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Review

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Modulation of Thermoreceptor TRPM8 by Cooling Compounds

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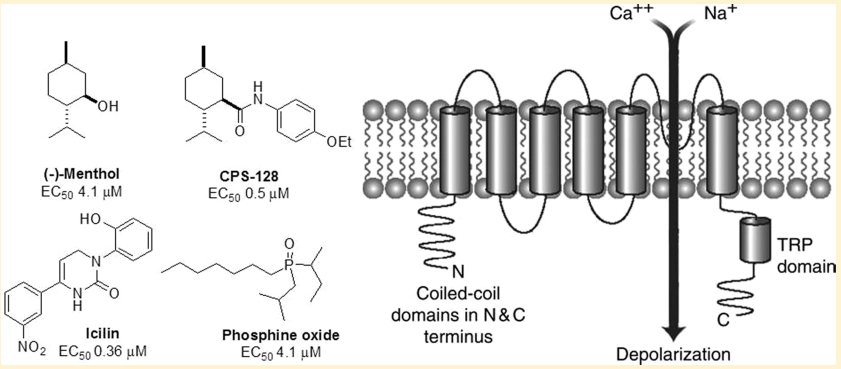

ABSTRACT: ThermoTRPs, a subset of the Transient Receptor Potential (TRP) family of cation channels, have been implicated in sensing temperature. TRPM8 and TRPA1 are both activated by cooling. TRPM8 is activated by innocuous cooling (<30 °C) and contributes to sensing unpleasant cold stimuli or mediating the effects of cold analgesia and is a receptor for menthol and icilin (mint-derived and synthetic cooling compounds, respectively). TRPA1 (Ankyrin family) is activated by noxious cold (<17 °C), icilin, and a variety of pungent compounds. Extensive amount of medicinal chemistry efforts have been published mainly in the form of patent literature on various classes of cooling compounds by various pharmaceutical companies; however, no prior comprehensive review has been published. When expressed in heterologous expression systems, such as *Xenopus* oocytes or mammalian cell lines, TRPM8 mediated currents are activated by a number of cooling compounds in addition to menthol and icilin. These include synthetic *p*-menthane carboxamides along with other class of compounds such as aliphatic/alicyclic alcohols/esters/amides, sulphones/sulphoxides/sulphonamides, heterocyclics, keto-enamines/lactams, and phosphine oxides. In the present review, the medicinal chemistry of various cooling compounds as activators of thermoTRPM8 channel will be discussed according to their chemical classes. The potential of these compounds to emerge as therapeutic agents is also discussed.

KEYWORDS: *Thermoreceptors, TRPM8, TRPA1, cooling compounds, menthol, icilin*

Recognition of temperature is a critical element of sensory perception and allows us to evaluate both our external and internal environments.[1,2] In vertebrates, the somatosensory system can discriminate discrete changes in ambient temperature, which activate nerve endings of primary afferent fibers. These thermosensitive nerves can be further segregated into those that detect either innocuous or noxious (painful) temperatures; the latter neurons being nociceptors.[3] Thermosensitive afferents express ion channels of the transient receptor potential (TRP) family that respond at distinct temperature thresholds, thus establishing the molecular basis for thermosensation.[4−13] The mammalian sensory system is capable of discriminating thermal stimuli ranging from noxious cold to noxious heat. Principal temperature sensors belong to the TRP cation channel family, but the mechanisms underlying the marked temperature sensitivity of opening and closing (gating) of these channels are unknown. Temperature sensing is tightly linked to voltage-dependent gating in the cold-sensitive channel TRPM8 (a transient receptor potential melastatin 8, the minty-cool ion channel) and the heat-sensitive channel TRPV1. Both channels are activated upon depolarization, and changes in temperature results in graded shifts of their voltage-dependent activation curves.[14−17] C. C. Galopin from Givaudan SA published a review discussing why cold and hot sensations are neither aroma nor taste but belong to a different class, often called chemesthetics.[18]

A wide range of temperatures span from cold to heat. Within this range, temperatures over about 43 °C and below about 15 °C evoke not only a thermal sensation but also a feeling of pain. In mammals, six thermosensitive ion channels have been reported, all of which belong to the TRP (transient receptor potential) superfamily. These include TRPV1 (VR1), TRPV2 (VRL-1), TRPV3, TRPV4, TRPM8 (CMR1), and TRPA1 (ANKTM1). These channels exhibit distinct thermal activation thresholds (>43 °C for TRPV1, >52 °C for TRPV2, > ∼34−38 °C for TRPV3, > ∼27−35 °C for TRPV4, < ∼25−28 °C for TRPM8, and <17 °C for TRPA1) and are expressed in primary sensory neurons as well as other tissues (Figure 1). The involvement of TRPV1 in thermal nociception has been demonstrated

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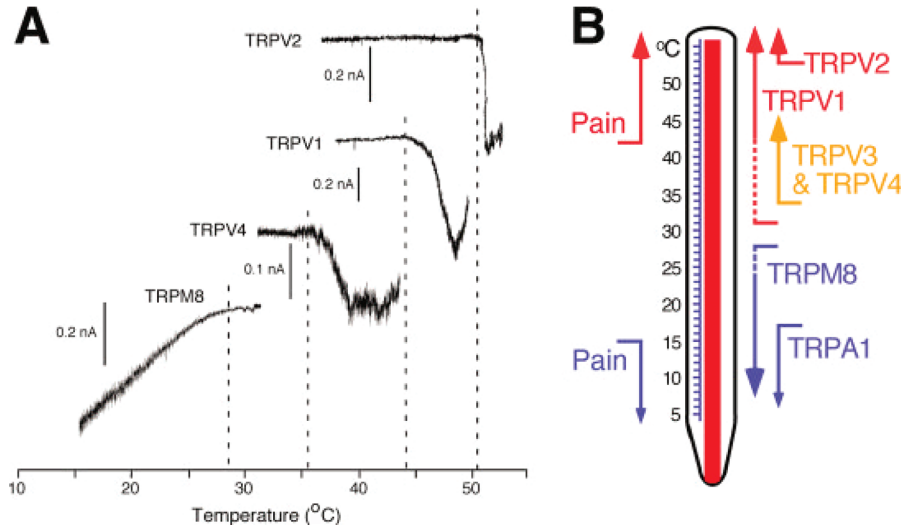

Figure 1. (A) Temperature response profiles of heat- or cold-induced activation of TRPV1, TRPV2, TRPV4, and TRPM8 at a holding potential of 60 mV in HEK293 cells expressing these ion channels. Dotted lines indicate the threshold temperatures for activation. (B) Temperatures causing pain and activating six TRP channels. Dotted lines indicate that threshold temperatures for activation of TRPV1 and TRPM8 are not fixed but changeable in the presence of other stimuli.

by multiple methods, including the analysis of TRPV1-deficient mice. TRPV2, TRPM8, and TRPA1 are also very likely to be involved in thermal nociception because their activation thresholds are within the noxious range of temperatures.[4,8,19,20] Thermo-TRP channels have a modular C-terminal domain that is able to determine channel phenotype regarding temperature sensitivity, channel gating kinetics and PIP2 (phosphatidylinositol-4,5-bisphophate) modulation. Thus, thermo-TRP channels contain an interchangeable specific region, different from the voltage sensor, which allows them to sense temperature stimuli.[21] TRP channels are directly involved in sensory modalities such as vision, taste, olfaction, hearing, touch, thermal perception, and nociception. Among several thermoTRPs, primarily TRPM8 and TRPA1 have been implicated in cold sensation.

Extensive amount of medicinal chemistry efforts have been published mainly in the form of patent literature on various classes of cooling compounds as activators of these TRPs. In recent years, several excellent reviews have been published on the physiology and functions of these cold TRPs.[22−27] Furthermore, several studies have shown TRP channels as potential therapeutic targets for a variety of diseases such as pain, cancer, respiratory diseases, etc.[28−36] Viana and Ferrer-Montiel[37] reviewed the patent literature of TRPA1 modulators which are in preclinical development. McKemy[38] reviewed the therapeutic potential of TRPM8 modulators, and Harteneck et al.[39] discussed various blockers of TRP channels. In the present review, a critical account on various cooling compounds and their activity toward activation of TRPM8/TRPA1 ion channels have been reviewed. The review is restricted to only cooling compounds and does not cover other thermoTRP activators. We systematically searched, analyzed, and summarized the publication and patent literature for various cooling compounds as activators of thermoTRPs until December, 2011. The patent information for this review is drawn from Scifinder, Esp@cenet, and freepatentonline.com databases.

■ COLD SENSORY CHANNELS AND COOLING COMPOUNDS

Ion channels belonging to the transient receptor potential (TRP) superfamily that are activated by distinct temperature thresholds are referred to as thermo-TRPs, and their targeting represents a novel and promising strategy in pain relief. Four of these channels (TRPV1−TRPV4) respond to heat, and two others (TRPA1 and TRPM8) are sensitive to cold. TRPM8 is an ion channel that alters how much sodium and potassium crosses the membrane of these nerve cells. Concentrations of these ions control the cells release of glutamate. Glutamate released from the TRPM8-containing nerve cells can inhibit other nerve cells that pass along pain messages to the central nervous system. Activating TRPM8 nerve cells with cooling compounds therefore blocks the transmission of pain messages. In cases of chronic pain, the central nervous system becomes sensitized, so an ordinary touch, such as that from a bed sheet, can be turned into a painful experience. Application of cooling compounds icilin or menthol to the skin of a person with chronic pain has been proved to be an effective pain-killer treatment.[40] All thermoTRPs are gated Ca^{2+} channels consisting of six transmembrane domains (TM1−TM6) flanked by large N- and C-terminal cytoplasmic domains. A schematic representation is shown in Figure 2 with the putative ion channel between TM5−TM6 in TRPM8, which is activated by menthol and other cold stimuli.

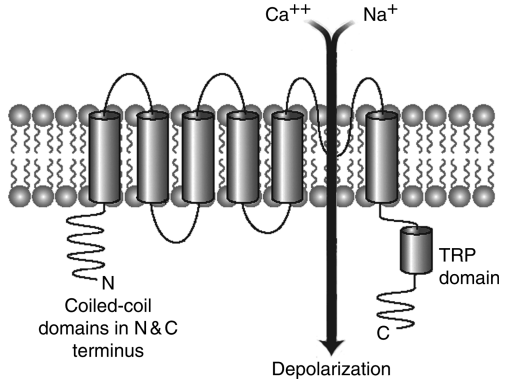

Figure 2. TRPM8 receptor channel.

TRPM8 is a cold and menthol receptor, and is activated at a temperature threshold of ~28 °C, with currents increasing in

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magnitude down to 8 °C,[41−43] thus spanning both innocuous cool to noxious cold temperatures. It is a calcium-permeable cation channel activated by cold, cooling compounds (such as menthol) and voltage, and is the main molecular entity responsible for the detection of cold temperatures in the somatosensory system. The activation mechanism involves voltage sensing of membrane potential, phosphatidylinositol 4,5-bisphosphate (PIP2), and Ca^{2+}.[44−46] In addition to menthol, a number of other cooling agents, including icilin, eucalyptol, and WS-3 activate TRPM8 in vitro.[41] The former of these compounds, icilin, is considered a supercooling agent since it has higher potency and efficacy than menthol in cellular and behavioral studies.[41,47] However, icilin appears to activate the channel in a manner that is divergent from other agonists, including cold.

