Small Molecule Inhibitors of NF-kB and JAK/STAT Signal Transduction Pathways as Promising Anti-Inflammatory Therapeutics

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Abstract: In the current review, we discuss the role of NF-kB and JAK/STAT signaling pathways and their small molecule regulators in the therapy of inflammatory diseases. Considering potential harmful effects directly assigned to the COX-2 inhibition, novel therapeutically-relevant biological targets such as NF-κB and JAK/STAT signaling pathways have received a growing attention. Here we summarize recent progress in the identification and development of novel, clinically approved or evaluated small molecule regulators of these signaling cascades as promising anti-inflammatory therapeutics. In addition, we illustrate key structural modifications and bioisosteric transformations among these inhibitors to provide a helpful basis for further development of novel small molecule anti-inflammatory agents.

Keywords: NF-kB, JAK/STAT, signalling, inhibitors, anti-inflammatory, therapy.

1. INTRODUCTION

Inflammation (Latin, *inflammatio*, to set on fire) is an infinitely complex biological response of the human organism to a variety of harmful stimuli, such as pathogens, damaged cells and wounds, infection, foreign `living` substances (bacteria and viruses), and irritants. From the functional point of view the organism painstakingly attempts to remove these stimuli as well as initiate the self-healing protective defense against unauthorized intrusion. Inflammation can broadly be classified as either acute or chronic. Acute inflammation is the initial response of the human body to harmful stimuli and is generally achieved by the increased movement of leukocytes from the blood plasma directly into the injured tissues. This process is accompanied by a cascade of various biochemical events regulating inflammatory response. Chronic inflammation leads to a progressive abnormal shift in the type of cells which are present around the nest of inflammation. This reaction, in turns, is invariably accompanied by simultaneous destruction and healing of the tissue from the inflammatory process. In some diseases, the immune system can inappropriately trigger an inflammatory response with no foreign substances or infection presence. In these conditions the body's defense mechanism causes huge damage to its own tissues sustaining the perpetual or transitory activation of the inflammatory process. Abnormalities associated with uncontrolled inflammation comprise a large, unrelated group of disorders which underlie a variety of dangerous human diseases, such as rheumatoid arthritis (RA), ischaemic heart disease, atherosclerosis, cancer, fibrosis, hay fever as well as several neurodegenerative disorders including Parkinson's and Alzheimer's diseases. To avoid these harmful outcomes, inflammation is normally tightly controlled by a variety of regulating factors which, in turn, can be potential targets for small molecule drug compounds.

The `new-age` treatment of diseases accompanied or initiated/sustained by harmful inflammation have been revolutionized by the discovery of common pro-inflammatory effector mechanisms involving a large variety of signaling molecules, specific proteins and transcription factors. Among a huge number of pro-inflammatory mediators cyclo-oxygenase 1 and 2 (COX-1/-2), mitogen-activated protein kinases (MAPKs), janus protein tyrosine kinases (JAKs), nuclear transcription factor (NF-κB) and signal transducers and activators of transcription (STAT) are the most principal effectors that directly or indirectly lead to the production of a vast number of pro-inflammatory cytokines and regulatory proteins such as IL-1/6, TNF-α, MIF, IFN-γ, MMPs. These mediators, in turn, jointly support inflammatory processes providing both homeostatic as well as pathological outcomes. In the early '90s, anti-cytokine therapies started and fully confirmed the critical role of these molecules in autoimmune diseases. During the last decade, our knowledge about the molecular basis of inflammation has been considerably broadened and much is actually known about the key role of pro-inflammatory mediators [1,2]. The accumulated findings are currently opening exciting doors to new horizons for the drug treatment of inflammatory-associated diseases through selective targeting of specific pro-inflammatory factors.

Historically, anti-inflammatory drugs had their origins in the serendipitous discovery of certain plants and their extracts being applied for the relief of pain, fever and inflammation. When salicylates were discovered in the mid-19th

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century, this enabled these compounds to be synthesized and later modified in acetyl-salicylic acid or Aspirin. Subsequent period has been inaugurated by the `triumph` of steroid-based anti-inflammatory compounds. Likewise, the chemical advances of the 19^{th}-20^{th} centuries led to development of the non-steroidal anti-inflammatory drugs (NSAIDs). The most prominent members of this group of drugs are aspirin, ibuprofen, and naproxen partly because they are available over-the-counter in many areas. However, many of clinically approved drug compounds were recently found to have significant side effects leading to increased clinical risk and various dangerous complications including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. Thus, a number of developed anti-inflammatory agents such as COX-1/-2 inhibitors showed a high incidence of gastrointestinal diseases (esophagitis, peptic ulcer, peptic ulcer complications, etc.) and renal side effects (the principal adverse reactions directly associated with NSAIDs usage) [3-5]. NSAIDs and aspirin can also damage the small bowel and the colon. These effects are dose-dependent, and in many cases severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding, and death, significantly limiting the clinical application of many NSAIDs. For example, one of the main drawbacks of many COX inhibitors lies in the curtailed production of gastroprotective PGs accompanied with the concurrent increased production of the gastro-damaging and bronchoconstrictive leukotrienes (LTs). Moreover, the adverse effects directly associated with the clinical usage of COX inhibitors are not only limited by gastro-intestinal and renal side effects. An alarming turn of events took place three years ago when in 2004 Vioxx (Rofecoxib) and some later, in 2005, Bextra (Valdecoxib) were both withdrawn worldwide because of serious cardiovascular damage. A recent through meta-analysis of many launched and clinically evaluated NSAIDs has found an 80% increase in the risk of myocardial infarction with nearly all of the classical and novel COX-2 antagonists as well as traditional anti-inflammatory agents compared with placebo [6,7]. It was also shown that nonselective NSAIDs may have deleterious effects on knee cartilage inducing knee osteoarthritis [8]. Lack of responsiveness, various side effects as well as resistance to many of the currently existing anti-inflammatory drugs mean that the design and development of novel anti-inflammatory agents is still absolutely necessary. Thus, reasonable efforts are currently underway to partly or completely overcome fatal cardiovascular reactions associated with coxibs usage, as well as elucidate the roles of COX-1 and COX-2 in cardiovascular diseases and stroke in the hope that there may be some basis for developing novel agents (e.g. nitric oxide-donating NSAIDs) to appropriately control these conditions. Considering all the potential risks listed an advanced generation of novel anti-inflammatory drugs primarily acting against pro-inflammatory mediator production are being discovered and developed based on their effects on alternative signal transduction pathways. These drugs are currently being heralded as the `new-age` therapies to control those diseases where these factors and signaling molecules as well as other non-prostaglandin components of chronic inflammatory and neurodegenerative diseases are becoming manifest. What started out as drugs to control inflammation, pain and fever in the last two centuries now has exploded to reveal an enormous range and type of anti-inflammatory agents and discovery of new prominent therapeutic targets to treat a whole range of conditions that were never hitherto envisaged. Among these biological targets NF-κB and JAK/STAT signaling cascades represent the most promising avenue for achieving optimal therapeutic response with minimal side effects.

2. SMALL MOLECULE INHIBITORS OF PRO-INFLAMMATORY SIGNALING CASCADES

The pro-inflammatory signaling pathways and cellular mechanisms that initiate the normal inflammatory response have become increasingly well characterized. However, the therapeutic arms by which uncontrolled and destructive inflammation can be temporarily or permanently switched off still remain unclear because of hugely complex and intricate interrelationships among a heap of pro-inflammatory mediators. Recent data clearly indicate that the resolution of inflammation is an active process controlled by a vast number of endogenous factors that can either activate or suppress pro-inflammatory gene expression and cell trafficking as well as strongly regulate inflammatory-cell apoptosis and phagocytosis. Recent progress that has been made in our understanding towards inflammatory signaling cascades has already identified a series of novel promising targets, notably in pathways involving NF-κB and JAK/STAT transcription factors, p38 MAP, GSK3 and IκB kinases activation, PLA2, caspases and various growth factors and GF-Rs, HMGB1, PDE4, mast cells and related TRPV channels, T-lymphocyte and platelet activation, MMPs, glucocorticoids, TIM-3, ILs-17/18/27, TWEAK, Toll (TLR) and Toll-like (TLR-like) receptors as well as Wnt/Frizzled signaling route. For example, it was clearly demonstrated that in addition to the proteolytic activity of caspases they can participate *via* their CARD domain in signaling complexes leading to NF-κB and p38 MAP kinase activation [9]. Other biological targets such as the key components and messenger molecules implicated in pathways activated *via* TNFR, GPCR, BCR, LTβR and Nod-like receptors also represent promising therapeutic targets for management of inflammation, and might show efficacy without being limited by effects on host defense. The prime challenge is to better understand the principal role of each targets, and to devise effective strategies to modulate their activities through small molecule agents or naturally derived compounds.

2.1. NF-κB Inhibitors

The NF-κB intracellular signaling system seems to be becoming the dominant paradigm for specific signal transduction molecules, regulatory proteins and gene activation in response to inflammatory and menacing stimuli. The spectrum of NF-κB target genes include primarily those that are responsible for mediators and effectors of both innate and adaptive immunity and inhibitors of apoptosis, growth promoting factors and virus-encoded proteins involved in viral replication, as well as self-regulatory proteins for NF-κB actions. In addition to the original inflammatory conditions, NF-κB signaling pathway deeply involves in the onset of various inflammatory-related autoimmune disorders such as leukemia, inflammatory bowel disease, arthritis, sepsis, asthma, multiple sclerosis (MS), colitis, diabetic neuropathy,

AIDS as well as different types of cancer [10,11]. For example, RA pathology was found to be thoroughly mediated by a number of cytokines (TNF-α, IL-1/6/17, IFN-γ, etc.), chemokines (MCP-1/4, CCL18, etc.), cell adhesion molecules (ICAM-1, VCAM-1, etc.) and MMPs. Thus, in patients diagnosed with RA, activation of NF-κB signaling pathway results in the transcription of a multitude of responsive genes that contribute to the inflammatory phenotype, including TNF-α, IL-6 and MMPs that, in turn, recruit immune cells to the inflamed pannus. This is largely a consequence of activation of the canonical NF-κB pathway that leads finally to the formation of heterodimeric transcriptional units composed of different p/p complexes which directly initiate gene expression [12]. Recent animal studies and data derived from genetic analysis in humans overwhelmingly indicate that signaling *via* NF-κB is a key process controlling inflammation and thereby constitutes an attractive target for anti-inflammatory therapeutic interventions [13].

Being severely constrained by the available space of current review we have elucidated solely the key aspects of the NF-κB signaling system. A schematic diagram of the canonical and alternative NF-κB pathways is shown in Fig. (**1**). While the canonical route is directly activated by many well-known receptor systems such as GPCR and TNFR, signaling through a subset of unique receptors including LTβR, CD40 and BR3 activates the alternative NF-κB signaling pathway *via* the activation of NIK kinase, which in turn directly

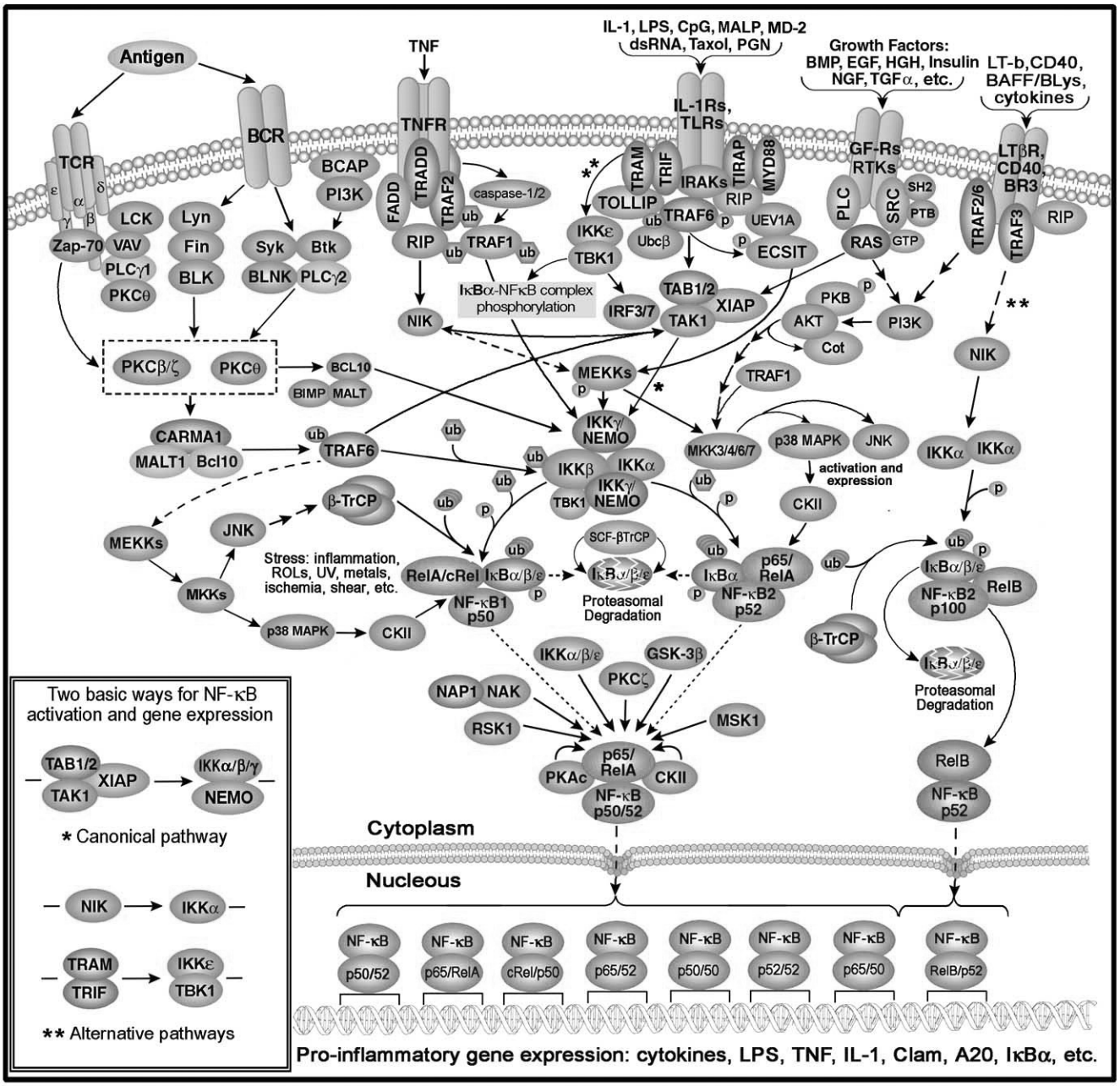


Fig. (1). Canonical and alternative NF-κB signaling pathways implicated in inflammation.

