VR_1 Receptor Modulators as Potential Drugs for Neuropathic Pain

María L. López-Rodríguez*[,a], Alma Viso[b] and Silvia Ortega-Gutiérrez[a]

[a]*Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense, 28040 Madrid, Spain;* [b]*Instituto de Química Orgánica, Consejo Superior de Investigaciones Científicas, C/ Juan de la Cierva 3, 28006 Madrid, Spain*

Abstract: The involvement of VR_1 in the endogenous pain signalling has converted this receptor into a promising therapeutic target for the development of a new family of potent analgesics devoid of the shortcomings of other analgesics commonly used. The desensitisation induced after VR_1 activation points to the utility of VR_1 agonists for the treatment of various nociceptive disorders including mitigation of neuropathic pain, inhibition of neurogenic inflammation and suppression of urinary bladder hyperreflexia, whereas VR_1 antagonists have been described as valuable agents for the treatment of inflammatory hyperalgesia and pain. Structure of the main classes of VR_1 ligands developed to date, their molecular mechanisms of action and their promising utility for the management of diverse nociceptive alterations, specially neuropathic pain, are discussed in this review.

Keywords: Vanilloid receptor type 1 (VR_1), analgesia, neuropathic pain, structure-affinity relationship studies.

1. INTRODUCTION

Complete control of pain is still one of the most ambitious pending objectives for current pharmacology. In keeping with this, during the past decades several successful strategies to face acute pain have been developed. However, chronic pain lacks to date satisfactory therapeutic treatments.

Among the different nociceptive disorders included in chronic pain, neuropathic pain has become a focus of major attention due to the clear need for the development of new agents able to induce amelioration of the unsustainable living conditions of many patients affected by this disorder.

Therefore, many efforts have been devoted to gain insights in both the understanding of the mechanisms and the identification of proteins, which could become targets for the treatment of neuropathic pain. Among these different targets, many of them reviewed along this special issue, the vanilloid receptor type 1 (VR_1) has arised as one of the most interesting, since VR_1 agonists represent a novel alternative to traditional analgesics bearing the advantage of impairing sensory neurotransmission without causing significant adverse effects in the central nervous system (CNS).

This review is focused on the involvement of vanilloid receptors in the regulation of pain neurotransmission pathways and on their promising utility for the treatment of neuropathic pain. Some aspects, such as the molecular pharmacology of vanilloid receptor, its anatomical localization as well as its tissue and species specificities, are only briefly summarized here as their details are clearly beyond the scope of this contribution and have already been widely discussed in previous reviews [1-3]. Instead, a major emphasis is laid on the structure-activity relationship (SAR) studies which have led to the development of structurally different classes of VR_1 ligands which may serve both as pharmacological tools to study its physiological implications and as regulators of the vanilloid receptor mediated effects.

*Address correspondence to this author at the Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense, 28040 Madrid, Spain; Tel: 34-91-3944239; Fax: 34-91-3944103; E-mail: mluzlr@quim.ucm.es

2. VANILLOID RECEPTOR

Analgesic potential of capsaicin, the main pungent principle present in hot peppers, has been known and exploited by human beings since ancient times all over the world, from the native americans to the chinese culture [2]. However, the molecular characterization of the specific membrane recognition site responsible for the effects induced by capsaicin, named VR_1, took place only six years ago, in 1997 [4].

The early studies, carried out mainly during the 70´s and 80´s, determined some important features of the putative binding site for capsaicin, such as the strict structural requirements involved in the capsaicin-like induced responses as well as remarkable tissue and species differences in sensitivity to capsaicin [5-12]. This body of indirect evidence was consistent with the existence of a specific receptor for capsaicin but only the identification of resiniferatoxin (RTX) as an ultrapotent capsaicin analogue allowed to confirm unequivocally the existence of vanilloid receptors, demonstrated by the specific [^{3}H]-RTX binding in rat dorsal root ganglion (DRG) membranes [13]. Finally, the definitive evidence of the existence of the specific binding for capsaicin came out in 1997, when a functional cDNA encoding a capsaicin receptor was isolated and cloned from a rat cDNA library [4]. This first vanilloid receptor, termed VR_1, supposed to be a novel and defined target for the development of new strategies for the pharmacological treatment of pain, and in particular, for neuropathic pain. In this context, the understanding of the localization, the molecular features and the key residues involved in the VR_1 activation has become a focus of current research. Therefore, in this contribution we will begin by summarizing the most important molecular characteristics of VR_1 reported to date

1389-5575/03 $41.00+.00

as well as the recent advances, to provide the rational principles for structure-based design of a new generation of more effective antinociceptive agents.

2.1. Biochemical Characterization

The rat VR_1 receptor, protein constituted by 838 amino acids with a molecular mass of 95 kDa, was described as a non-selective cation channel with a preference for Ca^{2+} which can be activated by different classes of stimuli including chemical (such as vanilloid ligands or protons) and physical (such as noxious heat (> 43 ºC)) [4]. Soon after, the identification of its human ortologue was reported [14]. The human VR_1 has a chromosomal location of 17p13 and consists of 839 residues, displaying a 92% of homology with rat VR_1. Although both proteins share an important number of pharmacological and structural features, subtle differences among them, regarding their tissue distribution and pharmacology have also been reported [14, 15].

The VR_1 is structurally characterized by six membrane-spanning domains, mainly β sheets, and the poreforming loop, a short hydrophobic stretch between transmembrane regions 5 and 6, which contributes to the ion permeation path. Both C- and N- terminal domains are cytoplasmic, and whereas the former (154 amino acids) lacks any recognizable motif, the latter, constituted by 432 residues, is characterized by a relatively proline-rich region followed by three ankyrin repeat domains (Fig. (**1**)). Also, VR_1 possesses three potential protein kinase A (PKA) phosphorylation sites, which could be involved in receptor desensitization [4].

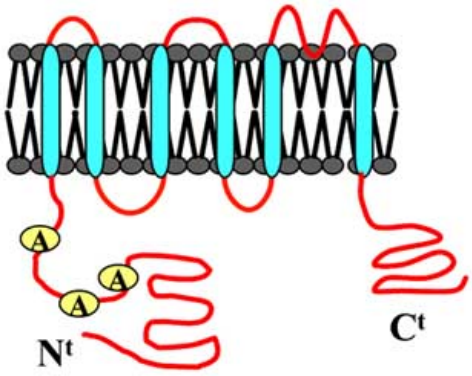


Fig. (1). Schematic structure of VR_1. Ankyrin repeat domains are indicated by the A.

By means of structural homology studies, VR_1 has been related to members of the transient receptor potential (TRP) family of store-operated calcium channels (SOCs), proteins that also display the same topological organization of their transmembrane domains as well as the multiple amino terminus ankyrin repeats [4].

Regarding the native quaternary structure of VR_1, recent evidence, obtained using different biochemical approaches, indicates that this channel is a multimer, most probably constituted by four identical subunits which can exist in different glycosilation states [16].

After receptor activation in the presence of agonist, the subunits of the homotetramer undergo covalent cross-linkage by a mechanism which is probably mediated by cellular transaminases [16].

2.2. VR_1 Homologues

Although to date only one functional vanilloid receptor called VR_1 has been cloned, the great diversity of vanilloid-induced biological responses seems to indicate the existence of isoforms and subtypes. In particular, a possible heterogeneity of vanilloid receptors in rat sensory fibers has been suggested [12, 17] and vanilloid receptor homologues, which do not respond to vanilloids, being instead sensitive to different physical stimuli, are also known.

Among these homologues we can mention the vanilloid receptor-like protein 1 (VRL-1) and the vanilloid receptor-related osmotically activated channel (VR-OAC). The VRL-1 [18] was obtained from rat brain and it functions as a sensor of high temperatures (≥ 52 ºC). The VR-OAC, cloned from rat, mouse, human and chicken, constitutes the first osmotically activated channel to be characterized in vertebrates. This cation-selective channel, structurally similar to VR_1 and distributed mainly in neurosensory cells in both CNS and the periphery, is activated under hypotonic conditions causing increased cytoplasmic calcium concentrations [19].

In addition, splicing variants of VR_1, such as the stretch-inactivated channel (SIC) and the vanilloid receptor 5′-splice variant (VR.5′sv) have also been reported. The SIC, cloned from rat kidney, consists of 563 amino acids and shares the same structural features that of VR_1 although with marked differences in the C-terminal tail. The SIC is one of the mechanosensitive cation channels of mammals, being permeable to calcium, inhibited by membrane stretch and blocked by the inorganic cation Gd^{3+} [20]. The VR.5′sv [21] lacks the majority of the intracellular N-terminal domain and ankyrin repeat elements and is insensitive to capsaicin, RTX, hydrogen ions or noxious stimuli. Consequently, it could represent a novel mechanism whereby nociceptive transduction could be modulated.

The different vanilloid receptors originated either through splice variants or through post translation modification, could provide a mechanism to generate complexity by means of different individual subunits which associate to form the functional tetramer [16].

2.3. Localization of VR_1

VR_1 receptors are mainly located in primary sensory neurons. Thus, the presence of VR_1 transcripts has been established by northern blot analysis in dorsal root (DRG) and trigeminal ganglia [4, 22] and by VR_1-like immunoreactivity in the dorsal horn of the spinal cord, among other primary afferent neurons [23]. Besides these cells, a subset of nodose ganglion neurons also expresses VR_1 [24]. Regarding the expression of VR_1 in other CNS areas not associated with primary sensory neurons, it has also been detected in several brain areas such as the hypothalamus and the basal ganglia [17]. Additionally, some peripheral tissues, such as urinary bladder [25], urethra

[26] or nasal mucosa [27], among others [2], also express VR_1.

Whereas its presence in primary sensory neurons is involved in nociception and neurogenic inflammation, the existence of vanilloid-sensitive neurons in some brain regions could imply an important role of vanilloids on memory formation, appetite regulation and emotions [28, 29].

Finally, the presence of VR_1 in nonneuronal tissues is still an unclear aspect, since although capsaicin is able to induce different responses in nonneuronal cells such as mast cells and glia, these effects have been attributed to non-specific mechanisms. However, several pieces of evidence suggest the possibility for nonneuronal cells, such as heart [4] or human lymphocytes [30], to express VR_1, facts which make this subject a current matter of debate.

3. ACTIVATION OF VR_1

3.1. Direct and Indirect Stimuli

VR_1 functions as polymodal transducer in nociceptive neurons, acting as a molecular integrator of three distinct types of noxious stimuli, either chemical, such as vanilloid ligands or protons, or physical, such as heat. In addition to these direct activators, the sensitivity of VR_1 towards some intracellular second messenger systems has also been reported.

Considering the direct stimuli, VR_1 functions as a molecular thermometer in response to heat [31]; it recognizes vanilloid ligands, as capsaicin, suffering specific conformational changes different from those caused by other stimuli [32]; and, finally, it responds also to the concentration of protons in the milieu. Thus, extracellular protons have a dual role, a merely regulatory function -since they increase the potency of heat or capsaicin as VR_1 agonists without inducing channel activation by themselves- together with a direct agonist capacity at higher concentrations ($pH < 6.0$) which provokes channel opening even at room temperature [33].

Both responses, proton potentiation and proton evoked receptor activation, imply different sites on VR_1. This behaviour has led to the hypothesis that VR_1 may exist in two distinct conformations with protons acting as allosteric switch. According to this, VR_1 can alternate between two conformers of differing affinities for vanilloids depending on the extracellular pH. Thus, at higher pH (6.5-7.5) the dissociation of protons from their binding sites yields a conformer with lower affinity toward vanilloids whereas an increase in the concentration of protons (pH 5.5-6.5) will provoke the change to the other conformer, the protonated form of VR_1, which displays an increased affinity toward vanilloid ligands [34].

Regarding the activation of VR_1 by second messenger systems, it appears to occur through multiple signalling pathways, involving mainly to PKA and protein kinase C (PKC). Phosphorylation of the channel by these protein kinases has attracted recent attention as a putative mechanism to modulate its agonist sensitivity.