The TRPM8 channel has been emerging as a therapeutic target for variety of diseases. TRPM8 is overexpressed in a range of cancers, including prostate, breast, lung, and colon. In prostate cancer cells, Ca^{2+} and Na^{+} inflow through TRPM8 is necessary for survival and function, including secretion at the apical membrane, but the function of TRPM8 in these cells is not really known. It may well differ from the role of TRPM8 as a cool sensor in sensory nerve cells. Androgen unresponsive prostate cancer is difficult to treat effectively, and there are limited diagnostic and prognostic markers available. TRPM8 is a potential tissue marker in differential diagnosis and a potential prognostic marker for androgen-unresponsive and metastatic prostate cancer. As a consequence of its ability to convey Ca^{2+} and Na^{+} and its expression in only a limited number of cell types, TRPM8 is considered to be a promising target for pharmaceutical, immunological, and genetic interventions for the treatment of prostate cancer.[48−52] Furthermore, the TRPM8 channel is also expressed in human melanoma G-361 cells, and activation of the channel produces sustainable Ca^{2+} influx. Menthol elevates cytosolic Ca^{2+} concentration in a concentration-dependent manner with an EC_{50} value of 286 μM in melanoma cells. Menthol-induced responses were significantly abolished by the removal of external Ca^{2+}. Moreover, inward currents at a holding potential of −60 mV in melanoma cells were markedly potentiated by the addition of 300 μM menthol. The viability of melanoma cells was dose-dependently depressed in the presence of menthol. A functional TRPM8 protein is expressed in human melanoma cells to involve the mechanism underlying tumor progression via the Ca^{2+} handling pathway, which indicates TRPM8 as a promising target for drug development for malignant melanoma.[53,54] The TRPM8 channel is also emerging as a therapeutic target for the diagnosis and/or treatment of respiratory diseases[55,56] as well, as its role is implicated in human urinary bladder disorders.[57]

TRPA1, a noxious cold sensor, displays broad specificity to many chemically damaging molecules both in vitro and in vivo, and functions as a central chemical nocisensor.[58,59] The cooling compound icilin, a known agonist for TRPM8,[41] also activates TRPA1 currents, although with reduced potency compared to that of TRPM8.[60] Thus, due to its expression pattern and temperature threshold, TRPA1 has been proposed to be a detector of noxious cold in nociceptive afferents.[60] Pungent compounds such as isothiocyanates, cinnamaldehyde (cinnamon), methyl salicylate (wintergreen oil), eugenol (clove oil), gingerol (ginger), allicin (garlic), and linalool (pepper) activate TRPA1 in vitro.[61−63] In addition to these pungent compounds, the inflammatory peptide bradykinin also activates TRPA1 currents in a G-protein-coupled receptor-dependent manner, presumably via phospholipase C (PLC).[61] Moreover, it has also been postulated that TRPA1 plays an important role in inflammatory hypersensitivity in that the channel may be activated in a receptor-operated mechanism, perhaps through activation of PLC, by proalgesic or pro-inflammatory mediators.[61,64] Recently, Karashima et al. provided several lines of evidence to establish that TRPA1 acts as a cold sensor in vitro and in vivo.[65]

In the 1970s, Wilkinson Sword Ltd. conducted an extensive research program under the company leadership of Roy Randolph. During this period, Watson and co-workers designed and evaluated about 1200 compounds for their cooling activity. The interest in such compounds relates to cooling sensation without the minty and volatile side effects of menthol such as eye irritation from aftershave lotions, etc. Of these original Wilkinson Sword compounds, three have now been successfully commercialized viz. WS-23 (2-isopropyl-*N*,2,3-trimethylbutyramide; FEMA-3804); WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide; FEMA-3455); and WS-5 [ethyl 3-(*p*-menthane-3-carboxamido)-acetate; FEMA-4309]. Four more related carboxamides have recently received FEMA-GRAS status viz. *N*-(4-cyanomethylphenyl)-*p*-menthanecarboxamide, FEMA-4496; *N*-(2-(pyridin-2-yl)ethyl)-3-*p*-menthanecarboxamide, FEMA-4549; cyclopropanecarboxylic acid (2-isopropyl-5-methyl-cyclohexyl)-amide, FEMA-4558; and *N*-ethyl-2,2-diisopropylbutanamide, FEMA-4557.

These initial landmark discoveries of Wilkinson Sword Ltd. were followed in subsequent years by several other pharmaceutical companies, which primarily include Givaudan SA, Millennium Specialty Chemicals, Takasago International Corp., Symrise GMBH & Co KG, International Flavors & Fragrances Inc., Firmenich SA, and several others. All WS compounds along with compounds discovered in recent years by different companies have been discussed in subsequent sections. For the purpose of this review, cooling compounds have been divided in 8 different chemical classes: menthol and its natural analogues, menthol-inspired synthetic analogues, carboxamides/ureas, sulphones/sulphoxides/sulphonamides, aliphatic/alicyclic alcohols, carboxylic acids/esters/amides, heterocyclics, cyclic ketoenamines/*N*-aryl lactams, and miscellaneous. A short account of TRPM8 antagonists is also provided at the end.

MENTHOL AND ITS NATURAL ANALOGUES

Menthol (**1**), a cyclic terpene alcohol found in leaves of the genus *Mentha*, is used in a wide range of products, such as confectionary, candy, toothpastes, vaporubs, and aromatherapy inhalations. Among 8 possible stereoisomers of menthol, only the (−)-menthol enantiomer possesses the clean desirable minty odor and intense cooling properties.[66] Historically, menthol is well-known cooling agent, and a large number of menthol based cooling agents are known in the literature. When applied at low concentrations to the skin or mouth, menthol (**1**) elicits a pleasant cool sensation, while higher doses cause burning, irritation, and pain.[67−69] Menthol has been reported to evoke pain in humans through activation and sensitization of C-fibers.[70] Conversely, prolonged exposure to large doses of menthol adapt or desensitize cold-sensitive neurons, a process analogous to that of capsaicin and heat-sensitive fibers. Menthol application in the mouth transiently prevents the irritancy of concomitant or subsequent capsaicin exposure, but very few studies have directly assessed the analgesic properties of menthol, and it remains to be seen if menthol or other cooling compounds can be used as effective analgesics.[71] One of the studies shows that (−)-menthol

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exhibits analgesic properties through selective activation of k-opioid receptors.[72]

A number of studies are reported toward the identification of the molecular mechanism of action of menthol for producing the cold sensation. Menthol exerts its actions on cold-sensitive fibers by raising their temperature threshold.[41,73−75] Cold and menthol have a common molecular site of action, activating a Ca^{2+} permeable channel.[76] Reid et al.[77] discovered an inward ionic current that is activated by moderate cooling in a small number of rat sensory neurons. This current has features that are found in intact cold receptors, including sensitization by menthol, adaptation upon sustained cooling, and modulation by calcium, and is likely to be important in cold sensing. Menthol stimulates entry of Ca^{2+} and increases intracellular Ca^{2+} concentration in cold-sensitive neurons; thus, stimulation of cold receptors by menthol can be explained more simply by sensitization of the cold-induced inward Ca^{2+} current.[78] Tsuzuki et al.[79] reported that menthol can act directly on presynaptic Ca^{2+} stores of sensory neurons to release Ca^{2+} resulting in the facilitation of glutamate release and the modulation of neuronal transmission at sensory synapses.

Bandell et al.[80] investigated the mechanism by which menthol activates mouse TRPM8. Using a high-throughput mutagenesis approach, Bandell and co-workers screened a random mutant library consisting of ∼14,000 individual TRPM8 mutants for clones that are affected in their response to menthol while retaining channel function. They identified determinants of menthol sensitivity in two regions: putative transmembrane segment 2 (S2) and the C-terminal TRP domain. Analysis of these mutants indicated that activation by menthol involves a gating mechanism distinct and separable from gating by cold, voltage, or PIP_2. Notably, TRP domain mutations mainly attenuated menthol efficacy, suggesting that this domain influences events downstream of initial binding. In contrast, S2 mutations strongly shifted the concentration dependence of menthol activation, raising the possibility that S2 influences menthol binding. Further, it has been reported that, a lipid second messenger, PIP2 plays a central role in both the activation and desensitization of TRPM8.[81]

Menthol (**1**) is also known to activate TRPA1 channel. In whole-cell and single-channel recordings of heterologously expressed TRPA1, submicromolar to low-micromolar concentrations of menthol cause channel activation, whereas higher concentrations lead to a reversible channel block. Further, TRPA1-mediated menthol responses were also observed in mustard oil-sensitive trigeminal ganglion neurons.[82] Xiao et al.[83] found that only human TRPA1 is activated by menthol, whereas TRPA1 from nonmammalian species are insensitive to menthol. In addition, Macpherson et al.[84] show that menthol could also stimulate heat-activated TRPV3 but at concentrations well above that needed to activate TRPM8.

Topical menthol application has recently been proposed as a possible model for the study of cold pain. Hatem et al.[85] characterized the psychophysical effects of 30% L-menthol in ethanol on glabrous skin in 39 healthy subjects, using a double-blind, randomized, crossover design, with ethanol as a control. Psychophysical testing included an assessment of pain thresholds and detection of mechanical, cold, and heat stimuli, and of the sensations induced by suprathreshold stimuli. Most subjects (90%) perceived a cooling sensation with menthol. Menthol decreased cold pain thresholds and enhanced pain responses to suprathreshold noxious cold stimuli, without affecting responses to other stimuli. Menthol therefore has selective effects on noxious cold processing. No subject displayed signs of skin irritation or redness. This data suggests that 30% menthol application may be a useful experimental model for studies of cold hyperalgesia in humans. The absence of local skin reactions also makes this test potentially suitable for use in patients. Namer et al.[86] studied psychophysical effects of both TRPA1 and TRPM8 activation in humans by application of either cinnamaldehyde or menthol. This study concluded that agonists of TRPA1 and TRPM8 channels produce strikingly different psychophysical patterns. Menthol has also been reported to activate the $GABA_A$ receptor ($GABA_AR$) in hippocampal neurons in culture.[87]

Apart from menthol (**1**), several other natural terpenes exhibit cooling properties. (−)-Isopulegol (**2**) produces cooling sensation, imparts a feeling of freshness, crispness, and coolness to citrus type fragrances and is sold under the name Coolact P (FEMA-2962) by Takasago International.[88] Further, *cis*- and *trans-p*-menthane-3,8-diols (**3a** and **3b**) (PMD38 or Coolact 38D in a ratio of 62:38, FEMA-4053),[89,90] cubebol (**4**, FEMA-4497),[91,92] and 1,8-cineole (**5**, eucalyptol)[93−95] have been patented by Takasago International, Firmenich, and Bayer Corp., respectively, as cooling agents. Peppermint oil is currently used to impart cooling in oral products such as toothpaste, mouthwash, chewing gum, candy, and other food products. Peppermint oil generally comprises about 45−55% menthol, 20−25% menthone, 5% menthyl acetate, 5% eucalyptol, and many other constituents. Peppermint oil is even used in non-peppermint products, such as spearmint or wintergreen flavored products, in order to create the desired cooling effect. Menthone (**6**) (the precursor of menthol in monoterpene biosynthesis), elicits very small currents in TG neurons or TRPM8-expressing cells. L-Carvone (**7**) (present in spearmint or Kuromoji oil), geraniol (**8**) (an acyclic monoterpene alcohol found in lemongrass and aromatic herb oils), *cis*- and *trans-p*-menthane-3,8-diol (**3a** and **3b**) (*E. citriodora*), isopulegol (**2**, *M. pulegium* or *Lilium ledebourii*; patented as Coolact P), hydroxy-citronellal (**9**) (from citronella oil, volatile oils, such as lemon, lemongrass, or melissa oil), and linalool (**10**) (an acyclic monoterpene alcohol found in the floral scents of Onagraceae species) produce an increase in intracellular Ca^{2+} levels in mTRPM8-HEK293 cells.[96] Among various natural cooling agents, only (−)-isopulegol (**2**) and PMD38 (**3**) showed a significant effect on TRPM8 channel activation in HEK cells, with EC_{50} values of 66 and 31 μM, respectively.[97] Chemical structures of cooling agents **1**−**10** are shown in Figure 3.

