Functions and Modulation of MAP Kinase Pathways

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Background

The transmission of extracellular signals into intracellular responses is a complex process which often involves the activity of one or more mitogen-activated protein kinases (MAPKs).[1] The activation of a MAPK employs a core three-kinase cascade consisting of a MAPK kinase kinase (MAP3K or MAPKKK) which phosphorylates and activates a MAPK kinase (MAP2K, MEK, or MKK) which then phosphorylates and increases the activity of one or more MAPKs. Upon activation, MAPKs can phosphorylate a variety of intracellular targets including transcription factors, nuclear pore proteins, membrane transporters, cytoskeletal elements, and other protein kinases.

Discovery of MAPKs

The MAPKs extracellular signal regulated protein kinases 1 and 2 (ERK1/2) were first identified as mitogen-stimulated ~42 kDa phosphoproteins in the early 1980s, and later as insulin and nerve growth factor (NGF)-stimulated activities that retained the ability to phosphorylate the model substrates microtubule-associated protein-2 (MAP2) and myelin basic protein (MBP).[2-8] The activities of ERK1/2 were shown to reactivate phosphatase-treated ribosomal protein S6 kinase (RSK or p90).[4,9]

In the following years, the MAPK family was discovered to include three c-Jun N-terminal kinases (JNK), four p38 isoforms, ERK3 isoforms, ERK5 and ERK7. The first JNK family members were independently identified as cycloheximide-activated MBP kinases and purified due to their ability to interact with the N-terminus of the transcription factor c-Jun.[10,11] p38α was identified as an inflammatory cytokine-stimulated tyrosine phosphoprotein, a target of an inhibitor of tumor necrosis factor α (TNFα) production, and a re-activating kinase for MAPK-activated protein kinase-2 (MAPKAP2 or MK2).[12-14] PCR-based cloning strategies and a two-hybrid screen led to the discovery of additional JNK and p38 isoforms and ERKs 5 and 7 reviewed by Chen *et al*[15] and Lewis *et al*.[16] A summary of the cellular processes involving

these MAPKs is shown in Figure 2. Detailed reviews by Adnane *et al*, Daaka *et al* and Dang *et al* are also recommended for further information.[1,16,17]

Upstream regulation of ERK1/2

The collaborative findings from a number of laboratories led to the connection of ERK1/2 to their upstream regulators MAPK/ERK kinase 1 and 2 (MEK1/2); the identification of Raf isoforms as upstream activators of these MAP2Ks; and the observation that Raf is an effector of Ras small GTP binding proteins.[18-24] Isoforms of Ras and Raf are found mutated in a variety of human tumors, implicating ERK1/2 in proliferation and oncogenic growth.[25,26] Although regulation through this pathway is exceedingly complex, the potential of this MAPK pathway to promote tumorigenesis was later supported by the demonstration that an activated mutant of MEK1 transformed cells and promoted growth of tumors in nude mice.[27] Subsequently, through the use of dominant interfering mutants, pharmacological inhibitors of MEK1/2, gene disruption and RNA interference, these ubiquitous kinases have been shown to be intimately involved in normal processes including embryogenesis, cell differentiation, glucose sensing and synaptic plasticity.[28-33]

MAP2Ks

MEK1, an exemplary MAP2K, was purified as a ~45 kDa biological activator of ERK1/2.[34-36] The identification of additional MAP2Ks (MEKs 2-7), all of roughly equivalent size, employed DNA-based molecular techniques as opposed to protein purification reviewed by Chen *et al*[15] and Lewis *et al*.[16] These kinases are unusual in that they are dual-specificity kinases, phosphorylating both tyrosine and serine/threonine residues. Unlike MAPKs, which phosphorylate a wide range of proteins, MAP2Ks are highly specific: they are dedicated to phosphorylation of only one or a couple of MAPKs and few, if any, other substrates. MAP2Ks integrate signals from multiple regulatory inputs and serve as points of signal integration, in part through scaffolding proteins and docking site-mediated protein-protein interactions.

