TRPing the switch on pain: an introduction to the chemistry and biology of capsaicin and TRPV1

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Capsaicin has elicited great interest for many centuries due to its noticeable culinary and medical properties. The discovery of its receptor, TRPV1, sparked an explosion of interest in TRPV1 and the development of TRPV1 agonists and antagonists. This *tutorial review* provides an introduction to the history of both capsaicin and TRPV1. Two TRPV1 antagonists that are undergoing clinical trials are highlighted, as are some light-activated molecular tools that are enabling the intracellular study of this protein. This article will be of interest to chemists and biologists with an interest in TRPV1, cell signalling, or medicinal and biological chemistry.

Introduction

Since the discovery and cloning of the cellular target of capsaicin (**1**), transient receptor potential vanilloid subtype 1 (TRPV1) in 1997,[1] this protein has been a focus of interest for both the biological and chemical communities. TRPV1 has been shown to be important in both nociception (the perception of pain) and the inflammatory response, and hence has rapidly become a significant therapeutic target.[2] Activity in the study of TRPV1 has been intense; therefore, it is not possible for a *tutorial review* to cover all aspects of this research in detail. Hence, the aim of this review is to introduce the reader to the salient points of this intriguing field. Further reading is available in the form of a number of excellent reviews, in particular, the pharmacology and biochemistry of TRPV1 action has been reviewed in detail by Szallasi and Blumberg.[3] The medicinal chemistry of TRPV1 and especially the development of therapeutically important TRPV1 antagonists has been reviewed by Szallasi and Appendino,[4] by Breitenbucher and colleagues,[2] by Gharat and Szallasi[5] and also by Westaway.[6] Szallasi and colleagues have also recently reviewed the progress in the field since the cloning of TRPV1 in 1997.[7] Gharat and Szallasi have reviewed the patent literature in the TRPV1 field.[8] This review provides an introduction to the biology and chemistry of TRPV1 and highlights some important agonists and antagonists that have proved useful as molecular tools to study this fascinating protein. The article concludes by discussing some more recently developed and therapeutically important compounds.

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Historical uses of chilli peppers and capsaicin

Capsicum or chilli peppers are thought to have originated in Mexico and were cultivated there from as early as 5000 BC and from around 2000 BC in Peru. It is likely that Christopher Columbus introduced chilli peppers to Europe and there are records of a letter from Columbus' fleet surgeon to the court surgeon of King Philip of Spain, which mentions chilli peppers. The spice trade was at its peak during this time and the use of chilli peppers soon spread around the world, leading to their inclusion in spicy culinary dishes from a number of cultural backgrounds.[9]

There is also a long history of chillies being used in traditional medicine by peoples from diverse geographical locations including the Aztecs and chillies also feature in early British medical history. Complaints that have been treated with a chilli preparation of some form include: tonsillitis, sore throat, coughs, cholera, backache, rheumatism, gout, abdominal complaints, paralysis, water retention and ulcers. In addition, chilli preparations have been applied as antiseptics, enhancing wound healing and treating parasitic infestations. It should be noted that it is not clear as to whether chilli preparations were effective in all of the above cases.[9]

More recently, capsaicin has been used topically to treat a number of human pain disorders. These treatments were developed prior to the identification of TRPV1 and are based on the ability of capsaicin to deplete substance P (a neuropeptide involved in pain transmission) from local sensory

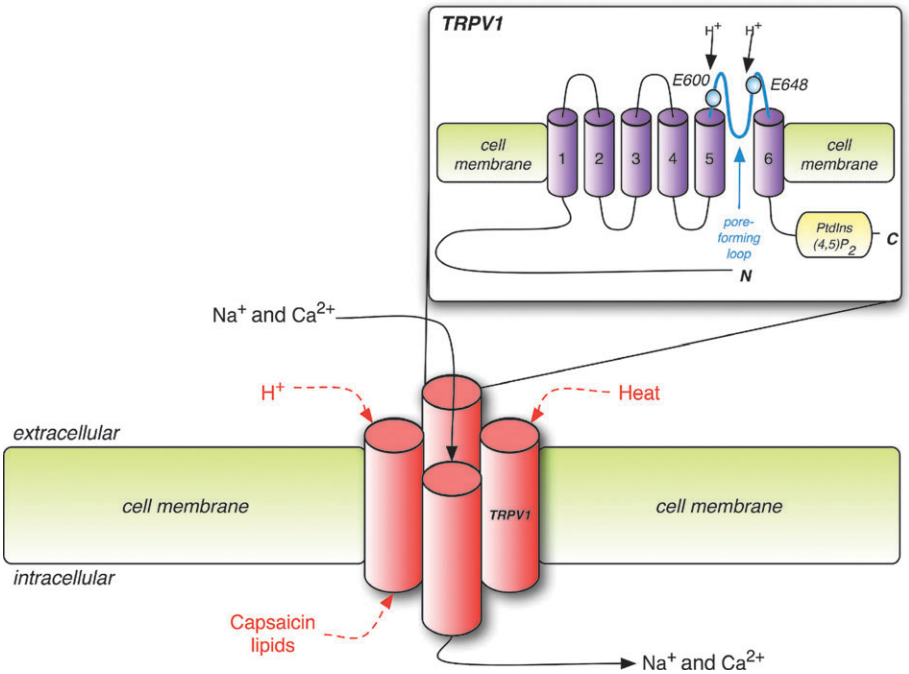


Fig. 1 A cartoon representation of TRPV1 depicting the proposed tetrameric structure of the receptor, each TRPV1 unit is shown as a red cylinder. It is thought that the receptor is activated extracellularly by heat above ~45 °C or protons (pH below 5.4). In addition, TRPV1 is activated by lipids, including capsaicin and anandamide. The inset shows a cartoon representation of a single TRPV1 protein, highlighting the six transmembrane domains, the two glutamate (E) residues involved in proton binding, the PtdIns(4,5)P_2 binding site and the pore-forming loop.

terminals. Minimal systemic absorption of capsaicin occurs by this manner of application and no permanent damage to tissues is known to occur. Topical capsaicin has been found to be effective in the treatment of a number of skin complaints, shingles and relief of the pain associated with arthritis or muscle strain. Treatment of neck pain, joint pain, osteoarthritis, diabetic neuropathy, with capsaicin shows improvement over placebo or vehicle.[9] Consequently, a number of companies market topical capsaicin under brand names including Capzasin-P, Menthacin and Zostrix.

Structure and function of the capsaicin receptor: TRPV1[10]

Despite the long history of capsaicin in medicine, its molecular target was not identified until 1997. David Julius and co-workers isolated and cloned the ''capsaicin receptor'', which was shown to be a non-selective cation channel that also responds to heat (above ~45 °C) and low pH (below ~pH 5.5). The channel is a member of the transient receptor potential (TRP) channels[11] and hence was named transient receptor potential vanilloid subtype 1 (TRPV1) although it is also known as VR1 (vanilloid receptor 1).[1]

TRPV1 comprises six transmembrane sections (TM1–TM6), with the N- and C-termini on the cytosolic side of the cell membrane[12] and a short, pore-forming hydrophobic region between TM5 and TM6 (Fig. 1). There are three ankyrin domains on the long N-terminus region. In other membrane proteins, ankyrin repeat domains are known to bind to many cytosolic proteins. To date, only calmodulin has been demonstrated to bind to an ankyrin repeat domain of TRPV1 (first ankyrin repeat domain). The functional cation channel is thought to be formed from tetramers of TRPV1 (Fig. 1). Most evidence to date supports a homomeric tetramer, containing only TRPV1, and TRPV1 expressed alone in human embryonic kidney-derived HEK293 cells or *Xenopus* oocytes can account for the majority of electrophysiological properties demonstrated by native TRPV1 in sensory neurons. However, it is possible that TRPV1 also forms heteromers with another member of the TRPV family, TRPV3. Thus it is plausible that only homomeric channels are functional, or that the homomeric and heteromeric channels possess similar pharmacology. TRPV1 is the prototypical member of a family of TRPV channels, which is thought to comprise six TRPV channels (TRPV1–6), which are divided into four groups.[11,13] These proteins all share common structural features, with 6 transmembrane sections and a pore-forming region between TM5 and TM6.

TRPV1 is activated by numerous exogenous ligands of which capsaicin and the ultrapotent daphnane diterpene, resiniferatoxin (RTX, **2**), are the most well known. There is increasing evidence to support the role of various lipids as endogenous TRPV1 ligands. Putative endogenous TRPV1 agonists include the lipids anandamide (**3**), 12-(hydroperoxy)-eicosatetraenoic acid (12-HPETE, **4**) and *N*-arachidonoyl dopamine (NADA, **6**), which are structurally related to capsaicin (Fig. 2).

Modulation of TRPV1 by exogenous and endogenous lipids

Anandamide (*N*-arachidonoyl ethanolamide, **3**), which was first isolated from pig brain in 1992, is defined as an endocannabinoid because it is an endogenous molecule that can

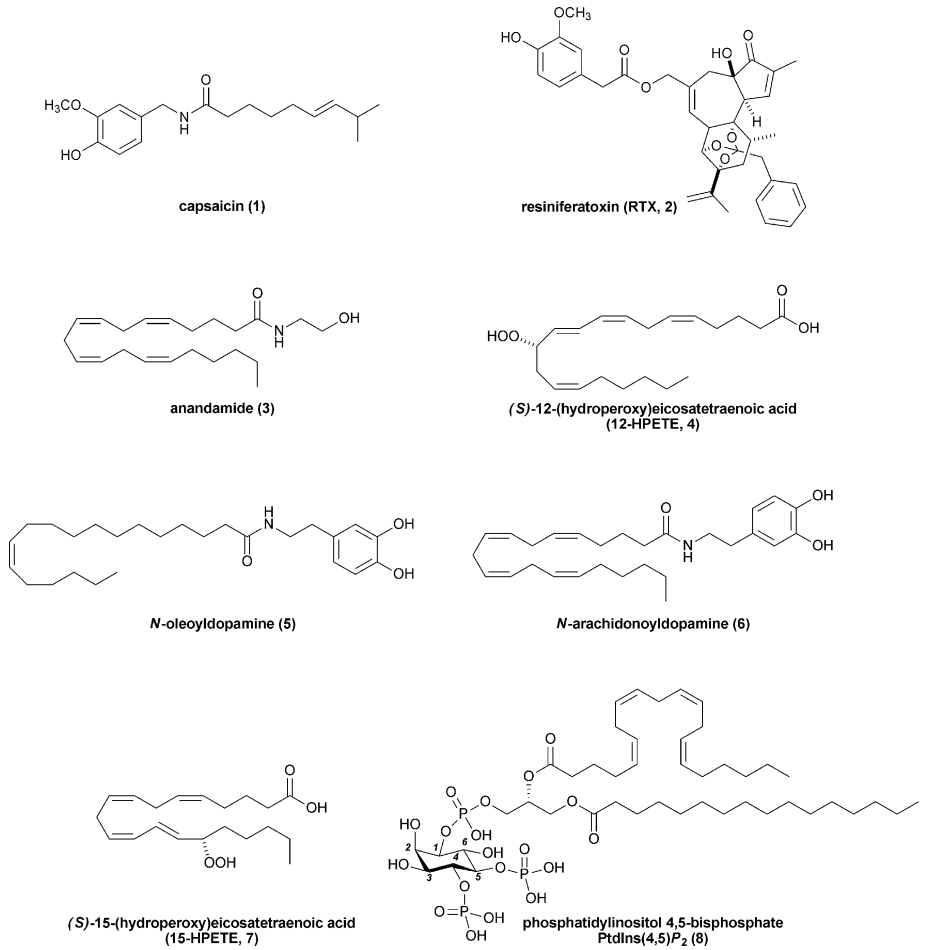


Fig. 2 The structures of some compounds that interact with TRPV1.

