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Anti-Inflammatory Compounds of Plant Origin. Part I. Action on Arachidonic Acid Pathway, Nitric Oxide and Nuclear Factor κ B (NF- κ B)

Abstract

Over the last 10 years, a significant body of evidence has emerged indicating that chemically diverse classes of naturally-occurring substances derived from higher plants are of potential interest for therapeutic interventions in several inflammatory diseases. Part I of this review article focuses on our current knowledge of the mechanisms by which a large range of plant-derived constituents interfere with three relevant targets involved in the inflammatory process, namely arachidonic acid metabolite pathways, nitric oxide and NF- κ B, and discusses their potential therapeutic use in the management of relevant inflammatory diseases.

Key words

Medicinal plants · plant constituents · inflammation

Abbreviations

AP-1: activator protein-1
CAPE: caffeic acid phenethyl ester

COX: cyclooxygenase
ELAM-1: endothelial-leukocyte adhesion molecule-1
ICAM-1: intercellular adhesion molecule-1
IL: interleukin
iNOS: inducible nitric oxide synthase
LO: lipoxygenase
LPS: lipopolysaccharide
LT: leukotriene
MPO: myeloperoxidase
NF- κ B: nuclear factor kappa B
NO: nitric oxide
PGE₂: prostaglandin E₂
TXA₂: thromboxane A₂
PLA₂: phospholipase A₂
PKC: protein kinase C
PMA/TPA: phorbol myristate acetate
TGF- β 1: transforming growth factor- β 1
TNF- α : tumour necrosis factor
CAM-1: vascular cell adhesion molecule-1

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Introduction

Inflammation is the response of the organism to invasion by a foreign body, such as bacteria, parasites and viruses. In this context, the inflammatory response is a critical protective reaction to irritation, injury, or infection, characterised by redness, heat, swelling, loss of function and pain [1]. Redness and heat result from an increase in blood flow, swelling is associated with increased vascular permeability, and pain is the consequence of activation and sensitisation of primary afferent nerve fibres [1]. Under normal conditions, these changes in inflamed tissue serve to isolate the effects of the insult and thereby limit the threat to the organism [1].

Our understanding of the molecular and cellular mechanisms involved in the inflammatory process has increased dramatically in recent decades and this has permitted the discovery of many promising targets for the development of new drugs to treat chronic inflammatory diseases, such as rheumatoid arthritis, allergy, asthma, inflammatory bowel disease, and others. A great number of inflammatory mediators including kinins, platelet-activating factor, prostaglandins, leukotrienes, amines, purines, cytokines, chemokines and adhesion molecules, has been found to act on specific targets (e. g., the microvasculature), leading to the local release of other mediators from leukocytes (e. g., mast cells and basophils) and the further attraction of leukocytes, such as neutrophils, to the site of inflammation [1].

A continuous flow of new findings from immunohistochemical, biochemical, molecular and functional animal models, together with clinical studies, has greatly increased the interest in the study of the mechanisms that underlie the inflammatory process. In recent years, roles have been identified for several inflammatory cells and for a large number of inflammatory mediators in important pathologies not previously known to be linked to inflammation, such as Alzheimer's disease and cardiovascular disorders including atherosclerosis, as well as cancer (see for recent review [2], [3]). Such findings have significantly increased the importance of, first, understanding the cellular and molecular mechanisms forming the basis of the inflammatory process and, second, identifying new targets for the development of innovative and safe therapeutic strategies to manage inflammatory diseases.

Natural products, including those derived from higher plants have, over the years contributed greatly to the development of modern therapeutic drugs. Most plant-derived secondary metabolites are known to interfere directly or indirectly with the following molecules and/or mechanisms: various inflammatory mediators (e. g. arachidonic acid metabolites, peptides, cytokines, excitatory amino acids, etc.), the production and/or action of second messengers (such as cGMP, cAMP, various protein kinases, and calcium, among others), the expression of transcription factors such as AP-1, NF- κ B, and proto-oncogenes (*c-jun*, *c-fos*, and *c-myc*), and the expression of key pro-inflammatory molecules such as inducible NO synthase (iNOS), cyclooxygenase (COX-2), cytokines (IL-1 β , TNF- α , etc.), neuropeptides and proteases.

In the first part of this review we highlight recent contributions of naturally occurring substances from higher plants acting on

