

Natural Inhibitors of Tumour Necrosis Factor- α Production, Secretion and Function

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Abstract: Tumour necrosis factor- α (TNF), originally discovered by its antitumor activity, is one of the most pleotropic cytokines acting as a host defence factor in immunologic and inflammatory responses. Although the antitumour activity and mediation of inflammation by TNF could be beneficial to the host, unregulated TNF is now known to be the basis for development of various diseases including septic shock, the wasting disease, cachexia, and various inflammatory and/or autoimmune diseases. With an attempt to find potential therapeutic agents for TNF-mediated diseases, research during the last decade has led in the identification of well over one hundred natural inhibitors of either TNF production/secretion or function. This review summarises the structures, mechanism of action and therapeutic potential of these natural products.

Key words: TNF, natural inhibitors, adhesion molecules, NF- κ B, cAMP, inflammation, cytotoxicity.

Introduction

Tumour necrosis factor- α (TNF; also known as cachectin) was originally discovered by its antitumour activity, but it is now recognised to be one of the most pleotropic cytokines acting as a host defence factor in immunologic and inflammatory responses (1), (2). Among its many different activities, TNF has effects on the vascular endothelium which leads to upregulation of various cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial-leucocyte adhesion molecule-1 (ELAM-1) (3). The regulated expression of these adhesion molecules and their counter-receptors on leucocytes mediates the adhesion and extravasation of white blood cells during inflammatory reaction. While mediation of inflammation and anti-tumour activity by TNF could be beneficial to the host, overproduction of TNF is the basis for the development of various diseases. There is now overwhelming evidence to suggest that TNF mediates the wasting disease, cachexia, associated with chronic diseases such as cancer and AIDS (4), (5). TNF also plays pivotal roles in the development of pathologies such as disseminated intravascular coagulation

and death in septic shock and cerebral malaria (6), (7) and a range of inflammatory diseases including asthma (8) and dermatitis, multiple sclerosis, inflammatory bowel disease, cystic fibrosis, rheumatoid arthritis, multiple sclerosis and immunological diseases (9). It is thus clear that suppression of TNF production/release or inhibition of its function could benefit in the treatment of these TNF-mediated diseases.

Potential Targets for Natural Products

The various target sites for modulation of TNF production and function by antibodies and pharmacological agents have been reviewed recently (9), (10). Potential target sites for inhibitory small molecular weight natural products could be broadly divided into three areas: i) inhibition of TNF production and secretion, ii) TNF receptor antagonism, and iii) inhibition of TNF function through modulation of its signal transduction pathway(s). During the 1990's, research in our laboratories and many others world-wide has identified a number of anti-TNF natural products belonging to various structural groups. In this article, the structures and biological activities of these natural anti-TNF compounds are systematically reviewed with reference to the above three modes/sites of action.

Inhibition of TNF Production and Secretion

Monocytes and macrophages are the principal TNF factories in the body but it has now emerged that other cell types including T lymphocytes, mast cells, neutrophils, keratinocytes, astrocytes, microglial cells, smooth muscle cells and many others are known to produce TNF. The induced (e.g., by bacterial endotoxins and proinflammatory cytokines) release of TNF from most of these cells requires *de novo* protein synthesis which can be targeted by drugs at transcriptional, translational and post-translational levels. A large number of natural anti-TNF compounds reviewed in this article act through this mode of action. Most of these bioactive compounds were assessed *in vitro* by using culture of either monocytes/macrophages isolated from human peripheral blood (11–13); alveolar macrophages (12) or most commonly thioglycolate-elicited peritoneal macrophages from experimental animals (mice or rats) (14–17) or established monocyte/macrophage cell lines such as RAW 264.7 cells (18–20). Treatment of monocytes/macrophages for 6–24 hours with proinflammatory agents, but most commonly with lipopolysac-

charide (LPS; *E. coli* endotoxin, 10 ng/ml – 10 µg/ml), results in substantial amount of TNF release which can be quantified by either ELISA (19–21) or using TNF-sensitive target cells such as L929 cells (14), (16), (18). Similarly, the effects of compounds on TNF production *in vivo* is commonly studied by administering LPS in experimental animals such as mice (about 100 µg/mouse) and monitoring the level of TNF as described above.

