CO_2CH_3	7.28
86	O	H	H	CO_2CH_3	H	10.22
87	CH_2	H	H	$CO_2C_4H_9$	H	8.07
88	CH_2	H	CO_2CH_3	H	H	9.24
89	O	H	H	$CONH_2$	H	10.12
90	O	H	$CONH_2$	H	H	9.94
91	O	H	CO_2CH_3	H	H	9.93

and 2-chlorobenzylidene malononitrile (CS) display their effects by causing acute eye pain and irritation, excessive tearing, and blepharospasm. These molecules have been recognized as electrophilic alkylating agents, readily reacting with nucleophilic sites in different enzymes. A recent study showed that practically all of the known electrophilic tear gases used as riot control or incapacitating agents are powerful activators of the human TRPA1 (hTRPA1) channel.[48,129] The physiological symptoms promoted by tear gases can be considered as an extreme form of the effects observed with the exposure to TRPA1 agonists of garlic and onions. CN (EC_{50} = 30 nM), CS (EC_{50} = 0.9 nM), and **81** (EC_{50} = 308 nM) are among the most potent TRPA1 activators so far known, displaying low-nanomolar potency.[129] Other typical tear gas agents, such as benzyl bromide (EC_{50} = 12 μM), bromoacetone (EC_{50} = 1.1 μM),

Table 9. Trichloro(sulfanyl)ethylbenzamides as hTRPA1 Antagonists and rTRPA1 Partial Agonists[50,133]



94, R_1 = H, R_2 = 4-Cl-Ph; X = S
95, R_1 = OCH_3, R_2 = 4-Cl-Ph; X = S
96, R_1 = NO_2, R_2 = 4-Cl-Ph; X = S
97, R_1 = Br, R_2 = 4-Cl-Ph; X = S
98, R_1 = CH_3; R_2 = 4-NO_2-Ph; X = S
99, R_1 = CH_3; R_2 = 4-Cl-Ph; X = S
100, R_1 = Cl; R_2 = $COCH_3$; X = S
101, R_1 = Br; R_2 = 3-CH_3-Ph; X = NH

compd	IC_{50}, μM (hTRPA1)	EC_{50}, nM (rTRPA1)
94	0.021	66 (E_{max} 50% compared to **5**)
	0.12	
	0.007	
95	2.23	115 (E_{max} 35% compared to **5**)
	0.091	
	0.26	
	0.029	
96	0.035	
	0.167	
	0.105	
97	0.051	
	0.252	
	0.028	
98	2.0[f]	4.0 (E_{max} 84% compared to **5** and 91% compared to **50**)
	0.85	
99	1.4	3.8
100	1.1	15.0
101	no effect	no effect

ethyl bromoacetate (EC_{50} = 39 nM), and bromobenzyl cyanide (EC_{50} = 10 nM), showed different degrees of hTRPA1 activation.

In a very recent patent by Janssen Pharmaceutica a series of substituted dibenzoazepines and dibenzo[*b*,*e*]azepine

92, Camphor **93**, Mecamylamine

Figure 8. Camphor and mecamylamine as TRPA1 specific blockers.

(morphanthridine, **82**, Table 8) derivatives have been claimed as TRPA1 agonists.[130] To our knowledge, these are the most potent TRPA1 agonists known to date (pEC_{50} values ranging from 7.28 to 10.22 as shown in Table 9). The need of the electrophilic component of **82** for TRPA1 activation (5,6-dihydromorphanthridine showed a potency of at least 1000 times lower than that of morphanthridine) and the electrophilic nature of all these chemicals clearly indicate that channel activation by these chemicals takes place in a covalent fashion. In addition, CS and **81** proved to react readily in vitro with thiols in a reversible and covalent mode. The search for selective TRPA1 inhibitors may a promising source of valuable methods for the treatment of the effects of tear gas exposure or of prophylactic agents against tear gas actions.

3. TRPA1 Antagonists

TRPA1 channels, activated by allyl isothiocyanate, proved to be blocked by very different organic and inorganic chemicals such as gentamicin, gadolinium (Gd^{3+}), amiloride, and ruthenium red (RR,[9] an inorganic dye originally reported to prevent capsaicin-evoked responses and now recognized as a nonspecific blocker of TRP channels). Here we summarize some very recent advancements in the identification of TRPA1 antagonists which may offer useful perspectives for medicinal chemistry purposes.

