MAP Kinase Pathways: Functions and Modulation

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Background

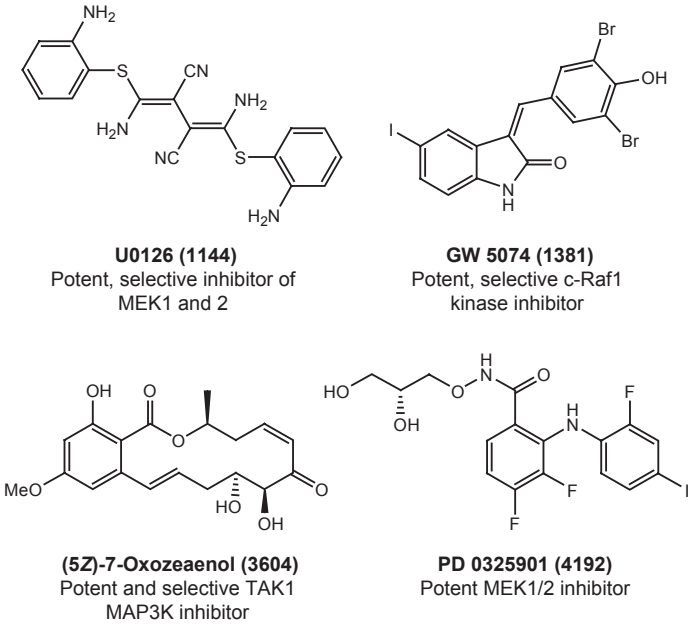
Mitogen-activated protein kinase (MAPK) networks are critical for the transmission of extracellular signals into appropriate intracellular responses. Prototypical MAPK activation employs a three-kinase core module consisting of a MAPK kinase kinase (MAPKKK or MAP3K) that phosphorylates and activates a MAPK kinase (MAP2K, MEK, or MKK) that in turn phosphorylates and dramatically increases the activity of one or more MAPKs (Figure 2). Once activated, MAPKs can phosphorylate a wide array of intracellular targets that include cytoskeletal elements, membrane transporters, nuclear pore proteins, transcription factors, as well as other protein kinases.

Discovery of MAPKs

The MAPKs extracellular signal regulated protein kinases 1 and 2 (ERK1/2) were initially identified as mitogen-stimulated ~42kDa phosphoproteins in the late 1980s.[1,2] Early studies demonstrated preparations of ERK1/2 retained the ability to phosphorylate the model substrates microtubule-associated protein 2 (MAP2) and myelin basic protein (MBP), and reactivate phosphatase-treated ribosomal protein S6 kinase (RSK or p90).[3-6]

In following years, the MAPK family was discovered to include three c-Jun N-terminal kinases (JNKs), four p38 isoforms, ERK3 isoforms, as well as ERK5 and ERK7 (Figure 2). The first JNK family members were independently identified as cycloheximide-activated MBP kinases and purified for their ability to interact with the N-terminus of the transcription factor c-Jun.[7,8] p38α was determined to be an inflammatory cytokine-stimulated tyrosine phosphoprotein, a target of an inhibitor of tumor necrosis factor α (TNFα) production, and a reactivating kinase for MAPK-activated protein kinase 2 (MAPKAP2 or MK2).[9-11] PCR-based cloning strategies and a yeast two-hybrid screen identified additional JNK and p38 isoforms, as well as ERKs 5 and 7 (reviewed by Chen *et al*, Sabapathy, Cuadrado & Nebreda, Nithianandarajah-Jones *et al*, and Drew *et al*; a summary of the cellular processes involving these MAPKs is shown in Figure 2, and detailed reviews by Raman *et al*, Dhillon *et al*, and Kyriakis & Avruch are also recommended for further reading).[12-19] Notably, ERK5 has become a recent target of therapeutic interest[20] because of its roles in cancer and structural differences from ERK1 and 2.[21,22] Although similar stimuli affect ERK1/2 and ERK5, particularly growth factors, activation of ERK5 is dependent on different

Figure 1 | MAP3K and MAP2K inhibitors



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

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

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

PD 0325901 (4192)
Potent MEK1/2 inhibitor

upstream factors.[21] Indeed, regulated cross-talk and insulation are both hallmarks of most related MAPK pathways.

