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Review

Natural products as targeted modulators of the nuclear factor-κB pathway

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Abstract

The use of plant extracts to alleviate inflammatory diseases is centuries old and continues to this day. This review assesses the current understanding of the use of such plants and natural products isolated from them in terms of their action against the ubiquitous transcription factor, nuclear factor kappa B (NF-κB). As an activator of many pro-inflammatory cytokines and inflammatory processes the modulation of the NF-κB transduction pathway is a principal target to alleviate the symptoms of such diseases as arthritis, inflammatory bowel disease and asthma. Two pathways of NF-κB activation will first be summarised, leading to the IKK (IκB kinase) complex, that subsequently initiates phosphorylation of the NF-κB inhibitory protein (IκB). Natural products and some extracts are reviewed and assessed for their activity and potency as NF-κB inhibitors. A large number of compounds are currently known as NF-κB modulators and include the isoprenoids, most notably kaurene diterpenoids and members of the sesquiterpene lactones class, several phenolics including curcumin and flavonoids such as silybin. Additional data on cellular toxicity are also highlighted as an exclusion principle for pursuing such compounds in clinical development. In addition, where enough data exists some conclusions on structure–activity relationship are provided.

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Introduction

The promotion of inflammatory conditions and the initiation of the innate immune response requires the synthesis of many special effector proteins. Numerous signalling cascades have been elucidated, involving as one of the last steps the activation of inducible transcription factors that bind to the promoter regions of their respective genes. Such targets include the genes for adhesion molecules (chemokines) and cytokines (tumour necrosis factor alpha (TNF-α), interleukins). Nuclear factor kappa B (NF-κB) is one of the principal inducible transcription factors in mammals and has been shown to play a pivotal role in the mammalian innate immune response (Hoffmann et al 1999) and chronic inflammatory conditions such as rheumatoid arthritis (Ghosh et al 1998). The signalling mechanisms of NF-κB involves an integrated sequence of protein-regulated steps and many are potential key targets for intervention in treating inflammatory conditions and some cancers (Baeuerle & Baichwal 1997; Barnes & Karin 1997; Miagkov et al 1998; Bours et al 2000; Yamamoto & Gaynor 2001).

Other mediators of inflammation that are under the influence of activated NF-κB include inducible nitric oxide synthase, iNOS (or NOS-2), the subsequent production of nitric oxide (NO) (D'Acquisto et al 1998) and prostaglandin synthase (cyclooxygenase), especially COX-2 (D'Acquisto et al 1997; Newton et al

1997; Willoughby et al 2000; Surh et al 2001). Prostaglandins have also been shown to inhibit NF-κB activation (D'Acquisto et al 1998), and cyclopentenone prostaglandin can directly inhibit the IκB kinase (IKK) (Rossi et al 2000). COX-2 may also have an anti-inflammatory role in the later stages of inflammation through the induced synthesis of anti-inflammatory cyclopentenone prostaglandin (Gilroy et al 1999). Consequently, a compound shown to interfere with NO/iNOS, COX-2, or both, may well act via the inhibition of NF-κB (D'Acquisto et al 2000).

The treatment of inflammatory conditions with plants is widely reported (Barberan et al 1987; Bingöl & Şener 1995; Heinrich et al 1998). Natural products are already providing lead compounds in the search for inhibitory small molecules but only a few are beginning to be commercially used. One example is a new veterinary product under development for the treatment of canine osteoarthritis (Phytopharm 2001a) and inflammatory bowel disease (Phytopharm 2001b).

There are a multitude of approaches for identifying new pharmaceuticals. In the field of natural product biology, ethnopharmacological as well as bioprospecting approaches have received renewed attention in recent years. The concept of ethnopharmacology specifically aims to develop plant-based drugs for more widespread local use either as pure compounds or plant extracts (phytotherapy) (Heinrich & Gibbons 2001). Concurrent with this analysis is the requirement for the documented use of useful plants by indigenous peoples (ethnobotany). Such data aids an ethnopharmacological approach by allowing the selected targeting of plants for analysis. For example, here at the School of Pharmacy we are currently involved in an EU funded project to identify plant extracts and compounds as inhibitors of NF-κB. The selection of plant material, mostly European in origin, has been made based on ethnobotanical data documenting the use of plants, or their extracts, against inflammatory conditions[1]. This research has led to our interest in reviewing the scientific literature on plant-derived modulators of NF-κB activation.

This review will summarise the principal stages of NF-κB activation, possible sites along that cascade for inhibition, inhibitory natural plant metabolites already discovered and will focus upon those chemicals showing specific activity against identified targets. We shall also attempt to highlight those compounds we believe to be the most potent in terms of a selective activity with no or little associated toxicological effects.

[1]The collection of plant material must proceed with the necessary legislative permission from host countries and local authorities as well as with protocols agreed for an economic return to the donor community or country if any financial return is produced from the research. The protocols of the Convention on Biological Diversity and how they relate to ethnobotany/pharmacology or drug discovery are beyond the scope of this paper and have been reviewed recently (Baker et al 1995; Heinrich & Gibbons 2001).

The NF-κB pathway

NF-κB and IκB

NF-κB consists of different combinations of Rel proteins (e.g. p65, p50, p52, RelA, RelB, c-Rel) in various heterodimers and homodimers and is generally represented by subunits p65/p50 (Verma et al 1995). All the Rel proteins share a conserved region of 300 amino acids at the N-terminal – known as the Rel homology domain. This region is responsible for DNA-binding, dimerisation and interaction with the NF-κB inhibitory protein IκB. Inactive NF-κB resides in the cytoplasm bound to the inhibitory protein IκB. IκB masks the nuclear localisation signal of NF-κB and it constitutes a number of proteins including IκBα, IκBβ, IκBε IκBγ and Bcl-3 (Ghosh et al 1998) and IκBζ (Yamazaki et al 2001). An analogous system of innate immunity is, for example, also present in *Drosophila* (Schuster & Nelson 2000), based on Toll cell surface receptors (Imler & Hoffmann 2000) and possessing NF-κB and IκB equivalent proteins in Dorsal/Dif and Cactus, respectively (Hoffmann et al 1999; Silverman & Maniatis 2001).

Specific cell surface receptors and upstream signalling from NF-κB activation

Two of the most important signalling cascades associated with the mammalian immune response, and specifically NF-κB activation, are those of interleukin/lipopolysaccharide (IL/LPS) (Bowie & O'Neill 2000c; Akira et al 2001) and TNF (Baud & Karin 2001). These pathways are summarised in Figure 1. However, elements of the pathways are continuing to be elucidated and some aspects remain cell-type specific (Zapata et al 2000).

Upon stimulation by various agents, including UV light, LPS, HIV-1 or pro-inflammatory molecules (TNF-α, interleukins i.e. IL-1) (Baeuerle & Baichwal 1997; Schmid & Adler 2000), various cell surface receptors are stimulated and include IL-1/Toll-like receptors and TNF receptor (TNFR) (Kopp et al 1999). The IL-1/Toll-like receptor family, such as IL-1R and IL-1RacP (O'Neill 2000), are stimulated by interleukins (O'Neill & Greene 1998) and LPS (Zhang et al 1999). Upon activation they recruit various adaptor proteins, including MyD88, to the IL-1R (Medzhitov et al 1998)