bind to and activate the cannabinoid receptors CB_1 and CB_2.[14] In 1999, anandamide was shown to activate TRPV1 and hence was classified as the first endogenous agonist of TRPV1 or "endovanilloid".[14] The action of anandamide at TRPV1 is selective as its actions at this receptor are blocked by TRPV1 antagonists, but not by antagonists of CB_1 or CB_2. Desensitisation of TRPV1 *via* pretreatment with capsaicin also prevents the activation of TRPV1 by anandamide. Anandamide has a lower EC_{50} at TRPV1 than capsaicin, with values in the μM range, although varying between tissues and systems. In addition, whether anandamide behaves as a full or a partial agonist appears to be tissue dependent.[15]

Anandamide is biosynthesised by the phospholipase D (PLD)-mediated hydrolysis of *N*-arachidonoyl phosphatidylethanolamine (NAPE). Metabolism of anandamide occurs *via* one of several routes. It is possible that anandamide is removed from the cell by a selective transporter protein, maybe the same protein that is responsible for the extracellular uptake of anandamide. Intracellularly, anandamide can be metabolised by either oxygenation or hydrolysis. Little is known about the oxidative metabolic pathway, although lipoxygenation and cyclooxygenation-catalysed reactions have been shown to produce a large number of anandamide derivatives, all of which may have some biological activity. Fatty acid amide hydrolase (FAAH) catalyses the hydrolysis of the anandamide amide bond, to produce arachidonic acid and ethanolamine. Arachidonic acid itself is not a TRPV1 agonist, however, the action of 12-lipoxygenase or 15-lipoxygenase on arachidonic acid leads to the formation of (*S*)-12-(hydroperoxy)eicosatetraenoic acid (12-HPETE, **4**) or (*S*)-15-(hydroperoxy)eicosatetraenoic acid (15-HPETE, **7**), respectively. Of the various oxygenated species that have been shown to activate TRPV1, 12-HPETE and 15-HPETE are the most potent. As would be expected, the hydroperoxy moiety in 12-HPETE and 15-HPETE is highly reactive and is rapidly reduced to a hydroxyl group. This may form an inactivation pathway, as the 12- and 15-hydroxy derivatives are much less potent activators of TRPV1.[14]

Other endovanilloids include the dopamine-derived lipid *N*-arachidonoyl dopamine (**6**) and related analogues, such as *N*-oleoyl dopamine (**5**). Little is known about the biosynthesis or metabolism of these lipids, although it seems likely that *N*-arachidonoyl dopamine is synthesised from arachidonic acid. Both lipids may be metabolised to their methyl ether derivatives through the action of a catechol-*O*-methyl transferase.

Unlike the other lipids that interact with TRPV1 to activate the ion channel, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] (**8**), is constitutively associated with TRPV1 and inhibits the action of the protein. Residues 777–820 of TRPV1, which include eight positively charged amino acids,

have been identified as the PtdIns(4,5)P_2-binding region, presumably through interaction of the positively charged residues with the negatively charged phosphate head group. The importance of PtdIns(4,5)P_2-binding has been demonstrated by replacement of the TRPV1 PtdIns(4,5)P_2-binding domain with a higher affinity PtdIns(4,5)P_2-binding domain, which resulted in the activation temperature for TRPV1 being increased.[10] The interaction of TRPV1 with PtdIns(4,5)P_2 potentially links this channel with the complex pathway of intracellular signalling that is mediated by a variety of inositol-derived messengers.[16]

The location and structure of the TRPV1 agonist-binding site

The agonist-binding site or sites of TRPV1 are not clearly identified at this time and presently there are a number of differing views in the literature as to their precise location. Work using a synthetic water-soluble capsaicin analogue suggests the existence of an agonist-binding site on a cytosolic TRPV1 domain. This appears to be supported by the observation of a time-lag between photolysis of caged capsaicin and TRPV1 activation when capsaicin was released extracellularly,[17] whereas intracellular photolysis of a caged TRPV1 agonist gave an instantaneous response.[18] In addition, anandamide has been shown to be more effective when applied intracellularly.[15] In the development of TRPV1 antagonists, Rami *et al.* have synthesised a quaternary ammonium salt of the potent competitive TRPV1 antagonist SB-452533 (**9**, Fig. 3).[19] This salt (**10**) demonstrated modest antagonist activity when applied extracellularly, but no antagonist activity when applied to the inside of the cell. These data have been interpreted as evidence for an extracellular agonist-binding site, as the ammonium salt is unlikely to be very membrane permeant.

Mutation studies have identified certain residues that are important in the binding of ligands to TRPV1. Julius and Jordt demonstrated that tyrosine 511 is vital for capsaicin-activation of TRPV1, as mutation to either alanine or cysteine resulted in a channel that was unresponsive to capsaicin, but had the same responses to pH and heat as the wild type (non-mutated) protein. It seems that the aromatic nature of residue 511 is important for its interaction with capsaicin, as replacement of the tyrosine residue with phenylalanine had only a moderate effect on capsaicin sensitivity. The tyrosine to alanine mutation of residue 511 (Y511A mutant) was also insensitive to activation by anandamide, suggesting that the anandamide and capsaicin binding sites are in the same or similar locations on TRPV1. Serine 512 also appears to play an important role in the TRPV1–capsaicin interaction. Mutation of serine 512 to phenylalanine resulted in a channel with reduced responses to both capsaicin and protons. However, mutation of residue 512 to alanine or threonine had less effect on the channel behaviour, indicating that perhaps residue 512 is less critical than residue 511 in the binding of capsaicin.[20] It has also been shown that residue 512 has a function in the recognition of at least some TRPV1 antagonists. A serine to tyrosine mutation of residue 512 (S512Y mutant) converts 5′-iodoresiniferatoxin (**45**, 5′-I-RTX, see below and Fig. 13 for details) from an antagonist into an agonist.[21]

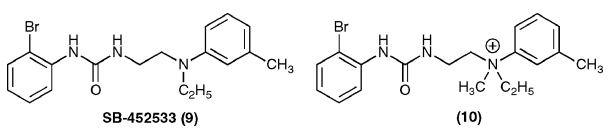


Fig. 3 The structures of SB-452533 and its membrane impermeant derivative **10**.

Rat and human TRPV1 show 86% sequence identity but show a difference in their response to RTX, with RTX showing greater potency at rat TRPV1. Johnson *et al.* have demonstrated that residue 547 (leucine in human and methionine in rat) plays an important role in the interaction of TRPV1 with RTX.[21] A methionine to leucine mutation of residue 547 (M547L mutant) in rat TRPV1 leads to a small reduction in function. Conversely, an L547M mutation in human TRPV1 leads to an increase in function, when activated by RTX. Residue 547 would be expected to play a role in the interaction with 5′-I-RTX, and this is shown to be the case. An L547M mutation in human TRPV1 caused an increase in the potency of 5′-I-RTX. The effects of the two above mutations were also mirrored in the response of the mutant TRPV1s to capsaicin and protons, indicating that residue 547 plays an important role in the binding of TRPV1 to ligands.[21]

Julius has proposed a model of the capsaicin receptor that is consistent with the above data and is based on the structural similarity observed between the TRP channels and voltage-gated potassium channels. This model places the residues that interact with capsaicin between TM2 and TM3, suggesting that capsaicin interacts with TRPV1 from within the cell (Fig. 4).[20]

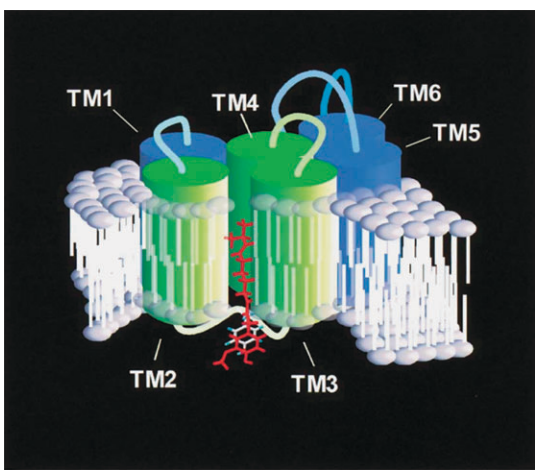


Fig. 4 Structural model of capsaicin bound to TRPV1. The transmembrane portion of the receptor monomer is rendered after a helix-packing model derived from structurally related K_v channels. Similar to voltage-gated potassium channels, TRPV1 is likely to form a tetramer with the ion-conducting pore in the center, faced by TM6. The vanillyl moiety of capsaicin (red) is shown to interact with an aromatic residue (such as Y511) located in the cytosolic region linking TM2 and TM3. Within the plane of the membrane, the ligand interacts with the TM2–3 region at the channel periphery (green). Structural rearrangements induced by ligand binding may be transduced through TM4 (green) into the channel core. In K_v channels, TM4 (S4) represents the voltage sensor that interacts *via* salt bridges with TM2 and 3. Reprinted from ref. 20, copyright (2002), with permission from Elsevier.