As deduced from its primary sequence, VR_1 possesses three putative phosphorylation sites for PKA [2] and eighteen consensus motifs for potential PKC phosphorylation [34]. Phosphorylation by PKA appears to play an important role in desensitization which probably reflects rapid agonist-induced internalization of the receptor [3, 35]. By contrast, intracellular signalling by PKC is able to open the VR_1 channel in the absence of any other extracellular ligand although it is not necessary for ligand direct activation of VR_1 [36]. Considering that the term PKC comprises more than eleven different serine-threonine kinases, considerable attention has been paid to elucidate the exact role played by the different isozymes to the nociceptive processing by VR_1: an aspect which has started to clarify with very recent studies that suggest the specific involvement of PKCα [34], PKCγ [2, 37] and PKC$\in$ [37] isozymes. Additionally, a series of current studies have addressed the interesting question of which residues are phosphorylated. Among the different residues of VR_1 which could be targeted by PKA, Ser 116 has revealed as one of the firmest candidates [35]. On the other side, direct phosphorylation of VR_1 by PKC$\in$ seems to occur at the first intracellular loop and at the carboxyl terminus, at serines 502 and 800, respectively [37].

Full clarification of the molecular details of these indirect regulation processes will likely provide new useful procedures in the treatment of pain by interfering with phosphorylation-mediated sensitization and activation events.

3.2. Independence of the Mechanisms of Activation

Recently, a growing body of evidence suggest that VR_1 has independent gating mechanisms for its stimuli, that is capsaicin, protons and heat. This fact makes possible the independent modulation of the domain involved in the receptor activation by capsaicin from the domains involved in the gating by protons or by heat [32].

This model of independent activation is supported by the reported results which indicate that the vanilloid-binding site is intracellular [31, 38] whereas the proton-binding site is extracellular [31]. Also, the fact that neither the avian vanilloid receptor ortholog from chicken sensory neurons nor the two more distantly related mammalian homologs, VRL-1 and VR-OAC, display sensitivity to capsaicin although being proton and heat-activated further strengths this hypothesis [39].

3.3. Key Residues Involved in VR_1 Activation

The model of independent activation has progressed significantly with the identification of the different domains of the receptor involved in the transduction of the signal evoked by ligand binding, pH changes or increased temperatures.

Site-directed mutagenesis studies indicate the existence of at least three key structural zones in the VR_1 channel which account for the binding and transduction of different stimuli able to activate the receptor, namely the third transmembrane domain (TM3), the cytosolic N- and C-

terminal tails and the pore region. Each of these zones is responsible, alone or in combination with the other domains, for the recognition and transduction of any of the stimuli.

The structural elements in VR_1 involved in the specific recognition of vanilloids have been partially clarified. The activation of VR_1 requires, as occurs for many of the ligand-gated channels, interaction of the agonist with different domains in the channel complexes. Taking as representative of the vanilloid ligands, the structure of capsaicin displays zones able to establish both hydrophobic and hydrophilic interactions with different domains of the receptor. According to this, the region in VR_1 spanning the third transmembrane domain (TM3) seems to be essential for ligand binding, presumably by hydrophobic interactions with capsaicin [39], whereas both N- and C- cytosolic domains are crucial in the hydrophilic ones. Both terminal domains are necessary for the sensitivity to vanilloids [40].

3.3.1.-Third Transmembrane Domain (TM3)

The TM3 is one of the main structural elements in VR_1 involved in the recognition of vanilloids, having attributed the specific sensitivity to capsaicin to eight amino acids located at the vicinity of this domain. In particular, mutation of the arginine located at 491 and the serine 512, belonging to TM2 and TM3, respectively, resulted in dramatic decreases in proton and capsaicin activation. Among the other conserved amino acids adjacent to this serine, the aromaticity of the residue located at position 511 seems to be an important factor for ligand binding as nonaromatic residues at this position preclude capsaicin sensitivity. The location of the amino acids 511 and 512 at the transition between an intracellular loop and TM3 is consistent with the vanilloid ligands binding site at the intracellular face of the membrane [39].

All these data indicate that the aromatic residue at position 511 establishes π-electron interactions with the aromatic vanillyl moiety of capsaicin at the inner side of the membrane, amino acids 491 and 512 are involved in hydrogen bonds and, finally, other lipophilic residues of TM3 would contribute to hydrophobic interactions with the aliphatic side chain of capsaicin [39].

3.3.2.- N- and C-terminal Cytoplasmic Domains

Two key residues have been identified within these domains as involved in the ligand-binding site, the arginine 114 and the glutamic of position 761. These two residues provide crucial hydrophilic interactions with capsaicin [40]. Thus, in addition to the TM3 region in VR_1, implicated in hydrophobic interactions, these two zones of the cytosolic terminus also constitute a part of the capsaicin-binding pocket.

Regarding the influence of these domains of the receptor over the other VR_1 stimuli, the two cytoplasmic terminal tails do not seem to be involved in the sensitivity to heat, although they do appear to be functionally linked to the activation of VR_1 by acids in addition to vanilloids [40].

3.3.3.- Putative Pore

Three residues, situated near the putative pore of VR_1, have been identified as contributing to the activation pathway for capsaicin, two of them extracellular (aspartic and glutamic of positions 646 and 648, respectively) and another glutamic residue located intracellularly, at position 636. Probably this region along with the sixth transmembrane domain (TM6) transduces capsaicin binding to channel gating and they likely constitute the selective filter which is located just upstream of the TM6, similarly to other channels [32]. In particular, mutagenesis studies suggest that the aspartic at position 646 is a molecular determinant of the pore properties such as blockade by ruthenium red and divalent cations permeability. However, the contribution of other amino acids determining these pore properties cannot be ruled out as the neutralization of the residue neither completely prevented ruthenium red blockade nor drastically changed the ionic selectivity, a result which suggests the existence of additional structural determinants [41].

Mutations near the pore of VR_1 channel can alter capsaicin activation properties without changing the effects produced by protons or temperature, an aspect which is confirmed by the fact that VRL-1 is activated by heat but not by capsaicin [32].

Additional recent site-directed mutagenesis studies have also clarified the molecular determinants involved in the recognition of protons as VR_1 agonists, agents which modulate VR_1 activity by interacting with specific amino acids on the extracellular surface of the channel protein. Two residues of glutamic have been pinpointed as responsible for the sensitivity of VR_1 towards protons. Glutamic 600, located in the region linking the TM5 domain with the putative pore region of VR_1, functions as a proton-modulatory site over a physiological pH range (pH 6-8). Also, glutamic 648 reduces proton-activated currents without altering heat or capsaicin-evoked responses, or without eliminating the ability of protons to potentiate responses to these stimuli.

Mutation of these charged residues on the extracellular surface or at the pore region of VR_1 provoked reduced responses to acid compared with those evoked by capsaicin, whereas deletion or disruption of the cytosolic tails dissipated capsaicin-sensitive currents and acid-gated responses. Therefore, cytosolic regions are linked functionally to the activation of VR_1 by protons and vanilloids.

The identification of these key residues represents a major step forward in the understanding of the molecular features of VR_1. However, the involvement of other zones of VR_1 cannot be ruled out as mutations at other domains, such as the TM6, have also revealed as important for binding affinity, so it is quite conceivable that there are regions different from TM3 or the terminal tails that also control VR_1 ligand binding [42]. Also the rest of the intracellular domains may play an important role in controlling folding or stabilization of the channel complex [40], so further research is still required to unequivocally define the residues which new vanilloid ligands should target.

These studies of site-directed mutagenesis represent the starting point for the development of molecular modeling models, one of the most powerful tools in the rational design of new compounds with predetermined and controlled affinities, selectivities and activities.

4. DESIGN OF VR_1 LIGANDS

4.1. Agonists

4.1.1. Plant-Derived Ligands

The general term vanilloids was coined in reference to the 4-hydroxy-3-methoxybenzyl ring, termed vanilloid moiety, the only structural element shared by the two firstly identified and even today the most representative VR_1 agonists: capsaicin (Fig. (**2**)) and RTX (Fig. (**4**)). This vanillyl-like fragment was considered essential for VR_1 affinity until 1996, when a series of compounds lacking the recognizable vanillyl-like motif was also identified as ligands of VR_1 channel. These studies led to the classification of the naturally occuring VR_1 ligands into four structural classes: capsaicinoids, resiniferanoids, unsaturated dialdehydes and triprenyl phenols.

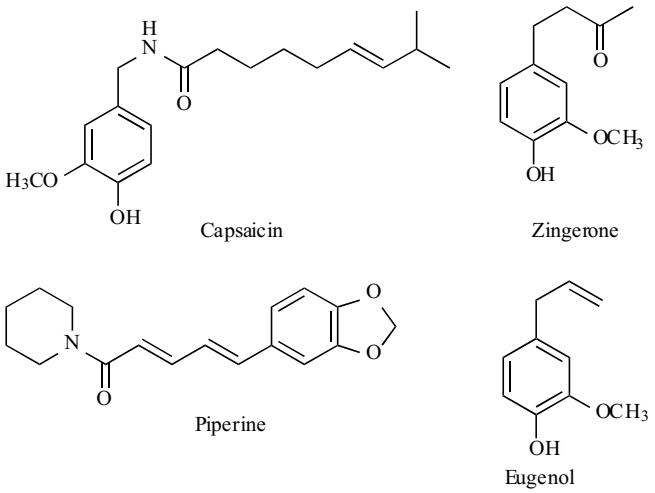


Fig. (2). Structure of representative capsaicinoids.

4.1.1.1. Capsaicinoids

The archetypal ligand belonging to this structural class is the well-known capsaicin (*N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide), the pungent principle of chilli peppers and related plants of the *Capsicum* family. Other important capsaicinoids are piperine, the active principle of black pepper, zingerone, which accounts for the piquancy of ginger and eugenol, obtained from the oil of cloves [2, 28]. The structure of these compounds is shown in Fig. (**2**).

In order to identify the structural requisites involved in the recognition of ligands by VR_1, a series of structure-activity relationship (SAR) studies was developed. These studies undertook the elucidation of the requirements

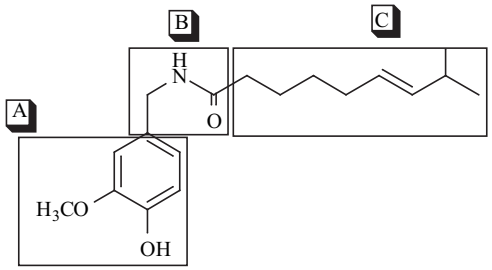


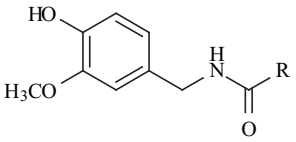
Fig. (3). Key regions of capsaicin.

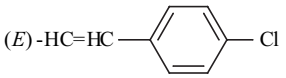
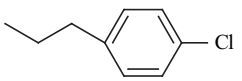
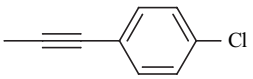
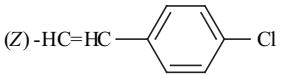
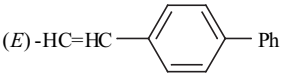
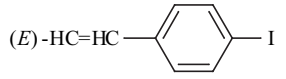
subdividing the capsaicin molecule into three regions (Fig. (**3**)), the aromatic ring (A moiety), the connecting amide bond (B region) and the hydrophobic side chain (C fragment). An important number of compounds, synthesised by systematic modification of each region while holding the others constant, were allowed to outline a putative pharmacophore for VR_1 agonism.

i) Modifications in the Hydrophobic Side Chain (C moiety)

The influence of the elimination of the branching as well as the double bond were tested as a first step in the

Table 1. Modifications in the Hydrophobic Side Chain



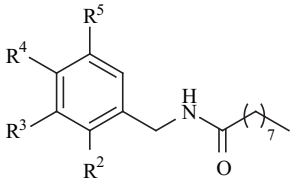
Compd.	R	Ca^{2+} Influx EC_{50} (μM)	Ref.
Capsaicin (1)	$(CH_2)_4(E)$-CH=CHCH$(CH_3)_2$	0.30±0.04	[9]
2	$(CH_2)_6$-CH$(CH_3)_2$	0.19±0.02	[9, 43]
3	$(CH_2)_7$-CH_3	0.55±0.08	[7]
4	CH_2-Br	>100	[9, 44]
5	CH_2-Cl	>100	[9, 44]
6	$(CH_2)_{16}$-CH_3	>100	[9, 45]
7	$(CH_2)_5$-NH-Boc	79.80±10.70	[9]
8	$(CH_2)_{10}$-COOH	>100	[9]
9	$(CH_2)_7(Z)$-CH=CH-$(CH)_7$-CH_3	0.17±0.03	[9, 46]
10		1.24±0.11	[9]
11		3.09±0.07	[9]
12		4.10±0.25	[9]
13		50.10±0.80	[9]
14		0.24±0.03	[9]
15		0.35±0.05	[9]
16	$(CH_2)_7(Z)$-CH=CH-CH_2-CH(OAc)-$(CH)_5$-CH_3	9.4±0.5[a]	[47]