Collectively, these findings established that prototypical three-kinase cascades culminating in activation of a MAPK are versatile signaling modules capable of integrating a multitude of inputs into a variety of responses.

Upstream regulation of ERK1/2

The observation that Ras and Raf mutations are prevalent in a variety of human tumors served as early evidence for ERK1/2 having roles in proliferation and oncogenic growth.[23] Work from multiple laboratories has led to the understanding that Raf isoforms are effectors of Ras small GTP binding proteins and are upstream kinases for MAPK/ERK kinase 1 and 2 (MEK1/2), which in turn activate ERK1/2 via dual phosphorylation.[24] Consistent with these early reports, the capacity to drive tumorigenesis was further demonstrated by the ability of an activated mutant of MEK1 to transform cells and promote tumor growth in nude mice.[25] Subsequent work employing dominant interfering mutants, pharmacological inhibitors of MEK1/2, and disruptions in component gene expression all revealed ERK1/2 to be intimately involved in normal processes including embryogenesis, cell differentiation, glucose sensing, and synaptic plasticity.[26-31]

MAP2Ks

MAP2Ks are dual-specificity kinases capable of phosphorylating both tyrosine and serine/threonine residues, and serve as points of signal integration, largely through docking site-mediated protein-protein interactions and the activity of scaffolding proteins. In contrast to MAPKs, which have a wide range of substrates, MAP2Ks are highly specific; they are primarily (but not exclusively) dedicated to phosphorylation of a small number of MAPK proteins.[25]

The quintessential MAP2K protein, MEK1, was initially purified as a ~45 kDa activator of ERK1/2, and DNA-based molecular techniques led to the subsequent identification of MEKs 2-7 (reviewed by Chen *et al* and Lewis *et al*).[12,32-35] While pharmacological inhibition of MEK1/2 is sufficient to effectively shut down ERK1/2 signaling, a recent paper has revealed that MEK1/2 have an additional substrate: the proteotoxic stress response master regulator heat shock factor protein (HSF1).[36] In addition to uncovering a connection between MEK1/2 and regulation of proteomic stability, this study also identified opposing roles of ERK1/2 and MEK1/2 within the proteotoxic stress response, raising the possibility that ERK1/2 and MEK1/2 exert divergent influences on other cellular processes. RNA-sequencing experiments in mouse embryonic stem cells under conditions of ERK1 depletion or pharmacological inhibition of MEK1/2 provide some support for the notion that MEK has both ERK-dependent and independent functions, although these experiments are complicated by the continued presence of ERK2.[37] Nevertheless, it remains to be seen whether other MAP2Ks also have targets outside their respective MAPKs and, further, how alternative substrates contribute to MAPK signaling regulation.

Raf isoforms and other MAP3Ks

The three-kinase cascade, a module conserved from yeast to humans and comprising a MAP3K, MAP2K and a MAPK, is the most readily identifiable feature of MAPK signaling. One of the best-characterized mammalian MAP3Ks is Raf, originally identified as a retroviral oncogene known for its role in cancer promotion. Three isoforms, c-Raf (or Raf-1), B-Raf, and A-Raf, have been identified in mammals.[38] In addition to the core ~30 kDa kinase domain, Raf proteins contain an N-terminal regulatory region of roughly the same size that can directly bind Ras. Raf proteins specifically phosphorylate the MAP2Ks MEK1/2 and were initially thought to function in a tissue-specific manner. Subsequent studies, aided by the development of B-Raf inhibitors, led to the understanding that Raf isoforms can dimerize to modulate signal transmission.[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 display different behaviors.[41-43] More recent work suggests that B-Raf dimerization is subject to feedback control by ERK1/2, potentially explaining why Raf mutants unable to dimerize maintain inappropriately elevated levels of ERK1/2 signaling.[44, 45]

Although no direct counterpart to Raf has been found in yeast, the highly conserved nature of MAPK signaling led researchers to look for homologs of the yeast MAP3K Ste11 in mammals. MEKK1 was the first mammalian Ste11 homolog discovered, and despite being able to phosphorylate multiple MAP2Ks *in vitro*, numerous cell-based studies indicate MEKK1 predominantly coordinates downstream signaling through MEKs

4 and 7 and the JNK pathway.[46-51] Subsequent isolation of related cDNAs have led to the discovery of a family of enzymes (i.e. MEKKs 1-4; ASK1/2; TAK1) that are reviewed by Raman *et al* and Johnson *et al*.[18,52] As a group, these enzymes display broader substrate specificity than Raf and presumably regulate multiple MAPK pathways in a context-dependent manner.

