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Modulating TNF-α signaling with natural products

Atish T. Paul, Vikrantsinh M. Gohil and Kamlesh K. Bhutani

Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), Sector-67, S.A.S. Nagar, Punjab 160062, India

Natural products have been, and continue to be, a major source of pharmacologically active substances from which drugs can be developed. Currently, tumor necrosis factor-α (TNF-α) inhibitors from natural origins are being advanced for the treatment of inflammatory disorders. Elevated TNF-α synthesis has been associated with the development of diabetes, septic shock, tumorigenesis, rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease. Currently, only protein-based drugs are available for the clinical inhibition of TNF-α activity. Small-molecule drugs that can regulate TNF-α levels or activity might provide a cost-effective alternative to protein-based therapeutics. This review briefly highlights the physiological and pathological roles of TNF-α, and covers those natural compounds capable of interfering with TNF-α activity.

Since the identification of tumor necrosis factor-α (TNF-α) as a major proinflammatory cytokine, which regulates inflammation and related disorders, two decades ago, there have been enormous research efforts related to defining its precise biological action, judging by the volume of published articles on the subject, which number in the thousands. Unfortunately, there has not been a concomitant explosion in the number of drugs that have been developed and approved for the treatment of diseases mediated by TNF-α, and those that are available are protein-based and, hence, there are significant cost implications for widespread clinical use.

TNF-α was identified in the mid-1970s by Lloyd Old and colleagues [1] as an endotoxin-induced serum factor that caused the necrosis of certain murine tumors *in vivo*. This biological phenomenon had actually been observed in the latter half of the 19th century when heat-killed bacteria, or products derived from them, were used to induce tumor regression in patients with inoperable neoplastic diseases [2]. These bacterial products (TNF-α and TNF-β) were first isolated in 1984, and research over the past two decades has identified a large superfamily of TNF ligands and receptors.

TNF-α: structure, biosynthesis and receptors

TNF-α is a homotrimeric protein encoded within the MHC. It was first identified as a 17 kDa secreted protein, but subsequent research showed that it exists as a transmembrane protein with a molecular weight of 27 kDa in its uncleaved form [3]. TNF-α mediates its diverse biologic effects through two distinct receptors known as TNF-α receptor type 1 (TNFR1; also known as p60, p55 and CD120a) and TNF-α receptor type II (TNFR2; also known as p80, p75 and CD120b), with apparent molecular masses of 55–60 kDa and 75–80 kDa, respectively. Stimulated macrophages produce 27 kDa TNF-α, which can either bind directly to TNFR1 and TNFR2 receptors through cell-to-cell contact or undergo cleavage and bind to these receptors in its soluble form. Structurally, it resembles a jelly roll, a feature it shares with viral coat proteins, and it has been hypothesized that all these proteins originated from a common ancestor cell [4]. TNF-α only has 36% amino acid sequence homology with TNF-β – also known as lymphotoxin (LT) [5]. Although the sequence homology at the amino acid level is low, the tertiary structures of the two proteins are remarkably similar and both bind to TNF-α receptors. TNFR1 expression is constitutive on all nucleated cells, whereas TNFR2 is primarily restricted to cells of hematopoietic lineage.

TNF-α signal transduction

TNF-α signaling involves various pathways and signaling molecules, and this makes it an interesting and complex process to investigate (Figure 1). Binding of TNF-α to TNFR1 initiates a cascade of events involving the activation of a series of mitogen-activated protein

Corresponding author: Bhutani, K.K. (kkbhutani@usa.net)