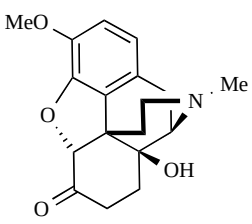
stimulates IKK complexes (see below). Recent enzymatic and genetic studies have shown that NF-κB family is composed of five principal members including NF-κB1/(p50), NF-κB2/(p52), RelA/(p65), RelB, and c-Rel/(Rel) (15 possible dimers). Hetero- and homo-dimerization observed among these nuclear factors which exhibit differential DNA-binding specificities and different transactivation potential lead to the formation of the following transcriptional complexes: p50/RelA, p50/c-Rel, p52/c-Rel, p65/c-Rel, RelA/RelA, p50/p50, p52/p52, RelB/p50 and RelB/p52. The activation of NF-κB is thought to be part of a stress response as it is activated by a variety of stimuli that include inflammatory cytokines such as TNF-α and ILs-1/6, growth factors, LPS (lipopolysaccharides, the main components of bacterial cell walls) or lymphokines, oxidant-free radicals, inhaled particles, viral infection or expression of certain viral or bacterial gene products, UV irradiation, B or T-Cell activation, pharmacological agents, different stress conditions as well as other physiological and non physiological stimuli [14]. Depending on the stimulus, the mechanism of activation involves several overlapping and nonoverlapping steps briefly overviewed in Fig. (**1**). Among the various stimuli known to date, IL-1- and TNF-induced activation of NF-κB is studied in greater detail. In general, TNF/NF-κB pathway involves the interaction of the ligand with specific transmembrane receptor (TNFR), which then recruits a protein called TRADD (TNF Receptor-Associated Death Domain). This protein binds to TRAF2 (TNF Receptor-Associated Factor-2), which, in turn, activates RIP (Receptor-Interacting Protein). RIP recruits and directly activates NIK (NF-κB-Inducing Kinase) and MEKKs (Mitogen-Activated Protein Kinase Kinases) thereby inducing a rapid phosphorylation of IKK (IκB kinase complex). NF-κB dimers are sequestered in the cytosol of unstimulated cells *via* non-covalent interactions with a class of inhibitor molecules, called IκBs, which form the protein conglomerate IKK. The IKK, in turn, includes three subunits: IKK-α (IKK1, a predominant IκB kinase), IKK-β (IKK2) and IKK-γ (NEMO) that has no catalytic activity but plays a critical regulatory role. To date seven IκBs have been identified: IκB-α, IκB-β, IκB-γ, IκB-ε, BCL3, p100 and p105. These complexes prevent nuclear translocation of NF-κB into the nucleus until signals that induce NF-κB activity cause the phosphorylation of IκBs, their dissociation and subsequent degradation, thereby releasing the NF-κB active complex. The degradation of IκB proteins is also carried out by the proteasome but only after prior phosphorylation of IκB by the IKK. The phosphorylated IκBs is further specifically recognized by β-TrCP, a component of the ubiquitin ligase complex, and TRAF6 which jointly regulate poly-ubiquitination of IκB and its subsequent proteasomal degradation. In the long run, the `shackle-free` NF-κB/Rel complexes translocate into the cell nucleus where, either alone or in combination with other transcription factor families including AP-1, Ets and STAT, induce pro-inflammatory gene expression such as cytokines IL-1/6, TNF-α and LPS which, in turn, restimulate and maintain inflammation.

In past decades, enormous resources have been recruited to invent, develop and apply novel therapeutics against inflammation. Among numerous compounds that were found to have considerable physiological and therapeutic significance against different inflammation conditions acting directly on the NF-κB protein complexes or NF-κB-related signaling pathways plant-derived agents (extracts and essence), steroid-based compounds and several small molecule mediators jointly compose a large therapeutic group. Recent advances achieved in different preclinical models have clearly identified a wide therapeutic potential of many small molecular NF-κB inhibitors (Fig. (**2**)), neutralizing antibodies/proteins or genetically altered gene functions against various inflammatory mediators. Several clinically approved drug compounds are currently launched onto the world pharmaceutical market. However, inhibition of the NF-κB signaling route can result in an exacerbation of inflammation if TNF-α production by macrophages is not completely controlled. In addition, there is a certain amount of risk due to induced immunodeficiency that may follow inhibitory treatment. Moreover, its primary anti-apoptotic function suggests that blockage of NF-κB activation has dramatic effects on cell functions and survival and eventually worsens the course of an inflammatory disease. Thus, it will be very important that such inhibitors are carefully monitored before their long-term use in chronic inflammatory conditions. Anyway, the relative *pros and cons* of NF-κB inhibition and corresponding drug therapy have already been critically reviewed [15].

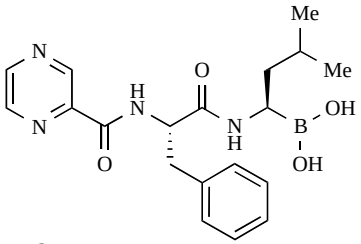
Table **1** summarizes a list of large-scale clinical trials that have been conducting by various pharmaceutical firms. Several inhibitors were found to have an improved therapeutic potential then they were used in combination.

As an illustration, Combunox™, a drug combination of Oxycodone hydrochloride **1** (a semi-synthetic opioid analgesic) and Ibuprofen **8** (NSAID with analgesic and antipyretic properties), was initially launched by Forest in 2005 for the short-term (up to 7 days) treatment of acute, moderate-to-severe pain [16]. Originally developed by BTG, the product was exclusively licensed to Forest for manufacturing, sales, and marketing in the U.S. The key anti-inflammatory component is Ibuprofen which mode of action is still not completely understood but, presumably, it is closely similar to other NSAIDs, and is thought to be related to its inhibition of COX activity and PGs synthesis. In addition, several recent studies strongly suggest that the prime activity of Ibuprofen is directly associated with its ability to inhibit NF-κB activation (see below, Ibuprofen) [17]. It should be especially noted that it is generally well tolerated after single or multiple doses and short-term usage is not expected to produce any of the serious adverse effects typically associated with the long-term treatment with opioids or NSAIDs (see above). Therefore, oxycodone/ibuprofen is an effective, convenient treatment option for the short-term management of acute, moderate-to-severe pain and different inflammatory conditions.

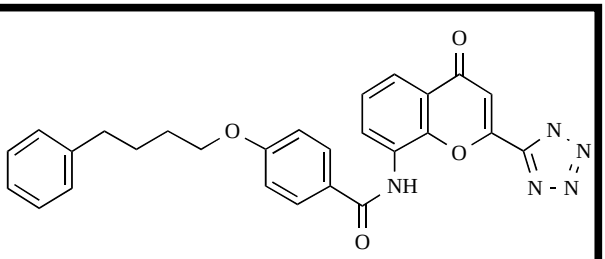
As a further example, Bortezomib **2** is a potent proteasome inhibitor originally launched in the U.S. in 2003 by Millennium for the treatment of multiple myeloma. During a recent clinical trial Bortezomib was found to effectively block multi-ubiquitinated protein degradation by inhibiting 26S proteasome activity involved in cell cycle regulation, cellular apoptosis, inflammation, and immune surveillance. Thus, Bortezomib-induced proteasomal inhibition leads to prolonged storage of NF-κB/IκB complexes resulting in



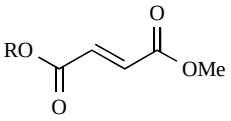
1
Oxycodone*



2
Bortezomib
(MLN-341)

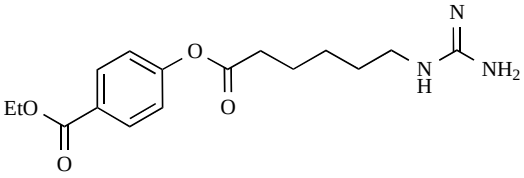


3
RS-411
(Pranlukast)

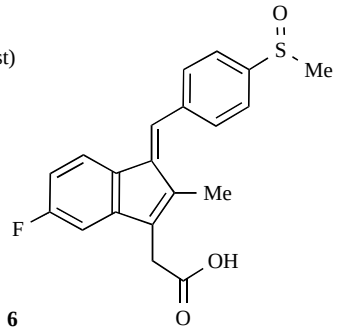


R=Me, Ca, Zn, Mg

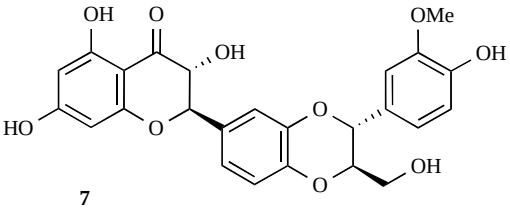
4
Fumaderm*



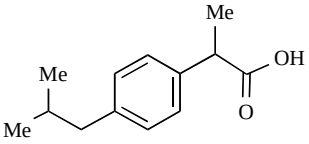
5
Gabexate



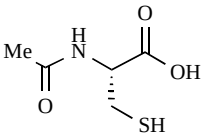
6
MK-231
(Sulindac, Arthrocine)



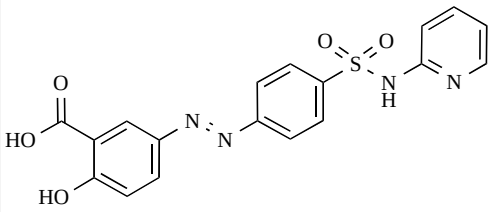
7
Silibinin
(Silymarin, Legalon)



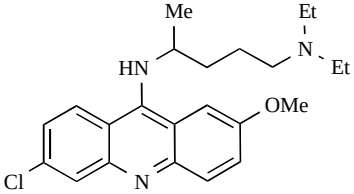
8
ST-1482
(ZAG-1701, Ibuprofen)



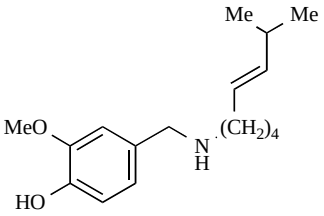
9
RK-0202
(NSC-111180, N-Acetylcysteine)



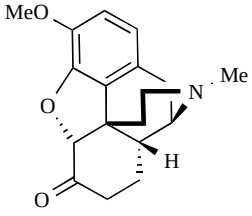
10
Salazosulfapyridine
(Sulphasalazine, Azulfidine)



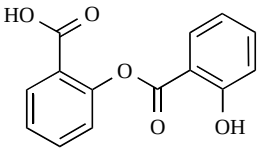
11
Curaxin CBLC-102
(Mepacrine, Tenicridine)



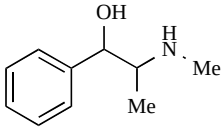
12
NGX-3781
(Capsaicin, TQ-1018, Axsain)



