Russian Chemical Reviews **77** (11) 967 – 981 (2008) © 2008 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2008v077n11ABEH003881

Low-molecular-weight inhibitors of NF-κB signalling pathways

N G Dolinnaya, E A Kubareva, E V Kazanova, N A Zigangirova, B S Naroditsky, A L Gintsburg, T S Oretskaya

Contents

I.	Introduction	967
II.	Signal transduction pathways leading to NF-κB activation	968
III.	Nuclear factor κB as a redox-sensitive transcription factor	969
IV.	The role of NF-κB in the development of inflammatory diseases and carcinogenesis	970
V.	Inhibitors of NF-κB activity	972
VI.	Conclusion	979

Abstract. The nuclear factor κB (NF-κB) is a transcription factor involved in inducible expression of cellular genes playing a key role in cardiovascular pathologies, carcinogenesis, inflammatory and viral diseases. The review describes the stimuli and processes inducing NF-κB activation and the components of a signalling cascade that could constitute targets for NF-κB inhibition. The molecular action and properties of various low-molecular weight inhibitors aiming to prevent NF-κB activity are summarised. The bibliography includes 120 references.

I. Introduction

The nuclear factor κB is one of the key switches in the signalling cascades related to the development of inflammatory and cardiovascular diseases, carcinogenesis and stress response. It is the main inducer of inflammatory response and negative regulator of apoptosis. The broad spectrum of biological action of NF-κB is due to its ability to regulate transcription of genes the products of which determine the cell response to environment conditions.

N G Dolinnaya, E A Kubareva, E V Kazanova, T S Oretskaya
Department of Chemistry and A N Belozersky Institute of Physico-Chemical Biology M V Lomonosov Moscow State University, 119991 Moscow, Leninskie Gory, Russian Federation. Fax (7-495) 939 31 81,
tel. (7-495) 939 31 53, e-mail: dolinnaya@hotmail.com (N G Dolinnaya),
tel. (7-494) 939 31 48, e-mail: kubareva@belozersky.msu.ru (E A Kubareva), evkazanova@mail.ru (E V Kazanova),
tel. (7-495) 939 54 11, e-mail: oretskaya@belozersky.msu.ru (T S Oretskaya)
N A Zigangirova, B S Naroditsky, A L Gintsburg N F Gamaleya Research Institute for Epidemiology and Microbiology, Russian Academy of Medical Sciences, ul. Gamaleya 18, 123098 Moscow, Russian Federation. Fax (7-499) 193 61 83,
tel. (7-499) 193 61 36, e-mail: zigangirova@mail.ru (N A Zigangirova),
tel. (7-499) 190 74 69, e-mail: bsnar@yahoo.com (B S Naroditsky),
tel. (7-499) 193 30 01, e-mail: gintsburg@riem.ru (A L Gintsburg)

Received 4 June 2008
Uspekhi Khimii **77** (11) 1036 – 1052 (2008); translated by Z P Svitanko

The transcription factor NF-κB was first described in 1986 as a heterodimeric protein of B lymphocytes, which is bound to an enhancer of immunoglobulin κ-light chain genes.[1] During subsequent studies it was shown that this protein is a prototype of a family of transcription factors comprising a site of high homology with the oncogen Rel product.[2] In mammal cells, the family of transcription factors NF-κB/Rel is represented by proteins p65 (RelA), p50, c-Rel, RelB, and p52, and precursor proteins p105 and p100. All these proteins share a highly homologous N-terminal domain composed of 300 amino acid residues that are responsible for DNA specific recognition, dimerisation and nuclear localisation.[3] Proteins of the NF-κB/Rel family form homo- and heterodimers possessing different abilities to initiate gene expression, this function being dependent on the nature of the target gene.[4] The heterodimer comprising p50 and p65 subunits is the most common form *in vivo*.

The NF-κB/Rel-dependent signalling pathway is among the most important and best studied regulator systems in eukaryotic cells. The normal function of NF-κB is regulation of cellular processes such as immune response, cell adhesion, apoptosis and proliferation. The central role of NF-κB as an immune regulator is indicated by its conservation during evolution. In the cell nucleus, the transcription factor NF-κB is bound through transactivation domains to the DNA κB-site comprising 10 base pairs 5′-GGGRNNYYCC-3′ (where R = A or G, Y = T or C, N = A, G, T or C).[5–7] Note that the sequence of some active DNA κB-sites can differ from the above-presented one. According to X-ray diffraction data (resolution 2.3 Å) for the complex of p50 homodimer and an oligonucleotide duplex containing the κB site 5′-GGGAATTCCC-3′, the peptide loops of the protein form a contact surface with heterocyclic bases in the DNA major groove and the sugar phosphate backbone (Fig. 1).[5,6] The precise protein – nucleic acid contacts in the NF-κB – DNA complex in solution were characterised by means of chemical modification of biopolymers, by using mutant proteins and covalent protein – nucleic acid binding followed by isolation of the peptide fragment involved in the complexation.[8,9]

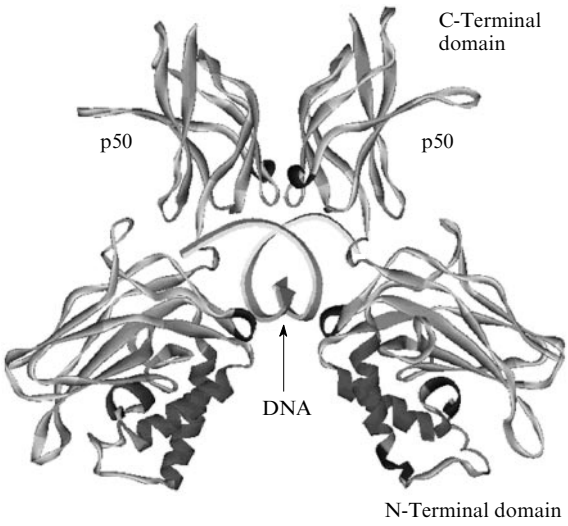


Figure 1. Structure of the complex formed by p50 homodimer of the human nuclear factor κB with κB-containing oligonucleotide duplex (X-ray diffraction data).[5]

It was found that κB sites are located in promoters of many genes encoding proinflammatory proteins and immune and stress response proteins such as cytokines (including interleukins IL-1, IL-2, IL-6, IL-8 and other), interferon γ, tumour necrosis factor (TNF), cell adhesion molecules, histocompatibility class I molecules, enzymes, growth factors, transcription factors, apoptosis regulators [10,11] including inhibitors of apoptosis proteins (IAP). Antiapoptosis proteins actively affect the signalling pathways that regulate programmed cell death and innate immunity, which in turn results in a decrease in the activity of the key apoptosis regulator, p53 protein.[12] Such external stimuli as hormones, cytokines, growth factors and physical treatment are the main NF-κB activators. Being bound to the promoters of its target genes, activated NF-κB regulates the biosynthesis of tens of proteins and factors responsible for physiological and pathophysiological cellular processes.[13] Its main purpose is to switch the cells from one developmental program to another in order to retain the functions of organs and organism as a whole.

The nuclear factor κB and components of its signalling cascade are currently regarded as promising therapeutic targets the inhibition of which contributes to preventing cardiovascular and oncological diseases, immune disorders, and inflammatory pathologies. In this review we concentrated attention on the biological action of low-molecular-weight inhibitors of NF-κB both isolated from natural sources and prepared by synthesis.

II. Signal transduction pathways leading to NF-κB activation

It is known that only B-cells and blood plasma cells of mammals use NF-κB for their development.[14] In other cells, the NF-κB resides in cytoplasm in an inactive form bound to an inhibitory proteins IκB. The family of inhibitory proteins IκB includes the proteins IκB-α, IκB-β, IκB-γ, IκB-δ, IκB-ε and Bcl-3. In non-stimulated cells, the proteins IκB bind specifically to both NF-κB subunits, which prevents them from being transported to the nucleus.[15,16] Presumably, the IκB-α protein not only masks the NF-κB site responsible for the translocation of the latter to the nucleus, but also contains a specific site of nuclear export owing to which the newly synthesised IκB-α molecules penetrating into the nucleus can 'retrieve' NF-κB already located there and take it back to the cytoplasm. The IκB-β and IκB-ε proteins are unable to perform such nuclear export. The role of each inhibitory protein in the regulation of NF-κB was studied for mouse embryonic fibroblasts knocked-out for different types of IκB.[17]

The complex of the inhibitory protein IκB with NF-κB dissociates upon phosphorylation of serine residues (Ser32 and Ser36) of IκB with protein kinases.[18–20] The liberated NF-κB enters the nucleus where it initiates the transcription of target genes encoding the signalling and protective proteins, proinflammatory cytokines and so on. Phosphorylation of IκB triggers its ubiquitation (with protein ubiquitin, Ub) and degradation by 26S-proteasomes. The high-molecular-weight complex of kinases (IKK) comprising IKKα and IKKβ catalytic subunits and IKKγ regulatory subunit (or NEMO, NF-κB Essential Modulator), is responsible for phosphorylation of IκB needed for activating NF-κB. By knock-out for different types of kinase complex subunits, their role for activation of NF-κB was identified. It was shown that IKKβ gene disruption is fatal, whereas IKKα gene suppression results in considerable violation of biological processes. The IKKβ and IKKγ

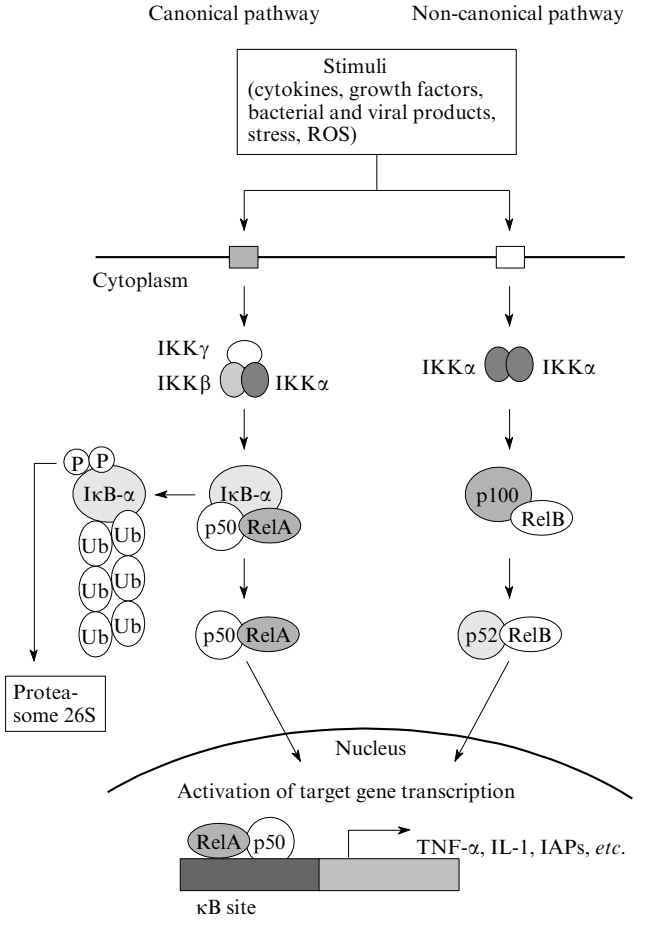


Figure 2. Canonical and non-canonical pathways of NF-κB activation.

pair ensures the canonical NF-κB activation pathway *via* phosphorylation and degradation of IκB,[21–23] whereas IKKα is responsible for the alternative pathway the components of which are other subunits of the transcription factor NF-κB; in particular, nuclear translocation takes place for the p52/RelB dimer.[24,25] The non-canonical pathway for NF-κB activation is essential for the development of lymphoid organs and adaptive immune response but not for other NF-κB-dependent functions such as apoptosis inhibition and innate immunity. The general NF-κB activation scheme is shown in Fig. 2.