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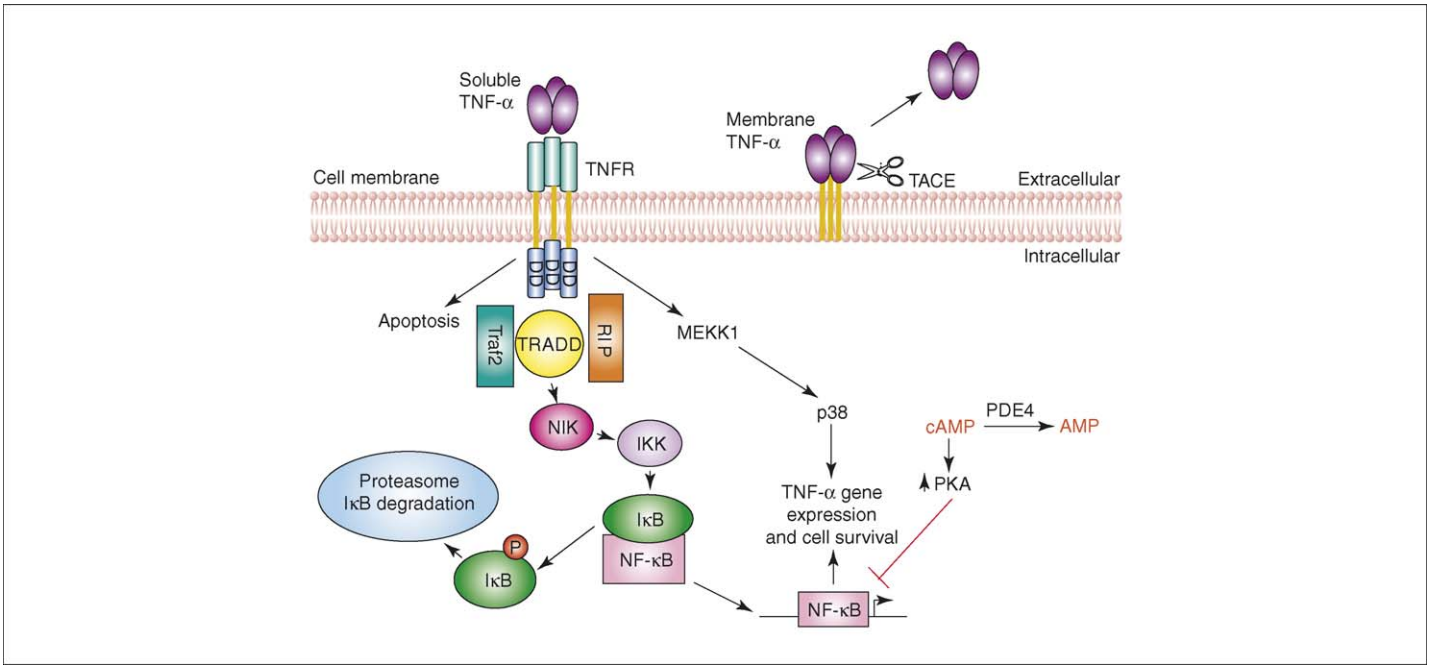

FIGURE 1

Simplified summary of the signaling pathway: The mechanism involves the binding of soluble tumor necrosis factor-α (TNF-α) to TNF-α receptor (TNFR), which leads to upstream signaling. Upstream events involve activation of mitogen-activated protein kinase kinase 1 (MEKK1), nuclear factor-κB-inducing kinase (NIK), inhibitor of nuclear factor-κB (IκB) kinase kinase (IKK), receptor-interacting protein (RIP), TNFR1-associated deathdomain-containing protein (TRADD) and TNFR-associated factor 2 (TRAF2). Activation of p38 results in activation of nuclear factor-κB (NF-κB). Inhibition of the activation of NF-κB prevents the synthesis of NF-κB-inducible genes, which include many proinflammatory cytokines as well as other important inflammation-related proteins. Figure reproduced, with permission, from Ref. [18]. Other abbreviations: DD, death domain; PDE4, phosphodiesterase 4; PKA, protein kinase A; TACE, TNF-α-converting enzyme.

kinase kinases (MEKKs) that further phosphorylate and activate a dual-specificity protein kinase (MEK). This, in turn, activates a mitogen-activated protein (MAP) kinase (e.g. p38 MAPK). Activated p38 MAPK then phosphorylates downstream kinases and nuclear factor-κB (NF-κB). The other major event is the activation of NF-κB. Binding of TNF-α to TNFR1 activates inhibitor of nuclear factor-κB (IκB) kinase kinase (IKK) via TNFR1-associated deathdomain-containing protein (TRADD). Activated IKK phosphorylates IκBα in the NF-κB–IκBα complex. This process releases activated NF-κB from the complex, which then translocates to the nucleus and binds in a sequence-specific manner to target genes, such as cytokines, chemokines and proteases.

Pathological roles of TNF-α

The inappropriate activation of NF-κB in diseases such as diabetes, rheumatoid arthritis, ischemia-reperfusion injury, adult respiratory-distress syndrome, endotoxic shock, tumorigensis and systemic inflammatory response has been attributed to TNF-α and other members of its superfamily. Through the activation of NF-κB, TNF-α induces the expression of various genes such as urokinase plasminogen activator, cyclooxygenase II (COX II) and vascular endothelial growth factor (VEGF) that can be involved in tissue invasion and metastasis. Moreover, activation of NF-κB can suppress apoptosis, which is likely to enhance tumorigenesis. It has also been shown that TNF-α interferes with insulin-signaling by inhibiting the tyrosine kinase activity of the insulin receptor and the serine phosphorylation of insulin receptor substrate 1 (IRS-1) [6]. TNF-α is thought to act on endothelial cells during the inflammatory process, exerting multiple biological effects such as induction of leukocyte adhesion molecules [7,8], proinflammatory cytokines [9,10] and the deposition of fibrin [11,12], in addition to modulating nitric oxide (NO) production [13,14]. Moreover, it might also induce alterations of the endothelial actin cytoskeleton that could lead to the formation of intercellular gaps, hence increasing the permeability to macromolecules [15,16]. Regulation of vascular permeability, together with the induction of leukocyte adherence and procoagulant activity on the vessel surface, are crucial events in the physiological response to several inflammatory or immunological stimuli, as well as in the pathogenic response in several conditions, including endotoxic shock, systemic inflammatory response and adult respiratory-distress syndrome [17].