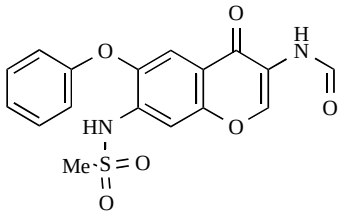
13
Hydrocodone*



14
NSC-49171
(Salsalate, Salflex)

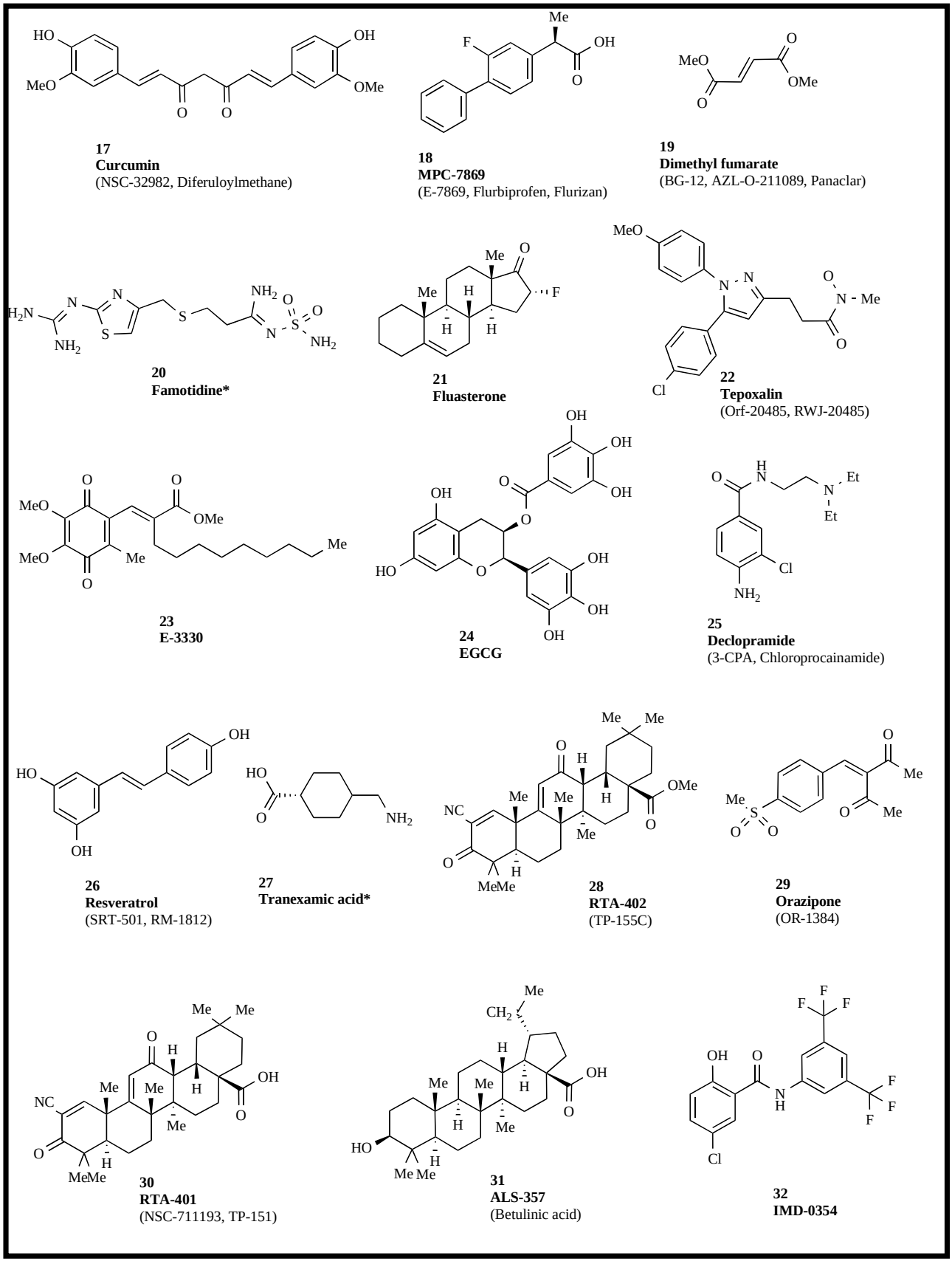


15
Pseudoephedrine*



16
T-614
(Iguratimod, Kolbet)

(Fig. 2). Contd…..



17
Curcumin
(NSC-32982, Diferuloylmethane)

18
MPC-7869
(E-7869, Flurbiprofen, Flurizan)

19
Dimethyl fumarate
(BG-12, AZL-O-211089, Panaclar)

20
Famotidine*

21
Fluasterone

22
Tepoxalin
(Orf-20485, RWJ-20485)

23
E-3330

24
EGCG

25
Declopramide
(3-CPA, Chloroprocainamide)

26
Resveratrol
(SRT-501, RM-1812)

27
Tranexamic acid*

28
RTA-402
(TP-155C)

29
Orazipone
(OR-1384)

30
RTA-401
(NSC-711193, TP-151)

31
ALS-357
(Betulinic acid)

32
IMD-0354

(Fig. 2). Contd…..

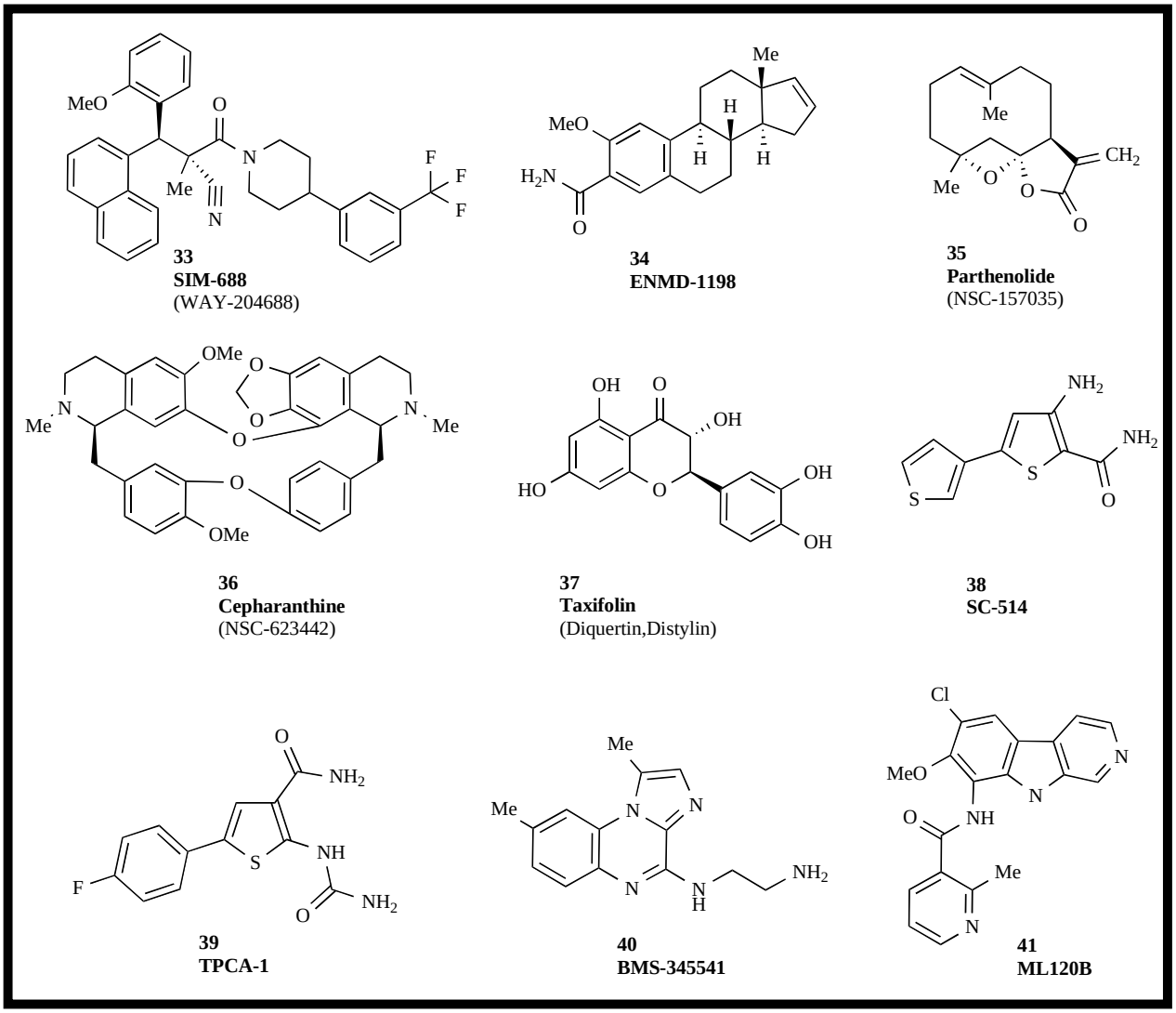


Fig. (2). Small molecule NF-κB inhibitors already released onto the pharmaceutical market or currently entered in Phase I-III clinical trials (* Compounds which are commonly used in combination).

Table 1. The Main Characteristics of Clinically Proven Small Molecule NF-κB Inhibitors

Compound (Fig. (2))	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
1*	Launched-2005	Non-specific inflammation	Multi-targeted inhibitor, especially against NF-κB and COX-1/2/3	BTG
2	Launched-2003	Non-specific inflammation and cancer	NF-κB, AP-1 and Proteasome inhibitor	Millennium Pharmaceuticals and Janssen-Cilag
3	Launched-1995	Upper respiratory tract disorders, chronic obstructive pulmonary diseases (COPD), asthma, allergic rhinitis	Multi-targeted inhibitor, especially against NF-κB and Leukotriene CysLT2/ CysLT1	Ono
4*	Launched-1994	Psoriasis and multiple sclerosis	NF-κB targeted inhibitor	Biogen Idec
5	Launched-1978	Pancreatic disorders	Multi-targeted inhibitor, particularly against NF-κB and AP-1	Ono

(Table 1). Contd…..

Compound (Fig. (2))	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
6	Launched-1976	Ankylosing spondylitis, rheumatoid arthritis, gout, osteoarthritis	NF-κB inhibitor and ABCC1/3 expression enhancer	Merck
7	Launched-1972	Liver and biliary tract disorders, lipoprotein disorders, disorders of the coronary arteries and atherosclerosis, diabetes, viral hepatitis	Multi-targeted inhibitor especially against NF-κB, HMG-CoA reductase, Reverse transcriptase as well as ApoB secretion	Madaus
8	Launched-1969	Ankylosing spondylitis, rheumatoid arthritis, osteoarthritis	NF-κB and COX-1/2/3 inhibitor	Zambon and Merckle GmbH
9	Launched-1968	Renal failure, interstitial lung diseases, inflammatory bowel disease, obsessive-compulsive disorder (COPD), metabolic disorders (not specified), psychiatric disorders (not specified), mucolytics, cardiovascular diseases, COPD, cocaine dependency, preterm labor, mucositis	NF-κB targeted inhibitor	Zambon and Yale University
10	Launched-1944	Inflammatory bowel disease and rheumatoid arthritis	NF-κB targeted inhibitor	Pfizer
11	Launched-1932	Prostate and renal cancer therapy, malarials, protozoal diseases, prion diseases	Multi-targeted inhibitor, particularly against NF-κB and Secretory phospholipase A2 (sPLA2)	Bayer
12	Launched	Neuropathic pain and diabetic neuropathy	Multi-targeted inhibitor, especially against NF-κB, TRPV1 and tNOX	NeurogesX
13*	Launched	Non-specific inflammation	NF-κB and COX-1/2/3 inhibitor	Abbott
14	Launched	Rheumatoid arthritis, diabetes, osteoarthritis	NF-κB targeted inhibitor	Roche
15*	Launched	Upper respiratory tract disorders	NF-κB and COX-1/2/3 inhibitor	SCOLR Pharma
16	Pre-Registered	Rheumatoid arthritis	NF-κB targeted inhibitor	Toyama
17	Phase III	Ocular genetic disorders, arthritis, alzheimer's dementia, psoriasis, cystic fibrosis, premalignant conditions, malarials, myelodysplastic syndrome, pancreatic cancer, multiple myeloma, mucositis	Multi-targeted inhibitor, especially against NF-κB, EGFR and CCND1 expression, Glucose-6-phosphatase, HIV Integrase as well as COX-2	Johns Hopkins University
18	Phase III	Non-specific inflammation, arthritis, alzheimer's dementia, colorectal and prostate cancers, oncolytic disorders	NF-κB modulator and γ-Secretase inhibitor	Loma Linda University and Encore
19	Phase III	Psoriasis and multiple sclerosis	NF-κB targeted inhibitor	Biogen Idec
20*	Phase III	Non-specific inflammation	Multi-targeted inhibitor, especially against NF-κB and COX-1/2/3. Histamine H2 receptor antagonist	Horizon Therapeutics
21	Phase II	Psoriasis, arthritis, systemic lupus erythematosus, diabetes, actinic keratoses, multiple sclerosis, oncolytic disorders	NF-κB targeted inhibitor	Temple University
22	Phase II	Psoriasis, ophthalmic inflammation, allergy and asthma	NF-κB and COX-1/2/3 inhibitor	Ortho-McNeil

(Table 1). Contd…..

