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Hot cuisine as a source of anti-inflammatory drugs

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Abstract

The shift in nutritional sciences from survival and safety to the promotion of well-being has led to systematic investigations on the biological activity of natural products of dietary origin, questioning the assumption that food plants contain little if any secondary metabolites apart those revealed by our senses and responsible for their colour, taste, and flavour. With 25% of the human population consuming chilli pepper every day, capsaicin is the most important pharmacological agent we get from our diet, and the study of its pungency set in motion a multidisciplinary investigation that ultimately led to the discovery of vanilloid receptors (TRPVs), a class of ion channels involved in thermo-, chemo-, and mechanosensation, and whose malfunctioning is implicated in neurogenic inflammation and a host of other pathological conditions. A series of studies centred on the modification of capsaicin will be described, focusing on a) the preparation of a library of unnatural natural capsaicinoids and the identification of leads with the lipophilic C-moiety amenable to structure-activity study, and b) the reversal of the biological activity of capsaicin from a TRPV1 agonist into an antagonist by modification of its vanillyl moiety.

Abbreviations: AE – Arachidonoylethanolamine (=Anandamide); COX-1 (2) – Cyclooxygenase-1 (2); CPS – Capsaicin; GRAS List – Generally Recognized as Safe List; NADA – *N*-Arachidonoyldopamine; NSAID – Non Steroid Anti-Inflammatory Drug; OLDA – *N*-Oleoyldopamine; PABA – *para*-Aminobenzoic Acid; PPAA – Prophyl Phosphonic Acid Anhydride; RTX – Resiniferatoxin; SERCA – Sarcoendoplasmatic Reticulum Ca(II) ATPase; TRPV – Transient Receptor Potential Vanilloid

Introduction

The modern clinical trial was first described in the Bible (Daniel, 1), but only carried out 2000 years later by the navy surgeon James Lind as part of a study on the prevention of scurvy (Appendino and Taglialatela- Scafati, 2003c). Several explanations have been given as to why medicine was so slow to adopt the scientific method (Sneader, 1996). Nevertheless, edible- and not medicinal plants were used in both cases (legumes and *Citrus* fruits, respectively). This observation is hardly surprising, since medicine and nutrition have long shaded into each other. Their divorce is essentially a modern artefact, since in all cultures a considerable share of food plants have also been used in traditional medicine (Pieroni et al., 2002). Scouring the planet for the most exotic and expensive remedies has always been a sort of fashionable trend in natural products chemistry, even finding its way into the plot of a successful Hollywood movie (Medicine Man, directed by J. McTieman and starring Sean Connery, 1992). Food plants are trivial, easily available, and generally contain low concentration of secondary metabolites. Unsurprisingly, they were long overlooked as a source of bioactive

compounds. However, over the past decades, there has been a growing awareness that food plants, apart from macronutrients (proteins, lipids, sugars) and essential micronutrients (vitamins, minerals) also contain secondary metabolites that can play a role in the maintenance of human health. This has led to a shift in nutritional sciences from survival and safety to the promotion of well-being, challenging the long-held assumption that food plants contain little if any secondary metabolites apart those revealed by our senses and responsible for their colour, taste, and flavour.