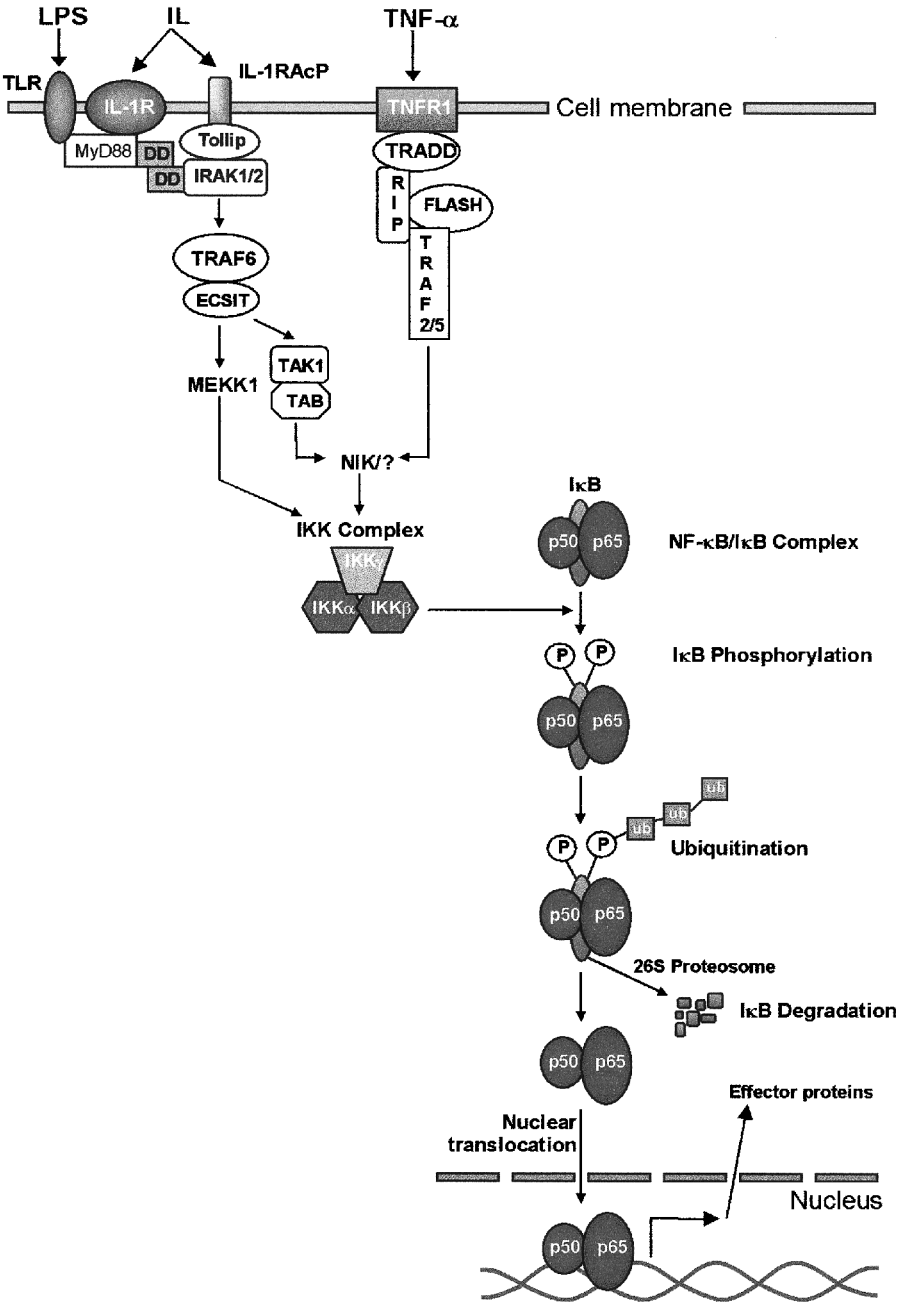


Figure 1 Selected pathways of NF-κB activation. A schematic representation of signalling cascades for LPS, IL and TNF-α stimulation and activation of NF-κB (p50/p65). The activation of NF-κB begins with stimulation of specific receptor families at the cell surface and recruitment of adaptor proteins, and leads to specific pathways of transduction controlled by various kinases. These pathways converge upon the IKK complex that, in turn, promotes the phosphorylation of IκB. This activation targets IκB for ubiquitination and degradation under the control of the 26S proteosome. As a consequence, the NF-κB inhibitory protein is removed and free NF-κB is rapidly translocated to the nucleus where it binds to specific promoter regions of various genes encoding, for example, inflammatory cytokines, adaptor molecules, IκB and p105.

DD, death domain; ECSIT, evolutionary conserved signalling intermediate on Toll pathways; FLASH, Fas ligand-interacting cell effector (FLICE)-associated huge protein; IκB, NF-κB inhibitory protein; IKK, IκB kinase; IL, interleukin; IL-1R, IL-1 receptor; IRAK1/2, IL-1R-associated kinase; LPS, lipopolysaccharide; MEKK1, mitogen-activated protein kinase/extracellular response kinase (MAPK/ERK) kinase kinase 1; MyD88, myeloid differentiation factor; NF-κB, nuclear factor kappa B; NIK, NF-κB-inducing kinase; RIP, receptor interacting protein; TAB, TAK binding protein; TAK, transforming growth factor-β-activated kinase; TLR, Toll-like receptor; TNF-α, tumour necrosis factor-α; TRADD, TNF-receptor-associated death domain protein; TRAF, TNF-receptor-associated factors; Tollip, Toll-interacting protein.

and Toll-interacting protein (Tollip) to IL-1RAcP (Burns et al 2000) in a receptor complex. Tollip and MyD88 trigger phosphorylation of IL-1R-associated kinase (IRAK) (Muzio et al 1997) via an N-terminal protein–protein interaction, termed the death domain (Feinstein et al 1995). IRAK then disassociates from the receptor complex and binds to TNF-receptor associated factor 6 (TRAF6) (Cao et al 1996), a member of a family of adaptor proteins (Arch et al 1998; Bradley & Pober 2001). At this point the pathway diverges on the binding of evolutionary conserved signalling intermediate on Toll pathways (ECSIT), a newly discovered protein in IL-1/Toll NF-κB signalling (Kopp et al 1999). NF-κB is activated by the mitogen-activated protein kinase (MAPK) kinase kinase (MAP3K) pathways of either MAPK/extracellular response kinase (ERK) kinase kinase 1 (MEKK1) (which also leads to Jun amino-terminal kinase activation), or via transforming growth factor-β-activated kinase 1 (TAK1) and its coactivator TAK binding protein (TAB) (Irie et al 2000; Wang et al 2001), to activate further MAP3Ks and induce the NF-κB pathway. Analysis of these MAP3Ks, based on transfection assays in cell lines, implicated NF-κB-inducing kinase (NIK) (and MEKK1) as the bridging kinases from TRAF6 to the IKK (Nemoto et al 1998). However the hypothesis has not been fully supported by subsequent analysis using knockout mice for these genes (Shinkura et al 1999). Therefore NIK could represent one of many upstream routes to the IKK complex with further kinases or novel phosphorylation routes to IKK awaiting elucidation (Israël 2000; Imler & Hoffmann 2001; Schmitz et al 2001).

The TNFR-induced cascade utilises separate adaptor proteins from the IL-1/Toll-like pathway but may utilise common MAP3K inducers of IKK (Baud & Karin 2001) (Figure 1). The TNFR's members number at least 20 (Wajant et al 2001) and includes receptor activator of NF-κB (RANK) (Lee et al 2000). The NF-κB signalling cascade from TNFR is a separate transduction pathway to that of IL-1/Toll receptors. Two types of TNFR have been characterised as TNFR1 and TNFR2. TNFR1 activity is more widely characterised and the transduction of the pathway begins with recruitment of cytosolic adaptor protein, TNFR-associated death domain protein (TRADD) (Hsu et al 1996a). This complex then serves as a platform to recruit a number of structurally and functionally diverse protein kinases that include Fas-associated protein with death domain (FADD) (Hu et al 2000), cIAP1 (cellular inhibitor of apotosis protein), TRAF2 (TNF-receptor associated factors) (Shu et al 1996), receptor interacting protein (RIP) (Hsu et al 1996a) and death receptor (Fas) ligand-interacting cell effector (FLICE)-associated huge protein (FLASH) (Choi et al 2001). Here the pathway diverges into two branches that lead to either apoptosis or NF-κB activation (Hsu et al 1995, 1996b; Van Antwerp et al 1998). The TRADD-FADD/cIAP1-mediated pathway leads to caspase activity and apoptosis (Schulze-Osthoff et al 1998), whereas the TRADD-TRAF2/RIP/FLASH-associated factors promote the phosphorylation of NIK and the activation of NF-κB (Choi et al 2001) (Figure 1). Activation of NF-κB is the dominant pathway from TNFR1 with NF-κB being able to regulate the induction of not only pro-inflammatory genes but also those genes controlling the expression of anti-apoptotic proteins (Beg & Baltimore 1996; Van Antwerp et al 1996; Wang et al 1998a). TNF-α can therefore negatively regulate its own cytotoxicity by the up-regulation of anti-apoptotic genes under the transcriptional control of NF-κB (Van Antwerp et al 1998). Conversely, the inhibition of NF-κB activation can reinstate cytotoxicity. This function of inhibiting TNF-α-induced NF-κB activation is currently under intense investigation as a means of sensitising targeted cancer tumours in chemotherapy treatments (Wang et al 1996; Weldon et al 2001), particularly in prostate cancer (Sumitomo et al 1999) and breast cancer (Keane et al 2000; Biswas et al 2001).

