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Novel natural vanilloid receptor agonists: new therapeutic targets for drug development

Olov Sterner and Arpad Szallasi

The discovery that compounds lacking a recognizable vanillyl-like motif might act as vanilloids has given new impetus to a search for novel vanilloid receptor agonists and antagonists in compound libraries. The availability of cell lines transfected with a cloned human vanilloid receptor will further expedite this search. In this article, the pharmacological properties of unsaturated dialdehydes and triprenyl phenols that represent two newly discovered chemical classes of vanilloids will be discussed. The existence of vanilloid receptors in several brain nuclei as well as in non-neuronal tissues predicts novel, innovative therapeutic indications for vanilloids. However, these findings also suggest that vanilloids might cause side-effects. An exploration of the uses of unsaturated dialdehydes in indigenous medicine might help identify new therapeutic targets for vanilloids and avoid unwanted actions.

For decades, the presence of a vanillyl-like moiety was believed to be an essential requirement of capsaicin-like activity[1–3]. This was the very reason why capsaicinoids and resiniferanoids (Fig. 1) were collectively termed vanilloids, and the receptor at which they interact was named the vanilloid (capsaicin) receptor[4,5].

In 1996, pungent terpenoid unsaturated 1,4-dialdehydes (Fig. 2) were identified as a novel class of naturally occurring vanilloid receptor agonists[6]. Subsequently, triprenyl phenols (Fig. 2) have been shown to function as non-pungent vanilloids[7]. These novel 'vanilloids' are of great interest because they apparently lack any vanillyl-like group, which suggests that the ligand-recognition width of vanilloid receptors is much broader than previously thought. This discovery suggests the existence of endogenous vanilloid receptor modulators lacking a vanillyl motif. In fact, the first such vanilloid was identified as anandamide (arachidonylethanolamide) earlier this year; it was originally isolated as an endogenous cannabinoid receptor ligand[8].

Although unsaturated dialdehydes and related terpenoids possess diverse bioactivities[9], these compounds are relatively unknown to pharmacologists. A brief overview of the natural sources of terpenoid unsaturated dialdehydes and triprenyl phenols, and of the bioactivities of these unusual compounds will be presented here, with special emphasis on their interaction at vanilloid receptors. Some of these natural products are used extensively in indigenous medicine, which implies that they might generate new leads for drug development.

Molecular pharmacology of vanilloid receptors

A functional vanilloid receptor termed VR1 (vanilloid receptor subtype 1), which is activated not only by vanilloids but also by noxious heat (48°C) and low pH (Ref. 10), has recently been cloned[11] (Box 1). Surprisingly, it is only the heat stimulus that seems able to gate the VR1; vanilloids and low pH instead reduce the temperature threshold for VR1 activation[12]. An important consequence of this phenomenon is that even room temperature can open the channel under mildly acidic conditions[10]. The VR1 protein is a distant relative of the transient release potential (TRP) family of store-operated Ca^{2+} channels, comprising six transmembrane domains and ankyrin repeats at the N-terminal. As predicted, VR1 is a nonselective cation channel with a preference for Ca^{2+} (Ref. 11). Although binding and Ca^{2+} uptake were initially believed to detect independent vanilloid receptor subtypes[13], it is now clear that VR1 can account for both the binding and functional activity of vanilloid ligands that show R (resiniferatoxin)- and C (capsaicin)-type structure–activity relations in rat dorsal root ganglion (DRG) neurones expressing native vanilloid receptors[14].

The presence of VR1 is well documented in DRG (Refs 10, 11, 15) and nodose ganglion[16,17] neurones, both at the mRNA and protein levels. In addition, a VR1-like band was described using RT–PCR (reverse transcriptase polymerase chain reaction) in several brain areas including

O. Sterner, Professor, Department of Organic Chemistry 2, University of Lund, S-221 00 Lund, Sweden. Email: Olov.Sterner@orgk2.lth.edu
and
A. Szallasi, Resident Physician, Department of Pathology, Saint Louis University School of Medicine, 3635 Vista Ave, St Louis, MO 63110, USA. Email: arpads@thalamus.wustl.edu