It is now well established that one crucial step in the transcriptional activation of TNF gene is the transcription factor, nuclear factor κB (NF-κB) mobilisation (22). NF-κB is sequestered in the cytoplasm by inhibitory proteins IκB, which are phosphorylated by a cellular kinase leading to degradation and translocation of NF-κB to the nucleus (23). The potential target of NF-κB in TNF-mediated diseases is evident as inappropriate regulation of NF-κB has been shown to be associated with diseases such as septic shock, graft versus host reaction, acute inflammatory condition, acute phase response, radiation damage, atherosclerosis and cancer (24). Of the known natural inhibitors of TNF production (see also Figs. 1 and 2), the two classical anti-inflammatory agents, aspirin and salicylic acid, have been shown to act *in vitro* through inhibition of IκB degradation and the subsequent inhibition of NF-κB activation (25), (26). Other anti-inflammatory agents reported for inhibition (at µM concentration range) of NF-κB activation and the subsequent TNF release include resveratrol (1) and quercetin (19), (21), (27), nordihydroguaiaretic acid (2), butylated hydroxyanisole and tetrahydropapaverolone (6) (11), curcumin (3) (12), (28), myricetin (27), epigallocatechin-gallate (29), 4-hydroxy-2-nonenal (30), and capsaicin (7) (31). In experiments using BALB/c mice, however, dietary capsaicin (7) did not show the expected immunosuppression and a rather high level of TNF was detected following capsaicin (7) feeding suggesting possible enhancement of the immune functions (32). Large doses (µM concentration) of morphine have also been shown to suppress NF-κB activation and TNF release both *in vitro* and *in vivo* (33), (34). This effect of morphine is in agreement with a high incidence of immunosuppression in opioid addicts (33). Other important observations related to immunosuppression were those of nicotine (maximum effect at 10 nM) and cigarette smoking, both of which were reported to suppress TNF release *in vitro* through inhibition of NF-κB mobilisation (35), (36). The other constituent of cigarette smoke is hydroquinone which is also known to suppress NF-κB activation (37).

Elevation of the intracellular cyclic adenosine 3',5'-monophosphate (cAMP) level in monocytes/macrophages and many other cell types results in inhibition of TNF release and downregulation of the immune response (38). Intracellular cAMP levels can be elevated by activation of cAMP-generating adenylate cyclase (AC). Thus, prostaglandin E₂ (PGE₂) that elevates cAMP through action resulting in the activation of AC has been shown to suppress TNF release *in vitro* and protects animals from septic shock (39). As expected, forskolin (74, at concentrations less than 10 µM), a direct activator of AC, has been shown to inhibit TNF production in various cell types (40 and references therein). Another means of elevating cAMP is through inhibition of cAMP cleavage by phosphodiesterase (PDE) enzymes. PDE type IV has been identified to be the major isoform in the catabolism of cAMP in inflammatory functions in various blood cells (41). While several synthetic PDE

IV inhibitors have been demonstrated to suppress TNF release *in vitro* and inflammation *in vivo* (9), (10), not many natural products have been identified with a similar mechanism. One natural product, theophylline with non-selective action on PDE inhibition has been demonstrated to suppress TNF release but its clinical use is limited due to its weak effect and narrow therapeutic window (42). A very recent work on biological activity studies of citrus flavonoids revealed that several polymethoxylated flavonoids (19–29) potentially inhibited (with IC₅₀ values between 5 and 30 µM) the release of TNF from monocytes *in vitro* (Table 1) (43). The further demonstration of PDE IV inhibition associated with a high level of intracellular cAMP following treatment of cells by these compounds (43) established their possible mechanism of action, although other effects could not be ruled out.

Although the underlying mechanism has not yet been clearly established, many other natural products have been shown to inhibit TNF production (see also Fig. 2). These include the antipsoriatic phenolic compound, anthralin (30) (13); lignans – woorenosides II–V (31–34), lariciresinol glycoside (35) and pinoresinol (36) (44) and eudesmin (37) and to some extent magnolin (38) and lirioreosinol-B dimethyl ether (39) (45); flavonoids – naringin (46), epicatechin (47), some isoprenoid-substituted flavones of *Artocarpus* spp., namely, heterophyllin (42), artonin E (43), artobioxanthone (44), cycloartobioxanthone (45), cycloheterophyllin (46) and morusin (47) (48), and silymarin (49) which is known to be a mixture of silydianin and silibinin; alkaloids – anisodamine (48) (39), (50), quinine (at concentrations that block potassium channels) (51), fangchinoline (49) and isotetrandrine (50) (52) and colchicine (51) (14), (53); terpenoids – cynaropicrin (54), reynosin (55) and santamarin (56) (64) and dehydrocostus lactone (53) (20) and the anti-inflammatory saponin, esculentoside A (57) (15), both of which have also been shown to suppress the serum level of TNF *in vivo*; long chain fatty acids – ω-fatty acids of fish oil origin, docosahexaenoic acid (DHA) and eicosahexaenoic acid (EPA) but also to some extent saturated fatty acids, stearic acid, palmitic acid (55) and anandamide (56). The hepatoprotective compound, tetrahydroswertianolin (40), which inhibits the D-galactoseamine (DGalN)/LPS liver injury in mice has been shown to suppress the serum level of TNF *in vivo* (57). Of the fungal metabolites, the immunosuppressive agent, cyclosporin A has been demonstrated to suppress TNF production both *in vitro* and *in vivo* (58). Another fungal metabolite of interest is trichodimerol (58) (59) as well as tetracycline (60), both of which have been shown to inhibit TNF secretion *in vitro*. Inhibition of TNF release at higher concentrations (mM range) of the antibiotic tetracycline has been shown to be associated with retention of membrane associated TNF (61) suggesting possible metalloproteinase blocking activity. Processing of the TNF precursor (pro-TNF) to mature biologically active TNF by metalloproteinases has been one of the anti-inflammatory target sites for synthetic agents (9), (10). The other possible natural metalloproteinase inhibitor is the immunosuppressive and psychactive agent, δ⁹-tetrahydrocannabinol (41), which has been reported to inhibit TNF production through action at a posttranscriptional level (62). Other natural products of fungal origin with TNF production inhibitory effects are the antibiotic erythromycin (63), cytochalasin D (52) (16), the toxic metabolite aflatoxin B₁ (59) (17) and known kinase inhibitors herbimycin A and staurosporin (see below).