It should be noted that the common inducer hypothesis of IKK by NIK in the IL-1/Toll-like and TNF pathways is now under re-evaluation in the light of recent research findings (Israël 2000; Baud & Karin 2001; Imler & Hoffmann 2001) including an alternative activation route for TRAF6 acting as a ubiquitin ligase, together with co-factors, and mediating polyubiquitin chains required for IKK activation (Deng et al 2000).

IKK complex

Following the cascades from TNF and IL-1/Toll receptors NF-κB activation is under the control of the IKK complex (Israël 2000). This complex consists of three subunits, two catalytic subunits in IKKα (IKK1) and IKKβ (IKK2) and a regulatory subunit, IKKγ (NF-κB essential modulator; NEMO) (Karin & Delhase 2000). Further IKK complexes have recently been characterised and include the phorbol 12-myristate 13-acetate (PMA)-induced IKKε (Peters et al 2000), identical to IKK-*i* (Shimada et al 1999), and TRAF-associated NF-κB activator binding kinase 1/NF-κB-activating kinase (TBK1/NAK) (Pomerantz & Baltimore 1999; Tojima et al 2000) (see review of Peters & Maniatis 2001). The IKKα/IKKβ/IKKγ kinase complex is linked to the induction cascade by activation from a number of MAP3K's that could include NIK, MEKK1/2/3 or TBK1/NAK (Nemoto et al 1998; Zhao

& Lee 1999; Peters & Maniatis 2001). Thus the activation of IKK, and so the NF-κB pathway, is the trigger responsible for the phosphorylation of IκB (Figure 1).

Phosphorylation/ubiquitination of IκB and nuclear translocation of NF-κB

Phosphorylated IκB is recognised by a specific ubiquitin protein ligase (E3) and undergoes poly-ubiquitination that targets the protein for rapid degradation under the control of the 26S proteasome (Karin & Ben-Neriah 2000). The free NF-κB is then translocated into the nucleus where it binds to various target genes (see below). Additionally, cytoplasmic NF-κB subunits can also be a target for phosphorylation. The Rel protein p105 (NF-κB1) is the precursor to the mature protein p50. In association with other Rel proteins (e.g. p65) p105 is phosphorylated and targeted for degradation in a similar manner to IκBα, but the degradation is only partial and thus generates p50. The free NF-κB (p65/p50) is then translocated to the nucleus or associates with IκB. NF-κB binding sites are found in the promoter regions of genes for cytokines (IL-1, IL-6, IL-8 and TNF-α), acute phase response proteins, anti-apoptotic genes and cell adhesion molecules (Schmid & Adler 2000). NF-κB promotes the up-regulation of these proteins and, in a self-regulatory manner, also IκB and p105. This nuclear transport model for NF-κB has recently come under scrutiny and the emerging data suggests a shuttling of NF-κB and IκB between the cytoplasm and nucleus (Carlotti et al 2000; Huang et al 2000; Tam et al 2001). As this dynamic transport system is elucidated further it may offer additional targets for studies in NF-κB modulation.

NF-κB inhibitory compounds from plants

The current understanding of the NF-κB cascade provides the biochemist and natural product scientist with a tantalising opportunity of potential targets. The first plant-derived modulators of NF-κB were reported nearly a decade ago by Kopp & Ghosh (1994) who identified sodium salicylate and its semi-synthetic derivative, aspirin. Following this discovery, a number of new natural products, from various chemical classes, have demonstrated NF-κB-inhibitory activity. Such studies also require good control experiments to determine the target(s) of inhibition: a range of stimulants to activate the cascade; assaying related inflammatory pathways; employing different cell types; specificity tests targeting different sites within the cascade, including IκB degradation, IKK complex, nuclear translocation of NF-κB and DNA binding of NF-κB.

The purpose of this review is to summarise our current state of knowledge of these naturally based NF-κB modulators.

Isoprenoids

Classical pharmacological data indicates that some species and extracts of *Sideritis* (Lamiaceae) have anti-inflammatory activity (Barberan et al 1987) and has led to the identification of andalusol (**1**, Figure 2), a known labdane diterpenoid from *Siderites foetens* Clemen. with anti-inflammatory effects (Navarro et al 1997). This compound is non-toxic (J774 macrophages, thiazolyl blue (MTT) test) and has been shown to reduce NO synthesis (IC50 value (50% inhibitory concentration) of 10.5 μM)[2], via iNOS inhibition, a gene which in turn is under the control of NF-κB. The NF-κB inhibitory concentration of andalusol was recorded as 50 μM (electrophoretic mobility shift assay; EMSA) (de las Heras et al 1999). This activity was found to be more potent when the period of lipopolysaccharide (LPS) stimulation took place for 1 h rather than 2 h.

Recently, members of the kaurene family of diterpenoids (Figure 2) were also found to inhibit NF-κB using the same J774 cell line (Castrillo et al 2001). A number of assays were employed to compare the inhibitory activity of three kaurene and three clerodane diterpenoids based on the level of iNOS in LPS-stimulated J774 macrophages. Here, only the kaurenes (Figure 2) were found to be effective inhibitors of iNOS with IC50 values for structures **2** and **4** of 3–5 μM and 19 μM for structure **3**. EMSA also showed effective inhibition of NF-κB activity based upon the κB consensus sequence of the iNOS promoter.

The kaurene diterpenoids (**2–4**) were further found to act via diminishing IKK activity, specifically IKK-β (25 μM: 70–80% inhibition). However, the Jun amino-terminal kinase pathway was unaffected and immunoprecipitation experiments with FLAG-IKK-β showed no effect on kinase activity by the kaurenes (50 μM) following LPS stimulation. These data suggested that the kaurenes have a specific inhibitory effect on the IKK complex but that it was actually occurring at a step preceding IKK. This hypothesis was tested in transfected cells containing a Myc-NIK expression vector, which triggers IKK-β activation. The kaurenes inhibited the activation of IKK-β in the absence of LPS stimulation. Secondly in LPS-stimulated cells, containing a co-transfection with Myc-NIK and $(\kappa B)_3$ConA.LUC, the kaurenes significantly inhibited the activation of NF-κB in

[2]Where concentrations for compounds in the literature have been quoted in μg mL^{-1} they have, in this review, been quoted in μM for comparative purposes.