Natural compounds as potential TNF-α inhibitors

Several protein-based TNF-α inhibitors, including Etanercept (Enbrel®), infliximab (Remicade®) and adalimumab (Humira®), have been approved (http://www.clinicaltrials.gov) for clinical use in various inflammatory diseases (Table 1).

As a class, these protein-based TNF-α inhibitors have demonstrated efficacy and several potentially serious adverse effects that include greater predisposition towards infection, congestive heart failure, neurologic changes (e.g. demyelination), lymphomas, re-exacerbation of latent tuberculosis and problems related to autoimmunity, for example lupus-like syndrome [18] (http://www.fda.gov/cder/present/DIA2004/Tauber_files/frame.htm). Thus, it has become important and essential to develop safer and perhaps more-cost-effective TNF-α inhibitors. In nature, many natural compounds belonging to various classes have been found

TABLE 1

Current status of some of the protein-based tumor necrosis factor-α (TNF-α) inhibitors[a]

Drugs	Indications	Status
Etanercept	Asthma	Phase II
	Ankylosing spondylitis	Phase IV
Infliximab	Ankylosing spondylitis	Approved
	Crohn's disease	Approved
	Dermatomyositis	Phase II
	Polymyositis	Phase II
Adalimumab	Psoriatic arthritis	Phase IV
TNFR:Fc	Uveitis	Phase III
	Arthritis	
	Juvenile rheumatoid arthritis	
Golimumab	Ankylosing spondylitis	Phase III

[a] Data source: http://www.clinicaltrials.gov.

to reduce TNF-α levels. These natural compounds (Figures 2 and 3; Table 2) have been found to interfere with various proinflammatory mediators and upstream targets, such as NF-κB and other signaling molecules, involved in TNF-α expression and, thus, could provide an alternative means of treating inflammatory disease by modulating production, rather than activity, of TNF-α.

Polyphenolic modulators of TNF-α action

Flavonoids are naturally occurring polyphenolic compounds (Figure 2) found throughout the plant kingdom. Flavonoids possess a wide-range of biological activities (e.g. in cancer, as antioxidants) in addition to their anti-inflammatory properties. It has been observed that flavones, flavonols and chalcones are potent inhibitors of the production of TNF-α. Flavanones naringenin **(1)**, anthocyanidin, pelargonidin **(2)** and cyanidin **(3)** exhibit moderate inhibitory activity. By contrast, genistein **(4)**, an isoflavone, possesses weak inhibitory properties, whereas eriodictyol **(5)**, another flavanone, was found to be inactive. Furthermore, it was found that the double bond between carbons 2 and 3, as well as the ketone group at position 4, of flavonoids might be necessary for a potent TNF-α inhibitory activity [19].

However, eriodictyol **(5)**, which was previously found to be inactive in the inhibition of TNF-α synthesis, was found to be capable of inhibiting TNF-α release. Luteolin **(6)**, luteolin-7-glucoside **(7)**, quercetin **(8)** and the isoflavonoid genistein all inhibited lipopolysaccharide (LPS)-stimulated TNF-α and interleukin-6 (IL-6) release, in RAW 264.7 cells. Hesperetin **(9)**, however, only inhibited TNF-α release. Luteolin and quercetin were the most potent at inhibiting cytokine production, with IC_{50}s $<1\,\mu M$ and $<5\,\mu M$, respectively, for TNF-α release. Pretreatment of the cells with luteolin was found to attenuate LPS-induced tyrosine phosphorylation of various proteins. Moreover, luteolin has been found to inhibit LPS-induced phosphorylation of Akt. Treatment of macrophages with LPS resulted in increased IκBα phosphorylation and reduced levels of IκBα. Treating cells with luteolin abolished the effects of LPS on IκBα. In addition, luteolin also inhibited protein tyrosine phosphorylation, NF-κB-mediated gene expression and proinflammatory cytokine production in murine macrophages [20].