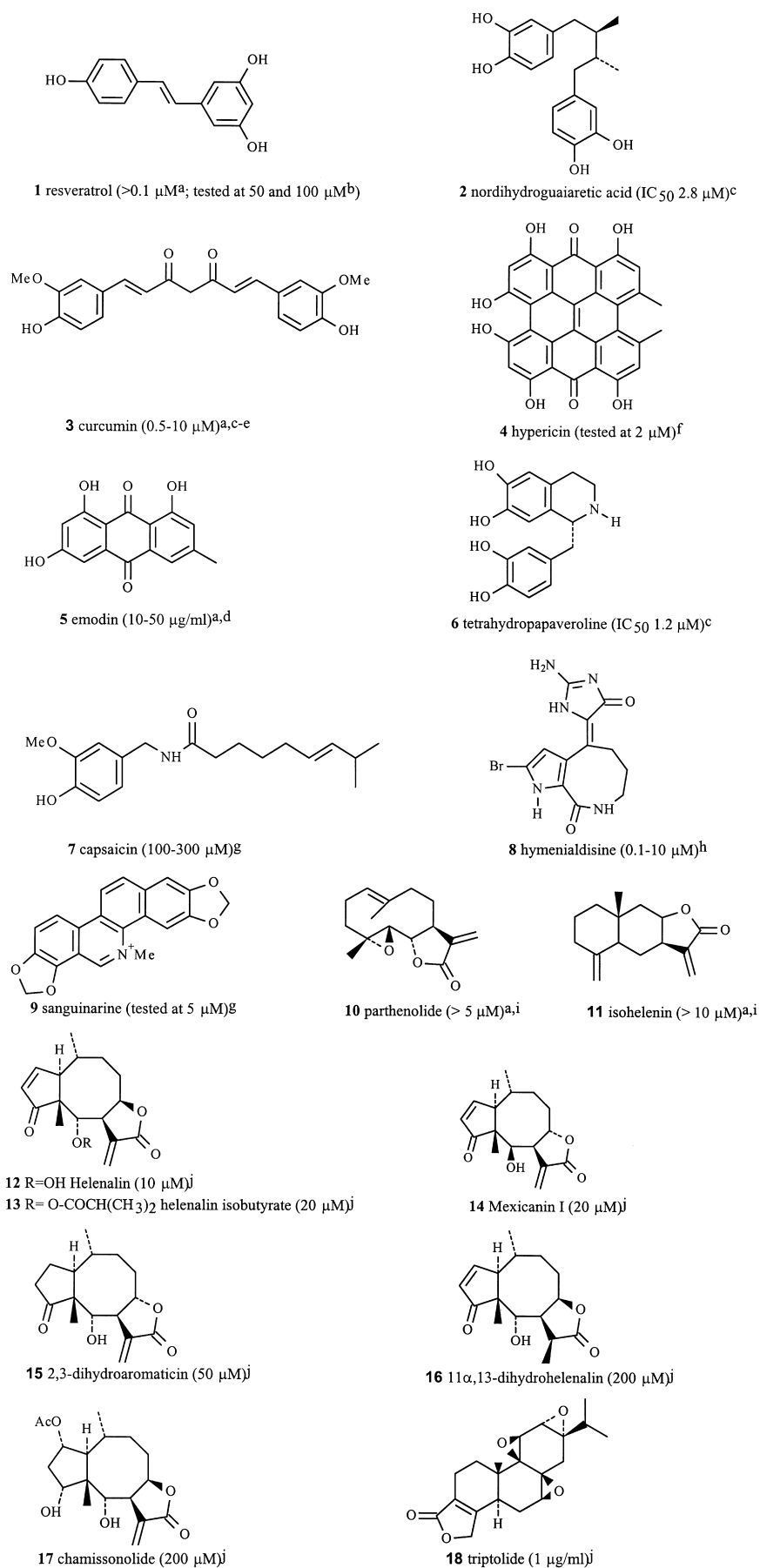


Fig. 1 Structure and activity of some inhibitors of TNF production/function *in vitro*: Inhibitors of NF- κB mobilisation (**1–18**).