III. Nuclear factor κB as a redox-sensitive transcription factor

The redox-sensitive transcription factors, in particular NF-κB, AP-1, *etc.*, are responsible for the change in the redox balance of the cells. This is estimated as the ratio of reduced and oxidised SH groups in proteins or, in a more general case, the ratio of pro- and antioxidants.[26] The activation of redox-sensitive cell elements by external stimuli and agents that induce oxidative stress and stimulate production of reactive oxygen species (ROS) changes expression of several hundreds of genes and, correspondingly, activates many enzymatic processes. Therefore, it is not surprising that these regulatory elements serve as essential units of cell proliferation and differentiation, induction of apoptosis and development of multiple drug resistance.

Reactive oxygen species are generated endogeneously by aerobic cells as intermediate products of metabolic transformations. The incorporation of oxygen into metabolic pathways of living organisms is related to its activation to form a number of more reactive compounds. The action of internal and external factors such as UV radiation, electromagnetic fields, X-rays, sonication, radiation, of pharmaceutical agents, smoking gives rise to excited singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{-\bullet}$), hydroxyl radicals ($HO^\bullet$), peroxide radicals ($HO_2^\bullet$), peroxide ions (HO_2^-), hydrogen peroxide (H_2O_2), nitrogen(II) oxide (NO), organic peroxides and radicals in the cell.[27]

A route for the formation of superoxide radicals is reduction of oxygen in the reaction with NADPH catalysed by plasmatic membrane oxidases:

$$O_2 \xrightarrow{\text{oxidase}} O_2^{-\bullet}.$$

In addition, superoxide radicals arise in reactions involving xanthine oxidase, microsomal monooxygenases, lipoxygenase and cyclohexenase and upon self-oxidation of thiols, flavins, quinones, catecholamines, which are the products of reductive cycle of xenobiotics.[28]

The superoxide radicals are inactivated by superoxide dismutase:

$$2\,O_2^{-\bullet} + 2\,H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2.$$

Hydroxyl radicals are formed in the following chemical reactions:

$$H_2O \longrightarrow H^\bullet + {}^\bullet OH \text{ (hydrolysis)},$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^\bullet OH + OH^- \text{ (the Fenton reaction)},$$

$$H_2O_2 + O_2^{-\bullet} \longrightarrow O_2 + {}^\bullet OH + OH^- \text{ (the Haber–Weiss reaction)}.$$

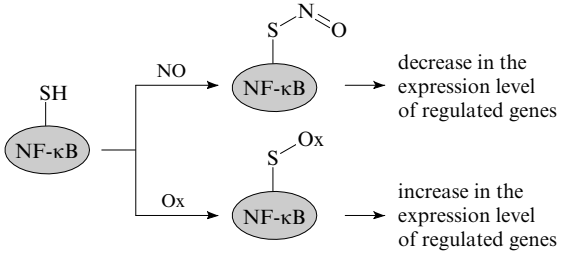
Nitrogen oxide is generated upon oxidation of L-arginine by NO synthase (NOS). The NO molecules having one unpaired electron are highly reactive and easily react with oxygen, superoxide radicals or transition metals. Reactive nitrogen oxide species, $NO^\bullet$, NO^+ and NO^-, are stabilised in biological complexes with thiols ($RS^-\cdots{}^+NO$), nitrites ($O_2N^-\cdots{}^+NO$), and other targets and intermediates.[29]

It is known that ROS promote organism functioning. They are involved in cell proliferation and differentiation, transfer of external signals, ion transport, immune response. Reactive oxygen species play the important role in protecting the organism from microbial and viral infections, in the fight against tumour cells, and in supporting intracellular communication.[30]

However, pronounced oxidative stress results in cell death or its malignant transformation. Ageing of cells and organism as a whole is also related to the activity of free-radical processes involving ROS, which cause non-specific changes of biopolymers. For survival under enhanced oxygen content, mammal cells developed a protection system, which includes the use of low-molecular-weight antioxidants (glutathione, vitamins A, C, E and so on) and ROS-inactivating enzymes such as superoxide dismutase, selenium-dependent glutathione peroxidase, catalase, glutathione reductase. The oxidative stress conditions enhance the expression of genes encoding both the antioxidative protection enzymes and enzymes involved in drug metabolism.[31,32] The level of proteins involved in the immune and antiinflammatory protection system also increases.

During evolution, organisms have learned to use unique properties of ROS to regulate the activity of many enzymes and transcription factors.

Hydrogen peroxide generated during breathing process or exogeneous can itself activate NF-κB in macrophages. This process is highly specific, as hydrogen peroxide in the concentrations used does not affect the proteins.[33] It was also found that the family of Rel proteins contains a conserved cysteine residue (Cys62) in the N-terminal domain; its oxidation with hydrogen peroxide increases, and nitrosylation decreases, binding of NF-κB to the recognition site in DNA and, hence, the expression of genes it regulates.[34]

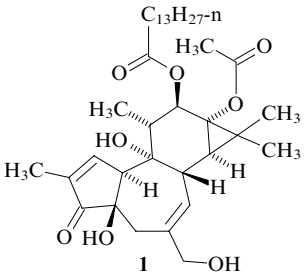


Ox is an oxidant, S–Ox is the oxidised form of the cysteine SH group.

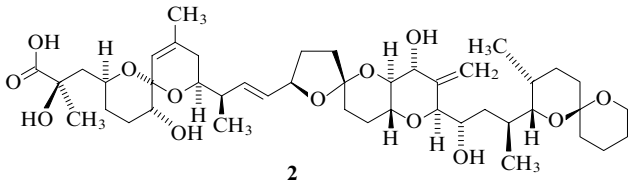
Presumably, nitrosylation of the Cys62 residue in the p50 protein and other proteins of the Rel family is a mechanism of physiological regulation of NF-κB. It is also possible that under the action of NO radicals, the activity of IκB kinase decreases and this decreases the efficiency of phosphorylation of IκB.[26]

There is a multitude of NF-κB activators, which use hydrogen peroxide as a secondary messenger. These include phorbol 12-myristate 13-acetate [below referred to as phorbol ester (**1**)], lipopolysaccharides, okadaic acid (**2**), low-

density lipoproteins†, phenobarbital, *tert*-butylhydroquinone (2-*tert*-butyl-1,4-dihydroxybenzene, TBHQ), *etc*.



1



2

Phorbol ester and lipopolysaccharides affect the transcription factor by activating protein kinase C, which catalyses phosphorylation of IκB, while okadaic acid, a toxin, which belongs to the class of fatty acids, activates NF-κB indirectly through production of reactive oxygen species.

Low-density lipoproteins in the oxidised form can activate the NF-κB transcription factor through formation of free radicals.[35] Oxidative modification of lipoproteins includes destruction of polyunsaturated fatty acids and the formation of oxidation products of lipids such as aldehydes. The activation of NF-κB by phenobarbital is also based on oxidative stress.[36]

The activation of NF-κB under the action of various cytokines, lipopolysaccharides, hydrogen peroxide, *etc*. is inhibited by antioxidants: *N*-acetylcysteine, glutathione and compounds capable of forming coordination bonds with metal ions. An increase in the glutathione content in endotheliocytes decreases the efficiency of IκB phosphorylation and suppresses activation of NF-κB by the tumour necrosis factor TNF-α.[37] This effect of glutathione and *N*-acetylcysteine is attributable to IKKα and IKKβ kinase inhibition.[38]

In some cases, a compound with antioxidant action can function as a prooxidant upon changes in the concentration or environmental conditions. For example, *tert*-butylhydroquinone able to form superoxide radicals during transformation into *tert*-butylquinone (TBQ)

$$TBHQ + O_2 \longrightarrow TBQ^{-\bullet} + O_2^{-\bullet},$$

$$TBQ^{-\bullet} + O_2 \longrightarrow TBQ + O_2^{-\bullet},$$

can activate the transcription factor NF-κB.[32] Its prooxidant activity is confirmed by inhibition of NF-κB induction in the presence of antioxidants such as acetylcysteine and glutathione. The TBHQ precursor, butylated hydroxyanisole, functions as a prooxidant only when present in low concentrations (about 30 μmol litre^{-1}). Concentrations of 200–400 μmol litre^{-1} start to exhibit antioxidant activity, resulting in inhibition of NF-κB activation in T-lymphocytes and monocytes.

† Lipoproteins are spherical particles with a hydrophobic core consisting of cholesterol esters and triacylglycerols; the surface of lipoproteins accommodates phospholipids, free cholesterol and apoproteins.

Cytokines that cause prolonged activation of NF-κB are produced by the immune system cells and affect the growth and vital activity of other cells. Cytokines such as interleukin and TNF-α, being bound to cellular receptors, induce a reaction cascade resulting in NO generation. The cytokine TNF-α produced by activated macrophages plays an important role in regulation of immune and antiinflammatory protection of the organism and stimulates generation of thymocytes. The TNF-α signal is transferred by means of external cell receptors, TNFR1 and TNFR2.[39,40] Their intracellular domains have different amino acid sequences, which suggests different mechanisms of action. The TNFR1 receptor is known as the main signal transducer of most TNF-α functions including cytotoxicity. Both receptors are able to activate independently the transcription factor NF-κB.[41,42] As regards interleukins, additional stimulation of interleukin IL-1β with cycloheximide (protein synthesis inhibitor) and actinomycin D (transcription inhibitor) results in superinduction of NF-κB, apparently due to a decrease in the *de novo* synthesis of the inhibiting subunit of IκB-α.[20]

Excited singlet oxygen plays an important role in NF-κB activation. It can be generated in biological systems upon photoexcitation under the action of endogeneous photosensitiser molecules such as flavins or porphyrins and also upon UV irradiation. Singlet molecular oxygen is also produced by phagocytes. Upon inflammation, these cells produce H_2O_2, HOCl and HOBr, which can together generate singlet molecular oxygen. Although $^1O_2$ is highly reactive, its contribution to the cytotoxic effect is not entirely clear. The possible oxidation targets include guanine residues in nucleic acids (this gives 8-oxoguanine), unsaturated hydrocarbon chains of lipids as well as proteins where oxidation of amino acids gives rise to cross-links.

The mechanism of activation of the transcription factor NF-κB by $^1O_2$ has been studied using various photosensitising agents, in particular, methylene blue and proflavine.[43] It was found that in the presence of proflavine, activation of NF-κB in lymphocytes and monocytes in the UV irradiation was much slower than under the action of TNF-α, the effect observed being independent of the presence of such antioxidant as *N*-acetylcysteine. Probably, this is due to the fact that *N*-acetylcysteine molecules, which serve as good traps for ROS in cell compartments, cannot hamper accumulation of oxygen radicals in the cell nucleus. It is assumed that oxidative destruction of DNA under the action of reactive oxygen species serves as a signal for one more mechanism of NF-κB activation.