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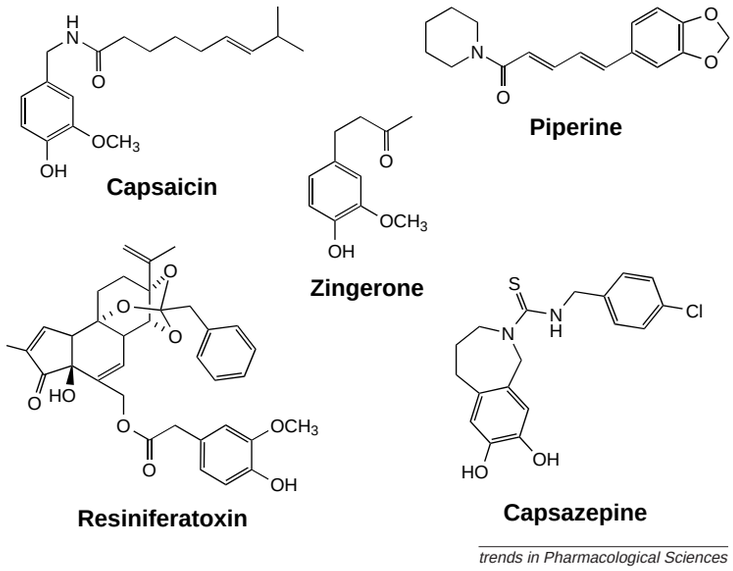

Fig. 1. Structures of typical vanilloids. Capsaicin is the principal irritant in hot pepper (*Capsicum*) extracts. Important capsaicinoids include piperine, the active ingredient in black pepper, and zingerone, which is responsible for the piquancy of ginger. Resiniferatoxin, isolated from the cactus-like plant *Euphorbia resinifera*, functions as an ultrapotent capsaicin analogue. Because all these compounds share a vanillyl (like) moiety essential for bioactivity, they are collectively termed vanilloids. Capsazepine is a synthetic competitive vanilloid receptor antagonist.

the preoptic area, cerebral cortex, hippocampus, cerebellum, striatum and hypothalamus[18]. If vanilloid-sensitive neurones are present in the brain, there would be obvious far-reaching implications linking vanilloids to memory formation, appetite regulation and emotions, among others. The existence of VR1-expressing neurones in the brain is, however, still controversial[5].

Natural sources, selected bioactivities and ethnobotanical uses of unsaturated 1,4-dialdehydes

To date, approximately 80 terpenoids containing an α,β-unsaturated 1,4-dialdehyde (3-formyl-3-butenal) functionality have been isolated from natural sources: the majority have been isolated from terrestrial plants and fungi[19], but also from algae[20], liverworts[21], arthropods[22], sponges and molluscs[23]. In general, these compounds are believed to protect the producing organism from parasites and predators. Because attacking organisms range from bacteria to mammals, it is not surprising that unsaturated dialdehydes have an extremely broad spectrum of bioactivities (Table 1), which ranges from a potent antibiotic action to repellency to mammals[9]. Unsaturated 1,4-dialdehydes as vanilloids will be the focus of this article; a detailed analysis of other actions probably not mediated by vanilloid receptors is beyond the scope of this review. However, it should be noted that several biological effects of unsaturated dialdehydes are of pharmacological interest regardless of their primary target. For example, terpenoid unsaturated dialdehydes have been shown to reduce blood cholesterol[24] and to inhibit platelet aggregation[25,26]. Clearly, these actions might increase the therapeutic value of future unsaturated dialdehyde-based vanilloid drugs. Of course, not all unsaturated dialdehyde actions are therapeutically beneficial[9]. Fortunately, structure–activity relations for terpenoid unsaturated dialdehyde-induced responses are distinct. For example, the mutagenic activity of merulidial (Fig. 2) disappears completely after acetylation[27], whereas the affinity of acetylmerulidial towards vanilloid receptors remains unchanged[6].

Unsaturated 1,4-dialdehydes play a pivotal role in the chemical self-defence system of their host organisms. To meet this goal, unsaturated dialdehydes attack seemingly diverse targets depending on the predator in question. For example, warburganal protects warburgia trees from the larvae of the African army worm by interfering with their stimulus transduction process[28,29]. Warburganal is also pungent[30], a property which might help to repel herbivores. But are the targets for warburganal in worms and mammals really unrelated? Recent evidence suggests that this might not necessarily be the case. Warburganal is hot-tasting by activating capsaicin-sensitive nerves in a vanilloid receptor-mediated fashion[6]. VR1 is, however, homologous to OSM-9, a protein involved in olfaction, mechanosensation and olfactory adaptation in the worm *Caenorhabditis elegans*[31]. Assuming that the African army worm also possesses a protein similar to OSM-9, warburganal might be a versatile self-defence agent acting on an evolutionary conserved segment of VR1.