Two more enzymes that function as MAP3Ks in the ERK1/2 pathway are Mos and Tpl2 (Cot), both originally identified as proteins able to transform cells, and both found to function in specialized situations to activate the core cascade [53,54]. Mos is expressed primarily in oocytes where translational recruitment of c-*mos* mRNA and subsequent MAPK pathway activation is critical for pronuclear formation, while Tpl2 is stabilized in response to lipopolysaccharide.[55,56] Another MAP3K family contains homologs of the yeast protein Ste20, TAOs 1-3, which serve as activators of the p38 signaling cascade.[18]

Figure 2 presents a simplified model of the organization of MAPK cascades with attention drawn to the number of MAP2K-MAPK combinations that a given MAP3K can regulate, and highlights putative nodes of cross-talk.

Properties of MAPK cascades

A large body of work on yeast MAPK cascades and the mammalian ERK1/2, p38, and JNK signaling pathways has provided insight into the primary features of MAPK core modules, most notably their versatility in processing a wide range of stimuli into a variety of responses. Efforts to understand how three-kinase cascades achieve such robust signaling have revealed that feedback mechanisms at multiple levels underlie the evolutionary success of these pathways. Moreover, multiple biochemical mechanisms involving pathway effectors, scaffolds, and targets serve to regulate MAPK signaling dynamics such that one core module can effectively regulate intracellular responses in a context-dependent manner. (For a detailed review on how signaling dynamics can shape cellular responses, refer to Purvis & Lahav.[57])

One major feature of ERK1/2 signaling is that two phosphorylation events on the activation loop are required for full activation, the first on a tyrosine residue and a second on a proximal threonine residue. In conjunction with scaffolding proteins that tether the three-kinase cascade together, and owing to its dual-specificity, MEK1/2 can behave either as a single switch via rapid double phosphorylation or can generate a pool of monophosphorylated ERK1/2, thereby increasing the number of contexts that can ultimately achieve full pathway activation.[4,58-61] The role of monophosphorylation of MAPKs may not be limited to potentiating a switch-like response to a graded stimulus, as work by Ashwell and colleagues has demonstrated with p38 in T-cells.[62] In this system, p38 phosphorylated on the threonine activation site, but not the tyrosine, displays altered substrate specificity, although it is important to note that this mode of activation is specific to T-cells and involves a non-canonical mechanism of p38 activation.