IV. The role of NF-κB in the development of inflammatory diseases and carcinogenesis

1. Inflammatory diseases

The transcription factor NF-κB plays the key role in the development of an inflammatory process. Presumably, activated NF-κB participates in progression of acute and chronic inflammatory diseases such as inflammatory bowel disease, Crohn's disease, colitis, rheumatoid arthritis, asthma. Many proinflammatory proteins (for example, interleukin IL-1 and TNF-α) induce nuclear translocation of NF-κB. The latter, in turn, activates genes whose products participate in the inflammatory response, angiogenesis and cell adhesion. These include chemokines, cytokines (interleukins, tumour necrosis factors TNF-α and TNF-β, interferon γ), apoptosis regulators [proteins of the

Bcl-2 and p53 families, 12-lipoxygenase, cyclooxygenase (COX)], acute phase proteins (angiotensinogen, complement factors B and C4, urokinase type plasminogen activator), adhesion molecules (platelet adhesion receptor, vessel cell adhesion molecule, endothelial leukocyte adhesion molecule).[26]

Such proinflammatory mediators as nitrogen oxide, prostaglandins, IL-1 and TNF-α, the synthesis of which involves NF-κB-dependent enzymes, are included in the regulation of blood pressure, body temperature, *etc*. Among genes that are exceptionally important in the development of inflammatory processes, note the gene of inducible NO synthase (iNOS), which generates nitrogen oxide (in particular, in human cells).[44] It was shown that the promoter of murine iNOS comprises two NF-κB binding sites whose activation is critical for gene expression; mutations in these κB-sites result in inhibition of the synthesis of NO synthase mRNA.[45] In mammalian cells, nitrogen oxide is generated by three isoforms of NO synthase: neuronal NO synthase (nNOS), endothelial NOS (eNOS) and iNOS. The isoforms nNOS and eNOS are constitutively active in the cell (for example, eNOS participates in maintenance of the vascular homeostasis), and iNOS does not function in normal resting cells. This enzyme isoform is expressed only in response to the action of some stimuli, namely, proinflammatory cytokines, interferon γ and lipopolysaccharides. It is iNOS that participates in the development of acute and chronic diseases and malignant tumours.[29]

The gene of cyclooxygenase COX-2, another important regulator of the inflammatory process and carcinogenesis, is also an NF-κB target. In the early 1990s, two different COX were identified. The isoform COX-1 is constitutively expressed in almost all tissues. This enzyme accomplishes the synthesis of prostaglandins PGE_2, which function as cytoprotectors. Unlike COX-1, the isoform COX-2 (an inducible form of prostaglandin-H-synthase) is activated in immune system cells such as macrophages in response to infection, injury or other stresses. The enzyme COX-2 directs the prostaglandin synthesis during inflammations[46] and is selectively superexpressed in tumour tissues, thus playing an important role in carcinogenesis.

The activation of NF-κB during chronic inflammations can initiate a tumour, as the products of antiapoptotic genes activated by this factor are favourable for survival of altered cells that would otherwise undergo apoptosis. Thus *Helicobacter pylori* infection enhanced by activation of NF-κB is a risk factor for the development of gastric cancer.[47] It was reliably established that NF-κB plays an important role in the development of some viral diseases, in particular, it regulates replication of HIV-1 and participates in the development of some pathological conditions such as Alzheimer's disease.[48] The activation of NF-κB occurs in cardiovascular pathologies,[49] and inhibition of this factor was shown to result in regression of cardiac hypertrophy.[50]

Generally, for any diseases that include an inflammatory component, a decrease in the NF-κB activity should have a positive effect.

2. Malignant transformation of cells and apoptosis

Malignant tumours appear as a result of multistage carcinogenic process including tumour initiation, promotion and progression stages. Tumour initiation is most difficult to prevent as it is determined by random events of carcinogen capture, DNA damage induced by adverse environmental conditions, chronic inflammations and hormonal unbalance. Inflammation is the key regulator in the stimulation of initiated cells probably, due to provision of cells with proliferation signals and prevention of apoptosis.[51] The role of inflammation in tumour induction and subsequent malignant progression is described in a review.[52] In the context of the present review, it is important that inflammatory reactions promote the production of ROS and reactive nitrogen oxides by activated neutrophiles and macrophages.[53] The disadjustment of the signalling pathway involving NF-κB activation makes a considerable contribution to the development of the second, slower stage of neoplastic transformation of cells and to the tumour progression.

When considering the complex relationship between NF-κB activation and malignant transformation of cells and the programmed cell death associated with this process, it should be borne in mind that the NF-κB protein functions as a part of network of coordinatively regulated DNA-binding proteins and transcription factors. Altogether they determine the mode of gene expression required for the response to particular cellular stimuli. No unambiguous answer to the questions of whether NF-κB is a promoter or suppressor of malignant tumours, whether it exhibits proapoptotic or antiapoptotic activity can be found in the literature.[54]

The role of transcription factors of the NF-κB family as tumour growth promoters was established reliably.[55] The first proof for the role of NF-κB in carcinogenesis follows from the fact that c-Rel protein from human cells is an analogue of v-Rel of the viral oncogene, which induces leukemia and lymphoma in chickens, mice and transgenic animals.[56] In addition, many kinds of cancer are associated with gene amplification and chromosomal rearrangements of genes encoding p65, c-Rel, *etc*. proteins. In recent years, direct relationship between NF-κB and carcinogenesis was revealed. Active NF-κB was found in the tumour tissues of breast and prostate cancer patients and in patients with malignant hematological diseases, multiple myeloma, acute and chronic leukemias.[57, 58] Suppression of NF-κB in these tumours inhibited cell proliferation, caused termination of cell cycle and resulted in apoptosis.[59] It was also noted that some viral oncoproteins activate NF-κB during malignant transformation of cells.

However, there are data indicating that under certain conditions NF-κB can inhibit tumour growth. Moreover, the activity of NF-κB is modulated by well known tumour suppressors such as p53 and ARF, whereas the proper NF-κB subunits rather inhibit the expression of carcinogens.[54] This suggests a dual function of NF-κB during malignant tumour progression. In early stages, NF-κB inhibits the neoplasm growth but when the level of biosynthesis of tumour suppressors decreases during accumulation of mutations, oncogenic functions of NF-κB come to the forefront. Note that the two-stage mechanism determining the effect of NF-κB on carcinogenesis is specific to both the tumour and cell types.[54]

This dual behaviour is directly related to the dual role of NF-κB in the regulation of the apoptotic program. Apoptosis, programmed cell death, is characterised by morphological changes in cells and includes activation of a family of cysteine proteinases, caspases. These enzymes exist in non-stimulated cells as inactive zymogens and are activated by proteolytic cleavage. Two routes for caspase activation are known: the external route involving death receptor and the internal or mitochondrial route.[60] The transcription factors of the NF-κB family play the key role in the regulation of

apoptosis in different cell lines. In some types of cells under certain conditions, NF-κB can exhibit activity stimulating cell apoptosis. Thus inhibition of NF-κB partly protects human embryonic liver cells from death.[61] However, the key function of NF-κB is stimulating the expression of anti-apoptotic genes. The expression products of some of them are closely tied with the blockage of the apoptotic cascade (for example, IAP and Bcl-2 protein family). The NF-κB factor inhibits apoptosis induced by TNF-α by activation of the synthesis of IAP proteins and the factor associated with the TNF receptor. Thus it inhibits the activity of caspase 8.[62] Proteins of the Bcl-2 family inhibit secretion of cytochrome C from mitochondria and, hence, prevent the formation of apoptosome and further development of the apoptotic cascade. The activation of NF-κB results in repression of some proapoptotic genes and interferes with the proapoptotic function of the p53 protein as both transcription factors, NF-κB and p53, can inhibit the activity of each other.[63]

Thus, by activating the gene transcription of antiapoptotic and inflammatory regulators of cell proliferation and angiogenesis, NF-κB triggers the promotion and progression of malignant tumours.

V. Inhibitors of NF-κB activity

Since NF-κB is the crucial factor responsible for the development of inflammatory processes, cardiovascular diseases, tumour progression and resistance to apoptosis, the interest in the search for its effective and specific inhibitors of having immunosuppressor, antiinflammatory, antiviral and antitumour properties is quite understandable. The inhibition may affect different steps of the NF-κB signal transduction pathway, in particular, IKK activation, IκB phosphorylation and degradation, nuclear translocation of NF-κB, DNA-binding ability of NF-κB and transcription activity. A classification of inhibitors of the factor NF-κB was reported in a review.[58]

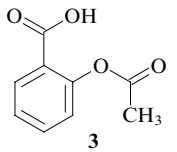
In this paper the attention is focused on low-molecular-weight inhibitors of NF-κB activity, which usually exhibit a broad spectrum of biological action. These include natural chemical compounds and their synthetic analogues capable of preventing development of diseases, stopping or reverting the disease (so-called chemopreventors). Many of these have long been known as antiinflammatory, analgesic and antitumour agents. As a rule, they possess antioxidant activity and affect not only the NF-κB signalling pathway, but also other well-known factors and mediators. Recent studies gave insight into molecular basis of their biological activity and identification of particular cell targets to which their action is directed.

According to modern views, there exist at least two levels of redox regulation of the NF-κB signalling pathway: in the nucleus and the cytoplasm.[64] The former includes redox modification of specific cysteine residues in DNA-binding domains of NF-κB, in particular, oxidation of the Cys62 residue in the p50 subunit, which inhibits the DNA-binding activity of the factor. The oxidation of NF-κB in cytosol (for example, upon addition of H_2O_2 or generation of ROS), conversely, leads to activation of NF-κB through activation of IKK. Treatment of cells with antioxidants inhibits the transcription activity of NF-κB. However, it should be noted that intracellular generation of ROS does not always result in NF-κB induction. Thus in epithelial cell lines no correlation was observed between NF-κB activation and increase in the production of ROS. Moreover, antioxidants and molecules forming chelates with metal ions are often inactive as NF-κB inhibitors in these cells. Meanwhile, there is a tight relationship between the oxidative stress and activity of NF-κB in monocytes and lymphoid cells.[64]

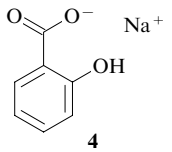
1. Low-molecular-weight inhibitors of NF-κB having antiinflammatory and antioxidant properties

As shown by recent studies, most antiinflammatory agents having antioxidant properties exhibit their activity through inhibition of IKK functions. Their action is reduced, at least partly, to removal from the organism of reactive oxygen species, which are secondary messengers in the activation of NF-κB by various stimuli. These compounds include *N*-acetyl-L-cysteine, aspirin, sodium salicylate, vitamin E derivatives, 2-mercaptoethanol, dithiocarbamates, butylated hydroxyanisol, α-lipoic acid, glutathione, pyrrolidinedithiocarbamate and iron and copper ion chelators. Below we consider some of them in more detail.

Aspirin [acetylsalicylic acid (**3**)] and sodium salicylate (**4**) are pharmaceutical agents with broad range of action. Their analgesic and antiinflammatory properties are based on inhibition of transcription of NF-κB-controlled genes.



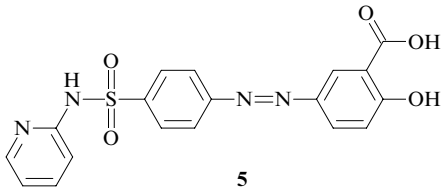
3



4

In particular, their action results in the regulation of the synthesis of proteins involved in pathogenesis of inflammatory response (for example, COX-2). In the organism, aspirin is rapidly deacetylated to salicylic acid, which effectively inhibits the synthesis of prostaglandins formed at the inflammation sites. In addition, salicylic acid derivatives block binding of NF-κB to the long terminal repeat of the human immunodeficiency virus in infected cells.[65] Presumably, the mechanism of action of aspirin and sodium salicylate is based on blocking of the phosphorylating function of the IKK complex due to competitive inhibition of ATP-binding IKKβ site.[66]

The most efficient agent for treating inflammatory bowel diseases and rheumatoid arthrites is sulfasalazine (**5**), a derivative of 5-aminosalicylic acid.