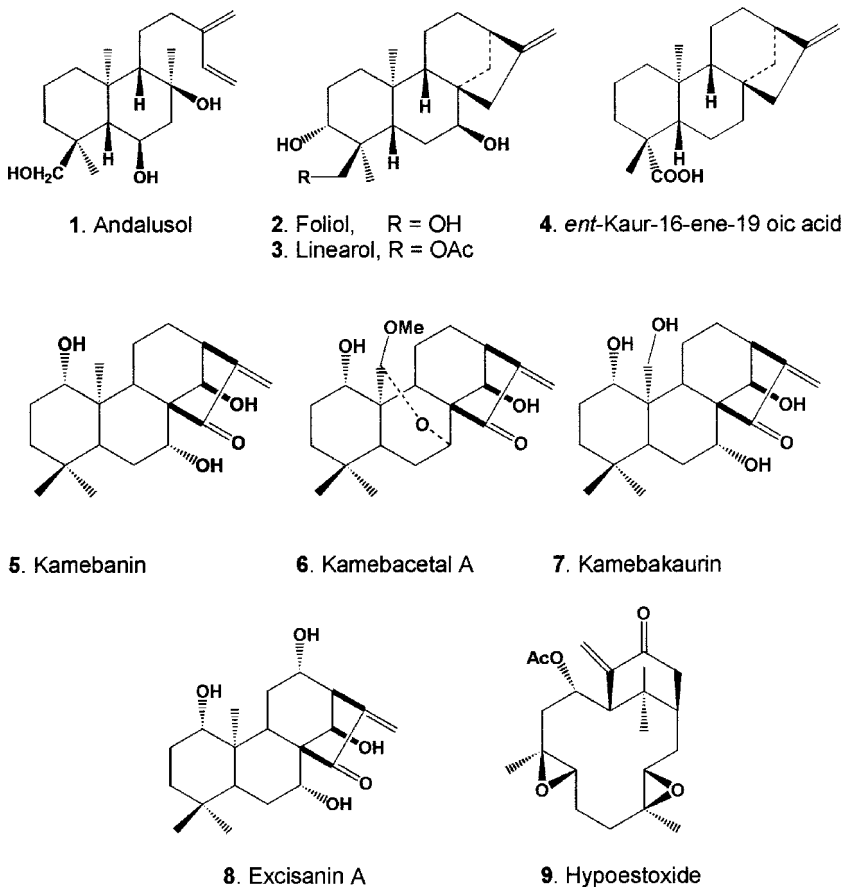


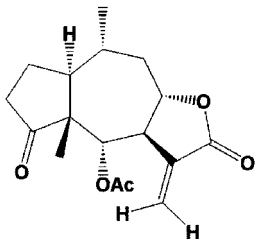
Figure 2 NF-κB inhibitory isoprenoid compounds: kaurene diterpenes (**1–4**), kaurane diterpenes (**5–8**) and cyclic diterpene (**9**).

this reporter gene assay. Taken together, these results show that the inhibitory effect of kaurenes, first identified as IKK inhibitors, operate upstream of IKK as NIK inhibitors and thereby interfere with downstream IKK phosphorylation.

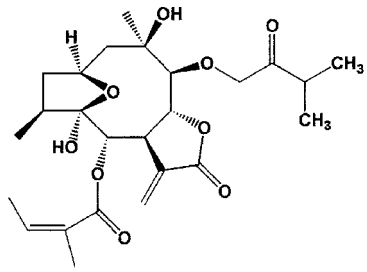
Four known compounds structurally related to diterpenoids **2–4** have recently been identified as inhibitors of NF-κB (Hwang et al 2001). Kaurane diterpenoids **5–8** were isolated from *Isodon japonicus* (Lamiaceae) and found to inhibit NO production and NF-κB activation in LPS-stimulated RAW 264.7 cells. NO production was strongly suppressed by all four compounds at very low IC50 values (0.06 μM, 0.58 μM, 0.15 μM and 0.35 μM, respectively, for **5**, **6**, **7**, and **8**). However, **5** was found to inhibit$<$ 50% cell growth and therefore **7** was identified as the strongest inhibitor of NO production. The most potent inhibition of NF-κB was demonstrated in EMSA by **6** and **7**. These compounds significantly suppressed NF-κB activation at 13.3 μM, with complete inhibition at 26.6 μM.

The diterpene class of isoprenoids are continuously proving to be a fruitful source of inhibitory compounds and models of NF-κB inhibition. Ethnobotanical data was again directly responsible for the discovery of hypoestoxide (**9**, Figure 2), from *Hypoestes rosea* (Acanthaceae), as an anti-inflammatory diterpene specifically targeting IKK (Ojo-Amaize et al 2001). In TNF-α-stimulated HeLa cells, hypoestoxide showed NF-κB inhibitory activity in an EMSA at 50–100 μM and had an IC50 value of 11 μM. However, some specificity for NF-κB was shown by the lack of inhibitory activity (100 μM) against the transcription factors AP-1 (Karin et al 1997) and Sp-1 (Black et al 2001). Additionally this compound at 10 μM inhibits the production of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in LPS-stimulated peripheral blood mononuclear cells. Hypoestoxide was also effective at significantly inhibiting NO production in interleukin-stimulated (IL-1β and IL-17) human articular chondrocytes in a dose-dependent fashion (5, 10 and 50 μM).

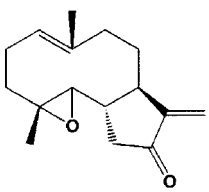
This set of data confirmed the targeted in-vitro anti-inflammatory activity of hypoestoxide but the authors



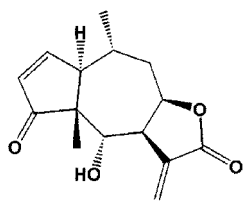
10. Ergolide



11. 2β,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-isobutyloxy-germacran-8α,12-olide



12. Parthenolide



13. Helenalin

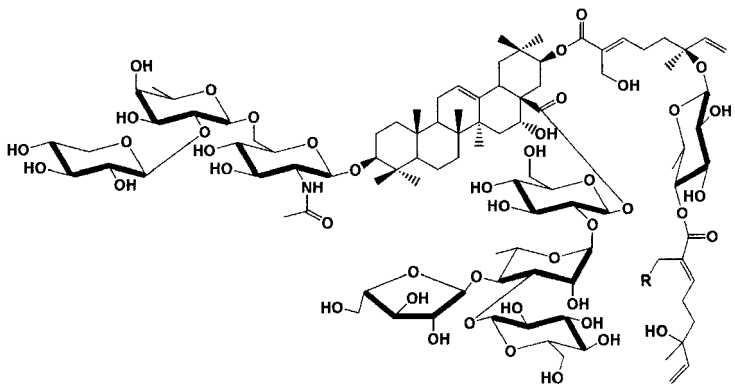
Figure 3 Examples of sesquiterpene lactones as potent inhibitors of NF-κB.

also showed in-vivo efficacy. Topically applied hypoestoxide was shown to possess some inhibitory activity (57%, 3 mg/ear) on mouse ear swelling following application of a phorbol ester. The potency of hypoestoxide was further supported by its lack of toxicity in cell viability assays in peripheral blood mononuclear cells (1.25–100 μM for 72 h).

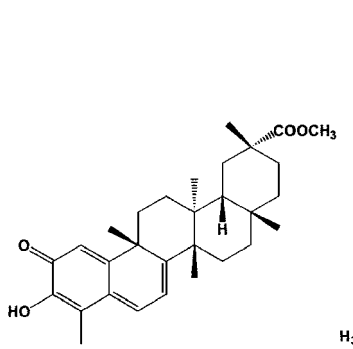
The most widely published class of natural products cited as inhibitors of NF-κB are the sesquiterpene lactones. Ethnopharmacological studies first yielded promising NF-κB inhibitory activity associated with sesquiterpene lactones (Bork et al 1996, 1997). Many reports of inhibitors within this class vary widely in their minimum inhibitory concentration (MIC) values for inhibition of NF-κB. These differences may be due to varying assay techniques and in the structural diversity of sesquiterpene lactone and, as such, various structure/function activities can be identified. The inhibitory effect of sesquiterpene lactones is very strongly enhanced by the presence of such groups as the isoprenoid ring system, a lactone ring containing a conjugated exomethylene group (α-methylene-γ-lactone) (Hehner et al 1998) and an α,β-unsaturated cyclopentenone or conjugated ester moiety (Rüngeler et al 1999). The presence of such groups in sesquiterpene lactones has been significant in eliciting potent inhibitory activity against iNOS-dependent NO synthesis monitored directly (Dirsch et al 2000) and via NF-κB inhibition (Figure 3: ergolide, **10** (Han et al 2001), and 2β,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-isobutyloxy-germacran-8α,12-olide, **11** (Kim et al 2001a)).