Compound (Fig. (2))	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
23	Phase II	Non-specific inflammation	NF-κB targeted inhibitor	Eisai
24	Phase II	Actinic keratoses, lipoprotein disorders, dermatologic disease, diabetes, ophthalmic disorders, parkinson disease, cancers and liver fibrosis	Multi-targeted inhibitor, especially against NF-κB, SGLT-1, PDGFR, BACE, VEGFR, VEGFR-2 (FLK-1/KDR), tNOX, AP-1, etc.	Kyushu University
25	Phase II	Inflammatory bowel disease and colorectal cancer	NF-κB targeted inhibitor	OxiGene
26	Phase II	Psoriasis, ocular disorders, diabetes, disorders of the coronary arteries and atherosclerosis, obesity, herpes virus, neuromuscolar genetic disorders	Multi-targeted inhibitor, especially against NF-κB, COX-1, Xanthine Oxidase, MAO-A and BACE1. APOA1 expression enhancer and SIRT1 activator	Royalmount Pharma
27*	Phase II	Non-specific inflammation	NF-κB and COX-1/2/3 inhibitor	Sawai
28	Phase I/II	Inflammatory bowel disease, autoimmune diseases, rheumatoid arthritis, melanoma, solid tumors, renal diseases, pancreatic cancer	Multi-targeted inhibitor, especially against NF-κB, Bcl-2, IKK-1 (IKK-α) and NOX production. PPARγ agonists, NADPH and Heme Oxygenase activator	Dartmouth College and M.D. Anderson Cancer Center
29	Phase I	Inflammatory bowel disease, allergy, asthma	NF-κB targeted inhibitor	Orion Corp.
30	Phase I	Inflammatory bowel disease, stroke, solid tumors, leukemia	Multi-targeted inhibitor, particularly against NF-κB and NOX production. PPARγ agonists	Dartmouth College and National Cancer Institute (US)
31	Phase I	Melanoma and severe acute respiratory syndrome (SARS)	Multi-targeted inhibitor, particularly against NF-κB, DGAT, SARS Coronavirus 3C-like protease and DNA Topoisomerase-I. Caspases 3/8 activator	University of Illinois
32	Phase I	Interstitial lung diseases, disorders of the coronary arteries and atherosclerosis, atopic dermatitis	NF-κB and IKK-2 (IKK-β) inhibitor	Institute of Medicinal Molecular Design
33	Phase I	Rheumatoid arthritis, non-specific inflammation, sepsis	NF-κB inhibitor and Estrogen receptor (ER) α/β ligand	Wyeth Pharmaceuticals
34	Phase I	Non-specific inflammation and solid tumors	Multi-targeted inhibitor, particularly against NF-κB, STAT-3 and HIF-1α factors	EntreMed
35	Clinical	Atherosclerosis therapy, leishmaniasis, oncolytic disorders, septic shock	NF-κB targeted inhibitor	Ashbury Biologicals
36	Clinical	Non-specific inflammation, HIV infection, cancers	NF-κB targeted inhibitor	Tohoku Pharmaceutical University
37	Clinical	Atherosclerosis, lipoprotein disorders, ischemic stroke, hepatitis virus, HIV infection	Multi-targeted inhibitor, especially against NF-κB, HMG-CoA reductase, Reverse transcriptase and ApoB secretion	Sigma Chemical and National Yang-Ming University
38	Early clinical trials	Non-specific inflammation and anti-tumor promoting effects, particularly against Adenocarcinoma	Selective inhibitor of IKK-2 activity	Pfizer

(Table 1). Contd…..

Compound (Fig. (2))	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
39	Early clinical trials	Corneal ulcer, COPD and related airway inflammation	Inhibitor of human IKK-2 activity	GlaxoSmithKline
40	Early clinical trials	Lung inflammation including airway inflammation in asthma, arthritis, inflammatory bowel diseases and cancer. It also suppresses graft rejection	Highly selective and potent inhibitor of IKK-2 activity. It bounds to an allosteric binding site	Bristol-Myers Squibb
41	Early clinical trials	Rheumatoid arthritis, COPD (particularly chronic airway inflammation) as well as cancer	Selective, reversible, and ATP-competitive small molecule inhibitor of IKKβ	Millennium Pharmaceuticals

*Clinically validated drug combinations: **1**. Combunox (Oxycodone/Ibuprofen); **4**. Fumaderm (Dimethyl fumarate/(Ca,Mg,Zn) Monoethyl fumarates; **13**. Vicoprofen (Hydrocodone/Ibuprofen); **15**. Rhinadvil (Pseudoephedrine/Ibuprofen); **20**. HZT-501 (Famotidine/Ibuprofen); **27**. SMS-113 (Tranexamic acid /Ibuprofen).

significant decrements of mRNA expression of TNF-α and IL-6 and decreased production of iNOS and COX2 [18]. In addition, Bortezomib as well as several other NF-κB inhibitors including dexamethasone, thalidomide also showed inhibitory effects on LPS-induced GADD45β (growth arrest and DNA damage-inducible family of genes) expression involved in the regulation of cell cycle progression and apoptosis as well as acute inflammation [19].

Pranlukast **3** was originally launched in Japan in 1995 as potential small molecule antagonist of leukotriene CysLT1 and CysLT2 activities deeply implicated in many inflammatory disorders such as chronic airway inflammation, bronchial asthma and allergic rhinitis [20]. It was also found that cysteinyl leukotrienes can directly activate the NF-κB signaling pathway [21]. Thus, Pranlukast strongly inhibits NF-κB signaling pathways leading to the reduction of RANTES, TGF-β, TH2, ILs-4/6/11/13, IFN-γ and VEGF gene expression, LPS production and activity which in turn might cause migration of eosinophils and activated T-lymphocytes into the inflammatory nidus. However, a series of recent studies have strongly suggested that Pranlukast also inhibits NF-κB signaling route and the expression of related pro-inflammatory genes including IL-5, MUC2 and TNF-α *via* a mechanism distinct from leukotriene-associated antagonism [22].

Gabexate **5** is an effective NF-κB, AP-1 (activator protein-1) and protease inhibitor that was launched in 1978 by Ono as a powder for injection for the treatment of disseminated intravascular coagulation and for the treatment of acute pancreatitis. Particularly, this compound was considered to be a key mediator in the regulation of immune response to infection, inflammatory-related diseases including cancer and autoimmune disorders, septic shock, viral penetration and improper immune development. For example, in human pancreatic cancer cell lines, Gabexate strongly inhibits TNF-α-induced NF-κB activation and enhances cellular apoptosis mainly through the activation of caspases 3 and 7 as well as dramatically suppresses the invasive potential of cells studied [23]. It has been shown to reduce endotoxin-induced pulmonary vascular injury in an animal model of sepsis by inhibiting leukocyte activation. It also inhibits the expression of leukocyte adhesion molecules by blocking the NF-κB-mediated transcription in HUVECs. Presumably, the actual mechanism of action is based on the inhibition of IκB degradation, results in the suppression of TNF-α production in monocytes stimulated by LPS [24]. In addition, Gabexate mesilate might significantly reduce LPS-induced pulmonary vascular injury not only by inhibiting monocytic TNF-α production but also by inhibiting the expression of endothelial leukocyte adhesion molecules [25].

MK-231 (Sulindac) **6** is widely used to relieve pain and prevent inflammation as well as tenderness, swelling, and stiffness caused by osteoarthritis, RA, ankylosing spondylitis, etc. From the functional point of view, MK-231 (Sulindac) **6** strongly inhibits IκB-β degradation and Ras signaling pathway thereby reducing Akt1 and NF-κB activities [26]. For example, in endometriotic cells, MK-231 dramatically decreased NF-κB activation and RANTES protein secretion as well as diminished TNF-α and IL-1β-induced NF-κB DNA binding activity [27]. In mouse bone marrow-derived mast cells, Sulindac significantly decreased IKKα phosphorylation and degradation, NF-κB phosphorylation and activation, as well as TNF-α production [28]. This compound also inhibited IFNγ-induced expression of chemokine CXCL9 that is very essential for activated T-cells, whereas the interferon-induced expression of CXCL10 or IFN regulatory factor-1 was not affected by MK-231 [29]. Surprisingly, Sulindac had no inhibitory effect on IFNγ-induced STAT1 activation; however, constitutive NF-κB activity was powerfully suppressed. In addition, MK-231 was found to strongly inhibit growth and progression of various cancer cells [30]. Thus, summarising these results, it can be concluded that Sulindac exerts strong anti-inflammatory effects mainly by suppression of NF-κB translocation, inhibition of NF-κB-mediated gene transcription, RANTES gene expression, and protein secretion.

RK-0202 (*N*-Acetylcysteine, NAC) **9** is a promising drug compound with powerful antioxidant and related anti-inflammatory effects. For example, NAC was recently found to be very effective in suppressing styrene-induced MCP-1 secretion *via* NF-κB inhibition and was capable of inhibiting the upregulation of GST expression [31]. In endothelial cells, low concentration of NAC dramatically activates IKKα activity, thereby inhibiting the downstream NF-κB and ICAM-

1 induction by TNF-α while high doses of NAC cause glutathionylation of IKKα, thereby inhibiting its activity that in turn enhances the downstream NF-κB activation and ICAM-1 expression by TNF-α [32]. Immunoblotting analysis has clearly shown that NAC treatment leads to a significant decrease in NF-κB expression [33]. It was recently demonstrated that in mice with traumatic brain injury NAC significantly inhibited NF-κB activity and related IL-1β, TNF-α, and ICAM-1 production with no effect on IL-6 level [34]. In pancreatitis rats, NAC powerfully blocks NF-κB activation and associated TNF-α expression [35]. LPS-induced inflammatory reaction can also be suppressed by NAC treatment that significantly reduces IL-6, IL-1β, TNF-α, MIP-1 and IRAK-1/4 production with no detectable effect on IL-10 production [36,37]. NAC can also modulate neutrophil activity *via* NF-κB inhibition *in vitro* (IC_{50}=10 μM) [38]. Acting as a strong NF-κB inhibitor NAC also abrogated acetaminophen-induced increases in TNF-α, KC/gro, and IL-10 levels, but enhanced expression of the anti-inflammatory cytokines IL-4 and TGF-β [39]. This compound also regulates hepatocyte activity by inhibition of both the NF-κB activity and NO synthase expression thereby providing preliminary evidence that NAC may have hepatoprotective actions of potential relevance in chronic inflammatory conditions [40]. In patients with sepsis, NAC significantly decreased NF-κB activation as well as related levels of IL-6/8 and ICAM-1 [41].

Dimethylfumarate (DMF) **19**, an NF-κB activation inhibitor, is currently undergoing phase III clinical trials at Biogen Idec for the treatment of relapsing-remitting multiple sclerosis. The company had also been investigating this compound for the oral treatment of mild to moderate psoriasis. The product is a second-generation fumarate derivative with promising anti-inflammatory potential. The early study has shown that DMF potently inhibits ICAM-1, VCAM-1, and E-selectin expression as well as reduces adhesion of U937 cells to stimulated HUVEC through the inhibition of NF-κB signaling pathway [42]. It was also found that DMF effectively blocks the TNF-induced translocation of NF-κB/p65 transcriptional complex into the nucleus of human endothelial cells [43]. Recent studies provide a detailed profile of DMF action. Thus, it was shown that DMF inhibits nuclear entry of NF-κB in rat heart endothelial cells (RHEC) and significantly reduces myocardial infarct size after ischemia and reperfusion in rats *in vivo*. [44]. A recent study demonstrates that DMF can effectively prevent the TNF-α-induced increase in M-CSF (macrophage colony stimulating factor) production by the inhibition of NF-κB transcriptional activity [45]. DMF was found to significantly inhibit both the MSK1 and MSK2 activities in cultured human keratinocytes [46]. Also, the study has provided strong evidence that DMF decreases phosphorylation of NF-κB/p65 complex, which is known to be transactivated by MSK1. Furthermore, a significant decrease in NF-κB binding to the IL-8/20 DNA domains leading to related decrease in IL-8 and IL-20 mRNA expression were also estimated. The obtained results strongly suggest that DMF specifically inhibits MSK1/2 activations and subsequently blocks NF-κB-induced gene-transcriptions, which are hugely important in the pathogenesis of inflammation. Other study highlights that the suppressive effect of DMF on pro-inflammatory cytokines and chemokines production is directly associated with selective inhibition of NF-κB signaling pathway [47]. In addition, DMF was also shown to induce apoptosis in various cells, particularly in human T-cells [48].

At least three well-known plant-derived flavonoids, Silibinin **7**, Curcumin **17** and Resveratrol **26**, were found to be key mediators of NF-κB signaling pathway [49]. Extensive research over the last decade has clearly shown that Silibinin can effectively suppress the proliferation of a variety of tumor cells; this is accomplished through cell cycle arrest at the G1/S-phase, induction of cyclin-dependent kinase inhibitors (such as p15, p21 and p27), down-regulation of anti-apoptotic gene products (e.g., Bcl-2 and Bcl-xL), inhibition of cell-survival kinases (AKT, PKC and MAPK) and inhibition of inflammatory transcription factors (e.g., NF-κB). This compound can also down-regulate gene products involved in the proliferation of tumor cells (cyclin D1, EGFR, COX-2, TGF-beta, IGF-IR), invasion (MMP-9), angiogenesis (VEGF) and metastasis (adhesion molecules). In general, the anti-inflammatory effects of Silibinin are mediated through suppression of NF-κB-regulated gene products, including COX-2, LOX, inducible iNOS, TNF-α and IL-1/6 [50]. For instance, it was recently suggested that Silibinin could be quite effective in the treatment of multiple sclerosis [51]. Thus, Silibinin significantly reduced the histological signs of demyelination and inflammation in experimental autoimmune encephalomyelitis (EAE) by down-regulating the secretion of pro-inflammatory Th1 cytokines and up-regulating the anti-inflammatory Th2 cytokines *in vitro*. It was also found that Silibinin inhibited UVB-caused phosphorylation and nuclear translocation of STAT3 and p65, as well as NF-κB DNA binding activity in SKH1 hairless mice providing a strong protective effect against photocarcinogenesis [52]. It was also suggested that Silibinin may exert its anti-inflammatory effect through the inhibition of IKK enzymatic activity and overexpression of the dominant-negative mutant IκB and Brg-1 [53,54].

