Gene-regulating protein kinases as important anti-inflammatory targets

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Protein phosphorylation is a key cellular regulatory mechanism. Protein kinases and phosphatases regulate cell-cycle progression, transcription, translation, protein sorting and cell adhesion events that are critical to the inflammatory process. Two of the best-characterized immunosuppressants, cyclosporin and rapamycin, are also effective anti-inflammatory drugs. They act directly on protein phosphorylation and, as such, validate the concept that small-molecule modulators of phosphorylation cascades possess anti-inflammatory properties. The authors describe studies that outline progress in defining specific protein kinase signal-transduction cascades, the key drug discovery targets in these cascades and progress towards developing selective agents that have potential in treating numerous inflammatory diseases.

The human genome encodes more than a thousand protein kinases[1]. These regulatory molecules phosphorylate as much as 30% of cellular proteins. In eukaryotes, two broad classes of protein kinases are well described: tyrosine protein kinases and serine/threonine protein kinases. In general, tyrosine protein kinases respond to growth factors or mitogenic signals to initiate rapid signal transduction. Serine/threonine protein kinases integrate and amplify signals and, in a number of cases, regulate signaling cascades at the level of transcription factors and gene expression.

A number of important transcription cascades are implicated as therapeutic targets for anti-inflammatory agent intervention (Fig. 1). Cytokines and growth factors, including interleukins, activate mitogen-activated protein (MAP) kinase signal cascades and result in the stimulation of c-Jun N-terminal kinase (JNK) and p38 protein kinases, which activate transcription factors such as Jun and ATF-2 (Ref. 2). Other proinflammatory agents, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα), also activate signaling cascades, but in this case involve the IκB kinases (IKK) that regulate IκB turnover and nuclear factor-κB (NF-κB) nuclear localization[3]. Engagement of antigen to the T-cell receptor activates signaling to the nuclear factor of activated T cells (NFAT), a transcription factor that is regulated by serine/threonine phosphorylation and which associates with calcineurin, the target for cyclosporin[4]. Proinflammatory cytokines such as IL-4 bind to cell-surface receptors that activate the JAK–STAT (Janus-associated kinase–signal transducers and activators of transcription) protein kinase–transcription factor cascade[5]. Inflammatory agents such as IL-2 stimulate rapamycin-sensitive signaling cascades that employ phosphoinositide 3-kinase (PI 3-kinase) or protein kinases such as mammalian target of rapamycin (mTOR) and activate ribosomal S6-kinase and cyclin-dependent kinases (CDKs)[6]. Finally, in osteoarthritis, transforming growth factor-β (TGFβ) and bone morphogenic proteins (BMPs) activate receptor serine/threonine kinases[7], which subsequently mediate the regulation of SMAD transcription factor activity. Clearly a plethora of protein kinases is important and essential for

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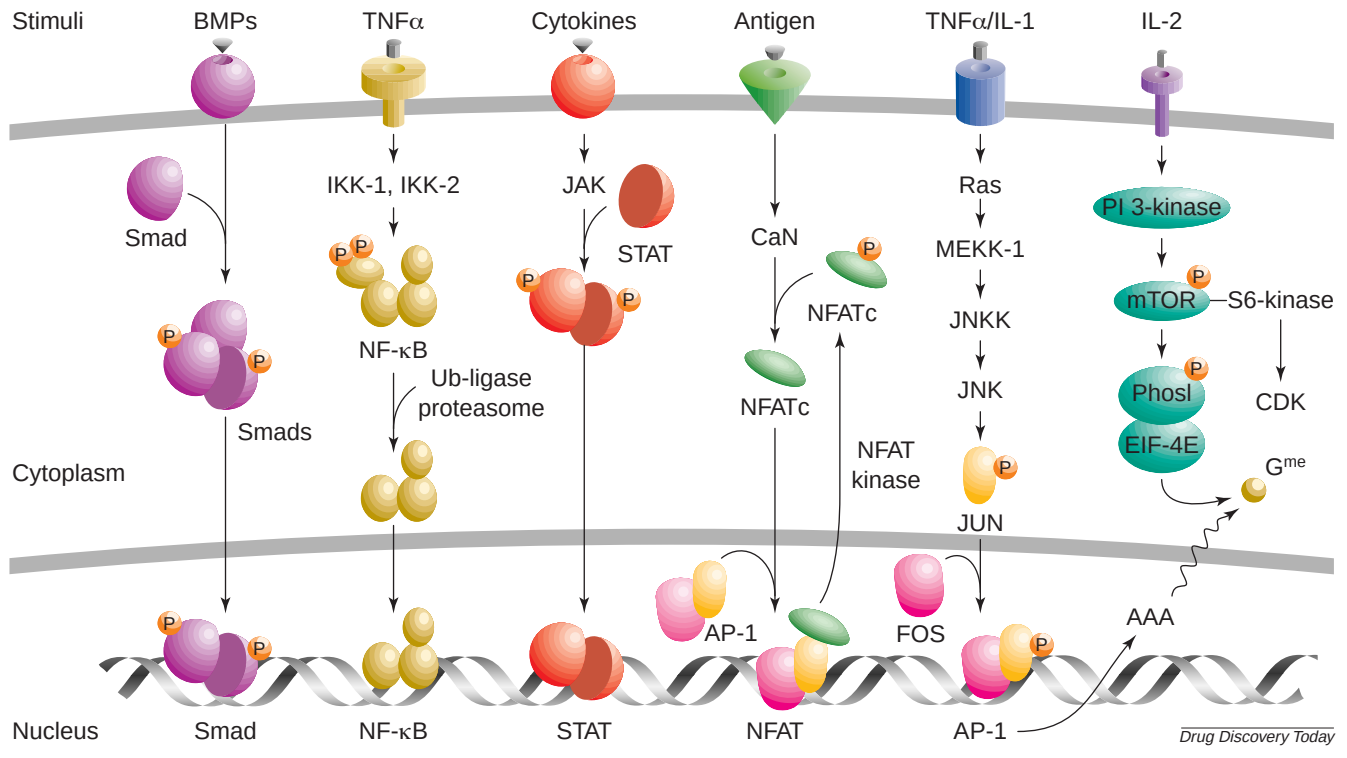