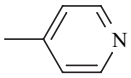
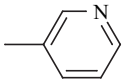
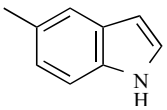
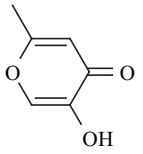
[a] pEC_{50} value is reported.

modification of capsaicin. The three compounds (**1**-**3**, see Table 1) showed a comparable VR_1 activity, a fact which indicated that the overall size and/or the hydrophobicity were the crucial factors for VR_1 agonism instead of the presence of insaturations or branchings in the chain [9]. The importance of both factors, size and hydrophobicity, was confirmed by the lack of activity displayed by compounds bearing shorter and polar side chains (**4**, **5**), very longer chains (**6**) or terminal polar groups (**7**, **8**). However, an important exception to this trend was observed in the case of olvanil (**9**), which exhibited a remarkable activity both *in vitro* and *in vivo* although possessing a long aliphatic monounsaturated chain. These results suggested the existence of a hydrophobic binding site able to accommodate either aliphatic hydrophobic fragments with optimum size (chain length of 8-12 carbon atoms) or aromatic moieties, as shown by compounds **10**-**15**, where constrained but not linear (**10** vs **11** and **12**) and extended (*E* isomer **10** vs its geometric *Z* isomer **13**) conformations with two-carbon spacer units are optimal. Additionally, the increase of both size and hydrophobicity of substituents in the *para* position (compounds **14** and **15**) enhances the activity at VR_1, result which further strengthens the hypothesis of the existence of a hydrophobic binding site responsible for the interaction of the hydrophobic side chain of the capsaicin [9].

Very recently a new series of vanillamides derived from a variety of fatty acids and isoprenoids have been synthesized and tested as potent VR_1 agonists, being **16** (pEC_{50} = 9.4; Table 1) the most potent compound within these analogues [47].

Table 2. Modifications in the Aromatic Ring Moiety of Capsaicin



Compd.	R^2	R^3	R^4	R^5	Ca^{2+} Influx EC_{50} (μM)	Ref.
3	H	OCH_3	OH	H	0.55±0.08	[7]
17	H	OH	OH	H	0.63±0.03	[7, 48]
18	OH	OH	OH	H	7.64±1.09	[7]
19	OCH_3	H	OH	OCH_3	>100	[7]
20	OCH_3	H	OH	H	>100	[7]
21	H	OCH_3	H	H	>100	[7]
22	H	OCH_3	NH_2	H	>10	[7]
23	H	OCH_3	SH	H	>100	[7]
24	H	OCH_3	NO_2	H	7.91±1.95	[7]
25	H	-OCH_2O-		H	>100	[7, 49]
26					28±3	[7]
27					>100	[7]
28					>100	[7]
29					>100	[7]

ii) Modifications in the Aromatic Ring (A moiety)

Consistently with the equipotent activity reported for compounds **1**-**3** (Table 1), the octyl side chain was used as the constant C moiety for the sake of simplicity. The modifications carried out in the aromatic region of the molecule (Table 2) indicate that the compound bearing the classical vanillyl-like motif, the 4-hydroxy-3-methoxybenzyl ring, compound **3**, was the most potent one, with an EC_{50} value of 0.55 μM. Also, its catechol analogue (**17**) displayed a high activity at VR_1 with comparable potency in the *in vitro* tests of calcium influx (with an EC_{50} value of 0.63 μM) and guinea pig ileum contractions (EC_{50} values of 0.40 μM for compound **3** and 0.22 μM for compound **17**, respectively), although with a reduced *in vivo* analgesic activity (ED_{50} (**3**) = 5.00 μmol·kg^{-1}; ED_{50} (**17**) = 20.00 μmol·kg^{-1}), probably due to the faster metabolic inactivation of catechols [7]. Substitution in other positions of the phenyl ring (R^2 and R^5), either single or in combination (compounds **18**-**20**), involved important decreases (compound **18**, with an EC_{50} value of 7.64 μM) or even complete abolition of VR_1 activity (compounds **19** and **20**). In general, both elimination of the hydroxy group attached to position four of the aromatic ring or its replacement for other groups lead to inactive derivatives (**21**-**23**, with EC_{50} values greater than 100 μM, vs EC_{50} (**3**) = 0.55 μM). However, compound **24** constitutes a remarkable exception since the electrowithdrawing nitro substituent is able to maintain a residual activity (EC_{50} (**24**) = 7.91 μM). Major modification in the substituents of the phenyl ring (**25**) or its complete replacement with other aromatic rings (**26**-**29**) yielded only poorly active (EC_{50} (**26**) = 28 μM) or completely inactive compounds with EC_{50} values greater than 100 μM.

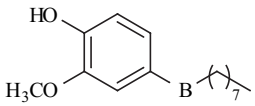
iii) Modifications in the Amide Fragment (B moiety)

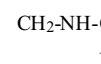
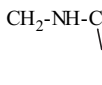
Once identified the vanillyl-like motif as the optimal for the A region and keeping the octyl side chain constant as the C moiety for the sake of simplicity, structural modifications were carried out in bridging B group. Among the modifications analyzed (Table 3), amides and esters were both able to activate VR_1, although amides being more potent (**3** vs **30** and **31** vs **32**). The *reverse* analogues were not only able to activate VR_1 but also exhibited better EC_{50} values (**31** vs **3** and **32** vs **30**). *N*-Methylation (**33**), shorten of the length of B fragment (**34**) and sp^2 hybridization of the atom next to the aromatic ring (**35**) led in all cases to the loss of activity. Analyzing the effects of other B groups, thiourea **36** allowed to obtain the major affinity at VR_1 (EC_{50} value of 0.06 μM) whereas the substitution for other conventional bioisosteres such us urea, *N*-cyanoguanidine and *C*-nitroethenoamidine (**37**-**39**, respectively) involved a lesser degree of activity at VR_1, although exhibiting a wide range of EC_{50} values, from the quite potent urea **37** (EC_{50} = 0.36 μM) to the inactive derivative **39** (EC_{50} > 100 μM). Finally, elimination of the dipolar functions in B fragment completely abrogated activity at VR_1, as shows compound **40**, being completely inactive (EC_{50} > 100 μM).

The most important conclusions drawn from these studies can be summarized as follows: i) a vanillyl-like fragment is optimal in the A region, ii) Amide, thiourea and ester, albeit this last one in a lesser extent, are groups exhibiting good ability to interact with the receptor in B region and iii) a hydrophobic moiety of limited size (molar refractivity, MR < 55) is required to obtain high potencies in C region, with chain lengths of 8-12 carbon atoms resulting the most suitable [7-9]. These data allowed to propose a two-dimensional model of VR_1 agonism based on multiple hydrogen-bonding interactions between the B region of the ligand and the binding site of VR_1. For activity in this model, compounds must possess functionality with a dual hydrogen-bonding behaviour, that is, a hydrogen-bond-donor-acceptor pair at this position which additionally adopts an extended *trans* coplanar configuration in the optimal cases [8].

These exhaustive studies allowed the identification of novel capsainoid compounds such as the thiourea **36**, more potent at VR_1 than capsaicin itself, supposed to be the first in-depth SAR studied devoted to VR_1 even before this receptor was molecularly characterized and opened the development of the rational design, with the help of molecular modeling, of new potent VR_1 ligands. In this line, Klopman and Li later used this numerous series of compounds in 1995 to set up an approach which led to the proposal of the first three-dimensional model of the capsaicinoid pharmacophore [52]. This model, obtained using a multiple computer automated structure evaluation (MULTICASE) method, identified three biophores with high probability of relevance which outlined the key structural requirements for VR_1 agonism and which were in close accordance with the conclusions drawn by Walpole and collaborators [7-9].

Table 3. Modifications in the Amide Moiety of Capsaicin



Compd.	B	Ca^{2+} Influx EC_{50} (μM)	Ref.
3	CH_2-NH-CO	0.55±0.08	[7]
30	CH_2-O-CO	14.20±0.06	[8]
31	CH_2-CO-NH	0.30±0.01	[8, 50]
32	CH_2-CO-O	0.67±0.11	[8, 51]
33	CH_2-N(CH_3)-CO	>100	[8]
34	CO-NH	>100	[8]
35		>100	[8]
36	CH_2-NH-CS-NH	0.06±0.01	[8]
37	CH_2-NH-CO-NH	0.36±0.04	[8]
38		3.28±0.63	[8]
39		>100	[8]
40	CH_2-CH_2-CH_2	>100	[8]

4.1.1.2. Resiniferanoids

Resiniferatoxin (Fig. (**4**)), a tricyclic diterpene isolated from *Euphorbia resinifera*, is the prototypical representation of this structural class of ligands. Its identification as an ultrapotent VR_1 agonist [13] has converted it into a new lead compound for the development of a new series of VR_1 agonists more potent than capsaicinoids and structurally different from them.

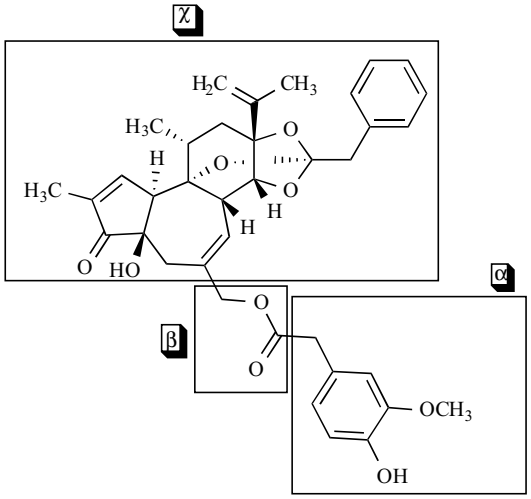
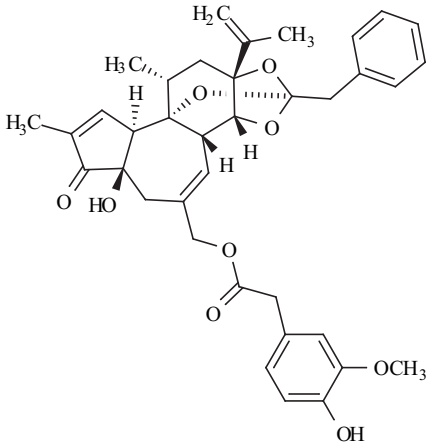
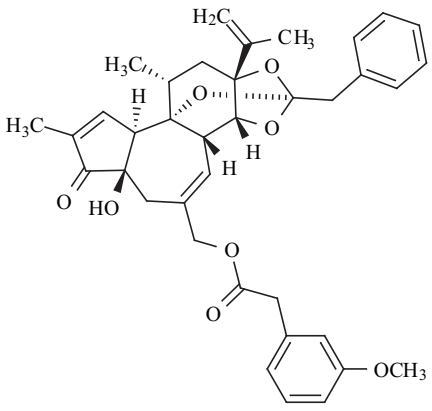


Fig. (4). Important fragments in resiniferatoxin.