Resveratrol **(10)**, an antioxidant phytoalexin from grapes, has been reported to exert anti-inflammatory activities on macrophages. Exposure of cultured rat cortical microglia and a mouse microglial cell line (N9) to LPS enhanced release of TNF-α and NO from both cell types, a phenomenon that was significantly inhibited by resveratrol. Resveratrol appears to suppress the LPS-induced degradation of IκBα, expression of inducible NO synthase (iNOS) and phosphorylation of p38 MAPKs in N9 microglial cells. Thus, resveratrol demonstrates a potent suppressive effect on proinflammatory responses of microglia, suggesting

TABLE 2

Natural compounds interfering with proinflammatory mediators and upstream targets through different mechanisms[a]

Mediators and targets	Compounds that reduce synthesis	Compounds that reduce release	Compounds that suppress activation	Compounds that inhibit phosphorylation	Compounds that inhibit expression	Compounds that activate expression
Tumor necrosis factor-α (TNF-α)	**1,2,3,4,11,12,13, 14,16,20,21,22, 23,25,26,28,30**	**4,6,7,8,9,10,15, 17,18,19,22,25,33**			**20,31,33**	
Interleukin-1β (IL-1β)	**16,28,30**	**15**				
IL-6	**20,30**	**4,6,7,8,15**			**31**	
Nitric oxide (NO)	**21,22,23,25,29,30**	**25**				
Nuclear factor-κB (NF-κB)			**16,20,24,25,29,31**			
Inducible nitric oxide synthase (iNOS)		**33**			**10,30,31,33**	
Cyclooxygenase-II (COX-II)					**15,21,31**	
c-fos and/or *c-jun*	**32**					
p38 Mitogen-activated kinase (MAPK)			**8,32**	**10**		
Prostaglandin E_2 (PGE_2)	**21,25**	**15,25**				
c-Jun amino-terminal kinase (JNK)	**32**		**8,32**	**8**		
Peroxisome proliferator-ctivated receptor-γ (PPARγ)						**27**

[a] See Figures 2 and 3 for compound structures, listed here by their corresponding numbers.