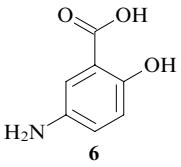


5

It was shown that micromolar amounts of sulfasalazine inhibit the NF-κB activity in the cells stimulated by TNF-α, lipopolysaccharides or phorbol ester by inhibiting the degradation of IκB-α. Presumably, sulfasalazine, like aspirin, acts on the kinase complex and suppresses its catalytic function.[67]

It is known that many stimuli that activate NF-κB by inducing degradation of IκB also cause phosphorylation of the p65 subunit, which furnishes another activation mechanism for NF-κB. In particular, an antiinflammatory agent, aminosalicylate, mesalamine (**6**), inhibits in a certain dose

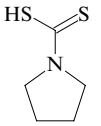
the IL-1-stimulated transcription activity of NF-κB by blocking the phosphorylation of the p65 subunit but not through degradation of IκB, nuclear translocation of the transcription factor or change of its DNA-binding properties.[68]



6

According to obtained data, it is possible to regulate the transcription activity of NF-κB by controlling the phosphorylation of the RelA (p65) protein.

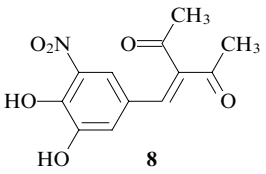
Pyrrolidinedithiocarbamate (**7**), which forms chelates with metal ions, has both antioxidant and prooxidant activities. Detailed mechanism of its action on NF-κB is unclear.



7

It was assumed that inhibition of NF-κB activation in IL-1- or TNF-α-stimulated cells can be related to modification of cysteine residues in p50 and/or p65 subunits, which form specific contacts with the phosphate groups of the DNA κB-site.[69, 70] According to experimental data obtained *in vitro*, it is not pyrrolidinedithiocarbamate itself that reacts with NF-κB, but its metabolite or cell glutathione. In the presence of pyrrolidinedithiocarbamate, the ratio of the reduced and oxidised forms of glutathione is changed, thus affecting the redox potential of the cells.

Nitecapone, 3-(3,4-dihydroxy-5-nitrophenylmethylidene)pentane-2,4-dione (**8**), possessing antioxidant properties promotes removal of peroxide, superoxide and hydroxyl radicals from the solution and cell membrane, prevents oxidation of lipids and suppresses activation of NF-κB induced by okadaic acid.[71]

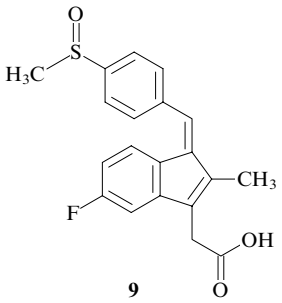


8

Apparently, like other antiinflammatory antioxidants, nitecapone either blocks phosphorylation of the IκB-α subunit that inhibits NF-κB or prevents binding of NF-κB to the promoter DNA sites in the nucleus.

2. Sulindac and its metabolites

Sulindac, {2-methyl-1-[(4-methylsulfinyl)benzylidene]-5-fluoroinden-3-yl}acetic acid (**9**), refers to the class of nonsteroidal antiinflammatory and analgesic agents, which resemble indometacin in the structure and pharmacological properties but, unlike indometacin, sulindac and its metabolites (sulfide and sulfone) inhibit the NF-κB signalling cascade.

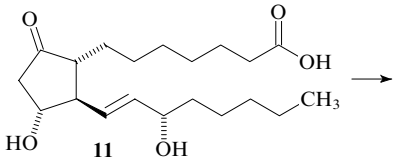


9

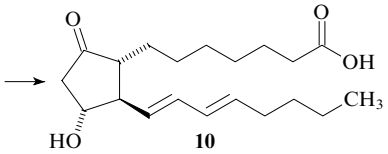
The antiinflammatory action of sulindac is related to its ability to decrease the level of the prostaglandin synthesis by inhibiting the activity of cyclooxygenase and other NF-κB-dependent enzymes. The driving force of the process is blocking of the catalytic activity of IKKβ (but not IKKγ) and concomitant inhibition of the nuclear translocation of NF-κB. Apart from the antiinflammatory action, sulindac and its metabolites have an antiproliferative action, induce cell apoptosis and play an important role in preventing colon cancer.[72] The concentrations at which these agents inhibit the NF-κB activation are beyong the cytotoxic level. Since sulindac is metabolised under the action of intestinal microflora, the concentration of the reduced form (sulfide) in the intestine epithelial cells is at least 20 times as high as the concentration of sulindac. It is this metabolite that is the main inhibitor of the formation of prostaglandins and is responsible for the pharmacological action of the drug.

3. Cyclopentenone prostaglandins

Prostaglandins, bioregulators of the lipid nature, are oxygenated derivatives of polyenoic fatty acids containing five- and six-membered rings. Prostaglandins possess exceptionally high and diverse physiological activities. They are synthesised by cell enzymes through oxidation of arachidonic acid in response to external stimuli. The reactivity and chemical lability of prostaglandins is related to the presence of an allylic hydroxyl group, which is rather easily epimerised, oxidised or eliminated to give a diene [as for example, in the formation of diene **10** from prostaglandin E1 (**11**)].



11



10

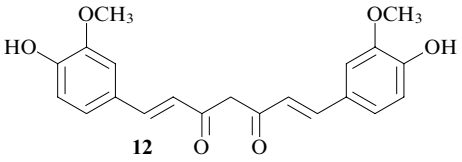
The NF-κB signalling pathway is known to be involved in the pathogenesis of inflammatory response by inducing genes responsible for the synthesis of COX-2 and inflammatory prostaglandins. However, at late inflammation stages, COX-2 regulates also the synthesis of antiinflammatory cyclopentenone prostaglandins. The antiinflammatory activity of prostaglandin PGJ(2) and its metabolites, in particular, 15-deoxy-δ-(12,14)-PGJ(2) [15d-PGJ(2)], is related to activation of the PPAR-γ receptor (Peroxisome Proliferator-Activated Receptor γ).[73] Being high-affinity

PPAR-γ receptor, the 15d-PGJ(2) metabolite inhibits the induction of the inflammatory response genes including iNOS and TNF-α genes. There is a number of proofs that the inhibitory properties of 15d-PGJ(2) are also related to chemical modification of the cysteine residues in IKKβ and in DNA-binding domain of the p50-subunit of NF-κB.[74] Moreover, for inhibiting the DNA-binding activity of the factor NF-κB, the prostaglandin molecule should contain a cyclopentenone fragment. By point replacement of particular amino acid residues in the p50 subunit of the transcription factor NF-κB, it was shown that the inhibitory effect of 15d-PGJ(2) is due to modification of the Cys62 residue. The formation of a conjugate between the p50 subunit and a cyclopentenone prostaglandin was confirmed by reversed-phase HPLC and mass spectrometry. Notably, the presence of glutathione or dithiothreitol in the medium prevented blocking of the DNA-binding ability of the factor associated with oxidation of cysteine residues.[75] The prostaglandin 15d-PGJ(2) decreased the DNA-binding activity of NF-κB in nuclear extracts, while treatment of the HeLa cell line with biotinylated 15d-PGJ(2) gave rise to a p50 subunit adduct with prostaglandin, which was proved by immunoprecipitation analysis.[75]

4. Flavonoids and phenolic compounds

Flavonoids are natural phenolic compounds exhibiting antioxidant, antiproliferative, antiinflammatory and antitumour properties. It was shown in some studies that the biological activity of flavonoids is caused, at least partially, by inhibition of NF-κB activation, mainly through inhibition of the IKK complex.

Curcumin, diferuloylmethane, 1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (**12**), is an active component of turmeric, which is widely used as a spice in the Asian kitchen and in the traditional Indian medicine Ayurveda.

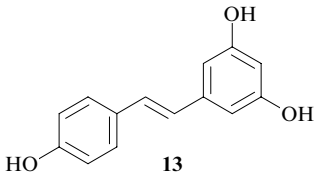


12

Curcumin has a broad spectrum of pharmacological action. It exhibits protective properties at all stages of carcinogenesis; in addition, unlike other chemopreventors, curcumin is non-toxic.[51,76] Curcumin modulates functions and expression of a large number of proteins not connected to each other including transcription factors NF-κB, PPARγ, AP-1, STAT3, antiapoptotic proteins, cytochrome *c*, caspase 9, Bcl-2, membrane proteins such as ion pumps, receptors and channels.[77] We will consider NF-κB-dependent molecular targets.

Curcumin decreases proliferation of colon, blood, liver, *etc.* cancer cells through negative regulation of iNOS in activated macrophages, interleukins, oncoprotein β-catenine, COX-2, cyclin D1, matrix metalloproteinases, glutathione-S-transferase.[78–80] Being a potential free radical scavenger, curcumin intensifies the synthesis of ornithine decarboxylase, glutathione, antioxidant proteins, *etc.*, thus protecting kidneys from oxidative damage. Curcumin is one of most potent inhibitors of NF-κB.[81] The mechanism of inactivation of NF-κB with curcumin includes the blockage of signal transduction resulting in activation of the IKK complex, which prevents phosphorylation of the Ser32 residue, degradation of the inhibitory protein IκB-α and nuclear translocation of the RelA subunit. Preclinical tests of curcumin on cell cultures and on animals confirmed its high potential as an antitumour drug and as a metastatic progression inhibitor.

The phytoalexin resveratrol, 3,5,4′-trihydroxystilbene (**13**), was found in diverse plant specimens including arachis, mulberries, grapes and products of their processing (red wine).



13

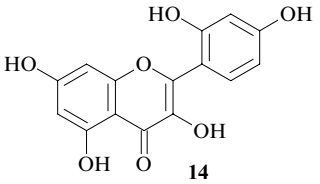
This compound, like other stilbene derivatives, is fomed in response to pathogenic attack, UV irradiation and ozonisation.[82] Resveratrol has long been used in Chinese and Japanese folk medicine. It was found that resveratrol decreases the risk of atherosclerosis, inhibits chemical carcinogenesis and possesses antiinflammatory and antiallergic action. Noteworthy are also its antioxidant and anticoagulation properties. Since the transcription factor NF-κB is incorporated into regulation of proinflammatory proteins and carcinogenesis, it was investigated whether resveratrol can change the activity of this factor. It was found that resveratrol inhibits both the activity of NF-κB and the expression of NF-κB-dependent genes by blocking the kinase function of the IKK complex, the main regulator of the activity NF-κB (see Fig. 2).[83]

These data may account for some aspects of the so-called 'French Paradox'‡ attributed to resveratrol present in red wine. The cause of atherosclerotic pathogenesis is known to be accumulation of lipoproteins in the arterial wall and, as a consequence, appearance of a local inflammation. The phagocytosed cells attracted by the inflammatory process generate ROS such as superoxide anion, hydrogen peroxide and hydroxyl radicals. The inflammatory process is accompanied by liberation of arachidonic acid and its metabolites, prostaglandins, *etc.* In turn, prostaglandin synthesis is regulated by the expression of COX-1 and COX-2 genes. These separate gene products have similar cyclooxygenase and peroxidase activities but differ in the type of regulation. Resveratrol was shown to interfere with a complex cascade of chemical transformations, resulting in a decrease in atherogenic changes in plasma and in arterial wall and mitigating the consequences of ischemic damage.[84] Resveratrol protects lipids from oxidative degradation and inhibits iNOS induction, thus preventing cytotoxic effects of nitrogen oxides.[85] It is able to trap peroxide and hydroxyl radicals in the region of ischemic damage (shown for an isolated rat heart) and restrict the size of the infarct area.