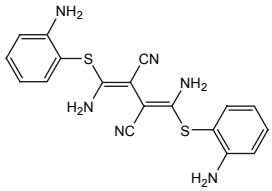
Raf isoforms and other MAP3Ks

The most readily identifiable feature of MAPK signaling is the three kinase cascade consisting of a MAP3K, a MAP2K and a MAPK. The three-kinase organization of this cascade is identical to that of the three-kinase cascade of Ste11 (MAP3K) - Ste7 (MAP2K) - Fus3/Kss1 (MAPK) in the yeast pheromone response pathway.[37] MAP2Ks and MAPKs are related in sequence throughout metazoans, although Raf proteins do not seem to have counterparts identified in yeast. Interestingly, Raf was originally discovered as a retroviral oncogene.[38] Three isoforms, c-Raf (or Raf-1), B-Raf and A-Raf, are found in mammals. In addition to the core ~35 kDa kinase domain, Raf proteins contain an N-terminal regulatory region, also about 35-40 kDa, which can bind Ras. Raf proteins specifically phosphorylate only the MAP2Ks MEKs1 and 2, and were initially thought to function in a tissue-specific manner. More recent studies, aided by the development of B-Raf inhibitors as anticancer agents, led to the understanding that Raf isoforms dimerize.[39,40] The unanticipated actions of these B-Raf inhibitors provoked more in-depth molecular analysis showing that dimerization can enhance Raf activity and that Raf heterodimers have different activities.[41-43]

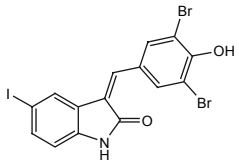
Two other enzymes that function as MAP3Ks in the ERK1/2 pathway are Mos and Tpl2 (Cot), both originally identified as proteins that could transform cells.[44,45] These enzymes function only in specialized situations and when present, they activate the cascade; Mos is expressed primarily in oocytes, while Tpl2 is stabilized in response to lipopolysaccharide.[46,47]

The parallels between yeast and mammalian signaling led investigators to search for Ste11 homologs in mammals. MEKK1 was the first mammalian MAP3K identified from its homology to Ste11.[48,49] In contrast to the selectivity displayed by Raf MAP3Ks, MEKK1, a large protein of 195 kDa, displayed the ability to phosphorylate several MAP2Ks (MEKs 1-4, 6 and 7) *in vitro*. Early evidence suggested that MEKK1 was a regulator of MEK1/2, but gene disruption experiments and numerous biochemical analyses indicate that MEKK1

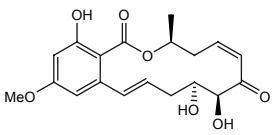
Figure 1 | MAP3K and MAP2K compounds



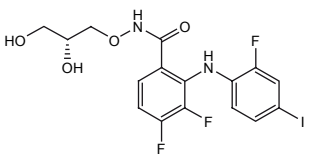
U0126 (1144)
Potent, selective inhibitor of MEK1 and 2



GW 5074 (1381)
Potent, selective c-Raf1 kinase inhibitor



(5Z)-7-Oxozeaenol (3604)
Potent and selective TAK1 MAP3K inhibitor



PD 0325901 (4192)
MEK inhibitor

predominantly coordinates downstream signaling through activation of MEKs 4 and 7 and the JNK pathway.[50-53] Subsequent isolation of related cDNAs have led to the discovery of a family of related enzymes (i.e. MEKKs 1-4; ASK1,2; TAK1) reviewed by Raman *et al*[1] and Johnson *et al*.[54] As a group, these enzymes also display broader substrate specificity than Raf and probably regulate multiple MAPK pathways in context-dependent processes. Non-Ste11 homologs, such as the Ste20 homologs TAOs 1-3 are MAP3Ks that regulate the p38 pathway.[1]