Figure 1. *Schematic representation of kinase-mediated signal-transduction cascades of interest in anti-inflammatory drug discovery. Bone morphogenic proteins (BMPs) activate receptor serine/threonine kinases for SMAD-based signaling. Tumor necrosis factor-α (TNFα) activates IκB kinases (IKK) for nuclear factor-κB (NF-κB) signal transduction. Likewise, TNFα/IL-1 (interleukin-1) can affect Ras/MAP (mitogen-activated protein) kinase signaling for activator protein-1 (AP-1). Cytokines affect the Janus family kinases (JAK) to regulate signal transducer and activator of transcription (STAT) activity. Antigen binding affects phosphorylation of the nuclear factor of activated T cells (NFAT). Interleukin-2 (IL-2) affects translation regulation through a rapamycin-sensitive mammalian target of rapamycin (mTOR) pathway. Other abbreviations: EIF-4E, eukaryotic initiation factor-4E; JNK, c-Jun N-terminal kinase; JNKK, JNK kinase; MEKK, MAP kinase kinase kinase.*

signal transduction in cells involved in inflammation, and the current challenge in protein kinase drug discovery is to identify agents that are potent, specific and selective for individual enzymes. This review focuses on gene-regulating protein kinases as discovery targets for selective therapeutic agents.

MAP kinases

Diverse stimuli modulate gene transcription via MAP kinase phosphorylation cascades[2]. MAP kinase cascades consist of tiered signaling modules in which the MAP kinase (MAPK) is activated by a MAP kinase kinase (MEK), which in turn is activated by a MAP kinase kinase kinase (MEKK) (Fig. 1). Three MAPK signaling cascades, culminating in activation of the epidermal growth factor regulated kinase (ERK), JNK and p38 MAP kinase families, have been described. ERKs are activated by mitogens and growth factors via a Ras-dependent pathway. JNKs and p38 kinases are activated in response to the proinflammatory cytokines and by cellular stress (e.g. heat shock, osmotic shock, reactive oxygen metabolites, protein synthesis inhibitors, UV irradiation).

MAP kinase pathways regulate the activities of a variety of transcription factors and other cellular proteins involved in gene expression. Briefly, MAP kinase pathways directly regulate activator protein-1 (AP-1)-dependent transcription, both at the level of *de novo* synthesis of AP-1 family proteins and by controlling their transactivation functions[5,8,9]. AP-1 is a nuclear partner of NFAT proteins, and MAP kinase pathways indirectly and directly regulate NFAT activity[4]. Recent work suggests that MAP kinase pathways are also intimately involved in NF-κB activation, although the mechanistic basis for this crosstalk has not been elucidated[10]. Lastly, MAP kinase pathways might be implicated in modulating the transcriptional functions of some STAT family proteins, which are activated in response to cytokine stimulation of cells[8,9].

Activation of MAP kinase pathways has been repeatedly documented in inflammatory settings both *in vitro* and *in vivo*, and therefore these pathways have become the focus of attention for drug discovery.