^a Adhesion molecules expression (12), (19), (21), (75), (88), (94).

^b TNF release from Kupffer cells (23) and RAW 264.7 macrophages (19).

^c TNF release from peripheral blood mononuclear cells (11), (12).

^d Monocyte-endothelial cell adhesion (88), (94).

^e Endothelial tissue factor expression (IC_{50} 5 μM) (101).

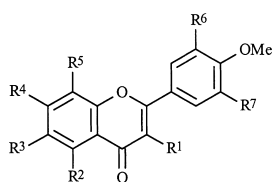
^f IL-6 production in stably transfected HeLa cells (97).

^g NF- κB activation in human ML-1a cells (31), (105).

^h IL-8 production by U937 cells (98).

ⁱ Enhance TNF cytotoxicity in L929 cells (75).

^j Total inhibition of NF- κB activation in Jurkat cells (99), (106).

Table 1 Biological activity of *Citrus* flavonoids.

	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	R ⁷	IC ₅₀ , μM*
19	H	OMe	OMe	OMe	H	H	H	22
20 (tangeretin)	H	OMe	OMe	OMe	OMe	H	H	30
21	OMe	OMe	OMe	OMe	OMe	OMe	H	5
22 (nobiletin)	H	OMe	OMe	OMe	OMe	OMe	H	10
23 (sinensetin)	H	OMe	OMe	OMe	H	OMe	H	8
24	H	OH	OMe	OMe	OMe	OMe	H	5
25	H	OMe	H	OMe	OMe	OMe	H	16
26	OMe	OMe	OMe	OH	OMe	OMe	H	22
27	OMe	OH	OMe	OMe	OMe	OMe	H	10
28	H	OMe	OMe	OMe	H	OMe	OMe	20
29	H	OMe	OMe	H	H	OMe	H	25

*effects of intracellular cAMP elevating flavonoids on LPS (10 ng/ml)-stimulated TNF release from human peripheral blood monocytes *in vitro* (43).

In some instances, the ubiquitous biological activities displayed by some compounds, for example, non-selective inhibition of various enzymes including kinases, inhibition of tyrosine phosphorylation and inositol phosphate metabolism displayed by polyphenols [flavonoids, curcumin (**3**), etc.] and these often seen in combination with antioxidant activity, made it difficult to pinpoint the exact mechanism of action of compounds. It is also worth noting that protein tyrosine kinase activity has been demonstrated to be essential for the activation of NF-κB by various agents and thus inhibition of TNF release by kinase inhibitors is expected (22), (64). For example, the well known natural protein tyrosinase (PTK) inhibitors, genistein and herbimycin A (65), (66) and a non-selective kinase inhibitor, staurosporine (22), have been shown to inhibit TNF production both *in vitro* and *in vivo*.

TNF Receptor Antagonists

As with other protein mediators, the binding surface between TNF and its protein receptors (TNF-R55 and TNF-R75) is expected to be large making it difficult to find a small molecular weight receptor antagonist. Since only a few complementary amino acids have been shown to be critical for TNF activity (67), small molecular weight receptor antagonists, though not available at the moment, could still be the ultimate goal of the TNF-based therapies.

Inhibition of TNF Function

Inhibitors of TNF-mediated cytotoxicity

While TNF toxicity to tumour cells and augmentation of this effect by drugs could be beneficial, the unwanted systemic cytotoxicity of TNF associated with various diseases needs to be modulated. As with TNF release, the cytotoxic response of TNF could be targeted at the various levels of TNF signalling. Experimentally, potential inhibitors of TNF-cytotoxicity could easily be studied using TNF-sensitive cell lines including by far the most commonly used, L929 cells (68–70). In the presence of protein synthesis inhibitors such as actinomycin D (usually 2 μg/ml), TNF induces DNA fragmentation within 6 hours followed by cell death within 24 hours. Based on this