Curcumin is a polyphenol present in the spice turmeric, which can directly scavenge free radicals such as superoxide anion and nitric oxide and modulate important signaling cascades mediated *via* NF-κB, MAPK and JAK/STAT pathways [55]. This compound is in Phase III clinical development against various inflammatory diseases, including fibrosis, asthma and COPD [56,57]. Curcumin down-regulates the expression of many pro-inflammatory mediators and specific proteins, including cytokines, MMPs, adhesion molecules, and growth factor receptor genes [58-62]. In general, Curcumin acts through the inhibition of phosphorylation of the IκB, which in turn reduces the nuclear translocation of NF-κB. The mechanism of action was strongly associated with decreased production of pro-inflammatory chemokines CXCL1, CXCL2 and CXCL-8 as well as chemokine receptor CXCR4 [63,64]. In addition to the typical NF-κB pathway, Curcumin was found to strongly modulate p38 MAPK, STAT-3 and JNK signaling cascades mainly through the blockage of TNF-α precursor activity in human umbilical vein endothelial cells (HUVECs) [65]. It was also estimated that the expression of ICAM)-1, monocyte chemoattractant protein (MCP)-1 [66], and IL-8 can be slightly reduced by Curcumin; however, it does not affect the expression of TNF

receptor I and II. It was previously concluded that orally ingested Curcumin reverses many of the inflammatory and metabolic derangements associated with obesity and improves glycemic control in mouse models of type 2 diabetes [67]. The observed effect was strongly associated with perturbations in NF-κB signaling. It was also found that Curcumin potently inhibits both the NF-κB and STAT3 activities in H-RS cells leading to a decreased expression of proteins involved in cell proliferation and apoptosis, e.g. Bcl-2, Bcl-xL, cFLIP, XIAP, c-IAP1, survivin, c-Myc and Cyclin D1 [68]. Curcumin also suppresses both ligand-induced and lauric acid-induced Nod2 signaling route which results in the suppression of NF-κB activation and target gene IL-8 expression [69]. Chen *et al*. [70] have shown that the interruption of NF-κB and ERK signaling by Curcumin may result in the complete suppression of connective tissue growth factor (CTGF) expression in activated hepatic stellate cells (HSC) *in vitro*.

In peripheral blood mononuclear cells (PBMCs), Curcumin was found to effectively prevent the degradation of IκB-α, which in turn inhibits the ALA-mediated activation of NF-κB and the related upregulation of MMP-9 [71]. It was also demonstrated that Curcumin down-regulates P-gp expression, encoded by the MDR gene, in multidrug-resistant L1210/Adr cells, presumably, through the inhibition of the PI3K/Akt/NF-κB signaling pathway [72]. The spectrum of the anti-inflammatory action attributed to Curcumin is not restricted solely by the activities mentioned above. A large number of scientific papers published during the last decade highlight the prominent role of Curcumin-based treatment of various chronic inflammatory diseases [73].

Starting from the end of the 90's, Resveratrol **26** began to receive considerable attention as novel promising anti-inflammatory agent which specifically acts against NF-κB signaling pathways. Thus, in distant 1999, Wadsworth and Koop [74] had shown that Resveratrol had inhibited the production of TNF-α by the blockage of LPS-stimulated activation of NF-κB route. Subsequent analysis has clearly indicated that Resveratrol inhibits the phosphorylation as well as degradation of IκBα, which hampers nuclear translocation of the transcriptionally active subunits of NF-κB [75]. Later Pellegatta *et al*. [76] have speculated that instead of serine phosphorylation Resveratrol increases tyrosine phosphorylation of IκBα, p50/NF-κB, and p65/NF-κB suggesting the involvement of such alterations in the modulation of NF-κB transcription activity. Similar anti-inflammatory action was also attributed to Piceatannol, a stilbene which is structurally homologous to Resveratrol [77]. It was also observed in lymphoid (Jurkat) and epithelial (HeLa and H4) cells that Resveratrol significantly inhibited NF-κB activation induced by different stimuli [78]. The suppression of NF-κB was accompanied by a decrease in AP-1 activity. Resveratrol also inhibited the TNF-induced activation of MAPKs and c-Jun kinase as well as abrogated TNF-induced cytotoxicity and caspase activation. In addition, both Resveratrol and Quercetin were suggested to have a powerful nonsteroidal anti-inflammatory activity that may have applications for the treatment of inflammatory diseases [79].

During the past five years the anti-inflammatory activity of Resveratrol was vigorously described in numerous scientific papers. Thus, several recent studies revealed that Resveratrol significantly suppressed LPS-induced degradation of IκBα guardian complexes, expression of iNOS and phosphorylation of p38 MAPK in N9 microglial cells [80]. Experiments performed in severe acute pancreatitis (SAP) rats have clearly indicated that the activation of NF-κB is deeply involved in the related inflammatory response [81]. Thus, Resveratrol was found to effectively inhibit the expression of NF-κB activation, alleviate the severity of SAP and regulate the activity of various inflammatory mediators. Gene analysis has clearly shown that Resveratrol can entirely suppress the expression of various pro-inflammatory factors, including ICAM-1, RANTES and MCP-1 proteins, TNFRSF and TLR3 receptors, pro-inflammatory cytokines that attract monocyte-granulocyte cells such as M-CSF, GM-CSF and G-CSF as well as TGF-β and IL-1α through the inhibition of NF-κB signaling pathway [82]. The NF-κB-associated mechanisms of anti-inflammatory action of Resveratrol have been comprehensively reviewed in [83]. Interestingly, Resveratrol was also found to reduce LPS-induced airway inflammation [84], but the reduction does not appear to be due to an impact on NF-κB activation or the expression of the respective genes as suggested by various *in vitro* studies [85]. The obtained results strongly suggest that Resveratrol may exert a beneficial anti-inflammatory effect *via* a novel mechanism of action. More recently, Resveratrol was found to significantly suppress NF-κB activation and COX-2 expression in RAW264.7 cells following TLR3 and TLR4 stimulation, but not TLR2 or TLR9 [86]. Recently, Zhu *et al*. [87] have amply confirmed the declared ability of Resveratrol to inhibit DNA binding activity of the NF-κB complex and subsequently suppress TNF-α-induced MCP-1 gene expression.

A wealth of data gathered over the past 15 years of intensive research in this field strongly suggest that Curcumin and Resveratrol hold the top position in the world rating of potent anti-inflammatory therapeutics [88].

SC-514 **38** is a selective inhibitor of IKK-2 activity of the native IKK complex or recombinant human IKK-1/IKK-2 heterodimer. It should be especially noted that this compound does not inhibit other IKK isoforms or other serine-threonine and tyrosine kinases. The inhibition is selective, reversible, and competitive with ATP molecules. Particularly, SC-514 inhibits transcription of NF-κB-dependent genes in IL-1β-induced RA-derived synovial fibroblasts in a dose-dependent manner. It was found that SC-514 did not inhibit the phosphorylation and further activation of the IKK complex directly. Thus, it was suggested that the effect of SC-514 on cytokine gene expression and corresponding pro-inflammatory mediators production lies generally in the combination of inhibiting IκBα phosphorylation/degradation, affecting NF-κB nuclear import/export as well as the phosphorylation and transactivation of p65 unit [89]. The perturbations in the NF-κB signaling pathway lead to attenuation of NO, CXCL2, and TNF-α release; the last one is a pivotal precursor for augmenting TLR2 and IL-6 expression [90]. Thus, in human adipocytes it was found that IL-6 release stimulated by TSH (Thyroid-stimulating hormone) was inhibited by 60% with SC-514 [91]. This compound was also found to strongly inhibit IKKβ activity and related TNF-

α-induced activation of NF-κB as well as the TNF-α-promoted metastasis of murine colon adenocarcinoma cells especially through the blocking of MMP-9 enzymatic activity [92]. In mice models SC-514 (1 μM) significantly attenuates TPA-induced activation of Akt and NF-κB, and also the expression of COX-2 in hairless mouse skin [93]. The rational use of SC-514 was found to be in the therapeutic combination with 9Z,11E-CLA (cis-9,trans-11-conjugated linoleic acid). The drug combination leads to a drastic inhibition of NF-κB-driven-COX-2 expression by blocking the IKK and PI3K-Akt signaling in TPA-treated hairless mouse skin *in vivo*, which may account for its previously reported anti-tumor promoting effects. In RAW 264.7 murine macrophage-like cells stimulated with lipopolysaccharide (LPS) SC-514 was found to inhibit LPS-induced NF-κB DNA binding. However, it does not inhibit LPS-induced HMGB1 secretion (HMGB1 - `high-mobility group box 1`, a nuclear protein that is secreted by immunostimulated macrophages and acts as a key pro-inflammatory mediator) [94]. Pretreatment of RASMCs (rat aortic smooth muscle cells) with SC-514 significantly reduced iNOS induction, NF-κB translocation and IκBα loss in a concentration-dependent manner [95]. It was also found that SC-514 and BMS-345541 **40** significantly blocked IgE/TNP-induced TNF production by mouse bone marrow-derived MC (BMMC) [96]. In turn, IgE and TNP jointly induce a rapid phosphorylation of IKKα but not IKKβ in BMMC. It was accompanied with phosphorylation and degradation of IκBα, subsequent NF-κB activation, and TNF production. Interestingly, IgE/TNP-induced IKKα and IκBα phosphorylation was completely inhibited by a protein kinase C (PKC) inhibitor, Ro-31-8220. The obtained results highlight a role of the IKK-IκB-NF-κB pathway in the regulation of MC function in allergy.

TPCA-1 **39** is a novel, highly potent (IC_{50} = 17.9 nM), and selective small molecule inhibitor of human IKK-2 activity [97]. This compound inhibits LPS-induced human monocyte production of several pro-inflammatory mediators including TNF-α, IL-6, and IL-8 with an IC_{50} values in the range of 170-320 nM. In mice with collagen-induced arthritis (CIA) TPCA-1 administration at the dosage of 3, 10, or 20 mg/kg (i.p., b.i.d.) leads to reduction and delay in disease severity. The maximum therapeutic action was observed at 20 mg/kg. It was found that nuclear localization of p65, as well as levels of IL-1β, IL-6, TNF-α, and IF-γ were significantly reduced in the paw tissue of TPCA-1- and etanercept-treated mice. In addition, administration of TPCA-1 *in vivo* resulted in significantly decreased collagen-induced T-cell proliferation *ex vivo*. The therapeutic potential of TPCA-1 against corneal ulcer caused by collagen degradation in the corneal stroma was recently investigated in [98]. Thus, it was found that IL-1β-induced collagen degradation was powerfully inhibited by TPCA-1 in a concentration- and time-dependent manner through the IKK-2 blockage. This compound inhibits both the IL-1β-induced expression of MMP-1, -3, and -9 in the cells studied at both the mRNA and protein levels as well as the IL-1β-induced activation of pro-MMP-2. In contrast to dexamethasone, TPCA-1 prevents the phosphorylation and degradation of IκBα as well as the nuclear translocation of NF-κB induced.

Since the severity of COPD strongly correlates with increased numbers of cytotoxic CD8(+) T-lymphocytes in the lung parenchyma, which in turn stimulate IFN-γ release leading to CXCR3 production in airway epithelial cells, TPCA-1 as a strong inhibitor of CXCL9, CXCL10, and CXCL11 release can be reasonably regarded as potential therapeutic agent against COPD. Thus, in human bronchial epithelial cell line BEAS-2B stimulated by IFN-γ, TPCA-1 was found to inhibit the listed activities *via* reducing the IκB kinase-2 activity with EC_{50} values of 0.50, 0.17, and 0.45 μM, respectively [99].

In the LPS model of airway inflammation, TPCA-1 was found to significantly block the increase in NF-κB DNA binding. This inhibition was directly associated with a reduction in inflammatory mediator release, principally for TNF-α, IL-1β, MMP-9, and in lung inflammatory cell burden (neutrophilia/eosinophilia) [100]. It was the first study to examine the effect of an IKK-2 inhibitor in well validated models that mimic aspects of the inflammatory lesion evident in diseases such as COPD. In result, the authors have demonstrated that animal models with similar profiles of airway inflammation can be IKK-2 inhibitor/steroid-sensitive or –insensitive; both profiles of inflammation are widely distributed in the clinic. Therefore, the finding is extremely exciting and may lead to greater understanding of disease pathology and the discovery of novel anti-inflammatory targets. In addition, soon afterwards Birrell and co-workers have reported that the therapeutic impact of TPCA-1 (in rats) can be effectively monitored using exhaled nitric oxide (eNO) that has been shown to be a reproducible, noninvasive indicator of the inflammatory status of the airway in the clinic [101].