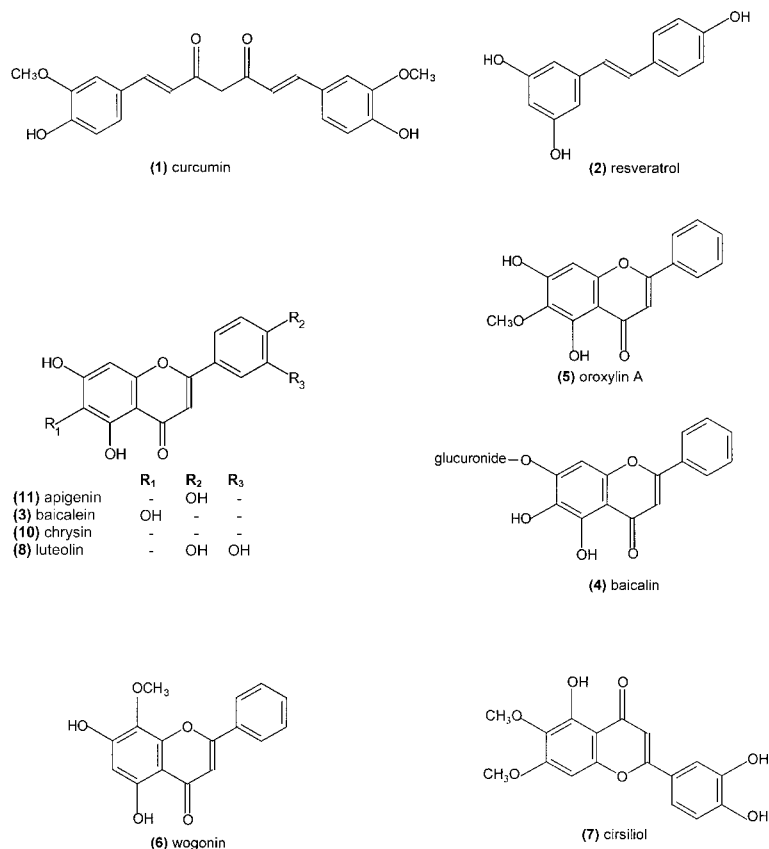
the arachidonic acid pathway, nitric oxide and the transcription factor NF- κ B, to the development of new drugs to treat acute and chronic inflammatory processes.

Prostanoid Inhibitors Derived from Plants

Curcumin (**1**) is a naturally-occurring yellow pigment present in the rhizomes of the plant *Curcuma longa* L. (Zingiberaceae), found in southern Asia. Apart from its use as a colouring and flavouring spice in foods, curcumin has also been widely used in the management of distinct inflammatory disorders and wound healing. It has been reported that curcumin possesses antioxidant, hepatoprotective and antitumoural actions [4], [5], [6], [7], [8], [9], [10], [11], [12], [13]. More recently, there have been a number of pre-clinical reports suggesting that curcumin exerts an anti-inflammatory action in models of atherosclerosis, Alzheimer's disease, arthritis and pancreatitis [6], [7], [8], [9], [10]. Several mechanisms have been proposed to explain the reported anti-inflammatory action of curcumin, including the inhibition of macrophage activation, and inhibition of lipoxygenase (LO) and cyclooxygenase 2 (COX-2), metabolite production via arachidonic acid pathways [11], [12], [13], [14], [15]. For a recent review of the anti-inflammatory action of curcumin see [6], [16].

Resveratrol (**2**) is a phytoalexin polyphenol present in grape skins and, consequently, red wines, and most other plants, and it is known to have a wide range of biological actions, including anti-allergic, anti-inflammatory, antioxidant, anticarcinogenic and antiplatelet activities [17], [18], [19], [20], [21]. In relation to its anti-inflammatory actions, resveratrol has been found to inhibit both COX-1 and COX-2 enzymes and prostanoid product generation via the LO pathway. Furthermore, resveratrol inhibits apoptosis in K562 cells through a mechanism involving inhibition of both LO and COX activities [22], [23]. Recent molecular studies have revealed that resveratrol acts at a broad range of sites and this seems likely to account for its pharmacological properties. Among other effects, resveratrol causes a pronounced reduction in the *c-fos* and TGF- β 1 expression in mouse skin stimulated by phorbol myristate acetate (PMA) [24]. Furthermore, resveratrol inhibits COX-2 transcription and activity associated with inhibition of AP-1-mediated gene expression in PMA-treated mammary epithelial cells, by inhibiting signal transduction through protein kinase C (PKC) [25]. Although the precise mechanisms by which resveratrol exerts its effects are unknown, these findings may explain, at least in part, the basis by which resveratrol exerts its anti-inflammatory actions and its favourable effects in the treatment of cardiovascular diseases and cancer. For a recent review on the anti-inflammatory actions of resveratrol see [17], [18], [26].

Flavonoids are broadly distributed in plants and have been reported to display marked *in vitro* and *in vivo* anti-inflammatory properties (see for recent reviews [27], [28]). The flavonoid baicalin (**3**), isolated from the roots of *Scutellaria baicalensis* Georgi (Lamiaceae) has been shown to selectively inhibit 5-LO and also LTC₄ (IC₅₀ = 9.5 μ M) production in rat resident peritoneal macrophages [29]. Oral application of baicalin (but not baicalin and wogonin) reportedly ameliorates several inflammatory symptoms of experimental colitis, such as body-weight loss, low blood

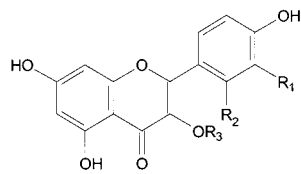


haemoglobin content and rectal bleeding [30]. Topical application of baicalin inhibits TPA-induced ear oedema as well as ornithine decarboxylase and myeloperoxidase activity in mouse skin, suggesting its potential as an anti-cancer agent [31]. In addition, the three flavonoids baicalein, baicalin and wogonin exhibit anti-oedematogenic properties in rats [32]. Furthermore, baicalin (4) inhibits the production of prostaglandin E₂ in C6 rat glioma cells, and leukotriene B₄ (LTB₄) biosynthesis [29, 33], while oroxylin A (5), baicalein and wogonin (6) inhibit 12-LO without affecting the levels of cyclooxygenase in human platelets [34].