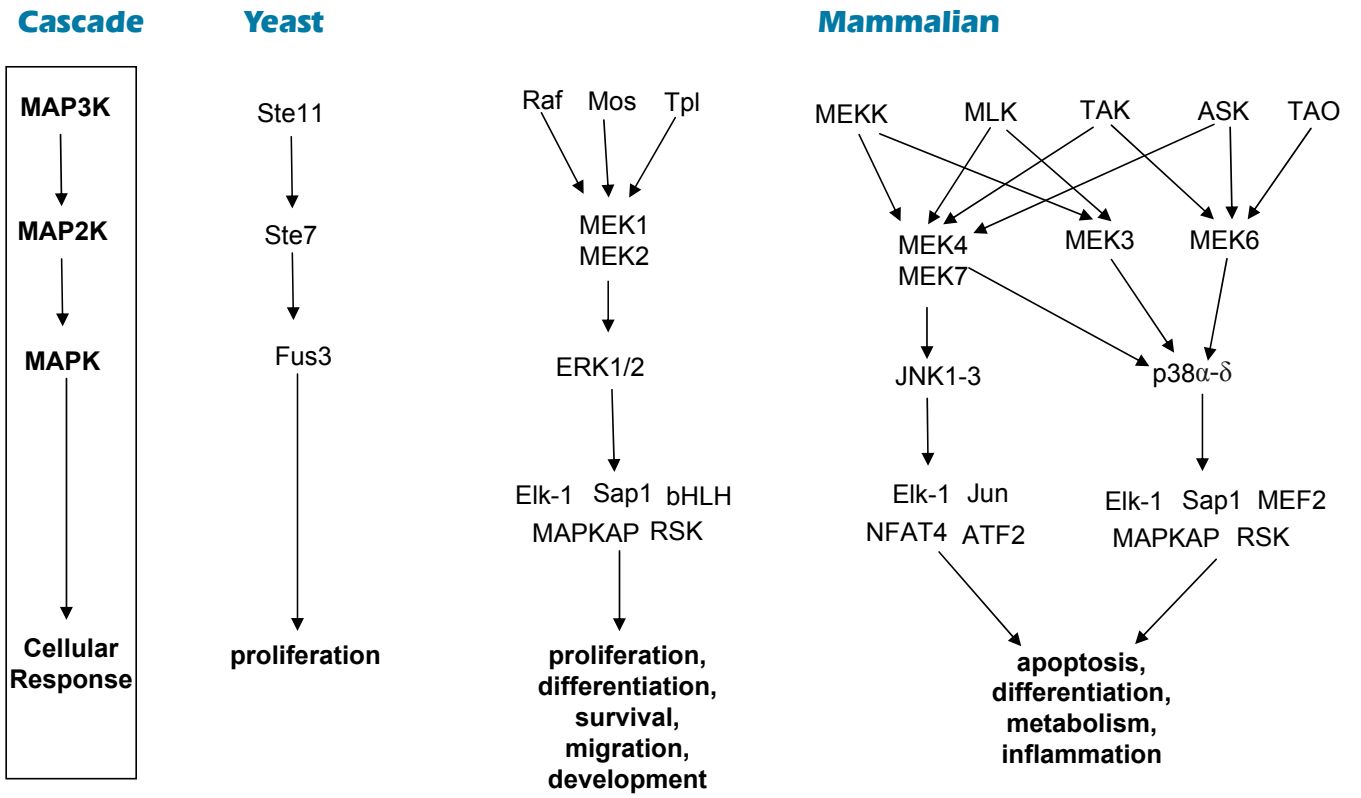
Figure 2 presents a simplified model of the organization of MAPK cascades. Of note are the number of MAP2K-MAPK combinations a given MAP3K can regulate and the resulting points of cross-talk. How the organization of MAPK cascades affects their function will be discussed next.

Properties of MAPK cascades

Signaling features of both mammalian Raf-MEK1/2-ERK1/2 and the yeast cascades initially provided an insight into the primary features of MAPK signaling. Generally similar mechanisms of regulation exist in other MAPK cascades, although it is expected that additional novel features will be found. The impact of scaffolds and cascade localization are still not well integrated into current models. As mechanistic insight has accumulated, the complexity of these pathways, in spite of the apparent simplicity of the three-kinase unit, must be acknowledged. Also the regulatory plasticity that accrues from the three-kinase cascade should not be underestimated.

The conventional view of a signaling cascade was developed from the first studied kinase pathway, cAMP-dependent protein kinase (PKA). Amplification occurred because components were more abundant moving down the cascade. The cooperative, switch-like behavior in this pathway derives from the requirement of four cAMP molecules to activate PKA.[55] This requirement may not apply unilaterally to MAPK pathways. MEK1/2 are much more abundant than Raf proteins, but MEK1/2 are in some cases as abundant as ERK1/2.[56,57] The MAPK pathway exhibits a similar switch-like behavior that guarantees a threshold to prevent activation of the pathway by noise in the system. This behavior is mechanistically distinct and partly derives from nonprocessive dual phosphorylation of MAPKs by MAP2Ks. MAPKs contain a poorly conserved loop that lies C-terminal to the catalytic residues, referred to as the activation loop. This loop contains a TXY motif. Phosphorylation of both the threonine and tyrosine residues of this motif by MAP2Ks is required to activate MAPKs.[58] In cells, the phosphorylation of tyrosine before threonine introduces the activation threshold and rapid cooperative activation.[59,60]

Figure 2 | MAP Kinase Networks



The existence of three proteins in series provides for multiple points of regulatory input. For example, Raf isoforms are phosphorylated on numerous sites by several protein kinases that increase or decrease activity and influence protein-protein interactions.[61] Prominent among these regulatory inputs are sites of feedback phosphorylation by ERK1/2 that interfere with re-activation of Raf by Ras.[62,63] Raf isoforms also interact with a variety of adaptor proteins.[64] MAP2Ks are also phosphorylated at other sites in addition to activating sites in their activation loops.[65-67] For example, phosphorylation of MEK1/2 in unique insert regions disrupts their ability to interact with Raf.[65,68,69] To summarize, the fidelity of signaling to ERK1/2 is dictated by the integration of a broad collection of signals that can be communicated at multiple levels in the pathway.