The JNK forms of MAP protein kinases are encoded by three genes: *JNK1*, *JNK2* and *JNK3*. *JNK1* and *JNK2* are ubiquitously expressed, whereas *JNK3* is selectively expressed in the brain, heart and testis. Inhibitors of *JNK*-mediated AP-1 activation might prove to be novel anti-inflammatory or anti-immunosuppressive agents that will inhibit inducible expression of inflammatory genes without affecting AP-1-mediated housekeeping functions. In T cells, *JNK* activation by costimulation through the antigen and CD28 receptors correlates with IL-2 induction[11]. Recently, examination of *JNK*-deficient mice revealed that the *JNK* pathway is induced in Th1 cells [producers of interferon-γ (IFNγ) and TNFα] but not in Th2 effector cells (producers of IL-4, IL-5, IL-6, IL-10 and IL-13) upon antigen stimulation[12,13]. This suggests that *JNK1* and *JNK2* do not have redundant functions in T cells and that they play different roles in the control of cell growth, differentiation and death.

Mice with a homozygous disruption for the *JNK3* gene are viable[14]. However, they are resistant to the excitotoxic stress response elicited by kainic acid, a glutamate receptor agonist. Kainic acid causes neuronal damage, especially within the hippocampus. The neurotoxicity of kainic acid possibly results from the induction of c-Jun and increased AP-1 DNA binding activity. *JNK3* inhibitors might be potentially useful in treating epilepsy and other neurodegenerative diseases such as stroke.

IκB kinases

IκB kinases regulate activation of NF-κB. This transcription factor was first described as a B-cell-specific factor for immunoglobulin-κ light-chain enhancer. However, NF-κB is expressed in all cell types and plays a broader role in gene transcription, regulating in excess of 70 genes that are associated with immune functions.

Activation of NF-κB

NF-κB is activated by a large number of signals such as UV light, IL-1 and TNFα (Refs 11,15). The same stress signals that activate the JNK and p38 MAP kinase pathways also activate NF-κB. Nevertheless, the basic steps of NF-κB activation appear similar in all cases. NF-κB exists in the cytoplasm in an inactive form associated with the IκB inhibitory proteins. The IκB family, which share common ankyrin-like repeating domains, regulate DNA binding and subcellular localization of NF-κB by masking a nuclear localization signal (NLS) located near the C-terminus. NF-κB activation is achieved through signal-induced proteolysis of IκB (Fig. 1). Extracellular stimuli initiate a signaling cascade, leading to activation of two IκB kinases, IKK-1 (IKKα) and IKK-2 (IKKβ), which phosphorylate IκB at specific residues[16–20]. Phosphorylated IκB is selectively ubiquitinated, and degraded by the 26S proteasome. This process exposes the NLS, freeing NF-κB to translocate to the nucleus.

The IκB kinases are members of a family of kinases containing an N-terminal protein kinase domain and a C-terminal region with a leucine zipper and a helix–loop–helix motif. These motifs mediate the dimerization of IKK-1 and IKK-2 and association of IKKs with other proteins. IKK-1 and IKK-2 are phosphorylated and activated by upstream kinases, which are members of the MEKK family of enzymes. One such upstream kinase, NIK, binds directly to TRAF2, an adapter protein for TNFα and IL-1 receptors[21]. A second MEKK, MEKK-1, is present in the IKK complex[19]. Coexpression of NIK or MEKK enhances IKK phosphorylation of IκB and NF-κB activation.

Additional kinase targets are associated with the upstream activation of NF-κB (Ref. 15). Receptor-interacting protein (RIP) is a part of the TNF receptor-associated signaling complex along with the TNF receptor-associated death domain (TRADD) and TNF receptor-associated factor 2 (TRAF2). RIP is a serine/threonine protein kinase and the only component of the TNF–R1 signaling complex with enzymatic activity. Analogous signaling components exist for the IL-1 receptor, including IL-1 receptor-associated kinase (IRAK), which is also a serine/threonine kinase that autophosphorylates upon receptor activation. IRAK associates with TRAF6 to activate NF-κB.

NFAT kinases

Proteins belonging to the NFAT family of transcription factors play a central role in inducible gene expression during the immune response[22]. NFAT proteins are expressed and are functional in several immune-related cells, including endothelial cells, macrophages, natural killer (NK) cells, B cells and mast cells. NFAT is activated by stimulation of receptors coupled to calcium mobilization, such as the antigen receptors on T and B cells, the Fcε receptors on mast cells and basophils, the Fcγ receptors on macrophages and NK cells, and the histamine and thrombin G-protein-coupled receptors on endothelial cells. Ligand binding to these receptors leads, via activation of phospholipase C and generation of inositol trisphosphate, to calcium mobilization and activation of the calcium- and calmodulin-dependent phosphatase calcineurin, a major upstream regulator of NFAT. The functions of NFAT proteins are regulated by cyclosporin-sensitive, calcineurin-

mediated dephosphorylation[23]. In resting cells, NFAT proteins are phosphorylated and reside in the cytoplasm whilst in activated cells, they become dephosphorylated by calcineurin and translocate into the nucleus.