Figure 2 | MAP kinase networks

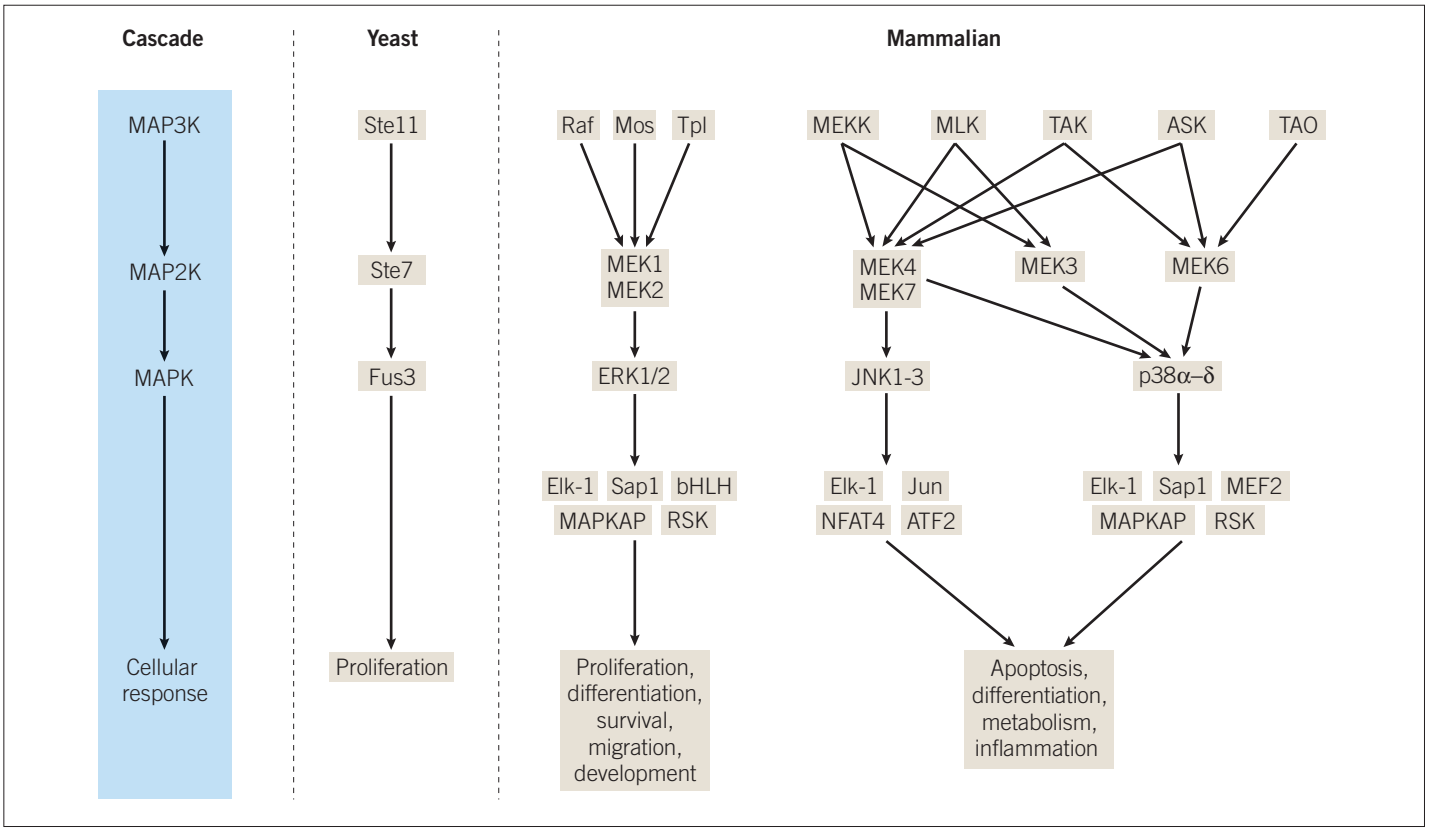


Table 1 | MAPK pathway components

	Gene	Aliases
MAPK		
ERK2	MAPK1	p42, p41
ERK1	MAPK3	p44, p43
ERK3α	MAPK6	rERK3, p97, p63
ERK3β	MAPK4	hERK3, ERK3-related, ERK4
JNK1	MAPK8	SAPK1, SAPKγ
JNK2	MAPK9	SAPKα, p54
JNK3	MAPK10	SAPKβ
p38α	MAPK14	CSBP, Mxi2 (alternative splice form)
p38β	MAPK11	SAPK2, p38-2
p38γ	MAPK12	ERK6, SAPK3
p38δ	MAPK13	SAPK4
ERK5	MAPK7	BMK1
ERK7	MAPK15	ERK8
MAP2K		
Mek1	MAP2K1	Mkk1, MAPKK1
Mek2	MAP2K2	Mkk2
Mek3	MAP2K3	Mkk3
Mek4	MAP2K4	Mkk4, Sek1
Mek5	MAP2K5	
Mek6	MAP2K6	
Mek7	MAP2K7	
MAP3K		
C-Raf	RAF1	
B-Raf	BRAF	
A-Raf	ARAF	
Mekk1	MAP3K1	MAPKKK1
Mekk2	MAP3K2	
Mekk3	MAP3K3	
Mekk4	MAP3K4	Mtk1
Mekk5	MAP3K5	Ask1
Mekk6	MAP3K6	Ask2
Tak1	MAP3K7	
Tpl-2	MAP3K8	Mekk8, Cot, Estf
Mlk1	MAP3K9	Mekk9,
Mlk2	MAP3K10	Mekk10, Mst
Mlk3	MAP3K11	Sprk, Ptk1
Zpk	MAP3K12	
Lzk	MAP3K13	Mekk13
NIK	MAP3K14	
Ask3	MAP3K15	Mekk5-like
TAO1	MAP3K16	TAOK1, PSK2, MARKK
TAO2	MAP3K17	TAOK2, PSK
TAO3	MAP3K18	TAOK3, DPK, JIK
MLK7	ZAK	AZK, MRK, MLTK