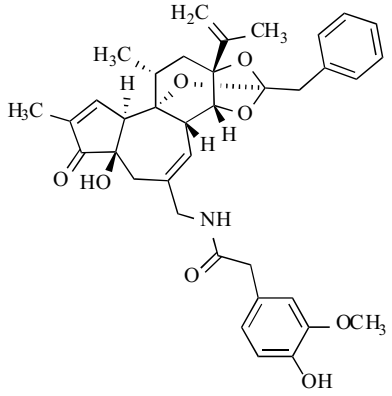
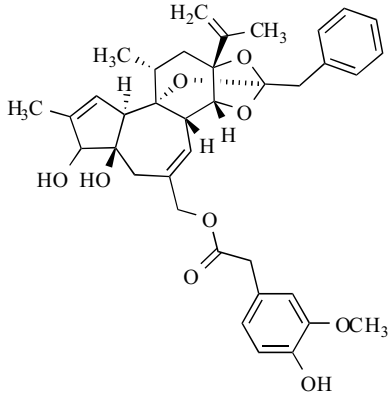
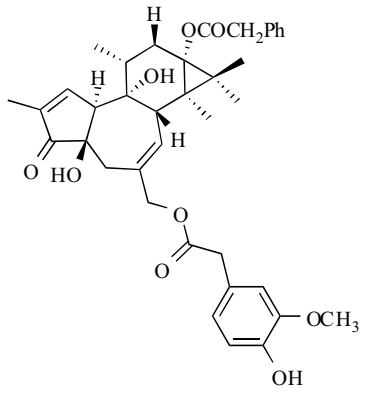
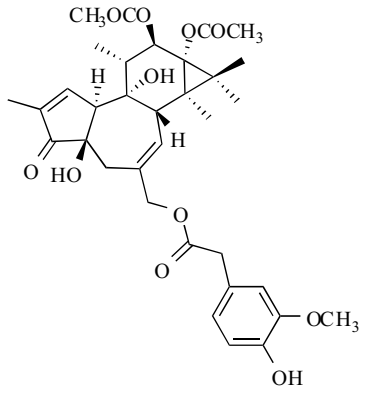
Although the structural similarities between both compounds, capsaicine and RTX, seem to be limited to the shared 4-hydroxy-3-methoxybenzyl ring, a further attempt of establishing homologies in the rest of the molecules has been addressed [10].

Analogously to capsaicin, the structure of RTX could be divided into three regions, α, β and χ, corresponding to the A, B and C moieties of capsaicin, respectively (Fig. (**4**)). Based on this approach, there developed an SAR study which made evident the important differences between capsaicinoids and resiniferanoids. In general, resiniferanoids tolerate modifications in the α region better than capsaicinoids, but are, however, extremely sensitive to variations of the diterpene skeleton (χ moiety). For instance, the phenolic group, which proved essential for capsaicinoids, can be removed in resiniferanoids derivatives without significant loss of activity, as shows compound **42** (Table 4) with an EC_{50} value of 7.89 nM vs the EC_{50} value of 1.6 displayed by RTX (**41**). Regarding β region, the most striking feature compared to capsaicinoids is the fact that replacement of the ester group for an amide link involves important decreases in activity, as indicated by compound **43** which experiments a more than 75-fold loss of activity

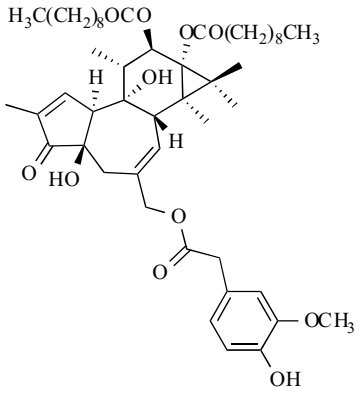
Table 4. SAR Studies on Resiniferanoids

Compd.		Ca^{2+} Influx EC_{50} (nM)	Ref.
RTX (41)		1.6±0.1	[10]
42		7.89±2.51	[10]

(Table 4). contd.....

Compd.		Ca^{2+} Influx EC_{50} (nM)	Ref.
43		122±19.0	[10]
44		57.5±21.2	[10]
45		2470±140	[10]
46		8510±1990	[10]

(Table 4). contd.....

Compd.		Ca^{2+} Influx EC_{50} (nM)	Ref.
47		>10000	[10]

compared to RTX (Table 4). The χ fragment seems to play a crucial role in the activity of RTX, far from being a merely hydrophobic anchor as suggested by the fact that small variations in its structure imply critical losses of activity. The most important role is attributed to the functional group substituting the 5-membered ring of RTX, specially the 3-keto group, whose reduction (compound **44**) involves a critical decrease in activity (EC_{50} (**44**) = 57.5 nM). Also the orientation adopted by this group is of importance as phorbol ester derivatives (**45**-**47**), substituents that induce conformational changes which markedly affect orientation of the 3-keto group, result nearly inactive at VR_1 [10].

In 1997, the totally enantiocontrolled synthesis of RTX and its conformational analysis were reported [53, 54]. These studies, considered together with the findings reported earlier by Walpole *et al.* [10], lead to the proposal of a three-points pharmacophore model which maintained the essential biophores previously suggested: the C_{20}-homovanillic moiety, the C_3-keto and the phenyl orthoester joined through a variable linker (Fig. (**5**)) but in a series of simplified RTX analogues [55]. The SAR study developed for these compounds (Table 5) indicated that the vanillyl-like moiety had to be linked to the rest of the molecule by an amide group since all the esters tested resulted inactive (see compound **48** in Table 5 as an illustrative example, K_i (**48**) > 100 μM). Amides exhibited better affinity values, ranging from moderate (K_i (**49**) = 19.3 μM) to more potent than capsaicin when the steric volume of R^2 is increased as shown by compound **50** (K_i (**50**) = 0.404 μM), with an enhancement in affinity of nearly 50-fold compared to **49**. The most critical factor in this series of compounds seems to be the amide bond (Y = NH) since other changes such as variations in R^2 or X do not appreciably affect the affinity and activity exhibited by compounds, as shown by compound **51**, which displays a K_i value and an agonist capacity similar to **50** (K_i (**50**) = 0.404 μM, K_i (**51**) = 0.48 μM).

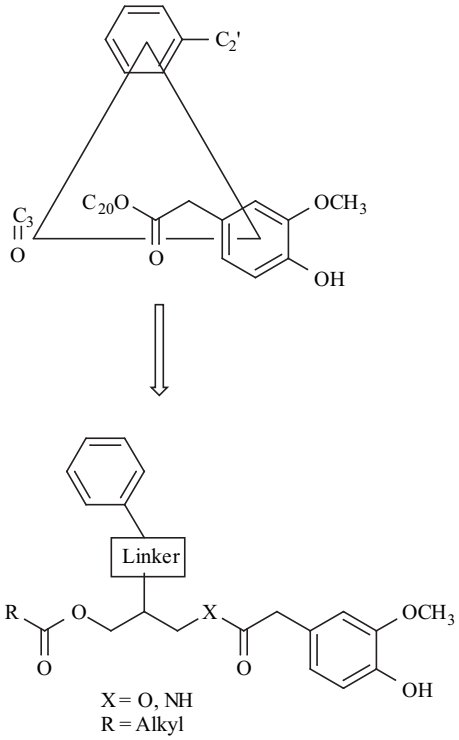


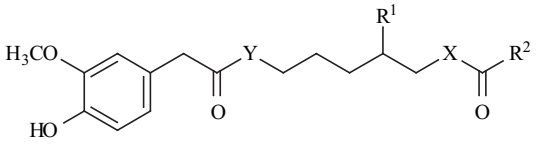
Fig. (5). Proposed pharmacophore model of RTX and design of a series of simplified RTX analogues.

Further research based on this pharmacophore model of simplified RTX analogues led to the development of the recently reported potent VR_1 agonists (compounds **52** and **53**, with K_i values of 19 and 11 nM, respectively, see Table 5), characterized by the 3-acyloxy-2-benzylpropyl moiety designed to maintain the key structural requirements of the diterpene fragment (χ region) in RTX [56]. Since all these compounds were tested as racemates, actual higher affinities cannot be ruled out for the pure enantiomers. In particular, compounds **55** (K_i = 18.4 nM) and **56** (K_i = 74 nM), the two optically active enantiomers of **54**, showed substantial differences in receptor binding affinity relative to its racemate (K_i (**54**) = 96 nM; Table 5), result which highlights the high stereospecifity showed by the thiourea binding site in VR_1 receptor [56].

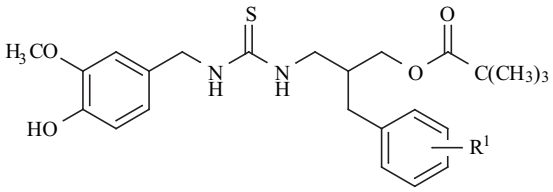
This approach allowed obtaining a new series of compounds with higher affinity and activity than capsaicin, which, although being less potent than RTX at VR_1, displayed the important advantage of greater synthetic

Table 5. New Simplified RTX Derivatives

Compd.	K_i (μM)	Ca^{2+} Influx[a]	Ref.
Capsaicin (1)	5.31±0.37	1	[55]
RTX (41)	$2.3 \cdot 10^{-5}$	100	[55]



Compd	R^1	R^2	X	Y	K_i (μM)	Ca^{2+} Influx[a]	Ref
48[b]	CH_2Ph	CH_3	O	O	>100	Inactive	[55]
49[b]	CH_2Ph	CH_3	O	NH	19.3±6.8	<1	[55]
50[b]	CH_2Ph	$C(CH_3)_3$	O	NH	0.404±0.037	10	[55]
51[b]	CH_2Ph	$C(CH_3)_3$	CH=	NH	0.48±0.05	10	[55]



Compd.	R^1	K_i (nM)	Ref.
52[b]	3,4 $-(CH_3)_2$	19±4.3	[56]
53[b]	4 $-C(CH_3)_3$	10.8±4.0	[56]
54[b]	H	96±31	[56]
55[c]	H	18.4±5.1	[56]
56[d]	H	74±16	[56]

[a]The data of Ca^{2+} influx is expressed as the ratio between the activity of compound and the activity induced by capsaicin. [b]Racemic mixtures were assayed. [c]*R*-isomer. [d]*S*-isomer.

accessibility compared to RTX. Therefore, the most potent compounds within this series (**52** and **53**) could represent new lead compounds for the development of vanilloid agonists with analgesic capacity.

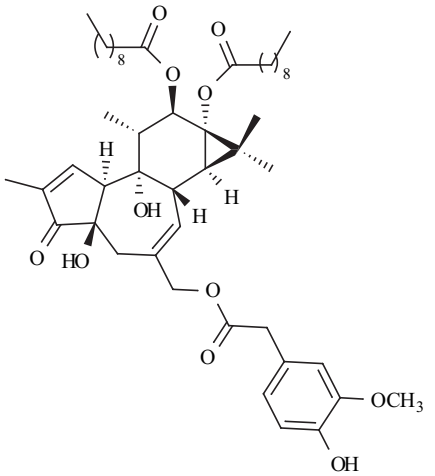


Fig. (6). Structure of phorbol 12,13-didecanoate 20-homovanillate (PDDHV).

4.1.1.3. Differences in the Relationship Between the Structure of Vanilloids and Affinity and Activity Responses

Whereas capsaicinoids are generally less potent for inhibiting RTX binding than for inducing calcium currents (for instance, capsaicin shows an affinity for VR_1 of K_i = 4900 nM and an EC_{50} value for calcium influx of 340 nM), resiniferanoids exhibit important differences in their relative potency for both responses, ranging from the behaviour of RTX, with higher affinity for VR_1 than capacity to activate it (K_i = 0.04 nM; EC_{50} value for calcium influx of 0.9 nM) to the opposite extreme constituted by phorbol 12,13-didecanoate 20-homovanillate (PDDHV, Fig. (**6**)), unable to bind VR_1 but, however, with an important agonist activity at this receptor (K_i > 10000 nM; EC_{50} value for calcium influx of 60 nM) [2]. These findings were originally interpreted in terms of at least two independent vanilloid receptor subtypes, one responsible for binding and other for calcium uptake. However, this hypothesis has been discarded by the recent evidences reported by Szallasi *et al.* [57] so the

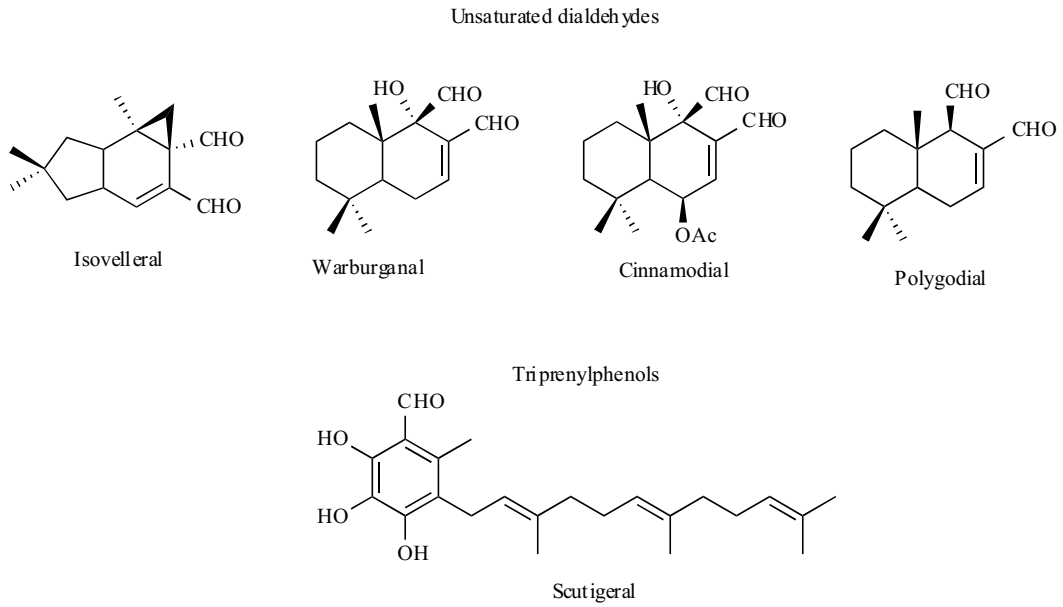


Fig. (7). Structure of vanilloids lacking a recognizable vanillyl motif.

current explanations for these results point to the existence of different but overlapping binding domains in VR_1 for resiniferanoids and capsaicinoids or, eventually, of different receptor conformations [2].