The mechanisms of action of some principal sesquiterpene lactones on the NF-κB activation pathway has been investigated and different conclusions drawn. Parthenolide (**12**) from feverfew (*Tanacetum parthenium*, Asteraceae) is a potent inhibitor of NF-κB at low-μM concentrations (5–10 μM, HeLa cells; Hehner et al 1998, 1999). The mechanism of action has been demonstrated in HeLa cells to prevent IκBα and IκBβ degradation (Hehner et al 1998), to act against the IKK complex (Hehner et al 1999) and specifically IKKβ by modification of cysteine 179 (Kwok et al 2001). Parthenolide has also recently been reported to decrease the chemoresistance of breast cancer cells exposed to paclitaxel. This action is related to the over-expression of NF-κB in breast cancer cells that code for anti-apoptotic genes and therefore inhibition could be useful in increasing sensitivity to chemotherapeutic drugs (Patel et al 2000).

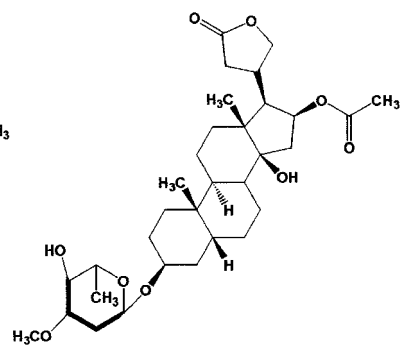
A further sesquiterpene lactone mechanistically studied for NF-κB inhibitory activity is helenalin (**13**) from *Arnicae flos* (Lyss et al 1997), previously shown to be highly cytotoxic (Woerdenbag et al 1994). Helenalin has been postulated to act directly against p65 by alkylation of the NF-κB subunit (Rüngeler et al 1999). No interference with DNA binding has been demonstrated for parthenolide (Hehner et al 1999). Therefore the



14. Avicin D, R = OH
15. Avicin G, R = H



16. Pristimerin



17. Oleandrin

Figure 4 Triterpenoids.

alkylation activity of helenalin could explain its high activity and cytotoxicity (Beekman et al 1997). This class of natural products seems to interfere with a large number of potential target proteins by modifying cysteine residues. Sesquiterpene lactones as a class, although structurally diverse and with many associated therapeutic uses, do possess unspecific toxicity as alkylating agents and presumably this will preclude any useful medical application (Schmidt 1999).

Considerable interest has recently been shown in dichloromethane/methanol extracts of *Acacia victoriae* from which triterpenoid saponins were isolated and termed avicins. A fraction (FO35) of the extract and avicins D (**14**) and G (**15**) were shown to selectively induce apoptosis and decrease tumour-cell proliferation in Jurkat T cells and breast cancer cells (Mujoo et al 2001). Avicin G has also been shown to inhibit activation of NF-κB in TNF-α-induced Jurkat T cells (Haridas et al 2001). Within 4 h of applying 2 μg mL^{-1} (1 μM) of avicin G to TNF-α-stimulated Jurkat T cells, the activation of NF-κB was inhibited by 90%. Avicin G was further shown to act specifically by suppressing the nuclear translocation of NF-κB (the p65 subunit) and NF-κB's binding to the DNA. In addition, the induction of iNOS and COX-2 was also inhibited by avicin G in LPS-treated RAW264.7 cells. Avicins have been highlighted as natural products with the potential in new anticancer therapy to be effective in inducing targeted apoptosis in malignant cells while leaving normal cells unaffected (Croce 2001; Mujoo et al 2001).

A potent inhibitor of NF-κB in macrophages is the triterpenoid, pristimerin (Dirsch et al 1997 and references therein). This compound (**16**, Figure 4) was effective at reducing the induction of iNOS, with IC50 values of 0.2–0.3 μM, in LPS stimulated RAW264.7 macrophages. This activity was ascribed to the inhibition of NF-κB in an EMSA where 0.5 μM pristimerin was sufficient to inhibit NF-κB activation. Here also, some toxicity was noted at 1 μM pristimerin in MTT (37%) and sulforhodamine B (20%) assays. The concentration at which cytotoxicity occurs is only two-to-three times higher than the concentration required to inhibit NF-κB and would give sufficient cause for concern that pristimerin's activity is largely due to cytotoxic effects. A

broad cytotoxicity has been shown for pristimerin in an earlier study with IC50 values of 0.25, 0.45 and 0.1 μM in V-70, KB and P388 cells, respectively (Itokawa et al 1991) – see also Ankli et al (2000) for similar results in KB cells. Pristimerin has also been shown to have some toxicity in MT-29 cells but at a concentration 20- or 30-fold higher than the active concentration for antimalarial activity (Figueiredo et al 1998). Additionally, synthetically derived triterpenoids have been shown to suppress the activation of iNOS, COX-2 and NF-κB (Suh et al 1998).

One recently cited potent NF-κB inhibitor is the cardenolide glycoside oleandrin (**17**, Figure 4) isolated from *Nerium oleander* (Apocynaceae) (Manna et al 2000b). Inhibition of NF-κB activation was recorded at 8.5 μM (90% at 1.7 μM) in U937 cells. A similar level of inhibition (1.7 μM) was found in different cells of human origin (HeLa, CaOV3 and Jurkat) and murine L-929 fibroblasts. A downstream target within the NF-κB cascade was indicated by the potency of oleandrin to inhibit NF-κB in U937 cells stimulated with either TNF-α, PMA or LPS. Inhibition of IκBα was confirmed together with a specificity to suppress induced reporter gene expression of TRAF2 and NIK at a concentration of only 0.85 μM. Therefore oleandrin was postulated to be inhibiting NF-κB via the IKK complex and, consequently, IκBα phosphorylation. Most agents that inhibit NF-κB activation also act against AP-1 and this activity was confirmed for oleandrin by its inhibition of AP-1 in TNF-α-, PMA- or LPS-stimulated U937 cells. The latter data point to an unspecific inhibitory action upon the NF-κB cascade and suggest that oleandrin could act as a kinase inhibitor.

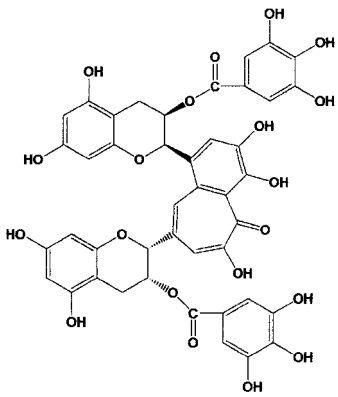
Phenolics

Phenolics have provided numerous examples of compounds with anti-inflammatory activity mediated by inhibition of NF-κB or iNOS in various cell types (Surh et al 2001). However, descriptions of activity do not necessarily equate to potency and the following discussion will illustrate wide variations in cited levels of biological effects. In addition, phenolics often possess antioxidant activity which could make them a non-specific inhibitor of NF-κB by reducing reactive oxygen species, for example quercetin (Musonda & Chipman 1998) and resveratrol (Manna et al 2000a), which could otherwise activate NF-κB (Oettinger et al 1999). However, the evidence of a role for reactive oxygen species in NF-κB activation is limited to a few cell lines and only well characterised in lymphocytes (Ginn-Pease & Whisler 1998; Schoonbroodt & Piette 2000). The experimental evidence for antioxidant compounds, such as vitamin C, inhibiting NF-κB activation in human endothelial cells independently of an antioxidant action (Bowie & O'Neill 2000a) has recently brought into further question the role of reactive oxygen species/antioxidant action in NF-κB activation (Bowie & O'Neill 2000b).

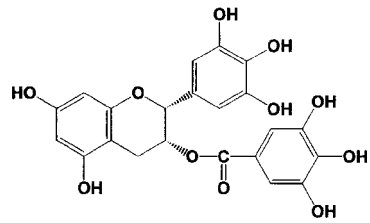
Black tea (*Thea sinensis*, Theaceae) polyphenols of theaflavin derivatives and epigallocatechin-3-gallate (EGCG), were studied for their ability to suppress NF-κB activation in LPS-stimulated RAW267.4 cells (Pan et al 2000b). The authors found that one derivative, theaflavin-3,3′-digallate (**18**, Figure 5), showed a strong inhibition of NF-κB at 30 μM in EMSAs. This activity had the consequence of reducing NO production and iNOS protein in LPS-stimulated cells. Theaflavin-3,3′-digallate was also found to be a potent inhibitor of IKKα expression in RAW264.7 cells (30 μM). Other compounds with moderate activity were EGCG (**19**), geraniin, penta-*O*-galloyl-β-D-glucose and theaflavin. Thearubigin and pyrocyanidin were found to be inactive. In addition, EGCG has been shown to possess NF-κB inhibitory activity against TNF-α-induced activation in normal human epidermal keratinocytes (NHEK) (80 μM) and human epidermal carcinoma (A431) cells (20 μM) (Ahmad et al 2000).