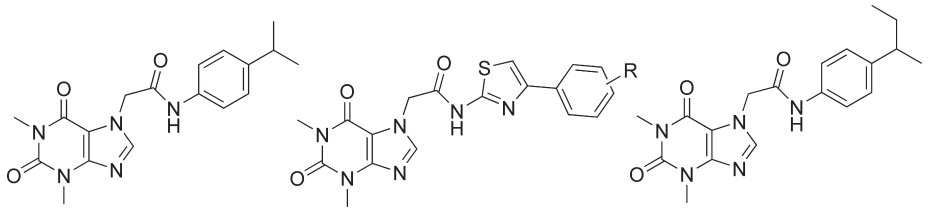
3.a. Camphor and Mecamylamine. Camphor (**92**, Figure 8), isolated from the wood of the camphor laurel tree (*Cinnamomum camphora*), has been used for its decongestant action on airways, and it is still much appreciated as an active component of skin balms and liniments because of its antipruritic, analgesic, and counterirritant properties.[131] In spite of the long history and extensive use of this compound, the molecular target of camphor remains partially unknown. Capsaicin, another topical agent traditionally used for similar purposes, is known to excite and desensitize sensory nerves by acting on TRPV1 (see above). Camphor, in addition to stimulating TRPV1 and TRPV3 channels,[131] has been reported to activate TRPA1.[131] However, at 10 mM, **92** completely suppressed TRPA1-mediated currents previously activated by mustard oil (200 μM) with half-maximal inhibition (IC_{50}) at 660 μM. Camphor was also shown to block thymol-induced activation of hTRPA1 with an IC_{50} value of 400 μM.[110] In whole-cell voltage-clamp experiments using primary cultured trigeminal neurons, the molecule inhibited current activation promoted by acetaldehyde.[132] Moreover, intradermal pretreatment with **92** significantly suppressed cinnamaldehyde and acetaldehyde-evoked nociceptive behaviors (licking and flicking) in mice.[132] Therefore, inhibition of TRPA1 might provide an additional molecular explanation for the analgesic action of camphor. Mecamylamine (**93**, Figure 8), a general inhibitor of nicotinic acetylcholine receptors sharing structural similarities with **92**, has been reported to block TRPA1, and this finding offers a partial explanation for its inhibitory effect on the mucosal irritation caused by high concentrations of nicotine.[54]

3.b. Trichloro(sulfanyl)ethylbenzamides. The activity of a small series of trichloro(sulfanyl)ethylbenzamides (TCEBs, compounds **94**−**97**, Table 9) as potent and selective antagonists of human TRPA1 has recently been recognized by a high-throughput screening program performed at Amgen Laboratories.[133] Derivatives **94**−**97** potently inhibited allyl isothiocyanate induced increase in $[Ca^{2+}]_i$ in hTRPA1. The IC_{50} values determined for **94** (AMG9090[133]), **95** (AMG5445[133]), **96** (AMG2504[133]), and **97** (AMG7160[133]) were 21, 91, 35, and 51 nM, respectively. The compounds have also been evaluated using whole-cell voltage-clamp configuration. In this assay TCEBs inhibited allyl isothiocyanate induced currents with IC_{50} values of 120, 260, 167, and 252 nM for **94**, **95**, **96**, and **97**, respectively. Moreover, all four compounds inhibited human TRPA1 activation by noxious cold (3.5 °C) with IC_{50} values for **94**, **95**, **96**, and **97** of 7, 29, 105, and 28 nM, respectively. The trichloro(sulfanyl)ethylbenzamide moiety seems to promote remarkable selectivity for TRPA1 vs closely related TRP channels (TRPV1, TRPV3, TRPV4, TRPM8). However, when tested at the rat isoform of TRPA1, results obtained by TCEBs were quite surprising, as **94** and **95** increased $[Ca^{2+}]_i$ in a concentration dependent manner, with half of the efficacy of allyl isothiocyanate. Similarly, maximum efficacy of **95** was approximately 35% (EC_{50} = 115 nM) of allyl isothiocyanate, suggesting a partial agonist behavior. Compounds **96** and **97** were instead practically inactive at the rat receptor, also as antagonists.

A limited SAR analysis can be delineated because there is little available data. Unsubstitution of the phenyl on the amide moiety gave the best result in terms of the inhibition of human TRPA1 activated by allyl isothiocyanate. In addition, 4-substitution with an electron-donating group (4-OCH_3, compound **95**) seems significantly detrimental for inhibition activity if compared with 4-substitution with electron-withdrawing groups (4-NO_2 and 4-Br, compounds **96** and **97**, respectively). All three substitutions (4-OCH_3, 4-NO_2, and 4-Br) did not alter the antagonist behavior of the compound at the hTRPA1 channels, while the same modifications dramatically affected the action of these molecules at rat TRPA1. For example, unsubstituted phenyl (**94**) and 4-OCH_3 phenyl (**95**) on amide promoted partial agonism at rTRPA1 while 4-bromophenyl (**97**) and 4-nitrophenyl (**96**) on amide resulted in substantial ineffectiveness.