BMS-345541 **40** is a highly selective and potent inhibitor of IKK-2 (IC_{50} = 0.30 μM), which however was considerably less potent against IKK-1 (IC_{50} = 4.0 μM) [102]. It should be especially noted that it was the first example of an inhibitor of IκB kinase with anti-inflammatory activity *in vivo*. In cell models, this compound failed to inhibit a set of 15 other kinases, including c-Jun and STAT3, as well as MAPK-activated protein kinase-2 [103]. Consistent with the role of IKK/NF-κB in the regulation of pro-inflammatory cytokine production, BMS-345541 inhibited LPS-stimulated TNF-α, IL-1β, IL-8, and IL-6 release in THP-1 cells with IC_{50} values in the 1- to 5-μM range. The constructed binding model shows that BMS-345541 binds to similar allosteric sites located in both the IKK-1 and IKK-2 catalytic complexes, which then affects the active sites of the subunits differently. BMS-345541 was also shown to have excellent pharmacokinetics in mice, and peroral administration showed the compound to dose-dependently inhibit the production of serum tumor necrosis factor alpha following intraperitoneal challenge with LPS. Thus, the compound is effective against NF-κB activation in mice and represents an important tool for investigating the role of IKK in disease models. It was also found that BMS345541 inhibits IL-6 and COX-2 production and activity with reduction in PGE2 level in LPS/TLR4-stimulated Chicken thrombocytes *in vitro* [104]. In addition, it has previously been shown that BMS-345541 blocks both inflammation and joint destruction in murine collagen-induced arthritis [105].

BMS-345541 was also tested in mice for its ability to inhibit anti-CD3-induced IL-2 and TNF-α production, T-cell proliferation in an *in vivo* mixed lymphocyte reaction as well as to suppress graft rejection in a murine nonvascularized heterotopic cardiac allograft model [106]. The compound was tested as a single agent and in combination with other immunomodulators for inhibition of T-cell proliferation and graft rejection *in vivo*. The best therapeutic outcome was achieved in the case of using the combined drug therapy with cytotoxic T-lymphocyte antigen-4 immunoglobulin. In result, it was found that at a dose of 100 mg/kg BMS-345541 suppressed the production of both IL-2 and TNF-α (up to 70%) in mice stimulated with an injection of anti-CD3 antibody in a dose-dependent manner resulting in 77% inhibition of $CD4^+$ T-cell proliferation.

Immune-complexes (ICs) are key players in a variety of autoimmune diseases, they stimulate monocytes recruitment through the interaction with Fcγ-receptor (a high affinity receptor for IgG) triggering the secretion of several pro-inflammatory mediators and favoring their tissue accumulation by inhibiting the apoptosis. BMS-345541 was found to abolish the apoptosis protection conferred by ICs through the inhibition of IKK complexes thereby preventing the inflammation progress [107]. The same therapeutic effect of BMS-345541 was also registered in IFN-γ-stimulated macrophage recruitment. Particularly, it was clearly shown that IFN-γ induction of Fcγ upregulation is strongly dependent on the IKK/NF-κB pathway [108].

Besides TNF-α plays a pivotal role in cytoprotection and inflammation, it is also regarded as a key necrotic death precursor in tumors *in vivo*. Thus, BMS-345541 was found to increase TNF-α killing in TNF-α resistant tumor cell lines by increasing cellular apoptosis, suggesting that in addition to inflammation the inhibition of NF-κB could be an effective strategy to enhance the tumoricidal effects of TNF-α [109].

It was recently disclosed that Airway smooth muscle (ASM) cells can act as effector cells towards the initiation and/or perpetuation of airway inflammation in asthma by producing various pro-inflammatory mediators, such as IL-6, IL-8, and eotaxin. Particularly, this action is accompanied by an enhanced secretion of TNF-α and IFN-γ or endogenous IFN-β results in a synergistic induction of various pro-inflammatory genes, including CD38. These genes were also found to be NF-κB-dependent in that TNF-α-induced expression of IL-6, IL-8, and eotaxin was dose-dependently inhibited by BMS-345541 (1-30 μM) [110].

It was recently suggested that NF-κB-dependent expression of MMP-9 in response to CpG-ODN plays an important role in the recruitment of immune cells into the inflammation nidus. In particular, an MMP-9 precursor was activated by the stimulation of CpG-ODN and ectopical expression of NF-κB transcription factor. BMS-345541 was found to dramatically inhibit the expression of MMP-9 gene induced by CpG-ODN [111]. In addition, in SW-1353 human chondrosarcoma cells, BMS-345541 inhibited IL-1-dependent expression of matrix metalloproteinases: MMP-1, MMP-3, and MMP-13 in a concentration-dependent manner thereby blocking aggrecan and collagen degradation [112].

BMS-345541 was also used to determine whether intervention in the NF-κB pathway could prevent progression to lung injury in the LPS pump model. The compound was administered into the model cells to down-regulate NF-κB activation following the onset of inflammation. In result, treatment with BMS-345541 significantly reduced lung NF-κB activation, concentration of KC and MIP-2 in lung lavage, neutrophil influx, and lung edema. Therefore, it was suggested that sustained NF-κB activation strongly correlates with severity of lung injury, and the blockage of NF-κB pathway by BMS-345541 is quite beneficial even after the onset of lung inflammation [113].

Inflammatory bowel diseases including ulcerative colitis and Crohn's disease are frequently accompanied by chronic relapsing inflammation supported by the transcription of various pro-inflammatory mediators which regulate the pathogenesis in inflammatory bowel disease (e.g., TNF-α, ICAM-1, and VCAM-1). Such expression is meticulously maintained by NF-κB signaling system activated *via* IκB kinase. Thus, it was found that BMS-345541 strongly inhibits the TNF-α-induced expression of both ICAM-1 and VCAM-1 in human umbilical vein endothelial cells with IC_{50} = 5 μM [114]. BMS-345541 administered orally at doses of 30 and 100 mg/kg was effective in blocking both clinical and histological endpoints of inflammation and injury. It clearly indicated that inhibitors of IκB kinase activity show the promise of being highly efficacious in inflammatory disorders such as inflammatory bowel disease.

Finally, BMS-345541 was also tested against collagen-induced arthritis (CIA) in mice [115]. Thus, BMS-345541 (in a dose range of 10-100 mg/kg) has showed exciting potential in reducing the incidence of disease and inhibiting clinical signs of CIA. Histologic evaluation of the joints showed that both inflammation and joint destruction were effectively blocked by BMS-345541.

ML120B **41** is a potent, selective, reversible, and ATP-competitive small molecule inhibitor of IKKβ with an IC_{50} of 60 nM (IκBα kinase complex assay) [116]. ML120B does not inhibit other IKK isoforms or a panel of other kinases. This compound strongly inhibits TNFα-stimulated NF-κB signaling *via* inhibition of the target IκBα phosphorylation, degradation, and NF-κB translocation into the nucleus [117]. As mentioned above, the IKK/NF-κB signaling pathway is deeply implicated in the rheumatoid arthritis disease process. For example, in rats, oral administration of ML120B inhibited paw swelling in a dose-dependent manner, thereby leading to significant protection against RA-induced weight loss as well as cartilage and bone erosion. It was clearly shown that NF-κB activity in arthritic joints was reduced dramatically after ML120B administration. In addition, it was observed that down-regulation of the IKKβ/NF-κB signaling pathway results in significant inhibition of the chronic inflammatory process associated with rat adjuvant-induced arthritis. Similar results were also obtained in the murine model of antibody-induced arthritis [118]. ML120B blocked numerous NF-κB-regulated cell responses that are involved in inflammation and destructive processes in the RA joint. Thus, *in vivo* imaging technique has demonstrated that NF-κB activity in inflamed arthritic paws was strongly inhibited by ML120B, resulting in significant suppression of several

pro-inflammatory genes including KC (murine IL-8), epithelial neutrophil-activating peptide 78, JE, intercellular adhesion molecule 1, CD3, CD68, TNF-α, IL-1β, IL-6, iNOS, COX-2, MMP-3, cathepsin B, and cathepsin K. It was also found that in mice ML120B sensitized bone marrow progenitors and granulocytes and induced cellular apoptosis in the bone marrow and spleen after oral administration [119]. In particular, inhibition of IKKβ by ML120B resulted in depletion of thymocytes and B cells in all stages of development in the bone marrow but did not deplete granulocytes. In result, ML120B-based therapy leads to rapid TNF-dependent depletion of T and B cells. This observation has several important implications for potential use of ML120B for the treatment of inflammatory disease and cancer.

Asthma and COPD are characterized by chronic airway inflammation and the airway epithelium is critical in the pathogenesis of these chronic inflammatory diseases. It is invariably accompanied by the expression of various pro-inflammatory genes associated with the NF-κB signaling route. Thus, ML120B was tested in two cell models: human A549 pulmonary cells and primary human bronchial epithelial (HBE) cells [120]. In the first system, interleukin IL-1β and TNF-α significantly increased phosphorylation of IκBα, and this was followed by loss of IκBα, induction of NF-κB DNA binding, and the induction of NF-κB-dependent transcription. The observed activity were strongly inhibited by ML120B in a dose-dependent manner resulted in loss of ICAM-1 expression. Similarly, IL-1β - and TNF-α-induced expression of IL-6, IL-8, GM-CSF, RANTES, GRO-α, and MCP-1 was also significantly repressed. Similar results were previously obtained by Catley and co-workers [121].

2.2. Inhibitors of JAK/STAT Pathway

The rapidly expanding knowledge underpinning the cytokine transcription factor network has "shed a bright light" on the acute and chronic inflammatory response. JAKs (Janus tyrosine Kinases) and STAT (Signal Transducer and Activator of Transcription) are critical components of many cytokine receptor systems that regulate cell growth, survival, proliferation, haematopoiesis and pathogen resistance. Thus, it was recently uncovered that JAK/STAT signaling network observed in three major cell types involved in inflammatory responses: T-cells, neutrophils, and macrophages, plays a critical role in the pro-inflammatory cytokine production. JAK belongs to a family of non-receptor PTKs comprising of JAK1, JAK2, JAK3 and TYK2 (non-receptor Protein Tyrosine Kinase-2). STATs are latent cytoplasmic transcription factors that become activated after recruitment to an activated receptor complex. A series of seven separate STAT proteins are present in a wide variety of cell types, including cells of epithelial origin [122]. These include STAT1 to 6, including STAT5a and STAT5b, which are encoded by distinct genes. For example, STAT-1 mediates a pro-inflammatory response to the activation of the interferon gamma receptor on the cell surface by IF-γ. STAT-3 participates in the signaling pathways for many cytokines in various cells and organs that are regulated by the suppressor of cytokine signaling (SOCS) family, including SOCS3 (see below) [123]. In addition, different isoforms of several STATs have been identified to date. The JAK/STAT signaling pathways are regulated by a vast array of intrinsic and environmental stimuli, which can add plasticity to the response of a cell or tissue.

Mechanistically, JAK/STAT signaling is relatively simple, with only a few principal components (Fig. (**3**)). A variety of ligands including cytokines, hormones and growth factors, as well as their specific receptors such as cytokine receptors, EGFR, GPCR and INF-Rs activate the JAK/STAT pathway through the multimerization of receptor subunits such as STATIP and Tyk2. For some ligands, such as erythropoietin and growth hormone, these subunits are bound as homodimers while, for others, such as interferons and ILs, the receptor subunits are heteromultimers. For signal propagation, the cytoplasmic domains of two receptor subunits must be associated with JAK tyrosine kinases. JAK activation occurs upon ligand-mediated receptor multimerization because two JAKs are brought into close proximity, allowing trans-phosphorylation. The activated JAKs subsequently phosphorylate principal targets, including both the receptors and the major substrates, STATs, results in dimerization of STATs through interaction with a conserved SH2 domain. As shown in Fig. (**3**), different JAKs and STATs are activated by different ligands *via* corresponding receptors. Phosphorylated STATs dimmers then translocate into the nucleus by a mechanism involving Importin and the Ran nuclear import pathway. In the nucleus, STAT transcriptional complexes bind to specific regulatory regions on DNA to activate or repress transcription of target pro-inflammatory genes. Thus the JAK/STAT cascade provides a direct mechanism to translate an extracellular signal into a transcriptional response. In addition, RTK family that normally regulates the Ras/Raf/MEK/ERK-related signaling cascades including p38 MAPK pathway can also induce the JAK/STAT signal transduction *via* cytokine or/and interleukin receptors.

In addition to JAK/STAT pathway effectors, there are three major classes of negative regulator: SOCS (suppressors of cytokine signaling), PIAS (protein inhibitors of activated STATs) and PTPs (protein tyrosine phosphatases) including CD45 and SHP-1. For example, SHP-1 is the best characterized member of PTPs containing two conserved SH2 domains and can bind to either phosphorylated JAKs or phosphorylated receptors to facilitate dephosphorylation of these activated signaling molecules. SOCS proteins are a family of at least eight members containing an SH2 domain and a SOCS box at the C-terminus. The hallmark of the SOCS family is the SOCS Box, which mediates interaction with the Elongin-B/C complex and couples the SOCS and associated target proteins JAKs to the proteasomal protein degradation pathway.