L929 cell-based bioassay, a number of phenolic compounds (3,4-dihydroxybenzoic acid and caffeic acid and also neurotransmitters dopamine and noradrenaline) which possess the catecholic functional moiety have been shown to suppress TNF cytotoxicity (68). These effects of catechols [including that of nordihydroguaiaretic acid (**2**)] are not related to their antioxidant action as related antioxidants failed to protect cells from TNF cytotoxicity (68). Further studies on the mechanism of action of catechols revealed that inhibition of TNF cytotoxicity by catechols is related to their iron chelating activity and the subsequent inhibition of lipoxygenase enzymes (68), (69). On the basis of these findings, large numbers of flavonoids were tested for their inhibitory effect on TNF cytotoxicity *in vitro* (70). It appears that all flavonols tested (galangin, kaempferol, kaempferide, quercetin, myricetin, morin and rutin) inhibited TNF cytotoxicity and the C-3 free hydroxy group of these structural groups appears to play a pivotal role in the observed protective effect. In contrast to this protective effect of flavonols, no protective activity was observed for the flavones (chrysin, apigenin and luteolin) tested. In fact, apigenin and chrysin which possess one (C-4') hydroxy group or none on the B-ring, respectively, enhanced the TNF cytotoxicity (70). Epicatechin and flavanones which bears the catecholic functional moiety (eriodictyol and taxifolin) were protective while other flavanones without this *o*-dihydroxy functionality either failed to protect (pinocembrin, isosakuranetin, hesperetin) or enhanced (naringenin) the TNF cytotoxicity (70). It is worth noting that all protective flavonoids and other catecholic compounds were effective when they were added as post-TNF treatment (70). As some of the flavonoids showed enhancement of the TNF cytotoxicity, the clinical use of such compounds for inhibition of TNF cytotoxicity needs detail analysis of their structural moiety *ex vivo* and *in vivo*.

Together with activation of various cascades, TNF signalling during induction of cell death is also associated with activation of NF-κB. Unlike the TNF production and action on endothelial cells (see below), however, NF-κB activation by TNF antagonises the TNF cytotoxicity (71), (72). Thus, cells including tumour cells, which are capable of activating their NF-κB during TNF treatment are resistant to TNF cytotoxicity and compounds including salicylic acid (73), curcumin (**3**) (74), parthenolide (**10**) and isohelenin (**11**) (75) as well as morphine (76) which down regulate the TNF-mediated NF-κB activation have been shown to enhance while agents that activate NF-κB inhibit the TNF cytotoxicity (71–76). There are now several reports suggesting that PTK is involved in the pathway leading to NF-κB activation and gene expression by TNF (22). Genistein and herbimycin A could thus inhibit NF-κB mobilisation and subsequently make resistant cells susceptible to TNF cytotoxicity (77). Since PTK and/or NF-κB are linked to diverse biological activities, inhibition of TNF cytotoxicity by activating PTK or NF-κB is not an attractive therapeutic approach. TNF cytotoxicity is also modulated by cAMP. The induction of TNF cytotoxicity in neutrophils has been shown to be inhibited by cAMP analogues, intracellular cAMP elevating agent, forskolin (**74**), or phosphodiesterase inhibitors (78).

Other agents that have been reported to inhibit TNF cytotoxicity *in vitro*, but with as yet unknown mechanism of action (see also Fig. 3) include, butylated hydroxyanisole (79), pyridoxin (80), retinoic acid (vitamin A, both the *cis* and *trans* form) and to a lesser extent carotene (81). Two lignanamides,