MENTHOL INSPIRED SYNTHETIC COOLANTS

Menthol (**1**) is still widely used since it has a definite refreshing effect and is available at low cost both by extraction from plants or by chemical synthesis. In spite of this fact, the use of menthol holds some drawbacks, including high volatility, a strong mint odor, and a bitter and burning taste when used in high concentrations. Menthol exhibits some undesirable properties such as strong stinging smell or somewhat bitter taste and it has relatively high volatility. These disadvantages of menthol have somewhat limited its utility in various applications and therefore stimulated an intense search for suitable physiological cooling agents that possess low volatility and exhibit relatively low odor or even no odor at all.

Since the 1960s, many synthetic efforts have been focused toward the discovery of new substances with a powerful cooling effect to overcome menthol drawbacks. To this end, three main

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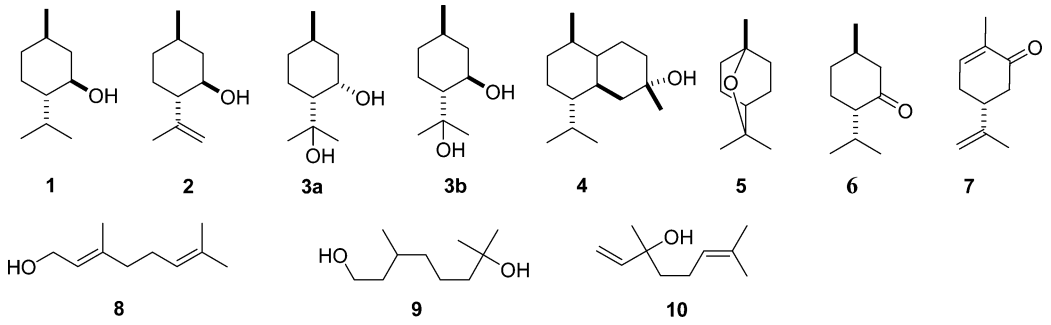

Figure 3. Naturally occurring monoterpenes with physiological cooling property.

approaches have been investigated: (a) study of terpenes and sesquiterpenes with an oxygenated *p*-menthane framework: a search within the menthol family produced two commercially successful coolants, Coolact P (FEMA-2962) and Coolact 38D (FEMA-4053). Most of other *p*-menthane derivatives are not free of disadvantages similar to L-menthol: strong smell, taste, volatility, etc. From this study, it was observed that the *p*-menthane carbon skeleton is not an absolute prerequisite for cooling properties, (b) modification of the L-menthol molecule making it "heavier." This approach produced at least 3 commercially successful physiological coolants of the menthoxy-type and several promising candidates, also at least one commercially successful N-monosubstituted amide type coolant. N-Monosubstituted amides are typically stronger coolants thanmenthoxy ones. (c) A search among molecules structurally unrelated to L-menthol. This approach produced at least 1 commercially successful physiological coolant of the amide type (WS-23) along with several promising candidates, including the MPF (methyl pyrrolidinyl furanone).[98]

Menthol Esters. (−)-Menthyl lactate (**11**, FEMA-3748, sold as Frescolat ML by Haarmann & Reimer) occurs naturally in peppermint oil, is faintly minty in odor, and is virtually tasteless, with a pleasant, long-lasting cooling effect.[99,100] It is commercially available as a white, low-melting point solid mass which needs to be physically broken down and melted before use in food or cosmetic applications. These physical properties make this compound difficult to work with. Consequently, attempts have been made to provide the compound in a more convenient form. These efforts led to invention of its crystalline form as well as its comixture with menthol carboxamide, which is stable and is present in liquid form.[101] Other menthol esters such as menthyl-3-hydroxybutyrate (**12**),[102] menthyl acetate (**13**),[103] and monomenthyl succinate (**14**, FEMA-3810, Physcool)[102] have been used for their cooling properties in various flavored and perfumed products.[104] Menthol ester **14** also occurs in nature in *Lycium barbarum* and *Mentha piperita*.[105] Two more menthol esters viz. monomenthyl glutarate (**15**, FEMA-4006) and dimenthyl glutarate (**16**) were identified in *Litchi chinesis*.[105] Monomenthyl succinate (**14**) was patented first as a tobacco additive in 1963[106] and later patented as a cooling agent in 1998.[107] Carbonate esters of menthol, **17**, and **18** intitially used as flavorants in tobacco[108] were later found to possess physiological cooling properties.[109] Menthol ethylene glycol carbonate (**17**) is known as Frescolat type MGC (FEMA-3805).[109] Another patented compound was menthol propylene glycol carbonate (**19**) (Frescolat type MPC), FEMA-3806.[109] Quest International patented menthyl pyrrolidone carboxylate (**20**, Questice, FEMA-4155) as a slow release coolant with the added benefit of skin conditioning. Its cooling property is considered not to be intrinsic, but on the skin, Questice is hydrolyzed by the skin's natural enzymes to produce menthol, giving an extended cooling sensation. Pyrrolidone carboxylic acid, the natural moisturizing factor (NMF), is also released during this process.[110] International Flavours and Fragrances Inc. (IFF) patented a series of menthyl half acid ester derivatives **21**−**27**, one of which is *N*,*N*-dimethyl menthyl succinamide (**21**, FEMA-4230), which showed cooling and refreshing effects on the tongue, palate, and front gums; fruity flavor with estery topnotes and sour undertones (cooling onset time = 25 s; and cooling duration = 11.25 min) was also shown.[111−113] (−)-Menthyl lactate (**11**) showed activation of the TRPM8 channel in an oocyte expression system at an EC_{50} of 163 μM (EC_{50} for menthol is 193 μM).[114] Structures of menthol esters **11**−**27** are shown in Figure 4.

Menthol Ethers and Other Related Acyclic Ethers. Bayer Corp. have reported several menthol ethers such as menthyl methyl ether (**28**, FEMA-4054), menthyl ethyl ether (**29**), menthyl propyl ether (**30**), menthyl isobutyl ether (**31**), and other ethers such as isopulegyl methyl ether (**32**) and acyclic ethers **33**−**35** as cooling compounds in the preparation of various confectionery products such as chewing gum, toothpaste, etc.[93−95] (−)-Menthoxypropane-1,2-diol (**36**, MPD, FEMA-3784, Coolact-10)[90,115] is another commercial cooling agent. Takasago reported a cooling threshold (in mouth) of **36** as 1 ppm (about 20−25% that of menthol) and the time of cold feeling maintenance as 20−25 min for a 100 ppm solution (about twice that of menthol). While the cooling strength of **36** is accepted as being about 20−25% that of menthol, interestingly, in a vaseline ointment, **36** showed a cool feeling 2.0 to 2.5 times stronger than that of menthol.[90,116] MPD (**36**) is produced from a reaction between glycerine with *l*-menthol by ether conjugation,[115−118] resulting in MPD being more hydrophilic (log*P* 2.5) than *l*-menthol (log*P* 3.2). MPD has also been used as a skin permeation enhancer for other therapeutic drugs.[119,120] MPD showed activation of the TRPM8 channel in MEK cells at an EC_{50} of 6 μM.[97] Takasago also claimed similar cooling compounds, hydroxyethyl-menthyl ether (**37**, Coolact-5, FEMA 4154), and an analogue of MPD with an additional methyl group in the glycerine part of molecule **38** (FEMA 3849).[90] Structures of menthol esters **28**−**38** are shown in Figure 5.

N-Substituted *p*-Methane-3-carboxylic Acid Esters and Amides. The commercial need for nonvolatile cooling agents devoid of menthol's negative attributes was originally addressed in the Wilkinson Sword Ltd. research laboratories in the seventies. Extensive studies led to the discovery and development of a new family of nonvolatile cooling agents, the menthane carboxamides.[121,122] Initially considered for cosmetic

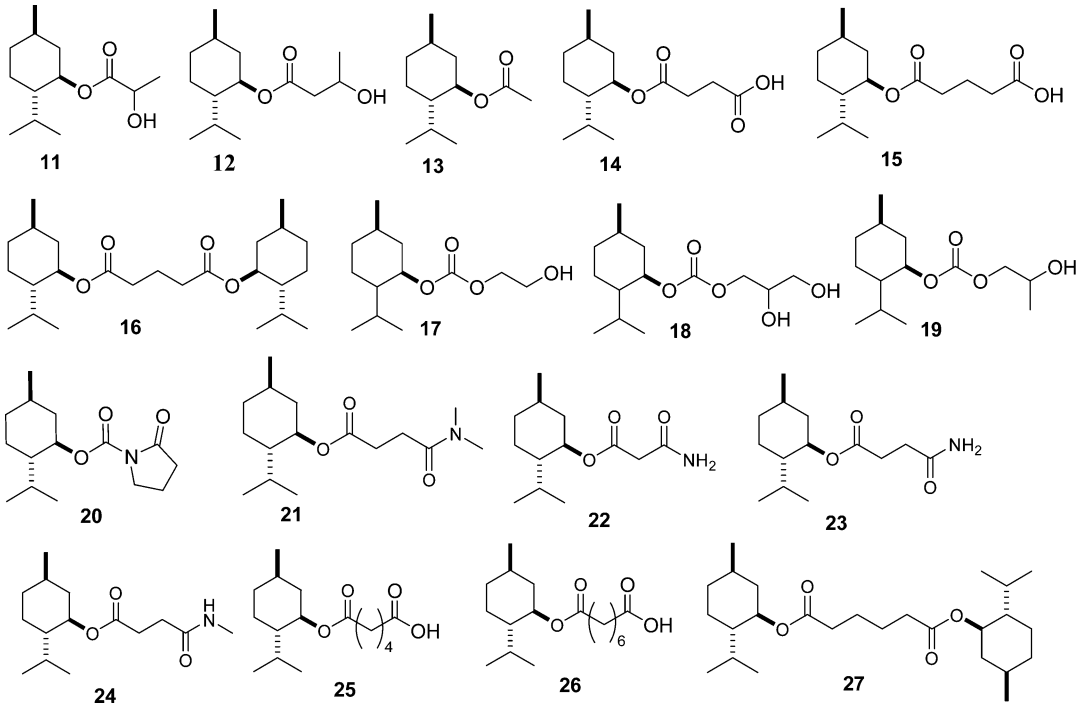

Figure 4. Menthol esters.