Scaffolding proteins

Scaffolds have a major influence on cascade function strongly impacting on the activities and outputs of MAPK pathways. Best described by the yeast example Ste5, scaffolds are paramount for achieving MAPK specificity.[64,70,71] The yeast MAP3K, Ste11, can activate either Ste7, a MAP2K for the MAPKs Kss1 and Fus3, in response to pheromone; or Pbs2, the MAP2K for Hog1 (the yeast p38 MAPK) in response to osmotic stress.[72] Scaffolding proteins dictate which signal activates Ste11 and which MAP2K is targeted for activation by Ste11. In the pheromone response, Ste5 scaffolds the interaction of Ste11 with Ste7, whereas the ability of Pbs2 to create a stable interaction of Ste11 with the osmosensor Sho1 allows Ste11 to act in the osmotic response.[73]

As noted above, mammalian signaling has similar complexities, suggested by the many observations showing that individual MAP3Ks can regulate multiple MAPK cascades. Nevertheless, no obvious Ste5 homolog has been identified in mammals. The scaffolding work is likely to be distributed among several different types of proteins in mammals and may often be “wrapped” within some of the upstream enzymes. Several MAP3Ks contain docking sites that allow them to bind stably to specific MAPKs. For example, MEKK1 binds tightly to JNKs through a docking motif.[74] Stable interactions with MAPKs may be mediated by at least two short sequence motifs, the docking (D or DEJL) motif and the FXF (DEF) motif.[75,76] One or more of these motifs are often present in scaffolds, activators, certain phosphatases and many substrates. JNK-interacting protein (JIP) scaffolds which organize JIP and sometimes p38 MAPK pathways have some functional parallels to Ste5s.[64,77] The scaffold kinase suppressor of Ras (KSR) was discovered in the sevenless eye development pathway in *Drosophila* and in the vulval induction pathway of *C. elegans*.[78,79] KSR proteins are members of the Raf family, but are pseudokinases because they lack the essential ATP-binding lysine residue. In mammals KSR1 and KSR2 bind ERK1/2, MEK1/2 and Raf isoforms. They interact with Raf proteins and can allosterically activate Raf.[41] Other proteins that influence assembly and activation of MAPK pathways include Sur8, CNK, MP-1 and IMP.[80-83]

Activation of MAPKs from the cell surface

Tyrosine kinase receptor activation of ERK1/2

ERK1/2 are activated by a wide variety of stimuli that act through cell surface receptors. Of all the signaling pathways emanating from these receptors, the pathway from receptor tyrosine kinases to ERK1/2 is the best delineated.[1,16,17] Ligand binding to receptor tyrosine kinases stimulates homo- and/or heterodimerization of the receptors and increases their tyrosine kinase activity. Activated receptors can then phosphorylate themselves and their dimerization partners, creating phosphotyrosine motifs. These motifs are recognized by SH2 domains that exist in a variety of proteins including the adaptor proteins Shc and Grb2. The SH3 domain of the Ras guanine nucleotide exchange factor son of sevenless (SOS) interacts with proline-rich regions on the receptor-bound adaptor proteins, completing the formation of a Ras-activating complex at the plasma membrane. After association with the receptor-adaptor protein complex, SOS stimulates the exchange of GDP for GTP on Ras. When GTP-bound, Ras interacts with a number of downstream effectors including Raf.[20,21] The direct interaction of Ras with Raf isoforms localizes them to the plasma membrane, which may serve to bring them in proximity to non-receptor kinases such as Src family members, and serine/threonine kinases including p21-activated kinases (PAKs) and protein kinase C (PKC) isoforms. These kinases may phosphorylate Ras-Raf isoforms and further increase their activity towards substrates or enhance their interactions with other proteins.[84-87] Upon activation, Raf isoforms can activate the MEK1/2-ERK1/2 pathway as discussed above.

Activation of MAPKs by G-protein-coupled receptors

Many hormones act through G-protein-coupled receptors (GPCRs) to increase ERK1/2 activity. Agonist binding to Gαs-coupled receptors results in the activation of adenylyl cyclase, which raises the intracellular concentration of cAMP. Elevated cAMP levels can increase, decrease or have no effect on the activity state of ERK1/2 in a manner dependent on cell type, and perhaps other factors yet to be defined.[1] Inhibition of ERK1/2 activity is believed to involve phosphorylation of serines 43 and 621 on

c-Raf by cAMP-activated protein kinase (PKA).[88] The N-terminal phosphorylation is reported to reduce the ability of c-Raf to interact with Ras.[89] Phosphorylation of serine 621 reduces c-Raf activity by disturbing binding to 14.3.3 protein.[90]