4.1.1.4. Unsaturated Dialdehydes and Triprenyl Phenols

The identification, in 1996, of compounds able to interact at vanilloid receptors despite lacking any recognisable vanillyl-like motif in their structure supposed the generation of putative new lead compounds for drug development in the interesting VR_1 pharmacology. Firstly, the pungent terpenoid unsaturated dialdehydes were characterized as a novel class of VR_1 agonists [58] and, few years later, the triprenyl phenols were reported as non-pungent vanilloids [59]. Among the former, the isovelleral is the most representative, although an important number of α,β-unsaturated 1,4-dialdehydes have been isolated from natural sources [2, 28], including warburganal, cinnamodial or polygodial (Fig. (**7**)). In spite of the capacity of these compounds to activate VR_1, they are not selective, possessing additional sites of action which includes, for example, the reported ability of polygodial to block tachykinin NK-2 receptors [60]. The prototypical triprenyl phenol is scutigeral (Fig. (**7**)) which is considered a non-pungent vanilloid. This compound does not display selectivity for VR_1 either, since it is able to bind also to dopamine D_1 receptors [2, 28].

This new class of VR_1 ligands could open up new possibilities enhancing the number of structures susceptible to displaying vanilloid activities with possible associated advantages with respect to those already characterized such as diminished undesirable pharmacological effects or easier synthetic accessibility.

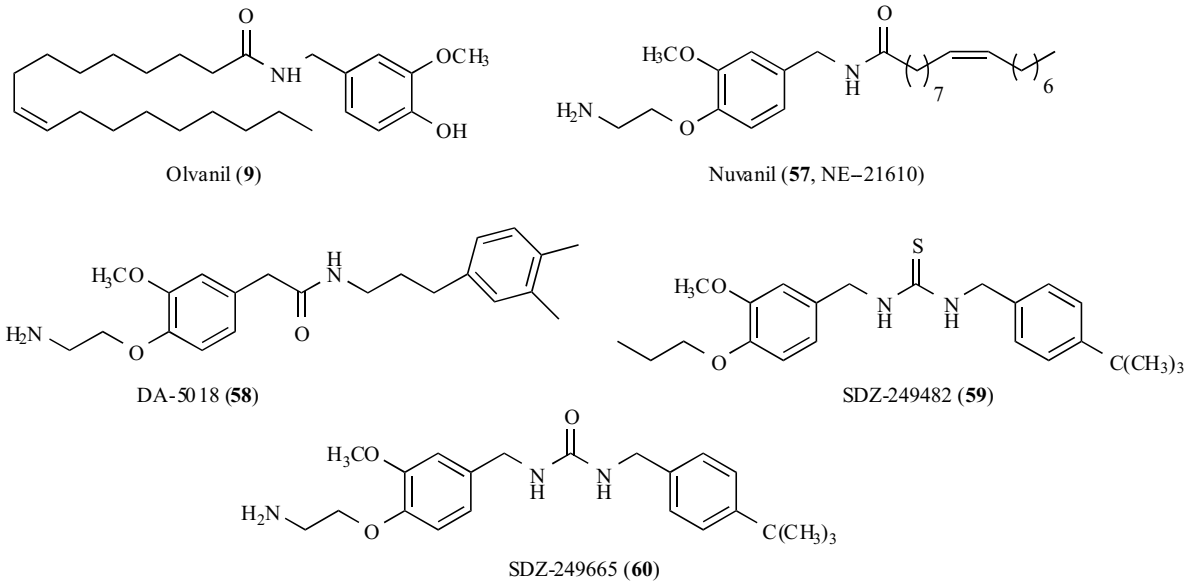
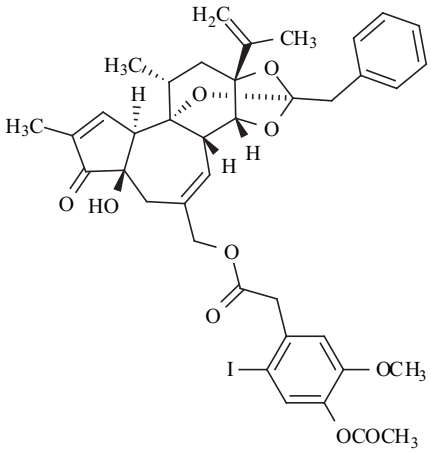
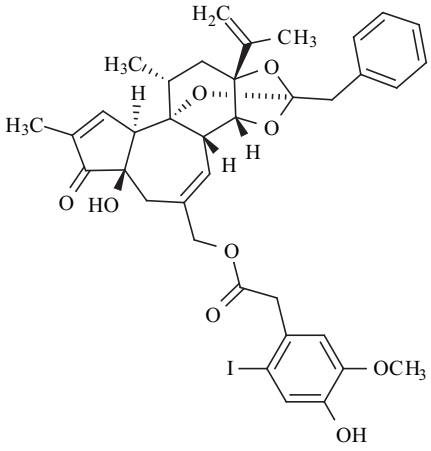


Fig. (8). Structure of some representative synthetic VR_1 ligands.

4.1.2. Synthetic Ligands

The promising therapeutic role of VR_1 ligands especially as putative analgesic agents, encouraged to different pharmacological laboratories to carry out studies aimed at exploring the structural features responsible for capsaicin-like activity. Among these attempts the studies set up by Sandoz deserve special attention, focused on establishing a comprehensive SAR of capsaicin and derivatives [7-9] as well as the research program lauched by Procter & Gamble [61], which led to the identification of two important synthetic VR_1 agonists (Fig. (**8**)), olvanil (**9**) and nuvanil (NE-21610, **57**). Olvanil is a non-pungent and orally active vanilloid which does not cause painful activation of the nociceptive afferent endings and, therefore, may be less inflammatory than other vanilloids [2, 62]. Nuvanil is a stable analogue of capsaicin with less agonist activity than this one but able to induce a certain antinociceptive activity at high doses and marked hypothermic side effects [63, 64]. Other synthetic vanilloid agonists with potential antinociceptive activities are DA-5018 (**58**) [65] and SDZ-249482 (**59**) (Fig. (**8**)), the latter characterized as an orally and topically effective analgesic, superior to others such as opioid or nonsteroidal antiinflammatory drugs in diverse animal models of pain [66]. Subsequently, the compound SDZ-249665 (**60**) was reported as a new addition to non-pungent capsaicin analogues [67]. SDZ-249665 displays an

Table 6. Synthetic VR_1 Agonists

Compd.		K_i (nM)	Ca^{2+} Influx EC_{50} (nM)	Efficacy (% capsaicin response)	Ref.
Capsaicin (1)		840±220	170±19	-	[67]
RTX (41)		0.88±0.41 0.48±0.07	0.97±0.6 1.9±1.0	- 116±6	[67, 68]
Olvanil (9)		340	180	-	[2]
DA-5018 (58)		1400±30	a	-	[55]
SDZ-249665 (60)		760±260	890±84	-	[67]
61		1.46±0.55	935±280	75±15	[68]
62		0.71±0.27	130±105	50±13	[68]

[a]Comparable to capsaicin

increased potency along with similar efficacy to capsaicin avoiding, besides, the appearance of some unwanted side effects such as bronchoconstriction, blood pressure changes or tolerance phenomena [67]. The affinity and activity data for some of these synthetic derivatives are shown in Table 6.

Very recently, a different structural approach, based on the RTX structure, has been explored with the aim to provide new VR_1 agonists susceptible, besides, to iodine radioactive labelling in order to improve the low specific activity of RTX, only available to date as tritiated radioligand [68, 69]. Two representative examples of this interesting new class of compounds, along with their affinity and activity data, are shown in Table 6 (**61** and **62**).

4.2. Antagonists

There are two different classes of VR_1 antagonists. The first group is termed competitive antagonists as they bind to the same site than capsaicin whereas the second class includes compounds which act in a non-competitive manner.

4.2.1. Competitive Antagonists

Among this class of VR_1 ligands, the most representative one is capsazepine (**63**, Fig. (**9**)), the first described competitive antagonist based on the structure of capsaicin [70]. Based on their previous studies [7-9], Walpole *et al.* led to the conclusion that the angle between the plane of the B region and the vanillyl-like moiety (A region) was critical for agonist activity in capsaicin (Fig. (**3**)). Therefore, they synthesized a series of compounds where the A and B regions were constrained by saturated ring systems of different sizes, from five to seven atoms. This approach allowed the identification of capsazepine, a compound able to antagonize capsaicin and RTX action both *in vivo* and *in vitro*. Additionally, the structural and conformational analysis studies, carried out using NMR spectroscopy, X-ray crystallography and molecular modeling techniques, made possible to propose different binding modes for agonists and antagonists [70].

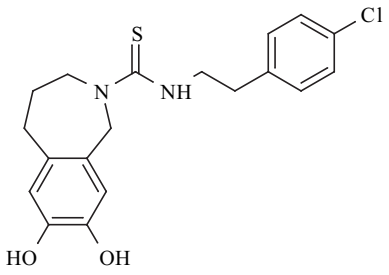


Fig. (9). Structure of capsazepine (**63**).

More recently, and in an attempt to overcome the main drawbacks of capsazepine, such as poor solubility or moderate metabolic stability, a new series of analogues were developed by Lee *et al.* [71], based on the idea of combining the key pharmacophoric points of capsazepine and their previous models [55] of simplified RTX analogues, as shown in Fig. (**10**). Among the compounds synthesized, three of them (**64**-**66**, see Table 7) resulted of special interest, as showing an antagonist activity comparable or even slightly better than capsazepine. The most important conclusions derived from this SAR study indicate that a free catechol moiety seems to be important for antagonist activity (as show compounds **65**, **66** and the proper capsazepine) and that it requires both the appropriate conformational restrictions of the 3,4-dihydroxybenzylamine (A region) and the 3-benzoyloxy-2-benzylpropyl group (C region). However, and in an attempt to evaluate the actual influence of the structural constraints in the A fragment, a series of ring opened trisubstituted thiourea derivatives, compounds that show an increased flexibility in this region, has been recently described [72]. Among these analogues, stands out compound **67** (Table 7) which showed the highest antagonist activity (IC_{50} = 0.32 μM). Additionally, a series of chain-branched phenethylthiocarbamates together with a structurally different set of antagonists, using pyrrolidine as scaffold, has also been reported [73, 74]. Within this structural class, compound **68** (Table 7) deserves special attention as being the most potent antagonist with an IC_{50} value of 3 μM.

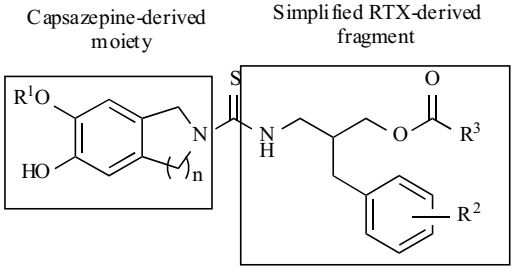


Fig. (10). Design of capsazepine-resiniferanoid hibrids as VR_1 ligands.