Resveratrol acts also on some factors involved in tumour initiation and progression. It was shown that it stimulates apoptosis in fibroblasts after induced expression of the H-Ras oncogen.[83] Antiallergic properties of resveratrol and its ability to regulate brain functions were also observed.[86]

‡ The French Paradox is the low rate of cardiovascular diseases in the French, despite large amounts of saturated fats in their food.

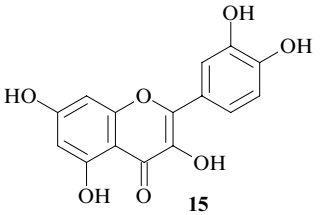
The flavonoid morin, 3,5,7,2′,4′-pentahydroxyflavone (**14**), was isolated from fig berry and mulberry, almond nut shell and Chinese herbs.



14

The fact that the antitumour and antiinflammatory properties of morin are due to modulation of NF-κB activity stimulated studies of the effect of morin on the NF-κB signalling cascade activated by inflammatory agents and carcinogens: TNF and its receptors, phorbol ester, lipopolysaccharides, ceramides, interleukin-1 and H_2O_2. It was shown that this flavonoid actually suppresses activation of NF-κB irrespective of the type of cells.[87] Morin inhibited phosphorylation and degradation of IκB-α and the subsequent nuclear translocation of the p65 subunit of NF-κB. Morin-induced negative regulation of expression of gene products involved in cell survival (apoptosis inhibitors IAP 1 and 2, Bcl-xL), proliferation (cyclin D1 and COX-2) and invasion (matrix metalloproteinase-9) was also noted. These effects were correlated with morin-induced enhancement of cell apoptosis in malignant tumours: cell carcinoma, leukemia, colon cancer.

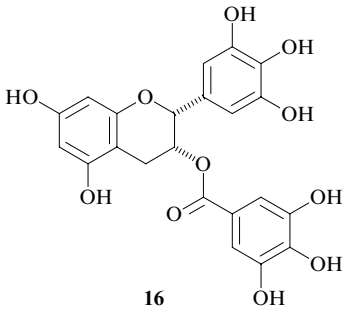
The plant metabolite quercetin (**15**) possessing, like other flavonoids, antioxidant and antiinflammatory properties, decreases the risk of cardiovascular diseases.



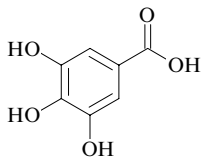
15

The effect of quercetin on gene expression and the secretion of the proinflammatory TNF-α cytokine, which is associated with the production of other cytokines, chemokines and inflammatory mediators, was studied on a human peripheral blood mononuclear cell (PBMC) culture stimulated by the phorbol ester and calcium ions. Quercetin inhibited TNF expression with an efficiency depending on the dose of the agent, the molecular mechanism of action being based on modulation of NF-κB and IκB activities.[88]

Endotoxin-stimulated TNF-α synthesis has also been inhibited by green tea polyphenols.[89] The mechanism of action of these compounds included blockage of functions of the IKK complex in the signalling pathway of NF-κB activation (see Fig. 2). The IKK activity was determined from the phosphorylation level of the IκB-α and glutathione-S-transferase fusion protein. Extracted green tea polyphenols were found to decrease the activity of the IKK complex and NF-κB, the most efficient inhibitory effect being found for (−)-epigallocatechin 3-gallate (**16**) containing a gallic acid (**17**) residue critical for the biological action of flavonoids.



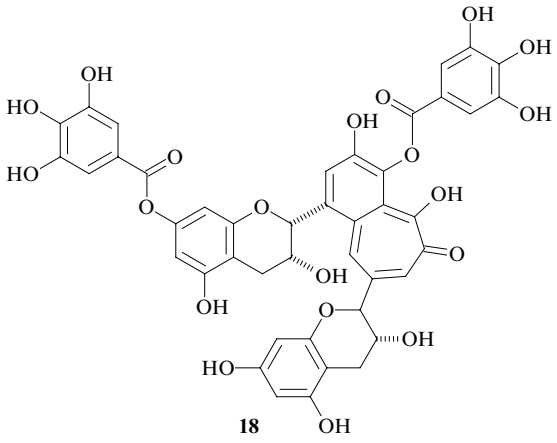
16



17

Further studies have shown that polyphenol **16** specifically affects the IKK complex without affecting functioning of other cell kinases.[89] It was shown that (−)-epigallocatechin 3-gallate suppresses inflammation, cell proliferation and angiogenesis caused by cigarette smoke,§ and prevents the development of lung, breast and prostate cancer.[91] Treatment of human bronchial epithelial cells stimulated by cigarette smoke condensate with (−)-epigallocatechin 3-gallate resulted in considerable negative regulation of NF-κB-dependent proteins: cyclin D1, MMP-9 (the protein involved in the degradation of type IV collagen), IL-8 and iNOS and in lower expression of a number of signalling molecules not involved in the NF-κB activation pathway.[92] The oncoprotector properties of polyphenol **16** are due to suppression of phosphorylation of IκB-α and nuclear translocation of the p65-subunit of NF-κB. When present in optimal amount, (−)-epigallocatechin 3-gallate creates different oxidative conditions in normal epithelial cells and in malignant tumour cells, which results in selective death of the neoplastic cells, while the cells of normal tissue remain intact.

The antitumour properties of a broad range of polyphenols including flavonoid **16** were studied in murine macrophages activated by lipopolysaccharides.[93] Of all polyphenols studied, TF-3 [theaflavin 3,3′-digallate (**18**)] proved to be the most potent inhibitor of the IKK activity.

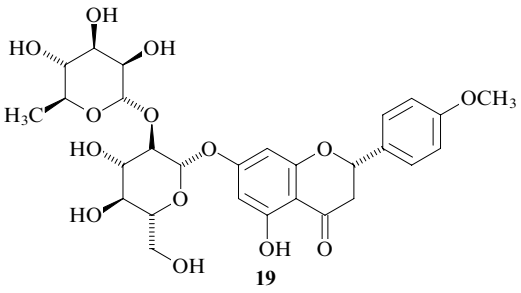


18

§ The cigarette smoke contains more than 40 compounds. These compounds have been identified as mutagens and carcinogens exhibiting their biological activities by forming DNA adducts, resulting in genetic mutations.[90]

Presumably, the action of polyphenol **18** can be directed on both the kinase complex itself and the components of the preceding stages of the NF-κB signalling pathway.

The flavonoid poncirin (**19**) was isolated from the ethanolic extract of desiccated immature fruit *Poncirus trifoliata* during the screening programme developed for identification of natural compounds with antiinflammatory properties.

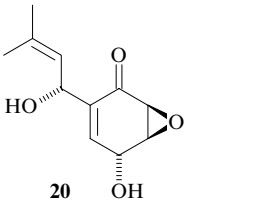


19

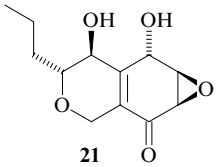
The desiccated immature fruits containing, along with poncirin, other biologically active flavonoids, coumarins and alkaloids have been traditionally used in China for obstetrics and for treating gastroenterological and cardiovascular diseases. The antibacterial, antithrombocytic and antiallergic action of the active component of poncirin is related to inhibition of the synthesis of prostaglandin PGE_2, interleukin IL-6, enzymes iNOS, COX-2 and the TNF-α factor in the macrophage cells activated by lipopolysaccharides.[94,95] Using molecular-biological approaches, it was shown that the production of COX-2 and iNOS decreases at the stage of transcription, as indicated by the decrease in the amount of their mRNA dependent on the drug dose.[95] As regards NF-κB, the inhibitory effect of poncirin is related to its inactivation by blockage of IκB-α phosphorylation, the nuclear translocation of the factor and the DNA-binding activity of the p65 subunit.

5. Biologically active components of microorganisms and plants and their synthetic analogues

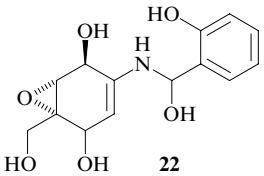
A number of compounds isolated from microorganisms and having immunosuppressor, antiinflammatory, antiviral and antitumour activities have been described in a review.[39] Panepoxydone (**20**) isolated from the fungi *Lentinus crinitus* inhibited phosphorylation and degradation of IκB in the COS-7 cells stimulated by TNF-α or phorbol ester.



20

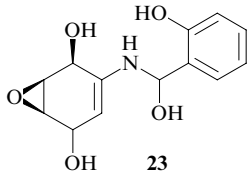


21

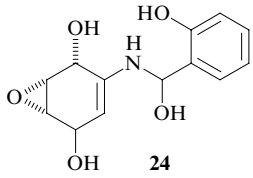


22

The activation of NF-κB was inhibited by cycloepoxydone (**21**) isolated from bacterial sources,[10,96] while the antibiotic epoxyquinomycin C (**22**), which also contains an epoxycyclohexenyl fragment, did not show this effect. Hence, this structural element is not responsible for inactivation of NF-κB.[10,39] However, specially designed and synthesised dehydroxymethylepoxyquinomycin (DHMEQ) having molecular structure close to that of epoxyquinomycin and existing as (−)-DHMEQ (**23**) and (+)-DHMEQ (**24**) enantiomers inhibited both the NF-κB activity stimulated by various methods and its constitutive activity in transformed cells (carcinoma and leukosis).[10,39]



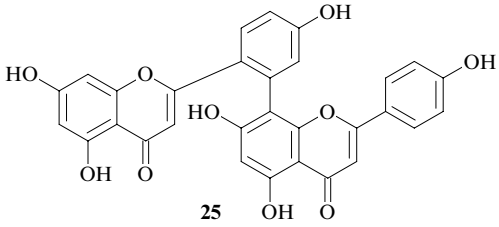
23



24

Dehydroxymethylepoxyquinomycin inhibited secretion of proinflammatory cytokines from cancer cells.[97] The action of DHMEQ enantiomers was studied in human leukosis Jurkat cells stimulated by phorbol ester. It was found that (−)-DHMEQ is only slightly more active than (+)-DHMEQ; therefore, in subsequent experiments a racemic mixture of the compounds was used. It was shown that DHMEQ inhibits the activity of NF-κB at the stages following the phosphorylation of IκB inhibitor with the IKK complex. In particular, these compounds act at the level of nuclear translocation of the factor and inhibit both the canonical and non-canonical NF-κB activation pathways without exhibiting pronounced toxicity (see Fig. 2).[39] In addition, DHMEQ blocks *in vitro* binding of NF-κB to DNA, but unlike the natural analogue, panepoxydone (**20**), it does not inhibit IκB phosphorylation and degradation stages.[10] Thus, although panepoxydone and DHMEQ comprise the same epoxycyclohexenyl fragment, the mechanisms by which they inhibit the NF-κB activity are different. Apart from the antitumour action, DHMEQ is useful in treating arthrites. Moreover, its efficiency is directly proportional to the level of inhibition of NF-κB activity.

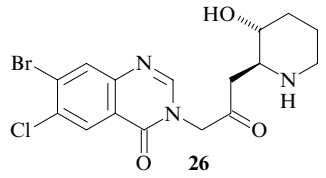
The biflavonoid 2′,8″-biapigenin (**25**), isolated from the plant *Selaginella tamariscina*, exhibits antiinflammatory and antitumour properties.