Increasing cAMP levels in cells of neuroendocrine origin typically stimulates ERK1/2 activity.[30,91] The activation of ERK1/2 may involve Ras itself or the Ras-related monomeric G-protein Rap-1, which can be activated by cAMP-activated guanine nucleotide exchange factors (GEFs).[92-94] It has also been suggested that Gαs coupled receptor activation of ERK1/2 involves Src.[95,96] Src may be activated by direct interaction with β-arrestin molecules that are engaged with internalized GPCRs.[96] In either case, activated Src, can directly influence c-Raf activity by phosphorylating sites that lie N-terminal to its kinase domain or by stimulating recruitment of SOS to receptor tyrosine kinases (RTKs).[97]

In vivo evidence indicates Gαi-coupled receptor activation of ERK1/2 most likely employs the βγ subunits of heterotrimeric G-protein complexes.[98,99] Through use of a βγ sequestering peptide, it has been suggested βγ subunits are necessary for Gαi-coupled receptor ligand stimulation of ERK1/2.[99] Consistent with this observation, exogenous expression of βγ is sufficient to activate ERK1/2.[98] Both Gαi-coupled receptor ligands and overexpressed βγ subunits require Src activity to stimulate ERK1/2.[98,100] Src activation by βγ subunits is not likely to involve direct interaction of the proteins; rather it has been proposed that PI-3 kinase γ serves as an intermediary in the activation mechanism.[101] Regardless of the mechanism employed, Src can activate ERK1/2 in the same manner as described for Src activation of ERK1/2 stimulated by Gαs.

Activation of ERK1/2 by Gαq-coupled receptors may require both Ras and PKC.[100,102,103] Upon activation, Gαq directly activates PLCβ, which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate inositol triphosphate (IP_3) and diacylglycerol (DAG). The DAG produced and the increase in intracellular Ca^{2+} resulting from IP_3 production can activate certain PKC isoforms. Phosphorylation of c-Raf by PKC can increase c-Raf activity.[87]

Inhibition of MAPK pathways

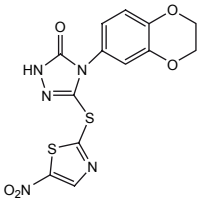
MAPK function studied by inhibition

Researchers utilize loss-of-function experiments such as dominant-negative mutants, gene silencing by RNA interference, and inhibitors of components of the MAPK signaling pathway to determine the dependence of a particular cellular function on a kinase pathway. Both a troublesome and a useful feature to the dominant-negative approach is that MAPKs are activated by overlapping upstream pathways and share common substrates causing the dominant-negative mutant to inhibit more than one target. Thus, a kinase-dead MAP2K may inhibit the activation of all the MAPKs it regulates. Gene silencing approaches using RNA interference may require targeting multiple closely related enzymes and rescue experiments are important to demonstrate that a phenotype is not due to off-target effects. Pharmacological inhibitors of components of the MAPK pathway are often a viable alternative or a complementary tool in understanding the functional requirement in a given pathway. Many inhibitors bind to the ATP binding pocket common to all protein kinases. Allosteric inhibitors are becoming increasingly valuable to achieve greater specificity. The specificity derived from interacting outside the ATP pocket has continued to spur the search for inhibitors of many kinases that bind to other pockets on kinase surfaces.[104] Some inhibitors are currently being developed that block essential protein-protein interactions.

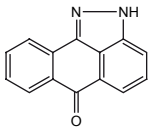
Inhibitors of the ERK1/2 pathway

Selective ERK1/2 inhibitors have only recently been described. One such inhibitor, FR180204, competes for the ATP-binding pocket of ERK2, with IC_{50} values of 0.14 and 0.31 μM for ERK2 and ERK1 respectively.[105] The utility of this compound is not yet certain. In the mid 1990's the MEK1/2 inhibitors PD 98059 and U0126, used to interfere with the

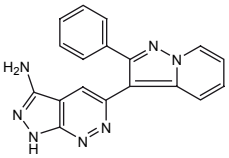
Figure 3 | JNK and ERK compounds



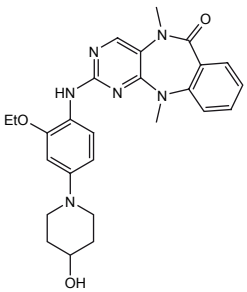
BI 78D3 (3314)
Selective, competitive JNK inhibitor