Finally, an iodinated form of RTX, named iodo-resiniferatoxin (I-RTX, **69**, Fig. (**11**)) was discovered [75] and confirmed [68] to compete with vanilloids for VR_1 binding but devoid of any agonist activity (Table 7). This result outlines the importance of the iodination site in the ability to activate VR_1, since iodinated compounds **61** and **62** (Table 6) were characterized as agonists whereas **69** was able to antagonize the action of capsaicin, RTX, protons and other VR_1 agonists in both efferent and afferent functions displaying even more selectivity and potency than capsazepine itself [76].

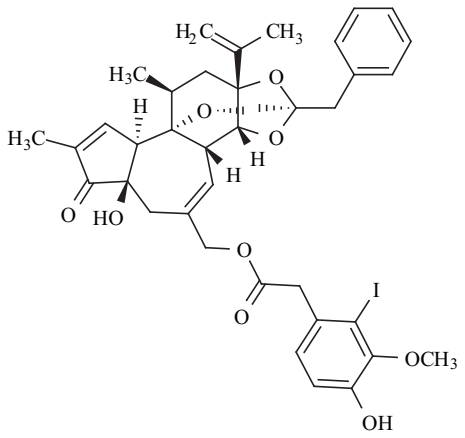


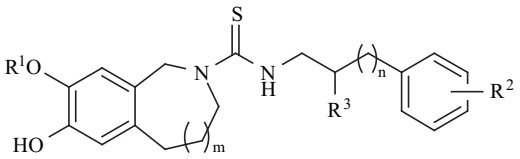
Fig. (11). Structure of iodo-resiniferatoxin (**69**, I-RTX).

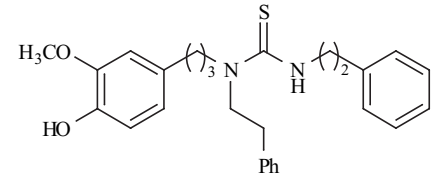
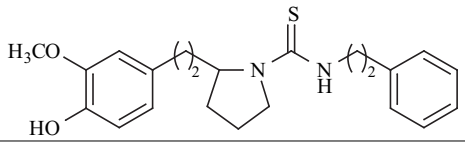
4.2.2.- Non-Competitive Antagonists

Ruthenium red can be considered, within the context of vanilloids, as the most classical functional capsaicin

Table 7. Competitive VR_1 Antagonists

Compd.	Ca^{2+} Influx		K_i (μM)	Ref.
	Agonist (ED_{50}, μM)	Antagonist (K_i, μM)		
Capsaicin (1)	0.081±0.011 0.038	Not effective	1.7	[71, 57]
Capsazepine (63)	Not effective	0.56±0.12 0.14	3.22±0.48 6.6	[71, 57]



Compd.	R_1	R_2	R_3	m, n	Ca^{2+} Influx: Agonist (ED_{50}, μM)	Ca^{2+} Influx: Antagonist (K_i, μM)	K_i (μM)		Ref.
64	CH_3	4 -Cl	H	m=1 n=0	Not effective		0.884±0.152	0.865±0.051	[71]
65[a]	H	H	CH_2OCOPh	m=1 n=1	Not effective		0.966±0.118	0.223±0.052	[71]
66[a]	H	H	CH_2OCOPh	m=0 n=1	Not effective		0.196±0.05	0.81±0.065	[71]
67					Not effective	0.32[b]	-		[72]
68					> 100	3[b]	-		[74]
I-RTX (69)					Not effective	$3.9 \cdot 10^{-3}$	$(4.8 \pm 0.6) \cdot 10^{-3}$ $(5.8 \pm 1.1) \cdot 10^{-3}$		[75]

[a]Absolute configuration not indicated. [b]These data were reported as IC_{50} value.

antagonist. These properties were known since the early nineties, and it was for years, until capsazepine was discovered, the only available VR_1 antagonist.

Ruthenium red is a polycationic inorganic dye able to block the agonist-evoked Ca^{2+} currents by interacting with a region in VR_1 different from the capsaicin-binding site [2, 3]. However, and although the molecular mechanisms by which it blocks vanilloid actions are not still fully understood, a role for the aspartic residue located at position 646 in the interaction with ruthenium red has been suggested [41].

More recently, the arginine-rich hexapeptides, a completely different structural set of compounds, have been reported as non-competitive VR_1 antagonists able to block the channel with submicromolar efficacies in a moderate voltage-dependent manner. These compounds are capable to induce an analgesic effect of comparable extent to that evoked by the endogenous arginine-rich hexapeptides opioids dynorphins [77]. These two findings may open new possibilities in the development of novel antinociceptive agents targeting VR_1 regions different from the capsaicin binding site as well as suggest a putative relationship between the opioid and the vanilloid system, since the well-known analgesic action of dynorphin could be partially mediated by interaction with VR_1.

5. ENDOGENOUS LIGANDS

The endogenous vanilloid activators are yet to be unequivocally characterized, making this area of research a current matter of debate. Molecular features displayed by VR_1 such as the high affinities exhibited towards ligands and the existence of positive cooperativity in ligand binding

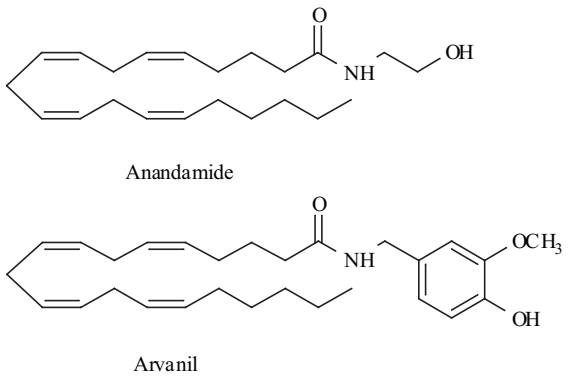


Fig. (12). Structure of anandamide and arvanil.

together with the indirect evidence which suggests a physiological role for a putative endovanilloid system strongly support the existence of endogenous vanilloids [2]. A challenging hypothesis was launched only four years ago, with the identification of anandamide, the endogenous cannabinoid ligand, as the potential endovanilloid ligand. Anandamide (Fig. (**12**)) shares a remarkable chemical resemblance with other known vanilloid agonists such as capsaicin or olvanil and it is capable to induce different effects mediated by VR_1 activation [78, 79], responses strongly reduced in the presence of capsazepine. However, the low affinity of anandamide for VR_1 continues being one of the most powerful arguments against this hypothesis, making this subject quite controversial yet [17, 80-82]. In spite of this, something beyond discussion is the fact that both cannabinoid and vanilloid receptors share some common ligand recognition properties as demonstrated by the endocannabinoid and vanilloid hybrid arvanil (Fig. (**12**)), compound able to activate both classes of receptors [83, 84]. In addition, several fatty acid ethanolamides which exhibit different degrees of selectivity upon VR_1, cannabinoid receptors and fatty acid amidohydrolase (FAAH) have been recently described [85]. The potential cross regulation between vanilloid and cannabinoid system suggested by these results constitutes an important area of research as it could shed light on diverse physiological roles played by these systems which are not completely understood yet. In particular, recent evidences point to one of the components of the endogenous cannabinoid system, the endocannabinoid transporter, as the physiological regulator of the effects evoked by anandamide by interaction either with VR_1 or cannabinoid receptors (for recent reviews on this aspect, see [86, 87]).

Additionally, some endogenous eicosanoids (Fig. (**13**)) such as 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and leukotriene B_4 (LTB_4) are also able to activate VR_1 with major potency than anandamide [88], and, very recently, the *N*-arachidonoyldopamine (NADA), has been identified as an endogenous VR_1 ligand able to activate this receptor with a potency and efficacy similar to those of capsaicin [89].

6. VR_1 AND NEUROPATHIC PAIN

Among the different classes of pain, neuropathic pain refers to the painful sensation, usually described by patients as “burning”, “stabbing”, “shooting”, “electric-like” or “throbbing”, resulting from injury to the central or the peripheral nervous system. It can be caused by different traumas (including surgery, amputation or radiation damage), pharmacological treatments such as antitumoral or anti-HIV drugs and diseases such as for instance herpes zoster, AIDS, multiple sclerosis, arthritis or diabetes.

The underlying biological mechanism of neuropathic pain has been attributed to the atypical discharges produced by the damaged nerve that follow to the nervous system injury. Thus, after the primary insult, the damaged nerves begin to discharge at atypical locations including neuromas and demyelinated nerve zones at the site of injury and associated DRG. These ectopic discharges result in spontaneous burning pain in the immediate area, while the increased barrage of impulses reaching the spinal cord leads to hyperexcitability and central sensitization of dorsal horn neurons [90, 91], resulting in hyperalgesia (exaggerated response to a normally mildly painful stimulus or even spontaneous pain sensation produced in the absence of external stimuli) and allodynia (pain that occurs in response to normally non-noxious stimuli).

The classical pharmacological approaches to treat these pathologies have consisted in the administration of diverse drugs with the objective of alleviating symptoms, using parameters such as pain intensity or relief or drug preference to evaluate the efficacy of the treatments. However, nowadays, the strategy to face neuropathic pain is directed to

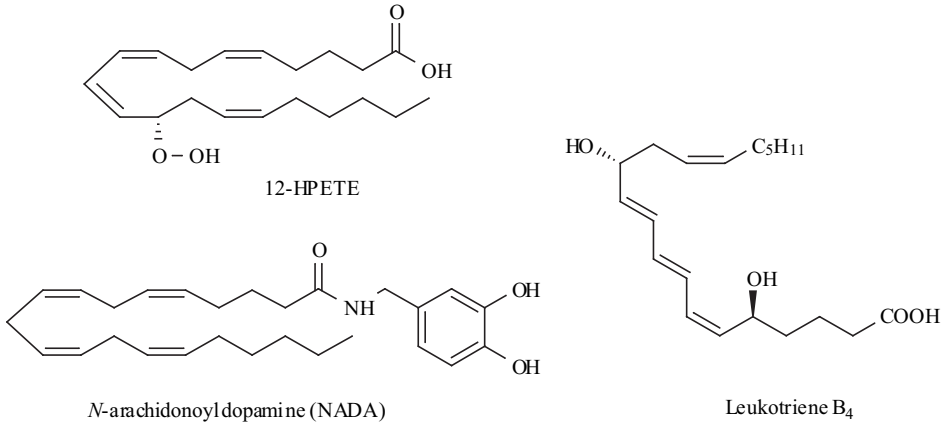


Fig. (13). Structure of some of the proposed endovanilloid ligands.

the understanding of the different pathophysiological mechanisms involved in the complex pain phenomena known as neuropathic pain in order to design optimal treatments for particular patients. Therapeutically, once elucidated the mechanisms that contribute to elicit neuropathic pain, it would be possible to use drugs which not only act on pain intensity but also on the specific pathways responsible for the origin of pain. This approach will provide the basis for a more efficacious and rationally founded treatment adapted to the individual needs of each patient.

At the present, the vanilloid system has arised as one of the most promising mechanisms in the management of some neuropathic pain conditions [92]. On one side, there has been reported the ability of vanilloid antagonists to reduce the pain perception by blocking VR_1 activation [2, 93]. However, also vanilloid receptor agonists could represent an attractive alternative to traditional analgesics considering their ability to silence sensory nerves by receptor desensitisation after prolonged exposure without causing significant CNS adverse effects [3]. These results indicate that vanilloid ligands, both agonists and antagonists, could represent new powerful analgesic drugs for the treatment of chronic and severe pain disorders. However, both types of agents not only have several advantages but also drawbacks.

6.1. VR_1 Antagonists as Antinociceptive Agents

The fact that VR_1 functions as the main receptor for protons and eicosanoids, some of the main inflammation mediators, implies that VR_1 antagonists may be of value in the treatment of inflammatory hyperalgesia and pain. This hypothesis has been further supported by the results obtained with animal models in which VR_1-null mice exhibit impaired nociceptive responses [94, 95] or in which the increased levels of VR_1 observed in rats with L5 spinal nerve ligation, a neuropathic pain model, seem to contribute to the exaggerated heat response observed [96].