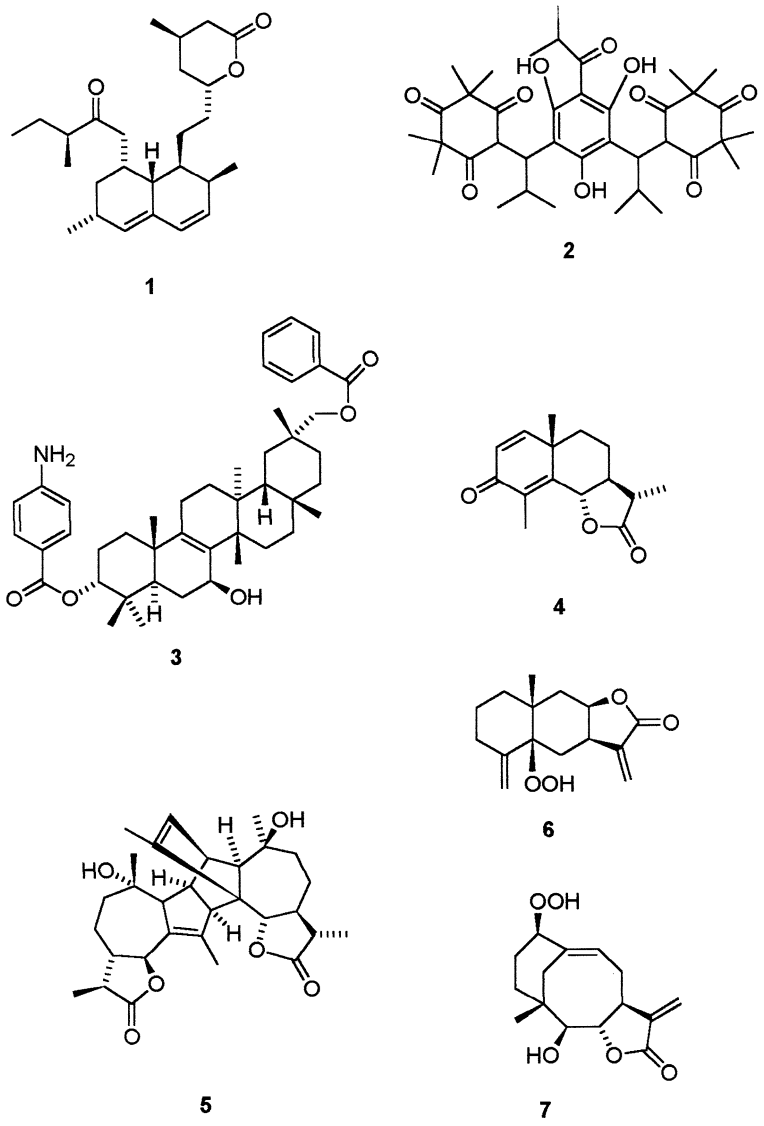
There is currently no comprehensive review or book on the chemistry and biochemistry of secondary metabolites from food plants. This article, while highlighting the relevance of the subject, will essentially summarise recent results on a single class of compounds (capsaicinoids). Though undoubtedly limiting, the examples discussed are nevertheless paradigmatic for the problems facing research in this field.

Food plants as a source of drugs, drug leads, and unusual secondary metabolites

Scouring the refrigerator or the cupboard for drugs leads might sound naïf in the age of -omic sciences, but this is the origin of statins. With annual sales over 15 billion dollars, statins are the commercially most successful drugs ever, and lovastatin (1) is their archetypal member. This compound was first isolated from the red yeast of rice, an ingredient of the oriental cuisine used to give the red colour and typical taste to the Pekinese duck, and this dietary origin has caused long and bitter legal litigation between the pharmaceutical- and the health food industries (Journoud and Jones, 2004). Statins are structurally unique compounds. They belong to the rare class of naturally occurring Diels-Alder adducts, and exemplify how our diet can be a real treasure throve of structurally unique and biologically active secondary metabolites. Another remarkable example is the occurrence of triterpene *p*-aminobenzoates (exemplified by 2) in the seeds of pumpkin and related plants (courgettes, cucumber, melon, water melon) (Appendino et al., 2000). Nature is rather conservative in the use of key metabolites, and the profligacy by which these plants accumulate esters of PABA in their seeds is unprecedented in living organisms, as is the occurrence of nonprenylated oligomeric phloroglucinols like 3 in the leaves of myrtle (Appendino et al., 2002a). While no bioactivity has so far been reported for pumpkin *p*-aminobenzoates, the oligomeric phloroglucinols from myrtle show a remarkable antibacterial activity, especially against super bugs (Appendino et al., 2002a). Myrtle is just one of the over 150 plants included in the GRAS (Generally Recognized As Safe) list of herbal ingredient for liqueurs (Duke, 1987). Many of these plants have never been investigated for their non-volatile constituents, and there is no shortage of interest for this matter, as shown by the fascinating correlation between the chromatic aberrations by van Gogh and the presence of santonin-like compounds in wormwood (*Artemisia absinthium* L.) (Arnold, 1992). α-Santonin (4) was once used in medicine as an anthelmintic drug, and can change the way colours are perceived, turning vision to yellow. Wormwood does not contain α-santonin, but the extremely bitter dimeric guaianolide absinthin (5), whose pharmacology is still totally unknown. Structurally unusual bitter sesquiterpene lactones are also contained in other plants used for the production of liqueurs, as exemplified by the peroxides 6 and 7 from mountain wormwood (*A. umbelliformis* Lam) (Appendino et al., 1983) and from curly tansy (*Tanacetum vulgare* L. var. *crispum* Fiori) (Appendino et al., 1982), two plants used for the production of alpine liqueurs (Genepy and Chartreuse, respectively).