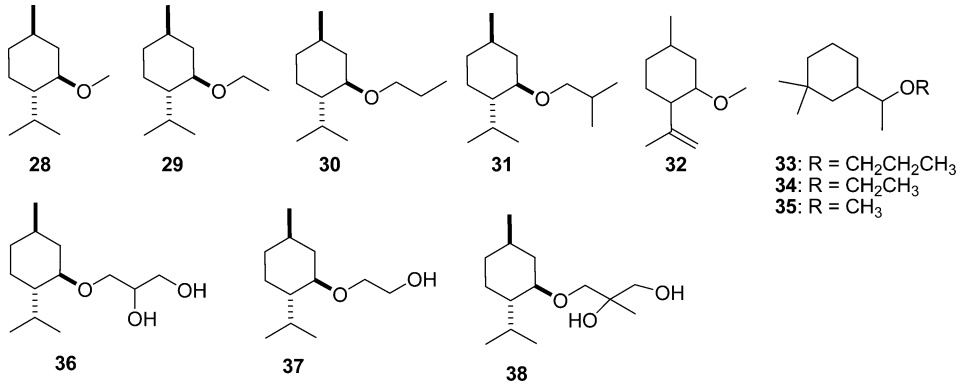

Figure 5. Menthol ethers and other related acyclic ethers.

applications, this class of molecules was further investigated for use in confectionary and oral care products. Several compounds were later on successfully brought to the market. Watson and co-workers designed and evaluated about 1200 compounds for their cooling activity. The interest in such compounds relates to cooling sensation without the minty and volatile side effects of menthol such as eye irritation from aftershave lotions, etc. WS-1 (**39**) was a key synthetic intermediate which is 16 times weaker than L-menthol (**1**).[123] Watson and co-workers synthesized several esters of WS-1 (**39**). Compounds WS-4 (**40**) and WS-30 (**41**) are ester analogues of coolact-5 (**37**) and coolact-10 (**36**), respectively. WS-30 (**41**) can be a less expensive alternative for coolact-10.[123] WS-30 (**41**) showed dose-dependent and reversible activation of the TRPM8 channel with an EC_{50} value of 5.6 μM.[124] Structures of *p*-menthane-3-carboxylic acid esters **39**−**41** are shown in Figure 6.

Researchers from Vector Tobacco Inc. reported the use of WS-3 (**42**, FEMA-3455) as a cooling agent in tobacco products.[99,100] It is white, crystalline, almost odorless, and mainly used as a coolant in medicinal preparations, oral care products, and confectionery products. Researchers from Wilkinson Sword Limited

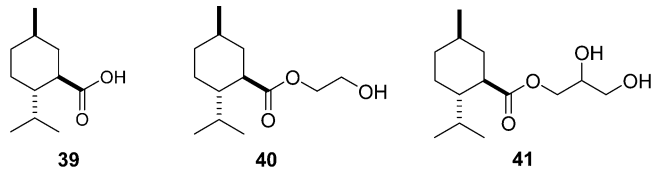

Figure 6. N-Substituted *p*-methane-3-carboxylic acid esters.

discovered several derivatives of WS-1 viz. **42**−**50**, all showing cooling thresholds less than 1 μg.[122,125−130] Compounds **42** (WS-3), **43** (WS-5, FEMA-4309), and **47**−**49** (**49**: WS-12) showed a cooling sensitivity threshold of $\geq$0.3 μg.[127] The absence of commercial interests in compounds **47**−**49** is recognized due to its toxicity considerations implicated by the benzene ring. However, it has been unclear as to why glycine derivative **43** (WS-5) has not attracted commercial interest as it is approximately 1.5 times stronger than WS-3 (**42**).[125,126] The reason for this was identified by German co-workers: compound **43** (WS-5) imparts a more bitter taste to a flavor composition when compared to the control. Recently, Sherkheli et al.[131]

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showed that WS-12 (**49**) selectively activates TRPM8 ion channels. WS-12 (**49**) robustly activated TRPM8 at low micromolar concentration (EC_{50} 12 μM) thereby displaying a higher potency and efficacy compared to menthol (EC_{50} 196 μM) ina *Xenopus oocytes* expression system. However, WS-12 activated TRPM8 at nanomolar concentrations (EC_{50} 193 nM) in HEK cells.[124] Other thermo-sensitive TRP ion channels including TRPV1, TRPV2, TRPV3, TRPV4, and TRPA1 were not activated at a concentration (1 mM) optimally effective for TRPM8 responses.[131] CPS-369 (**43**, WS-5) and CPS-368 (**44**) activated TRPM8 in an oocyte expression system with EC_{50} values of 84 and 104 μM, respectively.[114]

Menthane carboxamides are generally prepared from L-menthol in three steps.[132] Millennium Specialty Chemicals have reported the synthesis of an optically pure form of WS-5 (**43**).[133] Wilkinson Sword Ltd. synthesized WS-3 (**42**) and WS-14 (**46**) starting from WS-1,[125] while Millennium Specialty Chemicals synthesized WS-3 (**42**) using an alternative method starting from *p*-menthane nitrile.[133] Highly purified (1*R*,3*R*,4*S*)-WS-5 (**43**) showed cooling about 2.5−3.0 times stronger than that of WS-3 (**42**).[98] Philip Morris Inc. reported the synthesis of *N*-*t*-butyl-*p*-menthane-3-carboxamide (WS-14, **46**).[134,135]

Wei discovered *p*-menthane carboxamide CPS-128 (**51**), which is an ethyl analogue of WS-12, showing a cooling threshold of 0.1 μg and an activation of TRPM8 channel at EC_{50} 0.5 μM.[136] Bolddings et al.[124] characterized TRPM8 as a pharmacological receptor using effects of the carboxylic acid ester **41** and carboxamides **49**, **52**, and **44** by Ca^{2+} imaging experiments and whole-cell patch-clamp recordings on TRPM8 expressing human embryonic kidney (HEK), lymph node prostate cancer (LNCaP), and dorsal root ganglia (DRG) cells. Compounds **41**, **49**, **52** (CPS-124), and **44** (CPS-368) showed a dose-dependent and reversible activation of TRPM8 with EC_{50} values in the nanomolar to low micromolar range. Compounds **52** and **44** showed EC_{50} values of 1.2 and 3.6 μM. Carboxamide **49** (WS-12, CPS-112) is most potent (EC_{50} of 193 nM) in activating TRPM8. It is selective since other TRP proteins are not stimulated at micromolar concentrations, and its efficacy with respect to TRPM8 is similar to the one of icilin.[124] The structure **52** is named as CPS-113[124] as well as CPS-124[136] by two different research groups. Another *p*-menthane carboxamide CPS-125 (**53**) showed an activation of TRPM8 with an EC_{50} value of 30 μM in an oocyte expression system.[114]

Givaudan SA reported several analogues of N-monosubstituted *p*-methane-3-carboxamides among which **54** and **55** showed stronger activity than menthol, and *N*-(4-cyanomethylphenyl) *p*-menthane carboxamide (**55**, FEMA-4496) produced about 10 times more cooling effect as compared to menthol at 2 ppm. Compound **55** produced a cooling effect at 0.2 ppm for 93 min (menthol at 2 ppm produced a cooling effect for 35 min).[137−140] Givaudan SA discovered pyridyl ethyl substituted *p*-menthane analogue **56**, which exhibited a cooling effect at less than 2 ppm concentration.[141−144] Compound **56** (FEMA-4549) at 0.05 ppm concentration showed cooling equivalent to 2.0 ppm menthol (~40× more cooling).[145] The same group patented a series of new menthane carboxamides represented by structures **57** and **58** which had cooling strengths 100× stronger than menthol.[146] Further, phenylethyl carboxamides **59** and **60** have been discovered which showed cold receptor stimulant property with a cooling effect at <0.0005 ppm.[147] The relationship between the stereochemistry of the menthane core and the cooling profile was thoroughly studied in the case of menthol.[148] However, little is known about the influence of stereochemistry on the activity of menthane carboxamides. Recently, Furrer et al. discussed the effect of the stereochemistry on the cooling profile of menthane carboxamides. As menthane carboxylic acid is generally prepared from menthol (**1**) with retention of the stereochemistry, the concentration of neo-epimer is often negligible. Under harsh conditions, the acyl of the menthane carboxylic acid tends to epimerize. This epimerization was used to access a series of neo-epimers to investigate the effect of the stereochemistry on the cooling potency of menthane carboxamides. Throughout this series, a clear trend can be noticed. While the smaller molecules such as **39** still maintain comparable cooling intensities, the effect is much stronger for larger menthane carboxamides such as **54**−**56**[145] (Table 1).

Table 1. Activation of TRPM8 by Menthane Carboxamide and Neomenthane Carboxamide Derivatives

	TRPM8 activation[a]	
compd	(−)- (%)	neo- (%)
WS-1 (**39**)	130	50
54	127	1
55	131	3
56	127	15

[a]Values are expressed as percent of menthol (control).