This reasoning also identifies capsaicin as a chemical self-defence agent in hot peppers. Indeed, most mammals, including squirrels, dislike hot peppers. However, the capsaicin recognition site is an evolutionary recent addition to VR1 (Ref. 11) and birds do not find hot peppers repellent[32]. A practical application of this recognition is the recent development of a pepper-flavoured, squirrel-proof bird-seed[33].

The chemical defence system employing vanilloids, although effective, is not fool-proof. Native Africans find the hot-tasting sensation of warburgia bark pleasurable. Again, the parallelism with hot pepper is obvious: capsaicin, which keeps squirrels from eating bird seeds[33], is a culinary treat to many human beings. (Why it is that the same pungent sensation that repels most animal species is found attractive by humans is puzzling.)

Two prominent examples of unsaturated dialdehydes are polygodial and warburganal (Fig. 2). Both compounds are present in water pepper (*Polygonum hydropiper*)[9], a plant once used as a pepper substitute in Europe. In Japan, it is still a popular relish for 'sashimi' (raw fish). The Ethnobotany Database housed at the US Department of Agriculture (http://probe.nalusda.gov:8300/cgi-bin/browse/ethnobotdb.html) comprises a long list of uses for polygodial in indigenous medicine, ranging from an analgesic effect (to ameliorate toothache in Haiti or arthralgia in China) to an abortifacient (in Turkey) and a haemostat (in Spain), explaining why *P. hydropiper* is known as 'smart weed'.

Warburganal is also present in the bark of warburgia trees[34,35]. For millions of Mozambicans and South Africans, the bark of the pepper-bark tree (*Warburgia salutaris*) is still the only available medicine for treating various diseases including headache, toothache, respiratory ills,