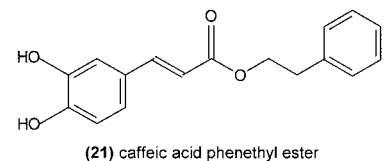
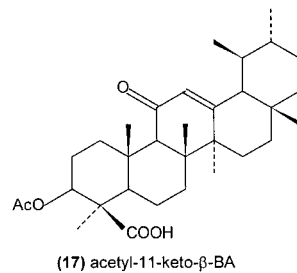
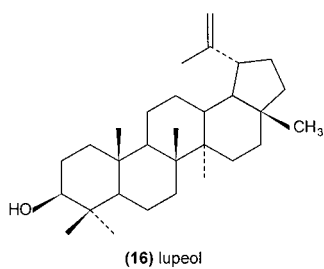
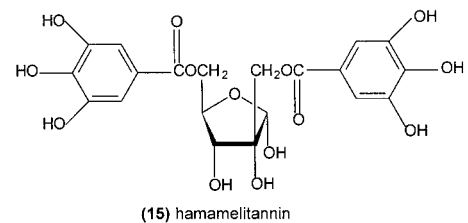
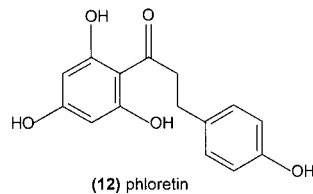
Another flavonoid compound, cirsiol (7) isolated from *Achillea fragrantissima* Forssk. (Asteraceae) has proven to be a potent inhibitor of 5-LO (IC₅₀ = 0.1 μM) in rat basophilic leukaemia cells and in guinea pig peritoneal polymorphonuclear leukocytes [35]. In addition, the oral administration of cirsiol to mice caused a dose-dependent inhibition of the paw oedema and leukocyte infiltration induced by injection of carrageenan [36]. Other plant-derived flavonoids, including luteolin (8) and morin (9), also exhibit moderate inhibition of the rat renal medulla COX-2 activity. There is evidence indicating that the flavones chrysin (10), apigenin (11) and phloretin (12) are effective in preventing COX-2 expression [37] and in inhibiting platelet aggregation [38]. Fiebrich and Koch [39] showed that the three pharmacologically active constituents denoted silymarin, namely, silybin, silydian and silychristin, from *Silybum marianum* L. (milk thistle) (Asteraceae) also inhibit both LO and COX activity. More recently, it has been reported that these compounds are capable of inhibiting the inflammatory response when assessed in several *in vivo* models, most probably through the inhibition of COX-2 expression [40], [41]. Likewise, the flavonoids rutin (13), quercetin (14), wogonin,

apigenin, galanin, morin and naringenin also inhibit COX-2 expression and activity in macrophages [37], [42], [43], [44]. Hamamelittannin (15) and the galloylated proanthocyanidins isolated from *Hamamelis virginiana* L. (Hamamelidaceae) exhibit pronounced inhibition of 5-LO (IC₅₀ values ranging from 1.0 to 18.7 μM) [45]. It has been recently reported that ginkgetin, a biflavone from *Ginkgo biloba* L. (Ginkgoaceae) leaves is effective in inhibiting phospholipase A₂ and reduces arthritic inflammation in rat adjuvant-induced arthritis as well as the abdominal constriction caused by acetic acid (ID₅₀ = 8.9 mg/kg) [46]. In addition, ginkgetin and the biflavone mixture inhibited croton oil-induced ear skin oedema by down-regulation of COX-2 [47]. The isoflavones tectorigenin and tectoridin (a glycosylated tectorigenin) isolated from the rhizomes of *Belamcanda chinensis* L. (Iridaceae) inhibit PGE₂ production in TPA-stimulated rat peritoneal macrophages [48], [49]. Very recently, Sadik et al. [50] have described the inhibition of 15-lipoxygenase by a series of flavonoids and have confirmed the great potency of luteolin and baicalein.

Sala et al. [51], [52], [53] have isolated several acetophenones from aerial parts of *Helichrysum italicum* (Roth) G. (Asteraceae) that were found to inhibit arachidonic acid metabolism. 4-Hydroxy-3-(3-methyl-2-butenyl)acetophenone and 4-hydroxy-3-(2-hydroxyl-3-isopentenyl)acetophenone caused a concentration-dependent inhibition of LTB₄ production in calcium ionophore-stimulated leukocytes with IC₅₀ of 24 and 111 μM, respectively. Some of these compounds also consistently inhibited myeloperoxidase (MPO) activity and showed *in vivo* anti-inflammatory activity against carrageenan and PLA₂-induced mouse paw oedema [52]. Tiliroside had the higher anti-inflammatory activity of three flavonoids (with gnaphaliin and pinocembrin)



	R ₁	R ₂	R ₃
(9) morin	-	OH	-
(13) rutin	OH	-	Glu-Rha
(14) quercetin	OH	-	-



isolated from *H. italicum* (Roth) G. and tested in several animals, inhibiting the mouse paw oedema induced by PLA₂ (ID₅₀ = 35.6 mg/kg) and the mouse ear inflammation induced by TPA (ID₅₀ = 357 μg/ear) as well as enzymatic and non-enzymatic lipid peroxidation (IC₅₀ = 12.6 and 28 μM, respectively) [53].