In addition to monophosphorylation, another emerging MAPK property is alternative phosphorylation of a threonine residue proximal to the canonical activation sites described above. In 2009 it was discovered that activated ERK2 can autophosphorylate upon dimerization resulting in the promotion of a distinct gene expression program in a model of cardiac hypertrophy. Subsequent biochemical characterization of a phosphomimetic mutant of the alternative phosphorylation site suggested divergent binding properties of ERK2 modified in that manner.[63,64] Although it is far from clear how different modes of phosphorylation contribute to pathway feedback regulation, these activities likely play some part in conferring network robustness.

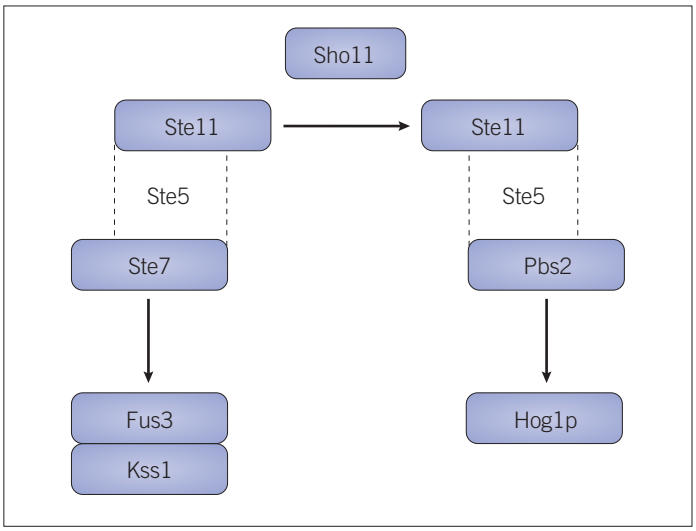
Free ERK1/2 can enter and exit the nucleus even in the absence of pathway activation because of its interactions with nuclear pore proteins.[65,66] However, full activation of ERK1/2 can promote dimerization and nuclear localization.[67] Recently, a small molecule, DEL 22379, able to interrupt dimer formation even when the pathway is activated has been identified, and interestingly, inhibition of dimerization seems to alter cytoplasmic ERK1/2 signaling predominantly by disrupting interactions between ERK dimers and scaffold proteins.[68,69] More work is required to delineate how this drug affects the extensive nuclear activities of ERK1/2, especially given the potential role of dimerization in driving specific gene expression programs in an ERK1/2-dependent manner.[63]

Once doubly phosphorylated, ERK1/2 have multiple substrates within the core-signaling pathway. As previously mentioned, ERK1/2 can phosphorylate Raf isoforms to inhibit reactivation by Ras and subsequent phosphorylation of MEK1/2, simultaneously buffering against inappropriate pathway activation and ensuring a linear response to stimuli.[70-75] In yeast it has been demonstrated that the p38 homolog Hog1p phosphorylates the signaling scaffold Ste20 (homologous to mammalian PAK) progressively over time, and the authors of a recent study implicated Ste20 in both negative and positive feedback loops because of its ability to act as a rheostatic effector of the MAPK pathway.[76]

Scaffolding proteins

Although the importance of scaffold proteins has been appreciated for some time, recent advances in computer-aided modeling, in combination with biochemical work, is shedding light on how these proteins exert major influence on cascade function and strongly impact the activities and outputs of MAPK pathways.[77] One of the best examples of scaffold-mediated signal specificity is the yeast protein Ste5 in concert with the MAP3K Ste11, which is required to activate the downstream MAPKs Kss1 and Fus3 via Ste7 in response to pheromone.[78-81] However, in response to osmotic stress, the sensor protein Sho11 enables Pbs2, the MAP2K upstream of Hog1p, to form a stable interaction with Ste5 and Ste11, thus funneling distinct stimuli through the same signaling components (Figure 3). More recently, a synthetic approach was taken to "rewire" Ste5 with highly modular PDZ domains, in order to better understand the effects of scaffolds on pathway dynamics.[82] Consistent with previous observations, the authors of this study found that scaffolds can act as logic gates by tethering core components together, as well as modulate pathway output through the recruitment of protein phosphatases.[83,84]