Potential kinase targets in the NFAT pathway include the $p56^{Lck}$ and ZAP-70 tyrosine kinases. The NFAT kinases that counteract the action of calcineurin are also interesting targets, as agents that enhance NFAT kinase activity would be expected to limit the total time spent by NFAT proteins in the nucleus[24]. These NFAT kinases have been variously proposed to be JNK kinases[25] and glycogen synthase kinase-3 (GSK-3)[26]. Inhibitors of AP-1 would be expected to inhibit NFAT transcriptional activity by interfering with the activity of cooperative NFAT–AP-1 complexes. Further elucidation of the NFAT regulatory pathway will enhance the potential for new immunosuppressive drug discovery.

Janus-associated kinases

Janus-associated kinases (JAKs) activate members of the STAT family of latent cytoplasmic transcription factors in response to stimulation of cells through various cytokine receptors (Fig. 1). STAT proteins mediate the intracellular effects of several cytokines[27], and as such, represent potential drug targets[28]. Seven mammalian STAT proteins have been identified and are activated by JAKs that associate with cell-surface cytokine receptors and are catalytically activated by transphosphorylation following ligand binding and receptor heteromerization. Activated JAKs phosphorylate tyrosine residues on the receptors, thus recruiting STATs. Subsequently, the STATs are phosphorylated, leading to their release from the receptor docking sites and the formation of dimers that translocate to the nucleus.

There are indications that serine phosphorylation of certain STATs is required for optimal function. In STAT1 and STAT3, phosphorylation occurs on a single serine in a conserved Pro-Met-Ser-Pro sequence in the C-terminal transactivation domain[27]. In STAT3 and STAT5, serine phosphorylation is reported to be required for optimal DNA binding, whereas in STAT1, serine phosphorylation does not influence DNA binding but enhances transactivation function. The Pro-Met-Ser-Pro sequence is similar to the consensus sequence for phosphorylation by MAP kinases, and it is possible that STAT function is influenced by MAP kinase activation in cytokine-stimulated cells.

Although no small molecule inhibitors of the STATs have been reported, the JAK2 antagonist AG490 inhibits leukemic cell growth *in vitro* and *in vivo*[29] and might work in synovial hyperplasia or the persistence of inflammatory cells in the inflamed joint as STAT proteins play a key role in lymphocyte differentiation and proliferation.

BMP receptor kinases

Like JAK–STAT signal transduction, bone morphogenetic proteins (BMPs) act to stimulate the phosphorylation and activation of SMAD transcription factors[7]. BMPs bind to a dimeric receptor complex containing activin–TGFβ family receptor serine/threonine protein kinases. Ligand binding stimulates receptor-mediated phosphorylation of SMAD proteins, which multimerize and translocate to the nucleus. Once in the nucleus, the SMAD complex associates with other transcription factors such as AP-1 to stimulate bone-specific gene expression. BMP signaling to SMADs is important in a variety of therapeutic settings such as bone density increase, bone formation, osteoporosis and osteoarthritis. Although specific compounds that affect BMP signaling have yet to be identified, it is expected that efforts in this area could lead to important therapeutics.

PI 3-kinase-related kinases

Approximately ten years ago, a novel lipid kinase activity associated with v-Src and other activated tyrosine kinases was identified. These studies led to the identification of PI 3-kinase, which phosphorylates the D-3-hydroxyl residues in the inositol headgroups of phosphatidylinositol (PtdIns), PtdIns(4)*P*, or PtdIns(4,5)P_2. The resulting 3-phosphorylated phosphoinositides are second messengers in mitogenesis, apoptosis, protein transport and cytoskeletal rearrangements. A number of novel, high-molecular mass kinases have now been identified as belonging to a family of PI 3-kinase-related kinases (herein referred to as PIKKs) because of the strong homology within their catalytic domains. Mammalian members of this family include DNA-PK, mTOR/FRAP (FK506-binding-protein rapamycin-associated protein) ataxia telangiectasia mutated protein (ATM) and the ataxia telangiectasia- and RAD3-related protein (ATR). The PIKKs play crucial roles in the development and function of the immune system, chromosome maintenance and DNA repair, cell cycle control and tumor suppression[30].