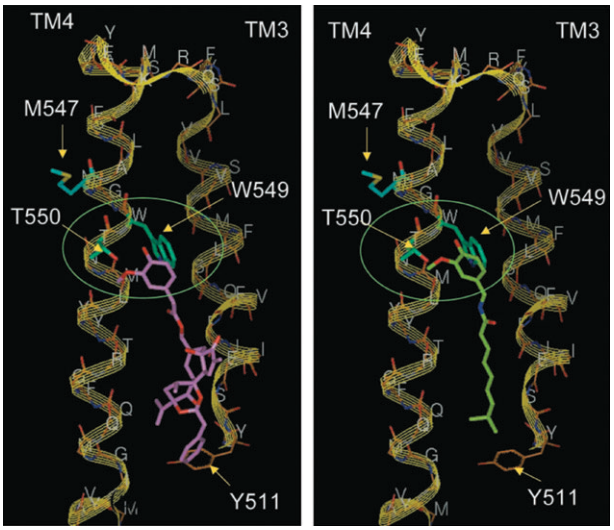


Fig. 5 Structural model of RTX (purple) and capsaicin (green) interacting with transmembrane helices TM3 and TM4 of TRPV1. The backbone of the complete structural model along with the side chains of residues considered to be involved in interactions is shown. The side chains of M547, W549, and T550 are shown as sticks (thick lines). Indicated interactions of the vanillyl moiety with T550 and T549 are highlighted in the green ellipses. Residues considered to be involved in interactions with the substituted phenyl portions of the two ligands are shown in cyan. Modeled hydrophobic contacts of Y511 with the hydrophobic ends of RTX and capsaicin are shown. Reproduced with the permission of the American Society for Biochemistry and Molecular Biology and the *Journal of Biological Chemistry*.[22]

An alternative model of capsaicin and TRPV1 binding has been proposed by Gavva and co-workers, in which the opposite orientation is adopted by capsaicin (Fig. 5). This model is consistent with the observation made by this group that threonine 550 plays an important role in the binding of TRPV1 to capsaicin and RTX and suggests that this occurs between TM3 and TM4.[22] This model is supported by molecular modelling studies that examined both the binding of TRPV1 agonists and also antagonists and suggested that both types of ligand bound between TM3 and TM4.[23]

However, neither model accounts for the observations of Vyklicky *et al*. who suggest that the N- and C-terminal cytosolic portions of TRPV1 are important for agonist interactions, especially the residues arginine 114 and glutamate 761. In addition, Vyklicky demonstrated that intracellular application of vanilloids is insufficient to activate TRPV1, and suggests that an interaction between at least one extracellular residue and a vanilloid molecule is required for channel activation.[24]

The fact that certain residues are repeatedly implicated in capsaicin binding gives credence to the idea that there is at least one defined point of interaction for agonists on TRPV1. However, it seems possible that there may be more than one interaction site for agonists and/or antagonists and there is currently little consensus on whether the site(s) are located intra- or extracellularly.

Activation of TRPV1 by heat

Heat-evoked and capsaicin-evoked TRPV1 currents show similar but not identical properties, suggesting that the responses to these stimuli involve different but overlapping mechanisms. TRPV1 is a voltage-gated channel that is activated upon depolarisation; the effect of heat is to move the voltage-dependent activation curve to be activated at a lower membrane potential.[10] It has been reported that the C-terminus is involved partly in thermosensitivity, however, the fact that no TRPV1 mutation has been described that selectively abolishes heat activation of TRPV1 suggests a more global effect of heat on TRPV1.

Activation of TRPV1 by protons

Lowering the surrounding pH affects TRPV1 by reducing the threshold for activation of TRPV1 by both heat and small molecule agonists; further acidification leads to channel opening at room temperature. Protons are thought to have the effect of increasing the probability of channel opening, rather than of altering the unitary conductance of the channel or interacting with the vanilloid binding site. Mutational analyses have shown that Glu600, located on a putative extracellular domain, is an important site for proton potentiation of TRPV1 activity. Glu648 is involved in direct proton-evoked activation of TRPV1.[10] These amino acids are located on the pore-forming region that is located between TM5 and TM6 (Fig. 1).

Historically important exogenous TRPV1 ligands

Early TRPV1 agonists: The capsaicinoids

Capsaicin was first isolated by Thresh in 1876, although the incorrect chemical formula was assigned at this stage. Further contributions towards assigning the correct structure were made by Micko, Lapworth and Royle.[25] However, it was Nelson who correctly assigned the structure of capsaicin in work conducted between 1919 and 1923, culminating in the synthesis of capsaicin and dihydrocapsaicin.[26] The capsaicin content of chillies is about 0.14% of mass (5 g of chillies contain 7 mg of capsaicin).[9] Analysis of chilli pepper extracts has led to the identification of a range of noxious compounds (**11**–**21**, Fig. 6) and it is likely that their biological activity derives, at least in part, from activation of TRPV1. The major components of the extracts are capsaicin (65%) and dihydrocapsaicin (**17**, 32%) with other derivatives isolated as minor components.[27] All of the compounds isolated from chilli extracts show some degree of noxious effects. Capsaicin, dihydrocapsaicin and *N*-vanillylnonamide (**14**) are approximately equipotent at TRPV1, suggesting that the exact composition of the lipid chain is less important than the overall hydrophobicity of the compound.[28,29] Capsaicin is the prototypical TRPV1 agonist although much early structure–activity work has been carried out on *N*-vanillylnonamide as this is synthetically more simple to construct.

Synthesis of capsaicin

Crombie *et al*. reported the first synthesis of capsaicin, in which the geometry of the C–C double bond was defined, in 1955 (Scheme 1).[30] Previously it had been unclear whether the C–C double bond was *E* or *Z*, however, IR analysis by Crombie *et al*. indicated that the *E* double bond was present,

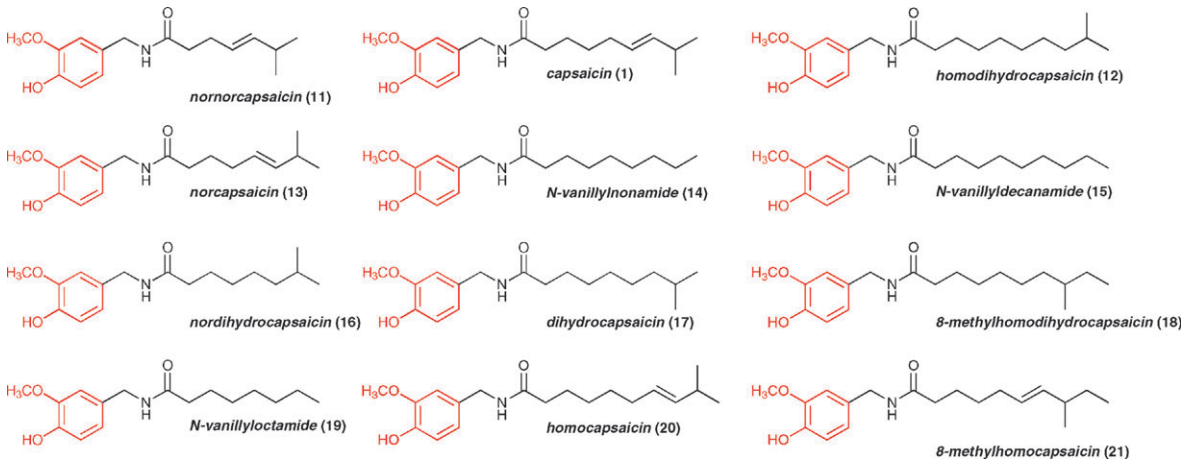
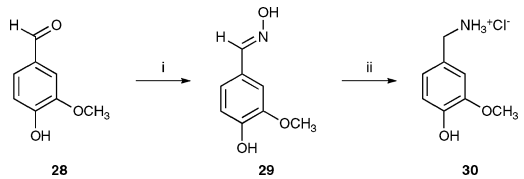


Fig. 6 The structure of capsaicin and related derivatives. The vanillyl moiety is highlighted in red.

hence they designed their synthesis to ensure that this geometry was present in their synthetic capsaicin. Reaction of isopropylmagnesium bromide (**22**) with 2,3-dichlorotetrahydropyran (**23**) afforded 3-chloro-2-(isopropyl)tetrahydropyran (**24**), which underwent reductive ring opening on treatment with powered sodium, to afford *E*-6-methylhepten-1-ol (**25**). Conversion to the bromide (**26**) and subsequent reaction with sodium malonate furnished the carboxylic acid **27**. The carboxylic acid was converted to the acid chloride and reacted with vanillylamine to afford material that was identical to capsaicin isolated from natural sources.

Gannett *et al*. reported a more general synthesis of capsaicin and its analogues (Schemes 2 and 3). Vanillylamine (**30**) was synthesised from 4-hydroxy-3-methoxybenzaldehyde (**28**) by formation of the oxime (**29**) and subsequent reduction, using Pd on carbon, to give the desired amine (**30**).[27]

The lipid chain portion of capsaicin was synthesised from the appropriate lactone (**31**, Scheme 3). Methanolysis of the lactone (**31**) afforded the alcohol (**32**), which was immediately oxidised to the aldehyde (**33**). A number of procedures were investigated to give the required double bond, with the Kocienski–Lythgoe–Julia procedure giving the optimum ratio of *E* to *Z* isomers (**35**). The methyl ester was hydrolysed, converted to the acid chloride and coupled with vanillylamine (**30**) to furnish capsaicin.

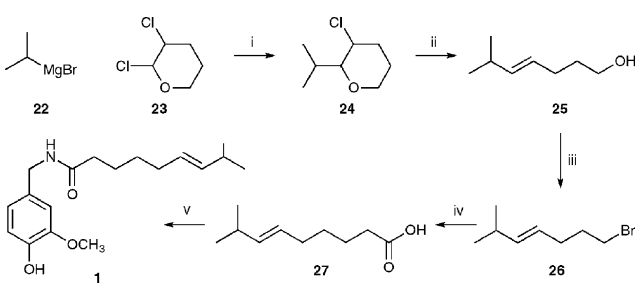


Scheme 2 The synthesis of vanillylamine (**30**) reported by Gannett *et al*. *Reagents and conditions*: (i) $NH_2OH{\cdot}HCl$, pyridine, EtOH, 83%; (ii) 10% Pd/C, H_2, EtOH, HCl, 98%.[27]

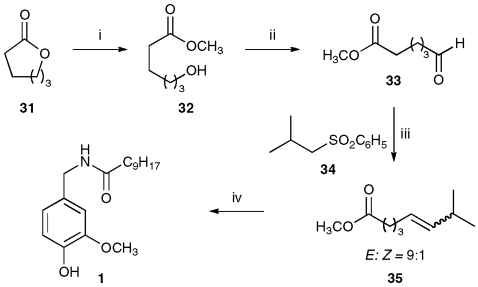
Resiniferatoxin (RTX)

Despite the long history of capsaicin, it is not the most potent TRPV1 agonist. RTX (**2**, Fig. 2), a daphnane diterpene component isolated from the latex of several members of the genus *Euphorbia*, is the most potent known TRPV1 agonist.[31] RTX had previously been identified as a compound with strong irritant properties, but in 1989 it was demonstrated that this compound caused capsaicin-like responses in rats.[32] However, the biological profiles of RTX and capsaicin are not identical and it is therefore thought that these two compounds do not bind at exactly the same binding site, although the binding sites may be similar, with overlapping regions.