Figure 3 | Regulation by Scaffolding



Mammalian signaling has similar complexities due to extensive crosstalk between upstream activators, and there have been many observations that a single MAP3K can regulate multiple MAPK cascades. However, because kinases themselves can act as binding hubs, tethering functions in mammalian MAPK cascades can be shared by both core pathway components as well as dedicated scaffolding proteins. Most interactions with MAPKs are mediated by two short sequence motifs: the docking (D or DEJL) motif and the FXF (DEF) motif, with one or more of these often present in scaffolds, activators, certain phosphatases, and many substrates.[85,86]

JNK-interacting protein (JIP) scaffolds, which organize JNK and sometimes p38 MAPK pathways, display some functional parallels to yeast Ste5. In a slight departure from the yeast situation, several MAP3Ks contain docking sites that promote stable interactions with specific MAPKs, such as the case with MEKK1 being able to bind tightly to JNKs through a docking motif.[87] Indeed, the scaffolding functions of kinases cannot be overstated, especially since some dedicated scaffolds are pseudokinases whose tethering functions were likely selected over enzymatic ones during the course of evolution.[83] The best-known example of this is the scaffold kinase suppressor of Ras (KSR), which was discovered in the sevenless eye development pathway in Drosophila and in the vulval induction pathway of *C. elegans*.[88,89] Although KSR proteins are members of the Raf family, they lack the essential ATP-binding lysine residue required for functional kinase activity. In mammals KSR1 and KSR2 bind ERK1/2 and MEK1/2, and can allosterically activate Raf.[43] Other proteins that influence assembly and activation of MAPK pathways include Sur8, CNK, MP-1 and IMP.[86,90-92]

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 multiple cell surface receptors, and of these receptor tyrosine kinase (RTK) pathways are among the best delineated.[14,18,34] Extracellular ligand binding triggers homo- and/or heterodimerization of RTKs, increasing kinase activity and generating multiple phosphotyrosine motifs on RTKs and their dimerization partners. These motifs are recognized by SH2 domains on a variety of factors including the adaptor proteins Shc and Grb2, which also contain proline-rich regions that can interact with the SH3 domain of the Ras guanine nucleotide exchange factor (GEF) son of sevenless (SOS). Upon association with the receptor-adaptor protein complex, SOS stimulates the exchange of GDP for GTP on Ras, promoting its interactions with a number of downstream effectors that include Raf. The direct interaction of Raf isoforms with Ras localizes these MAP3Ks to the plasma membrane and maintains proximity to non-receptor kinases, such as Src family members, p21-activated kinases (PAKs), and protein kinase C (PKC) isoforms, which in turn are able to phosphorylate Ras-Raf isoforms, altering their activity towards particular substrates or enhancing interactions with other signaling factors.[93,94] As discussed above, activated Raf isoforms can stimulate the MEK1/2-ERK1/2 pathway, thus forming the core MAPK module.

Activation of MAPKs by G-protein-coupled receptors

Many hormones act through G-protein-coupled receptors (GPCRs) to increase ERK1/2 activity, and as with RTK mediated stimulation, activation occurs primarily through Raf. Unlike RTK signaling to ERK1/2, however, GPCRs largely circumvent Ras and instead utilize a variety of mechanisms to regulate Raf activity.