A similar high-throughput screening of 700 000 compounds performed at Abbot Laboratories led to the identification of thioaminal-containing molecules (**98**, CMP1,[50] **99**, CMP2,[50] and **100**, CMP3;[50] Table 9) whose structures are closely related to those of TCEBs from Amgen (Table 10). Also, these derivatives showed a species-specific pharmacological profile as they activate rTRPA1 while inhibiting hTRPA1. The effects of **98** were characterized in greater detail. This compound activated rTRPA1 in a concentration-dependent manner (EC_{50} = 4.0 μM), but it was slightly less effective if compared to the known agonists **50** and allyl isothiocyanate (E_{max} of 91% and 0.84%, respectively). In contrast, **98** blocked hTRPA1 responses to **50** and allyl isothiocyanate (IC_{50} values of 0.85 ± 0.04 and 2.0 ± 0.4 μM, respectively). Moreover, this TCEB inhibited hTRPA1 activation by a variety of other stimuli, including 4-hydroxy-2-nonenal (IC_{50} = 1.7 μM), cynnamaldehyde (IC_{50} = 2.5 μM), trinitrophenol (IC_{50} = 0.36 μM), and hypertonic solution of 400 mOsm (IC_{50} = 0.56 μM).

Table 10. 7-Substituted Theophylline Derivatives as TRPA1 Antagonists



102 **103** **104**

TRPA1 antagonist	IC_{50} (μM)				
	TRPA1	hTRPV1	hTRPV3	hTRPV4	hTRPM8
102	6.2[47] 5.3[47] 0.7[47] 1.2[47] 1.8[57] 12.87[112] 4.9[135] 7.5[135]	> 20[47]	> 10[47]	> 10[47]	> 30[47]
103 (R = 4-Br, 2F, H, 4-Et, 3-F, 3-OMe, 2-OEt, 3-Cl)	< 1[136]				
104	14.3[137]				

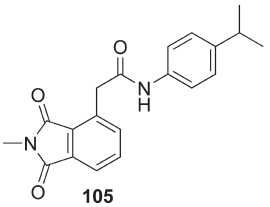
Therefore, **98** blocked hTRPA1 activation independent of the nature of agonists (reactive, nonreactive, or hypertonicity). The molecule did not activate TRPV1, TRPV4, or TRPM8 and did not inhibit TRPV1 activation by capsaicin, TRPV4 activation by hypotonicity, or TRPM8 activation by menthol, appearing to be relatively selective for TRPA1. Chen et al.[50] asserted that **98** activities on rTRPA1 would be related to the electrophilic nature of the thioaminal function. The electron withdrawing nitrophenyl substituent in **98** would particularly activate the thioaminal function, generating a high-reactive electrophilic moiety (data supported by ALARM NMR-based spectroscopy). In addition, the less electrophilic **101** (CMP4,[50] Table 9), in which the sulfur atom has been replaced by nitrogen, had no effect on rTRPA1 or hTRPA1 in terms of activation or inhibition.

3.c. Xanthine Based Antagonists. In the search for specific TRPA1 inhibitors, Hydra Biosciences screened a small-molecule library for inhibitors of the allyl isothiocyanate induced Ca^{2+} increase in TRPA1-expressing cells. One such compound **102** (HC-030031,[47] Table 10)[134] was found to antagonize allyl isothiocyanate evoked and formalin-evoked Ca^{2+} influx with IC_{50} values of 6.2 ± 0.2 and 5.3 ± 0.2 μM, respectively.[47] Moreover, electrophysiology experiments confirmed that both inward and outward currents elicited by allyl isothiocyanate or formalin were rapidly and reversibly blocked by **102**. The ability of **102** to block TRPA1 activation was tested in a parallel fluorescent imaging plate reader (FLIPR) Ca^{2+}-influx assay using HEK-293 cells stably expressing human TRPA1. Compound **102** dose-dependently blocked cinnamaldehyde-induced and allyl isothiocyanate induced Ca^{2+} influx with IC_{50} values of 4.9 and 7.5 μM, respectively.[135] The molecule was also shown to produce a potent ($IC_{50} = 1.8$ μM) and fully reversible inhibition of nifedipine-evoked $[Ca^{2+}]_i$ elevations.[57] Compound **102** confirmed its antagonistic action on other mammalian orthologues (rat and mouse) and on cultured rat-DRG neurons. The potency of **102** appeared similar in different assays regardless of the agonist used (reversible agonists, such as allyl isothiocyanate, or irreversible agonists, such as *N*-methylmaleimide).[47] Selectivity of **102** for TRPA1 was evaluated against 48 different enzymes, receptors, and transporters that have been reported to modulate pain signaling showing no significant activity in any assay at concentrations up to 10 μM.[135]