Chinese herb Huang Qui (*Scutellaria baicalensis*, Lamiaceae) has provided a number of structurally related polyphenols that inhibit NO production and NF-κB activation. Wogonin (**20**), baicalin (see also Krakauer et al 2001) and baicalein were all shown to reduce NO production in LPS-stimulated RAW264.7 macrophages with IC50 values of 9.5, 15.0 and 19.4 μM, respectively (Chen et al 2001). This activity was also found to be mediated via inhibition (20–40 μM) of iNOS gene expression, with wogonin also suppressing COX-2. Wogonin, again from *S. baicalensis*, has been shown to have similar activity as an iNOS inhibitor in LPS-stimulated C6 rat glial cells (Kim et al 2001b).

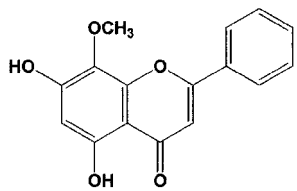
Among a number of compounds isolated from Huang Qui, oroxylin A (**21**) was the most potent inhibitor of NO production in LPS-stimulated RAW264.7 macrophages in a dose-dependent fashion (17.6, 35.2, 70.4 μM) (Chen et al 2000). The anthraquinone emodin had similar activity but also showed some toxicity (30%, 74 μM) against the cells. The same compound was also marginally toxic in human umbilical-vein endothelial cells and complete inhibition of NF-κB was measured in TNF-α-stimulated cells only at 185 μM (90% at 92.5 μM) (Kumar et al 1998). Oroxylin A activity (70.4 μM) was again mediated by iNOS gene expression and this compound, like wogonin, also suppressed COX-2 gene expression. Both these compounds were similar in structure and each contained a methoxy group on the A ring.



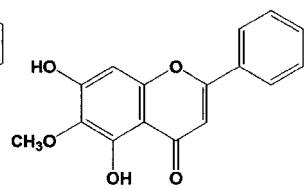
18. Theaflavin 3,3'-digallate



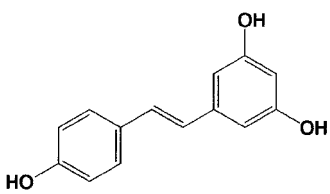
19. EGCG (epigallocatechin-3-gallate)



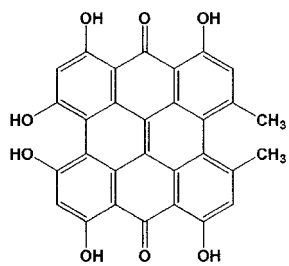
20. Wogonin



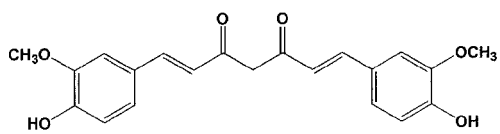
21. Oroxylin A



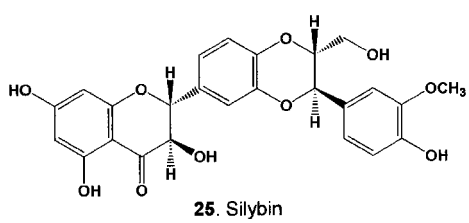
22. Resveratrol



23. Hypericin



24. Curcumin



25. Silybin

Figure 5 NF-κB-inhibiting phenolic compounds.

The gene expression of iNOS in macrophages is known to be under the control of NF-κB (Kim et al 1997) and oroxylin A showed inhibition (70.4 μM) of NF-κB in LPS-stimulated macrophages (Chen et al 2000).

Resveratrol (**22**) is a strong inhibitor (5 μM) of TNF-α-induced NF-κB activation in a number of different cell types, including U-937, Jurkat, HeLa and H4 cells (Manna et al 2000a). The authors found resveratrol to have a broad spectrum of activity with it inhibiting NF-κB activation following stimulation of U-937 cells with either TNF-α, PMA, H_2O_2, okadaic acid, LPS or ceramide. The wide variety of NF-κB activation routes inhibited by resveratrol indicates that the compound is acting in an unspecific manner. Resveratrol was also reported to have inhibitory action against the TNF-α-induced activation of AP-1, c-Jun kinase and MAPK kinase in U-937 cells. Some specificity was found within the TNF-α-induced cascade against the translocation of the p65 NF-κB subunit into the nucleus but independent of IκB phosphorylation/degradation or the binding of NF-κB to the DNA.

The medicinal plant St John's wort (*Hypericum perforatum*, Hypericaceae) has been used for centuries and is popular today as a treatment for mild depressive disorders (Barnes et al 2001). One of the principal constituents of the herb is hypericin (**23**) which has potent activity as a non-antioxidant inhibitor of NF-κB (Bork et al 1999). Hypericin was shown to inhibit NF-κB in HeLa and TC10 cells following stimulation with PMA and TNF-α, respectively. In HeLa cells the inhibitory concentration was 1.98 μM and in TC10 cells was 3.96 μM. However, H_2O_2-induction of NF-κB in HeLa cells was unaffected and therefore showed hypericin was not acting as a radical scavenger. Overall these data suggested that hypericin is acting upon upstream kinases of the NF-κB pathway, particularly protein kinase C, which is implicated in NF-κB induction (Lallena et al 1999), and is directly activated by PMA (Vincenti et al 1992). Hypericin was also effective (3.96 μM) at suppressing TNF-α-induced IL-6 expression and thus illustrated its ability to prevent induced expression of inflammatory genes targeted by NF-κB.

Curcumin (**24**), from *Curcuma longa* (Zingiberaceae), has been studied as an anticancer drug (Levi et al 2001) and has proven effective at inhibiting iNOS in an ex-vivo mouse cell model (Chan et al 1998) and in RAW264.7 murine macrophages via a mechanism of NF-κB inhibition (Pan et al 2000a). In both studies, 10 μM curcumin was sufficient to inhibit iNOS in LPS stimulated conditions against either the mouse ex vivo cells or the RAW264.7 cells. Once again the target of inhibition appeared to be the IKK complex and arresting of IκB phosphorylation (Pan et al 2000a). Curcumin has also been shown to inhibit the activation of NF-κB and AP-1 transcription factor following both IL-1α and TNF-α stimulation in bone marrow cells (Xu et al 1998). An earlier report of curcumin suppression of NF-κB activation placed the inhibitory concentration higher, at 40–60 μM (Singh & Aggarwal 1995). This may be due to the cell type employed (ML-1a vs human leukaemia cells). These authors also showed curcumin to be a non-specific inhibitor of NF-κB by suppressing its activation in cells stimulated with PMA or H_2O_2. The activity in H_2O_2-stimulated cells is in contrast to hypericin (**23**) and shows curcumin acts as a radical scavenger.

Recent evidence has shown that curcumin can also potentiate the apoptotic pathways in prostate cancer cells by inhibiting TNF-α induction of NF-κB (Mukhopadhyay et al 2001).

Silymarin is anti-hepatotoxic mixture of flavonoids widely used in clinical medicine, especially in Germany, and obtained from the seeds of the milk thistle (*Silybum marianum*, Asteraceae) (Valenzuela & Garrido 1994), the principal component of which is silybin[3] (**25**) (Saliou et al 1998). It should be noted that there is some contradiction in the literature over the definition of silymarin and its principal constituents. For example, Manna et al (1999) discuss anti-NF-κB activation activity of silymarin as a distinct compound, even quoting a molecular weight, but the data in their methods section refer to a mixture and in reality the authors were presumably working with the principal flavanone component, silybin. The lack of adequate characterisation of silymarin, either as a mixture or a pure compound, casts doubt on the usefulness of some of the work on compounds from milk thistle.