Further, Wei has shown that several compounds related to WS-5 (**43**) possess strong cooling properties with remarkable cooling longevity. For example, the methyl and ethyl ester analogues of WS-5 (**43**) referred to as D-Ala-O-Me (**61**) and D-Ala-O-Et (**62**), respectively produced from D-alanine (rather than glycine). Similarly, when D-homoserine lactone is employed, the resultant compound is **63** (referred to as "D-HSL"), which also is a potent long lasting coolant. Compounds **61**−**63** showed cooling longevities of 114, 142, and 85 min, respectively. By combining suitable sympathomimetic amine drugs that act as α-adrenergic receptor agonists to form the corresponding *p*-menthane carboxamides, Wei discovered L-phenylephrine *p*-menthane carboxamide CPS-195 (**64**) which is effective as a long lasting coolant and possessed additional therapeutic properties.[149] These long lasting compounds are used in topical applications to skin to relieve irritation, itching, and pain due to inflamed skin, and are used to alleviate discomfort associated with skin damage caused by antiaging therapies. Long lasting *N*-alkylcarbonyl-D-amino acid hydroxyalkyl esters (NACE) are also used to get relief from coughs and airway obstruction. Use of a potent NACE as a cough suppressant to reduce virus dissemination and transmission in an influenza epidemic is a new concept. It may be especially useful to help protect health workers who treat infected individuals. Further, these compounds can be used in smoking cessation therapy. Other applications of these compounds are in getting relief from nausea, heat stress, and fever.[150] Recently, Wei reported *N*-alkylcarbonyl-D-amino acid hydroxyalkyl esters (NACHE), compounds **65** and **66**, with cooling effect.[151] In early 2004, T. Hasegawa Co. Ltd. patented a series of cooling compounds based on alkyloxy amides represented by structure **67** (Hase-1), which showed a cooling effect without any bitterness.[152]

Dendreon Corp. claimed four series of N-substituted *p*-menthane carboxamides as TRPM8 agonists, varying by type of moiety linked to the carboxylic acid group viz. substituted aniline **68**, aminoalkyl-biheteroaryl moiety **69**, phenethyl moiety **70**, and dihydrobenzimidazole **71** showing

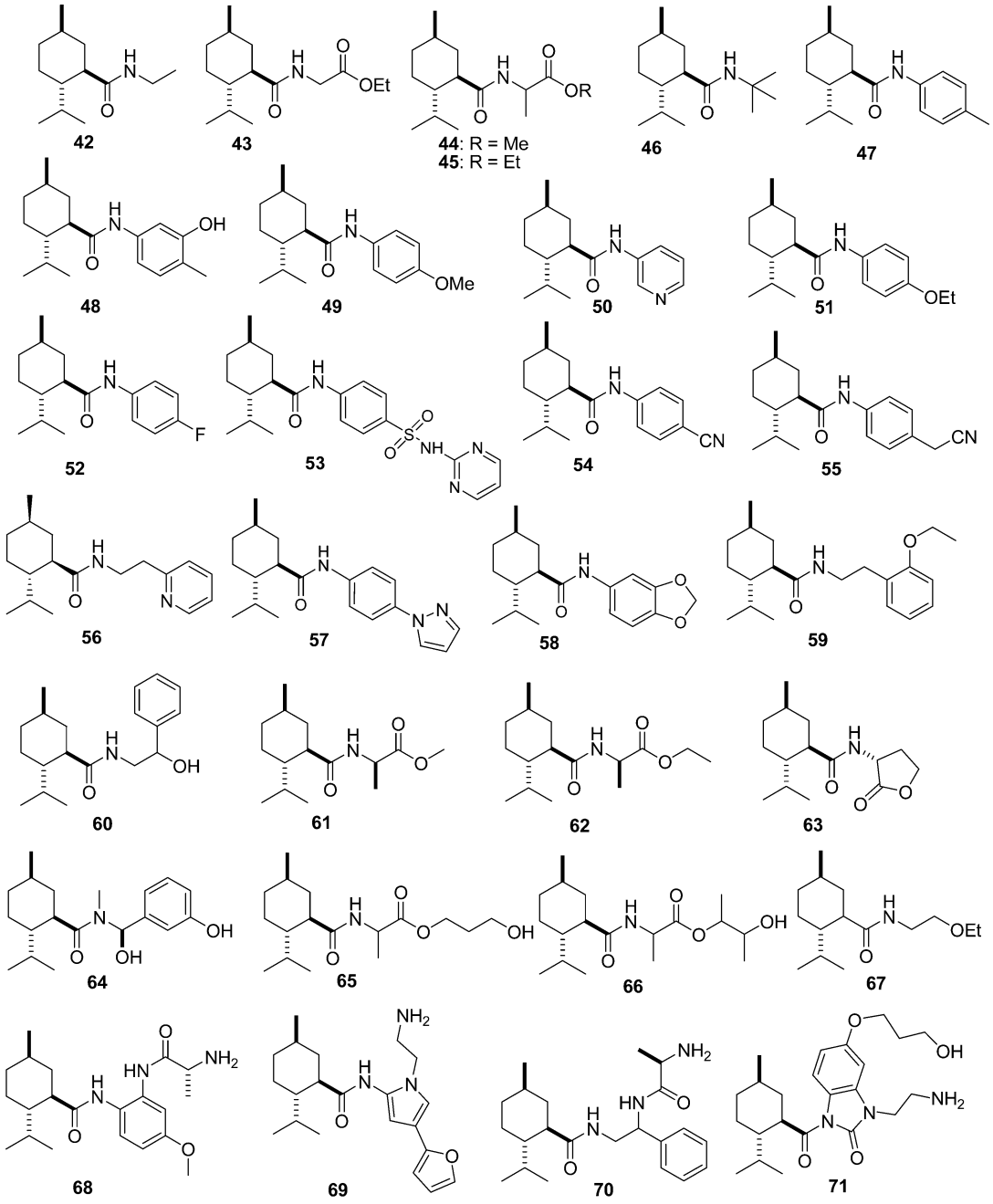

Figure 7. N-Substituted *p*-methane-3-carboxamides.

EC_{50} < 0.02 μM for TRPM8 activation.[153] Researchers from Dendreon Corp. identified Trp-p8, a prostate-specific gene which is up-regulated in prostate cancer and other malignancies (such as breast, colon, lung, and skin) and shares high homology with transient receptor potential calcium channel proteins.[154] Genentech Inc. claimed a series of *p*-menthane carboxamides as Trp-p8 agonists and observed an agonist specific killing of Trp-p8 expressing cells. One of the most potent *p*-menthane carboxamides, **49**, showed activity for 293/Trp-p8.c18 and 293/Trp-p8.c10 clones with IC_{50} < 0.5 μM.[155] Similarly, researchers from Dendreon Corp. discovered a series of *p*-menthane carboxamides as agonists of Trp-p8 activity which are capable of inhibiting the growth and/or induction of apoptosis in cells that express Trp-p8. Several *p*-menthane carboxamides showed EC_{50} values in the range of 0.05−0.20 μM (e.g., **60**) and 0.5−1 μM (e.g., **56**).[156] Structures of N-substituted *p*-methane-3-carboxamides **42**−**71** are shown in Figure 7.

2/3-Substituted *p*-Menthane-3-ols. Lever Brothers Co. reported hydroxymethyl or hydroxyethyl derivatives of *p*-menthane viz. 2-hydroxymethylmenthol (**72**), 2-(β-hydroxy ethyl)-menthol (**73**), 3-hydroxymethylcarvomenthol (**74**), and 3-(β-hydroxy ethyl)-carvomenthol (**75**) and their use in imparting a cooling property to a composition for the care of the oral cavity.[157] Bayer Material Science AG used 2,3-dihydroxy-*p*-menthane (**76**) as a cooling agent in flavored chewing foams along with several other coolants.[158] Recently, Fuganti et al. reported several 3-alkyl-*p*-menthan-3-ol derivatives and their their physiological cooling activity. In order to investigate the influence of the chemical structure on the cooling sensation, Fuganti and co-workers have accomplished stereoselective

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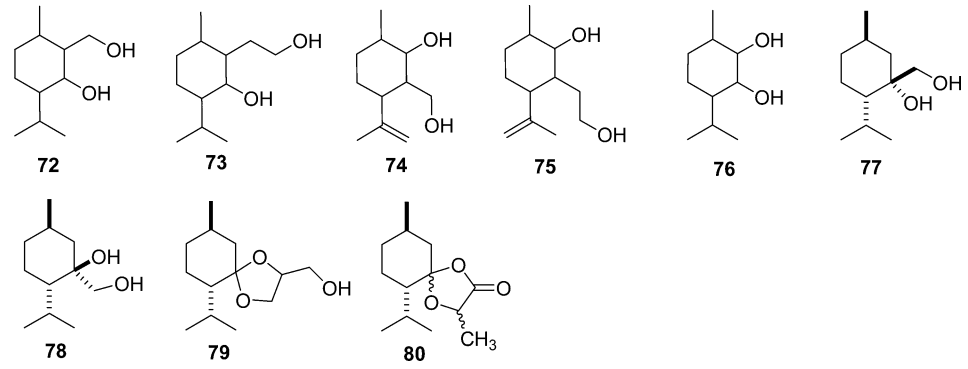

Figure 8. 2/3-Substituted *p*-menthane-3-ols.

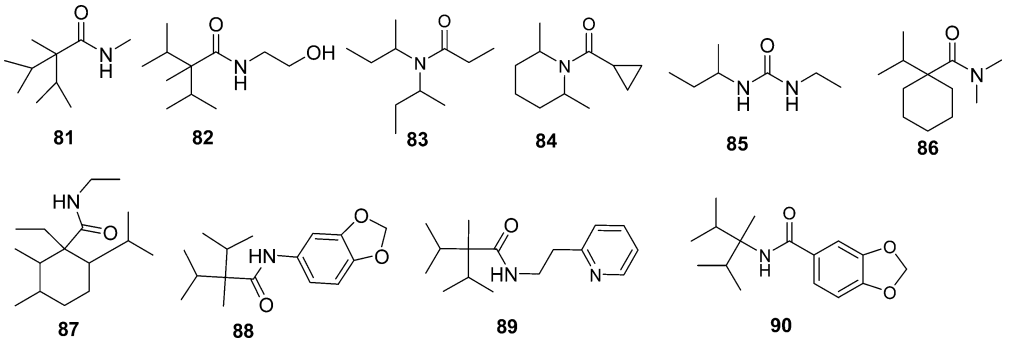

Figure 9. Acyclic/alicyclic carboxamides/ureas.

syntheses of 29 different 3-alkyl-*p*-methan-3-ol derivatives. All compounds obtained were odorless and were evaluated by taste, considering two sensations: a cooling effect and bitterness. A structure–activity relationship study highlights the idea that compounds with a (1*R*,4*S*)-configuration are the isomers with the more intense cooling effect and lower bitterness. In addition, the structure of the 3-alkyl chain affected the latter properties. Increasing the chain length over two carbon atoms does not change the cooling power, but enhances the bitterness with the additional feature that the branched isomers are considerably more bitter than the linear ones. Overall, the 3-alkyl-*p*-menthan-3-ol isomers with the best quality in terms of high cooling power and low bitterness are (1*R*,4*S*)-3-(hydroxymethyl)-*p*-menthan-3-ol diastereoisomers (−)-**77** and (−)-**78**.[159]

Further, *p*-menthane-3-ols with another oxygen at the 3-position as a part of another five-membered ring with 3-hydroxy of *p*-menthane forming ketals are reported with cooling activity. Bereman and Shi reported that menthane glycerol ketal (**79**, FEMA-3807) is capable of imparting cooling sensation to the oral cavity of a smoker upon inhalation of a smoke stream from the smoking article.[99,100] It is sold as Frescolat MGA by Haarmann & Reimer. Both the racemic and leavo-forms appear on the FEMA GRAS list, but the leavo-form appears to be the item of commerce.[160] Menthane glycerol ketal (**79**) activated the TRPM8 channel both in the oocyte expression system as well as in HEK cells with EC_{50} values of 184 μM[114] and 4.8 μM,[97] respectively. In 2006, Firmenich filed patent applications for the use of 6-isopropyl-3,9-dimethyl-1,4-dioxaspiro[4.5]decan-2-one (**80**, FEMA-4285), in the form of any one of its isomers or of a mixture thereof as having an enhanced refreshing cooling effect (with some minty, fruity notes).[161] Structures of 2/3-Substituted *p*-menthane-3-ols **72**–**80** are shown in Figure 8.