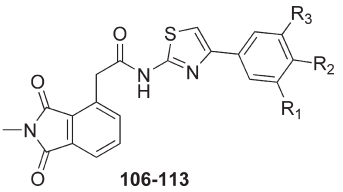
More than 100 analogues of **102** have been synthesized and screened at Hydra Laboratories in the search for more potent and selective TRPA1 antagonists,[136] most of the substitutions involving the *N*-phenylacetamide side chain at the 7-position of the xanthine nucleus. The most potent derivatives have been obtained by introducing sterically demanding moieties at the para-position of the *N*-phenyl ring (IC_{50} lower than 1 μM as measured in patch clamp assay in the case of *p*-adamantyl substitution or lower than 500 nM in presence of *p*-phenoxy group). This would suggest that the 7-side chain probably interacts with a hydrophobic pocket of the channel. A particularly successful modulation of the 7-acetamido chain is represented by compounds of general formula **103** (Table 10). In these derivatives the *N*-phenyl ring was replaced by a *N*-(4-(substituted)phenyl)thiazol-2-yl moiety. Different substitution of the aromatic ring at the 4-position of the thiazol nucleus led to TRPA1 antagonists endowed with submicromolar potency (see Table 10).

In the rat, oral administration of **102** reduced allyl isothiocyanate induced nocifensive behaviors at a dose of 100 mg/kg.[135] The effect of the compound (100 mg/kg, oral administration) was evaluated in the CFA (complete Freund's adjuvant) model of inflammatory pain, and the molecule was found to reverse CFA-induced mechanical hypersensitivity. In addition, it has been found that **102** (100 mg/kg, po) significantly reversed tactile hypersensitivity in the SNL (spinal nerve ligation) model of neuropathic pain. These data clearly suggest that oral administration of small molecule TRPA1 receptor antagonist could be a successful therapeutic strategy for the treatment of both inflammatory and neuropathic pain and support the development of potential novel analgesics addressing this target which plays an important role in nociceptive transmission. The TRPA1

Table 11. 4-Substituted Phthalimide Derivatives as TRPA1 Antagonists[139]



105



106-113

compd	R_1	R_2	R_3	% inhibition at 1 μM	% inhibition at 10 μM	IC_{50} (nM)
105				18	21	
106	H	Cl	H	39	93	500−1000
107	H	I	H	76	95	<250
108	H	H	CF_3	60	96	500−1000
109	H	Cl	Cl	86	98	250−500
110	H	Cl	CH_3	83	91	250−500
111	H	CF_3	F	97	100	
112	F	CF_3	F	92	100	250−500
113	F	$OCHF_2$	F	92	99	

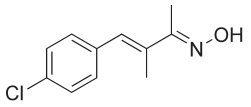
antagonist **102** is currently under preclinical investigation for its possible employment as an analgesic and antiasthmatic agent (Integrity source).

Diabetes mellitus is frequently associated with sensory neuropathy and chronic pain that are difficult to treat with commonly available methods. Chronic treatment of diabetic rats with a structural congener of **102** synthesized by ChemBridge Corporation, **104** (namely Chembridge-5861528,[137] Table 10), administered twice daily for 1 week showed it to be effective in preventing diabetic pain hypersensitivity. No marked side effects by acute or prolonged treatment were observed. The compound is now under preclinical investigation at Orion Pharma for diabetes (Integrity source). Compound **104** antagonized allyl isothiocyanate evoked (5 μM) and 4-hydroxy-2-nonenal (30 μM) evoked TRPA1 mediated increase of $[Ca^{2+}]_i$, with IC_{50} values of 14.3 ± 0.7 and 18.7 ± 0.3 μM, respectively. Neither **102** nor **104** showed any TRPA1 agonism up to 100 μM. In TRPV1-transfected cells, **102** and **104** were not able to attenuate capsaicin-induced responses and were not agonists themselves up to the highest tested dose of 100 μM. **102** concentration-dependently antagonized in the same test TRPA1 responses with IC_{50} values of 41.8 ± 3.5 and 48.4 ± 3.4 μM, when allyl isothiocyanate and 4-hydroxy-2-nonenal were agonists, respectively.

