Molecular Basis of the Anti-Inflammatory Effects of Terpenoids

B. de las Heras[1] and Sonsoles Hortelano[*,2]

[1]*Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain*

[2]*Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro 3, 28029 Madrid, Spain*

Abstract: Natural products play a significant role in human health in relation to the prevention and treatment of inflammatory conditions. Among them, terpenoids (also referred to as terpenes), are the largest and most widespread class of secondary metabolites. They are found in higher plants, mosses, liverworts, algae and lichens, and also in insects, microbes or marine organisms. Some terpenoids have been used for therapeutic purposes for centuries as antibacterial, anti-inflammatory, antitumoral agents, and in recent decades research activity into the clinical potential of this class of compounds has increased continuously as a source of pharmacologically interesting agents. In the present review, molecular basis of the anti-inflammatory action of diterpenoids is presented with special emphasis on their ability to modulate critical cell signaling pathways involved in the inflammatory response of the body such as nuclear transcription factor-kappaB (NF-κB) activation. NF-κB plays an important role in the regulation of immune and inflammatory responses. Indeed, deregulated NF-κB expression is a characteristic phenomenon in several inflammatory diseases and NF-κB has become a major target in drug discovery. Hence, this article also introduces our recently elucidated findings about the potential of labdane diterpenoids as anti-inflammatory agents due to their ability to inhibit NF-κB. The future development of this class of compounds as anti-inflammatory drugs requires the introduction of novel molecular targets of therapeutic relevance in addition to biotechnological approaches for the production of these molecules.

Keywords: Inflammation, innate immunity, terpenoids, NF-κB.

INTRODUCTION

Inflammation is the biological response of body to harmful stimuli, such as pathogens, physical injury or damaged cells. The first four cardinal signs of inflammation - redness (Latin *rubor*), heat (*calor*), swelling (*tumor*), and pain (*dolor*) - have been known since ancient times and were described by Celsus (1st century AD). The fith sign, the loss of function, was added by Virchow in the 19th century (1870). Redness is caused by the vasodilation in the affected area, heat reflects the increased blood flow, swelling or edema is caused by the accumulation of fluids and the pain results from the edema, and it is also induced by chemical mediators. Finally, loss of function may be a consequence of pain and swelling.

There are two fundamental types of inflammation: *acute* or *chronic*. *Acute inflammation* is a short-term process which is characterized by a vascular response and initial recruitment of polymorphonuclear granulocytes, typically neutrophils, followed by monocytes, which differentiate locally into macrophages. This leads to a coordinate activation of various signaling pathways that regulate expression of both pro- and anti- inflammatory mediators including chemokines, cytokines, vasoactive amines, eicosanoids and products of proteolytic cascades. Briefly, after a tissue is injured, there is an increase of blood flow into the area (vasodilation), following by an increased permeability of the capillaries that permit plasma proteins and leukocytes to leave the circulation (extravasation). Circulating leukocytes adhere to the endothelium *via* adhesion molecules, transmigrate across the endothelium, and migrate to reach the site of injury, where they remove the stimulus and repair the tissue.

Once inflammation has accomplished its function must be actively terminated to prevent unnecessary damage to tissues. Resolution of inflammation initiates in the first few hours after an inflammatory response begins and is mainly mediated by tissue-resident and recruited macrophages [1]. This active and coordinated program of resolution is controlled by endogenous 'pro-resolving' mediators such as lipoxins generated from arachidonic acid (AA), together with resolvins and protectins [2-4].

If the inflammatory process persists, chronic inflammation takes place. Indeed, chronic *inflammation* might be defined as a prolonged, dysregulated and maladaptive response that results in cellular destruction, and is associated with many chronic human conditions and diseases, including asthma, atherosclerosis, cancer, and autoimmune diseases. The hallmark of chronic inflammation is the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocyres, and plasma cells). These cells are recruited from the circulation by the steady release of chemotactic factors.

ROLE OF TRANSCRIPTION FACTOR NF-κB IN INFLAMMATION

One of the most important regulators of immune system and inflammatory response is the nuclear transcription factor κB (NF-κB) [5]. NF-κB is an ubiquitous transcription factor, evolutionarily conserved from flies to mammals [6]. NF-κB regulates the transcription of a number of genes involved in programmed cell death (apoptosis), cell adhesion, proliferation, immune and inflammatory pathways, cellular stress

*Address correspondence to this author at the Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro 3, 28029 Madrid, Spain; Tel: (34) 91-4531200; Fax: (34) 91-4531208;
E-mail: shortelano@cnic.es

1871-5281/09 $55.00+.00

Table 1. A List of Some Genes Regulated by NF-κB

Signaling Pathway	NF-κB Activated Genes
Programmed cell death (apoptosis)	TNF-receptor associated factors (TRAF) 1 and 2, inhibitor of apoptosis (IAP), Fas-ligand, CD95 (Fas), c-FLIP, Bcl-2, Bcl-xL
Cell adhesion molecules	E-selectin, intercellular adhesion molecule (ICAM-1), P-selectin, vascular cell adhesion molecule (VCAM-1)
Immune system and inflammatory pathways	Cytokines: IL1-α, IL-1β, lymphotoxin (LT)- α and β, TNF-α, interferons (IFN) β and γ Chemokines: eotaxin, Gro-1, macrophage chemotactic protein (MCP β), macrophage inflammatory proteins (MIP) 1β, 2, and 3α, regulated upon activation normal T lymphocyte expressed and secreted (RANTES), T-cell activation (TCA) gene 3
Stress response genes	Angiotensin II, cyclooxygenase-2 (COX-2), 12-lipoxygenase, inducible nitric oxide synthase (NOS-2), superoxide dismutase (Mn SOD)

response and tissue remodelling [7-13]. Table **1** shows a list of genes whose expression is regulated by NF-κB.

Dysregulation of NF-κB contributes to a variety of pathological conditions such as septic shock, acute inflammation, viral replication, and some malignancies [13, 14] (Table **2**). Hence, the extensive involvement of NF-κB activation in human inflammation and disease establishes this transcription factor as a relevant target for the pharmacological activity of anti-inflammatory molecules. Indeed, there is a growing interest in the search for molecules that inhibit activation of NF-κB.

In mammalian cells, there are five NF-κB family members, RelA/p65, RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). NF-κB is composed of homo- and heterodimers of different Rel family proteins but the most abundant and active forms of NF-κB in many cell types are dimeric complexes of p50/RelA (p50/p65). All NF-κB subunits share a Rel homology domain (RHD) of approximately 300 amino acids, that contains a nuclear localization sequence (NLS) and is involved in dimerization, sequence-specific DNA binding and interaction with the inhibitory IκB proteins [15]. p65, RelB, and c-Rel, are synthesized as active proteins, contain a terminal transactivation domain (TAD) at their C-terminal end which is required for the activation of transcription [16]. On the other hand, p50 and p52, are generated through a C-terminal proteolytic processing of longer precursors, NF-κB1 (p105) and NF-κB2 (p100) respectively and lack TAD which therefore means that they are transcriptionally inactive and they have to form hetero-complexes with other members of this family to transactivate defined target genes [16].

In unstimulated cells, NF-κB is sequestered in the cytoplasm by association with the inhibitory proteins IκB (IκBα, IκBβ, IκBε, IκBγ, Bcl3) that mask its NLS [7]. In response to an extracellular signal (e.g., inflammatory cytokines, mitogens, bacterial products, or oxidative stress), IκB is phosphorylated by IκB kinases (IKKs) at specific serine residues. The phosphorylation is followed by Lys^{48}-linked (Ub^{K48}) polyubiquitination of IκB, which marks the protein for degradation by the 26S proteasome [15]. Degradation of IκB allows the NF-κB to translocate to the nucleus, where it binds to the promoter region of various genes, including cytokines [*e.g.*, tumor necrosis factor (TNF)-α, interleukin (IL)-1β], cyclooxygenase-2 (COX-2), inducible nitric oxide-synthase (NOS-2), proteases [*e.g.*, matrix metalloproteases (MMPs)], and receptors involved in leukocyte adhesion and migration [15] (Fig. **1**).