Pentacyclic triterpenes constitute a class of naturally occurring substances that are widely distributed in plants and possess a broad range of biological actions, including pronounced anti-inflammatory effects, as confirmed by *in vivo* and *in vitro* experiments. These effects appear to be related to interference with key components of the inflammatory response. As a comprehensive review on the anti-inflammatory actions of pentacyclic triterpenes has been recently published [54], we will focus here only on the more recent and relevant findings regarding this topic.

Topical application of the lupane pentacyclic triterpene lupeol (16) inhibits TPA-induced ear oedema and myeloperoxidase activity in mice. These effects seem likely to involve decreased prostanoid production, because lupeol prevents PGE₂ generation in macrophages without affecting the levels of LTC₄ [55]. Suh et al. [56] screened more than 80 pentacyclic triterpenes and found that most of them prevented COX-2 and iNOS expression in RAW 264.7 macrophages. It has been recently reported that furanoligularenone, an eremophilane isolated from *Ligularia fischeri* var. *spiciformis* (Compositae), inhibits both the production of PGE₂ (IC₅₀ = 1.93 μM) and expression of COX-2 in RAW 264.7 stimulated by LPS [57].

The gum resin of *Boswellia serrata* Roxb. (Burseraceae) is reported to be beneficial in the treatment of patients with chronic colitis. Its positive effects may be, at least in part, related to the presence of the pentacyclic triterpene boswellic acid [58]. Boswellic acid has been found in *Boswellia carterii* Birdw. (Burseraceae) and it partially but selectively inhibits 5-LO in ionophore-stimulated peritoneal polymorphonuclear leukocytes [59]. Acetyl-11-keto-β-boswellic acid (17) inhibits the 5-LO in rat neutrophils in a non-competitive and specific manner (IC₅₀ = 1.5 μM) [60]. Using several pentacyclic triterpene analogues, Sailer et al. [60]

have shown that the inhibition of LO by these compounds depends on the presence of the pentacyclic skeleton.

Avicins (18), a family of triterpenoid saponins isolated from *Acacia victoria* Benth (Leguminosae) inhibit the expression of COX-2 through inhibition of NF-κB [61]. In addition, saikosaponins, isolated from *Scrophularia scorodonia* L. (Scrophulariaceae) and *Bupleurum rigidum* L. (Apiaceae), as well as two tirucallane-type lanostanoids (masticaienonic acid and masticadienolic acid) and oleanane (morolic acid), from *Pistacia terebinthus* L. (Anacardiaceae), have anti-inflammatory effects *in vivo*. Thus, they prevented ear oedema formation caused by PMA and also the synthesis of LO products, especially LTC₄ and, to a lesser extent, COX metabolites derived from arachidonic acid [62], [63], [64]. Similarly, platycodin D isolated from the roots of *Platycodon grandiflorum* A. DC. (Campanulaceae) suppresses PGE₂ production and inhibits the induction of COX-2, but not COX-1, in rat peritoneal macrophages [65]. Diaz Lanza et al. [66] have demonstrated that three phenylpropanoids (salidroside and syringin) and lignan (phillyrin), isolated from the leaves of *Phillyrea latifolia* L. (Oleaceae), are capable of inhibiting production of the COX metabolite PGE₂ (IC₅₀ = 72.1, 35.5 and 45.6 μM, respectively) and, to a lesser extent, reducing TXB₂ levels (IC₅₀ = 154, 29.3 and 168 μM, respectively); however, coniferin inhibits both COX (PGE₂ [IC₅₀ = 75.2 μM] and TXB₂ [IC₅₀ = 619 μM]) and 5-LO metabolites, especially LTC₄ (IC₅₀ = 63.6 μM), in calcium-stimulated mouse peritoneal macrophages and human platelets [66]. It has been recently shown that lignans, such as diphyllin acetylapioside, an aryl naphthalene-type, isolated from *Haplophyllum hispanicum* Spach. (Rutaceae) exhibit topical anti-inflammatory activity through inhibition of 5-lipoxygenase [67].

Ursolic acid (19) and the oleanic acid (20) isomers extracted from *Plantago major* L. (Plantaginaceae), have also been shown to selectively inhibit the COX-2 enzyme, although their mean IC₅₀ values are considerably high (130 and 295 μM) [68]. Ursolic acid also exhibits anti-inflammatory properties [69], [70]. However, Suh et al. [56] did not find any inhibition of COX-2 by either of the triterpenes. Subbaramaiah et al. [71], in a very interesting and elegant study, explored further the molecular mechanisms