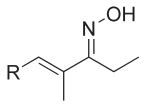
Consistent with the xanthine-based structures of **102** and **104**, it is not surprising that the naturally occurring xanthine, caffeine (1,3,7-trimethylxanthine), was recently identified as a TRPA1 antagonist at the human isoform of the channel,[138] whereas it showed agonistic properties for the mTRPA1 (EC_{50} between 1 and 2.5 mM), thus confirming the peculiar species-specific differences of TRPA1 pharmacology.

3.d. Phthalimide Derivatives. A recent invention by Glenmark Pharmaceuticals provides a series of phthalimide derivatives as TRPA modulators.[139] The claimed compounds were proposed to be useful for treating or preventing diseases, conditions, and/or disorders modulated by TRPAl. Forty-four compounds were prepared by coupling (2-methyl-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl)acetic acid with 4-isopropylaniline (**105**, Table 11) or with the appropriate 4-(substituted)phenylthiazol-2-ylamine (**106**−**113**). Selected compounds were screened for TRPA1 antagonist activity. IC_{50} values and percent inhibition at various concentrations have been reported in Table 11. The activity of the 4-isopropyl derivative (direct analogue of **102**) was quite modest. Different substituents of the 4-isopropyl moiety did not show it to improve the activity of the molecules, whereas among the 4-(substituted)phenylthiazol derivatives some antagonists with submicromolar potency have been identified. In particular, *N*-[4-(4-iodophenyl)thiazol-2-yl]-2-(2-methyl-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl)acetamide (**107**) exhibited an IC_{50} lower than 250 nM. The substitution with a $-CF_3$ at the para-position of the phenyl ring was particularly effective in increasing potency (see compounds **111** and **112**). Comparison between Hydra purinone derivatives (**102** and congeners) and molecules developed at Glenmark suggests a similar binding mode with the xanthine and phtalimide bicycles behaving as bioisosters.

Table 12. Oxime Derivatives as TRPA1 Inhibitors[42,140,141]



114



115-125

compd	R	IC_{50} (μM)
114		0.6[112]
		3.1
		4.5
115	6-F-pyridin-3-yl	1.25
116	2-thienyl	11
117	6-MeO-pyridin-3-yl	23.4
118	3-thienyl	1.32
119	3-pyridyl	17
120	4-pyridyl	8.61
121	6-Me-pyridin-3-yl	>100
122	quinolin-3-yl	>100
123	4-Me-pyridin-3-yl	>100
124	6-CF_3-pyridin-3-yl	>100
125	isoquinolin-4-yl	>100

3.e. Oxime Derivatives. The Scripps Research Institute screened more than 40 000 small molecules for their ability to block cinnamaldehyde activation of hTRPA1 in CHO cells.[42] Of the found hits, (*Z*)-4-(4-chlorophynyl)-3-methylbut-3-en-2-oxime (**114**, Table 12) has been chosen for further characterization.[140] The molecule blocked TRPA1 activation with IC_{50} of 3.1 and 4.5 μM for human and mouse clones, respectively. At concentrations up to 50 μM, **114** was unable to appreciably block activation of TRPV1, TRPV2, TRPV3, TRPV4, or TRPM8. This molecule was also shown to block mouse TRPA1 responses to iodoacetamide, mustard oil, and cold. Moreover, the compound blocked cinnamaldehyde-induced TRPA1 currents in excised patches from Xenoopus oocytes. If injected in hind paw of mice, the molecule significantly blocked cinnamaldehyde-induced but not capsaicin-induced nociceptive events, demonstrating efficacy and TRPA1 specificity. Some very recent findings indicate that the replacement of the chlorine atom at the para-position of **114** with other functions occasionally abolished the antagonistic properties of the compound inducing partial agonism.[140]