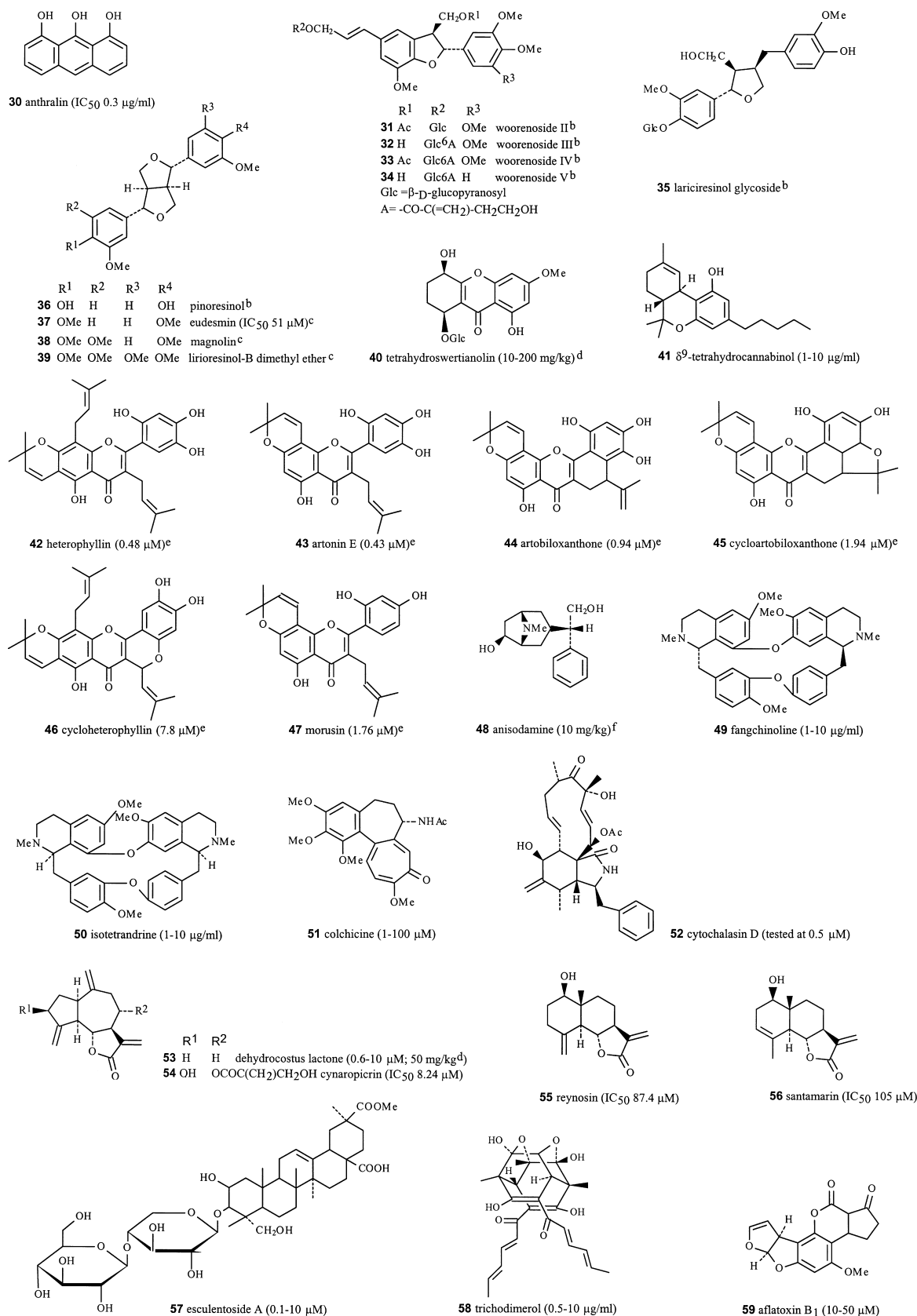


Fig. 2 Structures and activity of some inhibitors of TNF production through as yet unknown mechanism (**30–59**). ^a LPS-induced TNF release from monocytes/macrophages *in vitro* unless otherwise stated. ^b 20–55% inhibition (tested at 25 μ g/ml) of TNF release (44). ^c 20–40% inhibition (tested at 12.5 μ g/ml) of TNF release (45). ^d Effects on LPS-mediated increase in serum level of TNF in mice (57). ^e IC₅₀ values for inhibiting okadaic acid-induced TNF release from BALB/3T3 cells (48). ^f Inhibition of LPS-mediated increase in TNF mRNA in rats (39).