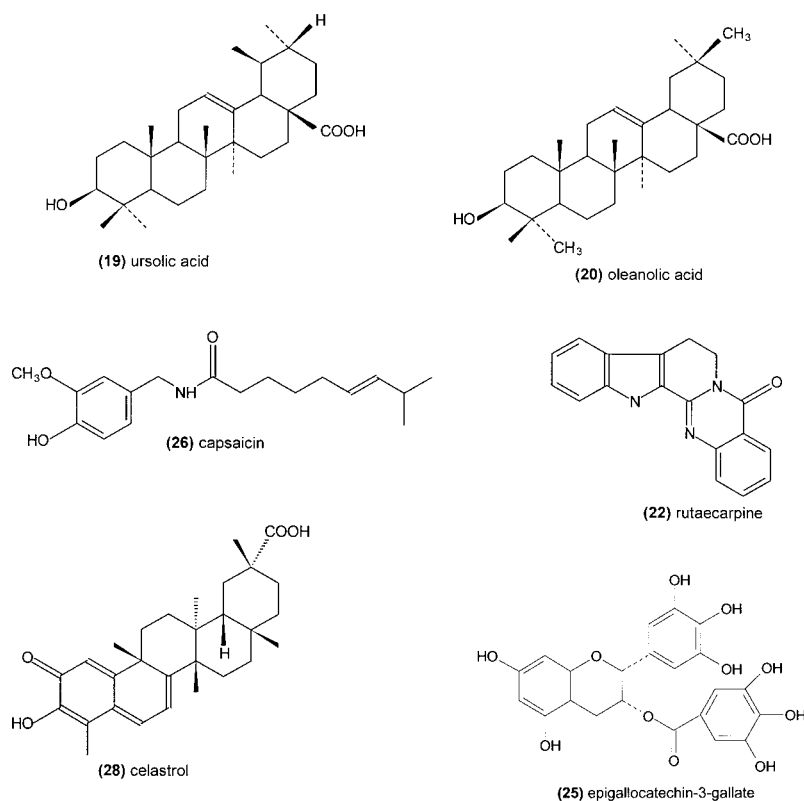
by which ursolic acid inhibits COX-2 in PMA-treated human mammary and oral epithelial cells, showing also that this is mediated by a cAMP response element in the COX-2 promoter, associated with inhibition of protein kinases [71]. The synthetic oleane triterpene 2-cyano-3,12-dioxolean-1,9-dien-28-oic acid also inhibits COX-2 expression and shows potent antiproliferative and anti-inflammatory activity [72].

β -Turmerone and α -turmerone are sesquiterpenes from *Curcuma zedoaria* L. (Zingiberaceae) that inhibit LPS-induced PGE₂ production in RAW 264.7 cells [73]. It has been demonstrated that several sesquiterpenoids, including ilicic acid and inuviscolide, isolated from *Inula viscosa* Ait. (Asteraceae) possess anti-inflammatory activity *in vivo*, being capable of preventing the ear oedema caused by TPA (ID₅₀ = 0.65 μ mol/ear), while also inhibiting the synthesis of LTB₄ (IC₅₀ = 94 μ M) in rat peritoneal neutrophils [74]. Rosas-Romero et al. [75] have demonstrated that two sesquiterpene lactones, 9 β -hydroxy-atrapiolicolide 8-O-tiglate and 9 β -hydroxyatrapiolicolide 8-O-(5'-acetoxytiglate) from *Lourteigia ballotaefolia* (H.B.K.) (Asteraceae) inhibit the ear oedema caused by croton oil in mice. Likewise, a new iridoid, pendunculariside and agnuside, from the stem bark of *Vitex penduncularis* (Verbenaceae) showed preferential inhibition of COX-2 (IC₅₀ = 0.15 and 0.026 mg/mL, respectively), while having only a small inhibitory effect on COX-1 activity [76].

Several fatty acids extracted from *Plantago major* L. (Plantaginaceae), including linoleic acid, myristic acid, palmitic acid and stearic acid, have been found to inhibit both COX-1 and COX-2 (IC₅₀ = 3.9 to 180 μ M) activities. However, they lack selectivity against these enzymes [77]. The phenolic antioxidant substance caffeic acid phenethyl ester (CAPE) (**21**), a compound produced by honeybees from the gum of various plants, has

been shown to possess anti-inflammatory, anticarcinogenic, antimitogenic and immunomodulatory properties. Evidence now indicates that CAPE inhibits the activity of both COX-1 and COX-2 (IC₅₀ of 58 and 82 μ M, respectively) along with COX-2 expression in human oral epithelial cells [78]. When tested in a rat carrageenan air pouch model of inflammation CAPE, administered systemically, caused dose-dependent inhibition of PGE₂ formation in the exudate with complete inhibition at 100 mg/kg (mean ID₅₀ of 23 mg/kg). In addition, CAPE markedly reduced the amount of COX-2 in the pouch, while indomethacin, a non-specific COX inhibitor, only inhibited the PGE₂ production [79].

There is evidence showing that some phenols, including 5,7,5'-trihydroxy-3,6,2',4'-tetramethoxyflavone, scopoletin and centaureidin from *Eupatorium buniifolium* H. et A. (Asteraceae) [80], as well as some acetylenes from the rhizomes of *Atractylodes lancea* DC. (Asteraceae), have anti-inflammatory actions. They prevented ear oedema caused by TPA and inhibited COX-1 and 5-LO activities [81]. Danz et al. [82], showed that the alkaloid tryptanthrin from *Isatis tinctoria* L. (Brassicaceae), reduces inflammation by blocking the production of several metabolites of arachidonic acid TXB₂ and 6-ketoPGF_{1 α} , as well as by decreasing the activity of both COX in monocytes Mac 6 and RAW 264.7 cells. In addition, tryptanthrin also reduces the production of 5-LO metabolites, especially LTB₄, in calcium-stimulated human neutrophils [82]. The quinazolinocarboline alkaloid rutaecarpine (**22**) from *Evodia rutaecarpa* Benham (Rutaceae) was reported to inhibit LPS-induced PGE₂ production in RAW 264.7 cells [83]. This compound also reduced the rat paw oedema induced by carrageenan and the conversion of exogenous arachidonic acid to PGE₂ in COX-2 transfected HEK293 cells. However, rutaecarpine did not change PLA₂ and