The silymarin mixture has been shown to completely inhibit NF-κB activation in a hepatoma cell line (HepG2) at 25 μM (Saliou et al 1998). This occurred in cells stimulated with okadaic acid and LPS but not TNF-α. Other transcription factors were unaffected by silybin in okadaic-acid-induced cells. Silybin also inhibited the gene expression of NF-κB at 12.5 and 25 μM, in cells stimulated with okadaic acid but not with TNF-α. Also the gene expression of cells exposed to PMA responded in a similar manner to those exposed to TNF-α whereas cells exposed to LPS responded in a similar fashion to that of okadaic acid exposure. It was postulated that NF-κB inhibitory activity is unconnected to the reducing potential of silybin at the concentrations used. In other cell lines silybin has shown NF-κB

[3]Merck Index 12 (1996): 8680. Silymarin comprised mainly of three isomers: silidianin, silicristin and the main component, silybin (formerly called silymarin). A synonym of silybin is silibinin.

inhibiting potential following stimulation by different activation mediators (Manna et al 1999). In TNF-α-stimulated U937 cells, NF-κB activation was completely inhibited at 50 μM, unlike that in HepG2 cells (Saliou et al 1998). This difference in activity was cited as not being a consequence of cell type because other myeloid cells showed inhibition of TNF-α-induced NF-κB activation, although at higher concentrations of silybin. Gene expression of NF-κB was also completely inhibited at 50 μM of silybin in a reporter gene assay using transfected U937 cells. In other cell types the TNF-α induction of NF-κB was also inhibited but at 10- to 100-fold increase in concentration. Silybin was also able to inhibit NF-κB activation in U937 cells whether stimulated with PMA/okadaic acid or ceramide but not with H_2O_2, indicating different pathways leading to NF-κB activation. Silymarin (50–63 mg kg^{-1}) has additionally been shown to reduce other inflammatory indicators in rat models (De La Puerta et al 1996; Cruz et al 2001).

Ethyl gallate (3–10 μM), present in red wine, has also shown potent activity to inhibit NF-κB in IL-1α- or TNF-α-stimulated vascular endothelial cells (Murase et al 1999). However, other reports of phenols with NF-κB inhibitory activity have all been attained at rather high concentrations. These include genistein (an isoflavone protein tyrosine kinase inhibitor from soybean), 100 μM in PMA-stimulated Jurkat cells (Imbert et al 1996) and 185 μM in a cell-free system (Ishikawa et al 1995), catechin (344 μM), epicatechin (344 μM) and taxifolin (164 μM – toxic at 328 μM) in TNF-α-stimulated RAW 264.7 macrophages (Park et al 2000) and quercetin (50 μM) in IL-1β-stimulated glomerular cells (Ishikawa et al 1999). Quercetin, also a protein tyrosine kinase inhibitor, and genistein inhibit TNF-α-induced NF-κB activation by halting the degradation of IκB but not DNA binding of NF-κB (Natarajan et al 1998). Finally, caffeic acid phenyl ester (CAPE) also inhibited NF-κB in U937 cells (87.5 μM) induced with TNF-α, PMA, ceramide, okadaic acid or H_2O_2 (Natarajan et al 1996), indicating that CAPE may be acting on a point where all these stimulation pathways merge in their activation of NF-κB. CAPE was also specific to NF-κB because the activation of transcription factors Ap-1, TFIID and Oct-1 were unaffected (see also Orban et al (2000) for a modulatory effect of CAPE on apoptosis and NF-κB).

Other compounds and plant extracts

Reports of other plant-sourced compounds possessing NF-κB inhibitory activity are limited but they do include the bisbenzylisoquinoline alkaloid tetrandrine from *Stephania tetranda* (Menisspermaceae) 50 μM in PMA-stimulated Jurkat cells (Ye et al 2000), the cannabinoid, cannabinol, 20 μM in thymocytes stimulated with PMA/Io (Herring & Kaminski 1999), glycyrrhizin from liquorice (*Glycyrrhiza glabra*, Fabaceae; Wang et al 1998b) and sesquiterpene-lactone-containing extracts from *Arnica* species (Lyss et al 1997) and other Asteraceae (Bork et al 1996).

Much of the initial work in the field is based on the systematic screening of collections of plants used in indigenous cultures (Bork et al 1996; 1997). Part of this work also elucidated the NF-κB inhibiting activity of pheophorbide A (92%, 2 μg mL^{-1}) from *Solanum diflorum* (Solanaceae) in PMA-induced HeLa cells (Heinrich et al 2001). Pheophorbide is also a photosensitiser and the authors found the compound to be toxic if the HeLa cells were exposed to light. As pointed out by the authors, the data on NF-κB inhibitory activity should therefore be interpreted with caution. Some plant extracts that have proven effective in inflammation or anti-NF-κB activity include the commonly used phytomedicine stinging nettle (*Urtica dioica*, Urticaceae; Riehemann et al 1999), *Siderites foetens* Clemen. (Lamiaceae) (Navarro et al 2001), a commercially obtained extract of *Ginkgo biloba* (Ginkgoaceae) (Wei et al 1999), *Siderites javalambrensis* (Godoy et al 2000), *Uncaria tomentosa* (Cat's claw, Rubiaceae; Sandoval-Chacón et al 1998), *Drosera madagascariensis* (Droseraceae; Melzig et al 2001), *Tripterygium wilfordii* Hook F. (Sylvester et al 2001) and a bioflavonoid extract from *Pinus maritima* (Pinaceae) (Cho et al 2000).

Non-plant sources of inhibitors

Natural products from marine (Kerr & Kerr 1999; Faulkner 2000) and microbial sources provide a rich source of biologically active or pharmacologically relevant compounds. NF-κB inhibitors from the marine environment include the metabolites cyclolinteinone (D'Acquisto et al 2000) and hymenialdisine (Breton & Chabot-Fletcher 1997; Roshak et al 1997).

A sesquiterpene from marine sponge, *Cacospongia linteiformis*, cyclolinteinone (**26**, Figure 6), was demonstrated to inhibit PGE_2, nitrite production and COX-2 expression in LPS-stimulated J774 macrophages (D'Acquisto et al 2000). This inhibition increased over a dosage range of 12.5–50 μM. Only at 50 μM did significant inhibition of NF-κB-DNA binding occur in an EMSA. A second NF-κB inhibitory marine metabolite, hymenialdisine (**27**), was able to inhibit luciferase activity from two different reporter constructs containing HIV and IL-8 promoter sequences, both of which are activated by NF-κB (Breton & Chabot-Fletcher 1997). The IC50 values were low, at 1.2–2 μM in the transfected U937 cells stimulated with LPS, TNF-α or PMA and

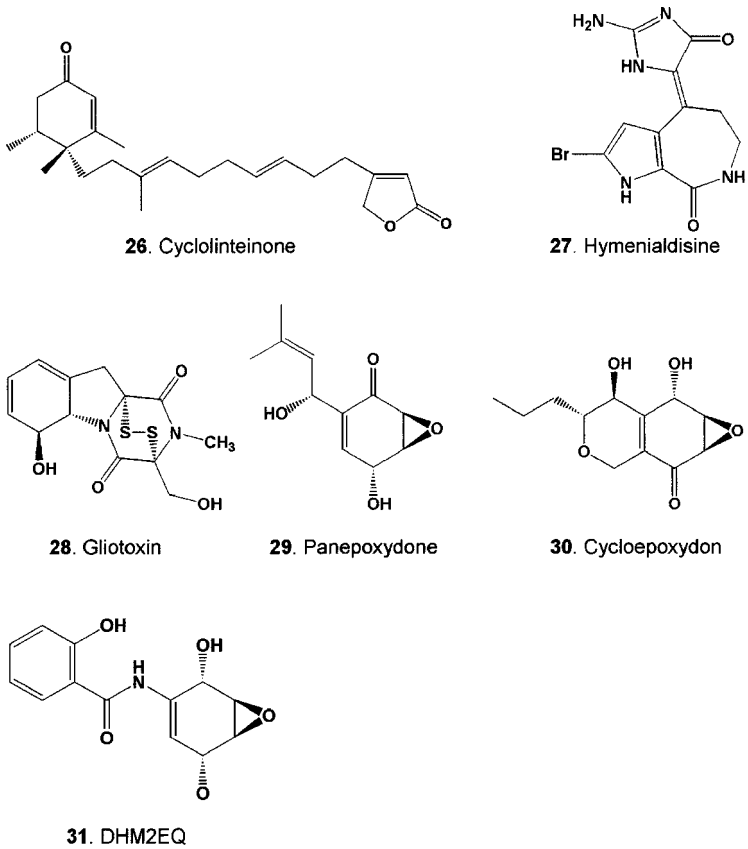


Figure 6 NF-κB inhibiting compounds from sponges (**26, 27**), fungi (**28, 29** and **30**) and a synthetically produced product (**31**).