The mTOR/FRAP protein kinases play a key role in the immune system and these proteins are the pharmacological targets for rapamycin[6]. Rapamycin inhibits mTOR function through binding to its intracellular receptor, the 12-kDa FK506-binding protein (FKBP12) and inducing binding of this complex to mTOR. The mechanism of rapamycin activity is well characterized. IL-2 stimulation leads to an increase in the translation of mRNAs whose protein products are required for progression through the G1-stage of the cell cycle. Rapamycin interferes with G1 progression in lymphoid and other cells by inhibiting mTOR function, leading to blockade of translation of a range of mRNAs and to the lack of activation of S6 kinase and CDKs. FRAP/mTOR is the terminal kinase in a

signaling pathway that couples mitogenic stimulation with phosphorylation of the eukaryotic initiation factor eIF-4E-binding protein, 4EBP1 or PHAS-1. mTOR phosphorylates PHAS-1 on serine and threonine residues, and these modifications inhibit the binding of PHAS-1 to eIF-4E. Efficient translation of mRNAs bearing long, highly structured 5′-untranslated regions (UTRs) is dependent on eIF-4F, a multisubunit complex containing eIF-4E (an N^7-methylguanosine cap-binding subunit), an RNA helicase, eIF-4A and a multifunctional scaffold protein, eIF-4G. The RNA helicase function of the complex is believed to melt secondary structure in the 5′-UTR of capped mRNAs, thereby facilitating ribosome binding to the AUG codon. In quiescent cells, PHAS-1 is hypophosphorylated and binds tightly to eIF-4E, inhibiting eIF-4E binding to eIF-4G. mTOR-mediated phosphorylation of PHAS-1 promotes dissociation of PHAS-1–eIF-4E complexes. The clinical utility of rapamycin is limited by its side-effects, which are believed to be caused primarily by the role of FKBPs in other cellular functions. Direct inhibitors of mTOR would be highly attractive agents for the treatment of autoimmune disease.

Protein kinase inhibitors

Drug discovery efforts targeting receptor tyrosine kinases have been conducted for nearly a decade and the experience gained has been valuable for discovery efforts in serine/threonine kinases. Thus, screening of natural products of different origin and collections of available compounds has led to the identification of research compounds that inhibit both classes of kinases. Compounds such as staurosporine and some of its analogs, genistein, quercetin and certain other flavanoids that were found to be receptor tyrosine kinase inhibitors have also been shown to inhibit serine/threonine kinases (see Fig. 2).

Two specific technologies are expected to have a significant impact on future drug discovery efforts targeting protein kinases. First, structure-directed synthesis of compounds will be key to developing selective agents. More than 40 protein kinase structures, derived from homology methods and X-ray crystallography, have been reported and this will continue to be an important tool for inhibitor development. Second, focused inhibitor libraries of chemical entities amenable to combinatorial explosion will be an important development and will be essential to the rapid identification of kinase inhibitor leads. The coupling of these two technologies should lead to a rapid explosion of kinase-based drugs.

MAP kinase inhibitors

PD098059 (see Fig. 3) was discovered in a screen for inhibitors of the ERK cascade[31]. PD098059 binds to the inactive form of MEK1, a primary MEK in the ERK cascade, and blocks the phosphorylation required for MEK activation. Specificity of action was indicated by the inability of PD098059 to inhibit phosphorylation mediated by c-Raf, JNK, p38, PKA, PKC, v-Src, active MEK1 and several other serine/threonine and protein tyrosine kinases (including receptor tyrosine kinases). U0126 is another MEK inhibitor that has potent *in vitro* and *in vivo* efficacy in models of inflammation and delayed-type hypersensitivity[32].

Although no specific JNK inhibitors have been described, isoform-specific inhibitors are being sought. The greatest effort and most progress has been made in the discovery of p38 MAP kinase inhibitors[33]. Elucidation of the important role of the p38 pathway in inflammatory processes resulted from studies using a series of pyridinyl imidazoles, exemplified by SK&F86002 and SB203580. These potent inhibitors