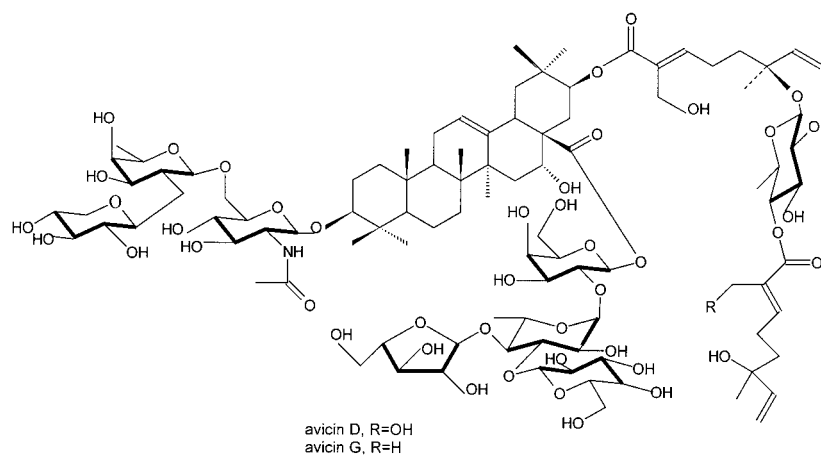
COX-1 activities nor COX-2 expression in bone marrow-derived mast cells [84]. It has been shown that rutaecarpine reduces human platelet aggregation probably by the initial inhibition of phosphoinositide breakdown, followed by inhibition of TXA₂ formation as well as [Ca²⁺]_i mobilization [85]. More recently, Sheu et al. [86] have shown that rutaecarpine has an antithrombotic effect in mice against ADP-induced acute pulmonary thrombosis and fluorescein sodium-induced platelet thrombus in mesenteric microvessels, as well as affecting haemostatic bleeding time in rat mesenteric arteries. Furthermore, rutaecarpine exerts vasodilatory and hypotensive effects in the rat by activating the endothelial Ca²⁺-nitric oxide-cGMP pathway, as well as exerting a minor direct inhibition of membrane Ca²⁺ channels and intracellular Ca²⁺ release in vascular smooth muscle cells [87]. Kobayashi et al. [88] described positive inotropic and chronotropic effects elicited by rutaecarpine on the guinea-pig isolated right atria, via interaction with vanilloid receptors and release of the neuropeptide CGRP. Recently, Hu et al. [89], [90], [91] have further extended these observations indicating that cardioprotective, depressor and vasodilator effects of rutaecarpine are associated with stimulation of endogenous CGRP release via activation of vanilloid receptors.

Finally, some standardised plant extracts currently used in most countries for the management of various pathologies are known to interfere with prostanoid synthesis, mainly via inhibition of COX and LO activities. Examples of plants producing such extracts are: *Echinacea purpurea* L. (Asteraceae), *Hamamelis virginiana* L. (Hamamelidaceae), *Tanacetum vulgare* L. (Asteraceae), *Urtica dioica* L. (Urticaceae), *Rheum palmatum* L. (Polygonaceae), *Arnica montana* L. (Asteraceae), *Allium sativum* L.

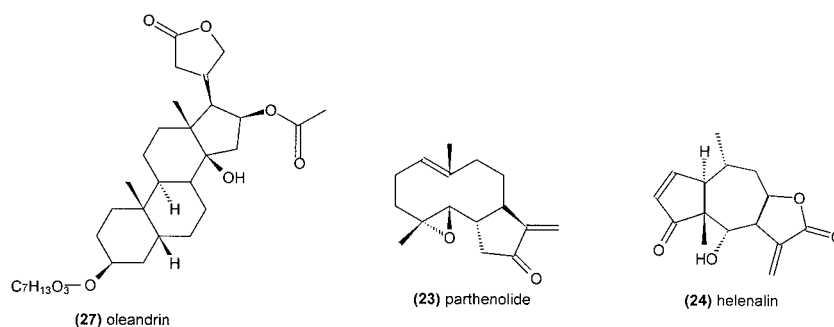
(Liliaceae), *Heterotheca inuloides* Cass. (Asteraceae), *Angelica sinensis* (Oliv.) Diels (Umbelliferae) and *Artemisia species* (Asteraceae) [92], [93], [94], [95], [96], [97], (for review see [98]).