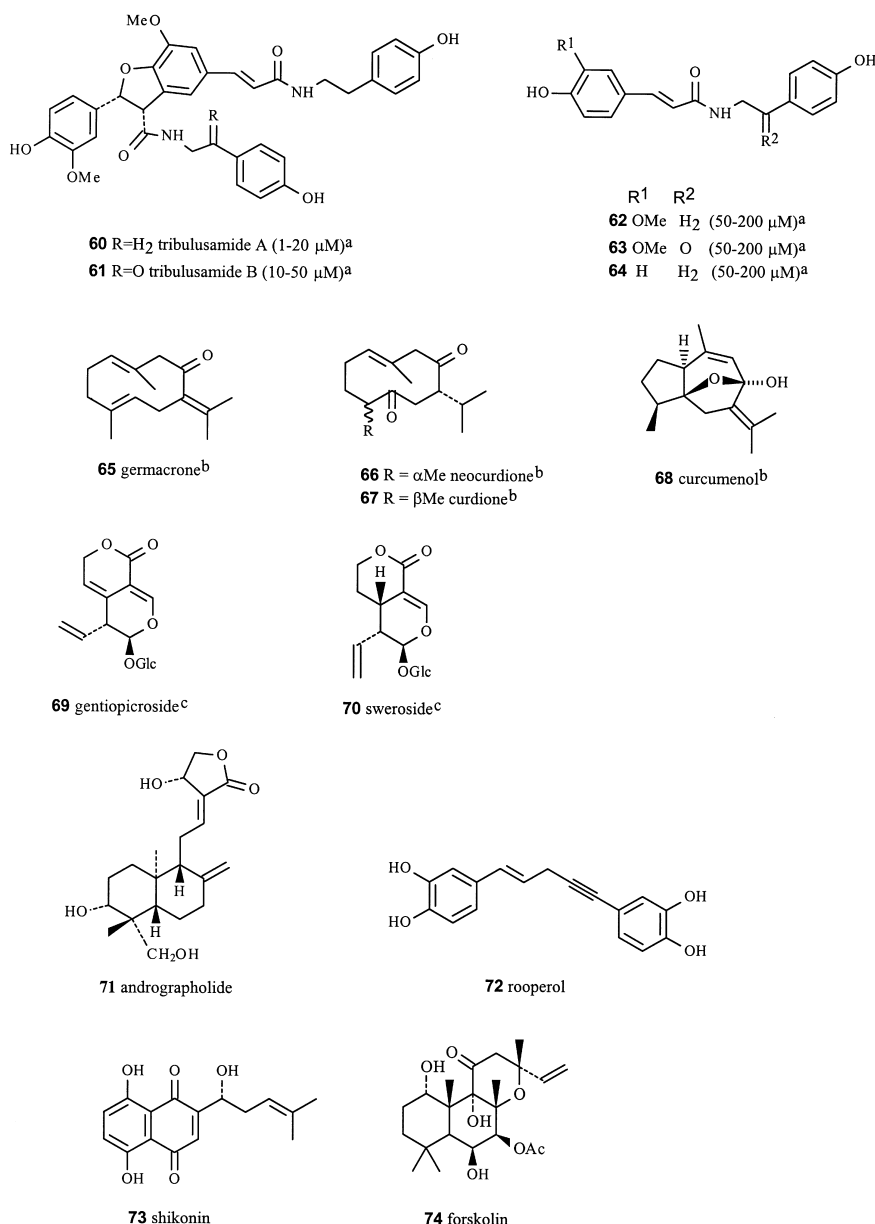


Fig. 3 Compounds with inhibitory effect on TNF toxicity in liver cells (**60–70**).

^a Inhibitory effects (IC₅₀ values) on TNF cytotoxicity in DGalN-sensitized cultured mouse hepatocytes (82).

^b Over 50% inhibition (at a dose of 50 mg/kg, *p.o.*) of liver injury in mice induced by TNF (10 μg/kg) and DGalN (350 mg/kg) (83).

^c Effect on LPS/DGalN-induced liver injury in mice (83), (84).

tribulusamides A (**60**) and B (**61**) and to a less extent compounds **62–64** have been shown to inhibit the TNF cytotoxicity in DGalN-sensitized cultured mouse hepatocytes (82) while sesquiterpene lactones germacrone (**65**), neocurdione (**66**), curdione (**67**) and curcumenol (**68**) have been reported to inhibit *in vivo* liver injury induced by co-administration of TNF and DGalN (83). These latter compounds (83) as well as gentiopicroside (**69**) and sweroside (**70**) (84) have also been shown to inhibit the endotoxin/DGalN-induced liver injury *in vivo*, which is now known to be TNF-dependent (85). Whether the compounds act through direct inhibition of the TNF-induced cytotoxicity in liver cells, however, remains to be proved.

Inhibitors of TNF-mediated protein expression and endothelial-leucocyte adhesion

The TNF-mediated expression of endothelial cell adhesion molecules (mainly ICAM-1, VCAM-1 and ELAM-1) has been

the focus of therapeutic targets in the last decade. Endothelial cell adhesion molecules expression and leucocyte-endothelial cell adhesion have been studied mainly by culturing human umbilical cord endothelial cells and leucocyte sub-populations freshly isolated from human peripheral blood (86–90). The recently characterised endothelial cell lines including EAhy 926 and ECV304 cells together with established monocytic (e.g., U937, HL60 cells) and other cell lines are now routinely used in cell adhesion and adhesion molecules expression studies (18), (27), (88–91 and references therein).

Upregulation of many endothelial cell surface adhesion molecules by TNF involves *de novo* protein synthesis requiring the activation of NF-κB (92). By inhibiting this key activation process, salicylic acid (93), gallates (methyl, ethyl, propyl and octyl gallates) (87), emodin (**5**) (88), curcumin (**3**) (94) and epigenin (95) treatments of endothelial cells have been shown to suppress the TNF-mediated expression of ICAM-1,

VCAM-1 and ELAM-1 and also endothelial-leucocyte adhesions *in vitro*. In contrast, another NF- κ B mobilisation and endothelial-monocyte adhesion inhibitor, aspirin, inhibits the TNF-mediated induction of VCAM-1 and ELAM-1 without altering the ICAM-1 level on endothelial cell surfaces (89). Sesquiterpene lactones, such as parthenolide (**10**) and isohelenin (**11**) (75) as well as several flavonoids (acetin, bicalein, chrysin, chrysoeriol, kaempferol, luteolin and quercetin; IC₅₀ 25–50 μ M) (95) have been shown to inhibit NF- κ B mobilisation and the subsequent expression of ICAM-1. Inhibition of NF- κ B activation has also been shown to account for the *in vitro* inhibition of TNF-mediated cytokine production by curcumin (**3**) (96), hypericin (**4**) (97), hymenialdisine (**8**) (98) and triptolide (**18**) (99); inhibition of monocyte chemoattractant protein expression by quercetin (100) and endothelial-monocyte adhesion inhibitory effect of myricetin (30 and 50 μ M) and other weakly active antioxidants [genistein, resveratrol (**1**) and quercetin] (27). In addition to inhibition of NF- κ B activation, curcumin (**3**) has been reported to inhibit the transcription factor AP-1 thereby suppressing the expression of a coagulation mediator, endothelial tissue factor (101). Other natural NF- κ B mobilisation inhibitors of anti-TNF compounds include DHA and EPA (both tested at 50 μ g/ml) which have been shown to selectively suppress the TNF-induced expression of VCAM-1 (with little effect on the level of ICAM-1 and ELAM-1) and lymphocyte-endothelial cell adhesion (102). In contrast, the monounsaturated fatty acid, oleic acid, suppresses the above three adhesion molecules equally (103). DHA is also known to suppress the TNF-mediated cytokines production in monocytes (104). TNF-mediated NF- κ B activation inhibitory effect of sanguinarine (**9**) (105) has recently been reported. It remains to be proved, however, whether the compound inhibits the TNF-induced gene/protein expression. It is interesting to note that, unlike the sesquiterpene lactones parthenolide (**10**) and isohelenin (**11**) which have been reported to inhibit TNF function through inhibition of I κ B phosphorylation (75), similar compounds **12–17** (Fig. 1) have recently been shown to act through direct alkylation of the activated NF- κ B (106).