Table 2. Human Pathologies Associated with NF-κB Dysregulation

System	Pathology
Immune system and inflammatory response	AIDS Arthritis Asthma Lupus Septic shock Viral infections Inflammatory bowl disease Crohn´s disease Cancer Multiple sclerosis Systemic inflammatory response syndrome
Cardiovascular system	Heart failure Ischemia/reperfusion Cardiar hypertrophy Atherosclerosis
Central nervous system (CNS)	Multiple sclerosis Alzheimer disease
Muscular system	Muscular distrophy
Skin	Incontinentia pigmenti
Renal system	Renal disease

Two major signaling pathways lead to activation of NF-κB, the classical or canonical and the alternative pathway (Fig. **2**). Both pathways converge on the degradation through the proteasome pathway of the inhibitory molecules, but are triggered by different stimuli, and signal through a different IKK complex. In the *classical canonical pathway*, various signals including proinflammatory cytokines such as TNF-α and IL-1β and activation of Toll-like receptors (TLRs) cause the activation of an IKK complex consisting of the catalitic subunits IKKα and IKKβ and the IKKγ regulatory subunit (also known as NF-κB essential modulator, NEMO) [7]. The activated IKK complex then phosphorylates IκB at 2

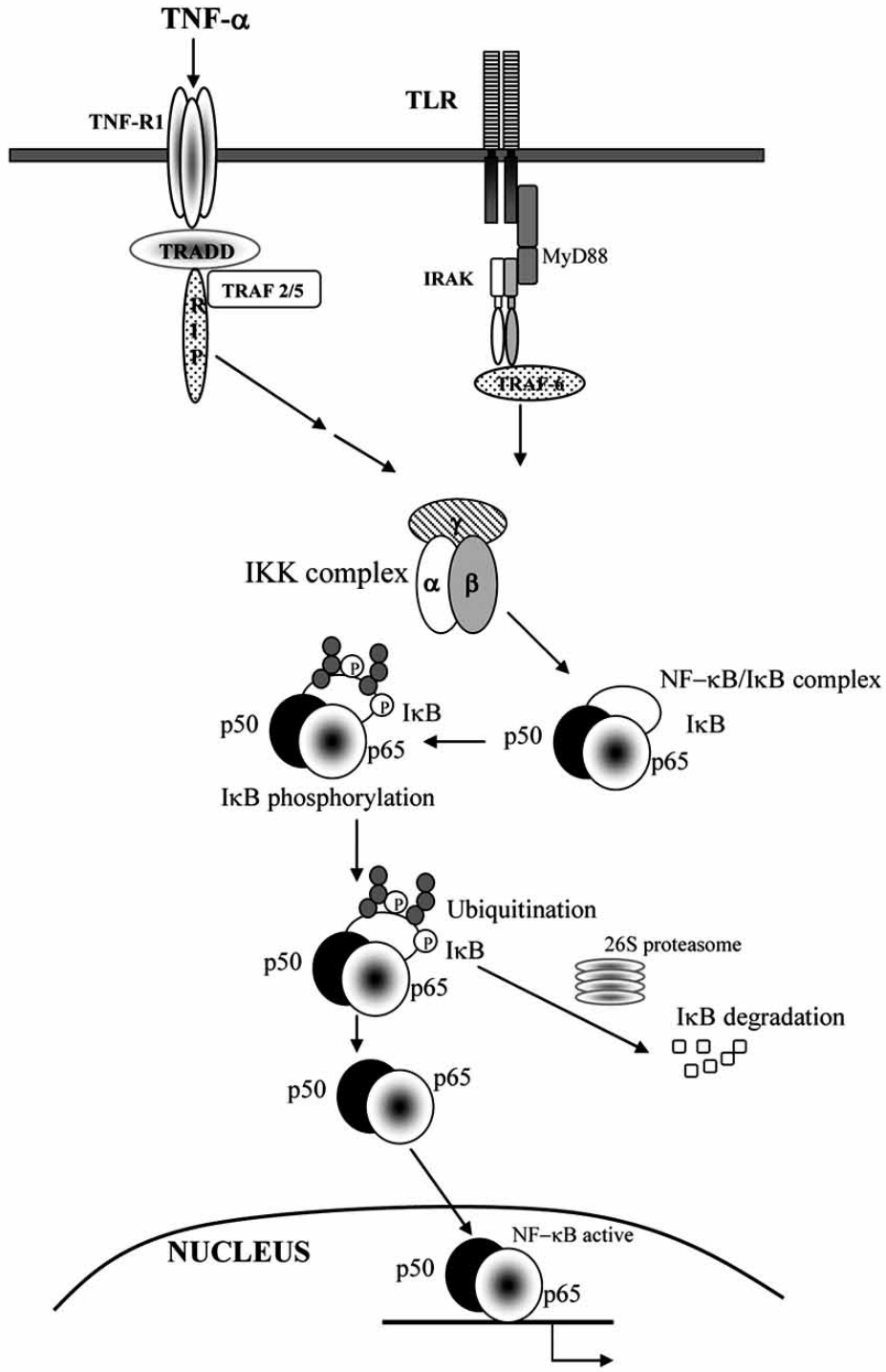

Fig. (1). Schematic overview of NF-κB signaling pathways.

Activation of NF-κB initiates with stimulation of different receptors (TLRs, TNFR1, IL-1R), leading to the recruitment of adaptor proteins, and activation of the IKK complex. IKK activation results in IκB phosphorylation, ubiquitination and degradation by the 26S proteasome. NF-κB translocates to the nucleus inducing the transcription of target genes. TLRs (Toll-like receptors), MyD88 (myeloid differentiation factor), Tollip (Toll-interacting protein), IRAK (interleukin-1R-associated kinase), TRAF6 (TNF-receptor associated proteins), MEKK (mitogen activated protein kinase).

N-terminal serines (Ser32 and Ser36 on IκBα, and Ser19 and Ser 23 on IκBβ), which signals it for ubiquitination and subsequent degradation by the 26S proteasome. The NF-κB released translocates to the nucleus, bind DNA and activates gene expression [15]. This pathway is crucial for the activation of innate immunity and inflammation as well as inhibition of apoptosis.

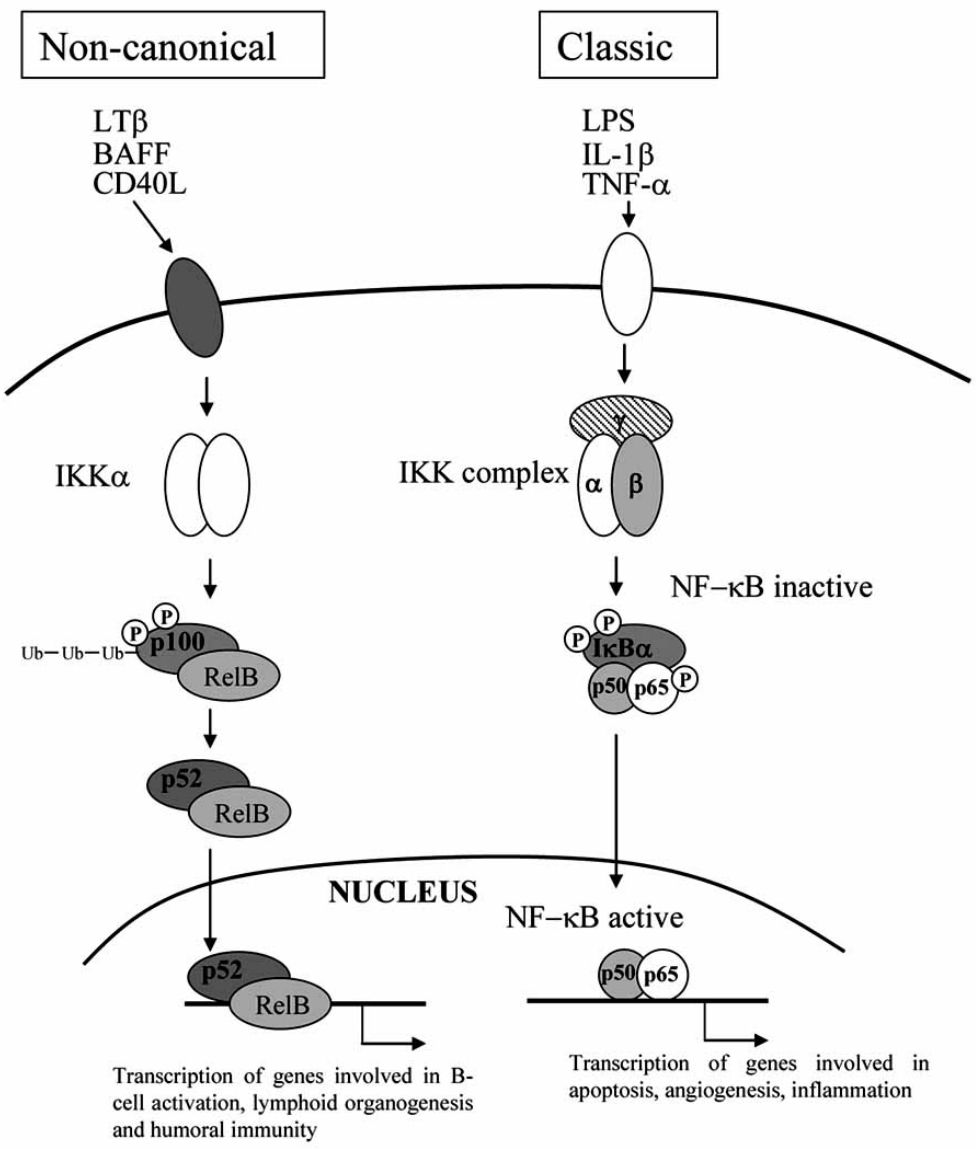

Fig. (2). Classical and alternative NF-κB pathways.
The classical pathway is induced by TNF-α, IL-1, ligands of TLRs such as LPS, etc, and results in IKK complex (IKKα,β,γ) activation. Activated IKK promotes phosphorylation of IκB, following by ubiquitination and degradation in the proteasome. Free NF-κB translocates to the nucleus and initiates the transcription of genes involved in angiogenesis, apoptosis and inflammation. In the alternative pathway, receptors such as BAFF, LTβ, CD40, signal through an IKKα complex, that mediate p100 phsophorylation and processing into p52. P52/RelB heterodimers move into the nucleus where they activate genes related to B-cell activation, lymphoid organogenesis and humoral immunity.

An *alternative pathway* that is involved in B-cell activation, lymphoid organogenesis, and humoral immunity, is activated by different stimuli, such as lymphotoxin-β (LTβ) [17], B-cell-activating factor (BAFF) [18] and CD40L [19]. This pathway is strictly dependent on IKKα homodimers that are activated by the NF-κB-inducing kinase (NIK) [17] and results in the processing of the cytosolic NF-κB2/p100 precursor to yield the mature p52 subunit, followed by translocation of p52 to the nucleus [8,20].