The kitchen connection. Vanilloid receptors as anti-inflammatory targets

No compound better than capsaicin (CPS, 8) exemplifies the blurred boundaries between food and medicine. With 25% of the human population consuming chilli pepper every day, 8 is the most important pharmacological agent we get from our diet (Szallasi and Blumberg, 1999), and the study of its pungency set in motion a multidisciplinary investigation that ultimately led to the discovery of vanilloid receptors (TRPVs). These ion channels are involved in thermo-, chemo-, mechanosensation, and their malfunctioning is involved in neurogenic inflammation, and, possibly, in other pathological conditions as well (Szallasi and Blumberg, 1999).



Capsaicin was first isolated in 1876, structurally elucidated in 1919, and first synthesised in 1930 (Szallasi & Blumberg, 1999). Despite all this chemical activity, it was ignored by pharmacologists for a long time, probably because too obnoxious to handle. Things changed in 1989, when an ultrapotent analogue was discovered, the phorboid resiniferatoxin (RTX, 9a) (Szallasi and Blumberg, 1989). By using radioactive RTX, it was possible to demonstrate a specific binding of capsaicin to nerve membranes, and this set in motion an intense research activity that culminated eight years later in the characterisation of a thermoreceptor specifically activated by CPS. Since **8** and **9** share a vanillyl moiety, this receptor was named the vanilloid receptor (VR1), but is now better known as TRPV1 (for a recent review, see: Appendino et al., 2003b). In 2000, knock-out mice for TRPV1 were created by genetic engineering. This animals showed an impaired pain sensation, but were otherwise normal, an observation that validated TRPV1 as a pharmacological target. Four further vanilloid receptors were cloned in the years 2000–2003. Though structurally related to TRPV-1, these receptors (TRPV-2/TRPV-5) do not bind capsaicin, nor does the avian and version

of TRPV1 (Appendino et al., 2003). TRPV1 is a heat-sensitive ion channel whose activation threshold is lowered by an increase of temperature, a decrease of pH, as well as by capsaicin and an heterogenous group of compounds named vanilloids. Activation is followed by a calcium influx, and is eventually translated into a painful stimulus on the proximal ending of the nerve, while inflammatory peptides are secreted on the distal nerve ending. Activation is followed by a long lasting refractory state known as desensitisation, a particular form of analgesia where only pain sensitivity is lost, and a unique property that makes capsaicin different from all other offensive agents. Vanilloid receptors are also expressed in the central nervous system, where temperature and pH are constant, and where receptor activation is provided by a series of lipid-derived endogenous compounds known as endovanilloids by analogy with the endogenous versions of opiodis and cannabinoids (endorphins and endocannabinoids, respectively) (Di Marzo et al., 2002). While anandamide (AE, **10**) and NADA (*N*-arachidonoyldopamide, **11a**) can also interact with cannabinoid receptors, OLDA (*N*-Oleyldopamide, **11b**) is more selective for vanilloid receptors. The analgesic properties of arachidoylated endovanilloids and endocannnabinoids might contribute to the beneficial effects of non-steroideal antiinflammatory drugs. By inhibiting COX-enzymes, these compounds might in fact redirect the metabolism of arachidonic acid from inflammatory prostanoids to anti-inflammatory fatty amides like AE and NADA. Alternatively, the inhibition of COXs might shut down one major way of degradation of AE ane NADA (Fowler, 2004). A more direct involvement was suggested for acetaminophen (12a), the most successful NSAID. This compound does not inhibit COX-1or COX-2, but is deacetylated and arachidonoylated in the brain, affording AM-404 (12b), a known inhibitor of anandamide degradation, and a powerful activator of TRPV1 and inhibitor of COX-1 and COX-2 (Hogestatt, 2003).