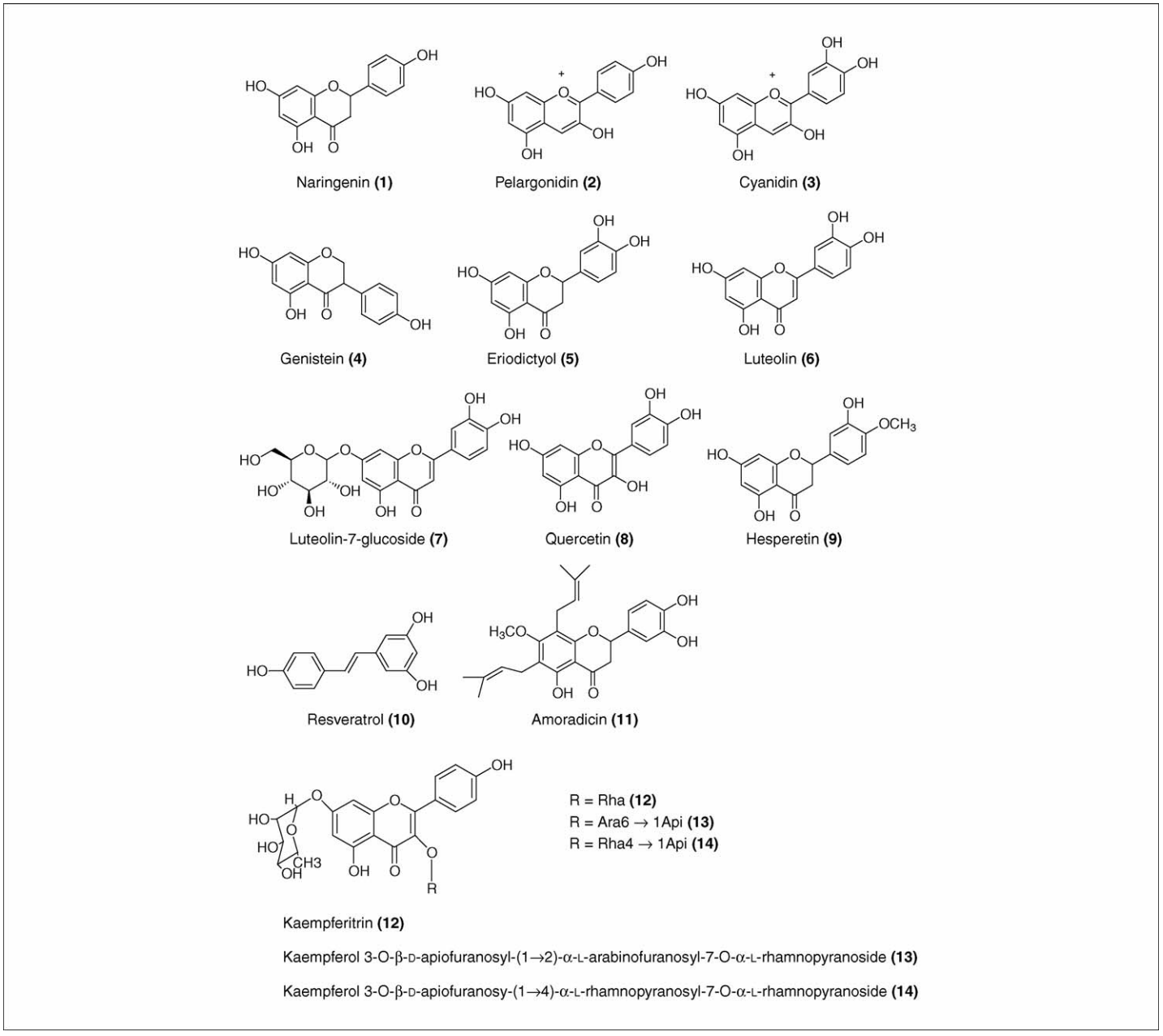

FIGURE 2

Structures of phenolic natural tumor necrosis factor-α (TNF-α) inhibitors (1–14).

potential for the treatment of neurodegenerative diseases that involve microglial activation [21].

Amoradicin **(11)**, a prenylated flavanone isolated from the extract of *Amorpha fruticosa* by bioactivity-guided fractionation, significantly inhibited TNF-α production in LPS-stimulated RAW 264.7 cells, with an IC_{50} value of 28.5 μM. The activity was comparable with or greater than activities of standard flavonoid compounds genistein and silybin – IC_{50}s of 24.9 μM and 140.3 μM, respectively [22].

Kaempferol glycosides, particularly kaempferitrin **(12)**, kaempferol 3-*O*-β-D-apiofuranosyl-(1 → 2)-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside **(13)** and kaempferol 3-*O*-β-D-apiofuranosy-(1 → 4)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside **(14)**, have been isolated from the leaves of *Cinnamomum osmophloeum* Kaneh [23]. Compound **13** has been found to significantly inhibit TNF-α production (41% inhibition) at 10 μM compared with controls, whereas the relative inhibitory effects of compound **12** (at 50 μM) and compound **14** (at 40 μM) were 44% and 21%, respectively.

Scopoletin

Scopoletin (Figure 3) **(15)**, isolated from the aqueous extract of *Artemisia feddei,* was found to inhibit the release of TNF-α, IL-1β, IL-6 and prostaglandin E_2 (PGE_2) over a dose range of 1–50 μg/ml. It also suppressed the expression of COX-II in a concentration-dependent manner. These results suggest that scopoletin prevents the release of these proinflammatory cytokines and exerts an inhibitory activity on LPS-induced PGE_2 production through the suppression of COX-II expression [24].