In a truly remarkable synthetic achievement, the total synthesis of RTX was reported by Wender and co-workers in 1997.[33] Their synthesis was all the more impressive as it



Scheme 1 The first synthesis of capsaicin, reported by Crombie *et al*. *Reagents and conditions*: (i) Et_2O, 0 °C, 31–38%; (ii) Na, Et_2O; (iii) PBr_3, pyridine, 0 °C; (iv) (a) sodium malonate, EtOH, reflux; (b) NaOH (aq), reflux; (c) 160–180 °C, 52%; (v) (a) $SOCl_2$, RT for 18 h then 100 °C for 30 min; (b) 4-hydroxy-3-methoxybenzylamine, Et_2O.[30]



Scheme 3 The synthesis of capsaicin reported by Gannett *et al*. *Reagents and conditions*: (i) MeOH, H_2SO_4, reflux, 85%; (ii) PCC, NaOAc, CH_2Cl_2; (iii) (a) **34**, BuLi, THF, −78 °C → −30 °C; (b) **33**, 0 °C; (c) BzCl, −78 °C → RT; (iv) (a) KOH, EtOH; (b) $SOCl_2$; (c) **30**, pyridine, Et_2O, RT.[27]

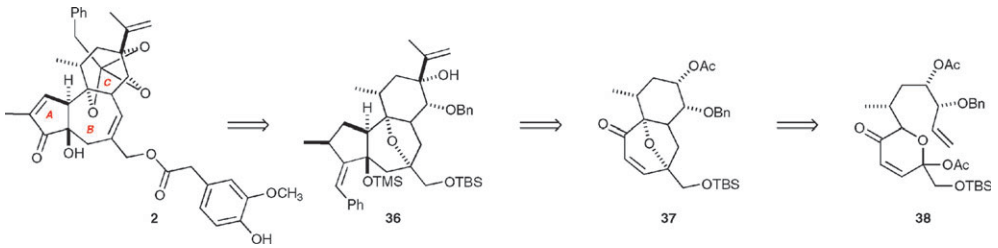


Fig. 7 The retrosynthetic approach employed by Wender and co-workers. The letters shown in red indicate the ring designation.[33]

represents the first total synthesis of a member of the daphnane family of alkaloids. The retrosynthetic approach employed by Wender is illustrated in Fig. 7. The homovanillyl chain was introduced late in the synthesis, a deliberate strategy to enable the synthesis of RTX analogues. The key reaction was an oxidopyrylium cycloaddition reaction, which was used to form the B and C rings with the correct stereochemistry. The absolute stereochemistry of the synthesis was controlled in the first step through the use of a Sharpless-type epoxidation. The synthesis was completed in 42 linear steps with an overall yield of 0.28%.

Despite representing a major synthetic achievement, the length of this synthesis prohibits the synthesis of a large number of RTX analogues and the cloning of TRPV1 has led to the discovery of many TRPV1 agonists and antagonists that are synthetically easier to obtain than RTX.

Basic structure–activity trends

One of the most comprehensive structure–activity relationship (SAR) studies on capsaicin analogues was carried out by Walpole and co-workers.[29,34,35] Despite the identity of the capsaicin receptor being unknown when this work was completed, the conclusions reached hold true for the TRPV1 activities of compounds that are based on the vanillyl core structure. Walpole *et al.* divided capsaicin into three regions: region A comprises the vanillyl moiety; region B contains the amide and region C is the lipid chain (Fig. 8). The following structure–activity deductions were made using Ca^{2+} uptake

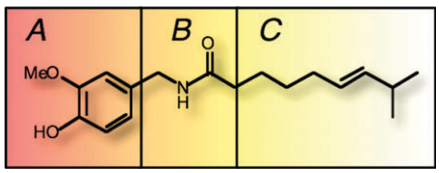


Fig. 8 The three regions of capsaicin as defined by Walpole and co-workers.

into a subpopulation of dorsal root ganglion (DRG) neurons as a primary assay. Those compounds that were active in the Ca^{2+} flux assay were further evaluated using *in vitro* assays.[34]

The general observations of region A SAR are shown in Fig. 9 and discussed below. These trends were identified through modification of the A region with the rest of the molecule being either the *N*-nonoyl amide or the "reverse" amide. The 3-methoxy-4-hydroxy motif present in capsaicin is the most potent combination in terms of Ca^{2+} flux (and therefore presumably TRPV1 agonist) activity. Substitution at the 2-position (R^2) or 5-position (R^5) abolishes activity, irrespective of the nature or pattern of the other substituents. Transposition of the 3-methoxy and 4-hydroxy groups led to retained but reduced activity.

Removal of the 3-position methoxy group led to reduced activity in the Ca^{2+} assay. Replacement of the 3-methoxy group with $-CH_3$, $-NH_2$, –NHAc or $-OC_2H_5$ led to loss of activity when the 4-position substituent was a hydroxyl group. Some activity was retained when a nitro group was introduced at the 3-position and high activity in the Ca^{2+} flux assay was

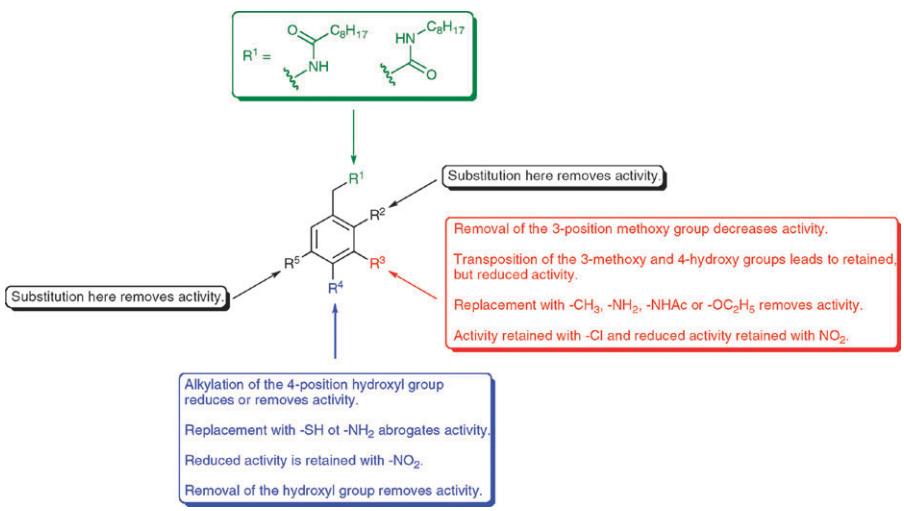


Fig. 9 The SAR of the A region of capsaicin, deduced by Walpole and co-workers.[34]

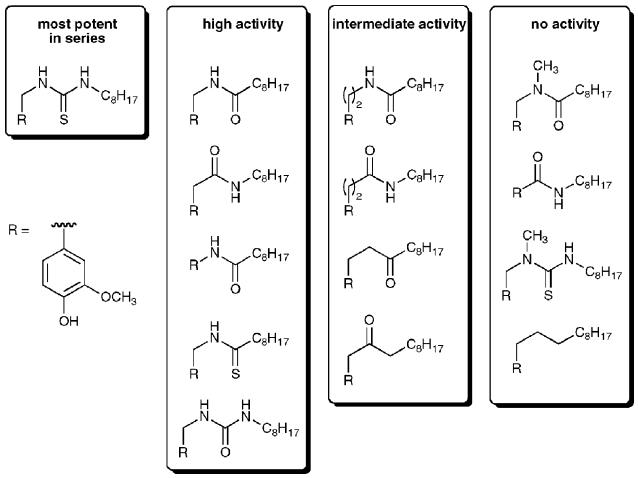


Fig. 10 The SAR of the B region of capsaicin, deduced by Walpole and co-workers.[35]

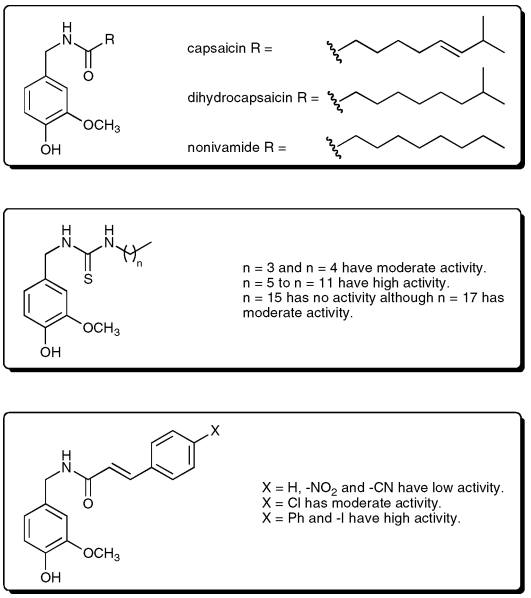


Fig. 11 The SAR of the C region of capsaicin, deduced by Walpole and co-workers.[29]

observed with a 3-chloro substituent. However, the high activity of the 3-chloro derivative did not translate into high *in vitro* activity as the compound proved to be a weak partial agonist.

The SAR of the B region of the vanilloid compounds was assessed using the vanillyl A region and an octyl chain as the C region (Fig. 10). The amide observed in capsaicin and the reverse amide, in which the nitrogen and carbonyl are transposed, are both highly potent in the Ca^{2+} flux assay. The thioamide and the urea also displayed high activity. Removal of the sp^3 carbon linking the phenyl ring to region B led to retention of activity in the case of the amide, but a loss of activity in the case of the reverse amide, and all other analogues.