25

It inhibits the activity of NF-κB by preventing the nuclear translocation of the p65 subunit of the transcription factor.[98] By inactivating NF-κB, biapigenin blocks the transcription of iNOS and COX-2 genes in macrophages. These enzymes are known to control the redox balance of the cells. They are responsible for the synthesis of NO and inflammatory prostaglandins and are involved in the pathogenesis of malignant tumours.

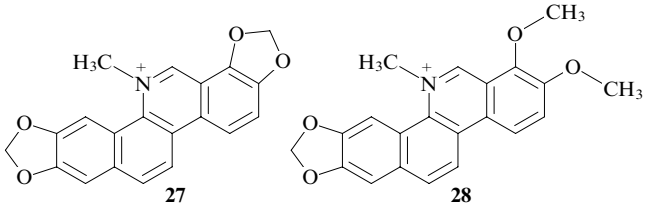
Halofuginone, 7-bromo-6-chloro-3-[3-(3-hydroxypiperidin-2-yl)-2-oxopropyl]-(3*H*)-quinazolin-4-one (**26**), is a low-molecular-weight plant alkaloid possessing antitumour and antiangiogenic activity.



26

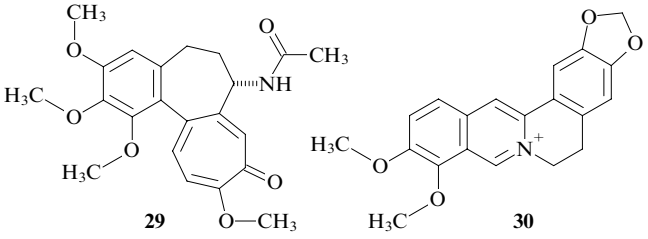
It is able to decrease the level of biosynthesis of cytokines and immunomodulators and secretion of TNF-α and interleukins in activated peripheral blood T-cells.[99] Halofuginone stimulates apoptosis of cancer cells,[100] while simultaneously inhibiting programmed cell death of normal T-cells. It has been assumed [99] that the biological activity of this alkaloid is partly due to inactivation of NF-κB at the nuclear translocation level.

The ability of various alkaloids with antiinflammatory properties to modulate the signalling pathways involved in the development of inflammatory process has been studied.[101] In particular, the effect of alkaloids on the transcription activity of NF-κB-dependent genes in the HeLa cells was studied. It was found that sanguinarine (**27**) and chelerythrine (**28**) forced the nuclear translocation of the p65 subunit of NF-κB, the nuclear import of p65 being markedly enhanced under the action of these alkaloids both in non-stimulated cells and in TNF-α-treated cells.[101]



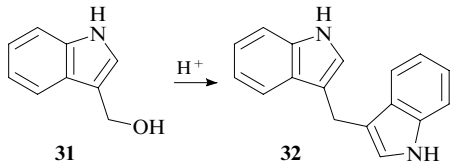
27 **28**

Meanwhile, colchicine (**29**) and berberine (**30**) were not involved in the signal transduction pathway for NF-κB activation. Moreover, all alkaloids studied in the cited work [101] remained transcriptionally inactive, *i.e.*, did not affect the expression of NF-κB-dependent genes.



29 **30**

Among the low-molecular-weight inhibitors of NF-κB isolated from plant sources, 3-hydroxymethylindole (**31**) deserves attention. This compound and its dimer, diindolylmethane (**32**) readily formed *in vivo* are known as agents preventing malignant transformation of tissues and initiating apoptosis in prostate and breast tumour cells.[102, 103]

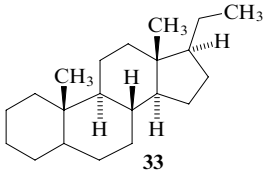


31 **32**

As shown in *in vitro* experiments, 3-hydroxymethylindole substantially inhibits NF-κB binding to DNA, although the exact mechanism of inactivation of NF-κB as the main therapeutic target remains unknown.[104]

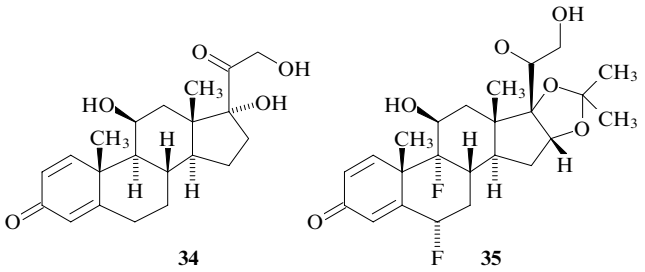
6. Glucocorticoid inhibitors

Yet another class of low-molecular-weight modulators of NF-κB activity are glucocorticoids, which are known by their antiinflammatory, antishock and antiallergic action and immunosuppressor activity. This is a special group of steroid hormones with a pregnane skeleton (**33**).



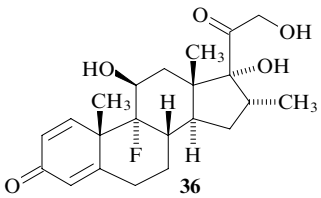
33

They participate in regulation of the carbohydrate, water and ion exchange and are used for treating bronchial asthma, eczema, infectious hepatitis, arthrites, asthenias, *etc*. Currently both natural corticoids isolated from adrenal cortex and their synthetic analogues [prednisolone (**34**), synalar (**35**), *etc*.] having higher biological activities are used.



34 **35**

It was shown that glucocorticoids are potential inhibitors of NF-κB activity in cell cultures and mice.[105] In the signal transduction pathway for NF-κB activation, glucocorticoids inhibit the expression of genes of proinflammatory proteins COX-2, TNF-α, almost all known cytokines and some cell surface molecules needed for exhibiting the immune properties. The synthetic glucocorticoid, dexamethasone (**36**), induces the synthesis of the IκB-α protein.



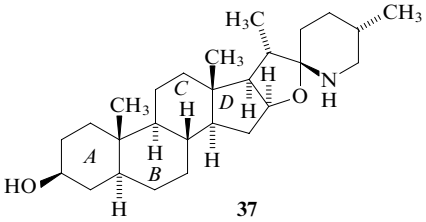
36

This newly synthesised inhibitor is complexed with NF-κB thus suppressing its activity. A decrease in the amount of NF-κB imported in the nucleus retards the cytokine secretion. Thus, dexamethasone efficiently blocks the activation of the immune system.[105, 106]

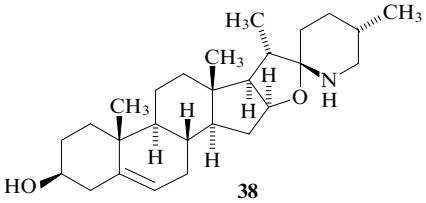
However, the mechanism of action of glucocorticoids is not only in inhibition of NF-κB activation. These compounds also control the catalytic activity of a number of enzymes whose regulation pathways include the p65 subunit.[107] According to experimental data, glucocorticoids inhibit the NF-κB-dependent expression of proinflammatory interleukin-8 genes and other products through the protein–protein interaction between the glucocorticoid receptors and NF-κBd subunits bound to the regulatory

sites of the target genes.[108] The glucocorticoid-mediated inhibition of NF-κB activity involves various combinations of intermediate proteins and factors used in the signalling pathways of the inflammatory and immune responses.

A recent study[109] considers the antiinflammatory effect of steroid alkaloids: tomatidine (**37**) and solasodine (**38**), which were found in tomatoes, potatoes and eggplants.



37

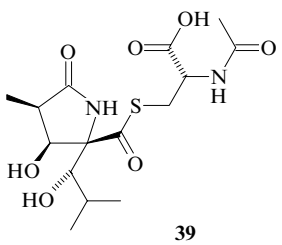


38

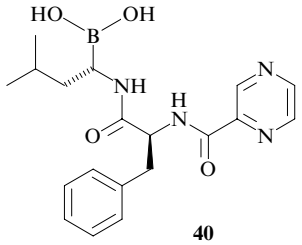
These compounds similar to glucocorticoids in their structure possess a broad spectrum of biological action, in particular, they decrease the cholesterol level, enhance the immune response, protect the organism from fungal and bacterial infections. It was shown that tomatidine and solasodine act as antiinflammatory agents by blocking the NF-κB signalling pathway at the stage of phosphorylation of the inhibitory protein IκB-α and nuclear translocation of the factor; tomatidine shows a more pronounced antiinflammatory effect.[109] It is based on the negative regulation of expression of two important proinflammatory proteins, iNOS and COX-2. A comparison of molecular structures of tomatidine and solasodine has shown that the key difference between them is the absence of the double bond in the 5,6-position of ring *B* in the case of tomatidine, which is believed to be the structural determinant dictating the intensity of biological response.

7. Proteasome inhibitors

The activation of the NF-κB factor and cell protection proteins in carcinogenesis may occur through externally induced phosphorylation or ubiquitin-mediated degradation of the IκB-α inhibitor in proteasomes (see Fig. 2). The ubiquitin-mediated degradation of proteins in proteosomes is a highly conserved intracellular process that controls the amount of short-lived proteins. The proteasome 26S is a large (1500–2000 kDa) multienzyme complex present in the nucleus and cytoplasm of eukaryotic cells.[110] It accomplishes degradation of a number of key cell cycle regulators and apoptosis-inducing factors such as p53, Bcl-2, p21, cyclins, cyclin-dependent kinase inhibitors, *etc*. Thus, being an important participant of the NF-κB signalling pathway, proteasome is a new promising biochemical target. The inhibitors of proteasome activity controlling the NF-κB-dependent synthesis of antiapoptotic and proinflammatory proteins have an obvious antitumour potential and prospects for treatment of chronic inflammations. In recent years, selective proteasome inhibitors based on dipeptidyl aldehydes[111] and boronic acid,[111,112] and also lactacystin (**39**) and vinyl sulfone tripeptides[112] have been proposed.



39

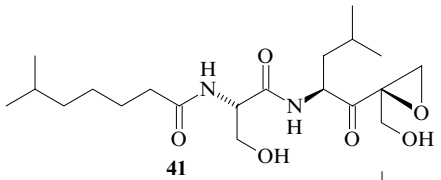


40

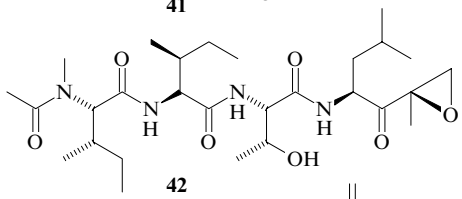
The studies were carried out both on cell cultures and in *in vivo* systems, for example, in human tumour models in mice. The action of selective proteasome inhibitors, their participation in cell response such as antigene presentation and degradation of regulatory or membrane proteins have been described in reviews.[112,113]

A dipeptidyl boronic acid derivative, bortezomib, {(1*R*)-3-methyl-1-[{(2*S*)-1-oxo-3-phenyl-2-[(pyrazin-3-ylcarbonyl)amino]propyl}amino]butyl}boronic acid (**40**) (Velcade, PS-341), is the prototype of a new class of therapeutic agents. This compound directly modulates the expression of NF-κB, cyclins, p53, Bcl-2, Bax and other regulatory proteins and affects a broad spectrum of intracellular processes associated with tumour progression and resistance against chemotherapeutic agents and irradiation.[113–116] It was shown that a combination of proteasome inhibitors with chemotherapeutic drugs results in a sharp increase in the treatment efficiency and enhancement of tumour cell apoptosis (the size of a solid tumour decreased by 94% and the apoptosis level reached 80%–90% *versus* 10% of the apoptosis response to the use of antitumour drugs alone).[116] The inhibitor PS-341 has passed the first stage of clinical trials on patients with advanced cancer forms. The antiproliferative and antiproteolytic properties of PS-341 were demonstrated for 60 cell lines obtained from different human tumours including multiple myelomas, lung cancer, prostate cancer and carcinomas.[113,117] The proteasome inhibitor PS-341 has also proved efficient in treating malignant blood diseases. The mechanism of action of peptide inhibitors is still little studied.