Abbott Laboratories recently claimed a series of (heteroaryl)alkenone oxime derivatives as TRPA1 antagonists structurally related to **114** useful in the treatment of various diseases.[141] Selected compounds of the series are reported in Table 12. (1*E*,3*E*)-1-(6-Fluoropyridin-3-yl)-2-methylpent-1-en-3-one oxime (**115**) exhibited an IC_{50} of 1.25 μM in

antagonizing hTRPA1. Replacement of the 6-fluoropyridin-3-yl moiety with a 3-thienyl group resulted in a substantial preservation of activity (**118**, $IC_{50} = 1.32\ \mu M$). The introduction of a pyridine ring substituted with a methyl or a trifluoromethyl group and the substitution of the six-membered heterocycle with the bicycles quinoline or a isoquinoline produced a dramatic loss of activity ($IC_{50} > 100\ \mu M$). The Scripps Institute also claimed compound *N*,*N*′-bis(2-hydroxybenzyl)-2,5-diamino-2,5-dimethylhexane[142] as a specific inhibitor of TRPA1. This compound suppresses TRPA1-mediated mechanical nociception but not heat hyperalgesia and was shown to have little or no effect on the other TRPs such as TRPV1, TRPV2, TRPV3, TRPV4, and TRPM8. The potency of the molecule in inhibiting TRPA1 activity was similar to that of **114**.

4. TRPA1 Ion Channel Activation/Inactivation Mechanism

So far, two classes of TRPA1 agonists have been distinguished on the basis of their interaction with the receptor.[143] The first group, represented by ligands such as Δ^9-tetrahydrocannabinol (**30**, Table 3), icilin (**52**, Figure 6), eugenol (**25**, Figure 4), methyl salicylate, and carvacrol (**24**, Table 2, Figure 4),[30] seems to stimulate the receptor via a temporary and reversible interaction with a binding pocket following the traditional Fischer lock and key principle. This is in line with the chemical activation of other members of the TRP family such as the activation of the TRPV1 channel by capsaicin. The second class of (generally more potent) TRPA1 agonists is characterized by the presence of an electrophilic carbon or sulfur that is subject to attack by nucleophilic groups, such as cysteine thiols or the lysine amino group of the receptor. For example, the nucleophilic mercapto group of cysteines can attack the β-carbon of the α,β-unsaturated bond of cinnamaldehyde (**15**, Figure 4) via a typical Michael addition. Other activators including isothiocyanates, such as allyl isothiocyanate (**5**, Table 1, Figure 3), or isocyanates, such as toluene diisocyanate (**39**, Table 4), could be conjugated with cysteines and/or lysins via an addition to form (di)thiocarbamates and (thio)ureas. These chemicals seem to establish the covalent bond with cysteine residues located at the cytoplasmatic N-terminus of TRPA1 aminoacid sequence.[44,47,49] Thus, membrane permeability is supposed to be a fundamental requirement of potential TRPA1 agonists, displaying their action through covalent interaction with the target.

Several findings support the covalent-based activation mechanism, as indicated, for example, by the finding that the agonistic potency increases with the increase of the electrophilic nature and/or the molecule reactivity as potential Michael acceptor (compare **49**, Figure 6, with **15**, Table 2). It has been pointed out that the disparate chemical nature of TRPA1-activating irritants suggests that reactivity rather than the structure by itself determines TRPA1 agonist activity. An example in support of such a conclusion is given by benzyl thiocyanate, which has no activity toward TRPA1, while its isoster, benzyl isothiocyanate (**7**, Table 1, Figure 3), showed agonistic activity at TRPA1. Both compounds possess thiocyanate functional groups of similar size, but the single-bond character of the S−C linkage in benzyl thiocyanate reduces its electrophilicity compared with **7**. Moreover, in vitro formation of adducts with the cysteine-containing tripeptide glutathione (Glu-Cys-Gly) has been proved for allyl isothiocyanate, cinnamaldehyde, the isatin derivative **65**, and iodoacetamide. Instead, nonelectrophilic activators of TRPA1, such as icilin, did not react in vitro with glutathione as measured by ESI-MS spectrometry. The covalent based mechanism has been further confirmed by a study of the capability of formalin to elicit pain.[47] Formaldehyde, the active ingredient in formalin, is indeed a fixative that covalently cross-links proteins in a nonspecific fashion.

Site directed mutagenesis studies revealed that replacement of three cysteine residues (C619, C639, and C663) located within the cytoplasmic N terminus of hTRPA1 resulted in partial, but significant and cumulative, decreases in electrophilic agonists-evoked responses.[44] As mentioned above, some TRPA1 ligands were shown to lose activity toward the TRPA1-3C mutant, demonstrating a specific requirement for these three cysteine residues in channel activation. In contrast, the TRPA1-3C mutant retained sensitivity to nonreactive structurally unrelated agonists. A lysine residue (K708), located close to the C619, C639 and C663 cluster, was shown to contribute to channel activation to a lesser extent. A parallel site directed mutagenesis study performed by Macpherson et al. on the mouse isoform of TRPA1 led to identification of C415, C422, and C622 as fundamental for channel activation.[49] Hinnman et al. and Macpherson et al. identified only one cysteine in common: C622 in mouse TRPA1 corresponding to C619 in the human isoform. This could partially explain interspecies differences observed in the pharmacological profile of some ligands, especially menthol (**27**, Table 2, Figure 4), thymol (**23**, Table 2, Figure 4), caffeine, and trichloro-(sulfanyl)ethylbenzamides (Table 9).