Inclusion of an extra methylene unit between the phenyl ring and region B led to retained but significantly reduced activity, as did removal of the nitrogen atom in either the reverse or standard sense. In all cases *N*-methylation led to a total loss of activity, as did removal of both the nitrogen atom and the carbonyl. The most significant finding was that the thiourea moiety is the most potent in terms of TRPV1 activation, and this has informed the design of numerous subsequent compounds.[35]

The SAR of the C region of the vanilloid compounds was assessed using the vanillyl A region and the standard amide as the B region (Fig. 11). Capsaicin, dihydrocapsaicin and the octylamide, nonivamide, are all approximately equipotent. This suggests that the overall size or hydrophobicity is more important than the double bond or the branching methyl group.

The importance of side chain hydrophobicity is further evident in the thiourea series, where it is demonstrated that those compounds with short (four- or five-carbon) chains display only moderate activity in the Ca^{2+} flux assay. Those compounds with carbon chain lengths of six to twelve carbons display high activity, with the eight-carbon chain analogue showing the highest activity. The compound with a sixteen-carbon chain shows no activity and the compound with an eighteen-carbon chain shows only moderate activity, suggesting that these chains are above the maximum size that can be tolerated by the TRPV1 capsaicin-binding site and the hydrophobic chain is being forced into unfavourable interactions with the water surrounding the protein.[29] Olvanil, which has an oleamide chain (17 carbon atoms with a *Z* double bond between carbons 8 and 9), is a TRPV1 agonist.

Aromatic substituents in the C region are tolerated by TRPV1, although their activity is dependent on the nature of their linkage to the B region. Studies with a 4-chlorophenyl substituent have shown that a two-carbon chain containing an *E* double bond is more active than a one-carbon chain, a two-carbon chain containing a *Z* double bond, a saturated two-carbon chain or a two-carbon chain containing an alkyne unit. In the reverse amide series the saturated two-carbon chain showed high activity. The nature of the 4-position ring substituent also plays an important role in determining the activity of the compound, with more hydrophobic substituents showing higher activity than more polar substituents. The least active compound possessed a nitrile group in the 4-position and the compound with a hydrogen atom in this position also showed low activity. A 4-chloro substituent gives moderate activity with a 4-iodo or a 4-phenyl substituent conveying high activity to the compound. These findings underline the importance of the overall hydrophobicity of the side chain in determining the activity of the compound. Walpole *et al.* discuss the importance of hydrophobicity *versus* size in more detail,[29] but conclude that hydrophobicity is the overriding important factor for the C region.

It is important to note that the SAR discussed above relate primarily to compounds based on the vanilloid scaffold. As will become clear, those compounds that are now considered to be of therapeutic importance have moved away from this structure, either as a result of further structure–activity studies or as a result of high-throughput screening. However, these studies represent some of the first rational investigations of ligand interactions at the capsaicin receptor and have guided subsequent work on more recently reported TRPV1 ligands.

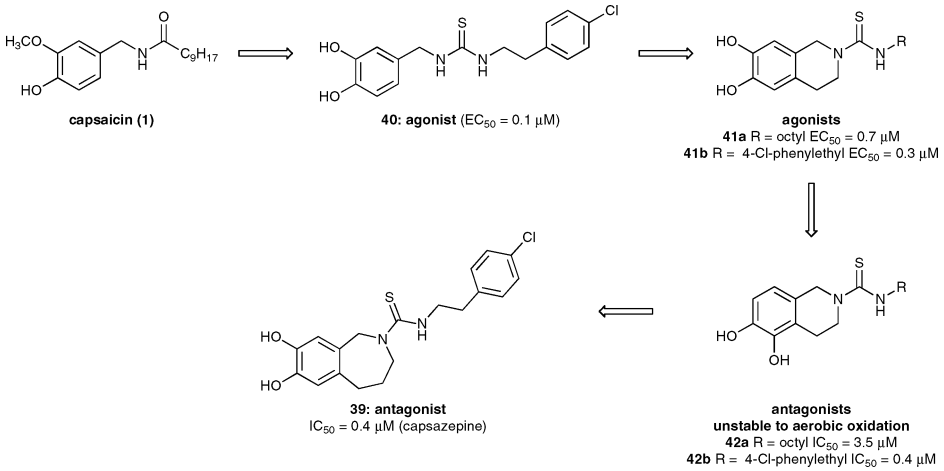


Fig. 12 The structure–activity studies conducted by Walpole and co-workers, which led to the discovery of the first competitive antagonist of capsaicin activity, capsazepine (**39**).[37]

This is evident in the following section on the development of TRPV1 antagonists.

Early TRPV1 antagonists

Although pain relief can be achieved through TRPV1 desensitisation as a result of TRPV1 agonist application, this ultimately leads to excessive Ca^{2+} influx and death of the nociceptive neurons on which TRPV1 is located. Hence, TRPV1 antagonists rather than agonists have emerged as the most likely drug candidates to treat pain and a number of other indications, in which TRPV1 is involved.

Capsazepine

The dye Ruthenium red was the first TRPV1 antagonist identified and was shown to reverse the activity of capsaicin, most likely by blocking the capsaicin-gated channel. However, the first competitive antagonist of capsaicin activity was capsazepine (**39**, Fig. 12), which was shown to compete with both capsaicin and RTX in binding to the capsaicin receptor.[36,37]

While investigating the SAR for capsaicin-like agonists (*vide supra*), Walpole and co-workers progressed through the compound development detailed in Fig. 12. It had previously been demonstrated that the 4-hydroxy 3-methoxy motif (such as that seen in capsaicin) could be replaced by the catechol (3,4-dihydroxy) motif without loss of agonist activity. A number of unconstrained thiourea structures (such as compound **40**) were synthesised, and it was demonstrated that these compounds retained agonist activity. The importance of the orientation of region C relative to region A was probed through the synthesis of conformationally restricted tetrahydroisoquinoline (THIQ) thioureas. This conformational restriction gives rise to two possible isomers exemplified by **41** or **42** (Fig. 12). Both isomers were synthesised, with two different C region chains, and it was shown that **41a** and **41b** retained activity as agonists, whereas **42a** and **42b** behaved as antagonists of capsaicin activity. However, these compounds were not suitable for further development, as they were susceptible to aerobic oxidation. A series of compounds were synthesised in which the size of the unsaturated ring was altered. Those compounds that contained a 5-membered ring were observed to have similar agonist activity to their unconstrained counterparts. Several compounds that contained a 7-membered ring were synthesised and were moderately potent antagonists of capsaicin activity. The most effective C region chain was the 4-chlorophenylethyl, which gave rise to capsazepine (**39**). This compound was demonstrated to be an antagonist of both capsaicin and RTX action, with a Schild† plot gradient of ≈ 1 in both cases, indicating that capsazepine was functioning as a competitive antagonist. From their SAR studies, Walpole and co-workers proposed that two modes of binding at the capsaicin receptor are possible, one for agonists and another for antagonists. Determination of the solution phase and crystal structures of a number of their compounds gave rise to the suggestion that the antagonist compounds bind in a more "bent" orientation, whereas the agonists adopt a more extended conformation.[37] Despite its relatively low potency as an antagonist, capsazepine represented the only capsaicin/TRPV1 antagonist until 2001 and is still used by many pharmacologists due to its ready availability. However, this compound is not fully selective and has been reported to have non-specific actions at voltage-gated calcium channels and nicotinic acetylcholine receptors at concentrations used to block TRPV1-mediated responses. In addition, it has been shown that capsazepine may not be effective in blocking TRPV1 activation caused by heat or low pH.[7]

† A Schild analysis plots log (concentration of agonist) against log (dose ratio −1). The gradient of the line gives information about the binding of the drug to the receptor, with a slope of ~ 1 indicating that the antagonist is competing with the agonist to bind with the receptor. The intercept of the line gives the pA_2 value where $pA_2 = -\log K_B$, and K_B is the equilibrium constant of the antagonist binding to the receptor. The dose ratio is the ratio by which the agonist concentration has to be increased in the presence of the antagonist in order to restore a given level of response. For further information see ref. 57.

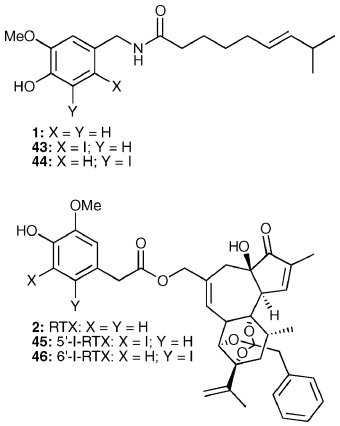


Fig. 13 The structure of the halogenated capsaicin and RTX derivatives.

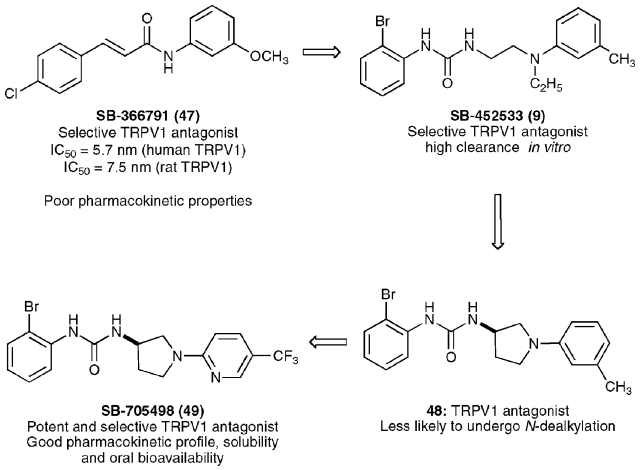


Fig. 14 The development of the potent and selective TRPV1 antagonist SB-705498 (**49**) by GSK.

Halogenated capsaicin and RTX derivatives

A more potent TRPV1 antagonist was discovered in 2001 through work towards an ^{125}I-labelled TRPV1 ligand.[38] 5′-Iodo-RTX (**45**, 5′-I-RTX, Fig. 13) and 5′-^{125}I-RTX were both synthesised by regioselective iodination of RTX. It was discovered that 5′-I-RTX, in which the vanillyl ring is iodinated at the 5-position, is not an agonist of TRPV1 but is a potent TRPV1 antagonist (IC_{50} = 3.9 nm *in vitro*). 5′-I-RTX was also effective *in vivo*, blocking capsaicin-induced pain responses in mice at intrathecal doses of 16 ng per mouse, and is approximately 40-fold more potent than capsazepine. It was subsequently shown that 6′-iodo-RTX (**46**) is a partial agonist of TRPV1. Although the aim of this work was the synthesis of a compound that would allow the mapping of TRPV1 locations within cells, it is the discovery of a potent and selective TRPV1 antagonist that has facilitated a large body of work on TRPV1 channels.