In line with this assumption, both vanilloid antagonists, capsazepine and ruthenium red, have been tested as potential analgesic agents. Capsazepine has demonstrated only modest antihyperalgesic effects [97], probably due to its limited receptor affinity combined with poor adsorption and delivery features, and ruthenium red, assessed in some animal models, showed severe limitations considering the convulsions it induced at doses needed to antagonise receptor activation [2]. However, it opened the possibility for the development of other non-competitive antagonists, such as the arginine-rich hexapeptides, which have shown important analgesic activities in different models of pain by modulation of sensory nerve fibers excitability [77, 98]. Despite their promising antinociceptive capacity, different aspects related to their physicochemical features, such as an increased water solubility, should still be improved in order to obtain better bioavailability properties.

6.2. VR_1 Agonists as Antinociceptive Agents

The three effects evoked by VR_1 agonists, named excitation, desensitization and neurotoxicity, could be of therapeutic value [2]; features which have led to the development of deep studies aimed at exploring the actual significance of the analgesic effects induced by capsaicin, as the most prototypical VR_1 agonist. The analgesic effect of capsaicin is mainly due to the insensitivity that follows the initial neuronal excitation induced by vanilloid agonists and is characterized for the down-regulation of VR_1 expression that lasts for several weeks [3]. Based on this, the powerful analgesic effects induced by capsaicin has been deeply studied in different animal models of human disease, and topical capsaicin administration has been used to treat an important variety of neuropathic pain states such as postherpetic neuralgia, painful diabetic neuropathy or postmastectomy pain syndrome [2]. However, this use of capsaicin is hampered by several drawbacks, such as a poor absorption and high metabolism, aspects which contribute to maintain the levels of capsaicin under effective concentrations [3] and, in consequence, making it uneffective to desensitize nerve endings in human skin [2]. Moreover, the most important side effect associated with the therapeutic use of capsaicin relies on the strong irritancy induced by capsaicin in the early moments of the treatment, found intolerable for many patients [2, 3].

According to this, the optimal vanilloid agonist should induce lasting desensitisation with minimal pungency devoid of long-term toxicity. In an attempt to overcome the initial burning pain sensation preceding the desensitisation phase induced by capsaicin, the pharmacological profile of RTX has been exploited. RTX is able to produce lasting desensitisation with much less initial irritation than capsaicin: a feature that has prompted an important number of assays focusing on establishing the potential therapeutic applications of RTX [2]. These studies are at present undergoing phase II placebo-controlled trials in US and Europe [3].

With the same objective, olvanil is also considered to be a non- (or at least less) painful capsaicin analogue that has the added benefit of oral activity. Although it has demonstrated satisfactory results in animal models, its clinical value in humans remains to be elucidated [3]. Additionally, recent studies suggest its potential use for the treatment of chronic pain conditions by the removal of nociceptive primary afferent neurons. Olvanil, in addition to being less inflammatory than other vanilloids, since it does not cause the initial painful activation of the nociceptive afferent endings, is able to induce citotoxicity selectively only in those VR_1-expressing cells at non-toxic doses for the rest of the cells. This controlled deletion of nociceptive neurons could represent a new and effective mean for chronic pain management [99].

7. FUTURE PERSPECTIVES

Our molecular understanding of the vanilloid system has progressed significantly during the last decade. The parallel efforts carried out by biochemists and synthetic chemists have allowed the molecular identification of VR_1 together with the development of a series of synthetic agonists and antagonists, which constitute the basic tools to improve our knowledge of the vanilloid system. Deeper structural studies on the receptor will help to the design and synthesis of new compounds, whereas the development of new agents able to

interact in potent and selective ways will allow to determine the biochemical pathways in which VR_1 is involved. Only the complementary research in both areas will make possible both to confirm the promising therapeutic applications of this target and to make use of the real efficacious drugs for its management.

Based on this, it is more than probable that much progress is yet to be reported in the following years. Regarding vanilloid ligands, serious challenges about improving bioavailability, metabolic stability, selectivity or potency still lie ahead. Additionally, probably reinterpretation of current data and more refined experimental approaches are still needed in order to clarify some of the most controversial points in this field, including the unequivocal identification of the endogenous vanilloid ligands as well as the relationship with cannabinoid system as one of the most important issues that remains, to date, to be successfully addressed.

All this knowledge will hopefully provide better bases for the understanding of the vanilloid system and will lead to the rational design of analgesic compounds of clinical utility more efficacious and completely devoid of the undesired side effects or other previously antinociceptive agents used such as opioids or nonsteroidal antiinflammatory drugs. These new compounds probably will open up new avenues regarding the pain therapy offering a new strategy, used alone or in combination with others, to face neuropathic pain, a chronic pain state whose prevalence will exceed 75 million worldwide in the next decade and which, at present, lacks any efficacious therapy for its treatment.

ACKNOWLEDGEMENTS

This work has been supported by the Ministerio de Ciencia y Tecnología (BQU 2001-1459). The authors thank MECD for a predoctoral grant to S.O.G.

ABBREVIATIONS

AIDS	=	Acquired immune deficiency syndrome
CNS	=	Central nervous system
DRG	=	Dorsal root ganglion
EC_{50}	=	Concentration of the compound which induces a 50% of the effect measured
ED_{50}	=	Dosis of the compound which induces a 50% of the effect measured
FAAH	=	Fatty acid amidohydrolase
HIV	=	Human immunodeficiency virus
12-HPETE	=	12-hydroperoxyeicosatetraenoic acid
IC_{50}	=	Compound concentracion which causes a 50% of inhibition in the target considered
I-RTX	=	Iodo-resiniferatoxin
K_i	=	Affinity constant
LTB_4	=	Leukotriene B_4
MULTICASE	=	Multiple computer automated structure evaluation
NADA	=	*N*-arachidonoyl-dopamine
MR	=	Molar refractivity
PDDHV	=	Phorbol 12,13-didecanoate 20-homovanillate
PKA	=	Protein kinase A (cAMP-dependent protein kinase)
PKC	=	Protein kinase C
RTX	=	Resiniferatoxin
SAR	=	Structure-activity relationship
SIC	=	Stretch-inactivated channel
SOCs	=	Store-operated calcium channels
TM	=	Transmembrane domain
TRP	=	Transient receptor potential
VR_1	=	Vanilloid receptor subtype 1
VR.5′sv	=	Vanilloid receptor 5′-splice variant
VRL-1	=	Vanilloid receptor-like protein 1
VR-OAC	=	Vanilloid receptor-related osmotically activated channel

REFERENCES

[1] Szallasi, A.; Blumberg, P. M. *Pain* **1996**, *68*, 195.
[2] Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51*, 159.
[3] Szallasi, A. *Drug Aging* **2001**, *18*, 561.
[4] Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816.
[5] Szolcsányi, A.; Jancsó-Gábor, A. *Arzneimittel. Forsch.* **1976**, *26*, 33.
[6] Hayes, A. G.; Oxford, A.; Reynolds, M.; Shingler, A. H.; Skingle, M.; Smith, C.; Tyers, M. B. *Life Sci.* **1984**, *34*, 1241.
[7] Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Capmbell, E. A.; Dray, A.; James, I. F.; Perkins, M. N.; Reid, D. J.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2362.
[8] Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Capmbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2373.
[9] Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Capmbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2381.
[10] Walpole, C. S. J.; Bevan, S.; Bloomfield, G.; Breckenridge, R.; James, I. F.; Ritchie, T.; Szallasi, A.; Winter, J. Wrigglesworth, R. *J. Med. Chem.* **1996**, *39*, 2939.
[11] Wrigglesworth, R.; Walpole, C. S. J.; Bevan, S.; Capmbell, E. A.; Dray, A.; Hughes, G. A.; James, I. F.; Masdin, K. J.; Winter, J. *J. Med. Chem.* **1996**, *39*, 4942.
[12] Holzer, P. *Pharmacol. Rev.* **1991**, *43*, 143.
[13] Szallasi, A.; Blumberg, P. M. *Brain Res.* **1990**, *524*, 106.
[14] Hayes, P.; Meadows, H. J.; Gunthorpe, M. J.; Harries, M. H.; Duckworth, D. M.; Cairns, W.; Harrison, D. C.; Clarke, C. E.; Ellington, K.; Prinjha, R. K.; Barton, A. J. L.; Medhurst, A. D.; Smith, G. D.; Topp, S.; Murdock, P.; Sanger, G. J.; Terrett, J.; Jenkins, O.; Benham, C. D.; Randall, A. D.; Gloger, I. S.; Davis, J. B. *Pain* **2000**, *88*, 205.
[15] Smart, D.; Jerman, J. C.; Gunthorpe, M. J.; Brough, S. J.; Ranson, J.; Cairns, W.; Hayes, P. D.; Randall, A. D.; Davis, J. B. *Eur. J. Pharmacol.* **2001**, *417*, 51.
[16] Kedei, N.; Szabo, T.; Lile, J. D.; Treanor, J. J.; Olah, Z.; Iadarola, M. J.; Blumberg, P. M. *J. Biol. Chem.* **2001**, *276*, 28613.
[17] Di Marzo, V.; Bisogno, T.; De Petrocellis, L. *Trends Pharmacol. Sci.* **2001**, *22*, 346.

[18] Caterina, M. J.; Rosen, T. A.; Tominaga, M.; Brake, A. J.; Julius, D. *Nature* **1999**, *398*, 436.

[19] Liedtke, W.; Choe, Y.; Martí-Renom, M. A.; Bell, A. M.; Denis, C. S.; Sali, A.; Hudspeth, A. J.; Friedman, J. M.; Heller, S. *Cell* **2000**, *103*, 525.

[20] Suzuki, M.; Sato, J.; Kutsuwada, K.; Ooki, G.; Imai, M. *J. Biol. Chem.* **1999**, *274*, 6330.

[21] Schumacher, M. A.; Moff, I.; Sudanagunta, S. P.; Levine, J. D. *J. Biol. Chem.* **2000**, *275*, 2756.

[22] Helliwell, R. J. A.; McLatchie, L. M.; Clarke, M.; Winter, J.; Bevan, S.; McIntyre, P. *Neurosci*. *Lett.* **1998**, *250*, 177.

[23] Guo, A.; Vulchanova, L.; Wang, J.; Li, X.; Elde, R. *Eur. J. Neurosci.* **1999**, *11*, 946.

[24] Szallasi, A.; Nilsson, S.; Farkas-Szallasi, T.; Blumberg, P. M.; Hökfelt, T.; Lundberg, J. M. *Brain Res.* **1995**, *703*, 175.

[25] Ács, G.; Palkovits, M.; Blumberg, P. M. *Life Sci.* **1994**, *55*, 1017.

[26] Parlani, M.; Conte, B.; Goso, C.; Szallasi, A.; Manzini, S. *Br. J. Pharmacol.* **1993**, *110*, 989.

[27] Rinder, J.; Szallasi, A.; Lundberg, J. M. *Pharmacol. Toxicol.* **1996**, *78*, 327.

[28] Sterner, O.; Szallasi, A. *Trends Pharmacol. Sci.* **1999**, *20*, 459.

[29] Mezey, E.; Tóth, Z. E.; Cortright, D. N.; Arzubi, M. K.; Krause, J. E.; Elde, R.; Guo, A.; Blumberg, P. M.; Szallasi, A. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3655.

[30] Lai, J.-P.; Douglas, S. D.; Ho, W.-Z. *J. Neuroimmunol.* **1998**, *86*, 80.

[31] Tominaga, M.; Caterina, M. J.; Malmberg, A. B.; Rosen, T. A.; Gilbert, H.; Skinner, K.; Raumann, B. E.; Basbaum, A. I.; Julius, D. *Neuron* **1998**, *21*, 531.

[32] Welch, J. M.; Simon, S. A.; Reinhart, P. H. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13889.

[33] Jordt, S.-E.; Tominaga, M.; Julius, D. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8134.

[34] Olah, Z.; Karai, L.; Iadarola, M. J. *J. Biol. Chem.* **2002**, *277*, 35752.

[35] Bhave, G.; Zhu, W.; Wang, H.; Brasier, D. J.; Oxford, G. S.; Gereau IV, R. W. *Neuron* **2002**, *35*, 721.

[36] Premkumar, L. S.; Ahern, G. P. *Nature* **2000**, *408*, 985.

[37] Numazaki, M.; Tominaga, T.; Toyooka, H.; Tominaga, M. *J. Biol. Chem.* **2002**, *277*, 13375.

[38] Jung, J.; Hwang, S. W.; Kwak, J.; Lee, S. Y.; Kang, C.-J.; Kim, W. B.; Kim, D.; Oh, U. *J. Neurosci.* **1999**, *19*, 529.