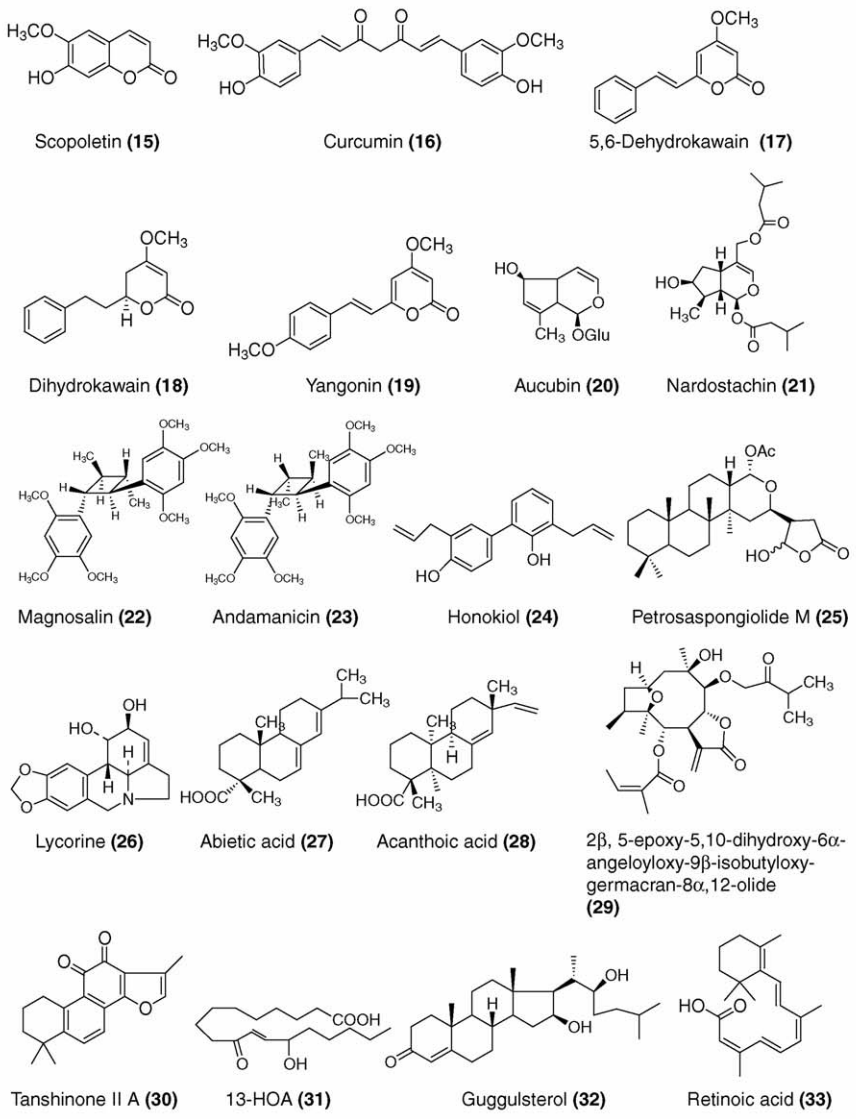

FIGURE 3

Structures of natural tumor necrosis factor-α (TNF-α) inhibitors. Phenolic compounds (15–24) and non-phenolic compounds (25–33) are shown.

Curcumin

The rhizome of the plant *Curcuma longa* Linn has been used widely in India and Indonesia for the treatment of inflammation since ancient times. Curcumin (Figure 3) **(16)** is a naturally occurring phytochemical present in these rhizomes. At a concentration of 5 μM, it inhibits LPS-induced production of TNF-α and IL-1β in a human monocytic macrophage cell line, MonoMac 6, *in vitro*. In addition, it has been demonstrated that curcumin, at the same concentration, also inhibited LPS-induced activation of NF-κB and reduced the biological activity of TNF-α in a fibroblast lytic assay in L929 cells [25].

Kawapyrones

Kawa (*Piper methysticum*) is a local beverage on the island of Fiji, which has been suggested to be responsible for the low incidence of cancer in natives of this island. Five known kawapyrones (kavapyrones), in addition to a new kawapyrone, 7,8-epoxyyangonin, have been isolated from methanol extracts of kawa [26]. 5,6-Dehydrokawain (desmethoxyyangonin) **(17)** and yangonin **(19)** were found to inhibit significantly TNF-α release from BALB/3T3 cells treated with okadaic acid, with IC_{50} values of 17 μM and 40 μM, respectively. Dihydrokawain **(18)** was the most potent inhibitor of TNF-α release in mice, but was relatively inactive in BALB/3T3 cells.

Aucubin

An iridoid glycoside, aucubin **(20)** has been reported to be a common constituent of many traditional oriental medicinal plants. The effect of aucubin on TNF-α and IL-6 expression has been studied in antigen-stimulated rat basophilic leukemia (RBL)-2H3 mast cells [27]. Aucubin inhibited antigen-induced TNF-α and IL-6 production in a dose-dependent manner with IC_{50}s of 0.101 μg/ml and 0.19 μg/ml, respectively. Maximal inhibition of TNF-α and IL-6 production was 73% (± 4.3) and 88.8% (± 5), respectively. Thus, aucubin was found to be a specific inhibitor

of NF-κB activation in mast cells, which might explain its beneficial effect in the treatment of chronic allergic inflammatory diseases.

Nardostachin

Nardostachin **(21)**, an iridoid isolated from *Patrinia saniculaefolia*, has been found to inhibit the production of NO and TNF-α in a dose-dependent manner, with IC_{50} values of 12.3 μM and 16.2 μM, respectively. In addition, this compound has been shown to reduce expressed COX-II protein levels and PGE_2 production in LPS-stimulated macrophages [28].