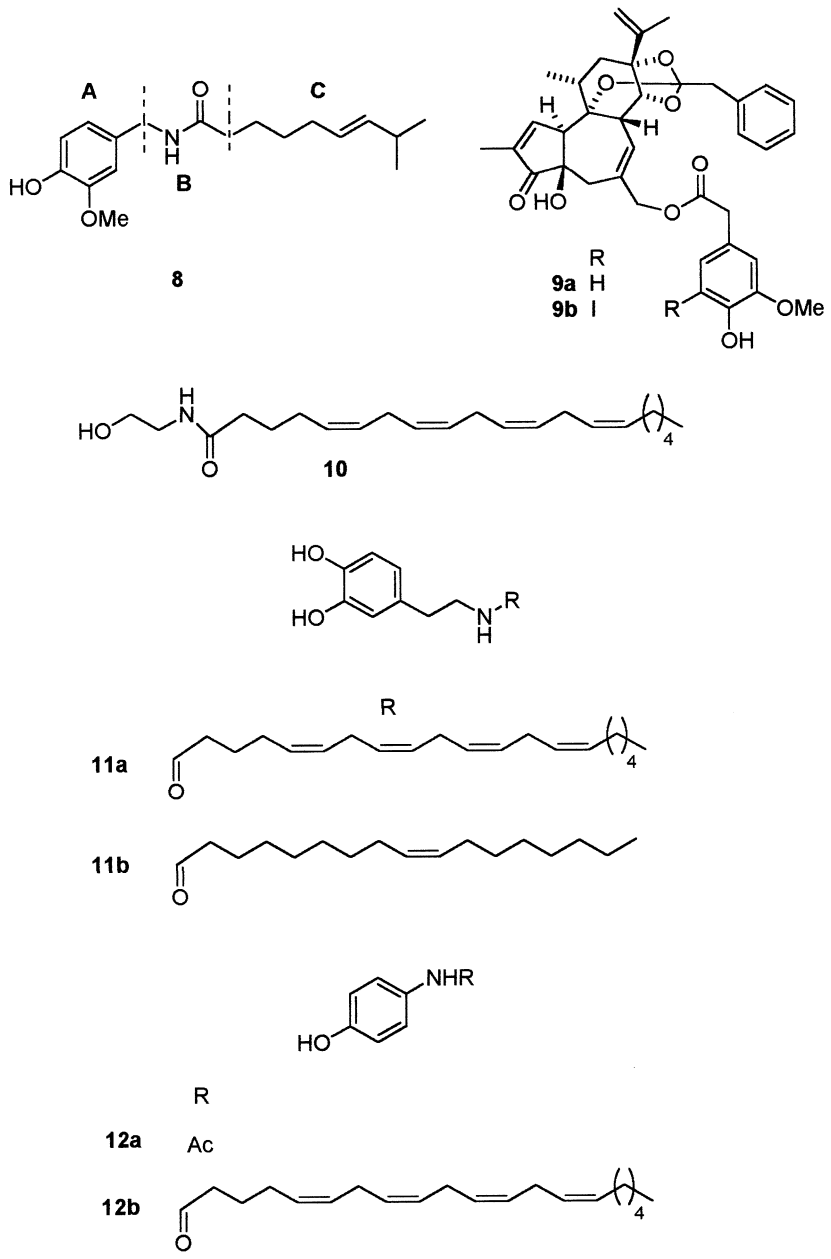
CPS-sensitive neurones are bi-modal. Besides the classical afferent algogenic function, they also have local efferent function, secreting neuropeptides that sustain a series of pathophysiological changes (vasodilatation, plasma protein extravasation, accumulation of inflammatory cells) that trigger neurogenic inflammation, a process involved several pathological conditions (rheumatoid arthritis, migraine, asthma, psoriasis, allergic rhinitis) whose current management is either modest or non-existing.