SP 600125 (1496)
Novel and selective JNK inhibitor



FR 180204 (3706)
Selective ERK inhibitor



XMD 8-92 (4132)
ERK5 inhibitor

ERK1/2 pathway, became available. PD 98059 was found in an *in vitro* kinase activation assay, while U0126 was identified in a cell-based assay as an inhibitor of AP-1 transcriptional activity.[106,107] In contrast to the usual ATP site inhibitors, U0126 and PD 98059 bind outside of the ATP binding site, selecting a low activity conformation and thereby keeping or shifting the protein to the inactive state. Due to their mode of binding, these drugs are among the most selective inhibitors available. The only other kinase affected by these drugs is the related MAP2K MEK5, which is inhibited at only slightly higher concentrations than MEK1/2.[108] Both drugs are useful at low micromolar concentrations and inhibit activation of MEK1/2, but require higher concentrations to block already activated MEK1/2 in cells.[109] Subsequently, a number of MEK1/2 inhibitors have been developed that are much more potent and have variable specificity relative to MEK5, including PD 198306 and PD 0325901.[104,110] PD 0325901 inhibits ERK1/2 activation in cells at concentrations as low as 25 nM, but fails to inhibit a large panel of other protein kinases at more than 100 times the concentration.[111] ERK5 activation was inhibited at low micromolar concentrations, consistent with a ~10-fold greater potency towards MEK1/2 than MEK5. ARRY-142886 (AZD6244) is another potent, noncompetitive inhibitor of MEK1/2 with a reported IC_{50} of 14 nM against purified MEK1.[112]

Another expanding array of compounds to block the ERK1/2 pathway is directed against Raf isoforms. B-Raf has been an attractive target particularly because a B-Raf mutant, V600E, is found in a large percentage of melanomas. The Raf inhibitors, overall, are less selective than MEK1/2-directed drugs and include GW 5074, ZM 336372, and BAY 43-9006 (Sorafenib). BAY 43-9006 inhibits c-Raf and B-Raf with IC_{50} values in the nanomolar range, but also has significant activity towards several receptor tyrosine kinases.[113]

Inhibitors of JNK pathways

SP 600125 is the most frequently used inhibitor of the JNK signaling pathway and blocks JNKs at concentrations in the range of 50-100 nM.[114] SP 600125 inhibits several other protein kinases with roughly equal potency.[115] CEP-1347 (KT-7515) blocks the JNK pathway but is specifically an inhibitor of the upstream mixed lineage kinases (MLKs).[116] Thus, it will not block JNKs that are regulated in an MLK-independent manner.

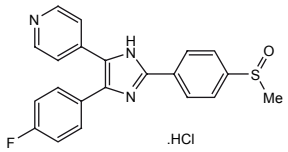
Inhibitors of p38 signaling pathway

p38 inhibitors including SB 203580 have been developed using pyridinyl imidazoles as lead compounds.[117] Crystallography showed that SB 203580 binds to the active site of p38, which prevents ATP binding.[118] SB 203580 and several related compounds inhibit p38α and β, but not p38δ and γ.[119] Structural studies of p38 identified a key feature of the ATP binding pocket that impacts inhibitor specificity. The threonine 106 residue of p38α and β is often larger than others, such as methionine or glutamine present in protein kinases such as p38δ and γ. This residue is called the gatekeeper.[120] Enzymes with small gatekeeper residues can accommodate larger compounds in their ATP sites. As a consequence, Raf isoforms (due to their small gatekeeper side chains) can interact with p38 inhibitors, while p38δ and γ cannot.[119] BIRB796, a diaryl urea compound, structurally unrelated to SB 203580, inhibits all four p38 isoforms by indirectly competing with the binding of ATP. BIRB796 binding requires a large conformational change in a conserved catalytic loop (DFG motif) of p38. This remodeled structure is unable to bind ATP.

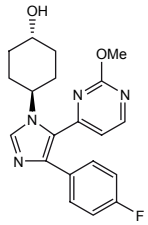
Future prospects

At present we have an ample amount of information on components involved in the MAPK signaling pathway. Likewise, many substrates have been identified and mapped to functions in specific processes. Unfortunately, our understanding of these pathways is unilateral and often excludes feedback mechanisms, spatio-temporal aspects and context-specific signaling. Finally, uncovering how the MAPK pathway regulates, or is regulated by, newly discovered processes is an exciting task. Increasing amounts of evidence points to a role for MAPK in disease. p38 MAPK and JNK are potential

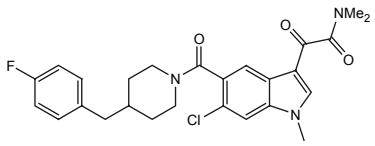
Figure 4 | p38 MAPK compounds



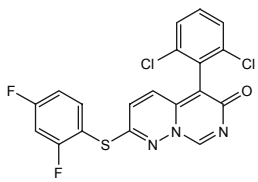
SB 203580 (1402)
Selective inhibitor of p38 MAPK