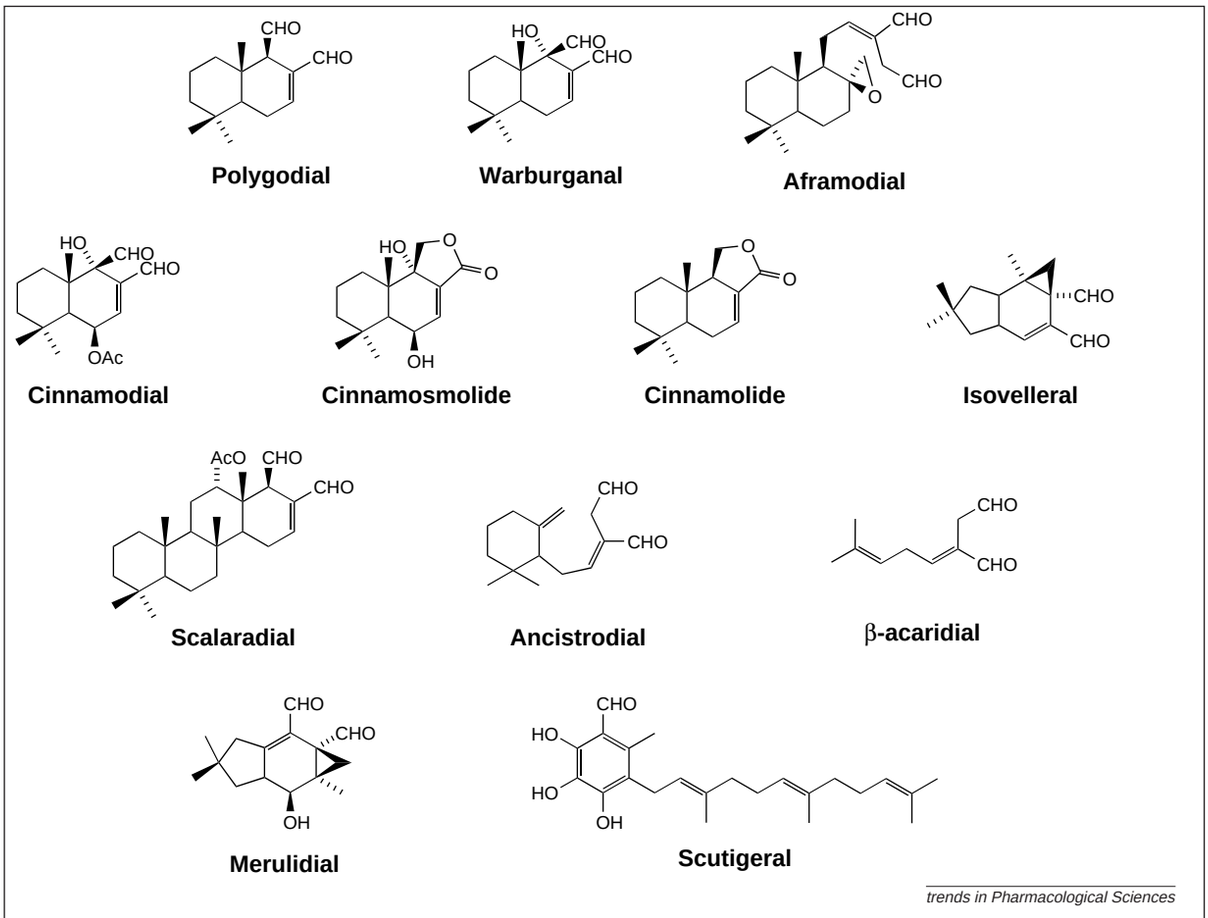

Fig. 2. Structures of novel vanilloids lacking a recognizable vanillyl motif. Polygodial is the active ingredient in marsh pepper; warburganal is present in the bark of warburgia trees growing in Africa and the Caribbeans; isovelleral and merulidial are typical fungal terpenoids; aframodial was isolated from the plant *Aframomum danielli*, a native of Cameroon. Polygodial and isovelleral act as full vanilloid receptor agonists despite the fact that they lack any recognizable vanillyl motif. Cinnamodial, cinnamosmolide and cinnamolide are present in the hot-tasting bark of *Cinnamosma fragrans*; cinnamodial and cinnamosmolide function as partial vanilloid receptor agonists only. Scalaradial is an example of an unsaturated dialdehyde produced by sponges; ancistrodial and β-acaridial were originally isolated from insects; scutigeral, isolated from the edible mushroom *Aframomum ovinus*, is a triprenyl phenol, chemically unrelated to either typical vanilloids or unsaturated dialdehydes (compare structures in Figs 1 and 2), and is also not pungent. Nevertheless, scutigeral interacts at vanilloid receptors, making it the prototype of a new chemical family of vanilloids.

hypertension and diabetes[36]. Unfortunately, the demand from the swelling market has already led to the extinction of *W. salutaris* in the wild (http://archive.outthere.co.za/outtherearchive/97/1211/wild2nov.html). At present, there are plans to cultivate the pepper-bark tree in South Africa. Moreover, the European Commission is funding a multinational project to find new ways for the synthesis of unsaturated dialdehydes and other bioactive terpenoids using abundantly available natural terpenes produced agriculturally or isolated from industrial waste (Project # FAIR3-CT96-1781). Unsaturated dialdehyde-producing trees (*Cannella winterana*) related to warburgia also grow in the Florida Keys and throughout the Caribbean region.

People of Cameroon use the seeds of *Afromomum daniellii* both medicinally and as a spice[37]. This seed is the natural source of the pungent dialdehyde aframodial[38] (Fig. 2). *A. daniellii* belongs to the family Zingiberaceae and is thus related to *Zingiber officinale*, the plant source of the capsaicinoid zingerone (compare the structures of aframodial and zingerone in Figs 1 and 2). The South American arboreal plant *Capsicodendron dinisii* (not to be confused with

Box 1. Vanilloid receptors and vanilloid receptor homologues

To date, only one vanilloid receptor called VR1 (vanilloid receptor subtype 1) has been cloned[1]. VR1 is activated by vanilloid receptor ligands and by noxious heat and acids[1,2]. However, vanilloid receptor homologues also exist; these homologues do not respond to vanilloids and are therefore not dealt with in this review. A homologue of VR1 was cloned from rat kidney[3] and is blocked by membrane stretch, hence its name SIC (stretch-inhibitable nonselective cation channel). Another VR1 homologue was cloned from rat brain and termed VRL-1 (vanilloid receptor-like protein 1)[4].