NATURAL PRODUCTS AS A SOURCE OF NEW THERAPEUTIC AGENTS: TERPENOIDS

Bioactive natural products can be considered very promising starting points for the development of new therapeutic agents. Indeed, synthetic medicinal chemistry has not replaced natural products as essential components of our therapeutic arsenal and many of the medicines prescribed today are natural products. Drugs such the antimalarial artemisin and quinine, digoxin (widely used in the treatment of various heart conditions), the anticancer taxol, the analgesics codeine, morphine and acetylsalicylic acid, among others have been developed from natural products, which demonstrates that natural products has a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases [21,22].

One of the most important families of natural compounds known for their medicinal value is the terpenoids. A few of

them have been used for therapeutic purposes for centuries as antibacterial, anti-inflammatory, antitumoral agents but in recent decades the level of research activity on these compounds has continuously increased.

Terpenoids are the largest and most widespread class of secondary metabolites, mainly in plants and lower invertebrates. These compounds are found in higher plants, mosses, liverworts, algae and lichens, as well as in insects, microbes or marine organisms. Terpenoids are derived from a common biosynthetic pathway based on mevalonate as parent, and are named terpenoids, terpenes or isoprenoids with the subgroup of steroids among them as a class [23-26]. In general, the term terpene is used to denote compounds containing an integral number of C5 units and chemically all terpenoids can be considered to be derived from the basic branched C5 unit isoprene (2-methyl-1,3-butadiene). It is useful to classify terpenoids according to the number of such C5 units present in the molecule, from which they are biogenetically derived. Thus, terpenoids are classified into **hemi-, mono-, sesqui-, di- sester-, tri- and tetraterpenoids** (carotenoids) having 1, 2, 3, 4, 5, 6 and 8 isoprenoid C5 residues, respectively.

In a previous review [27], we have reported the chemical features and biosynthesis pathways of terpenoids. Diterpenoids containing four isoprene units (C-20) constitute a large group of compounds found in higher plants, fungi, insects and marine organisms. Most of the diterpenoids found in recent years have been isolated from Asteraceae and Lamiaceae, and this class of terpenoids has one of the widest ranges of biological activities. Diterpenoids are classified according to their chemical structure [27] in labdanes, pimaranes, abietanes, kauranes, taxanes, etc.

TERPENOIDS AS MODULATORS OF NF-κB SIGNALING PATHWAYS

With the continuous emergence of new diseases, development of new drugs is needed. In the last years, an extensive literature on different types of diterpenoids has been published, describing their pharmacological potential. Several biological actions have been reported for diterpenes including antibacterial, antifungal, anti-inflammatory, antileishmanial, cytotoxic and antitumour activities [28]. Since the description by Kopp and Ghosh (1994) [29] of sodium salicylate and its semi-synthetic derivative as the first plant-derived NF-κB inhibitor, a large number of terpenoids have been tested for NF-κB inhibitory properties.

The molecular cascade of signaling events involved in NF-κB activation, provides several steps for specific inhibition of NF-κB activity. Inhibition of NF-κB activation can occur by different mechanisms including (a) inhibiting the activation of IKK complex, (b) targeting the proteasomal degradation of or (c) interfering the translocation of NF-κB to the nucleus, or the binding of NF-κB to DNA. Several agents including natural products such as sesquiterpene lactones (helenalin) [30-32], structural relative of flavonoids (caffeic acid phenethyl ester) [33], sesterterpenes (cyclolinteinone) [34], *ent*-kaurane diterpenes (oridonin and ponicidin) [35] and prostaglandins (15-deoxi-prostaglandin J_2) [36], have been shown to inhibit either nuclear translocation of p65 or binding of NF-κB to DNA. On the other hand, classic anti-inflammatory compounds such as salicylates and glucocorticoids either prevent the degradation of IκB [29] or activate the transcription of IκB [37]. Moreover, some naturally occurring inhibitors of ubiquitin-proteasome such as lactacystin, a streptomyces metabolite can block NF-κB activation by preventing IκB degradation [38]. Nevertheless, the most effective and selective approach for the inhibition of NF-κB activation is provided by inhibitors of the IKK activity [30,31]. Benzoquinones such as herbimycin A and geldanamycin isolated from marine and terrestrial bacteria [39], sesquiterpene lactones such as parthenolide [30,40], kaurane diterpenes such as kamebakaurin [41]; and labdane diterpenes such as hispanolone derivatives [42] are examples of the specifically targeting of IKK kinase activity.

BIOLOGICAL ACTIVITY OF DITERPENOIDS RELATED TO NF-κB INHIBITION

Labdane Diterpenoids: Andalusol, Andrographolide, Hispanolone-Derived Labdanes, Pinusolide

Labdane-type diterpenes have been reported to have broad spectrum of biological activities: antimicrobial, cytotoxic, anti-inflammatory, antiviral, antiprotozoal…[28,42-44]. These diterpenes have been found as secondary metabolites in tissues of fungi, insects, marine organisms, and in essential oils, resins and tissues of higher plants. Examples of diterpenes reported in the literature as NF-kB inhibitors are described below, with special emphasis on their mechanism of action.

Andalusol

Andalusol is a naturally occurring labdane-type diterpene, isolated from *Sideritis foetens* (Lamiaceae). This compound exhibited anti-inflammatory activity when evaluated in *in vivo* models of paw and ear inflammation [45]. Andalusol also inhibited expression of NOS-2 protein by inhibition of NF-κB activation in murine macrophage cell line J744. It inhibited the degradation of IκBα favouring the retention of the inactive NF-κB complexes in the cytosol. The exact inhibitory mechanism was not verified, but andalusol might inhibit a step leading to the activation of the IKK, or possibly the IKK itself, but further work is required to address this question [46].

Andrographolide

Andrographis paniculata is a shrub belonging to the Acanthaceae family, commonly used as a folk remedy for alleviation of inflammatory disorders in Asia for millennia. It currently is a prescribed medicine for treatment of rheumatoid arthritis in China. Andrographolide, a labdane diterpenoid isolated from this plant and related compounds have been investigated for their pharmacological activities in several animal studies, showing antipyretic and anti-inflammatory activity. Andrographolide has been shown to inhibit the production of TNF-α and IL-12 in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages [47]. Inhibition of NF-κB activation was described for andrographolide [48]. Mechanistically, it formes a covalent adduct with reduced cysteine (62) of p50, thus blocking the binding of NF-κB oligonucleotide to nuclear proteins [48]. Andrographolide suppressed the activation of NF-κB in stimulated endothelial cells, which reduced the expression of cell adhesion molecule E-selectin and prevented E-selectin-mediated leukocyte adhesion under flow [49]. It also abro-

gated the cytokine- and endotoxin-induced peritoneal deposition of neutrophils, attenuated septic shock, and prevented allergic lung inflammation *in vivo* [48]. Notably, it had no suppressive effect on IκBα degradation, p50 and p65 nuclear translocation, or cell growth rates.

Hispanolone-Derived Labdanes

Giron *et al*. (2007) [42] evaluated a series of 11 labdane-type diterpenoids with various patterns of substitution, consisting of the natural diterpenoids hispanolone and galeopsin and a series of nine hispanolone derivatives (2– 8, 10 and 11) as potential anti-inflammatory agents. Two of these diterpenoids (dehydroisohispanolone and 8,9-dehydrohispanolone-15,16-lactol) reduced the production of nitric oxide (NO), prostaglandin E_2 (PGE_2), and TNF-α in LPS-activated murine RAW 264.7 macrophages, with IC_{50} in the range 1–10 μM. Inhibition of these inflammatory mediators was related to inhibition of the expression of NOS-2 and COX-2 at the transcriptional level. The inhibition by both compounds of LPS-induced IκBα degradation, p65 nuclear translocation, and IKK activity indicates that their anti-inflammatory actions are at least partially mediated by inhibition of NF-κB-dependent gene transcription. Furthermore, the effects of these diterpenoids on MAPK signaling suggest that these kinases might participate in the transduction of the LPS signal to the IKK complex.

Pinusolide

Pinusolide, a labdane-type diterpene lactone isolated from *Biota orientalis* was found to be a potent anti-PAF receptor binding antagonist. Choi *et al*., [50] studied the inhibitory effect of 15-methoxypinusolidic acid (15-MPA) isolated also from *Biota orientalis* (Cupressaceae) on LPS-induced inflammation in murine microglial BV2 cells. 15-MPA significantly reduced the expression and activity of NOS-2, and the production of NO in LPS-stimulated BV2 cells. In addition, 15-MPA significantly suppressed the expressions of TNF-α, IL-6, and COX-2. However, 15-MPA did not affect LPS-induced degradation of IκB-α and translocation of NF-κB into the nucleus. LPS-activated p38 MAPK, ERK-1/2, and JNK were not affected by 15-MPA. Taken together, this study demonstrates that 15-MPA inhibits LPS-induced NOS-2 expression and NO production, independent on MAPK and NF-κB.

Hypoestoxide

Hypoestoxide (a bicyclo [9,3,1] pentadecane) is a diterpene from *Hypoestes rosea*, a tropical shrub in the family Acanthacea, several members of which are used in folk medicine in Nigeria. It was demonstrated that hypoestoxide abrogates the production of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in LPS-activated normal human peripheral blood mononuclear cells [51]. Moreover, hypoestoxide inhibits the production of NO by IL-1β- or IL-17-stimulated normal human chondrocytes [51]. *In vivo*, oral administration of hypoestoxide to mice significantly ameliorated hind paw edema induced by antibodies to type II collagen plus LPS. Furthermore, topical administration of this compound to mice also significantly inhibited phorbol ester-induced ear inflammation. The anti-inflammatory activity of hypoestoxide may be due in part to its ability to inhibit NF-κB activation through direct inhibition of IKK activity [51]. Antiogenic and antitumor activities have also been reported for this byciclic diterpenoid [52].