Elongin complex also recruits the RING finger protein Rbx-1 to form a multiprotein conglomerate that imitates the E3 ubiquitin ligase functions. Together with an ATP-dependent ubiquitin-activating enzyme (E1) and an ubiquitin-conjugating enzyme (E2), this complex acts to tag proximal proteins with polyubiquitin chains following by their subsequent degradation by the proteasome. In addition, JAK/STAT signaling also indirectly promotes Ras signaling through the transcriptional activation of SOCS3. Thus, SOCS3 binds RasGAP, a negative regulator of Ras signaling, and reduces its activity, thereby promoting activation of

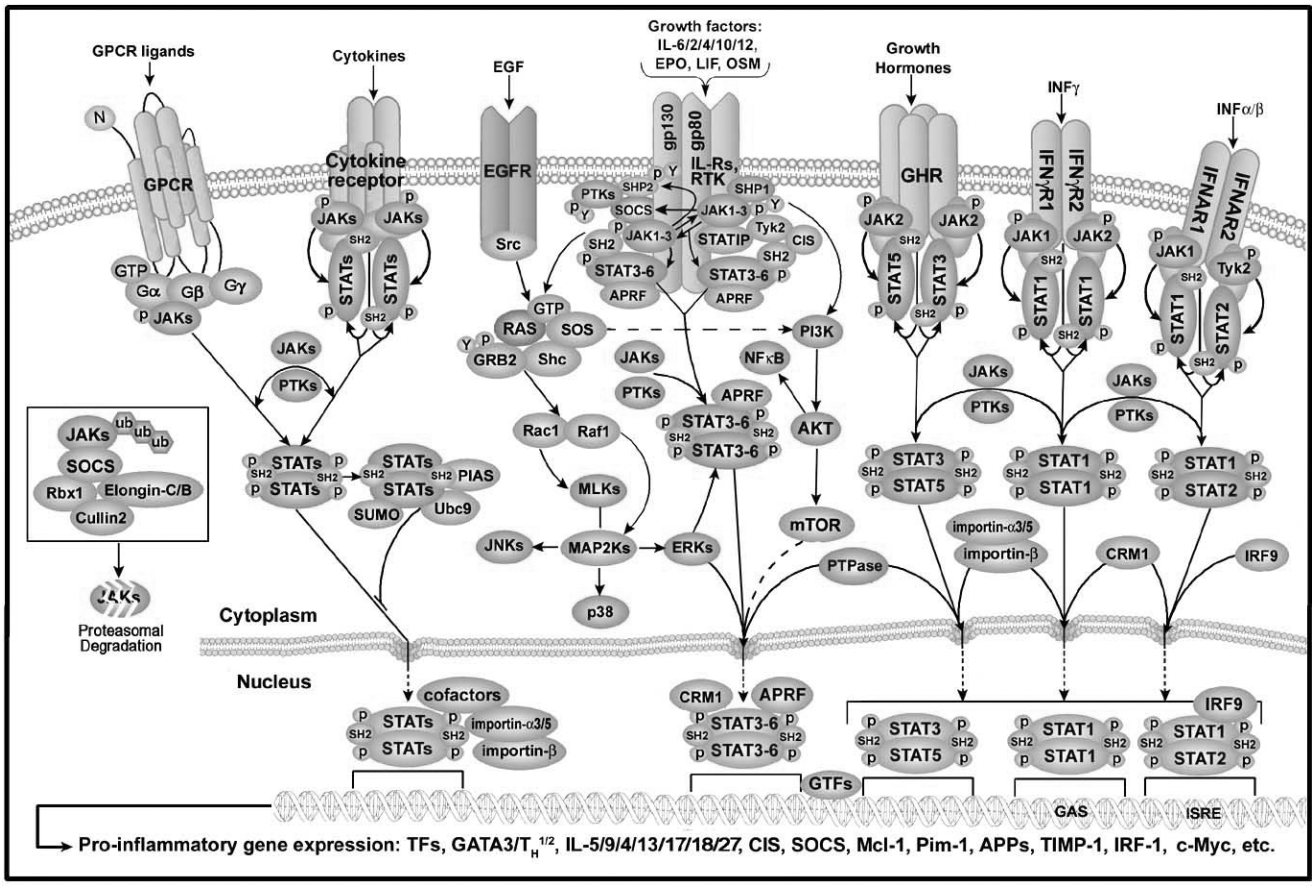


Fig. (3). The JAK/STAT signaling pathways implicated in inflammation.

the Ras pathway. RTK pathway also promotes JAK/STAT signaling by at least two mechanisms: first, the activation of some RTKs, including EGFR and PDGFR, results in the JAK-independent tyrosine phosphorylation of STATs, mainly by the Src kinase, and second, RTK/Ras pathway stimulation causes the downstream activation of MAPK which in turn specifically phosphorylates a serine near the C-terminus of most STATs leading to its activation and related transcription.

There are several small molecule agents that have been shown to regulate JAK/STAT signaling pathway (Fig. (**4**), Tables **2** and **3**). Several compounds are already launched on the market as drugs particularly acting against various inflammatory conditions while others are currently evaluated in different clinical trials.

Thus, Leflunomide **42** is widely used to treat rheumatoid arthritis and fibrosis. In the human keratinocyte cell line, HaCaT, Leflunomide was found to significantly inhibit the IL-4 and IL-13 enhanced production of CCL26 [124]. The obtained results suggest that keratinocytes are involved in the migration of CCR3 positive cells such as eosinophils in a Th2-dominant situation like atopic dermatitis. In addition, Leflunomide powerfully inhibited the deposition of type I collagen in hepatic stellate cells (HSCs) and the proliferation of primary HSC by interrupting the three proliferative signal transduction pathways *in vitro*, including the primary target JAK/STAT and related MAPK and (PI3K)/Protein kinase B (AKT) signaling cascades providing a novel insight into the mechanisms by which Leflunomide may exert in liver fibrosis [125].

Lestaurtinib **43** and MK-0457 **45** are being studied in a Phase II/III clinical trial in patients with treatment-resistant chronic myelogenous leukemia (CML), or Philadelphia chromosome-positive acute lymphoblastic leukemia. MK-0457 was originally developed as a small-molecule inhibitor of Aurora kinases A, B, and C. However, recent screening data shows that MK-0457 inhibits JAK2 with an IC_{50} of 123 nM for wild type JAK2 and 295 nM for the JAK2 V617F activating mutation [126]. Lestaurtinib (formerly known as CEP-701) also inhibits wild type JAK2 kinase activity (JAK2/STAT5 signaling pathway) in the same kinase assay with an IC_{50} of 1 nM *in vitro* [127]. Although these compounds are commonly targeted for the treatment of leukemia and myeloproliferative disorders they also being considered as a potential small molecule drug candidates against different inflammatory diseases.

CP-690550 **44**, a novel, selective small molecule inhibitor of JAK3 kinase activity with promising anti-inflammatory potential. JAK-3 has been shown to play a key role in pro-inflammatory cytokine signaling *via* IL-2/4/7/9/15/21. For example, in a murine model of allergic pulmonary inflammation, this compound potently inhibits IL-4 induced upregulation of CD23 (IC_{50}=57 nM) and class II major histocompatibility complex (MHCII) expression

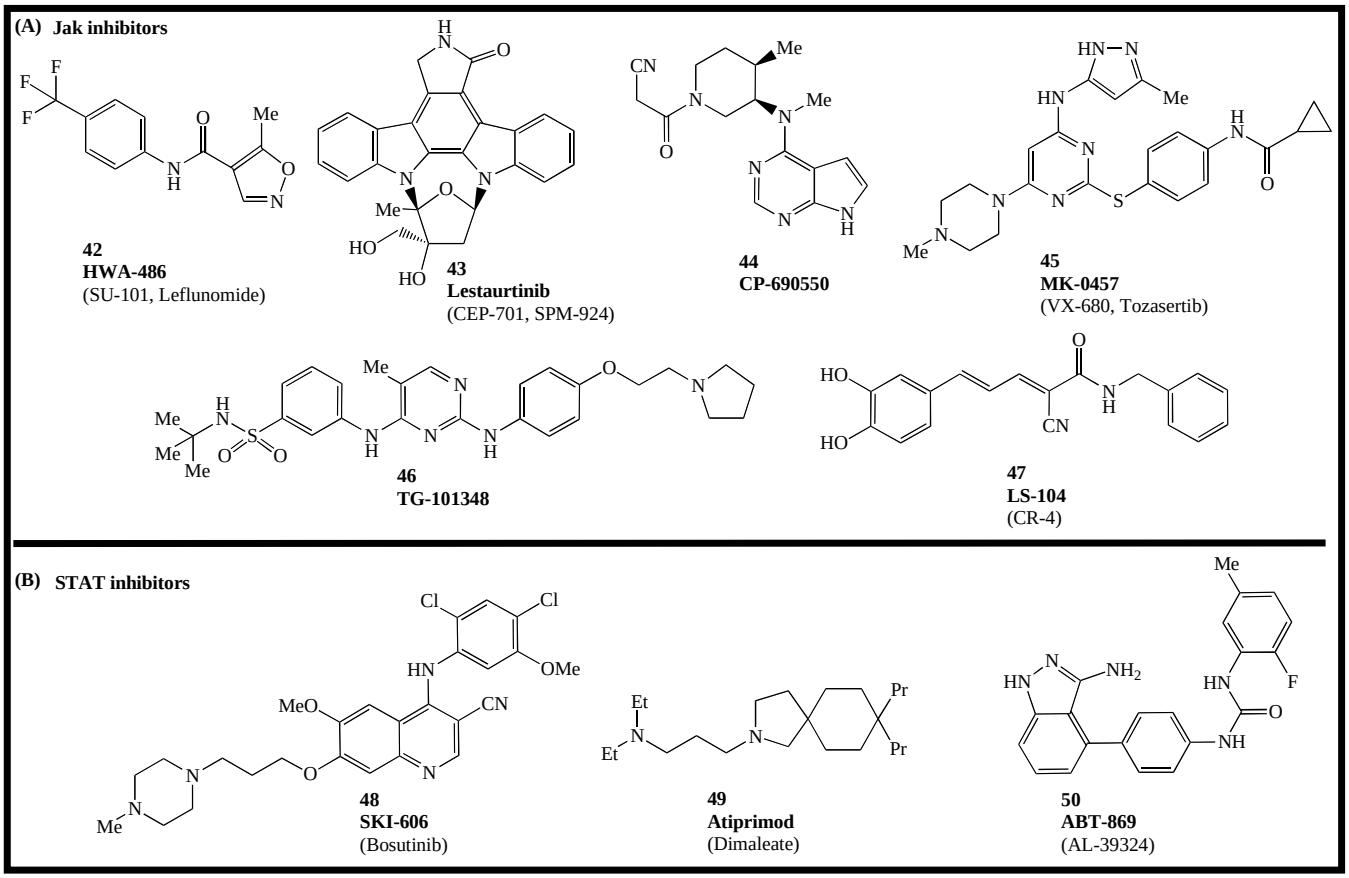


Fig. (4). Small molecule agents targeting JAK kinases (**A**) and STAT (**B**) that have already been approved or are currently in clinical trials.

(IC_{50}=71 nM) on murine B-cells [128]. Animals treated with CP-690550 have demonstrated significant reductions in bronchoalveolar lavage fluid (BAL) eosinophils and levels of IL-13 and eotaxin following ovalbumin aerosol exposure. Consequently, CP-690550 represents an intriguing novel therapy for treatment of allergic conditions associated with airway eosinophilia including asthma and rhinitis. CP-690550 effectively inhibited a murine mixed lymphocyte reaction (MLR) with an IC_{50} of 91 nm *in vitro* [129]. Furthermore, the ability of CP-690550 (1.5-15 mg/kg/day) to extend cardiac allograft survival in mice suggests that it may afford a new treatment for prevention of transplant rejection. In addition, this compound has demonstrated impressive immunosuppressive potency in nonhuman primates (NHPs) in combination with mycophenolate mofetil (MMF) leading to the treatment and prevention of kidney allograft rejection [130]. However, numerous and severe side effects (i.g.: mutations and perturbations across the whole signaling route) associated with all current immunosuppressive therapies and justify a search for drugs with better efficacy and safety profiles. For example, in several preclinical models CP-690550 showed high efficacy for the prevention of allograft rejection with a narrow side-effect profile [130].

TG101348 **46**, a potent, selective, and orally bioavailable inhibitor of JAK2 is currently in clinical trials against myeloproliferative and inflammatory diseases [131]. TG101348 was originally identified using rational design techniques and an extensive medicinal chemistry effort designed to optimize potency, selectivity, pharmaceutical and pharmacokinetic properties. Using a phylogenetically diverse panel of 223 kinases it was found that TG101348 inhibits JAK2 activity with an IC_{50} of 3 nM while in primary and cultured cells this compound inhibits JAK2 driven cellular activity with EC_{50} ranging from 50-300 nM. The effect of TG101348 on cell proliferation strongly correlated with reduced levels of phosphorylated STAT5 suggesting that the observed pharmacological effect is mediated by inhibition of JAK/STAT signaling pathway. In addition, TG101348 shows therapeutic efficacy in a murine model of myeloproliferative disease induced by the JAK2V617F [132] and JAK2V617F+ [133] mutations. Based on these promising efficacy results as well as valuable pharmaceutical properties and preliminary toxicity TG101348 is currently being evaluated in advanced clinical trials.

