Natural products and their role as inhibitors of the pro-inflammatory transcription factor NF-κB

Paul Bremner & Michael Heinrich*
Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX; *Author for correspondence (Tel: +44-20-7753-5844; Fax: +44-20-7753-5909; E-mail: phyto@ams1.ulsop.ac.uk)

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Abstract
Nuclear factor kappa B (NF-κB) is a transcription factor involved at the downstream stage of many signalling cascades and plays a crucial role in acute and chronic inflammatory conditions, induced and adaptive immunity, apoptosis and induced cell proliferation. For the last decade it has attracted intense research interest throughout the world. This review briefly expands on information on biogenic modulators of this transcription factor published since an earlier review in 2002 and goes into much more detail on parthenolide and curcumin – two natural products that have been at the centre of molecular and biochemical interest in identifying modulators of the NF-κB pathway. Both compounds have attracted considerable attention in regard to the mechanistic aspects of the compounds’ action. An attempt to further develop some compounds into leads for new medicines are also discussed, focusing on an EU-funded project on anti-inflammatory natural products, mostly from medicinal plants used locally in selected Mediterranean regions.

Introduction
In 2002 we published a comprehensive review of natural small molecule inhibitors of NF-κB (Bremner and Heinrich, 2002). More recently, Calixto et al. (2003) provided another broad review on natural compounds that interfere with the pro-inflammatory signalling cascades. Consequently, a review of the phytochemical diversity of NF-κB inhibitors would seem to have little relevance at this point. Instead, this review briefly expands on the data published since 2002 and deals in detail with the natural products that have been at the centre of molecular and biochemical interest as modulators of the NF-κB pathway – parthenolide and curcumin – highlighting the intensive research activities that focus on two natural product leads. Studies of the mechanistic aspects of the compounds’ action are discussed. Specifically, this review highlights three streams of research and current understanding in the role of plant natural products as inhibitors of the pro-inflammatory transcription factor, nuclear factor kappa B (NF-κB):

1. Since the discovery of the NF-κB family of rel-protein transcription factors and the associated transduction pathways, there has been intense research to discover inhibitory elements of the cascade, in the hope that this may lead to the discovery of anti-inflammatory therapeutic drugs. Some recent discoveries have increased this expectation after the discovery that NF-κB signalling also plays a role in cancer. The first part of this review outlines the basis of NF-κB signalling.
2. The potential that published natural products may act as inhibitors of NF-κB is broad. Published reports differ widely in their assay regimes, but a relatively small number of products are worth highlighting. This is particularly relevant in order to raise phytochemical research on particular products to a ‘higher’ level of pharmaceutical relevance.

3. What developments are there regarding new discoveries in small molecule inhibitors of NF-κB? A recent EU-funded project, which ran until January 2004, aimed to identify new inhibitors of NF-κB by combining different areas of European expertise in ethnobotany/ethnopharmacy, molecular pharmacology, phytochemistry and molecular biology. This project (Anti-Inflammatory Natural Products, AINP1) is outlined here as an example for this research area.

Biochemical and molecular biological summary of nuclear factor kappa B signalling

The nuclear factor kappa B (NF-κB) family of transcription factors in mammals is attracting intense research interest throughout the world (there is an analogous Drosophila system, see Hoffmann et al., 1999; Silverman and Maniatis, 2001). This is because NF-κB has a transcriptional regulatory role, the gene products of which are involved in induced and adaptive immunity. The transcription factor is also known to target genes whose products induce anti-apoptotic and cell proliferation processes. Therapeutic agents are therefore achievable in the arena of pro-inflammatory conditions and also cancers – if a modulation/inhibition of the activation pathway for NF-κB could be achieved. It is this therapeutic potential that drives research on this topic and has generated controversial patent activity (Ready, 2002).

NF-κB is the ‘family name’ of a group of proteins, all of which possess the Rel homology domain (~300 amino acids in length) in their N-termini. In mammalian cells there are five NF-κB subunits, divided into two groups. Group one consists of RelA (or p65 or NF-κB3), c-Rel and RelB. The second group includes p105 and p100, which are the larger proteins processed to produce the mature p50 (or NF-κB1) and p52 (or NF-κB2) proteins, respectively. The Rel domain mediates their DNA binding and dimerization; indeed, most combinations of homo- and hetero-dimers are possible within the family, but the most predominant is p50-RelA.

The principle route of NF-κB activation is based on the so-called canonical (or ‘classical’) NF-κB pathway. Activation stimuli (inflammatory cytokines e.g. TNF-α, IL-1, IL-6) at the cell surface begin the process of NF-κB activation and initiate a cellular cascade that results in the release of active NF-κB subunits. A detailed knowledge of this pathway of NF-κB induction (Ghosh et al., 1998; Ghosh and Karin, 2002) has revealed particular targets for intervention in a number of therapeutic areas (Karin et al., 2004), especially cancer (Lin and Karin, 2003; Perkin, 2004) and inflammation (Barnes and Karin, 1997; Zingarelli et al., 2003).

This pathway is mediated by the family of inhibitory IκB proteins of NF-κB, consisting of IκBz, IκBβ, IκBe and IκBγ. The cytoplasmic form of NF-κB (i.e. p50/RelA) is bound to IκB and is localised in the cytoplasm by the shielding of NF-κB’s nuclear localisation signal (NLS). More detailed structural studies have revealed that IκBz masks the NLS of RelA but not that of p50. Therefore a shuttling model of the RelA-p50/IκBz has been proposed to maintain a cytoplasmic concentration of RelA-p50/IκBz (Yamamoto and Gaynor, 2004), although its biological significance is still being investigated (Ghosh and Karin, 2002). In response to the activation stimuli, IκB is phosphorylated by the IκB kinase (IKK). IKK is composed of two catalytic subunits, IKKα and IKKβ, and a third regulatory unit, IKKγ (or NEMO). The canonical pathway depends on IKKβ which phosphorylates IκB, leading to ubiquitin-dependent proteolysis of IκB, mediated by the 26S proteosome (Karin and Ben-Neriah, 2000).

The non-canonical pathway involves processing of the p100 protein. p100 is retained in the cytoplasm as a RelB-p100 heterodimer. Upon activation, the processing of p100 depends on the NF-κB inducing kinase (NIK) and IKKα. Ubiquitin-controlled proteasomal partial degradation of p100 results in the generation of RelB-p52. This NF-κB active dimer can then translocate to the nucleus and bind to target genes on the DNA. The canonical pathway is essential in innate immunity, while the