Tanshinone IIA

Tanshinone IIA, a diterpene isolated from *Salvia miltiorrhiza* reduced the production of pro-inflammatory mediators in RAW 264.7 cells stimulated with LPS. Jang *et al*. (2006) [53] investigated the inhibitory effects of tanshinone IIA on the activation of NF-κB and IκBα phosphorylation, and also examined phosphorylation of NIK and IKK as well as the activation of MAPKs such as p38 MAPK (p38), extracellular signal-regulated kinases 1/2 (ERK1/2), and c-Jun N-terminal kinase (JNK) in RAW 264.7 cells stimulated with LPS. Tanshinone IIA inhibited NF-κB-DNA complex, NF-κB binding activity, and the phosphorylation of IκBα in a dose dependent manner. Tanshinone IIA also inhibited the translocation of NF-κB from cytosol to nucleus. Moreover, the phosphorylation of NIK and IKK as well as the phosphorylation of p38, ERK1/2, and JNK in the LPS-stimulated RAW 264.7 cells were suppressed by this compound in a dose-dependent manner. These results suggest that tanshinone IIA may inhibit LPS-induced IκBα degradation and NF-κB activation *via* suppression of the NIK-IKK pathway as well as the MAPKs (p38, ERK1/2, and JNK) pathway in RAW 264.7 cells and these properties may provide a potential mechanism that explains the anti-inflammatory activity of tanshinone IIA.

More recently, Xu *et al*. [54] evaluated the effect of sodium tanshinone IIA sulfonate (STS), a water-soluble derivative of tanshinone IIA, on concanavalin A (ConA)-induced hepatitis (CIH) in mice, an experimental model of immune-mediated liver injury. C57BL/6 mice pretreated with STS released much less alanine transaminase into plasma in response to ConA challenge and had reduced inflammatory infiltration and hepatocyte apoptosis in the liver compared with control mice pretreated with vehicle solutions. Thus, STS protected mice from CIH. In STS-pretreated mice induced with CIH, we found abrogated TNF-α and interferon-γ (IFN-γ) production. Moreover, mRNA expressions of IFN-inducible protein-10 and macrophage inflammatory protein-1α in these mice were decreased. The mechanism of anti-inflammatory effects of STS may be attributed to its modulation of crucial inflammatory signaling pathways, including NF-κB and IFN-γ/STAT1. In conclusion, STS was capable of protecting mice from immune-mediated liver injury *in vivo*, and the protection was associated with its suppressive effect on the production of important inflammatory mediators through modulating NF-κB and IFN-γ/STAT1 signaling pathways.

Pimarane Diterpenoids

Acanthoid Acid

The **acanthoic acid** is a pimarane diterpene that was isolated from the root bark of *Acanthopanax koreanum* Nakai (Araliaceae). Crude extracts of this plant have been used in traditional Korean medicine as a tonic and sedative, as well as a remedy for the treatment of rheumatism. Studies revealed that this compound suppresses the production of IL-1β and TNF-α at 10 mg/mL, is orally active and has no significant toxicity in a rodent model of chronic inflammation. A series of 16 non-natural acanthoic acid analogs was devel-

oped by Lam *et al*. [55,56], and were described as modulators of several proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in human peripheral blood mononuclear cells and murine RAW 264.7 macrophages. Further studies, through EMSA and Western blot analyses, indicated that these compounds exert their anti-inflammatory profile by inhibiting NF-κB-mediated cytokine synthesis [56].

Moreover, these diterpenes have also been described to be potent activators of liver X receptors (LXRα and LXRβ) in murine macrophages [57]. LXRs are nuclear receptors that play central roles in the transcriptional control of lipid metabolism and modulate immune and inflammatory responses. Indeed, LXRs repress a set of inflammatory genes after bacterial, LPS, TNF-α, or IL-1β stimulation [58], including NOS-2 and COX-2, IL-6 and IL-1β, the chemokines monocyte chemoattractant protein-1 (MCP-1) and MCP-3, and MMP9, through a mechanism that involved inhibition of the NF-κB pathway [58,59].

Abietane Diterpenoids: Carnosol and Carnosic Acid

Carnosol and Carnosic Acid

Carnosol and **carnosic acid** are abundant abietane diterpenes with aromatic ring C constituents in Rosemary extracts (*Rosmarinus officinalis*). Biological activities as antioxidant and anticarcinogen have been described for carnosol and carnosic acid [60,61]. In addition, carnosol has also anti-inflammatory properties. Carnosol is able to inhibit NOS-2 expression and reduced LPS-stimulated NO production by inhibiting NF-κB activation in mouse macrophage RAW 264.7 cell line in B16/F10 mouse melanoma cells [62]. Moreover, carnosol also inhibited LPS-induced p38 and ERK activation.

Kauranes: Foliol, Linearol, Siegeskaurolic Acid, *Ent*-Kaurane, Diterpenoids from *Croton Tonkinensis, Diterpenoids from Isodon Species*

Kaurane diterpenoids represent an important group of tetracyclic diterpenes. Diverse biological activities have been described for this type of diterpenoids, including plant growth regulating, antimicrobial, antiparasitic, insect antifeedant, cytotoxic, antitumour, anti-HIV, hipotensive and anti-inflammatory activities, among others [63]. More recently, attention has been paid to the study of the biological effects of tetracyclic *ent*-kaurane diterpenoids, because the number of these molecules isolated and characterized is continuously increasing [64]; *Ent*-kaurane is the main diterpene intermediate involved in the biosynthesis of gibberellins, a widespread family of plant hormones with isoprenoid structure that control various physiological plant functions as growth, germination and flowering [65].

Foliol and Linearol

Kaurenoic acid is one of the intermediate compounds involved in the biosynthesis of diverse kaurane diterpenes. Two kaurene diterpenes named **foliol, linearol,** hydroxylated molecules derived from the *ent*-kaur-16-en-19-oic acid (kaurenoic acid), and kaurenoic acid were reported as inhibitors of NF-κB pathway in macrophages [66]. These compounds inhibit the expression of NOS-2 and the release of TNF-α in murine J774 macrophages challenged with LPS. These diterpenes inhibit NF-κB and IKK activation *in vivo* but failed to affect *in vitro* the function of NF-κB, the phosphorylation and targeting of IκBα, and the activity of IKKβ. Transient expression of NIK activated the IKK complex and NF-κB, a process that was inhibited by kaurenes, indicating that the inhibition of NIK was one of the targets of these diterpenes. These results show that kaurenes impair the inflammatory signaling by inhibiting NIK, a member of the MAPK kinase superfamily that interacts with TNF receptor-associated factors, and mediate the activation of NF-κB by these receptors. Moreover, kaurenes delayed the phosphorylation of p38, ERK1, and ERK2 MAPKs, but not that of JNK, in response to LPS treatment of murine J774 cells. The absence of a coordinate activation of MAPK and IKK might contribute to a deficient activation of NF-κB that is involved in the anti-inflammatory activity of these molecules.

Siegeskaurolic Acid

Park *et al*. [67] reported the anti-inflammatory activity of the **diterpene *ent*-16α,17-hydroxy-kauran-19-oic acid (siegeskaurolic acid)** isolated from *Siegesbeckia pubescens* root. Pretreatment with siegeskaurolic acid (20 or 30 mg/kg/day, p.o.) exhibited anti-inflammatory and antinociceptive effects in animal models as acetic acid-induced abdominal constriction and hot plate tests in mice. Siegeskaurolic acid was also found to significantly inhibit the productions of NO, PGE_2, and TNF-α in murine RAW 264.7 macrophages. Consistent with these findings, NOS-2 and COX-2 proteins, and NOS-2, COX-2, and TNF-α mRNAs were found to be inhibited by siegeskaurolic acid. Furthermore, siegeskaurolic acid inhibited NF-κB activation induced by LPS, and this was associated with the prevention of IκB degradation, and subsequently with decreased nuclear p65 and p50 protein levels. The anti-inflammatory and antinociceptive properties of siegeskaurolic acid may be due to NOS-2, COX-2 and TNF-α inhibition *via* the down-regulation of NF-κB binding activity.

Ent-Kaurane Diterpenoids from Croton Tonkinensis

Four *ent*-kaurane diterpenoids isolated from the leaves of *Croton tonkinensis* have been recently described to be potent inhibitors of NO release. These *ent*-kauranoids, strongly inhibited the LPS-induced activation of NO production in murine RAW 264.7 macrophage cell line in a dose dependent manner. Moreover, these compounds inhibited NF-κB activation with an IC_{50} value in the range 0.07-0.42 μM. Although the exact mechanism involved in the NF-κB inhibition was not described, authors speculated that the presence of reactive centers, such as an exomethylene group conjugated to a carbonyl group in the cyclopentanone ring, may be the responsible of NF-κB inhibition [68].