■ ALIPHATIC/ALICYCLIC CARBOXAMIDES/UREAS

Wilkinson Sword discovered acyclic carboxamide, WS-23 (**81**) exhibiting physiological cooling property.[162−164] Their search among different classes of organic compounds resulted in another commercially successful amide type coolant: WS-23 (**81**, FEMA-3804). They synthesized WS-23 (**81**) from a corresponding nitrile derivative in three steps.[165] Later on after about 2 decades, IFF[166] and Millennium Specialty Chemicals[133] synthesized WS-23 (**81**) in a single step starting from diisopropyl propionitrile. WS-23 (**81**) is an odorless white powder and shows high cooling activity with no side effects such as burning, stinging, or tingling sensations. Typical applications include use as a coolant in medicinal preparations, oral care products, and confectionery products.[133] Qaroma Inc. reported a series of alkoxy carboxamides with pronounced cooling effect on the skin and on the mucous membranes of the body. These compounds also possess good taste quality and low melting points with no malodor. Alkoxy carboxamide **82** showed activity equal to or higher than WS-23 (**81**).[167] Wilkinson Sword also discovered acyclic reverse amides **83**–**84** along with substituted urea **85** showing a cooling threshold ≥2 μg.[99,100,168−171] The same group also discovered cyclohexanamides **86**[172,173] and **87**[174] with cold receptor stimulant activity.

Givaudan SA discovered aryl carboxamides **88**[175−177] and **89**[143,144] which showed cooling effect at a concentration of less than 2 ppm. Further, this group reported a series of aryl carboxamide analogues with reversed amide configuration **90**, many with cooling intensities equal to or greater than WS-23 (**81**). Analogue **90** showed ∼2.2 times more cooling property as compared to the 2 ppm of menthol.[178,179] WS-23 (**79**) activated the TRPM8 channel both in an oocyte expression system as well as in HEK cells with EC_{50} values of 1500 μM[114] and 44 μM,[97] respectively. Structures of acyclic/alicyclic carboxamides/ureas **81**–**90** are shown in Figure 9.

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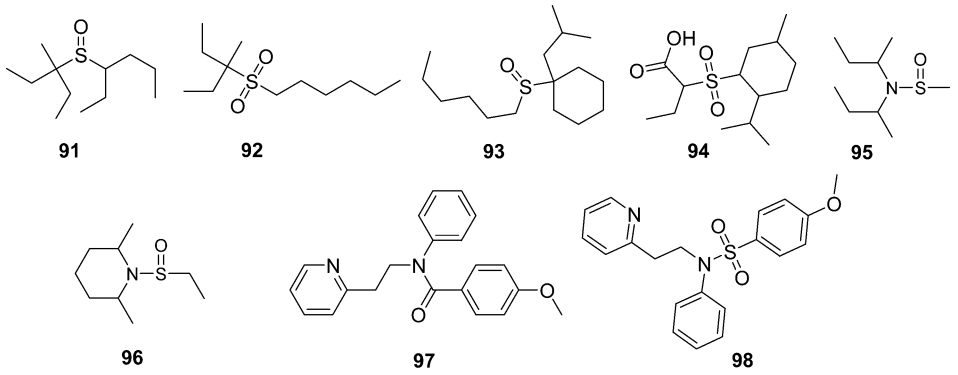

Figure 10. Sulphones, sulphoxides, and sulphonamides.

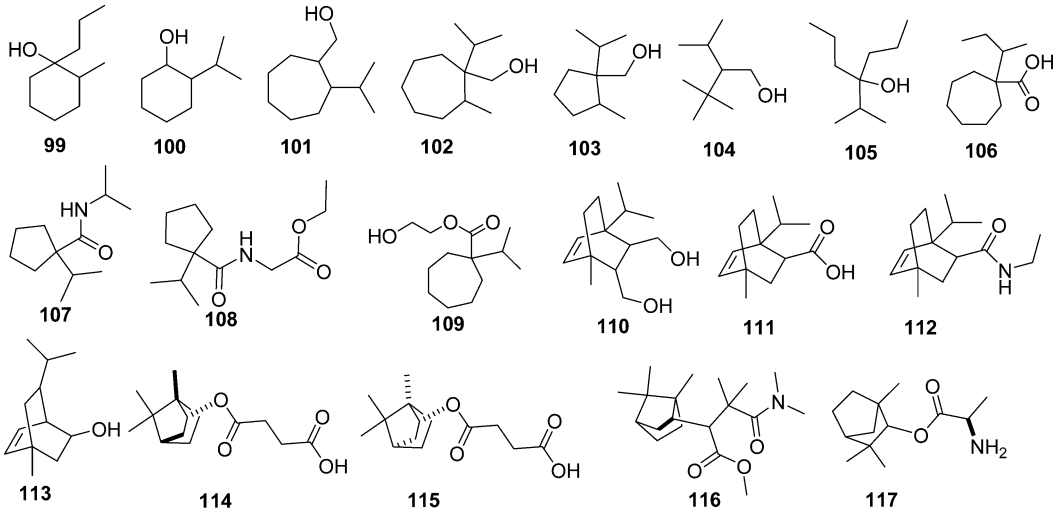

Figure 11. Alicyclic/cyclic alcohols, carboxylic acids, esters, and amides.

SULPHONES, SULPHOXIDES, AND SULPHONAMIDES

Wilkinson and Sword invented acyclic Sulphone **91**, sulphoxide **92**,[180] cyclic Sulphone **93**, and sulphoxide **94**[181] with promising physiological cooling properties. Sulphoxides were better than sulphones in their physiological cooling effect. Further, this group discovered several alkyl substituted sulphonamides represented by structures **95** and **96**, which showed a cooling threshold of 1 μg.[169−171] Givaudan SA discovered amide **97** and its sulphonamide analogue **98**, which exhibited slightly higher cooling intensity to that of menthol solution at 2 ppm.[182,183] Structures of sulphones, sulphoxides, and sulphonamides **91**−**98** are shown in Figure 10.

ALIPHATIC AND ALICYCLIC ALCOHOLS/ACIDS/ESTERS/AMIDES

Wilkinson Sword Ltd. discovered cyclohexane alcohols **99**−**100**,[184] cycloheptane, cyclopentane, and acyclic alcohols **101**−**104**[185] with physiological cooling properties. Further, a series of tertiary alcohols represented by structure **105** possessing physiological cooling property are also reported.[186] The same group discovered alkyl substituted carboxylic acids, esters, and amides **106**−**109** with a property for stimulating cold TRPs.[187,188] Givaudan SA reported a series of bicyclic [2.2.2] oct-5-ene derivatives **110**−**113** exhibiting cooling properties at a concentration of 2−5 ppm. Compound **110** possessed a cooling intensity similar to that of menthol at 2 ppm.[189] The same group claimed a series of terpene based cooling compounds represented by structures **114**−**117**. These compounds are useful in providing cooling sensations to the skin or mucous membranes of the body and have better cooling effects than menthol and WS-3. Compounds **114**−**117** showed a cooling effect at less than 0.001 ppm (menthol shows at 2 ppm).[190] Structures of alicyclic/cyclic alcohols, carboxylic acids, esters, and amides **99**−**117** are shown in Figure 11.

HETEROCYCLICS

Even though the research of Wilkinson Sword has resulted in the discovery of a wide variety of cooling chemicals, they all followed a similar trend, i.e., small modifications around the bulky hydrocarbon skeleton attached to the functional group. An interesting breakthrough was the discovery of icilin (**118**)'s cooling properties in the 1980s by E. Wei.[47] While this compound and its derivatives exhibited strong cooling activities and receptor activation of both TRPM8 and TRPA1, they bear no structural similarity to menthol (**1**). This result was not just an odd exception, as the cooling effect can be triggered by very diverse chemicals like thienopyrimidines, cyclic keto-enamines, *N*-aryl lactams, benzimidazoles, phosphine oxides, ureas, sulphonamides, etc. Among several substituted tetrahydropyrimidine-2-ones reported by Unilever, icilin (**118**) is approximately 2 times stronger than menthol (**1**), and compounds **119** and **120** have equal potency to that of menthol.[191,192]

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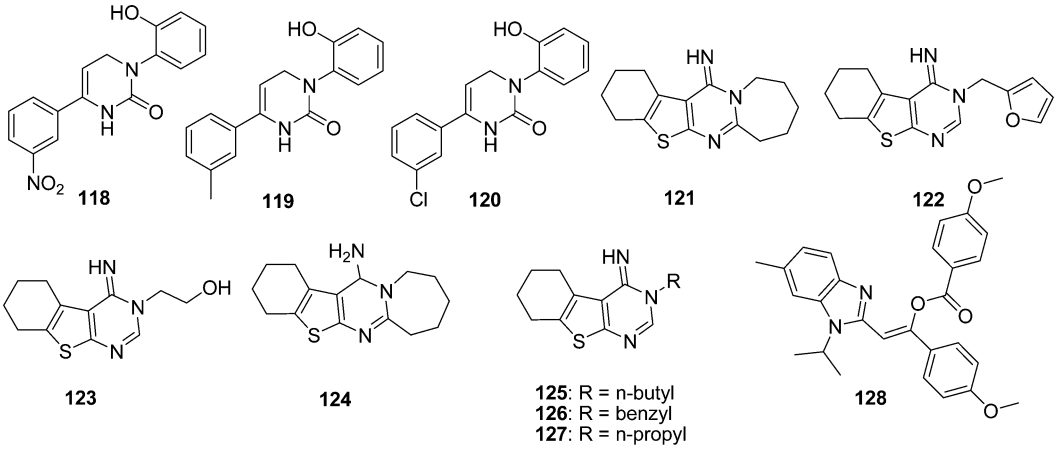

Figure 12. Heterocyclic cooling compounds.

Icilin (**118**) was discovered in 1983, which produced sensations of coldness when in contact with mucous membranes (nostrils, lips, and eyelids) of the researchers and also when ingested.[47] It is recognized that icilin (**118**) is a considerably more potent coolant than menthol. It is a super agonist that is 2.5-fold more efficacious and nearly 200-fold more potent than the reference cold thermosensory agonist, L-menthol (**1**). However, Andersson and co-workers at Novartis have shown that the activation of TRPM8 cold receptor by icilin (**118**) and cold, but not menthol, is modulated by intracellular pH in the physiological range. Their data suggests that activation by icilin (**118**) and cold involve a different mechanism than activation by menthol (**1**).[193]

Icilin activates TRPM8 in the presence of extracellular calcium.[41,194] Icilin (10 nM−10 μM) produced a concentration-dependent increase in intracellular Ca^{2+} in DRG neurons, which was attenuated by the nonselective TRP channel antagonist ruthenium red (10 μM). Ice and icilin produce differing effects on dorsal horn neurons indicating different mechanisms of action.[195] This requirement of a calcium rise for TRPM8 activity is not needed for cold- or menthol-induced channel activity, suggesting the channel can be activated by multiple mechanisms. Apart from TRPM8 activation, icilin also activates TRPA1 in CHO cells expressing TRPA1, TRPM8, and TRPV1.[60] Furthermore, icilin dose-dependently contracts the proximal and distal colon, and the contraction patterns observed for icilin are similar to those induced by allyl isothiocyanate.[196] Icilin at doses of 0.25, 0.5, and 0.75 mg/kg, i.p., elicited a dose- and time-dependent increase in glutamate within the striatum, indicative of icilin's neurochemical effect in rats.[197] Icilin also produces a dose-related hyperthermia in rats, which requires both NO production and NMDA receptor activation.[198] Additionally, a critical amino acid was identified: when mutated, it rendered icilin incapable of activating TRPM8. This residue was located between the second and third trans-membrane domains of the channel, a region known to be important for capsaicin sensitivity of TRPV1.[194]

Recently, Unilever claimed the thienopyrimidine class of compounds **121**−**127** as producing the sensation of cooling. These compounds showed activation of TRPM-8 with EC_{50} values of 9, 15, 20, 10, 25, 30, and 6.5 μM, respectively.[199,200] Givaudan SA reported benzimidazole **128** as possessing cooling intensities at 0.5 ppm equivalent to that of 2 ppm menthol.[201]

Structures of heterocyclic cooling compounds **118**−**128** are shown in Figure 12.