The molecular mechanism linking covalent binding to channel gating has not been fully elucidated. It is emphasized that covalent binding with the receptor is not a prerogative of channel activator. Thioaminal containing molecules such as **98** (Table 9) determined covalent modifications resulting in channel blocking rather than opening. Thus, covalent modification can cause opposite outcomes. Chen et al. established through the building of rTRPA1−hTRPA1 chimeras that a single amino acid modification (i.e., Ala-946 or Met-949) in the S6 region of rat TRPA1 results in important effects that may cause both channel activation and blockade.[50] One possible explanation is that energy of the covalent binding may be used for channel opening or closure. In the same study, however, the mutant channels were unable to switch the action of mustard oil to TRPA1 inhibition; moreover, it has been argued that the location of the critical domain for coupling may be ligand-related.

A soluble cytosolic factor appears to be essential for normal TRPA1 activation by both endogenous and exogenous ligands.[57] Some ligands indeed activate TRPA1 in cell-attached but not in inside-out patches. Inorganic polyphosphates have been identified as possible intracellular cofactors for TRPA1 activation.[144] Polyphosphates are in fact supposed to keep TRPA1 in the agonist-sensitive state for channel gating by pungent chemicals.

A particular mechanism of TRPA1 modulation has been ascribed to some amphipathic molecules such as trinitrophenol, chlorpromazine (antipsychotic drug marketed in the U.S. with the commercial name of Thorazine), and the peptide toxin GsMTx-4 recently isolated from the Chilean rose tarantula.[41] These molecules have been supposed to be able to partition in the inner or outer sheets of the lipid bilayer, determining a curvature of the membrane, which would activate or inhibit mechanosensitive ion channels.[41]

5. Conclusions

Chemonociception represents an important signaling mechanism of acute pain, but it may also contribute to persistent pain, especially in the context of peripheral tissue injury and inflammation. The primary role of TRPA1 as a general and nonspecific sensor of a variety of chemical species, generated by diverse enzymatic or nonenzymatic proinflammatory pathways, emphasizes the hypothesis that compounds that block channel activity represent a new class of analgesic medicine. In fact, although the generation of some of the newly identified TRPA1 stimulants and proalgesic agents, such as cyclopentenone PGs, is blocked by currently available drugs such as NSAIDs, this is not the case for other TRPA1 agonists such as α,β-unsaturated aldehydes or ROS and RNS. In this view, the design and development of selective TRPA1 antagonists may represent a novel perspective in the treatment of inflammatory pain. Only preliminary findings have been reported in experimental animals or human beings in this area of investigation. In patients with cold injury, the ensuing cold allodynia seems to be independent of TRPM8 or TRPA1 and differ therefore from neuropathic pain patients, which exhibit this type of hypersensitivity.[145] In contrast with these clinical observations, in a chronic constriction injury model of neuropathic pain, no role of TRPA1 and TPM8 channels in cold allodynia was observed.[146] However, in agreement with the hypothesis that TRPA1 blockade may be beneficial in the treatment of neuropathic pain, the TRPA1 antagonist **102** (Table 10)[47] inhibited mechanical hypersensitivity caused by both inflammatory and neuropathic pain models.[135] Clearly, much investigation is required before establishing a role for TRPA1 in neuropathic pain and for its antagonists in treating this condition. Nevertheless, it seems quite improbable that TRPA1 antagonists would not be carefully scrutinized for their ability to reduce not only inflammatory but also neuropathic pain either in animal models or in patients. Other potential therapeutic applications for TRPA1 inhibitors are increased compliance in smoking cessation therapies (by inhibition of nicotine patch-induced local irritation) and prevention of the pain evoked by general anesthetics.[51] In addition, the antagonist **104** (Table 10) is under preclinical investigation for the treatment of diabetic neuropathy.[137]