Magnosalin and andamanicin

Two neolignans, magnosalin **(22)** [1β,2α,3β,4α-1,2-dimethyl-3,4-*bis*-(2,4,5-trimethoxyphenyl)-cyclobutane] and andamanicin **(23)** [1α,2β,3β,4α-1,2-dimethyl-3,4-*bis*-(2,4,5-trimethoxyphenyl)-cyclobutane], isolated from the leaves of *Perilla frutescens*, inhibited NO synthases (IC_{50}s = 5.9 μM and 53.5 μM, respectively) and TNF-α in LPS-activated RAW 264.7 cells [29]. Compounds **22** and **23** have also been tested for their ability to reduce TNF-α activity and TNF-α levels in cell-culture media, determined in the L929 cell cytotoxicity assay. Administration of compound **22** (10 μM) to cells produced a relative cytotoxicity (26%), compared with LPS controls. This would suggest that compound **22** decreased TNF-α release from activated cells and, hence, reduced cytotoxicity against L929 cells. The inhibition of TNF-α production by compound **22** was greater than compound **23**, which gave 84% of control cytotoxicity at a concentration of 10 μM.

Honokiol

Honokiol **(24)** is a lignan isolated from *Magnolia officinalis* that has been shown to suppress NF-κB activation and NF-κB-regulated gene expression through the inhibition of IKKs. Honokiol has been found to inhibit the production of NF-κB-regulated inflammatory and carcinogenic gene products, including matrix metalloproteinase-9 (MMP-9), TNF-α, IL-8, intercellular adhesion molecule 1 (ICAM-1) and monocyte chemotactic protein-1 (MCP-1) [30].

Petrosaspongiolide M

Petrosaspongiolide M **(25)**, a marine metabolite from the Caledonian marine sponge *Petrosaspongia nigra,* reduced the production of nitrite, PGE_2 and TNF-α in a mouse air-pouch model of inflammation [31]. It was found to be a potent inhibitor of the NF-κB pathway at a concentration of 1 μM. Petrosaspongiolide M potently inhibited the release of nitrite, PGE_2 and TNF-α in a concentration-dependent manner.

EGb 761

EGb 761 [a standardized extract of *Gingko biloba* containing 24% flavonoid glycosides of mainly rutin and quercetin and 6% unique terpenes (3% bilobalide and 3% ginkgolides A, B and C)] and quercetin, its aglycone component, have selective effects on TNF-α and the MAPK cascade. Although both EGb 761 and quercetin **(8)** inhibit TNF-α secretion in LPS-stimulated RAW 264.7 macrophages, the results have suggested that quercetin is unique in its ability to inhibit TNF-α transcription by inhibiting phosphorylation and activation of c-Jun amino-terminal kinase (JNK)/stress-activated protein kinase (SAPK); therefore suppressing activation of the transcription factor AP-1. EGb 761 was found to diminish LPS-induced NF-κB transcriptional activity slightly, but it had no effect on TNF-α transcription. EGb 761 and quercetin can also inhibit TNF-α production at the post-transcriptional level. ERK1/2 and p38 MAPK activities, which are important in the post-transcriptional regulation of TNF-α mRNA, can also be inhibited by EGb 761 and quercetin [32].

Other chemical classes capable of modulating TNF-α action

Alkaloids

Lycorine **(26)** and lycoricidinol inhibited TNF-α production from murine macrophages stimulated with LPS (with IC_{50} values of 0.2 μg/ml and 0.002 μg/ml, respectively). Lycorine and lycoricidinol have also been reported to inhibit protein biosynthesis – at 1 μg/ml and 0.008 μg/ml. Although the inhibition of TNF-α production by lycoricidinol was mainly caused by an overall, non-selective inhibition of protein biosynthesis, lycorine was capable of inhibiting TNF-α production at lower concentrations than those required to inhibit overall protein synthesis in macrophages. These data suggest that inhibition of TNF-α production by lycorine and lycoricidinol is not necessarily just caused by the inhibition of protein translation, at least at lower concentrations [33].

Terpenes

Abietic acid **(27)** suppresses the expression of genes involved in inflammation, such as TNF-α and COX-II, in activated macrophages. At a concentration of 50 μM it inhibits TNF-α (16.3%) and COX-II (75.6%) protein expression through the activation of peroxisome proliferator-activated receptor-γ (PPARγ). Also, it was found to regulate the expression of PPARγ target genes including *αP2*, *LPL*, and *FAT/CD36* in 3T3-L1 adipocytes or RAW 264.7 macrophages [34].

Acanthoic acid **(28)** is (−)-pimara-9(11),15-dien-19-oic acid, a pimaradiene diterpene isolated from the Korean medicinal plant *Acanthopanax koreanum* [35]. Acanthoic acid (0.1–10.0 μg/ml) inhibited the production of IL-1β and TNF-α by up to 90% in human monocytes and macrophages stimulated with silica, but the production of IL-6 was not inhibited at all. At these concentrations there were no cytotoxic effects on human monocytes and macrophages.