As discussed above, PTK is involved in the pathway leading to NF- κ B activation and the subsequent gene expression by TNF (64). Genistein (10–100 μ M) and herbimycin A (0.1–1 μ M), have thus been demonstrated to suppress the TNF-mediated ICAM-1, VCAM-1 and ELAM-1 expression on endothelial cells and the subsequent monocyte-endothelial adhesion (107), (108). Plasminogen activator inhibitor-1 (PAI-1) expression in endothelial cells by TNF was also inhibited by genistein (109) while resveratrol (**1**), with a potent suppressive effect of TNF-mediated ICAM-1 and VCAM-1 expression, is reported for its PTK inhibitory activity (110). PTK inhibition is thus an important anti-inflammatory target site and may account for the anti-inflammatory activity of various non-selective kinase inhibitors such as many flavonoids. Unfortunately, PTK inhibitors have been shown to enhance or suppress the TNF-mediated adhesion molecules expression depending on the concentration used and cell types (91) and hence further research is required to clarify the potential of this therapeutic target. As with TNF production and cytotoxicity, elevation of the intracellular cAMP level in cells has been associated with suppressed expression of cell adhesion molecules by TNF which has recently been shown to be due to inhibition of NF- κ B mediated gene expression (111). The demonstration that forskolin (**74**, tested at 5 μ M), and PGE₂ (0.1–10 μ M) were able to

lower the TNF-mediated lymphocyte adhesion and upregulation of adhesion molecules (ICAM-1 and VCAM-1) expression in smooth muscle cells (112) was thus expected. PGE₂ and prostacyclin are also known to inhibit the TNF-mediated cytokines production (113).

Other compounds with suppressive effects on adhesion molecules expression include the anti-inflammatory diterpene, andrographolide (**71**; 1.8–16.7 μ g/ml), which has been shown to inhibit ICAM-1 expression and endothelial-monocyte adhesion (114); bicalein inhibiting ICAM-1 (IC₅₀ 40 μ M) and ELAM-1 (IC₅₀ 23 μ M) expression (90) and also inhibition of TNF-mediated expression of plasminogen activator inhibitor-1 (115); rooperol (**72**; 2.5–20 μ M) inhibiting VCAM-1 mRNA expression (116); retinoic acid inhibiting TNF-induced cell surface expression of VCAM-1 (117) and shikonin (**73**) inhibiting the $\alpha_v\beta_3$ integrin-dependent endothelial network formation *in vitro* and angiogenesis *in vivo* (118).

A very recent study on the cardioprotective effects of the polyphenolic components of red wine has demonstrated that resveratrol (**1**) inhibits not only TNF production but also the TNF-induced expression of tissue factor in endothelial cells (119). DHA and EPA suppress the TNF-induced production of another proinflammatory cytokine IL-6 in endothelial cells (120) while quercetin inhibits IL-8 and monocyte chemoattractant protein-1 production in synovial cells (121). The TNF-induced induction of inducible-nitric oxide synthase (iNOS) and the subsequent NO production have been shown to be inhibited by aspirin in vascular smooth muscle cells (122) while similar inhibitory effects were reported for curcumin (**3**) (123), quercetin (124), caffeine and theophylline (125), dehydrocostus lactone (**53**) (20), cyclosporin A (140), DHA and EPA (127), (128), and also rooperol (**72**) with inhibitory effect on iNOS mRNA (116).

Conclusions

While the complete biochemical pathways for TNF production and action have yet to be fully elucidated, several target sites have been identified. Of these targets, the transcription factor, NF- κ B is most interesting as NF- κ B regulates TNF production and, in turn, TNF regulates NF- κ B reciprocally to produce its biological effects. A number of natural products widely known for their anti-inflammatory activities has been shown to modulate TNF release and function through suppression of NF- κ B activation. Other targets for natural products that have been demonstrated to inhibit TNF production are kinase enzymes and the cAMP system. There appears to be very few compounds inhibiting TNF production/function at the post-translational level and the highly demanded TNF receptor antagonist is still not available.

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