The emerging role of neuronal TRPA1 in the orchestration of the inflammatory response in animal models of airway diseases, including asthma and COPD, is of much interest. The vast majority of the proinflammatory action mediated by sensory nerve activation in the airways is due to SP/NKA release, and the stimulation of tachykinin NK_1 and NK_2 receptors and their blockade by selective and high affinity NK_1 and NK_2 antagonists are both markedly effective in animal models of asthma.[147] However, these antagonists have failed to treat asthma in humans.[118,148] Thus, the possibility that TRPA1 antagonists may represent a novel therapy for asthma and other respiratory diseases remains disputable and requires severe scrutiny. Moving from the "efferent" to the "afferent" function of airway sensory nerves, it should be emphasized that two recent studies have focused on a novel role of TRPA1 in the airways.[116,117] In fact, a host of TRPA1 agonists have been found to act as potent protussive agents in guinea pigs, and cinnamaldehyde inhalation caused coughing in man.[116,117] A component of the tussive response to cigarette smoke inhalation is also most likely mediated by an action on the TRPA1 channel.[116] Thus, TRPA1 antagonists could be regarded as novel antitussive medicines.

An intrinsic flaw of the TRPA1 antagonists so far reported is their high reactivity which favors random interaction with other possible cellular nucleophilic components and increases the risk of drug toxicity and side effects. Hit-to-lead followed by pharmacokinetic optimization is therefore required. Another consideration of paramount importance for the drug discovery process is the in vitro/in vivo evaluation of potential TRPA1 ligands. Dramatic species-specific differences between human and rat TRPA1 responses (probably due to the relatively low amino acid homology (79.0%) between the two orthologues) have been reported. Considering that rats are widely used in models of pain conditions, the reported difference could significantly impair the drug discovery process. It is also emphasized that important differences (from 100- to 1000-fold in ligand potency) have been found when ligand-mediated effects in somatosensory neurons have been compared with those promoted by the same ligands in heterologous cells expressing cloned channel.[129]

Biographies

Pier Giovanni Baraldi received his degree in Chemistry in 1974 from the University of Ferrara, Italy, where he held a position of Lecturer in the Faculty of Pharmacy (1977−1980) and was Associate Professor of Medicinal Chemistry (1980−1987). In 1987, he became Full Professor of Medicinal Chemistry at the University of Bologna, Italy. In 1992, he returned to the University of Ferrara as Full Professor of Medicinal Chemistry. He has published 350 research papers including 40 patents in the following areas: synthesis of natural products possessing biological activity, prostaglandins, minor groove alkylating agents with antitumor activity (anthramycins, distamycins, and CC-1065 analogues), ligands for adenosine receptor subtypes (agonists and antagonists for A_{2A}, A_{2B}, and A_3 adenosine receptors), and TRP channel modulators.

Delia Preti received her B.S. degree in Medicinal Chemistry from Ferrara University, Italy, in 2001. In 2005 she obtained her Ph.D. in Pharmaceutical Sciences at the same university. Since 2005 she has worked at the Department of Pharmaceutical Sciences of Ferrara University in a postdoctoral research position in medicinal chemistry, focusing her research in the design, synthesis, and biological evaluation of adenosine receptors ligands, antitumor compounds inhibiting tubulin polymerization, and TRPA1 channel antagonists for the treatment of pain and inflammation. She has coauthored more than 50 publications including 3 international patents.

Serena Materazzi received her B.S. degree and her Ph.D. (with a thesis in the pathophysiology of aging) from the University of Florence, Italy. She has been Postdoctoral Fellow at the Departments of Phyisology and Surgery, University of California, San Francisco. She is currently Assistant Professor at the Department of Preclinical and Clinical Pharmacology of the University of Florence. Her research interest focuses on the molecular and translational medicine of byproducts of oxidative and nitrative stress and their role in the activation via transient receptor potential (TRP) channel stimulation of sensory neuronal pathways.

Pierangelo Geppetti received his M.D. from the University of Florence, Italy, and conducted postdoctoral studies at the Cardiovascular Research Institute of the University of California, San Francisco. He was Assistant Professor at the University of Florence and was Associate Professor at the University of Ferrara, Italy, until 2002. Since 2003 he has been Full Professor of Clinical Pharmacology at the Department of Preclinical and Clinical Pharmacology of the University of Florence. He is also the Director if the Headache Center of the Florence University Hospital of Careggi. The Geppetti research group focuses on pathophysiology of a subpopulation of peptidergic, primary sensory neurons that convey nociceptive signals and mediate

neurogenic inflammation and on the mechanisms, including TRP channels, that result in neuronal excitation or inhibition.

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