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Table 1. Biological activities of the fungal unsaturated 1,4-dialdehyde isovelleral

Activity	Effect[a]	Refs
Interaction at vanilloid receptors	Stimulation of Ca^{2+} uptake by rat DRG neurones: EC_{50} = 95 nM (Capsaicin: EC_{50} = 340 nM)	6 (73)
	Inhibition of [^{3}H]RTX binding to native vanilloid receptors in rat spinal cord: K_i = 5.2 μM (Capsaicin: K_i = 3.4 μM)	6 (5)
	Inhibition of [^{3}H]RTX binding to VR1-transfected HEK293 cells: K_i = 20 μM (Capsaicin: K_i = 4 μM)	14
	Pungency in the rat eye: threshold concentration = 4 μM (Capsaicin threshold concentration = 1 μM)	6 (74)
	Pungency on the human tongue: threshold dose = 2.2 nmol per tongue	6
Interaction at other receptors and toxicity to mammalian cells	Inhibition of [^{3}H]SHC23390 binding to dopamine D1 receptors in rat brain: K_i = 290 nM	51
	Stimulation of Ca^{2+} uptake by human neuroblastoma (SH-SY5Y) cells: EC_{50} = 2 μM	69
	Cytotoxicity to BHK 21, L 1210 or B16-F1 cells: LD_{100} = 2–5 μM	70
Phytotoxicity	Toxicity against *Setaria italica*: IC_{50} = 4 μM	71
	Toxicity against *Lepidium sativum*: IC_{50} = 20 μM	71
Antibacterial activity	Inhibition of the growth of *Bacillus subtilis* or *Brevibacillus brevis*: IC_{50} = 4 μM	71
	Inhibition of the growth of *Enterobacter dissolvens*: IC_{50} = 40 μM	71
Antifungal activity	Inhibition of the growth of *Penicillium notatum* or *Nematospora coryli*: IC_{50} = 4 μM	71
	Inhibition of the growth of *Paecilomyces variotii*: IC_{50} = 40 μM	71
Mutagenicity	*Salmonella typhymurium* strain TA98 assay, 244 revertants per μg. (Note: the (−)-enantiomer of isovelleral is ten times less mutagenic than natural isovelleral but shows an enhanced affinity towards vanilloid receptors[49]) (Capsaicin: 34 revertants per 40 μg)	72 (75)

[a]For comparison, capsaicin potencies in selected assays are also given. References to capsaicin are given in parentheses. Abbreviations: DRG, dorsal root ganglion; RTX, resiniferatoxin; VR1, vanilloid receptor subtype 1.

Capsicum) produces two pungent unsaturated dialdehydes, cinnamodial (Fig. 2) and capsicodendrin[39].

Some mushrooms can also synthesize unsaturated dialdehydes but have no culinary or medicinal uses. Nevertheless, these fungi provide a window of opportunity to study biology. A characteristic example of these fungal dialdehydes is isovelleral (Fig. 2), isolated from the injured fruit bodies of *Lactarius vellereus*, *Lactarius rufus* and *Lactarius quietus*[9]. Isovelleral has a wide range of bioactivities (Table 1) and structure–activity relations for these actions have been explored in depth with the aid of synthetic isovelleral analogues[9,40].

Unsaturated dialdehydes are also found in the animal kingdom. Interestingly, molluscs belonging to the subclass of Opisthobranchia lost their shell during evolution and instead produce deterrent unsaturated dialdehydes[41,42]. This new chemical defence system seems to be far more effective than the traditional mechanical system because only very few predators to these molluscs are known. During evolution, sponges tried to develop a similar chemical defence system using sesterpene unsaturated dialdehydes such as scalaradial[43] (Fig. 2). Insects are also capable of unsaturated dialdehyde synthesis. Examples include the termite *Ancistrotermes cavithorax*, which uses ancistrodial[44], and mites producing β-acaridial[45] (Fig. 2).

Natural sources and bioactivities of triprenyl phenols

When compared with the extremely rich pharmacology of terpenoid unsaturated dialdehydes, triprenyl phenols appear rather dull compounds. They are isolated from the fruit bodies of various *Albatrellus* species[46] and have a mixed, partly polyketide and partly isoprenoid, biosynthetic origin. *Albatrellus ovinus*, the major source for the triprenyl phenols, is considered delicious and is, in fact, used by the food industry as a substitute for truffles[47]. Scutigeral (Fig. 2) is the major component in the *A. ovinus* fraction containing triprenyl phenols. Unlike unsaturated dialdehydes, triprenyl phenols are not pungent[7]. Some triprenyl phenols bind to dopamine D1 receptors[48] and vanilloid receptors[7] (see below), but nothing else is known about their pharmacological actions. It is interesting to note that several terpenoid unsaturated 1,4-dialdehydes also bind to D1 receptors[49].