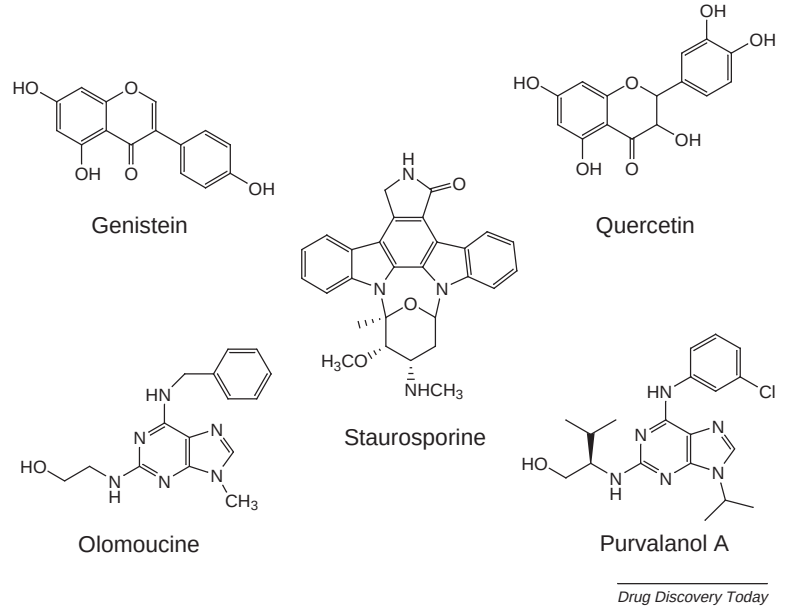

Figure 2. *Natural products reported to be inhibitors of serine/threonine kinases and cyclin-dependent kinase 2 (CDK2). Staurosporine, genistein and quercetin inhibit a variety of tyrosine and serine/threonine kinases. Olomoucine and purvalanol A (synthetic compound) inhibit CDK2 (the latter being a significantly more potent and selective inhibitor).*

of p38 activity block IL-1 and TNF production in lipopolysaccharide-stimulated monocytes. SB203580 competes with ATP and inhibits JNK2, but with a 10–20-fold lower potency than p38. SB203580 binds p38 by inserting into the ATP-binding pocket. Although the 4-fluorophenyl ring of the compound does not make contact with residues in the ATP-binding pocket, it is in near proximity to the Thr106 of the enzyme. Mutation of this amino acid to Met106 makes p38 insensitive to SB203580. Thr106 is conserved in p38β, another isoform of p38 that is sensitive to SB203580, but is replaced by methionine in p38γ, p38δ, JNK1 and JNK2 (all much less sensitive to SB203580). Mutation of p38β Thr106 to Met106 renders p38β almost resistant to SB203580, and the reverse mutation of Met106 to Thr106 in p38γ and p38δ results in SB203580 sensitivity.

Some of the initial p38 inhibitors, including SB203580, are inhibitory towards cytochrome P450 isoforms (1A2, 2C9, 2C19, 3A4, 2D6). This is because of the high-affinity binding of the 4-pyridyl group to heme iron. As a consequence, replacements for the 4-pyridyl ring were sought. The pyrimidine analog, as represented by SB226882 (J.L. Adams *et al.*, Inflammation Research Association Ninth International Conference, 1–5 November 1998), has equivalent p38 inhibitory potency *in vitro* and is effective in *in vivo* mouse models that measure circulating TNF levels. Several other p38 inhibitors have been reported, including L167307 (Ref. 34), VK19911 (Ref. 35), SC102 (R.J.M. Mourey *et al.*, Inflammation Research Association Ninth International Conference, 1–5 November 1998), RWJ67657 (S.A. Beers *et al.*, Inflammation Research Association Ninth International Conference, 1–5 November 1998) and RWJ68354 (Refs 36,37).