The inhibition of capsaicin-sensitive nerves is a logical approach to contrast neurogenic inflammation, and is an important pharmacological target (Szallasi and Appendino, 2004). There are two basic strategies to accomplish this. The first one is desensitisation, a long-lasting process, possibly mediated by phosphorylation, typical of TRPV-1 agonists. The second one is classical antagonism, namely the interference with the activation from agonists (low pH, endovanilloids). We pursued both of them.

The development of a library of unnatural natural capsaicinoids

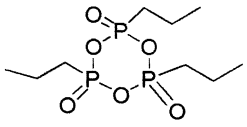
There is no shortage of activators of TRPV1 within the pool of natural products (Appendino et al., 2003), but capsaicinoids are those better investigated and more accessible from a synthetic standpoint. For structure-activity studies, capsaicin has been traditionally divided into three moieties, the aromatic A-region, the lipophilic C-region and the amide B-linker (see **8**). While the A and B regions have been thoroughly investigated, the lipophilic C-region is not, because of its poverty of functional groups (the branching and the unsaturation, both redundant for biological activity) and because of its conformational flexibility (rotation is possible around eight sigma C–C bonds). Capsacin is not therefore a suitable probe to investigate the effect of changes on the C-region, while an ideal lead should have this region decorated with functional groups non redundant in terms of biological activity, and versatile from the viewpoint of chemical manipulation.

In search for a convenient surrogate for capsaicin, we turned to Nature and got inspiration from its unmatched capacity to generate chemical diversity. The process of drug discovery has been compared to a lottery. In this lottery, natural products are special tickets, but are few and difficult to get. So, we turned to surrogate natural products, or 'unnatural' natural products (Appendino et al., 2001). The branched short fatty acid of CPS is found exclusively in plants from the genus *Capsicum*, but a host of fatty- and isoprenoid acids are easily available from the natural



products pool. Acylation of vanillamine with these acids would afford unnatural (as regards occurrence) natural (as regards the origin of their building blocks) capsaicinoids. Nature has deftly exploited this strategy, and its view as a molecular tinkerer should be credited to François Jacob. In a seminal conference on evolution held at Berkeley University, Jacob remarked that '*natural selection works like a tinkerer who does not known exactly what he is going to produce but uses whatever he finds around him to produce some kind of workable object. None of the material at the tinkerer's disposal has a precise and definite function. Each can be used in different ways. Novelty comes from previously unseen associations of old materials. To create is to recombine*' (Jacob, 1977).

Vanillamine is a phenolic amine, and to implement our strategy we first had to solve a



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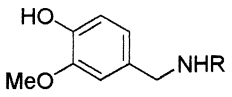

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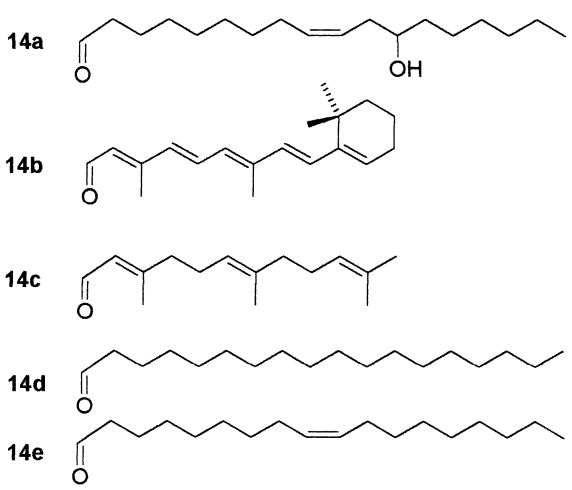
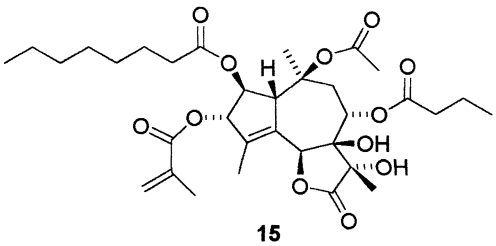