SB 239063 (1962)
Potent, selective p38 MAPK inhibitor; orally active



SX 011 (3639)
p38 MAPK inhibitor



VX 745 (3915)
Potent and selective p38α inhibitor

targets for drug development in neuronal disease as their inhibition may reduce the production of inflammatory cytokines known to be involved in a number of neural diseases such as cerebral ischemia, Alzheimer's disease and Parkinson's disease.[121] Mutations in signaling components that activate ERK have been found in many forms of cancer. Specifically, mutations in K-Ras are prominent in colon and pancreatic cancer; N-Ras mutations occur in melanomas; H-Ras mutations in cervical and bladder cancer; while B-Raf mutations are found in over 65% of malignant melanomas.[122,123] The ERK signaling pathway is a main component in several steps of tumorigenesis including cancer cell proliferation, migration, invasion and survival. A deeper understanding of MAPK signaling pathways is required for the development of new therapeutic drugs for various disease states.

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MAPK Compounds Available from Tocris

MAP3K- MAP2K- MAPK module

MAP3K

1381 **GW 5074**
Potent, selective c-Raf1 kinase inhibitor
3185 **L-779,450**
Potent Raf kinase inhibitor
3604 **(5Z)-7-Oxozeaenol**
Potent and selective TAK1 MAPKKK inhibitor
1321 **ZM 336372**
Potent, selective c-Raf inhibitor

MAP2K

1777 **Arctigenin**
Potent MKK1 inhibitor. Also inhibits IκBα phosphorylation
1213 **PD 98059**
Specific inhibitor of MEK
2605 **PD 198306**
Selective inhibitor of MEK1/2
4192 **PD 0325901**
Selective inhibitor of MEK1/2
1969 **SL 327**
Selective inhibitor of MEK1 and MEK2; brain penetrant
1868 **U0124**
Inactive analog of U0126 (Cat. No. 1144)
1144 **U0126**
Potent, selective inhibitor of MEK1 and 2

MAPK

JNK

2651 **AEG 3482**
Inhibitor of JNK signaling
3314 **BI 78D3**
Selective, competitive JNK inhibitor
1565 **JIP-1 (153-163)**
JNK-selective inhibitor peptide
1496 **SP 600125**
Novel and selective JNK inhibitor
3607 **SU 3327**
Selective JNK inhibitor
2827 **TCS JNK 5a**
Selective inhibitor of JNK2 and JNK3

ERK

3464 **Anti-ERK1**
Antibody recognizing ERK1
3465 **Anti-ERK2**
Antibody recognizing ERK2
3706 **FR 180204**
Selective ERK inhibitor
4132 **XMD 8-92**
ERK5 inhibitor

p38

1290 **Anisomycin**
Activates JNK/SAPK/p38 MAPK
2186 **CMPD-1**
Non-ATP-competitive p38α inhibitor
2908 **EO 1428**
Selective inhibitor of p38α and p38β_2
2657 **JX 401**
Potent, reversible p38α inhibitor
2999 **RWJ 67657**
Potent, selective p38α and p38β inhibitor
1264 **SB 202190**
Potent, selective inhibitor of p38 MAPK
1202 **SB 203580**
Selective inhibitor of p38 MAPK
1402 **SB 203580 hydrochloride**
Selective inhibitor of p38 MAPK; water-soluble
1962 **SB 239063**
Potent, selective p38 MAPK inhibitor; orally active
2938 **SD 169**
Selective, orally active p38α inhibitor
2008 **SKF 86002 dihydrochloride**
p38 MAPK inhibitor; anti-inflammatory agent
3639 **SX 011**
p38 MAPK inhibitor
3916 **VX 702**
Orally active p38α and p38β inhibitor
3915 **VX 745**
Potent and selective p38α inhibitor

Receptor and Upstream Signaling

FAK

3414 **FAK Inhibitor 14**
Selective FAK inhibitor
3239 **PF 573228**
Potent and selective FAK inhibitor

PI3K

3578 **AS 605240**
Potent and selective PI 3-kinase γ (PI3Kγ) inhibitor
3606 **BAG 956**
Dual PI 3-kinase and PDK1 inhibitor
1130 **LY 294002 hydrochloride**
Selective PI 3-kinase inhibitor
2418 **LY 303511**
Negative control of LY 294002 (Cat. No. 1130)
2930 **PI 103 hydrochloride**
Inhibitor of PI 3-kinase, mTOR and DNA-PK
2814 **PI 828**
PI 3-kinase inhibitor, more potent than LY 294002 (Cat. No. 1130)
1232 **Wortmannin**
Potent, irreversible inhibitor of PI 3-kinase. Also inhibitor of PLK1

FTI (Ras)