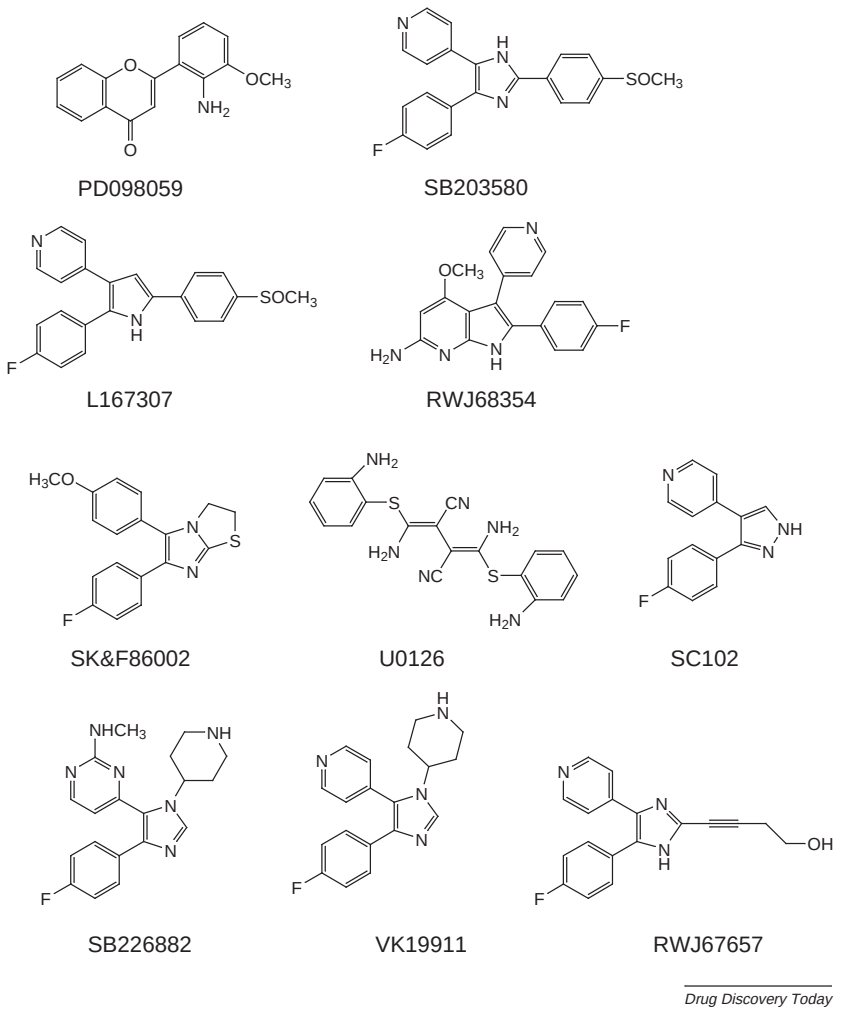

Figure 3. *Serine/threonine kinase inhibitors. PD098059 and U0126 inhibit mitogen-activated protein (MAP) kinase kinase 1 (MEK1). SB203580, L167307, RWJ68354, SK&F86002, SC102, SB226882, VK19911 and RWJ67657 inhibit p38 MAP kinase.*

NF-κB inhibitors

There are multiple targets that are amenable to small-molecule blockade within the NF-κB activation pathway. Several recent reviews have summarized the effects and the mechanism of action of many compounds reported to inhibit the activation or function of NF-κB (Ref. 15). No specific kinase inhibitors have been described. However, antioxidants and free radical scavengers such as *N*-acetylcysteine, curcumin and caffeic acid block NF-κB activation. The 26S proteasome contains a chymotrypsin-like activity that degrades IκB, an activity that can be blocked by peptide aldehydes, including PS115, PS341 and Z-LLF-CHO (Ref. 38). Furthermore, several clinically important anti-inflammatory drugs such as salicylates, gold and glucocorticoids also inhibit NF-κB-induced gene expression. Glucocorticoids might exert some of their anti-inflammatory effects by inducing the synthesis of IκB.

Directed screening of focused libraries

Recently, there has been a report on the design, synthesis and screening of libraries containing compounds that have the structural features of CDK2 inhibitors[39]. The rationale for this approach is based on the premise that when trying to inhibit a kinase at its ATP binding-site, use of the structural features of known ATP-competitive kinase inhibitors could increase the frequency of finding hits.

Olomoucine is a selective but weak inhibitor of CDK2. Examination of the mode of binding of olomoucine relative to ATP in the X-ray structure of CDK2 led to the prediction that variation of the substituent at the 2-position of olomoucine could lead to optimization of binding in the ribose binding region of ATP. In addition, optimization of the substituents at the 2- and 6-positions of olomoucine was predicted to improve binding. Iterative chemical library synthesis and biological screening led to potent and selective CDK2 inhibitors (Fig. 2). Some of the other members of the library were found to inhibit other kinases such as JNK and glycogen synthase kinase. A well-designed kinase-specific diverse library would be expected to provide hits for different kinases in a screening operation.

Structure-based drug design

Structure-based drug design (SBDD) is important for drug discovery efforts in search of kinase inhibitors. Determination of the X-ray structure of members of the MAP kinase family, ERK (Ref. 40), p38 (Ref. 41) and, more recently, JNK3 (Ref. 42), has revealed the active site-alignment of the conserved residues around the threonine and tyrosine residues within the activation loop that are phosphorylated by ATP. The MAP kinases ERK, JNK and p38 have the characteristic activation sequences TEY, TPY and TGY, respectively. Efforts are ongoing to design novel inhibitors in the active site of these enzymes using a variety of software packages. Drug design for other kinases for which the X-ray structure is not yet definitive has been aided by the construction of hypothetical three-dimensional homology models based on sequence conservation with a kinase whose X-ray structure has been solved. Such hypothetical models, known as homology models, are refined using the SAR of a known series of inhibitors. The X-ray structure of JNK3 has been valuable in building homology models of JNK1 and JNK2, which have 90% homology with JNK3 (Fig. 4). An inhibitor for any one of these enzymes is a candidate for SBDD of inhibitors for the other two JNKs. A combination of high-throughput screening (HTS), kinase-specific libraries and SBDD is expediting the drug discovery efforts for kinase inhibitors.