synthetic problem, since the acylation of phenolic amines shows low chemoselectivity with diimides, while activation via chlorides or mixed anhydrides is difficult with polyunsaturated and hydroxylated acids. To solve this problem, we developed an alternative protocol based on the *in situ* formation of mixed phosphoric anhydrides by treatment of acids with PPAA (propyl phosphonic acid anihydride, **13**). Compared to other condensing agents, PPAA is chemoselective for amino groups vs phenolic hydroxyls, and shows also a series of practical advantages, being cheap, relatively non toxic, easy to store, and essentially traceless, giving only water-soluble by-products (Appendino et al., 2002b). Having solved the synthetic problem, we could easily assemble a small library of unnatural natural capsaicinoids made from fatty acids as well acyclic, monocyuclic and polycyclic terpenoid acids. All compounds were assayed for vanilloid activity in cells transfected with h-TRPV1, measuring efficiency and potency. Three compounds emerged as powerful vanillloid ligands, over tenfold more active than capsaicin, namely the vanillamides of ricinoleic acid, retinoic acid, and farnesic acids (rinvanil (**14a**), retvanil (**14b**), and farnvanil (**14c**), respectively). Using fatty acyl vanillamides, some interesting insights on the recognition site for the lipophilic moiety of capsaicinoids could be obtained. Thus, while the vanillamide of stearic acid (**14d**) was essentially inactive, that of oleic acid (olvanil, **14e**) was more potent than capsaicin, showing that the double bond is critical for activity. Since the double bond could be replaced by a cyclopropane ring, its role is apparently conformational. Furthermore, hydroxy groups could be easily accommodated into the lipophilic moiety, as shown by the powerful activity of rinvanil (**14a**). The presence of a secondary hydroxyl on the C-region of this compound gives the opportunity for a variety of modification, currently actively pursued with the hope of improving the unnatural natural lead.

The taming of capsaicin

While the presence of offensive compounds within the pool of natural products is somewhat logical, secondary metabolites being essentially chemical weapons, the existence of vanilloid antagonists is apparently counterintuitive. The sesquiterpene lactone thapsigargicin (**15**) is the only known natural inhibitor of TRPV1 (Toth et al., 2002). This highly obnoxious, offensive, and tumour-promoting guaianolide is a known activators of sarcoplasmatic reticulum Ca^{2+}-ATPases (SERCAs), and it is not clear to what extent vanilloid antagonism can be dissected from this activity. Furthermore, thapsigargicin is structurally complex, difficult to obtain by isolation, and not easily amenable to total synthesis. A serendipitous observation at Novo-Nordisk paved the way to a different approach to get vanilloid antagonists from the natural products pool, namely the agonist-to-antagonist swap (Wahl et al., 2001). Thus, while attempting to prepare a radioactive version of RTX (**9a**), it was discovered that iodination *ortho* to the phenolic hydroxyl (C-5′) reverted the biological activity of the natural product, turning it into a powerful vanilloid antagonist (**9b**). Further studies at Johnson & Johnson confirmed these finding, and also disclosed that iodination at C-6′ could generate a partial agonist (McDonnel et al.,



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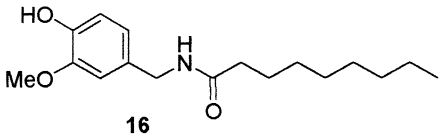

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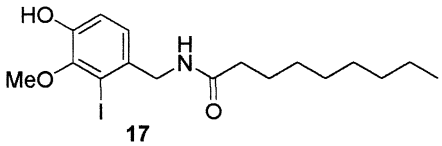

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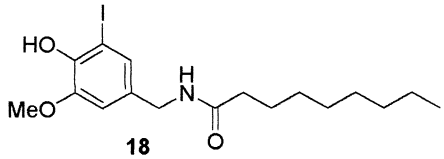