Plant-Derived Constituents that Interfere with the Nuclear Transcription Factor κ B (NF- κ B)

NF- κ B is a ubiquitous and well-characterised protein responsible for the regulation of complex phenomena, with a pivotal role in controlling cell signalling in the body under certain physiological and pathological conditions. Among other functions, NF- κ B controls the expression of genes encoding the pro-inflammatory cytokines (e.g., IL-1, IL-2, IL-6, TNF- α , etc.), chemokines (e.g., IL-8, MIP-1 α , MCP1, RANTES, eotaxin, etc.), adhesion molecules (e.g., ICAM, VCAM, E-selectin), inducible enzymes (COX-2 and iNOS), growth factors, some of the acute phase proteins, and immune receptors, all of which play a critical role in controlling most inflammatory processes (for reviews, see [99], [100]). In unstimulated cells, NF- κ B is present in the cytoplasm as an inactive heterodimer composed of two sub-units, p50 and p65 (also called I κ B). The heterodimer is complexed with an inhibitory protein I κ B- α , preventing it from moving into the cell nucleus. When activated by certain inflammatory agents, specific protein kinases phosphorylate the I κ B- α protein, causing its rapid degradation, and NF- κ B becomes dissociated from I κ B- α . Phosphorylated I κ B is then rapidly degraded by the proteasome, leading to the translation of NF- κ B to the nucleus, where it binds to specific DNA sequences present in the promoters of numerous target genes [99], [100].



(18) avicins



Since NF- κ B represents an important and very attractive therapeutic target for drugs to treat many inflammatory diseases, including arthritis, asthma, and the auto-immune diseases, most attention has been paid in the last decade to the identification of compounds that selectively interfere with this pathway. More recently, a great number of plant-derived substances has been evaluated as possible inhibitors of the NF- κ B pathway. Interesting and comprehensive reviews published recently discuss in detail some specific groups of naturally-occurring substances of plant origin capable of interfering with NF- κ B [101], [102]. Thus, we will discuss in this section distinct classes of plant-derived compounds that form part of this group, emphasising recently published data.

The NF- κ B pathway is affected by several distinct classes of plant-derived compounds including: curcumin (**1**), parthenolide (**23**), ergolide, 2 β ,5-epoxy-5,10-dihydroxy-6 α -angeloyloxy-9 β -isobutyloxy-germacran-8 α ,12-olide, andalusol, ent-kaur-16-ene-19-oic acid, kamebanin, kamebacetal A, kamebakaurin, exvisanin A, hypoestoxide, resveratrol (**2**), helenalin (**24**), pristimerin, epigallocatechin gallate (**25**), avicin (**18**), quercetin (**14**), capsaicin (**26**), silymarin, the diterpene hypostoxide, oleandrin (**27**), vesnarinone and caffeic acid phenethyl ester (CAPE) (**21**). The majority of these compounds are anti-oxidants and act by blocking the protein kinase-mediated I κ B degradation, thereby preventing NF- κ B activation.

Recently, Gukovsky et al. [10] reported that curcumin ameliorates ethanol- and non-ethanol-induced experimental pancreatitis (causing pronounced inhibition of the serum amylase and lipase increase, reduction of neutrophil influx and inhibition of intrapancreatic trypsin activation). They found that the inhibition of NF- κ B and AP-1, another important pro-inflammatory transcription factor, is largely responsible for this effect. The authors concluded that curcumin, which is currently in clinical trials for cancer prevention, has therapeutic potential for the management of pancreatitis. Jobin et al. [103] demonstrated that curcumin blocks cytokine-induced pro-inflammatory gene expression in intestinal epithelial cells by inhibition of the signal leading to IKK activation, subsequent I κ B phosphorylation/degradation, and NF- κ B activation. Furthermore, evidence also indicates that curcumin is able to inhibit the I κ B kinase 1 and I κ B kinase 2 activities induced after stimulation with LPS [104]. Very recently, Ukil et al. [105] shown that curcumin exerts beneficial effects on 2,4,6-trinitrobenzenesulphonic acid-induced colitis in mice, a model for inflammatory bowel disease. The anti-inflammatory effects of curcumin are associated with an inhibition of up-regulation of the pro-inflammatory Th1 cytokine response leading to the suppression of iNOS and attenuation of the recruitment of neutrophils and lipid peroxidation that in turn reduce tissue injury. The authors suggested that curcumin may be useful in the management of human inflammatory bowel disease [105].

Recent results suggest that silymarin inhibits nitric oxide production and iNOS gene expression by inhibiting NF- κ B/Rel activation in RAW 264.7 macrophages. Furthermore, the radical-scavenging activity of silymarin may explain its inhibitory effect on NF- κ B/Rel activation [106]. In a recent study, Dhanalakshmi et al. [107] revealed that silibinin (a component of silymarin) effectively inhibits constitutive activation of NF- κ B in DU145 human

prostate adenocarcinoma cells. The flavonoid apigenin (**11**) was shown to down-modulate the constitutive expression of NF- κ B/p65 in the human prostate adenocarcinoma cell line LNCaP. Such data suggest that apigenin has strong potential for the development of agents to prevent prostate cancer [108].

Parthenolide is a sesquiterpene lactone used in folk medicine for its anti-inflammatory and analgesic properties. Several *in vitro* studies have shown that a great part of the anti-inflammatory action of this compound appears to be related to its ability to inhibit the NF- κ B pathway. Furthermore, parthenolide has recently been reported to exert beneficial effects during endotoxic shock in rats through inhibition of NF- κ B DNA binding in the lung [109]. Kamebakaurin (a kaurane diterpene) also prevents the activation of NF- κ B by different stimuli in various cell types by directly targeting the DNA-binding activity of p50 [110], [111]. A novel triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid inhibits NF- κ B-mediated gene expression at some stage after translocation of activated NF- κ B to the nucleus in ML-1 leukaemia cells [112].