Figure 4 | JNK and ERK inhibitors

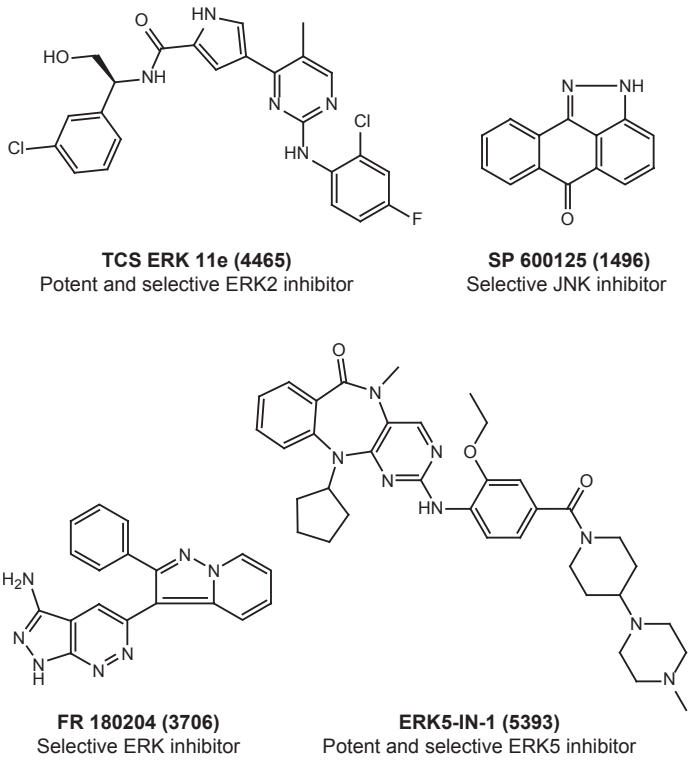
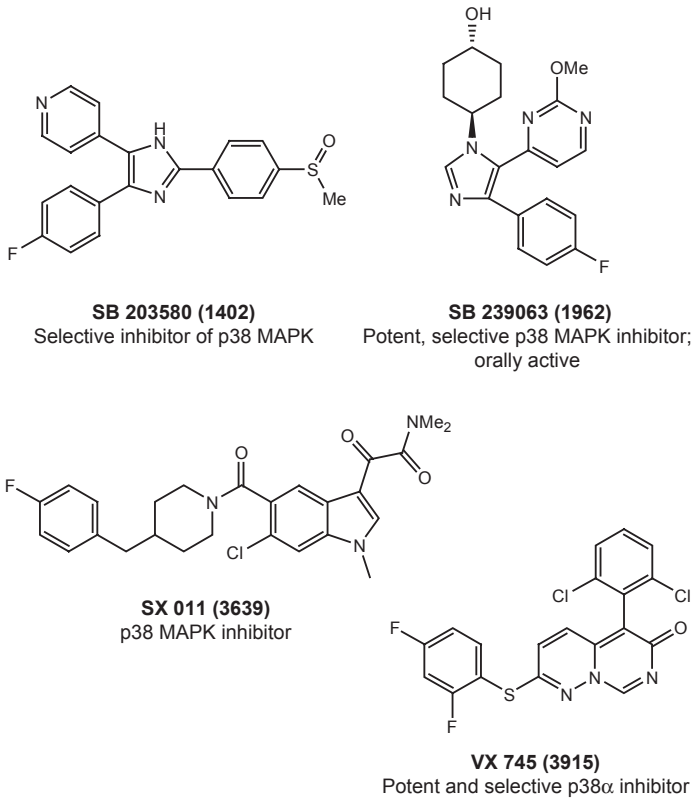


Figure 5 | p38 MAPK inhibitors



Agonist binding to $G_{\alpha s}$-coupled receptors results in activation of adenyl cyclase, which in turn raises the concentration of cyclic adenosine monophosphate (cAMP). While changes in cAMP concentration are known to affect ERK1/2 activity differentially, the pathway response appears cell-type specific or otherwise highly context-dependent.[95,96] An increase in cAMP stimulates protein kinase A (PKA), a signaling molecule whose activities employ extensive cross-talk with ERK1/2 signaling.[97] Inhibition of ERK1/2 activity is thought to involve phosphorylation of serine 41 and serine 621 on c-Raf by PKA, disturbing Raf associations with Ras and 14-3-3 protein, respectively.[98,99] Conversely, PKA can also augment ERK1/2 sensitivity, amplitude, and duration.[97]

$G_{\alpha i}$-coupled receptors can also regulate ERK1/2 signaling. Early *in vivo* evidence indicated that $G_{\alpha i}$-coupled receptors most likely employ βγ subunits [100,101], and indeed, recent work on GPCR regulation of ERK1/2 has identified a novel mode of signaling that results in an alternative phosphorylation event that plays a causative role in cardiac hypertrophy.[63]