Diterpenoids from *Isodon Species*

Oridonin, Ponicidin, Kamebanin, Kamabecetal, Kamebakaurin, Excisanin A and Inflexinol

Isodon species are an important source of bioactive diterpenoids [69]. The use of *Isodon* species in Chinese popular folk medicine has a long tradition for the treatment of respiratory and gastrointestinal bacterial infections, inflammation and cancer. In 1977, the standard extract of *I. rubescens* leaves was successfully developed into a drug product which was used in treating sore throats and inflammation in China. *Isodon* diterpenoids have attracted considerable attention as

antibacterial, antitumour, anti-inflammatory, and anti-feeding agents. Many natural *Isodon* diterpenoids from different species, as well as hemisynthetic derivatives have been tested as anti-inflammatory agents. *Ent*-kauranes is the largest group of known *Isodon* diterpenoids, and appears to be the most widely distributed.

Oridonin and Ponicidin

Oridonin (epoxy-*ent*-kaurane) and **ponicidin,** the two major constituents of *I. rubescens* have been the most frequently studied compounds among *Isodon* diterpenoids. More recently, *ent*-kauranes isolated from *I. rubescens* including oridonin, ponicidin and xindongnin A, were found to be potent inhibitors of NF-κB transcription activity and the expression of its downstream targets, COX-2 and NOS-2 in murine RAW 264.7 macrophage cell line. The mechanisms of action of diterpenoids against NF-κB are similar, but significant differences were also identified. All of the diterpenoids directly interfere with the DNA-binding activity of NF-κB to its response DNA sequence [35]. Oridonin and ponicidin have an additional impact on the translocation of NF-κB from the cytoplasm to nuclei without affect IκB-α phosphorylation and degradation. The effects of these compounds on the interaction of NF-κB with consensus DNA sequence are unique. Different inhibitory effects were observed when NF-κB bound to various DNA sequences. Both p65/p65 and p50/p50 homodimers, as well as p65/p50 heterodimer association with their responsive DNA, were inhibited.

Kinetic studies on the NF-κB DNA interaction indicate that the diterpenoids decrease the $B_{max\ app}$ but had no effect on $K_{d\ app}$. This suggest that this class of compounds interacts with both p65 and p50 subunits at a site other than the DNA binding site and subsequently modulates the binding affinity of the transcription factor toward DNA with different NF-κB binding sequences. E*nt*-kaurenoids are a novel and new class of NF-κB inhibitors that interfere with the binding between NF-κB and DNA with a unique sequence by a distinct mechanism [35,69].

Kamebanin, Kamabecetal, Kamebakaurin and Excisanin A

Plant extracts of *Isodon japonicus*, contain several highly oxidized kaurane diterpenoids as **kamebanin, kamabecetal, kamebakaurin and excisanin A.** It was found that all diterpene compounds inhibited LPS-induced DNA-binding activity of NF-κB dose-dependently and NF-κB activation was completely inhibited in the presence of 10 μg/ml of kamebakaurin or excesanin A [41,70]. Lee *et al.* [41] deeply investigated the mechanism of action of kamebakaurin, describing this diterpene as a potent inhibitor of NF-κB activation by directly targeting targeting DNA-binding activity of p50. Kamebakaurin prevented the activation of NF-κB by different stimuli in various cell types including murine RAW 264.7 macrophage cell line, human MCF-7 breast cancer cell line, human Jurkat T leukemia cells, and human monocytic THP-1 cell line. Kamebakaurin did not prevent either stimuli-induced degradation of IκB-α or nuclear translocation of NF-κB, however, it significantly interfered DNA binding activity of activated NF-κB in cell and *in vitro* and preferentially prevented p50-mediated DNA-binding activity of NF-κB rather than that of RelA as measured using *in vitro* translated p50 and RelA proteins. Moreover, a p50 mutant with a Cys-62 --> Ser mutation was not inhibited with kamebakaurin, indicating that the effect of kamebakaurin was probably due to its interaction with cysteine 62 in p50. The covalent modification of p50 by kamebakaurin was further demonstrated by mass spectrometry analysis that showed an increase in the molecular mass of kamebakaurin-treated p50, and this modification was not reverted by addition of dithiothreitol. These results suggested that kamebakaurin exhibited its inhibitory activity by a direct covalent modification of cysteine 62 in the p50. Also, treatment of cells with kamebakaurin prevented TNF-α-induced expression of antiapoptotic NF-κB target genes encoding c-IAP1 (hiap-2) and c-IAP2 (hiap-1), members of the inhibitor of apoptosis family, and Bfl-1/A1, a prosurvival Bcl-2 homologue, and augmented the TNF-α-induced caspase 8 activity, thereby resulting in sensitizing MCF-7 cells to TNF-α-induced apoptosis. Furthermore, kamebanin, kamebacetal and kamebakaurin also significantly inhibited LPS-induced NO and PGE_2 production but they did not influence the DNA binding of AP-1.

Inflexinol

Inflexinol, an *ent*-kaurane diterpenoid, was isolated from the leaves of *Isodon excisus* [71]. Inflexinol (1, 5, 10 μM) suppressed the expression of NOS-2 and COX-2 as well as the production of NO in LPS-stimulated RAW 264.7 cells and astrocytes. Consistent with the inhibitory effect on NOS-2 and COX-2 expression, inflexinol also inhibited transcriptional and DNA binding activity of NF-κB *via* inhibition of IκB degradation as well as p50 and p65 translocation into nucleus. These results suggest that inflexinol inhibits NOS-2 and COX-2 expression through inhibition of NF-κB activation, thereby inhibits generation of inflammatory mediators in RAW 264.7 cells and astrocytes.

Triptolide

In recent years there has been considerable interest in the use of *Tripterygium* extracts and of the main bioactive constituent, **triptolide** to treat a variety of autoimmune and inflammation-related conditions, such rheumatoid arthritis. Intensive medicinal research [72] have attempted to identify the target molecule(s) and understanding its mechanisms [73]. The main mode of action of the *Tripterygium* extracts and triptolide is the inhibition of expression of proinflammatory genes such as those for IL-2, NOS-2, TNF-α, COX-2 and IFN-γ. The efficacy and safety of certain types of *Tripterygium* extracts were confirmed in human clinical trials in the US and abroad. Triptolide is a naturally occurring diterpene triepoxide whose anti-inflammatory effects correlate with transcriptional inhibition of various cytokines. Several studies [74] have verified inhibition of NF-κB activation by triptolide in different cell types including human alveolar basal epithelial A549 cells and human Jurkat T leukemia cells [75]. In RAW 264.7 cells stimulated with LPS to mimic inflammation, triptolide inhibits NO production in a dose-dependent manner and abrogates NOS-2 gene expression. The mechanism by which triptolide inhibits murine NOS-2 gene expression was analyzed by examining activation of MAPK and NF-κB in these cells. Addition of triptolide inhibited phosphorylation of JNK but not that of ERK or p38 MAPK. In addition, triptolide significantly inhibited the DNA binding activity of NF-κB, suggesting that triptolide acts to inhibit inflammation through inhibition of NO pro-

duction and NOS-2 expression through blockade of NF-κB and JNK activation. Triptolide is also a potent inhibitor of NF-κB activation in T lymphocytes and this effect is partly due to the upregulation of IκBα mRNA expression [74].

Triptolide bound specifically and irreversibly to a single, 90 kDa protein in nuclear extracts from stimulated and non-stimulated monocytic and epithelial cell lines. Thiol reactivity of one or more of the epoxides on triptolide was necessary for the covalent binding. The correlation between the structure-activity relationship and observed binding suggests that identification of the triptolide binding protein could provide insight into the cellular mode of action of this anti-inflammatory natural product [76].

Pepluane Diterpenoids

From the whole plant of *Euphorbia peplus* L., a diterpene based on a rare pepluane skeleton, named **pepluanone** was isolated, and the ability of this compound to act as anti-inflammatory agent has been evaluated by *in vivo* tests on carrageenin-induced rat paw edema, an experimental model of acute inflammation. *In vitro* experiments performed on pepluanone allowed to hypothesize that its activity could be explained in reducing the production of NO, PGE_2, and TNF-α by inhibiting the expression of NOS-2, COX-2, and TNF-α mRNA through the down-regulation of NF-κB binding activity [77].

Marine Diterpenoids

Natural products isolated from marine organisms have also been shown a great potential in drug discovery. For several decades, marine organisms have provided marine natural products chemists with a rich source of unsual metabolites [78-81].