SKI-606 **48** was originally developed as promising antiproliferative agent against CML acting as dual inhibitor of Src and Abl/Bcr kinase activities [134]. It was also found that SKI-606 inhibits phosphorylation of several cellular proteins, especially STAT5 (IC_{50}=10-25 nM). Thus, simultaneous inhibition of the Ras, STAT5, and PI3K pathways directly coupled to Bcr/Abl by CrkL leads to a synergistic enhancement of apoptosis in CML cells and significant suppression of pro-inflammatory gene expression. In addition, it was found that in a variety of leukemic states Bcr/Abl may use a bypass mechanism to activate JAK/STAT signal

Table 2. Therapeutically Active Small Molecule Inhibitors of Jak Kinases and STAT Activities

Compound (Fig. (4))	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
38	Launched-1998	Ovarian, brain and prostate cancers, rheumatoid arthritis, HIV-infection and transplant rejection	Multi-targeted inhibitor, especially against Jak3 kinase and STAT-6	Lepetit and Sanofi-aventis
39	Phase II/III	Psoriasis, hematopoiesis, prostate and pancreatic cancers, neurologic cancer as well as myeloid leukemia	Multi-targeted inhibitor, especially against Jak2 kinase, RET, TRKA and Flt3 (FLK2/STK1)	Kyowa Hakko
40	Phase II	Psoriasis, inflammatory bowel disease, rheumatoid arthritis, asthma and transplant rejection	Jak3-targeted inhibitor	Pfizer
41	Phase II	Non-specific and cancer-associated inflammatory diseases	Multi-targeted inhibitor, especially against Aurora-C and Jak2 kinases	Vertex
42	Phase I/II	Cancer and cancer-associated inflammatory diseases	Jak2 and Flt3 (FLK2/STK1) inhibitor	TargeGen
43	Phase I	Cancer-associated inflammatory diseases	Jak2, Bcr-Abl and Flt3 (FLK2/STK1) inhibitor	Hospital for Sick Children
44	Phase III	Cancer and cancer-associated inflammatory diseases, ischemic stroke	Multi-targeted inhibitor, particularly against STAT-5 and Src, Bcr-Abl, Abl kinases	Wyeth Pharmaceuticals
45	Phase II	Bone resorption, endocrine and colorectal cancers, multiple myeloma, inflammatory bowel disease and rheumatoid arthritis	Multi-targeted inhibitor, particularly against STAT-3 (IL-6 production inhibitor) and PKB/Akt Apoptosis inducer (Caspase-3/9 activator)	AnorMED and GlaxoSmithKline
46	Phase II	Cancer and cancer-associated inflammatory diseases	Multi-targeted inhibitor, particularly against STAT-5, ERK, CSF1R (c-FMS), PDGFRβ, VEGFR-2 (FLK-1/KDR), Flt3 (FLK2/STK1)	Abbott

Table 3. Small Molecule Inhibitors of Jak Kinase and STAT which Structures are not Disclosed Yet

Compound Name(s)	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
INCB-018424 (INCB-18424)	Phase II	Psoriasis, thrombocytopenic anemia, hematological and prostate cancers, multiple myeloma, rheumatoid arthritis as well as PMF* and POST-PV/ET MF*	Jak2-targeted inhibitor	Incyte
AT-9283	Phase I/II	Cancer-associated inflammatory diseases	Multi-targeted inhibitor, especially against Jak2, Aurora-A/B, Bcr-Abl kinases	Astex
R-348	Phase I	Psoriasis, autoimmune diseases, multiple sclerosis, rheumatoid arthritis and transplant rejection	Jak2-targeted inhibitor	Rigel
XL-019	Phase I	Non-specific inflammation, hematopoiesis and oncolytic diseases	Jak2-targeted inhibitor	Exelixis

*PMF - Primary Myelofibrosis; Post-PV/ET MF - Post Polycythemia Vera/Essential Thrombocythemia Myelofibrosis.

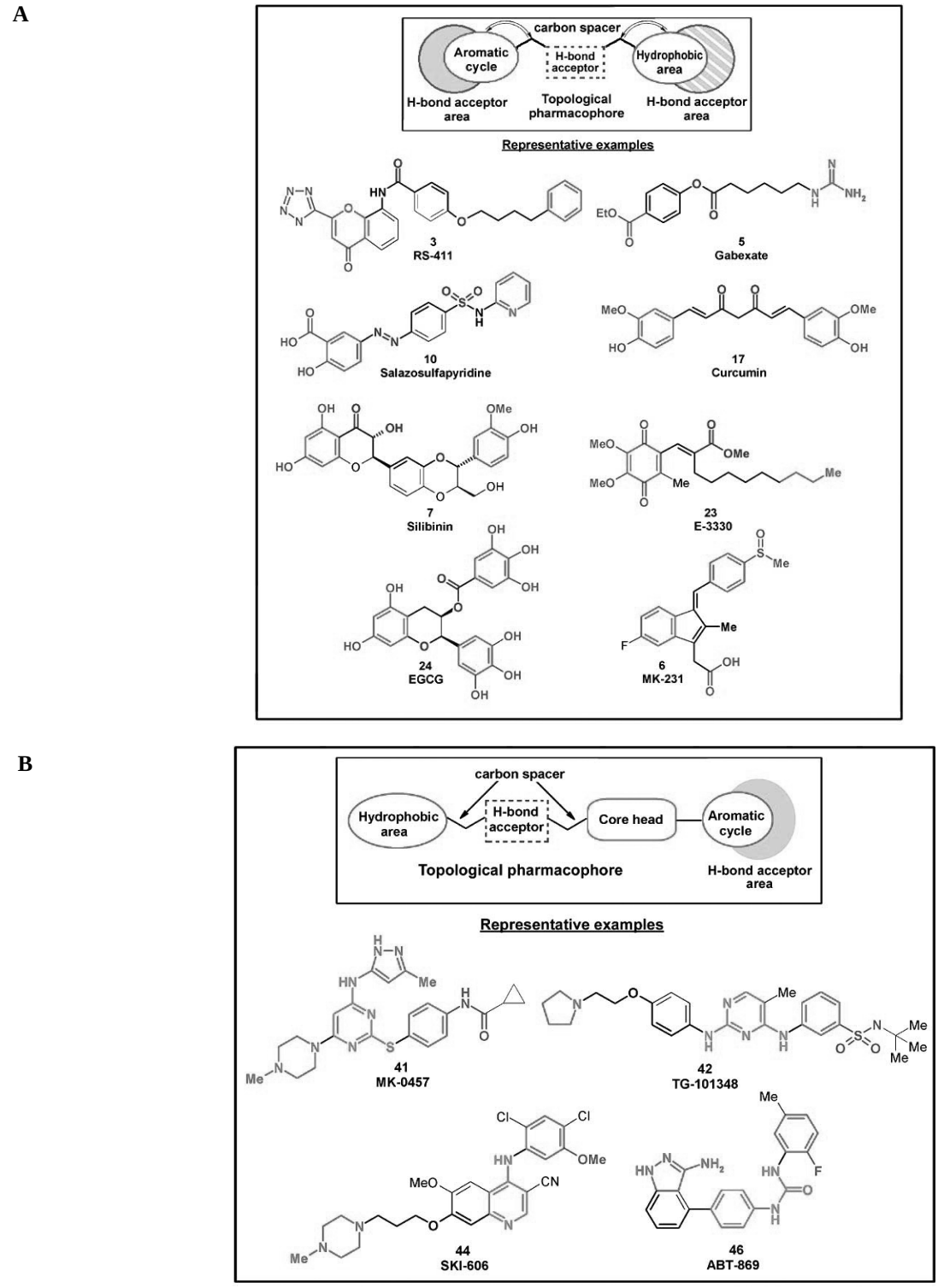


Fig. (5). A common topological pharmacophore and bioisosteric transformations of NF-kB (**A**) and JAK/STAT (**B**) inhibitors.

transduction pathways [135]. Similar activity was assigned to STI-571, a structural analog of SKI-606 [136].

In multiple myeloma cells (MM), Atiprimod **49** effectively blocks STAT3 phosphorylation and colony-forming cell proliferation as well as induces cellular apoptosis by the cleavage of caspase-3 and PARP [137]. During Phase I clinical trials, this compound has exerted potent anti-inflammatory activities in different animal models and in patients diagnosed with rheumatoid arthritis. For instance, in MM cells, this agent has greatly inhibited STAT3/5 activation thereby blocking the expression of IL-6, which in turn contributes to myeloma cell proliferation and survival. In addition, Atiprimod has downregulated the antiapoptotic

proteins Bcl-2, Bcl-X(L), and Mcl-1. Interestingly, in acute myeloid leukemia (AML) cells, Atiprimod significantly decreased phosphorylation of STAT3/5, and protein levels of JAK2, whereas gene expression of JAK2 was not affected [138].

ABT-869 **50** (a novel multitargeted RTKs inhibitor) has been found to effectively block the growth and progression of AML cells, particularly expressed a wild-type FLT3 receptor [139]. FLT3 mutations, in turn, trigger a strong autophosphorylation of the FLT3 kinase domain and constitutively activate several downstream effectors such as the PI3K/AKT, RAS/MEK/MAPK and mTOR/STAT5 signaling pathways. Thus, ABT-869 has been found to inhibit the phosphorylation of FLT3, STAT5, and ERK in MV-4-11 and MOLM-13 cells (IC_{50}=1-10 nM) [140]. In addition, ABT-869 treatment significantly downregulates the expression of cyclins D/E and Pim-1 but increases the expression of p21 and p27 as well as induces apoptosis through downregulation of Bcl-xL and upregulation of BAK, BID and BAD [141]. It was also found that ABT-869 strongly inhibits CSF-1 activity in both the enzymatic and cellular assays [142]. For example, in enzymatic assay, this compound competitively blocked CSF-1/CSF-1R signaling response with K_i values of 3 nmol/L. In turn, CSF-1/CSF-1R receptor system encoded commonly by the c-Fms proto-oncogene directly regulates macrophage differentiation, survival, and function thereby it is an attractive therapeutic target for chronic inflammation and malignant diseases. For example, autocrine regulation by CSF-1 has been reported in macrophages during inflammatory responses and in neoplastic cells [143].

3. A VALUABLE INSIGHT INTO THE TOPOLOGICAL COMPOSITION AND BIOISOSTERIC TRANSFORMATIONS OF NF-KB AND JAK/STAT INHIBITORS

In general, bioisosteric transformation allows for a better balancing between different lead-like parameters including specificity, physicochemical and PKPD properties. In addition, this approach provides insight into the patentability of lead candidates. Structural transformations occurred largely among a wide range of synthetic compounds are often based on bioisosteric modifications and specific topological skeleton of naturally derived compounds. Bioisosteric morphing provides a solid foundation for analysis of key structural elements which can further be combined in common topological pharmacophore. Typical examples of key bioisosteric modifications clearly observed among NF-kB and JAK/STAT inhibitors as well as topological pharmacophore are shown in Fig. (**5**). The modified structural fragments are highlighted in colour.

As shown in Fig. (**5**), many of NF-kB and JAK/STAT inhibitors belonging to different structural classes commonly contain several key structural motifs. For example, in the case of NF-kB inhibitors these include two H-bond acceptor areas (green), one hydrophobic and one aromatic area or two aromatic areas (red), carbon spacer (black), which can also be amplified by H-bond acceptor (blue) or double bonds. In turn, many of JAK/STAT inhibitors share the same topological elements with minor differences (e.g., two aromatic and/or one H-bond acceptor areas can be safely ignored). The "core head" includes pyrimidine fragment or its bioisosteric modifications such as pyridine (SKI-606 **48**) or various linear chains which are direct analogues of the core heterocyclic head (ABT-869 **50**). Structural elements which are not regarded as significant in the core topological pharmacophore are highlighted by dotted lines. The classical bioisosteric transformations (e.g., cyclic analogues of linear molecules, carboxyl group/tetrazole, carbocycle/heterocycle or carboxamide/sulfonamide) which can frequently be found in many medicinal chemistry studies are clearly shown by the example of RS-411 **3**, E-3330 **23** and Salazosulfapyridine **10**, Silibinin **7** and Curcumin **17** as well as TG-101348 **46**, SKI-606 **48** and ABT-869 **50**. The mirror composition of carbon spacer and hydrophobic cap was featured in several NF-kB inhibitors for example, as in the pair of Pseudoephedrine **15** and MPC-7869 **18**. Double bounds sited intermittently along the carbon spacer provide a rigid conformational state which, in a number of cases, is strongly correlated with activity and/or selectivity of drug compounds, for example, for Curcumin **17** and Resvertanol **26**.

CONCLUSION

It became increasingly clear that a range of inflammatory disorders include various diseases which are commonly aggravated by the uncontrolled expression of a broad spectrum of different pro-inflammatory mediators, such as cytokines and chemokines, growth factors and various immune response regulators. Their production and activity are, in turn, tightly controlled by different signaling systems including COX-2, NF-κB, MAPK, JAK/STAT, etc. Because of significant side effects currently revealed for many COX-2 inhibitors, the novel small molecule regulators of alternative NF-κB and JAK/STAT signaling cascades as well as related precursor molecules have already received a great deal of attention as promising drug candidates for the treatment of various inflammatory conditions, including rheumatoid arthritis, psoriasis, multiple sclerosis, COPD and diabetes. To date, many of these compounds have already launched in the market while others are currently being evaluated in different clinical trials in the hope of developing novel, effective, and at the same time, safe therapeutics. (based on a thorough analysis of the Prous Ensemble Database; the Internet site: http://www.prous.com)).

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