Unsaturated 1,4-dialdehydes and related terpenoids and triprenyl phenols as vanilloids

The extract of *Cinnamosma fragrans*, a native plant of Madagascar containing several sesquiterpenes such as cinnamolide, cinnamodial and cinnamosmolide, was described as having a 'distinct pepper-like taste'[50]. The similarity between the pungent sensation evoked in the human tongue by capsaicin and isovelleral, the active ingredient in the mushroom *L. vellereus*, was also noted[51]. Despite these observations, the possibility that unsaturated dialdehydes might be pungent by activating vanilloid-sensitive sensory neurones was not investigated until 1996.

Like capsaicin, the fungal terpenoid isovelleral causes protective eye-wiping movements in the rat upon intraocular instillation[6]. Moreover, there is cross-tachyphylaxis

between capsaicin and isovelleral actions both in the rat eye and in the human tongue[6], which implies a shared primary target for these compounds. Isovelleral, which is fully inhibited by the competitive vanilloid receptor antagonist capsazepine, induces Ca^{2+} uptake by rat DRG neurones in culture[6]. Isovelleral also inhibits specific [^{3}H]RTX (resiniferatoxin) binding to both native vanilloid receptors expressed by DRG neurones[6] and VR1-transfected mammalian Chinese hamster ovary (CHO) cells[14]. Taken together, these findings strongly suggest that isovelleral is pungent by activating vanilloid receptors on sensory neurones. For a series of 14 terpenoids with an unsaturated 1,4-dialdehyde moiety, a good correlation was found between pungency on the human tongue and affinity for vanilloid receptors in the rat spinal cord[6].

Isovelleral acts as a full vanilloid receptor agonist[6]. It is notable, however, that related terpenoids (e.g. cinnamodial and cinnamosmolide) behave only as partial agonists in vanilloid receptor assays[52]. These ligands display a complex behaviour in Ca^{2+}-uptake responses (the concentration-dependent Ca^{2+} uptake at low ligand concentrations is superceded by a blockade of the response at high concentrations), which might account for this partial agonism. When working with such compounds, one should be aware of this phenomenon, which can distort or even mask the vanilloid-receptor-mediated effects.

Consistent with its classification as a vanilloid, polygodial (the principal irritant in marsh pepper) inhibits the pain response evoked by intradermal capsaicin or formalin injection in the mouse[53]. In addition, like capsaicin or RTX (Ref. 54), polygodial blocks acetic acid-induced writhings in mice. Furthermore, polygodial has potent anti-allergic and anti-inflammatory activities[55], although, at present, the role played by vanilloid-sensitive neurones in these beneficial effects of polygodial is unclear. However, vanilloids do have anti-allergic and anti-inflammatory actions[3,5,13]: polygodial targets opioid, serotoninergic supraspinal pathways[53], or both, and blocks tachykinin NK_2 receptors[56] in addition to its interaction at vanilloid receptors[6]. Clearly, polygodial is an interesting new lead for drug development because it targets a variety of pathways involved in pain perception and inflammation (Table 2).

The archetypal triprenyl phenol is scutigeral, isolated from the edible mushroom *A. ovinus*[48]. Unlike unsaturated dialdehydes, scutigeral is not pungent[7]. Nevertheless, scutigeral induces Ca^{2+} uptake by rat DRG neurones in culture, a response prevented by capsazepine[7]. Scutigeral also inhibits specific [^{3}H]RTX binding to rat DRG neurones[7] and CHO cells transfected with the cloned rat vanilloid receptor[14]. Taken together, these observations are consistent with scutigeral being a vanilloid. The finding that scutigeral is non-pungent is surprising but not unprecedented. For example, olvanil is also considered non-pungent, although it mimics most capsaicin responses[57,58]. Consistent with its non-pungency, scutigeral fails to induce action potential formation in patch-clamped rat DRG neurones[7]. What makes scutigeral interesting is its ability to abolish the first, rapidly activating current by capsaicin, leaving the second, slowly activating current relatively intact[7]. Thus, scutigeral should be able to selectively block capsaicin responses mediated by the rapidly activating conductance.