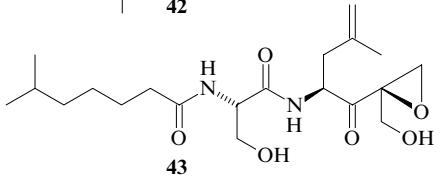
Dihydroeponemycin (**41**)[118] and epoxomycin (**42**),[119] analogues of antitumour and antiangiogenic natural product eponemycin (**43**), also affect proteosome-determined signalling pathway. These compounds modify some catalytic subunits and affect to different extents the main peptidolytic proteasome activities.[118]



41



42



43

The inhibition of proteasome functions by dihydroeponemycin results in morphological changes of cells and apoptosis. The potential oncoprotector, epoxomycin, unlike the peptidyl aldehyde inhibitors, does not block non-proteosomic proteases such as trypsin, chymotrypsin, papain or cathepsin B in concentrations of up to 50 μmol litre^{-1}. Epoxomycin effectively decreases the NF-κB activity *in vitro* and reduces inflammation in mice with otitis *in vivo*. This compound can be considered as a useful tool for the study of the role of proteasomes in various systems.[119]

8. Alkylating reagents

A cytotoxic alkylating reagent temozolomide (**44**) inducing the formation of O^6-methylguanine and other DNA adducts is often used for treating malignant gliomes.

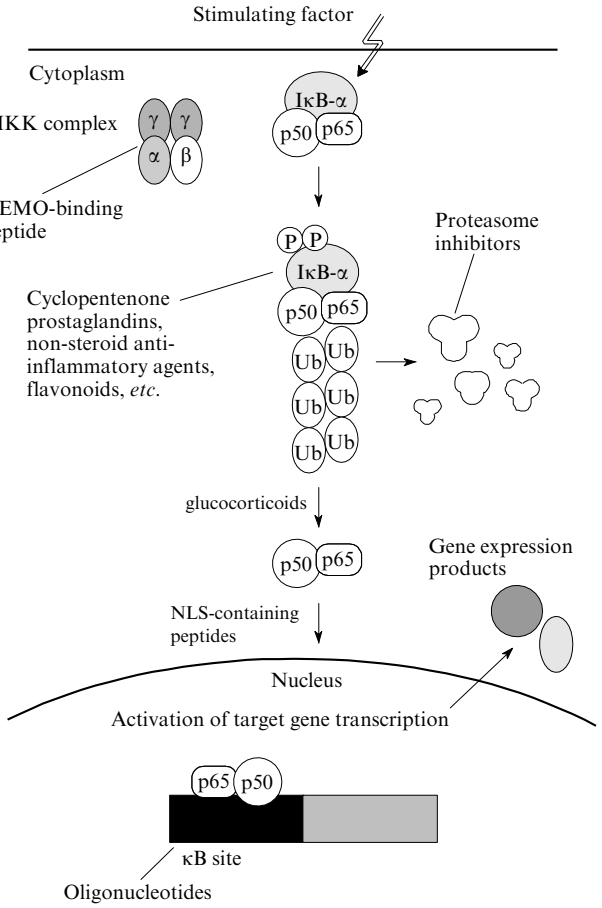
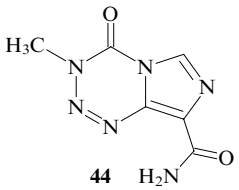


Figure 3. Various types of inhibitors aimed at different steps or particular molecular targets of the canonical signal transduction pathway leading to NF-κB activation.
NEMO-binding peptides prevent the IKK complex formation; cyclopentenone prostaglandins, non-steroid antiinflammatory agents and compounds from natural sources inhibit the IKK activation; proteasome inhibitors block the degradation of IκB, and glucocorticoids induce *de novo* IκB synthesis; peptides containing nuclear localisation signal (NLS) of the NF-κB inhibit the nuclear translocation of the factor subunits, whereas antisense oligonucleotides, DNA-decoys and alkylating reagents prevent transcription of target genes (a modified chart from the review [58]).



44

A study of the effect of temozolomide on the transcription activity of NF-κBB [120] has shown that it inhibits the NF-κB factor by an IκB-α-independent mechanism. Temozolomide blocks the binding ability of p65 subunit to the κB-site of the DNA target, without affecting its nuclear translocation. The inhibition of NF-κB by temozolomide requires a low cellular activity of O^6-alkylguanine-DNA-alkyltransferase and the presence of intact DNA mismatch repair system.

Figure 3 shows a scheme of signal transduction pathway leading to NF-κB activation under the action of external stimuli and indicates the types of inhibitors aimed at different steps and particular biotargets of the canonical pathway.

VI. Conclusion

The transcription factor NF-κB and products of NF-κB-directed gene expression are apoptotic and proinflammatory mediators and thus involved in various pathological conditions: chronic inflammatory processes, oncological and viral diseases. Therefore, the inhibition of the NF-κB activity is a very promising approach to fight those pathologies. During the last decade, numerous drugs inhibiting different steps of the NF-κB activation pathway have been proposed and tested. Nevertheless, it is obvious that more profound understanding of the biological processes resulting in the transcription activity of NF-κB in different types of cells and characteristics of the components of NF-κB signalling pathways regulating the programmed cell death and innate immunity would allow rational search, design and synthesis of efficient inhibitors. This work includes identification of promising biotargets in NF-κB signal transduction pathways, screening of libraries of chemical compounds for potential inhibitors of the selected target, development of cellular and cell-free systems for testing their efficiency and the use of experimental models *in vivo*. The diversity of low-molecular-weight NF-κB inhibitors in combination with oligonucleotide and protein target-specific molecules is a reliable basis for selecting real candidates for new-generation therapeutic agents.

This review was written with financial support of the Rosnauka Federal Target Programme (Project No. 2007-2-1.2-05-02-071) and the Russian Foundation for Basic Research (Project No. 06-04-49196).

References

1. R Sen, D Baltimore *Cell* **46** 705 (1986)
2. M L Schmitz, I Mattioli, H Buss, M Kracht *ChemBioChem*. **5** 1348 (2004)
3. G J Nabel, I M Verma *Genes Dev*. **7** 2063 (1993)
4. M L Schmitz, P A Baeuerle *EMBO J*. **10** 3805 (1991)
5. G Ghosh, G van Duyne, S Ghosh, P B Sigler *Nature (London)* **373** 303 (1995)
6. C W Mueller, F A Rey, M Sodeoka, G L Verdine, S C Harrison *Nature (London)* **373** 311 (1995)