[39] Jordt, S.-E.; Julius, D. *Cell* **2002**, *108*, 421.

[40] Jung, J.; Lee, S.-Y.; Hwang, S. W.; Cho, H.; Shin, J.; Kang, Y.-S.; Kim, S.; Oh, U. *J. Biol. Chem.* **2002**, *277*, 44448.

[41] García-Martínez, C.; Morenilla-Palao, C.; Planells-Cases, R.; Merino, J. M.; Ferrer-Montiel, A. *J. Biol. Chem.* **2000**, *275*, 32552.

[42] Kuzhikandathil, E. V.; Wang, H.; Szabo, T.; Morozova, N.; Blumberg, P. M.; Oxford, G. S. *J. Neurosci*. **2001**, *21*, 8697.

[43] Gannett, P. M.; Nagel, D. L.; Reilly, P. J.; Lawson, T.; Sharpe, J.; Toth, B. *J. Org. Chem.* **1988**, *53*, 1064.

[44] Jones, E. C. S.; Pyman, F. L. *J. Chem. Soc.* **1925**, *127*, 2588.

[45] Brand, L. M. EP 0306060, **1989**.

[46] Lahann, T. R.; Buckwalter, B. L. U.S. Pat. US 4493848 A, **1985**.

[47] Appendino, G.; Minassi, A.; Morello, A. S.; De Petrocellis, L.; Di Marzo, V. *J. Med. Chem.* **2002**, *45*, 3739.

[48] Hayes, A. G.; Oxford, A.; Reynolds, M.; Shingler, A. H.; Skingle, M.; Smith, C.; Tyers, M. B. *Life Sci.* **1984**, *34*, 1241.

[49] Chen, I. J.; Yang, J. M.; Yeh, J. L.; Wu, B. N.; Lo, Y. C.; Chen, S. J. *Eur. J. Med. Chem.* **1992**, *27*, 187.

[50] Lahann, T. R.; Buckwalter, B. L. Eur. Pat. Application 89710, **1982**.

[51] Loomans, M. E.; Janusz, J. M.; Buckwalter, B. UK Pat. Application GB2168976, **1986**.

[52] Klopman, G.; Li, J.-Y. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 283.

[53] Wender, P. A.; Jesudason, C. D.; Nakahira, H.; Tamura, N.; Tebbe, A. L.; Ueno, Y. *J. Am. Chem. Soc.* **1997**, *119*, 12976.

[54] Victory, S. F.; Appendino, G.; Vander Velde, D. G. *Bioorg. Med. Chem.* **1998**, *6*, 223.

[55] Lee, J.; Park, S.-U.; Kim, J.-Y.; Kim, J.-K.; Lee, J.; Oh, U.; Marquez, V. E.; Beheshti, M.; Wang, Q. J.; Modarres, S.; Blumberg, P. M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2909.

[56] Lee, J.; Lee, J.; Kim, J.; Kim, S. Y.; Chun, M. W.; Cho, H.; Hwang, S. W.; Oh, U.; Park, Y. H.; Marquez, V. E.; Beheshti, M.; Szabo, T.; Blumberg, P. M. *Bioorg. Med. Chem.* **2001**, *9*, 19.

[57] Szallasi, A.; Blumberg, P. M.; Annicelli, L. L.; Krause, J. E.; Cortright, D. N. *Mol. Pharmacol* **1999**, *56*, 581.

[58] Szallasi, A.; Jonassohn, M.; Ács, G.; Bíró, T.; Ács, P.; Blumberg, P. M.; Sterner, O. *Br. J. Pharmacol.* **1996**, *119*, 283.

[59] Szallasi, A.; Bíró, T.; Szabó, T.; Modarres, S.; Petersen, M.; Klusch, A.; Blumberg, P. M.; Krause, J. E.; Sterner, O. *Br. J. Pharmacol.* **1999**, *126*, 1351.

[60] El Sayah, M.; Filho, V. C.; Yunes, R. A.; Pinheiro, T. R.; Calixto, J. B. *Eur. J. Pharmacol.* **1998**, *344*, 215.

[61] Brand, L.; Berman, E.; Schwen, R.; Loomans, M.; Janusz, J.; Bohne, R.; Maddin, C.; Gardner, J.; LaHann, T.; Farmer, R.; Jones, L.; Chiabrando, C.; Fanelli, R. *Drugs Expl. Clin. Res.* **1987**, *13*, 259.

[62] Dickenson, A.; Hughes, C.; Rueff, A.; Dray, A. *Pain* **1990**, *43*, 353.

[63] Szallasi, A. *Drug News Perspect.* **1997**, *10*, 522.

[64] Dray, A.; Bettaney, J.; Rueff, A.; Walpole, C.; Wrigglesworth, R. *Eur. J. Pharmacol.* **1990**, *181*, 289.

[65] Wrigglesworth, R.; Walpole, C. S. J. *Drugs of the Future* **1998**, *23*, 531.

[66] Badger, A. M.; Lee, J. C. *Drugs Discuss. Today* **1997**, *2*, 427.

[67] Urban, L.; Campbell, E. A.; Panesar, M.; Patel, S.; Chaudhry, N.; Kane, S.; Buchheit, K.-H.; Sandells, B.; James, I. F. *Pain* **2000**, *89*, 65.

[68] McDonnell, M. E.; Zhang, S.-P.; Dubin, A. E.; Dax, S. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1189.

[69] Seabrook, G. R.; Sutton, K. G.; Jarolimek, W.; Hollingworth, G. J.; Teague, S.; Webb, J.; Clark, N.; Boyce, S.; Kerby, J.; Ali, Z.; Chou, M.; Middleton, R.; Kaczorowski, G.; Jones, A. B. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 1052.

[70] Walpole, C. S. J.; Bevan, S.; Bovermann, G.; Boelsterli, J. J.; Breckenridge, R.; Davies, J. W.; Hughes, G. A.; James, I.; Oberer, L.; Winter, J.; Wrigglesworth, R. *J. Med. Chem.* **1994**, *37*, 1942.

[71] Lee, J.; Lee, J.; Szabo, T.; Gonzalez, A. F.; Welter, J. D.; Blumberg, P. M. *Bioorg. Med. Chem.* **2001**, *9*, 1713.

[72] Park, H.-G.; Park, M.-K.; Choi, J.-Y.; Choi, S.-H.; Lee, J.; Park, B.-S.; Kim, M. G.; Suh, Y.-G.; Cho, H.; Oh, U.; Lee, J.; Kim, H.-D.; Park, Y.-H.; Koh, H.-J.; Lim, K. M.; Moh, J.-H.; Jew, S.-S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 601.

[73] Yoon, J. W.; Choi, H. Y.; Lee, H. J.; Ryu, C. H.; Park, H.-g.; Suh, Y.-g.; Oh, U.; Jeong, Y. S.; Choi, J. K.; Park, Y.-H.; Kim, H.-D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1549.

[74] Park, H.-G.; Park, M.-K.; Choi, J.-Y.; Choi, S.-H.; Lee, J.; Suh, Y.-G.; Oh, U.; Lee, J.; Kim, H. D.; Park, Y.-H.; Jeong, Y. S.; Choi, J. K.; Jew, S.-S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 197.

[75] Wahl, P.; Foged, C.; Tullin, S.; Thomsen, C. *Mol. Pharmacol.* **2001**, *59*, 9.

[76] Undem, B. J.; Kollarik, M. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 716.

[77] Planells-Cases, R.; Aracil, A.; Merino, J. M.; Gallar, J.; Pérez-Payá, E.; Belmonte, C.; González-Ros, J. M.; Ferrer-Montiel, A. *FEBS Lett.* **2000**, *481*, 131.

[78] Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.-h.; Sørgård, M; Di Marzo, V.; Julius, D.; Högestätt, E. D. *Nature* **1999**, *400*, 452.

[79] De Petrocellis, L.; Bisogno, T.; Maccarrone, M.; Davis, J. B.; Finazzi-Agrò, A.; Di Marzo, V. *J. Biol. Chem.* **2001**, *276*, 12856.

[80] Szolcsányi, J. *Trends Pharmacol. Sci*. **2000**, *21*, 41.

[81] Zygmunt, P. M.; Julius, D.; Di Marzo, V.; Högestätt, E. D. *Trends Pharmacol. Sci*. **2000**, *21*, 43.

[82] Szolcsányi, J. *Trends Pharmacol. Sci*. **2000**, *21*, 203.

[83] Di Marzo, V.; Griffin, G.; De Petrocellis, L.; Brandi, I.; Bisogno, T.; Williams, W.; Grier, M. C.; Kulasegram, S.; Mahadevan, A.; Razdan, R. K.; Martin, B. R. *J. Exp. Pharmacol. Ther.* **2002**, *300*, 984.

[84] Lo, Y.-K.; Chiang, H.-T.; Wu, S.-N. *Biochem. Pharmacol.* **2003**, *65*, 581.

[85] Vandevoorde, S.; Lambert, D. M.; Smart, D.; Jonsson, K.-O.; Fowler, C. J. *Bioorg. Med. Chem.* **2003**, *11*, 817.

[86] López-Rodríguez, M. L.; Viso, A.; Ortega-Gutiérrez, S.; Fernández-Ruiz, J.; Ramos, J. A. *Curr. Med. Chem.-Central Nervous System Agents* **2002**, *2*, 129.

[87] Di Marzo, V.; Blumberg, P. M.; Szallasi, A. *Curr. Opin. Neurobiol.* **2002**, *12*, 372.

[88] Hwang, S. W.; Cho, H.; Kwak, J.; Lee, S. Y.; Kang, C. J; Jung, J.; Cho, S.; Min, K. H.; Suh, Y. G.; Kim, D.; Oh, U. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6155.

[89] Huang, S. M.; Bisogno, T.; Trevisani, M.; Al-Hayani, A.; De Petrocellis, L.; Fezza, F.; Tognetto, M.; Petros, T. J.; Krey, J. F.; Chu, C. J.; Miller, J. D.; Davies, S. N.; Geppetti, P.; Walker, J. M.; Di Marzo, V. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 8400.

[90] Williams, M.; Kowaluk, E. A.; Arneric, S. P. *J. Med. Chem.* **1999**, *42*, 1481.

[91] Zimmermann, M. *Eur. J. Pharmacol.* **2001**, *429*, 23.

[92] Jensen, T. S.; Gottrup, H.; Sindrup, S. H.; Bach, F. W. *Eur. J. Pharmacol.* **2001**, *429*, 1.

[93] Caterina, M. J.; Julius, D. *Annu. Rev.* **2001**, *24*, 487.

[94] Davis, J. B.; Gray, J.; Gunthorpe, M. J.; Hatcher, J. P.; Davey, P. T.; Overend, P.; Harries, M. H.; Latcham, J.; Clapham, C.; Atkinson, K.; Hughes, S. A.; Rance, K.; Grau, E.; Harper, A. J.; Pugh, P. L.; Rogers, D. C.; Bingham, S.; Randall, A.; Sheardown, S. A. *Nature* **2000**, *405*, 183.

[95] Caterina, M. J.; Leffler, A.; Malmberg, A. B.; Martin, W. J.; Trafton, J.; Petersen-Zeitz, K. R.; Koltzenburg, M.; Basbaum, A. I.; Julius, D. *Science* **2000**, *288*, 306.

[96] Fukuoka, T.; Tokunaga, A.; Tachibana, T.; Dai, Y.; Yamanaka, H.; Noguchi, K. *Pain* **2002**, *99*, 111.

[97] Kwak, J. Y.; Jung, J. Y.; Hwang, S. W.; Lee, W. T.; Oh, U. A. *Neurosci*. **1998**, *86*, 619.

[98] García-Martínez, C.; Humet, M.; Planells-Cases, R.; Gomis, A.; Caprini, M.; Viana, F.; De la Peña, E.; Sánchez-Baeza, F.; Carbonell, T.; De Felipe, C.; Pérez-Payá, E.; Belmonte, C.; Messeguer, A.; Ferrer-Montiel, A. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2374.

[99] Olah, Z.; Szabo, T.; Karai, L.; Hough, C.; Field, R. D.; Caudle, R. M.; Blumberg, P. M.; Iadarola, M. J. *J. Biol. Chem.* **2001**, *276*, 11021.