CYCLIC KETO-ENAMINES AND *N*-ARYL LACTAMS

A series of cyclic keto enamines **129**−**139** as odorless or with only faint odors have been reported. Calculating the ratio of cooling threshold to odor threshold revealed values below 0.1 for compounds **137**, **138**, and **139** clearly demonstrating that these compounds might be used as cooling agents without exhibiting any odor. In comparison, for (−)-menthol, the odor theshold is lower by a factor of 9.5 than the cooling threshold, thus indicating that it is not possible to evoke a "cooling" effect in a food product without having a significant mint odor. Other keto enamines also showed faint odors besides the cooling effect (Table 2), e.g., the thresholds of the amine-like odor of

Table 2. Comparison of Cooling and Odor Thresholds of *r*-Keto Enamines

compd	cooling threshold	odor threshold	odor quality	ratio (cool/odor)
129	29.0−43.5	43.5−72.5	faintly amine-like	0.8
130	4.5−9.0	2.6−5.2	faintly mint-like	1.7
131	26.7−42.5	13.4−22.4	faintly mint-like	2.0
132	16.0−24.0	12.0−20.0	faintly mint-like	2.7
133	12.0−20.0	6.0−9.0	curcuma-like	2.1
134	48.4−67.8	2.4−4.8	mint-like	16.0
135	1605.0−2675.0	50.2−83.6	amine-like	32.0
136	26.9−44.8	3.4−5.6	rubber-like	8.0
137	2.0−4.0	32.0−64.0	faintly mint-like	≪0.1
138	1.5−3.0		odorless	≪0.1
139	0.02−0.06		odorless	≪0.1
(−)-**1**	0.9−1.9	0.1−0.2	mint-like	9.5

135, the mint-like note of **134**, and the carvone-like aroma of **133** were found to be 32- or 16-fold below the threshold concentration required for cooling. On the basis of these data, it might be concluded that, apart from the cooling effect, there is no common property of cooling compounds, thus illustrating that there is no physiological link between cooling activity and mint-like odors. Compounds **130**, **138**, **139**, and **137** were also tested for topical cooling activity. All compounds showed significant cooling effect on the skin, but compounds **139** and

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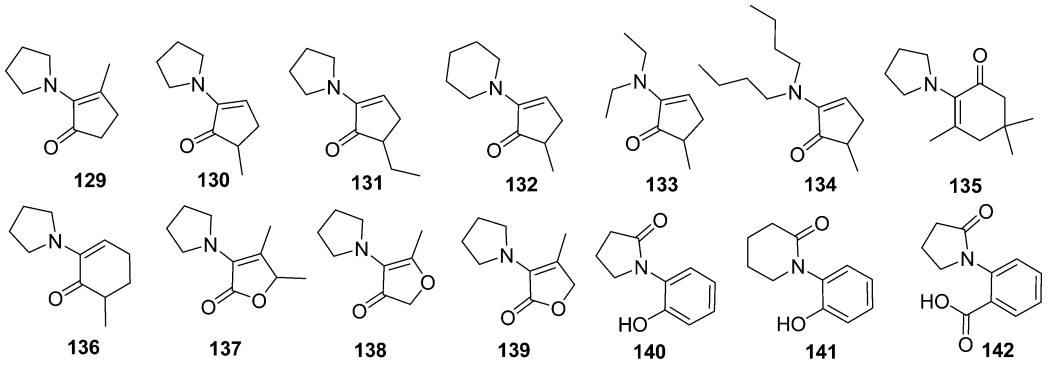

Figure 13. Cyclic keto-enamines and *N*-aryl lactams.

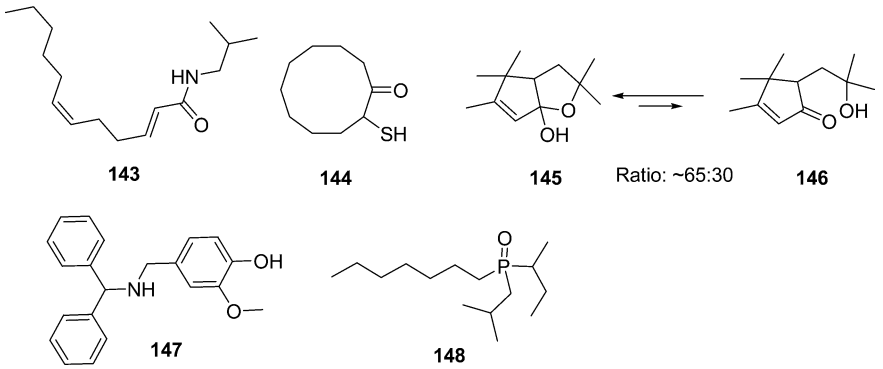

Figure 14. Miscellaneous cooling compounds.

137 showed the highest cooling activity than menthol. The 4-methyl-3-(1-pyrrolidinyl)-2(5*H*)-furanone (**139**) exhibited the strongest cooling effect at the low oral threshold concentration of 0.02−0.06 mmol/L, which is 35-fold below the value determined for (−)-menthol. Also, it is 512 times more powerful on the skin than menthol. The cooling effect also lasts twice as long. Compound **138** has a cooling threshold of 1.5−3.0 ppm. These investigations indicated that candidates of these cyclic *R*-keto enamine derivatives, in particular **139**, **138**, **129**, and **130**, might be used to evoke certain cooling effects during the consumption of nonmint food compositions such as drinking water, confectionary products, malted and citrus beverages, and fruity or browned flavors.[99,100,202−206]

Bassoli et al. synthesized a number of *N*-aryl lactams and tested for cooling properties. Three compounds **140**−**142** exhibited a cooling sensation. They contain two rings, one with an oxygenated function in the 2-position, and the other is a lactam ring. They have been obtained easily and in high yields by Goldberg arylation or lactam ring closure on the appropriate phenol or benzoic acid derivative.[207] Structures of cyclic keto-enamines and *N*-aryl lactams **129**−**142** are shown in Figure 13.

MISCELLANEOUS

IFF reported alkyldienamide **143** possessing a cooling/tingling effect and is used in different compositions such as alcoholic beverages, chewing gums, and toothpastes.[208] Symrise GMBH and Bayer Material Science AG used 2-mercaptocyclodecanone (**144**) as a cooling agent in flavoring compositions.[102,158] Aromagen Corp. patented a novel mixture of cyclic oxygenated compounds **145** and **146**, which had a strong long lasting cooling sensation and a faint menthol-like odor.[209,210] Givaudan SA reported a series of biphenyl methyl amines represented by structure **147** possessing cooling intensities stronger than menthol and WS-3.[211] Wilkinson and Sword claimed a number of phosphine oxides with varying alkyl substitution with cooling efficacy.[99,100,212] WS-148 (**148**) shows a dose-dependent and reversible activation of TRPM8 with an EC_{50} value of 4.1 μM.[124] Structures of miscellaneous cooling compounds **143**−**148** are shown in Figure 14. Table 3 provides the summary of cooling compounds as activators of thermoTRPs.

TRPM8 ANTAGONISTS

Along with the number of cooling agents that activate TRPM8, several antagonists have been identified (Table 4).[97,193] The latter of these findings has further supported the notion of differential modulation of TRPM8 by various mediators. Specifically, lowering of the intracellular pH to below 7 was able to completely block TRPM8 currents elicited by either cold or icilin but not menthol.[193] As discussed above, primarily cooling compounds exhibit activation of TRPM8. Among various discoveries of commericial cooling agents, the *p*-menthane scaffold has a major contribution. Interestingly, there also exist few reports on the *p*-menthane scaffold based TRPM8 channel antagonists. Dendreon Corp. identified Trp-p8 antagonists using a cell viability assay with CHO/Trp-p8 cells. Compounds belonging to the *p*-menthane scaffold **149**−**153** as well as heterocyclics **154**−**156** protected the cells from toxic effects of Trp-p8 agonists and thus were classified as Trp-p8 antagonists[156] (Figure 15). Recently, *p*-menthane esters **157**−**161** (Figure 16) have been reported as selective TRPM8 antagonists of icilin or menthol-induced activation. These compounds are potent antagonists of icilin and (−)-menthol induced TRPM8 activation with IC_{50} values in the range of 20 nM to 0.7 μM and showed 4- and ∼150-fold selectivity versus

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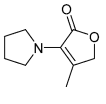
Table 3. Summary of Cooling Compounds as Activators of TRPM8 and TRPA1 Channel