7. I A Kozlov, E A Kubareva *Usp. Biol. Khim.* **38** 77 (1998)
8. A S Romanenkov, A A Ustyugov, T S Zatsepin, A A Nikulova, I V Kolesnikov, V G Metelev, T S Oretskaya, E A Kubareva *Biokhimiya* **70** 1474 (2005) [*Biochemistry (Moscow)* **70** 1212 (2005)]
9. V Metelev, A Romanenkov, E Kubareva, E Zubin, N Polouchine, T Zatsepin, N Molochkov, T Oretskaya *IUBMB Life* **58** 654 (2006)
10. K Umezawa, C Chaicharoenpong *Mol. Cell* **14** 163 (2002)
11. J T Wu, J G Kral *J. Surg. Res.* **123** 158 (2005)
12. V P Almazov, D V Kochetkov, P M Chumakov *Mol. Biol.* **41** 947 (2007) [a]
13. M Philip, D A Rowley, H Schveiber *Semin. Cancer Biol.* **14** 433 (2004)
14. P A Baeuerle, T Henkel *Annu. Rev. Immunol.* **12** 141 (1994)
15. R Lin, D Gewert, J J Hiscott *J. Biol. Chem.* **270** 3123 (1995)
16. J E Thompson, R J Phillips, H Erdjument-Bromage, P Tempst, S Ghosh *Cell* **80** 573 (1995)
17. H-C Liou, C Y Hsia *BioEssays* **25** 767 (2003)
18. J A Brockman, D C Scherer, T A McKinsey, S M Hall, X Qi, W Y Lee, D W Ballard *Mol. Cell Biol.* **15** 2809 (1995)
19. K Brown, S Gerstberger, L Carlson, G Franzoso, U Siebenlist *Science* **267** 1485 (1995)
20. C Kretz-Remy, P Mehlen, M-E Mirault, A-P Arrigo *J. Cell Biol.* **133** 1083 (1996)
21. D M Rothwarf, E Zandi, G Natoli, M Karin *Nature (London)* **395** 297 (1998)
22. C Makris, V L Godfrey, G Kröhn-Senftleben, T Takahashi, J L Roberts, T Schwarz, L Feng, R S Johnson, M Karin *Mol. Cell* **5** 969 (2000)
23. H J Kim, N Hawke, A S Baldwin *Cell Death Differ.* **13** 738 (2006)
24. E Dejardin, N M Droin, M Delhase, E Haas, Y Cao, C Markis, Z-W Li, M Karin, C F Ware, D R Green *Immunity* **17** 525 (2002)
25. B Barré, N D Perkins *EMBO J.* **26** 4841 (2007)
26. E B Men'shchikova, V Z Lankin, N K Zenkov, I A Bondar', N F Krugovykh, V A Trufakin, in *Okislitel'nyi Stress. Prooksidanty i Antioksidanty* (Oxidising Stress. Prooxidants and Antioxidants) (Moscow: Slova, 2006) p. 512
27. N N Gessler, A A Aver'yanov, T A Belozerskaya *Biokhimiya* **72** 1342 (2007) [*Biochemistry (Moscow)* **72** 1091 (2007)]
28. Ch D Georgiou, N Patsoukis, I Papapostolou, G Zervoudakis *Integr. Compar. Biol.* **46** 1 (2006)
29. F L M Ricciardolo, P J Sterk, B Gaston, G Folkerts *Physiol. Rev.* **84** 731 (2004)
30. C J Lowenstein, E Padalko *J. Cell Sci.* **117** 2865 (2004)
31. R Pinkus, L M Weiner, V Daniel *Biochemistry* **34** 81 (1995)
32. R Pinkus, L M Weiner, V Daniel *J. Biol. Chem.* **271** 13422 (1996)
33. N Kaul, J Choi, H J Forman *Free Radical Biol. Med.* **24** 202 (1998)
34. H E Marshall, J S Stamler *J. Biol. Chem.* **277** 34223 (2002)
35. C Maziere, M Auclair, M Djavaheri-Mergny, L Packer, J-C Maziere *Biochem. Mol. Biol. Int.* **39** 1201 (1996)
36. S Cho, Y Urata, T Iida, S Goto, M Yamaguchi, K Sumikawa, T Kondo *Biochem. Biophys. Res. Commun.* **253** 104 (1998)
37. Y Li, L K Leung, B T Spear, H P Glauert *Biochem. Biophys. Res. Commun.* **229** 982 (1996)
38. S Oka, H Kamata, K Kamata, H Yagisawa, H Hirata *FEBS Lett.* **472** 196 (2000)
39. K Umezawa *Cancer Sci.* **97** 990 (2006)
40. A S Baldwin Jr *Annu. Rev. Immunol.* **14** 649 (1996)
41. G Kruppa, B Thoma, T Machleidt, K Wiegmann, M Kroenke *J. Immunol.* **148** 3152 (1992)
42. M Rothe, S C Wong, W J Henzel, D V Goeddel *Cell* **78** 681 (1994)
43. S Legrand-Poels, V Bours, B Piret, M Pflaum, B Epe, B Rentier, J Piette *J. Biol. Chem.* **270** 6925 (1995)
44. Z Guo, L Shao, Q Du, K S Park, D A Geller *FASEB J.* **21** 535 (2007)
45. R Korhonen, A Lahti, H Kankaanranta, E Moilanen *Curr. Drug Targets Inflamm. Allergy* **4** 471 (2005)
46. V S Seybold, Y P Jia, L G Abrahams *Pain* **105** 47 (2003)
47. E M El-Omar, M Carrington, W H Chow, K E McColl, J H Bremm, H A Young, J Herrera, J Lissowska, C C Yuan, N Rothman, G Lanyon, M Martin, J F Fraumenti Jr, C S Rabkin *Nature (London)* **404** 398 (2000)
48. M P Mattson, S Camandola *J. Clin. Invest.* **107** 247 (2001)
49. S Gupta, S Sen *J. Mol. Med.* **83** 993 (2005)
50. S Gupta, D Young, R K Maitra, A Gupta, Z B Popovic, S L Yong, A Mahajan, Q Wang, S Sen *J. Mol. Biol.* **375** 637 (2008)
51. R L Thangapazham, A Sharma, R K Maheshwari *AAPS J.* **8** E443 (2006)
52. R K Maheshwari, A K Singh, J Gaddipati, R C Srimal *Life Sci.* **78** 2081 (2006)
53. J W Pollard *Nat. Rev. Cancer* **4** 71 (2004)
54. N D Perkins *Trends Cell Biol.* **14** 64 (2004)
55. T D Gilmore, M Koedood, K A Piffat, D W White *Oncogene* **13** 1367 (1996)
56. T D Gilmore *Cancer Treat Res.* **115** 241 (2003)
57. M Hinz, P Loeser, S Mathas, D Krappmann, B Doerken, C Scheidereit *Blood* **97** 2798 (2001)
58. S Delhalle, R Blasius, M Dicato, M Diederich *Ann. N. Y. Acad. Sci.* **1030** 1 (2004)
59. A C Bharti, B B Aggarwal *Ann. N. Y. Acad. Sci.* **973** 392 (2002)
60. W C Earnshaw, L M Martins, S H Kaufmann *Annu. Rev. Biochem.* **68** 383 (1999)
61. S Grimm, M K Bauer, P A Baeuerle, K Schulze-Osthoff *J. Cell Biol.* **134** 13 (1996)
62. C Y Wang, M W Mayo, R G Korneluk, D V Goeddel, A S Baldwin Jr *Science* **281** 1680 (1998)
63. G A Webster, M D Perkins *Mol. Cell Biol.* **19** 3485 (1999)
64. S Xiong, H She, H Takeuchi, B Han, J F Engelhardt, C H Barton, E Zandi, C Giulivi, H Tsukamoto *J. Biol. Chem.* **278** 17646 (2003)
65. E Kopp, S Ghosh *Science* **265** 956 (1994)
66. M J Yin, Y Yamamoto, R B Gaynor *Nature (London)* **396** 77 (1998)
67. C Wahl, S Liptay, G Adler, R M Schmid *J. Clin. Invest.* **101** 1163 (1998)
68. L J Egan, D C Mays, C J Huntoon, M P Bell, M G Pike, W J Sandborn, J J Lipsky, D J McKean *J. Biol. Chem.* **274** 26448 (1999)
69. P Brennan, L A J O'Neill *Biochem. J.* **320** 975 (1996)
70. A F G Slater, M Kimland, S A Jiang, S Orrenius *Biochem. J.* **312** 833 (1995)
71. Y J Suzuki, L Packer *Biochem. Mol. Biol. Int.* **32** 299 (1994)
72. Y Yamamoto, M-J Yin, K-M Lin, R B Gaynor *J. Biol. Chem.* **274** 27307 (1999)
73. A Rossi, P Kapahi, G Natoli, T Takahashi, Y Chen, M Karin, M G Santoro *Nature (London)* **403** 103 (2000)
74. D S Straus, G Pascual, M Li, J S Welch, M Ricote, C H Hsiang, L L Senqchanthalangsy, G Ghosh, C K Glass *Proc. Natl. Acad. Sci. USA* **97** 4844 (2000)
75. E Cernuda-Morollon, E Pineda-Molina, F J Canada, D Perez-Sala *J. Biol. Chem.* **276** 35530 (2001)
76. L Pari, D Tewas, J Eckel *Arch. Physiol. Biochem.* **114** 127 (2008)
77. H I Ingolfsson, R E Koeppe II, O S Andersen *Biochemistry* **46** 10384 (2007)
78. S M Plummer, K A Holloway, M M Manson, R J Munks, A Kaptein, S Farrow, L Howells *Oncogene* **18** 6013 (1999)
79. A Duvoix, F Morceau, S Delhalle, M Schmitz, M Schnekenburger, M M Galteau, M Dicato, M Diederich *Biochem. Pharmacol.* **66** 1475 (2003)
80. Y Takada, A Bhardwaj, P Potdar, B B Aggarwal *Oncogene* **23** 9247 (2004)
81. C Jobin, C A Bradham, M P Russo, B Juma, A S Narula, D A Brenner, R B Sartor *J. Immunol.* **163** 3474 (1999)
82. L Fremont *Life Sci.* **66** 663 (2000)
83. M Holmes-McNary, A S Baldwin Jr *Cancer Res.* **60** 3477 (2000)
84. E Ignatowicz, W Baer-Dubowska *Pol. J. Pharmacol.* **53** 557 (2001)

85. H Matsuda, T Kageura, T Morikawa, I Toguchida, S Harima, M Yoshikawa *Bioorg. Med. Chem. Lett*. **10** 323 (2000)
86. G Tredici, M Miloso, G Nicolini, S Galbiati, G Cavaletti, A Bertelli *Drug Exp. Clin. Res*. **25** 99 (1999)
87. S K Manna, R S Aggarwal, G Sethi, B B Aggarwal, G T Ramesh *Clin. Cancer Res*. **13** 2290 (2007)
88. M P Nair, S Mahajan, J l Reynolds, R Aalinkeel, H Nair, S A Schwartz, C Kandaswami *Clin. Vaccine Immunol*. **13** 319 (2006)
89. F Yang, H S Oz, S Barve, W J de Villiers, C J McClain, G W Varilek *Mol. Pharmacol*. **60** 528 (2001)
90. J D Patel *J. Clin. Oncol*. **23** 3212 (2005)
91. M X Doss, S P Potta, J Hescheler, A Sachinidis *J. Nutr. Biochem*. **16** 259 (2005)
92. D N Syed, F Afaq, M-H Kweon, N Hadi, N Bhatia, V S Spiegelman, H Mukhtar *Oncogene* **26** 673 (2007)
93. M H Pan, S Y Lin-Shiau, C T Ho, J H Lin, J K Lin *Biochem. Pharmacol*. **59** 357 (2000)
94. A-R Han, J-B Kim, J Lee, J W Nam, I S Lee, C K Shim, K T Lee, E-K Seo *Chem. Pharm. Bull*. **55** 1270 (2007)
95. J-B Kim, A-R Han, E-Y Park, J-Y Kim, W Cho, J Lee, E-K Seo, K-T Lee *Biol. Pharm. Bull*. **30** 2345 (2007)
96. A Gehrt, G Erkel, T Anke, O Sterner *J. Antibiot. (Tokyo)* **51** 455 (1998)
97. G Matsumoto, J Namekawa, M Muta, T Nakamura, H Bando, K Tohyama, M Toi, K Umezawa *Clin. Cancer Res*. **11** 1287 (2005)
98. E-R Woo, Y R Pokharel, J W Yang, S Y Lee, K W Kang *Biol. Pharm. Bull*. **29** 976 (2006)
99. M Leiba, L Cahalon, A Shimoni, O Lider, A Zanin-Zhorov, I Hecht, U Sela, I Vlodavsky, A Nagler *J. Leukocyte Biol*. **80** 399 (2006)
100. Z Gavish, J H Pinthus, V Barak, J Ramon, A Nagler, Z Eshhar, M Pines *Prostate* **51** 73 (2002)
101. Z Dvorak, R Vrzal, P Maurel, J Ulrichova *Chem. Biol. Interact*. **159** 117 (2006)
102. L M Howells, B Gallacher-Horley, C E Houghton, M M Manson, E A Hudson *Mol. Cancer Ther*. **1** 1161 (2002)
103. Y Li, X Li, F H Sarkar *J. Nutr*. **133** 1011 (2003)
104. F H Sarkar, Y Li *J. Nutr*. **134** 3493S (2004)
105. N Auphan, J A DiDonato, C Rosette, A Helmberg, M Karin *Science* **270** 286 (1995)
106. R I Scheinman, P C Cogswell, A K Lofquist, A S Baldwin Jr *Science* **270** 283 (1995)
107. K Ito, E Jazrawi, B Cosio, P J Barnes, I M Adcock *J. Biol. Chem*. **276** 30208 (2001)
108. R M Nissen, K R Yamamoto *Genes Dev*. **14** 2314 (2000)
109. F-L Chiu, J-K Lin *FEBS Lett*. **582** 2407 (2008)
110. P Brooks, G Fuertes, R Z Murray, S Bose, E Knecht, M C Rechsteiner, K B Hendil, K Tanaka, J Dyson, J Rivett *Biochem. J*. **346** 155 (2000)
111. J Adams, M Behnke, S Chen, A A Cruickshank, L R Dick, L Grenier, J M Klunder, Y T Ma, L Plamondon, R L Stein *Bioorg. Med. Chem. Lett*. **8** 333 (1998)
112. J Adams, V J Palombella, P J Elliott *Invest. New Drugs* **18** 109 (2000)
113. P C Mack, A M Davies, P N Lara, P H Gumerlock, D R Gandara *Lung Cancer* **41** S89 (2003)
114. J B Sunwoo, Z Chen, G Dong, N Yeh, C C Bancroft, E Sausville, J Adams, P Elliott, C van Waes *Clin. Cancer Res*. **7** 1419 (2001)
115. R J Bold, S Virudachalam, D J McConkey *J. Surg. Res*. **100** 11 (2001)
116. J C Cusack Jr , R Liu, M Houston, K Abendroth, P J Elliott, J Adams, A S Baldwin Jr *Cancer Res*. **61** 3535 (2001)
117. J Adams, V J Palombella, E A Sausville, J Johnson, A Destree, D D Lazarus, J Maas, C S Pien, S Prakash, P J Elliott *Cancer Res*. **59** 2615 (1999)
118. L Meng, B H Kwok, N Sin, C M Crews *Cancer Res*. **59** 2798 (1999)
119. L Meng, R Mohan, B H Kwok, M Elofsson, N Sin, C M Crews *Proc. Natl. Acad. Sci. USA* **96** 10403 (1999)
120. B Yamini, X Yu, M E Dolan, M H Wu, D W Kufe, R R Weichselbaum *Cancer Res*. **67** 6889 (2007)

[a] — *Mol. Biol. (Engl. Transl.)*