Table 2. Analgesic, anti-allergic and anti-inflammatory activities of polygodial

Activity	Refs
Analgesia (mouse)	
Inhibition of acetic acid-induced writhings	53
Inhibition of both phases of the formalin-induced paw-licking response	53
Block of capsaicin-evoked paw-licking response	53
No effect in the hot-plate test	53
Analgesia (rat)	
Inhibition of carrageenan-induced hyperalgesia	55
Anti-inflammatory and anti-allergic actions	
Block of rat paw oedema formation in response to carrageenan, dextran, bradykinin or PAF	55
Inhibition of anaphylactic shock in mice sensitized to ovalbumin	55
Inhibition of guinea-pig trachea contractions in response to bradykinin, tachykinins or capsaicin	56

Abbreviation: PAF, platelet-activating factor.

Unsaturated dialdehydes in clinical practice

The only current therapeutic use of an unsaturated 1,4-dialdehyde in the Western hemisphere that we are aware of is the use of polygodial (Kolorex Capsules) by practitioners to control localized candidiasis. According to the producer, polygodial damages the cell wall of *Candida albicans* and other fungi (http://www.forestherbs.co.nz/capsules.htm). It is notable that apart from a mild stomach discomfort or dizziness, or both, experienced by some people, most individuals tolerate polygodial remarkably well.

Capsaicin has been in clinical use for decades and is used for several therapeutic indications including amelioration of neuropathic pain or itching, inhibition of neurogenic inflammation and suppression of urinary bladder hyperreflexia[5,13,59]. Capsaicin is, however, poorly absorbed from the human skin and is extensively metabolized[5], which explains why capsaicin creams are less active than expected on the basis of animal experimentation[3,5,59]. When applied via a catheter into the human urinary bladder, capsaicin is a powerful drug that relieves detrusor hyperreflexia[60–62]. However, capsaicin is also very painful and, even worse, might activate undesirable autonomic reflexes if absorbed into the circulation through the bladder mucosa[61].

RTX seems to be devoid of the shortcomings of capsaicin. Unlike capsaicin, RTX is well tolerated by patients with a hyperactive urinary bladder[61,63,64]. Moreover, a single RTX treatment is followed by a remarkably lasting (several months) improvement in the urge frequency to void[65]. However, RTX has its own shortcomings: most importantly, RTX is expensive to isolate from natural sources

and is difficult to synthesize. Clearly, there is a need for structurally simplified, orally active vanilloids. Whether either unsaturated dialdehydes or triprenyl phenols could serve as templates for the synthesis of such improved vanilloids remains to be seen. Nonetheless, both scutigeral (which is orally active and non-pungent) and polygodial, a multifaceted antinociceptive natural product, possess features that could be exploited by medical chemists. As one consultant on medicinal plants put it: 'why market 20 different headache tablets when one plant [i.e. *W. salutaris*] could suffice?' (http://archive.outthere.co.za/outtherearchive/97/1211/wild2nov.html).

Human exposure to genetically engineered polygodial: implications for toxicology

Polygodial is one of the most potent plant-derived compounds active against aphids. Aphids are considered by many to be the most important insect pests in temperate-zone agriculture. Most of our present-day aphid-control methods depend heavily upon the use of chemical aphicides. Predictably, a growing number of aphid species have developed resistance to aphicides[66]. This, along with the increasing environmental concerns related to the persistence and toxicity of chemical aphicides, predict an increasing importance for biological control methods in crop protection in the foreseeable future. For example, barley sprayed with polygodial is more resistant to infection from barley yellow dwarf virus than untreated controls[67]. Polygodial is, however, unstable and is thus unsuited for lasting crop protection. Therefore, efforts are being made to develop transgenic crops capable of polygodial biosynthesis[68]. In sensitive individuals, capsaicin can cause dermatitis, provoke persistent cough or exacerbate asthmatic symptoms[5]. If transgenic crops synthesizing polygodial are cultivated, the sporadic occurrence of similar symptoms among farm workers is likely.