The antioxidant compound caffeic acid phenethyl ester (CAPE), an anti-carcinogenic, anti-inflammatory and immunomodulatory substance present in *Apis mellifera* propolis, is also reported to prevent activation of the NF- κ B pathway by a wide variety of inflammatory agents including TNF- α , phorbol ester, okadaic acid and H₂O₂ [79]. The action of CAPE is selective for the NF- κ B pathway since other transcription factors were not affected. An *in vivo* study has revealed that CAPE also inhibits formation of the neointima by inhibiting NF- κ B activation [79]. The mechanism by which CAPE inhibits the NF- κ B pathway appears to be related to its ability to suppress the interaction of NF- κ B with DNA, without affecting I κ B degradation [79]. These findings further support the reported anti-inflammatory and anticancer properties of CAPE and could certainly account for the anti-inflammatory and immunomodulatory effects described for propolis extract (see for review [113]). Because NF- κ B plays a central role in most disease processes, and since it can regulate the expression of many key genes involved in inflammatory as well as in a variety of human cancers [100], [101], [102], it represents a relevant and promising target for the development of new therapeutic agents. However, the ubiquitous nature of NF- κ B suggests that such drugs would exhibit some undesirable side effects.

Plant-Derived Constituents that Interfere with the Nitric Oxide Pathway

Nitric oxide (NO) is synthesised by many cell types involved in immune and inflammatory responses. The principal enzyme involved is the inducible (type-2) isoform of nitric oxide synthase (NOS-2 or iNOS) which produces high-level sustained NO synthesis. Inducible NOS is expressed after stimulation by, for example, cytokines, LPS and calcium ionophore [114]. In chronic inflammation (arthritis, gastritis, inflammatory bowel disease) the inhibition of NO release is beneficial. Since nitric oxide production by iNOS occurs several steps downstream of immune stimulation, it can be regulated at many levels including transcription, translation and post-translational modification [114]. A number of medicinal plants and their isolated compounds

have been screened for their ability to inhibit inducible NOS activity and expression.

Resveratrol (**2**) has been shown to suppress the iNOS expression and NO production in cultured cells by down-regulation of NF- κ B binding activity via blockade of I κ B α degradation [115], [116]. Structure-activity analysis of resveratrol and nine hydroxystilbenes suggests that the structural balance between oxygen functional groups on the benzene rings is important for inhibition of nitric oxide production in RAW 264.7 and J774 macrophage cells [117].

Curcumin (**1**) possesses anti-inflammatory activity and is a potent inhibitor of reactive oxygen species-generating enzymes such as LO/COX, xanthine dehydrogenase/oxidase and iNOS. Thus, curcumin blocks NO production and NOS activity and expression in macrophages [118] and in pancreatitis tissue of rats [10].

Recently, it has been demonstrated that silymarin reduces NO production and iNOS gene expression in macrophages, and attenuates nitric oxide production by peritoneal macrophages in LPS-treated mice, by inhibiting NF κ B/Rel activation [106].

Several other phenolic compounds decrease NO production, namely, wogonin (**6**), baicalein (**3**), baicalin (**4**) [119], quercetin (**14**) [116], broussonchalcone A [120], apigenin (**11**), luteolin (**8**) [121], morusin, kuwanon C, sanggenon D, bilobetin, ginkgetin, echinisoiflavanone, genistein, daidzein, glycitein, theaflavin 3,3'-digallate, and epigallocatechin 3-gallate (**25**) [122], [123].

Treatment with parthenolide (**23**) or isohelenin appears to reduce NO production and iNOS mRNA expression in cultured rat aortic smooth muscle cells treated with LPS and interferon-gamma by a mechanism involving stabilisation of the I κ B α /NF κ B complex [124]. Furthermore, the increase in iNOS expression caused by TPA is effectively suppressed by parthenolide in the human monocyte cell line THP-1 [124]. Other terpenes have been evaluated as inhibitors of NO production, such as yakuchinones A and B [125], celastrol (**28**) [126], avicins (**18**) (a family of triterpenoid saponins) [61], kaurane [110], prunioside [127], hypostoxide [128], ergolide [129], costunolide [130], pimobendan [131], andrographolide [132], auranofin [133], pristimerin [134], dehydrocostus lactone [135], alpha-terpinene [136], anthraquinone [137] and furanologularenone [57].

Conclusion

An overview of the recent literature reveals that a great number of plant-derived constituents exhibit a pleiotropic spectrum of anti-inflammatory actions. Although most, if not all of the compounds are still in the pre-clinical phase, pharmacological and molecular studies have revealed that different chemical classes of plant-derived substances show promising anti-inflammatory activity by interacting with important cellular targets, mainly by controlling the gene expression of relevant key pro-inflammatory substances involved in the genesis and maintenance of inflammation. It has become apparent that plant-derived compounds with anti-inflammatory actions represent a very active area of research. Thus, further investigation in this area may lead to the development of efficacious and safer drugs for the

management of inflammatory processes, including new drugs that can prevent or delay the onset of some of the increasingly prevalent diseases of ageing such as rheumatoid arthritis, Alzheimer's disease and atherosclerosis.

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