Paya *et al.* [82] described the inhibitory effect of a series of 6 cycloamphilectenes, novel marine diterpenes based on amphilectene skeletons and isolated from the Vanuatu sponge *Axinella* sp., on NO, PGE_2 and TNF-α production in murine peritoneal macrophages. These compounds reduced

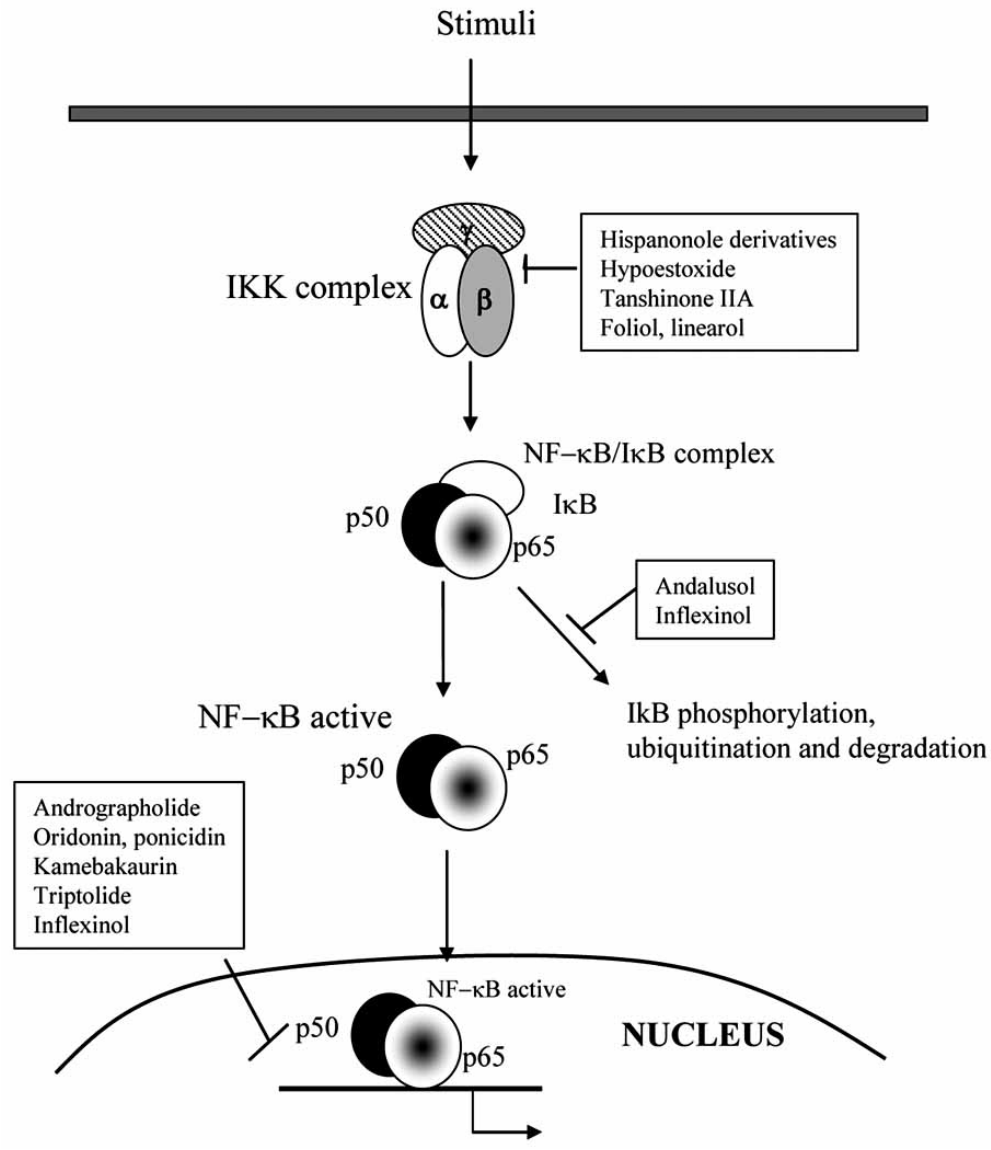

Fig. (3). Main signaling pathways inhibited by diterpenoids.

potently NO production in a concentration-dependent manner with IC_{50} values in the submicromolar range (0.1-4.3 μM). Studies on intact cells and Western blot analysis showed that the more potent cycloamphilectenes reduced the expression of NOS-2 without affecting COX-2 expression. Among them cycloamphilectene 2, the unique compound bearing an exocyclic methylene group, was able to reduce NO production without affecting TNF-α release. Cycloamphilectene 2, which is an inhibitor of the nuclear factor-κB pathway, exhibited topical anti-inflammatory activity.

CONCLUSION REMARKS

Since the discovery of NF-κB as a key transcription factor in the inflammatory response, inhibition of NF-κB activation has constituted a promising therapeutic approach to fight against many of the human inflammatory pathologies including arthritis, asthma, the auto-immune diseases and different types of cancer. Consistent with this, several commonly-used anti-inflammatory agents such as aspirin (sodium salicylate), and glucocorticoids have been shown to inhibit NF-κB activity. Natural products are very promising candidates for drug development. In addition, the therapeutic effects of many natural products as anti-inflammatory agents may be due to their ability to inhibit NF-κB-dependent inflammation. From an evolutionary point of view, plants produce secondary metabolites such as terpenoids as a mechanism of defence against pathogen attack. Similar to animal immune responses, induced plant defence responses involve a network of signal transduction and the rapid activation of gene expression following pathogen infection. Terpenoid release could be envisaged as a way of combating pathogens through the destruction of the immune system after NF-κB signaling suppression. This phenomenon may also provide effective therapeutic compounds for the treatment of inflammatory diseases and cancer in humans. In this review we have highligthed the potential of diterpenoids as potent and pathway-specific modulators of NF-κB pathways.

The better understanding of the mechanisms that govern NF-κB pathway, has contributed to the development of inhibitors that block NF-κB activation. Over 750 inhibitors of the NF-κB pathway have been identified, including a variety of natural and synthetic molecules that can act either directly on IKKs, on the proteosome machinery, or on the NF-κB-DNA binding [83]. However, regulation of IKK complex has been the prime target for the development of NF-κB signaling inhibitors. Indeed, a vast majority of diterpenoids described in this review exert their inhibitory effects signaling at this step (Fig. **3**). Noteworthy, a great number of diterpenoids that potently inhibit NF-κB, bear α-β-unsaturated carbonyl functions which are widely recognized as the most common NF-κB inhibitory pharmacophore. Methylene groups of α-β-unsaturated carbonyl compounds can react by Michael type addition to sufhydryl groups of cysteine residues in the DNA binding domain of NF-κB subunit [81].

There is no doubt that further studies remain necessary to fully understand the molecular mechanisms induced by diterpenoids as NF-κB inhibitors, however it could be concluded that diterpenoids are very promising candidates as leads for developing useful therapeutics.

ACKNOWLEDGEMENTS

S.H. is a FIS program investigator and is supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) and the Instituto de Salud Carlos III. This work was supported by grants from the FIS PI05.0050 (2005), and the Fundación Mutua Madrileña to S.H, and by a Santander-Complutense grant to S.H. and B.de las H. The CNIC is supported by the Spanish Ministry of Science and Innovation and the Pro-CNIC Foundation.

ABBREVIATIONS

AP-1 = Activating protein-1
BAFF = B-cell-activating factor
CIH = Concanavalin A (ConA)-induced hepatitis
COX-2 = Cyclooxygenase-2
ERK = Extracellular signal-regulated kinase
IL = Interleukin
IκB = Inhibitor of NF-κB
IKK = IκB kinase
IFN-γ = Interferon-γ
IP-10 = Inducible protein-10
JNK = c-Jun N-terminal kinase
MAPK = Mitogen-activated protein kinases
15-MPA = 15-methoxypinusolidic acid
NO = Nitric oxide
NOS-2 = Inducible nitric oxide-synthase
LPS = Lipopolysaccahride
LTβR = Lymphotoxin-β receptors
LXR = Liver X receptors
MIP-1α = Macrophage inflammatory protein-1α
MMPs = Matrix metaloproteases
NEMO = NF-κB essential modulator
NIK = NF-κB-inducing kinase
NF-κB = Nuclear transcription factor-κB
NLS = Nuclear localization sequence
PGE_2 = Prostaglandin E_2
RDH = Rel homology domain
STS = Sodium tanshinone IIA sulfonate
STAT = Signal transducers and activators of transcription
TAD = Terminal transactivation domain
TLRs = Toll-like receptors
TNF-α = Tumour necrosis factor-α

REFERENCES

[1] Serhan, C.N.; Savill, J. Resolution of inflammation: the beginning programs the end. *Nat. Immunol.*, **2005**, *6*, 1191-1197.
[2] Serhan, C.N.; Clish, C.B.; Brannon, J.; Colgan, S.P.; Chiang, N.; Gronert, K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids *via*

cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J. Exp. Med.*, **2000**, *192*, 197-1204.

[3] Serhan, C.N.; Hong,.; Gronert, K.; Colgan, S.P.; Devchand, P.R.; Mirick, G.; Moussignac, R.L. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J. Exp. Med.*, **2002**, *196*, 1025-1037.

[4] Serhan, C.N.; Chiang, N.; Van Dyke, T.E. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.*, **2008**, *8*, 349-361.

[5] Mercurio, F.; Manning, A.M. Multiple signals converging on NF-kappaB. *Curr. Opin. Cell Biol.*, **1999**, *11*, 226-232.

[6] Tickle, C. Worlds in common through NF-kappaB. *Nature*, **1998**, *392*, 547-549.

[7] Hayden, M.S.; Ghosh, S. Signaling to NF-kappaB. *Genes Dev.*, **2004**, *18*, 2195-2224.

[8] Bonizzi, G.; Karin, M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol.*, **2004**, *25*, 280-288.

[9] Gerondakis, S.; Grossmann, M.; Nakamura, Y.; Pohl, T.; Grumont, R. Genetic approaches in mice to understand Rel/NF-kappaB and IkappaB function: transgenics and knockouts. *Oncogene*, **1999**, *18*, 6888-6895.

[10] Pasparakis, M.; Luedde, T.; Schmidt-Supprian, M. Dissection of the NF-kappaB signalling cascade in transgenic and knockout mice. *Cell Death Differ.*, **2006**, *13*, 861-872.

[11] Pahl, H.L. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*, **1999**, *18*, 6853-6866.

[12] Baeuerle, P.A.; Baichwal, V.R. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. *Adv. Immunol.*, **1997**, *65*, 111-137.

[13] Baeuerle, P.A.; Henkel, T. Function and activation of NF-kappa B in the immune system. *Annu. Rev. Immunol.*, **1994**, *12*, 141-179.