6.4 μM in the IL-8 transfected, PMA-stimulated cells. In EMSA, following TNF-α stimulation, 1 or 10 μM of hymenialdisine was suffi cient for a 50% drop in NF-κB-binding activity. IL-8 production in U937 cells was also significantly reduced by hymenialdisine (IC50, 0.34–0.48 μM) in cells stimulated with LPS, TNF-α or PMA. In conjunction with this activity, hymenialdisine also inhibits COX-2 and the subsequent production of PGE_2, via inhibition of NF-κB, in an inflammatory in-vitro model consisting of rheumatoid synovial fibroblasts (Roshak et al 1997).

Known microbial-sourced inhibitors of NF-κB activation include the highly toxic gliotoxin (**28**) (Pahl et al 1996), panepoxydone (**29**) (Erkel et al 1996) and cycloepoxydon (**30**) (Gehrt et al 1998). Synthesized derivatives have also been prepared based upon the epoxydone structures and one, DHM2EQ (**31**), was shown to inhibit NF-κB activation without high cell toxicity (Umezawa et al 2000). Gliotoxin is a well known immunosuppressant compound of the epipolythiodioxopiperazine class (Waring & Beaver 1996). Pahl et al (1996) demonstrated the potency of gliotoxin in the mediation of this effect by significantly inhibiting NF-κB at 0.3 μM and almost completely at 2.1 μM in PMA/phytohaemagglutinin-stimulated Jurkat cells. Oct-1 transcription factor was unaffected by gliotoxin at similar concentrations. Gliotoxin (4.2 μM) also suppressed the DNA binding of NF-κB in a luciferase reporter assay upon stimulation with TNF-α, IL-1β and PMA. However, IFN-γ, which is induced by signal transducers and activators of transcription (Levy 1999), could not suppress the induction of intercellular adhesion molecule-1 (ICAM-1). This, and the data for Oct-1, inferred some specificity upon gliotoxin in its ability to inhibit NF-κB activation. Gliotoxin activity was reported to exert its effect through interfering with IκB degradation, although no congruent data was presented for gliotoxin's toxicity in Jurkat cells. The inhibitory effect of gliotoxin in RAW264.7 macrophages has also been studied and it has been found that the cells have 90% viability to the compound at 4.24 μM (Herfarth et al 2000). These authors took the work of Pahl et al (1996) further by showing the suppression of NF-κB activity and the reduced expression of NF-κB inducible genes in-vivo (mice: dextran sulfate

sodium-induced colonic mucosa inflammation). This suppression was seen at an equivalent of 2 mg kg^{-1} in mice as compared with the known LD50 (50% lethal dose) of gliotoxin in mice at ~10 mg kg^{-1}. This level of activity below the toxic dose is encouraging. However, some caution needs to be taken over gliotoxin's potency as an NF-κB inhibitor because it is also a known fungal toxin/pro-oxidant in other cell lines (Zhou et al 2000), toxic in animal models (Frame & Carlton 1988) and as such is precluded from further clinical development (Waring & Beaver 1996). Gliotoxin (**28**) (and curcumin (**24**)) have, though, both been very useful as model inhibitors of NF-κB in studies seeking to elucidate the signalling events of NF-κB activation (Ward et al 1999; Izban et al 2000).

Both panepoxydone and cycloepoxydon suppressed activation of NF-κB in a TNF-α- or 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced COS-7 cell reporter-based assay with IC50 values of 7–9 μM (Erkel et al 1996) and 4–8 μM (Gehrt et al 1998), respectively. AP-1-mediated gene expression was also inhibited by panepoxydone but not cycloepoxydon and therefore the former showed a higher degree of NF-κB inhibitory activity. The synthesized product, DHM2EQ (**31**) (Umezawa et al 2000) was found to be the most active at inhibiting NF-κB activation and had a low toxicity rating. This compound was also a potent inhibitor of type-II collagen-induced arthritis in mice (2–4 mg kg^{-1} body weight).

Conclusions

The NF-κB pathway provides exciting challenges both from a molecular biological as well as from a drug development perspective. In this review we highlight the potential of natural products as potent and pathway-specific modulators of the NF-κB pathway. Two groups of potential targets currently are of particular interest: the IKK complex involved in the integration of the various upstream pathways and the ubiquitination and subsequent degradation of IκB.

Additionally, for example, the nuclear import/export of NF-κB and IκBα and the interaction of NF-κB with the DNA may well prove to be noteworthy targets.

Specifically, the review highlights the potential of natural products and pharmacognostical research into plants as leads for developing potent modulators of the NF-κB pathway. Numerous plant-derived products have been identified as inhibitors of NF-κB activation and such research has shown the usefulness of a multidisciplinary approach.

The effect of some drugs used as standardised or unstandardised extracts can now be explained on a molecular level (e.g. medicinal plants containing sesquiterpene lactones or certain groups of diterpenoids). It is possible to show novel effects of such extracts or mixtures (e.g. *Ginkgo biloba*, silymarin from *Silybum marianum*, *Hypericum perforatum* preparations rich in hypericin and other naphtodianthrones). This research consequently helps in providing evidence for pharmacological effects of plant extracts (whether they are called health food supplements, phytomedicines, indigenous medicinal plants or just botanical drugs). This will aid the further development of plant-based pharmaceuticals for local and international usage as well creating additional possibilities for the small-scale production of medicinal plants with established pharmacological effects. *Curcuma longa* (turmeric), which has been used as a spice for many centuries, is now undergoing pharmaceutical development as a veterinary drug used in the treatment of arthritis in dogs (Phytopharm 2001a).

A large number of pure compounds have been shown to interfere with the cascade leading to NF-κB activation. Notably these compounds generally come from medicinal plants used in indigenous or other medical systems (including European phytotherapy). The biological and cultural diversity may still provide many exciting leads for developing useful pharmaceuticals.

Several of these pure natural products are of no interest to clinical development, but provide very useful tools for elucidating the biochemical mechanism of this and other pathways (e.g. gliotoxin).

A number of important challenges remain. Firstly, compounds which act only on a single target are unlikely to be identified because of the multiple effects generally observed. The pharmacological consequences of these actions have to be studied in detail. Secondly, in-vivo studies on the pharmacological effects of the extracts or plants will be required to assure that the effects are truly of pharmacological relevance. Thirdly, the cell-line specificity of NF-κB activation requires further detailed elucidation before clinically useful pharmaceuticals can be developed to interfere with this pathway. Fourthly, extracts or natural products provide a particular challenge in the field of molecular biology. The information provided must include the characterised or quantified ingredients of an active extract (providing at least an HPLC fingerprint) and for natural products, the evidence of compound purity from HPLC, NMR and MS data. Finally, truly novel natural inhibitors of NF-κB activation derived from local pharmaceutical knowledge require appropriate mechanisms of benefit sharing between the original keepers of traditional knowledge and

the investigators who further develop such products (Heinrich & Gibbons 2001).

The most important challenge remains the threatened loss of cultural and biological diversity due to over-exploitation of the environment and unsustainable use of natural and human resources as well the enormous threat to the cultural diversity of the world – a problem far beyond the scope of this academic review.

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