The compound 2β,5-epoxy-5,10-dihydroxy-6α-angeloyloxy-9β-isobutyloxy-germacran-8α,12-olide **(29)**, another terpene identified as sesquiterpene lactone from *Carpesium divaricatum,* also decreased NO production in LPS–IFN-γ-stimulated RAW 264.7 cells in a concentration-dependent manner, with an IC_{50} of ~2.16 μM; however it was found to have no direct effect on the iNOS activity of fully LPS–IFN-γ-stimulated RAW 264.7 cells. Treating cells with compound **29** resulted in reduced levels of iNOS protein and mRNA. These effects appeared to be caused by inhibition of NF-κB activation through a mechanism involving the concomitant stabilization of the NF-κB–IκBα complex, followed by a reduction in nuclear translocation of the p65 subunit of the NF-κB–IκBα complex [36].

Tanshinone II A **(30)**, a diterpene isolated from *Salvia miltiorrhiza* root, inhibited the production of TNF-α, IL-1β and IL-6 in activated RAW 264.7 cells in a dose-dependent manner

(0.34–34.0 μM). It also inhibited the expression of iNOS (3.4–34.0 μM) in a dose-dependent manner, and NO was also inhibited with an IC_{50} of 20 μM [37].

Fatty acids and their derivatives

The functionally novel fatty acid (±)-13-hydroxy-10-oxo-*trans*-11-octadecenoic acid (13-HOA) **(31)**, derived from linoleic acid (LA) by corn and rice lipoxygenase (LOX), markedly attenuates the expression of proinflammatory genes in LPS-stimulated macrophages via a blockade of the NF-κB and AP-1 pathways [38]. At higher concentrations, 10-ODO, 13-HOA and 9-HOA exhibited profound suppressive effects on the expression of iNOS, COX-II, IL-6 and TNF-α, with the following sensitivity: COX-II > IL-6 > iNOS > TNF-α. The ability of 13-HOA to attenuate highly the expression of proinflammatory genes such as COX-II, iNOS, TNF-α and IL-6 makes it an important lead for the development of TNF-α inhibitors.

Sterols

The anti-inflammatory properties of *Commiphora mukul* gum have been known since ancient times, and it has been used in various traditional systems, including Ayurveda and traditional Chinese medicine (TCM). It has been possible to demonstrate downregulation of inflammatory mediators such as interferon-γ (IFN-γ), IL-12, TNF-α, IL-1β and NO following administration of an ethyl acetate extract of the gum [39]. Guggulsterol **(32)**, isolated from this gum extract, did not inhibit MAP kinase (ERK), but it could reduce *c-fos* and *c-jun* mRNA levels in phorbol 12-myristate 13-acetate (PMA)-stimulated cells. This reduction in *c-fos* and *c-jun* levels, taken together with the inhibition of MAPK activation, provides a possible mechanism by which crude ethyl acetate extracts and purified guggulsterol might exert their actions.

Retinoids

Retinoic acid **(33)**, an active metabolite of vitamin A, attenuated TNF-α (29–97%) and iNOS (61–96%) mRNA expression in microglia exposed to either β-amyloid peptide (Aβ) or LPS, in a dose-dependent manner (0.1–10.0 μM). The inhibition of TNF-α and iNOS mRNA expression in activated microglia, induced by retinoic acid **(33)**, was accompanied by a concomitant reduction in the release of iNOS and TNF-α [40].

Conclusions

The clinically approved protein-based TNF-α inhibitors are capable of reducing TNF-α activity, but can have serious side effects. The recently reported side effects of the blockbuster COX-II inhibitor (COXIB) series of non-steroidal anti-inflammatory drugs (NSAIDS) (http://www.fda.gov/cder/drug/infopage/COX2/default.htm), combined with other disadvantages of these protein-based anti-TNF-α drugs, has been a driver for the natural-product chemist to search for and develop alternatives. Low-molecular-weight natural compounds can have many advantages, not least cost-effectiveness (compared with current protein-based drugs) and route of administration. Many natural compounds, particularly the phenolics, terpenes and, to a lesser extent, alkaloids, have been found to inhibit the upstream signaling molecules that are involved in TNF-α expression. To increase the number of leads from the natural-compound libraries for TNF-α modulating activities, there is a need to develop HTS protocols. Further, for better understanding of natural-compound library SAR, extensive *in silico* studies can and should be carried out. Thus, drugs derived from natural-compound leads, either alone or in combination (synergistically) might provide an alternative approach for the treatment of inflammatory diseases via modulation of the TNF-α signaling pathway.

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