[14] Siebenlist, U.; Franzoso, G.; Brown, K. Structure, regulation and function of NF-kappa B. *Annu. Rev. Cell Biol.*, **1994**, *10*, 405-455.

[15] Ghosh, S.; Karin, M. Missing pieces in the NF-kappaB puzzle. *Cell*, **2002**, *109* Suppl, S81-S96.

[16] Keutgens, A.; Robert, I.; Viatour, P.; Chariot, A. Deregulated NF-kappaB activity in haematological malignancies. *Biochem. Pharmacol.*, **2006**, *72*, 1069-1080.

[17] Dejardin, E.; Droin, N.M.; Delhase, M.; Haas, E.; Cao, Y.; Makris, C.; Li, Z.W.; Karin, M.; Ware, C.F.; Green, D.R. The lymphotoxin-beta receptor induces different patterns of gene expression *via* two NF-kappaB pathways. *Immunity*, **2002**, *17*, 525-535.

[18] Claudio, E.; Brown, K.; Park, S.; Wang, H.; Siebenlist, U. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. *Nat. Immunol.*, **2002**, *3*, 958-965.

[19] Coope, H.J.; Atkinson, P.G.; Huhse, B.; Belich, M.; Janzen, J.; Holman, M.J.; Klaus, G.G.; Johnston, L.H.; Ley, S.C. CD40 regulates the processing of NF-kappaB2 p100 to p52. *EMBO J.*, **2002**, *21*, 5375-5385.

[20] Perkins, N.D. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. *Oncogene*, **2006**, *25*, 6717-6730.

[21] Balunas, M.J.; Kinghorn, A.D. Drug discovery from medicinal plants. *Life Sci.*, **2005**, *78*, 431-441.

[22] Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.*, **2007**, *70*, 461-477.

[23] Bouvier, F.; Rahier, A.; Camara, B. Biogenesis, molecular regulation and function of plant isoprenoids. *Prog. Lipid Res.*, **2005**, *44*, 357-429.

[24] Bohlmann, J.; Keeling, C.I. Terpenoid biomaterials. *Plant J.*, **2008**, *54*, 656-669.

[25] Keeling, C.I.; Bohlmann, J. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytol.*, **2006**, *170*, 657-675.

[26] Tholl, D. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr. Opin. Plant Biol.*, **2006**, *9*, 297-304.

[27] De las Heras, B.; Rodriguez, B.; Bosca, L.; Villar, A.M. Terpenoids: sources, structure elucidation and therapeutic potential in inflammation. *Curr. Top. Med. Chem.*, **2003**, *3*, 171-185.

[28] Singh, M.; Pal, M.; Sharma, R.P. Biological activity of the labdane diterpenes. *Planta Med.*, **1999**, *65*, 2-8.

[29] Kopp, E.; Ghosh, S. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science*, **1994**, *265*, 956-959.

[30] Bremner, P.; Heinrich, M. Natural products as targeted modulators of the nuclear factor-kappaB pathway. *J. Pharm. Pharmacol.*, **2002**, *54*, 453-472.

[31] Karin, M.; Yamamoto, Y.; Wang, Q.M. The IKK NF-kappa B system: a treasure trove for drug development. *Nat. Rev. Drug Discov.*, **2004**, *3*, 17-26.

[32] Lyss, G.; Knorre, A.; Schmidt, T.J.; Pahl, H.L.; Merfort, I. The anti-inflammatory sesquiterpene lactone helenalin inhibits the transcription factor NF-kappaB by directly targeting p65. *J. Biol. Chem.*, **1998**, *273*, 33508-33516.

[33] Natarajan, K.; Singh, S.; Burke, T.R., Jr.; Grunberger, D.; Aggarwal, B.B. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc. Natl. Acad. Sci. US A.*, **1996**, *93*, 9090-9095.

[34] D'acquisto, F.; Lanzotti, V.; Carnuccio, R. Cyclolinteinone, a sesterterpene from sponge Cacospongia linteiformis, prevents inducible nitric oxide synthase and inducible cyclo-oxygenase protein expression by blocking nuclear factor-kappaB activation in J774 macrophages. *Biochem. J.*, **2000**, *346*, Pt 3, 793-798.

[35] Leung, C.H.; Grill, S.P.; Lam, W.; Han, Q.B.; Sun, H.D.; Cheng, Y.C. Novel mechanism of inhibition of nuclear factor-kappa B DNA-binding activity by diterpenoids isolated from Isodon rubescens. *Mol. Pharmacol.*, **2005**, *68*, 286-297.

[36] Straus, D.S.; Pascual, G.; Li, M.; Welch, J.S.; Ricote, M.; Hsiang, C.H.; Sengchanthalangsy, L.L.; Ghosh, G.; Glass, C.K. 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway. *Proc. Natl. Acad. Sci. USA.*, **2000**, *97*, 4844-4849.

[37] Scheinman, R.I.; Cogswell, P.C.; Lofquist, A.K.; Baldwin, A.S., Jr. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science*, **1995**, *270*, 283-286.

[38] Adams, J.; Palombella, V.J.; Elliott, P.J. Proteasome inhibition: a new strategy in cancer treatment. *Invest. New Drugs*, **2000**, *18*, 109-121.

[39] Ogino, S.; Tsuruma, K.; Uehara, T.; Nomura, Y. Herbimycin A abrogates nuclear factor-kappaB activation by interacting preferentially with the IkappaB kinase beta subunit. *Mol. Pharmacol.*, **2004**, *65*, 1344-1351.

[40] Garcia-Pineres, A.J.; Lindenmeyer, M.T.; Merfort, I. Role of cysteine residues of p65/NF-kappaB on the inhibition by the sesquiterpene lactone parthenolide and N-ethyl maleimide, and on its transactivating potential. *Life Sci.*, **2004**, *75*, 841-856.

[41] Lee, J.H.; Koo, T.H.; Hwang, B.Y.; Lee, J.J. Kaurane diterpene, kamebakaurin, inhibits NF-kappa B by directly targeting the DNA-binding activity of p50 and blocks the expression of antiapoptotic NF-kappa B target genes. *J. Biol. Chem.*, **2002**, *277*, 18411-18420.

[42] Giron, N.; Traves, P.G.; Rodriguez, B.; Lopez-Fontal, R.; Bosca, L.; Hortelano, S.; de las Heras, B. Suppression of inflammatory responses by labdane-type diterpenoids. *Toxicol. Appl. Pharmacol.*, **2008**, *228*, 179-189.

[43] Hanson, J.R. Diterpenoids. *Nat. Prod. Rep.*, **2007**, *24*, 1332-1341.

[44] Chinou, I. Labdanes of natural origin-biological activities (1981-2004). *Curr. Med. Chem.*, **2005**, *12*, 1295-1317.

[45] Navarro, A.; De las Heras, B.; Villar, A.M. Andalusol, a diterpenoid with anti-inflammatory activity from Siderits foetens Clemen. *Z. Naturforsch. [C].*, **1997**, *52*, 844-849.

[46] De las Heras, B.; Navarro, A.; Diaz-Guerra, M.J.; Bermejo, P.; Castrillo, A.; Bosca, L.; Villar, A. Inhibition of NOS-2 expression in macrophages through the inactivation of NF-kappaB by andalusol. *Br. J. Pharmacol.*, **1999**, *128*, 605-612.

[47] Qin, L.H.; Kong, L.; Shi, G.J.; Wang, Z.T.; Ge, B.X. Andrographolide inhibits the production of TNF-alpha and interleukin-12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinases. *Biol. Pharm. Bull.*, **2006**, *29*, 220-224.

[48] Xia, Y.F.; Ye, B.Q.; Li, Y.D.; Wang, J.G.; He, X.J.; Lin, X.; Yao, X.; Ma, D.; Slungaard, A.; Hebbel, R.P.; Key, N.S.; Geng, J.G. Andrographolide attenuates inflammation by inhibition of NF-kappa B activation through covalent modification of reduced cysteine 62 of p50. *J. Immunol.*, **2004**, *173*, 4207-4217.

[49] Jiang, C.G.; Li, J.B.; Liu, F.R.; Wu, T.; Yu, M.; Xu, H.M. Andrographolide inhibits the adhesion of gastric cancer cells to endothelial cells by blocking E-selectin expression. *Anticancer Res.*, **2007**, 27, 2439-2447.

[50] Choi, Y.; Moon, A.; Kim, Y.C. A pinusolide derivative, 15-methoxypinusolidic acid from Biota orientalis inhibits inducible nitric oxide synthase in microglial cells: implication for a potential anti-inflammatory effect. *Int. Immunopharmacol.*, **2008**, *8*, 548-555.

[51] Ojo-Amaize, E.A.; Kapahi, P.; Kakkanaiah, V.N.; Takahashi, T.; Shalom-Barak, T.; Cottam, H.B.; Adesomoju, A.A.; Nchekwube, E.J.; Oyemade, O.A.; Karin, M.; Okogun, J.I. Hypoestoxide, a novel anti-inflammatory natural diterpene, inhibits the activity of IkappaB kinase. *Cell Immunol.*, **2001**, *209*, 149-157.

[52] Ojo-Amaize, E.A.; Nchekwube, E.J.; Cottam, H.B.; Bai, R.; Verdier-Pinard, P.; Kakkanaiah, V.N.; Varner, J.A.; Leoni, L.; Okogun, J.I.; Adesomoju, A.A.; Oyemade, O.A.; Hamel, E. Hypoestoxide, a natural nonmutagenic diterpenoid with antiangiogenic and antitumor activity: possible mechanisms of action. *Cancer Res.*, **2002**, *62*, 4007-4014.