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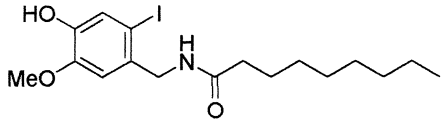

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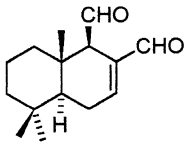

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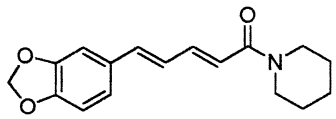

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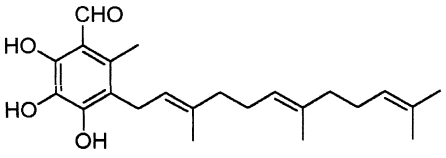

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2002). RTX is not commercially available in synthetically useful amounts, and a comprehensive investigation on the effect of aromatic substitution could not be carried out on this compound. On the other hand, synthetic capsaicin (nonivamide, *N*-nonanoylvanillammine, **16**) is commercially available, and we used this compound to assess these points. All three iodinated forms of nonivamide were synthesized. While the 2-iododerivative (**17**) lacked affinity for TRPV1, both the 5- and the 6-iododerivatives (**18** and **19**, respectively) showed antagonist activity, potency being higher for C-6 rather than C-5 iodination (Appendino et al., 2003a). The antagonistic potency was dependent not only on the location of the halogen group, but also on its nature, since, both for C-5 and C-6 substitution, the bromo- and chloro analogues were less potent than their parent iodinated derivatives. Replacement of the 6-iodo group with various carbon susbstituents (ethyl, ethenyl, ethynyl, acetyl) gave vanilloid antagonists less potent that the iododerivative **19**. Compared to capsazepine, the standard capsaicinoid antagonist, 6-iodononivamide (**19**) is easier to synthesise (three steps from a commercial product) and more potent (ca. 2-fold). Furthermore, it seems reasonable to assume that the optimisation of the acyl moiety in terms of affinity could be combined with that of the vanillyl moiety in terms of antagonism, generating even more powerful TRPV1 antagonists with capsaicinoid structure.

Conclusions

Despite investigations spanning over a century, capsaicin is still a versatile lead compound for induction and modulation of bioactivity. The capsaicinoid template has provided inspiration for the discovery of new potent TRPV1 agonists and antagonists, paving the way to the development of new compounds aimed at contrasting neurogenic inflammation by desensitisation or pharmacological antagonism of TRPV1. Capsaicin is not the only clinically-validated antiinflammatory compound of dietary origin, nor is the only TRPV1 agonist that hot cuisine has provided to the biomedical research. A search on the Drugs of the Future database evidenced other dietary leads undergoing clinical development as anti-inflammatory agents (luteoline, quercetine, salicilic acid,

curcumine) (Appendino and Taglialatela-Scafati, 2003c), while the sesquiterpene dialdehydes polygodial (**20**), the alkaloid piperine (**21**) and the prenylated phenol scutigeral (**22**) are examples of non-capsaicinoid TRPV1 activators of dietary origin (Sterner and Szallasi, 1999).

If food plants contain such a wealth of biologically active compounds, then there is also a flip side of the coin, related to the poverty in plant diversity of modern diet. Western diet is based on few domesticated plants bred for yield and succulence rather than biochemistry variety, and by adopting it, we are 'missing something'. Our diet is pharmacologically impoverished, being deprived of a host of 'quasi-vitamins' that our physiology has evolved to make us of. Cheap fats-and-sugars rich food, large portions, and persuasive advertisement have changed our diet, in a way whose impact on health is impossible to study in any kind of rigorous controlled scientific trial, but nevertheless worth considering. We are living in a toxic food environment, and, if nothing is done against the fast-food culture that sweeps youth '*for the first time in a hundred years, life expectancy will actually go down*' (Krebs, 2003). Knowledge about food plants is disappearing rapidly, and local culinary traditions are rapidly becoming 'exotic'. We do not need to travel to the tropic to see a massive loss of biodiversity, a look into our dish will probably suffice.

Acknowledgments

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