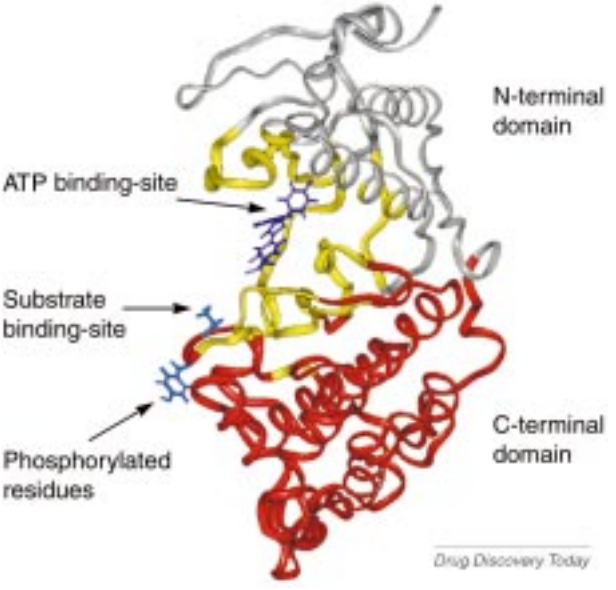

Figure 4. *Three-dimensional model of the kinase domain of c-Jun N-terminal kinase 2 (JNK2) displayed as a ribbon diagram. Highlighted in yellow are residues comprising the active site, where ATP and substrate are bound and phospho-transfer occurs. Highlighted in orange are residues of JNK2 which are phosphorylated by the upstream kinase, JNKK, leading to an alteration in protein confirmation that promotes catalytic activity. The predicted orientation of SB203580, an ATP-dependent inhibitor of JNK2, bound in the active site is depicted.*

The future

The recent new anti-inflammatory drug approvals in the USA (leflunomide, soluble TNF receptor and celecoxib) have generated great interest in the next generation of therapeutics to treat inflammatory diseases such as rheumatoid arthritis, asthma, multiple sclerosis and psoriasis. As these diseases are triggered by several proteins including cytokines, degradative enzymes and cell adhesion molecules, protein kinase strategies to control multiple gene and protein activities are being investigated. Furthermore, a number of key inflammatory cytokines activate intracellular signaling kinase cascades to elicit their actions and provide the opportunity to modulate these inflammatory proteins and avoid protein-receptor targets that are often intractable to small-molecule intervention. Protein kinase signal-transduction cascades thus provide a rich source of targets for small-molecule drugs that have the potential to control both multiple and individual inflammatory protein production and activity.

Issues that remain unanswered include the preferred site for targeted intervention on the kinase cascades. For example, is it better to develop a MEKK inhibitor or a MAP kinase inhibitor? There are reasons to suggest that inhibitors of each step of the cascade might provide a different profile of activity based on the cellular environment, activation and amplification steps. Likewise, protein kinases are challenging drug targets, but, despite the conventional dogma that the catalytic site inhibitors are nonspecific, the identification of very selective kinase inhibitors has created considerable optimism for the future. There is also the promise that protein substrate binding sites will provide additional opportunities for kinase inhibitor design. Finally, the rapid progress made in the production of crystal structures of a number of serine/threonine and tyrosine-specific protein kinases has identified the catalytic core of these important enzymes.

As greater knowledge of active site inhibitors for p38 as well as other kinases emerges, it is becoming clearer how to modify compounds to create greater potency and specificity. A debate also exists as to the benefits of developing reversible inhibitors that block the enzyme for only a few hours, in contrast to irreversible inhibitors that provide greater duration of inhibition. Because protein kinases are intracellular enzymes, issues related to cell penetration, selectivity and *in vivo* efficacy and safety remain the challenge for the medicinal chemist. In this age of increased chemical diversity, it is anticipated that to address these issues, new kinase inhibitor templates will emerge from chemical libraries and natural product screening, as well as from the availability of massive combinatorial libraries. Biologically enhanced screening capacities resulting from HTS together with the availability of multiple recombinant human kinases is expediting selective kinase inhibitor identification. There are numerous kinases within the cell and, consequently, rapid and broad profiling remains an important goal. Understanding of the secondary and tertiary events that are modified by selective kinase inhibition will be greatly facilitated by gene chip methodologies that will allow transcript profiles to be obtained. Such profiles will be extremely useful in evaluating the selectivity of drug candidates. It is anticipated that multiple inhibitors of gene-regulating kinases will be developed in a variety of immuno-inflammatory and proliferative diseases and it is hoped that our capability to generate kinase inhibitors will allow the rapid transition from novel kinase to validated target to clinical application and the marketplace. As more kinases are discovered and their activities identified, it can be anticipated that this large gene family will provide numerous therapeutic opportunities in multiple major diseases.

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