[53] Jang, S.I.; Kim, H.J.; Kim, Y.J.; Jeong, S.I.; You, Y.O. Tanshinone IIA inhibits LPS-induced NF-kappaB activation in RAW 264.7 cells: possible involvement of the NIK-IKK, ERK1/2, p38 and JNK pathways. *Eur. J. Pharmacol.*, **2006**, *542*, 1-7.

[54] Xu, Y.; Feng, D.; Wang, Y.; Lin, S.; Xu, L. Sodium Tanshinone IIA Sulfonate Protects Mice From ConA-Induced Hepatitis *via* Inhibiting NF-kappaB and IFN-gamma/STAT1 Pathways. *J. Clin. Immunol.*, **2008** [Epub ahead of print].

[55] Lam, T.; Ling, T.; Chowdhury, C.; Chao, T.H.; Bahjat, F.R.; Lloyd, G.K.; Moldawer, L.L.; Palladino, M.A.; Theodorakis, E.A.; Theodorakis, E.A. Synthesis of a novel family of diterpenes and their evaluation as anti-inflammatory agents. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3217-3221.

[56] Chao, T.H.; Lam, T.; Vong, B.G.; Traves, P.G.; Hortelano, S.; Chowdhury, C.; Bahjat, F.R.; Lloyd, G.K.; Moldawer, L.L.; Bosca, L.; Palladino, M.A.; Theodorakis, E.A. A new family of synthetic diterpenes that regulates cytokine synthesis by inhibiting Ikappa-Balpha phosphorylation. *Chembiochem.*, **2005**, *6*, 133-144.

[57] Traves, P.G.; Hortelano, S.; Zeini, M.; Chao, T.H.; Lam, T.; Neuteboom, S.T.; Theodorakis, E.A.; Palladino, M.A.; Castrillo, A.; Bosca, L. Selective activation of liver X receptors by acanthoic acid-related diterpenes. *Mol. Pharmacol.*, **2007**, *71*, 1545-1553.

[58] Joseph, S.B.; Castrillo, A.; Laffitte, B.A.; Mangelsdorf, D.J.; Tontonoz, P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat. Med.*, **2003**, *9*, 213-219.

[59] Castrillo, A.; Joseph, S.B.; Marathe, C.; Mangelsdorf, D.J.; Tontonoz, P. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J. Biol. Chem.*, **2003**, *278*, 10443-10449.

[60] Huang, M.T.; Ho, C.T.; Wang, Z.Y.; Ferraro, T.; Lou, Y.R.; Stauber, K.; Ma, W.; Georgiadis, C.; Laskin, J.D.; Conney, A.H. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Res.*, **1994**, *54*, 701-708.

[61] Huang, S.C.; Ho, C.T.; Lin-Shiau, S.Y.; Lin, J.K. Carnosol inhibits the invasion of B16/F10 mouse melanoma cells by suppressing metalloproteinase-9 through down-regulating nuclear factor-kappa B and c-Jun. *Biochem. Pharmacol.*, **2005**, *69*, 221-232.

[62] Lo, A.H.; Liang, Y.C.; Lin-Shiau, S.Y.; Ho, C.T.; Lin, J.K. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-kappaB in mouse macrophages. *Carcinogenesis*, **2002**, *23*, 983-991.

[63] Garcia, P.A.; de Oliveira, A.B.; Batista, R. Occurrence, biological activities and synthesis of kaurane diterpenes and their glycosides. *Molecules*, **2007**, *12*, 455-483.

[64] Tudzynski, B. Biosynthesis of gibberellins in Gibberella fujikuroi: biomolecular aspects. *Appl. Microbiol. Biotechnol.*, **1999**, *52*, 298-310.

[65] Yamaguchi, S. Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.*, **2008**, *59*, 225-51.

[66] Castrillo, A.; de las Heras, B.; Hortelano, S.; Rodriguez, B.; Villar, A.; Bosca, L. Inhibition of the nuclear factor kappa B (NF-kappa B) pathway by tetracyclic kaurene diterpenes in macrophages. Specific effects on NF-kappa B-inducing kinase activity and on the coordinate activation of ERK and p38 MAPK *J. Biol. Chem.*, **2001**, *276*, 15854-15860.

[67] Park, H.J.; Kim, I.T.; Won, J.H.; Jeong, S.H.; Park, E.Y.; Nam, J.H.; Choi, J.; Lee, K.T. Anti-inflammatory activities of ent-16alphaH,17-hydroxy-kauran-19-oic acid isolated from the roots of *Siegesbeckia pubescens* are due to the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages *via* NF-kappaB inactivation. *Eur. J. Pharmacol.*, **2007**, *558*, 185-193.

[68] Giang, P.M.; Jin, H.Z.; Son, P.T.; Lee, J.H.; Hong, Y.S.; Lee, J.J. ent-Kaurane diterpenoids from croton tonkinensis inhibit LPS-induced NF-kappaB activation and NO production. *J. Nat. Prod.*, **2003**, *66*, 1217-1220.

[69] Sun, H.D.; Huang, S.X.; Han, Q.B. Diterpenoids from Isodon species and their biological activities. *Nat. Prod. Rep.*, **2006**, *23*, 673-698.

[70] Hwang, B.Y.; Lee, J.H.; Koo, T.H.; Kim, H.S.; Hong, Y.S.; Ro, J.S.; Lee, K.S.; Lee, J.J. Kaurane diterpenes from Isodon japonicus inhibit nitric oxide and prostaglandin E2 production and NF-kappaB activation in LPS-stimulated macrophage RAW264.7 cells. *Planta Med.*, **2001**, *67*, 406-410.

[71] Lee, J.W.; Lee, M.S.; Kim, T.H.; Lee, H.J.; Hong, S.S.; Noh, Y.H.; Hwang, B.Y.; Ro, J.S.; Hong, J.T. Inhibitory effect of inflexinol on nitric oxide generation and iNOS expression *via* inhibition of NF-kappaB activation. *Mediators Inflamm.*, **2007**, 93148.

[72] Brinker, A.M.; Ma, J.; Lipsky, P.E.; Raskin, I. Medicinal chemistry and pharmacology of genus *Tripterygium (Celastraceae)*. *Phytochemistry*, **2007**, *68*, 732-766.

[73] Lin, N.; Sato, T.; Ito, A. Triptolide, a novel diterpenoid triepoxide from Tripterygium wilfordii Hook. f., suppresses the production and gene expression of pro-matrix metalloproteinases 1 and 3 and augments those of tissue inhibitors of metalloproteinases 1 and 2 in human synovial fibroblasts. *Arthritis Rheum.*, **2001**, *44*, 2193-2200.

[74] Liu, H.; Liu, Z.H.; Chen, Z.H.; Yang, J.W.; Li, L.S. Triptolide: a potent inhibitor of NF-kappa B in T-lymphocytes. *Acta Pharmacol. Sin.*, **2000**, *21*, 782-786.

[75] Jang, B.C.; Lim, K.J.; Choi, I.H.; Suh, M.H.; Park, J.G.; Mun, K.C.; Bae, J.H.; Shin, D.H.; Suh, S.I. Triptolide suppresses interleukin-1beta-induced human beta-defensin-2 mRNA expression through inhibition of transcriptional activation of NF-kappaB in A549 cells. *Int. J. Mol. Med.*, **2007**, *19*, 757-763.

[76] McCallum, C.; Kwon, S.; Leavitt, P.; Shen, D.M.; Liu, W.; Gurnett, A. Triptolide binds covalently to a 90 kDa nuclear protein. Role of epoxides in binding and activity. *Immunobiology*, **2007**, *212*, 549-556.

[77] Corea, G.; Fattorusso, E.; Lanzotti, V.; Di Meglio, P.; Maffia, P.; Grassia, G.; Ialenti, A.; Ianaro, A. Discovery and biological evaluation of the novel naturally occurring diterpene pepluanone as anti-inflammatory agent. *J. Med. Chem.*, **2005**, *48*, 7055-7062.

[78] Nagle, D.G.; Zhou, Y.D.; Mora, F.D.; Mohammed, K.A.; Kim, Y.P. Mechanism targeted discovery of antitumor marine natural products. *Curr. Med. Chem.*, **2004**, *11*, 1725-1756.

[79] Newman, D.J.; Cragg, G.M. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.*, **2004**, *67*, 1216-1238.

[80] Amigo, M.; Paya, M.; Braza-Boils, A.; De Rosa, S.; Terencio, M.C. Avarol inhibits TNF-alpha generation and NF-kappaB activation in human cells and in animal models. *Life Sci.*, **2008**, *82*, 256-264.

[81] Folmer, F.; Jaspars, M.; Dicato, M.; Diederich, M. Marine natural products as targeted modulators of the transcription factor NF-kappaB. *Biochem. Pharmacol.*, **2008**, *75*, 603-617.

[82] Lucas, R.; Casapullo, A.; Ciasullo, L.; Gomez-Paloma, L.; Paya, M. Cycloamphilectenes, a new type of potent marine diterpenes: inhibition of nitric oxide production in murine macrophages. *Life Sci.*, **2003**, *72*, 2543-2552.

[83] Gilmore, T.D.; Herscovitch, M. Inhibitors of NF-kappaB signaling: 785 and counting. *Oncogene*, **2006**, *25*, 6887-6899.

Received: September 1, 2008 **Revised: November 12, 2008** **Accepted: November 18, 2008**