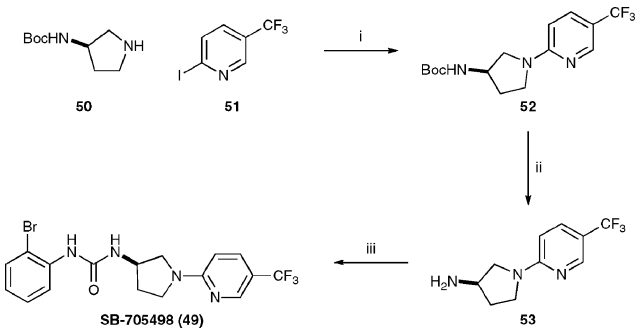
In a logical extension of the above work, Appendino and coworkers have demonstrated that halogenation of capsaicin and its close analogue, *n*-vanillylnonamide (**14**, nonivamide), gives rise to further TRPV1 antagonists.[28,39] The 6′-substituted compounds were observed to be more potent than the 5′-substituted compounds (I > Br ≫ Cl). In more recent work, Appendino has synthesised a range of compounds that were substituted with non-halogen moieties at the 6-position and it is interesting to note that most of these compounds demonstrated negligible antagonist activity. In addition, when the C region lipid chain was varied, the antagonist activity of the halogenated compounds did not correlate with the agonist activity of the unhalogenated parent compound. This suggests that although the agonists and antagonists occupy the same binding site in TRPV1, the modes of binding may be different.[28]

Therapeutically important compounds

Although some early topical ointments were TRPV1 agonists, current research towards a therapeutically relevant compound focuses on TRPV1 antagonists. There are currently no TRPV1 antagonists that have been registered as drugs; however, several compounds are in clinical trials. It is beyond the scope of this review to discuss all of these compounds so the most attention will be given to the most advanced compounds. Further examples of compounds that act at TRPV1 and which are currently in clinical trials can be found in a recent review.[7]

The discovery of SB-705498

SB-705498 (**49**) is a potent and selective TRPV1 antagonist that has been developed by GSK (Fig. 14). The development of SB-705498 can be traced back to the discovery of the first TRPV1 antagonist reported by workers at GSK, SB-366791 (**47**). SB-366791 is a selective TRPV1 antagonist but had poor pharmacokinetic (PK) properties. Structural modifications of SB-366791 led to SB-452533 (**9**), which was also a selective TRPV1 antagonist, but had high clearance *in vitro*. It was thought that the high clearance of SB-452533 could be due to dealkylation of the *N*-ethyl group. Initial attempts to address this problem involved the synthesis of dihydroindole analogues, however, these compounds were less potent than the parent compounds and exhibited no improvement in their PK profile. An alternative approach was taken, in which the *N*-ethyl group was cyclised onto the ethylidene linker to form pyrrolidine derivatives. This introduces a stereogenic centre into the molecule and it was demonstrated that the (*R*)-enantiomer (**48**) was at least 10-fold more active than its antipode. Addition of a methyl or trifluoromethyl group to the aromatic ring led to compounds with potency comparable to that of the SB-452533 parent compound. It was determined that the use of a trifluoromethyl-substituted pyridine ring gave rise to a compound that displayed potent and selective antagonist activity at TRPV1, and in addition, this compound also showed good solubility, PK properties and oral bioavailability. SB-705498 was recently reported to be in phase 2 clinical trials for migraine and phase 1 for dental pain.[7] Chizh *et al*. reported that SB-705498 reduced the area of capsaicin-evoked flare and increased heat pain tolerance at the site of UVB-evoked inflammation and therefore shows the first clinical example of antihyperalgesia (pain relief) by a TRPV1 antagonist.[40] Interestingly, it would appear that this compound is not only acting on peripheral TRPV1 channels, but that it is able to cross the blood–brain barrier and act on



Scheme 4 The synthesis of SB-705498 (**49**). *Reagents and conditions*: (i) K_2CO_3, DMF, 100 °C, 7 h, 83%; (ii) (a) HCl (4 M)–dioxane (1 : 1), 80 °C, 2 h; (b) pH 12, CH_2Cl_2; (iii) 2-(bromo)phenylisocyanate, Et_2O, 84%.

TRPV1 in the CNS. Furthermore, it appears that the CNS activity of this compound may be important for its clinical effects.

The synthesis of SB-705498 (**49**) commenced from Boc-protected 3-aminopyrrolidine (**50**) and 2-iodo-5-trifluoromethylpyridine (**51**, Scheme 4), both of which are commercially available. Nucleophilic substitution of the pyridine by the pyrrolidine afforded the Boc-protected pyridyl intermediate **52**. Deprotection of the Boc group under acidic conditions, followed by basification afforded the free amine **53**, which was used without purification in the next step. Reaction of the amine with 2-(bromo)phenylisocyanate afforded SB-705498 in an overall yield of 62%, without the need for chromatography.

The discovery of benzimidazole-based TRPV1 antagonists

A second example of a therapeutically important TRPV1 antagonist involves efforts by scientists at both Purdue Pharma and Amgen (Fig. 15). This work focused on compounds derived from the piperazine-1-carboxamide-based structure, such as **54**, which was furnished from a high-throughput screen. This compound is a selective TRPV1 antagonist with IC_{50} = 58 nM (capsaicin activation of rat TRPV1) and IC_{50} = 39 nM (H^+ activation of rat TRPV1);[41] however, **54** was not orally bioavailable in rats. Parallel synthesis optimisation of compound **54** led to the discovery that replacement of the trifluoromethyl group with a chlorine atom and substitution of

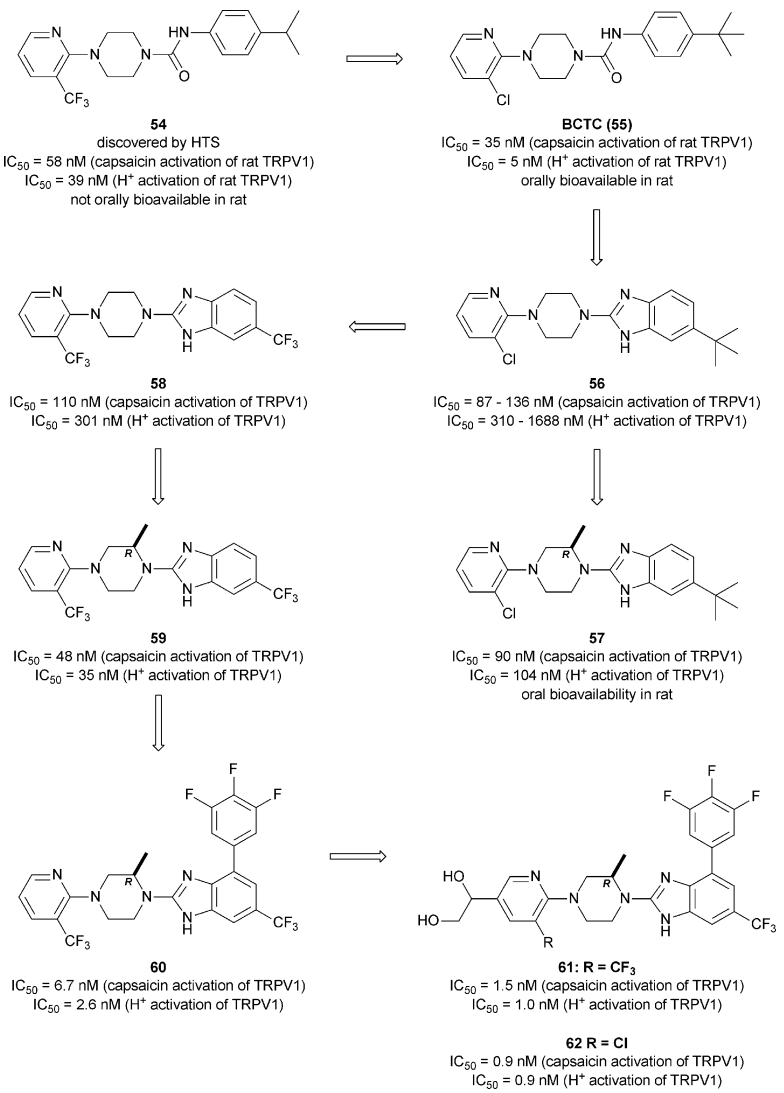


Fig. 15 The development of benzimidazole-based TRPV1 antagonists.

the isopropyl group with a *tert*-butyl group gave the optimum activity. Compound **55** had IC_{50} = 35 nM (capsaicin activation of TRPV1) and IC_{50} = 5 nM (H^+ activation of TRPV1) and in addition was seen to be orally bioavailable in rat.[41] Although the BCTC (**55**) template provides effective TRPV1 antagonists, its pharmacokinetic profile is not ideal, with suboptimal metabolic stability, half-life, water solubility and only moderate bioavailability.[42] Both Purdue Pharma[43] and Amgen[42] investigated conformationally restricted analogues of BCTC and hence developed a range of benzimidazole derivatives. Compound **56** was shown to be an effective antagonist (IC_{50} = 136 nM) of capsaicin-evoked activation of human TRPV1 (expressed in HEK 293 cells). However, this compound was less effective (IC_{50} = 1688 nM) as an antagonist of H^+-evoked activation of human TRPV1 (expressed in HEK 293 cells).[43] Amgen reported the same compound to have IC_{50} = 87 nM in their capsaicin-mediated assay and IC_{50} = 310 nM in their pH-mediated assay.[42] The scientists at Purdue Pharma investigated the substitution of the piperazine ring and demonstrated that inclusion of a methyl group on the carbon nearest to the benzimidazole ring gave improved activity against both capsaicin- and H^+-evoked TRPV1 activation (**57**, IC_{50} = 90 nM and 104 nM, respectively, against activation of human TRPV1 expressed in HEK 293 cells) when the configuration of the new stereogenic centre was *R*. The enantiomeric compound displayed reduced activity with IC_{50} = 948 nM against capsaicin activation of human TRPV1 (expressed in HEK 293 cells) and IC_{50} = 3608 nM against H^+-evoked human TRPV1 (expressed in HEK 293 cells).[43] Compound **57** was seen to have a moderate half-life, mainly due to relatively rapid clearance. Despite this rapid clearance, compound **57** displayed oral bioavailability in rats.