Entry	Name	Chemical class	TRPM8 (EC_{50})	References
(-)-1[a]	(-)-Menthol	p-menthane-3-ol	196 μM[b]	(*114, 131*)
			66.7 μM[b]	(*41*)
			4.1 μM[c]	(*97*)
(+)-1	(+)-Menthol	p-menthane-3-ol	600 μM[b]	(*114*)
			14.4 μM[c]	(*97*)
2	(-)-Isopulegol	p-menthane-3-ol	498 μM[b]	(*114*)
	(Coolact P)		66 μM[c]	(*97*)
3	PMD38	p-menthane-3-ol	31 μM[c]	(*97*)
5	1,8-Cineole	p-menthane	3400 μM[b]	(*41*)
			7700 μM[c]	(*97*)
8	Geraniol	acyclic alcohol	5900 μM[c]	(*97*)
9	Hydroxy-citronellal	acyclic alcohol	inactive	(*97*)
10	Linalool	acyclic alcohol	6700 μM[c]	(*97*)
11	Fresolat ML	p-menthane-3-ol ester	163 μM[b]	(*114*)
			3.3 μM[c]	(*97*)
36	MPD, Coolact-10	p-menthane-3-ol ether	6 μM[c]	(*97*)
41	WS-30	p-menthane-3-carboxylate ester	5.6 μM[c]	(*124*)
42	WS-3	p-menthane 3-carboxamide	216 μM[b]	(*114*)
			3.7 μM[c]	(*97*)
43	WS-5	p-menthane 3-carboxamide	26 μM[b]	(*114*)
44	CPS-368	p-menthane 3-carboxamide	104 μM[b]	(*114*)
			3.6 μM[c]	(*124*)
45	CPS-369	p-menthane 3-carboxamide	84 μM[b]	(*114*)
			3.6 μM[c]	(*124*)
49	WS-12, CPS-112	p-menthane 3-carboxamide	12 μM[b]	(*114, 131*)
			193 nM[c]	(*124*)
			30 μM[c]	(*51*)
51	CPS-128	p-menthane 3-carboxamide	0.5 μM	(*136*)
52	CPS-124	p-menthane 3-carboxamide	1.2 μM[c]	(*124*)
53	CPS-125	p-menthane 3-carboxamide	30 μM[b]	(*114*)
68	-	p-menthane 3-carboxamide	< 0.02 μM[c]	(*153*)
69	-	p-menthane 3-carboxamide	< 0.02 μM[c]	(*153*)
70	-	p-menthane 3-carboxamide	< 0.02 μM[c]	(*153*)
79	Frescolat MGA	p-menthane-3-ol ether	184 μM[b]	(*114*)
			4.8 μM[c]	(*97*)
81	WS-23	acyclic carboxamide	1500 μM[b]	(*114*)
			44 μM[c]	(*97*)
118[a]	Icilin	tetrahydropyrimidine-2-one	7 μM[b]	(*114*)
			0.36 μM[c]	(*41*)
			0.2 μM[c]	(*97*)
121	-	thienopyrimidine	9 μM[c]	(*199, 200*)
122	-	thienopyrimidine	15 μM[c]	(*199, 200*)
123	-	thienopyrimidine	20 μM[c]	(*199, 200*)
124	-	thienopyrimidine	10 μM[c]	(*199, 200*)
125	-	thienopyrimidine	25 μM[c]	(*199, 200*)
126	-	thienopyrimidine	30 μM[c]	(*199, 200*)
127	-	thienopyrimidine	6.5 μM[c]	(*199, 200*)
148	-	phosphine oxide	4.1 μM[c]	(*124*)
	5-Methyl-4-(1-pyrolidinyl)-3-*2H*-furanone	furanone	Inactive[b]	(*114*)

[a]These cooling compounds also activate TRPA1 ion channel. [b]*Xenopus oocytes*. [c]HEK cells.

TRPV1 and TRPA1 activation.[213] However, a large number of non-*p*-menthane based compounds have been known as potent TRPM8 antagonists, for example, capsazepine (**162**, 95% inhibition at 10 uM), BCTC (**163**, IC_{50} 143 nM), CTPC

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Table 4. TRPM8 Antagonists

entry	TRPM8 IC_{50} (μM)[a]	TRPM8 IC_{50} (μM)[b]	TRPV1 EC_{50} (μM)	TRPA1 EC_{50} (μM)
157	0.05	0.02	NM[c]	4.1
158	0.5	0.4	NM[c]	26.7
159	0.6	0.3	NM[c]	13.8
160	0.7	0.2	NM[c]	3.0
161	0.08	0.1	18.3	11.7

[a]TRPM8 antagonism against icilin induced activation. [b]TRPM8 antagonism against menthol induced activation. [c]EC_{50} values were not measurable as % efficacy values for these were <10%.

(**164**, IC_{50} 131 nM), and SB-452533 (**165**, IC_{50} 571 nM).[214] Few other recently reported TRPM8 antagonists include cannabinoids (cannabigerol and tetrahydrocannabivarin),[215,216] 5-benzyloxytryptamine,[217] AMTB,[218] sulfamoyl benzoic acid derivatives,[219] organophosphonates,[220] sulfamides,[221] sulfonamides,[222] benzimidazoles,[223] etc. However, there exists significant overlap in the antagonist pharmacology at different TRP channels. For example, TRPM8 antagonists **162**–**165** are also potent antagonists of TRPV1 channel. Thus, it is challenging to discover selective TRP channel antagonists.

CONCLUSIONS

The underlying process in thermoreception, whether hot or cold, is dependent on ion transport across cellular membranes. Cellular membranes consist of an oily phospholipid bilayer, which would be impermeable to ions such as K^+ or Ca^{2+} except for receptor protein ion channels. The diverse range of structures showed cooling properties and activation of thermoTRPs. It was observed that several newly discovered cooling compounds are potent and better activators of TRPM8 than menthol (**1**) (Table 3). *p*-Menthane carboxamides **68**–**70** have showed activation of this ion channel at EC_{50} <0.02 μM. Similarly, other *p*-menthane carboxamides such as **44** (CPS-368), **51** (CPS-128), and **52** (CPS-124) showed low micromolar EC_{50} values of 3.6, 0.5, and 1.2 μM, respectively. Compounds with totally different chemical scaffolds from *p*-menthane also exhibited promising EC_{50} values toward activation of TRPM8. These include icilin (**118**, 7 μM in oocytes, 0.36 μM in HEK) and other thienopyrimidines (**121**–**127**, 6.5–30 μM in HEK). Interestingly a phosphine oxide **148** showed an EC_{50} value of 4.1 μM (in HEK) toward TRPM8 activation. It was found that chemical derivatives of menthol such as CPS-368, CPS-369, CPS-125, WS-5, and WS-12 are

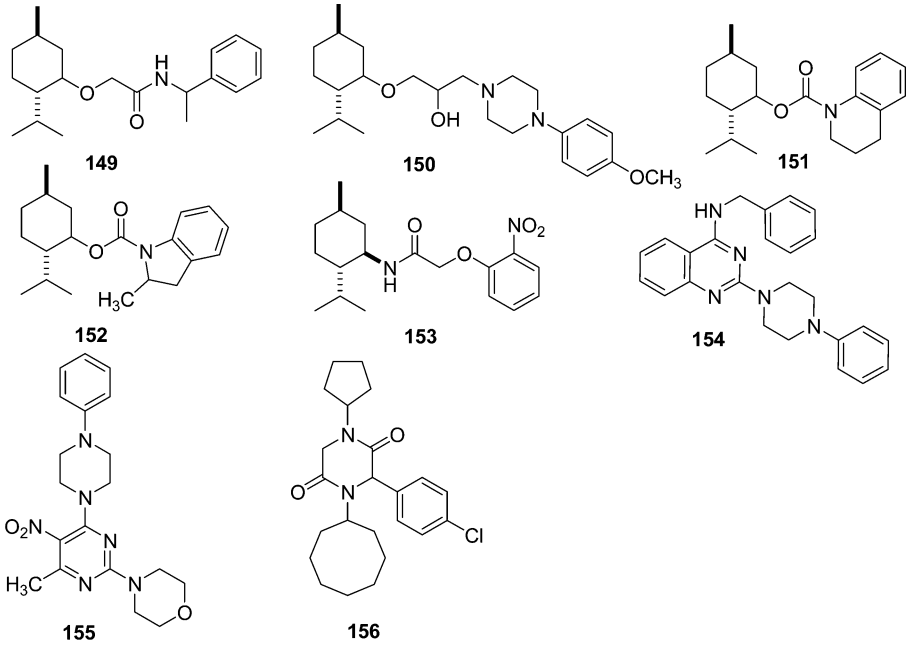

Figure 15. Trp-p8 antagonists.

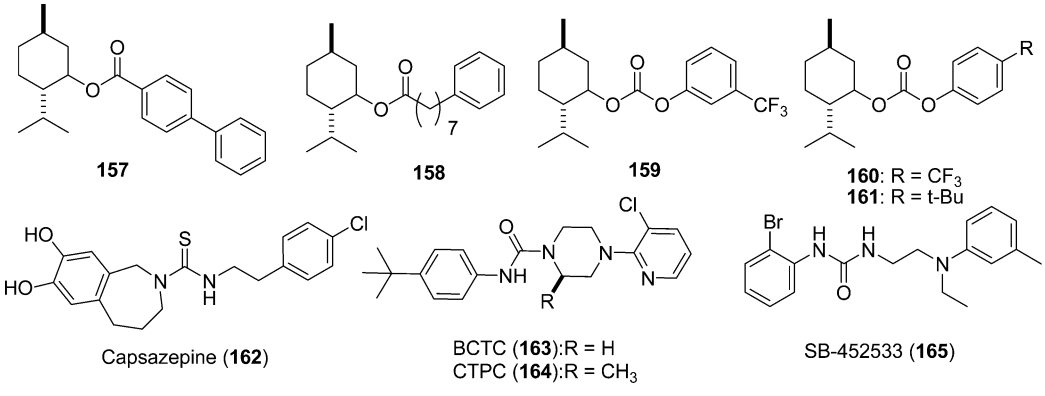

Figure 16. TRPM8 antagonists.

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the most selective ligands for TRPM8. The enhanced activity and selectivity seem to be conferred by the hexacyclic ring structure present in all ligands as substances such as WS-23 which lack this functional group activate TRPM8 with much lower potency (EC_{50} in mM), and those with pentacyclcic ring structure (furanone compounds) are totally inactive.

Furthermore, a large number of pharmaceutical compositions (chewing gum, toothpaste, candy mints, mouthwash, beverages, cosmetics, tobacco products, etc.) have been formulated by different pharmaceutical companies and have been patented. To satisfy the increasing demand on the market for cool feeling agents, researchers have made efforts to develop novel cool feeling agents. Also, attempts have been made to enhance an improved cool feeling by combining two or more cool feeling agents or combining cool feeling agents with other substances. In addition to the major interest in cooling agents from the flavor and fragrance industry, their use has expanded beyond oral care and confectionary applications. It has been found that cooling compounds show excellent insect repellency. Along with menthol (**1**), other TRP agonists have shown promise as therapeutic agents for pain, cancer, respiratory diseases, etc. Menthol and icilin are already in use to relieve pain; thus, several other cooling compounds acting via the TRPM8 ion channel activation will have great potential to emerge as analgesic treatment.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

BCTC, *N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2*H*)-carboxamide; CTPC, (2*R*)-4-(3-chloro-2-pyridinyl)-2-methyl-*N*-[4-(trifluoromethyl)phenyl]-1-piperazinecarboxamide; AMTB, *N*-(3-aminopropyl)-2-[(3-methylphenyl)methyl]oxy-*N*-(2-thienylmethyl) benzamide hydrochloride salt; FEMA, Flavor and Extract Manufacturer's Association; GRAS, Generally-Recognized-As-Safe; TRP, transient receptor potential; TRPM8, transient receptor potential cation channel subfamily M member 8; TRPA1, transient receptor potential cation channel, subfamily A, member 1; CMR, cold-and menthol-sensitive receptor; PIP2, phosphatidylinositol 4,5-bisphosphate; MPD, (−)-menthoxypropane-1,2-diol; PMD38, *p*-menthane-3,8-diol; MPF, methyl pyrrolidinyl furanone; NMF, natural moisturising factor; WS, Wilkinson Sword; NACHE, *N*-alkylcarbonyl-D-amino acid hydroxyalkyl esters; IFF, International Flavors and Fragrances; MSC, Millennium Specialty Chemicals

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