Concluding remarks

In an very short time, a rapid succession of breakthrough discoveries has transformed the vanilloid receptor from a pharmacological oddity (a receptor for pungent spices) to an attractive therapeutic target (a molecular integrator of various noxious chemical and physical stimuli). To date, one vanilloid receptor VR1 has been cloned[11], but the heterogeneity of vanilloid-induced biological responses predicts the existence of VR1 isoforms, additional receptor subtypes, or both[5]. With the availability of cell lines stably transfected with the cloned rat VR1, there can be little doubt that pharmaceutical companies are now actively searching their compound libraries for novel vanilloid receptor agonists and antagonists. Therefore, several new vanilloid drugs should undergo clinical trials in the foreseeable future. The naturally occurring ultrapotent vanilloid RTX seems to be devoid of the most important shortcoming of capsaicin, the modest potency to induce desensitization as compared with the initial pain response[5,13]. At present, RTX is undergoing clinical trials with promising results to suppress detrusor instability[61,63,64], an important cause of urinary incontinence. RTX is, however, expensive to isolate from natural sources, difficult to synthesize and cannot be given orally. Therefore, the synthesis of simplified and orally active vanilloids is an ongoing objective. Unsaturated 1,4-dialdehydes and triprenyl phenols might provide new clues for vanilloid drug development.

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Cyclooxygenase 2 inhibitors: discovery, selectivity and the future

Lawrence J. Marnett and Amit S. Kalgutkar

The recent marketing of two selective cyclooxygenase 2 (COX-2) inhibitors climaxes the first phase of an exciting and fast-paced effort to exploit a novel molecular target for nonsteroidal anti-inflammatory drugs (NSAIDs). Much has been written in the lay and scientific press about the potential of COX-2 inhibitors as anti-inflammatory and analgesic agents that lack the gastrointestinal side-effects of traditional NSAIDs. Although research on COX-2 inhibitors has focussed mainly on inflammation and pain, experimental and epidemiological data suggest that COX-2 inhibitors could be used in the treatment or prevention of a broader range of diseases. In this review, some key points and unresolved issues related to the discovery of COX-2 inhibitors, the kinetic and structural basis for their selectivity, and possible complications in their development and use will be discussed.

Cyclooxygenase 2 (COX-2) was discovered in 1991 and the first lead inhibitors were described in 1992 (Refs 1, 2). A mere seven years later, the first examples of selective inhibitors are on the market. This remarkably fast drug discovery and development effort did not happen in a vacuum but took advantage of a solid base of medicinal chemistry and clinincal experience related to non-steroidal anti-inflammatory drugs (NSAIDs). The history of NSAIDs is rich and has been well documented[3,4]; the discovery in 1971 that COX was their molecular target was a landmark finding but it did not provide an obvious strategy to separate the pharmacological effects from the undesired effects of NSAIDs (Ref. 5). Such a breakthrough awaited the discovery of a second form of COX that is differentially regulated from the originally studied isoform[6].

COX enzymes head a complex biosynthetic cascade that results in the conversion of polyunsaturated fatty acids to prostaglandins and thromboxane – a family of autocrine and paracrine mediators that contribute to many physiological and pathophysiological responses (Fig. 1)[7,8]. Inhibition of COX leads to a decrease in the production of all prostaglandins and thromboxane, which accounts for the beneficial anti-inflammatory, antipyretic, analgesic and cardiovascular effects of NSAIDs as well as their gastrointestinal side-effects. Both COX-1 and COX-2 are inducible in certain situations but, in general, COX-1 is constitutively expressed whereas COX-2 is inducible[6,9]. Like other immediate-early genes, COX-2 is rapidly upregulated in response to growth factors and cytokines but its mRNA and protein exist transiently. Thus, COX-2 is not commonly found in differentiated cells in the absence of stimulation, which explains why its existence remained undiscovered for three decades. Of particular importance is the fact that COX-1 is widely distributed throughout the gastrointestinal (GI) tract where it is believed to protect against gastric damage. COX-2 is undetectable or present only at low concentrations in the GI tract[10,11]. By contrast, COX-2 is detectable in leukocytes, synoviocytes and in the CNS – locations where it appears to play a role in inflammation, fever and pain, respectively[12–14]. This tissue specificity provided a new paradigm with which to approach the problem of NSAID-induced side-effects – that is, COX-2 is responsible for inflammation and hyperalgesia whereas COX-1 is critical for gastric cytoprotection.

Classes of selective COX-2 inhibitors

Enormous effort was expended in the development of NSAIDs between the 1960s and 1980s so there were numerous pharmacophores to test when COX-2 was discovered. (For an historical review of the medicinal chemistry

L. J. Marnett, Professor, Email: marnett@toxicology.mc.vanderbilt.edu and **A. S. Kalgutkar,** Research Associate, A. B. Hancock, Jr Memorial Laboratory for Cancer Research, Departments of Biochemistry and Chemistry, Center in Molecular Toxicology and Vanderbilt Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA. Email: amit_kalgutkar@grotin.pfizer.com

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