Amgen conducted significant SAR studies on the core of compound **56**.[42] Antagonist activity was retained when both the *tert*-butyl group and the chlorine atom were replaced with trifluoromethyl groups (**58**). Introduction of the (*R*)-methyl group on the piperazine ring (**59**) resulted in a 2-fold potency increase against capsaicin-evoked TRPV1 activation and a 9-fold potency increase against H^+-evoked TRPV1 activation, compared to the parent compound. Interestingly, addition of a methyl group on the alternative piperazine carbon, giving a stereogenic centre with the (*R*)-configuration, resulted in a similarly potent TRPV1 antagonist. As was observed by Purdue Pharma, either elongation of the alkyl chain or inversion of the stereochemical configuration to give the (*S*)-enantiomer resulted in reduced activity. Further structure–activity studies showed that increased potency was achieved when a 3,4,5-(trifluoro)phenyl group was introduced at the 4-position of the benzimidazole ring (**60**). The increased potency of this compound (**60**) is suggestive of a large hydrophobic pocket existing within the ligand-binding site of TRPV1. It was also shown that substitution at the 5′-position of the pyridine ring was tolerated. Inclusion of the 1,2-dihydroxyethyl substituent at this position resulted in a diastereomeric mixture of compounds (**61**), which was very potent in both the capsaicin- and H^+-mediated assays. In a similar compound, differing only in the replacement of the trifluoromethyl group with a chlorine atom (**62**), the same degree of potency was observed (IC_{50} = 0.9 nM in both capsaicin- and H^+-mediated assays). The latter compound (**62**) was taken forward into pharmacokinetic studies and was seen to have an elimination half-life of 5.6 h, with a low clearance rate and was shown to be orally bioavailable in rats.

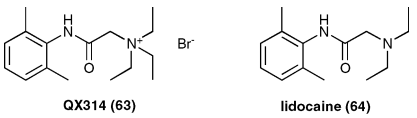


Fig. 16 The structures of QX314 (**63**) and lidocaine (**64**).

Amgen has recently reported comprehensive syntheses and pharmacological data on another range of compounds designed to act as TRPV1 antagonists, which are initially derived from *trans*-cinnamides.[44–46] However, these compounds will not progress past phase 1 clinical trials as it has recently been reported that the clinical candidate (AMG 517) causes a long-lasting increase in body temperature to 40 °C.[47]

TRPV1-mediated entry of impermeant sodium channel blockers

TRPV1 and capsaicin have recently been used in another clinically relevant application, which may have therapeutically significant potential. Most local anaesthetics are relatively hydrophobic and diffuse into the cell across the cell membrane and bind to sodium channels to exert their action. The major disadvantage of these local anaesthetics is that they block sodium channels in all neurons, not just those involved in sensing pain (nociceptive neurons). Hence administration of local anaesthetics not only blocks those nerve transmissions that are associated with pain, but also cause numbness and motor impairment. Co-administration of capsaicin with a membrane impermeant derivative of lidocaine (**64**), QX314 (**63**, Fig. 16), has been shown to circumvent this problem. As QX314 is not able to diffuse into cells, it is unable to block sodium channels when administered on its own. However, TRPV1 has a relatively large channel pore, which QX314 is able to pass through. Therefore, when both capsaicin and QX314 are applied, capsaicin activates TRPV1, allowing QX314 to pass through the TRPV1 channel and block sodium channels from within the cell. The major advantage of this method is that TRPV1 is expressed on most nociceptive neurons, but not other neurons, hence sodium channel block occurs only in those neurons involved in sensing pain and not those involved in motor control. Therefore the co-administration of QX314 and capsaicin may find application in situations where local anaesthesia but not motor impairment or numbness is required, for example some dental procedures and childbirth.[48]

The above examples demonstrate that there is a great deal of potential for compounds acting on TRPV1 to be important in a clinical setting. It is likely that compounds such as those described above will become important drugs in the near future.

Caged TRPV1 ligands

It is not only drugs that are important for the study of endogenous receptors such as TRPV1; chemical probes that enable the study of these proteins are also of great value. The use of photolabile protecting groups to release biologically

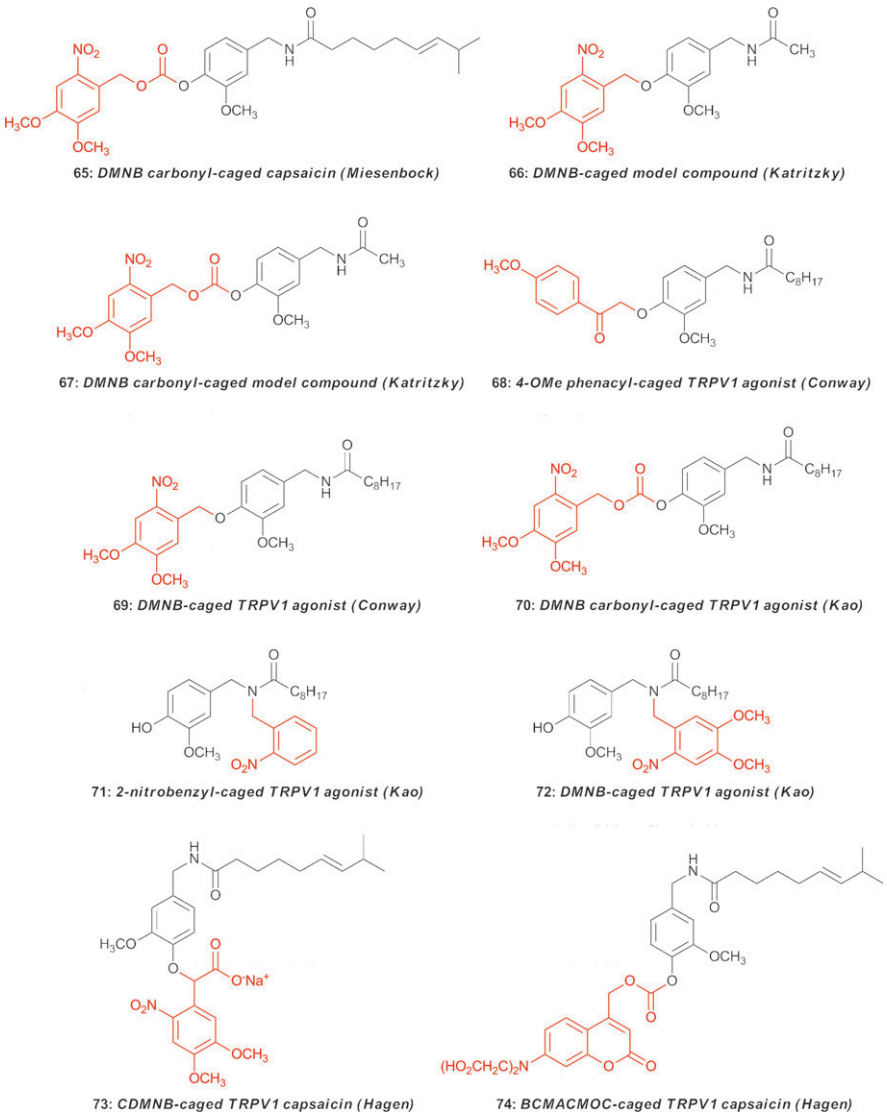


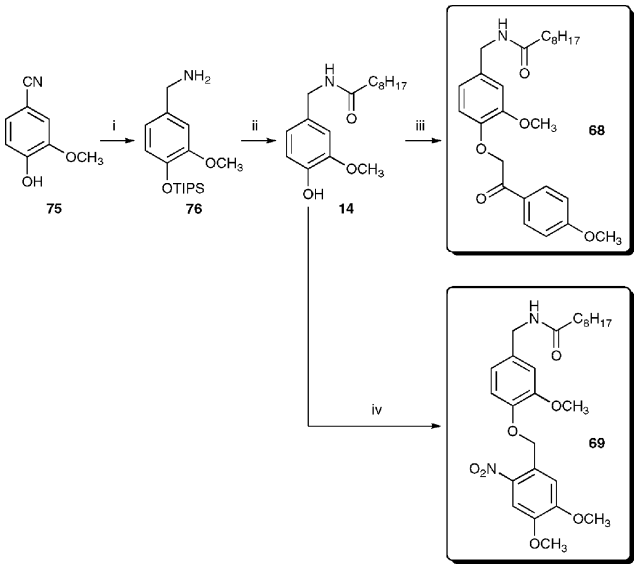
Fig. 17 The structure of the caged TRPV1 agonists. The caging group is highlighted in red.

active compounds within cells using light irradiation (caging) has found application in the study of many biological systems.[49,50] In order to be useful, the photolabile caging group must remove all biological activity of the compound in question. In the case of TRPV1 agonists, this is particularly attractive as this removes the noxious irritant properties of the compound and renders it easier to handle (Fig. 17). The inactive compound can then be introduced to the cells under study, but only when irradiated with light will the active compound be released. This provides increased control over when and where the compound is released, compared to simply applying the active compound directly to cells.

Miesenböck *et al.* were the first to report a caged capsaicin derivative (**65**) and demonstrate its use in the activation of TRPV1.[17] This compound (**65**) was synthesised by simple dimethoxynitrobenzyl (DMNB) protection of capsaicin. Katritzky *et al.* reported studies on the photolysis of a model caged capsaicin system (**66** and **67**). The compound used in these studies had an acetyl chain on the nitrogen, rendering it biologically inert.[51] Conway *et al.* reported the synthesis of a phenacyl (**68**) and DMNB (**69**) caged TRPV1 agonist and demonstrated that photolysis of the phenacyl compound in dorsal root ganglion neurons resulted in a response consistent with the activation of TRPV1.[18]

The syntheses of **68** and **69** commenced from the commercially available benzonitrile **75** (Scheme 5), which was TIPS protected and then reduced using $LiAlH_4$ to afford vanillylamine **76**. Acylation of the amine (**76**) with nonanoyl chloride and TBAF deprotection of the TIPS group afforded the TRPV1 agonist **14**. The phenol was alkylated with 2-bromo-4′-methoxyacetophenone or 4,5-dimethoxy-2-nitrobenzyl bromide using sodium hydride or potassium *tert*-butoxide as a base to afford the two caged TRPV1 agonists **68** and **69**, respectively. The phenacyl-caged compound **68** was demonstrated to activate TRPV1 after flash lamp photolysis *in vitro*; however, in general the responses were modest. ^{1}H NMR analysis of the photolysis of **68** and **69** demonstrated that **69** was more readily photolysed at the wavelengths employed.[18]

In all of the above cases the vanillyl phenol moiety was protected using the caging group. Kao *et al.* subsequently