2406 **FTI 276**
Farnesyltransferase (FTase) inhibitor; antitumor
2407 **FTI 277**
Prodrug form of FTI 276 (Cat. No. 2406)

G protein

3090 **Gallein**
Inhibitor of βγ signaling
3594 **GRK2i**
GRK2 inhibitory polypeptide. Gβγ antagonist

RTKs

EGFR

0414 **AG 490**
EGFR-kinase inhibitor. Also JAK2, JAK3 inhibitor
1276 **AG 1478 hydrochloride**
Highly potent EGFR-kinase inhibitor
2416 **BIBX 1382 dihydrochloride**
Highly selective EGFR-kinase inhibitor
3000 **Iressa**
Orally active, selective EGFR inhibitor

VEGFR/PDGFR

1819 **Demethylasterriquinone B1**
Selective insulin RTK activator
1683 **K 252a**
Protein kinase inhibitor
2542 **Ki 8751**
Potent, selective VEGFR-2 inhibitor
2693 **PHA 665752**
Potent and selective MET inhibitor
2956 **Picropodophyllotoxin**
Selective IGF1R inhibitor
3304 **SU 16f**
Potent and selective PDGFRβ inhibitor
1459 **SU 4312**
Potent inhibitor of VEGFR tyrosine kinase
3300 **SU 5402**
Potent FGFR and VEGFR inhibitor
3037 **SU 5416**
VEGFR inhibitor. Also inhibits KIT, RET, MET and FLT
3768 **Sunitinib malate**
Potent VEGFR, PDGFRβ and KIT inhibitor
2475 **ZM 323881 hydrochloride**
Potent, selective inhibitor of VEGFR-2

Regulators

14.3.3

2145 **Difopein**
High affinity inhibitor of 14.3.3 proteins; induces apoptosis

2144 **R18**
Inhibitor of 14.3.3 proteins; induces apoptosis

Src

1629 **Herbimycin A**
Src family kinase inhibitor. Also Hsp90 inhibitor

1397 **PP 1**
Potent, selective Src inhibitor

1407 **PP 2**
Potent, selective Src inhibitor

3063 **1-Naphthyl PP1**
Src family kinase inhibitor; also inhibits c-Abl

3642 **Src I1**
Dual site Src kinase inhibitor

Hsp90

1515 **17-AAG**
Selective Hsp90 inhibitor

2435 **CCT 018159**
Hsp90 inhibitor

1368 **Geldanamycin**
Selective Hsp90 inhibitor

1629 **Herbimycin A**
Hsp90 inhibitor. Also Src family kinase inhibitor

1589 **Radicicol**
Hsp90 inhibitor. Antifungal antibiotic

SGK

3572 **GSK 650394**
Serum- and glucocorticoid-regulated kinase (SGK) inhibitor

PAK1

3622 **IPA 3**
Group I p21-activated kinase (PAK) inhibitor

Akt

2151 **API-2**
Selective inhibitor of Akt/PKB signaling. Antitumor and antiviral

2558 **10-DEBC hydrochloride**
Selective Akt/PKB inhibitor

2926 **FPA 124**
Akt/PKB inhibitor

PKC

1626 **Calphostin C**
Potent, selective and photo-dependent PKC inhibitor

1330 **Chelerythrine chloride**
Potent protein kinase C inhibitor

0741 **GF 109203X**
Protein kinase C inhibitor

2253 **Go 6976**
Potent protein kinase C inhibitor; selective for α and β isozymes

2285 **Go 6983**
Broad spectrum PKC inhibitor

1201 **Phorbol 12-myristate 13-acetate**
Protein kinase C activator

1587 **Ro 32-0432 hydrochloride**
Potent, orally active PKC inhibitor

2549 **ZIP**
Cell-permeable inhibitor of atypical PKC isozyme PKMζ

PP2A

1336 **Calyculin A**
Protein phosphatase 1 and 2A inhibitor

1840 **Fostriecin sodium salt**
Potent PP2A and PP4 inhibitor

1136 **Okadaic acid**
Protein phosphatase 1 and 2A inhibitor

Downstream targets and effectors

1290 **Anisomycin**
Activates JNK/SAPK/p38 MAP kinase

2731 **CGP 57380**
Selective inhibitor of Mnk1

1989 **c-JUN peptide**
JNK/c-Jun interaction inhibitor

2358 **Anti-c-Jun (Clone CJ 4C4/1)**
Antibody recognizing c-Jun

3140 **PHA 767491 hydrochloride**
MK-2 inhibitor. Also inhibits cdc7/cdk9

2250 **SL 0101-1**
Selective p90 ribosomal S6 kinase (RSK) inhibitor

2476 **SR 11302**
Inhibitor of AP-1 transcription factor; antitumor agent

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