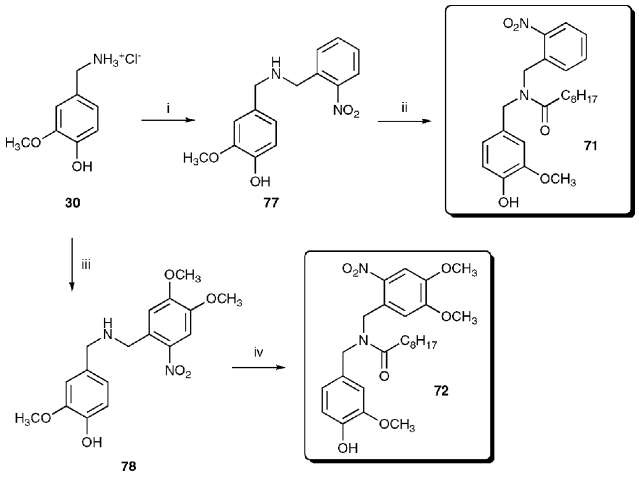
Scheme 5 The syntheses of two caged TRPV1 agonists reported by Conway and co-workers. *Reagents and conditions*: (i) (a) TIPSCl, imidazole, DMF, 91%; (b) $LiAlH_4$, THF, 90% crude yield; (ii) (a) nonanoyl chloride, 4-DMAP, pyridine, CH_2Cl_2, 0 °C → RT, 78%; (b) TBAF, THF, 81%; (iii) 2-bromo-4′-methoxyacetophenone, NaH, DMF, 0 °C → RT, 74%; (iv) 4,5-dimethoxy-2-nitrobenzyl bromide, ^{t}BuOK, THF, 66%.[18]

reported a range of caged TRPV1 agonists that contained compounds that were caged on the amide nitrogen. Kao *et al.* also demonstrated that the caged TRPV1 agonists could be released using 2-photon photolysis as well as 1-photon (standard) photolysis.[52]

Kao *et al.* commenced their synthesis of the nitrobenzyl-caged derivative (**71**, Scheme 6) from vanillylamine hydrochloride salt (**30**). Reductive amination of 2-nitrobenzaldehyde afforded the bis-*N*-benzyl derivative **77**, which was acylated with nonanoyl chloride to afford the caged TRPV1 agonist **71**. The dimethoxynitrobenzyl derivative **72** was also synthesised from vanillylamine hydrochloride (**30**). Alkylation with 4,5-dimethoxy-2-nitrobenzyl bromide to give **78**, followed by acylation with nonanoyl chloride afforded the 4,5-dimethoxy-2-nitrobenzyl caged derivative **72**.

One problem associated with caged TRPV1 ligands is the poor water solubility of these compounds. Gilbert *et al.* have addressed this problem with the synthesis of capsaicin protected by two water-soluble caging groups.[53] The α-carboxy-4,5-dimethoxy-2-nitrobenzyl (CDMNB) caging group was the first employed. This caging group was synthesised from dimethoxy-2-nitrophenylacetic acid (**79**) as shown in Scheme 7. The carboxylic acid (**79**) was coupled to 2-(trimethylsilyl)ethanol in the presence of DCC and 4-DMAP. Bromination of the benzylic carbon was achieved with AIBN and NBS furnishing **80**. The phenolic hydroxyl group of capsaicin was alkylated using **80** and potassium carbonate to give compound **81**. The TMS group was removed by treatment with TFA in dichloromethane and water. The carboxylic acid was subsequently converted to its sodium salt using an ion exchange resin affording **73**.

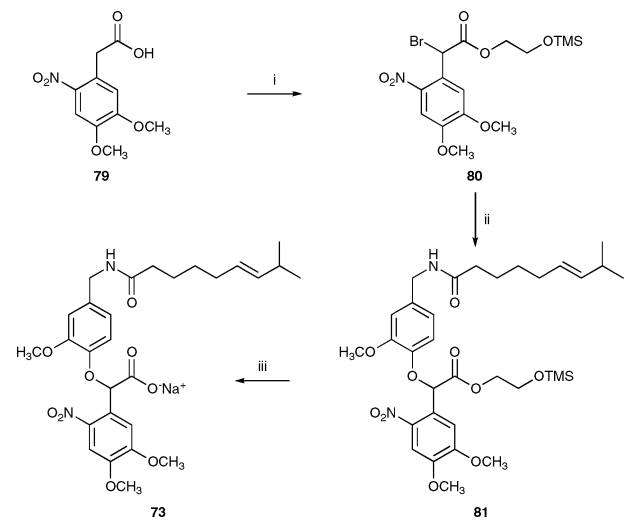
The absorption spectrum of caged capsaicin derivative **73** was measured in MeCN–HEPES buffer (pH 7.2) and showed



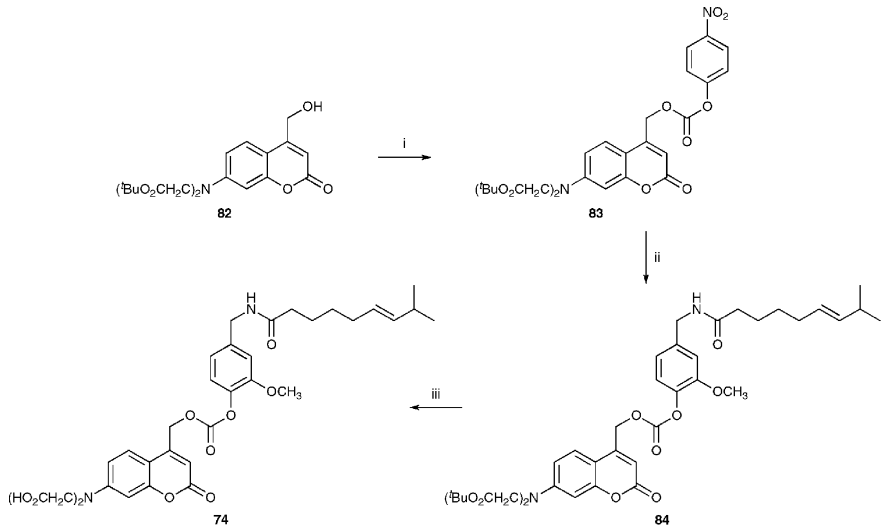
Scheme 6 The syntheses of two caged TRPV1 agonists reported by Zhao and co-workers.[52] *Reagents and conditions*: (i) 2-nitrobenzaldehyde, NaOAc, $Na(CH_3CO_2)_3BH$, 1,2-dichloroethane, 57% yield of hydrochloride salt; (ii) nonanoyl chloride, K_2CO_3, Et_3N, CH_2Cl_2, 56%; (iii) 4,5-dimethoxy-2-nitrobenzyl bromide, 1,2,2,6,6-pentamethylpiperidine, DMF, MeCN, 0 °C → RT, 65%; (iv) nonanoyl chloride, K_2CO_3, Et_3N, CH_2Cl_2, 69%.

λ_{max} at 349.5 nm and a molar extinction coefficient (ε) of 4700 M^{-1} cm^{-1}. The quantum yield of the compound (Φ) was measured as 0.05 in MeCN–HEPES buffer (pH 7.2). The photolysis of **73** was also investigated in dorsal root ganglion neurons (DRGs) using the patch clamp technique. It was observed that the photolysis of **73** gave responses that were consistent with TRPV1 activation.[53]

The second caged capsaicin derivative reported by Gilbert *et al.* employed the (7-[bis(carboxymethyl)amino]coumarin-4-yl)methoxycarbonyl (BCMACMOC) group (Scheme 8). The coumarin **82** was activated by formation of the 4-nitrophenyl carbonate (**83**) and coupled to capsaicin in the presence of 4-DMAP furnishing **84**. The *tert*-butyl ester was removed by



Scheme 7 The synthesis of the CDMNB-caged TRPV1 agonist reported by Gilbert and co-workers.[53] *Reagents and conditions*: (i) (a) 2-(trimethylsilyl)ethanol, 4-DMAP, DCC, ethyl acetate, 10 °C → RT, 66%; (b) NBS, AIBN, CCl_4, 76 °C, 55%; (ii) capsaicin, K_2CO_3, DMF, 83%; (iii) (a) TFA–CH_2Cl_2–H_2O (75 : 24 : 1), 70%; (b) Dowex W50-Na^+, 93%.



Scheme 8 The synthesis of the BCMACMOC-caged TRPV1 agonist reported by Hagen and co-workers.[53,54] *Reagents and conditions*: (i) 4-nitrophenyl chloroformate, 4-DMAP, CH_2Cl_2, 4 h; (ii) capsaicin, 4-DMAP, CH_2Cl_2, 28% over two steps; (iii) TFA–CH_2Cl_2–H_2O (75 : 24 : 1), 90%.

treatment with TFA in dichloromethane and water to give the desired product **74**.

The absorption spectrum of **74** showed λ_{max} at 383 nm and a molar extinction coefficient (A) of 18 750 M^{-1} cm^{-1}. The single photon photolysis quantum yield (Φ) was 0.12 in buffer (pH 7.2) at 383 nm. Isothermal titration calorimetry experiments demonstrated that **74** has very low membrane permeability and thus could be used to release capsaicin exclusively inside the cell or outside the cell. This experiment is interesting as it addresses the unsolved issue of whether TRPV1 is activated from within the cell or externally, or both. It was observed that intracellular photolysis of **74** elicited only small responses from the cell, whereas extracellular photolysis gave large responses and desensitisation of the receptor. These data indicate that the sensitivity of TRPV1 to activating ligands is distinctly different depending on the site of activation.[53,54]

That so many caged TRPV1 ligands have been developed in a relatively short space of time indicates not only the importance of TRPV1 but also the utility of caged compounds. Further development of tools, such as those highlighted above, is important as numerous aspects of TRPV1 function are not understood, notably the location of the ligand-binding site(s).

Summary and conclusion

This insight into some of the current areas of research involving capsaicin and TRPV1 highlights the intensive efforts towards the development of TRPV1 antagonists, and interest in this field seems certain to be sustained. With the discovery of new TRP channels, including TRPM8, which is a menthol and cold receptor,[55] it seems likely that our understanding of the TRP family of channels is vital for the development of new drugs and our improved understanding of many biological processes. There are certain to be significant challenges involved in developing compounds that act at TRPV1 as drugs, this is exemplified by a receptor report that TRPV1 channels are involved in mediating long-term depression (LTD) in the hippocampus, a process that is involved in learning and memory.[56] It is inevitable that synthetic chemistry has a vital role to play in this understanding through the development of novel molecular tools to probe the function of these proteins.

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