Inhibition of MAPK pathways

MAPK function studied by inhibition

Much has been learned about MAPK signaling pathways through loss-of-function experiments utilizing dominant-negative mutants, RNA interference, and small molecule inhibitors. However, because MAPKs are activated by overlapping upstream components and often share common substrates, the dominant negative approach can become confused owing to inhibition of more than one target pathway. Similarly, gene-silencing approaches rely heavily on rescue experiments to determine whether a phenotype is pathway-dependent or due to an off-target effect and may require targeting multiple closely related enzymes. In view of these considerations, pharmacological inhibitors of MAPK pathway components present both a viable alternative and a complementary tool in understanding the functional requirements of a given pathway.[102,103] Although many inhibitors bind to the ATP binding pocket common to all protein kinases, the success of more specific allosteric inhibitors has spurred the search for molecules that bind to alternative pockets on kinase surfaces.[104] MAPK pathway inhibitors are being developed that block essential protein-protein interactions, reflecting an improved understanding of how kinase signaling networks function.

ERK1/2 pathway inhibitors

Owing to the high degree of feedback control exhibited by the Raf-MEK-ERK pathway, direct inhibition of ERK1/2 is an attractive strategy to be used in conjunction with other inhibitors of upstream factors. The small molecule FR 180204 competes for the ATP-binding pocket of ERK1/2, with IC_{50} values of 0.31 and 0.14 µM, respectively, but its practical use is difficult to ascertain due to its targeting strategy.[105] In contrast, the allosteric inhibitor SCH772984 appears to both exquisitely inhibit the activity of ERK1/2 (with IC_{50} values of 0.004 and 0.001 µM, respectively) and prevent reactivation by MEK1/2, possibly by inducing a conformational change rendering ERK1/2 refractory to feedback-regulated stimulation.[106] Somewhat related to this is the inhibitor DEL-22379, which targets and interrupts ERK dimerization instead of its catalytic activity.[69]

In the mid 1990s, the MEK1/2 inhibitors PD 98059 and U0126 became available, allowing researchers to effectively interfere with the ERK1/2 pathway.[107] PD 98059 was discovered through an *in vitro* kinase activation assay, while U0126 was identified in a cell-based assay as an inhibitor of AP-1 transcriptional activity.[104,108,109] 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.[103] U0126 and PD 98059 bind outside of the ATP binding site of MEK1/2 and select for a low activity conformation, thereby shifting and maintaining the protein in an inactive state. Because of their binding mode, these drugs are among the most selective inhibitors available, with the only other kinase affected being the related MAP2K MEK5, which is inhibited at only slightly higher concentrations than MEK1/2. Subsequently, a number of MEK1/2 inhibitors have been developed that are much more potent and display variable selectivity toward MEK5, including PD 198306, PD 0325901, and ARRY-142886 (AZD6244).[110-113] PD 0325901 inhibits MEK1/2 activation of ERK1/2 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.[103] However, consistent with a ~10-fold greater potency towards MEK1/2 than MEK5, ERK5

activation is inhibited at low micromolar concentrations by PD 035901 (for more on ERK5 inhibition, see Chen *et al*).[114] The success of allosteric inhibitors of MEK1/2 has extended past the laboratory with several MEK1/2 targeting compounds currently in development as therapeutics.[110,113,115-119]

Another expanding group of compounds able to block the ERK1/2 pathway is directed against Raf isoforms. B-Raf in particular has emerged as an interesting target due to the high prevalence of B-Raf V600E mutations in melanomas. Several compounds have been identified that predominantly interact with this mutant form, including vemurafenib, encorafenib, PLX-4720, dabrafenib, and GDC-0879.[120-124] Other drugs that target Raf are less selective, with compounds like Sorafenib, RO5126766, and AZ 628 inhibiting multiple kinases.[125-127] Similarly, LY3009120, RAF265 (CHIR-265), TAK-632, ZM 336372, SB 590885 and GW5074 largely affect multiple isoforms of Raf.[118,128-133]

JNK pathway inhibitors

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, however, this compound also inhibits several other protein kinases with roughly equal potency.[134] CEP 1347 (KT-7515) blocks the JNK pathway, but is specifically an inhibitor of the upstream mixed lineage kinases (MLKs), so does not block JNKs that are regulated in an MLK-independent manner.[135]

p38 pathway inhibitors

Several p38 inhibitors including SB 203580 have been developed using pyridinyl imidazoles as lead compounds.[136,137] Crystallographic studies indicate that SB 203580 prevents ATP binding by interacting with the active site of p38, but only for the α and β isoforms and not p38 γ and δ.[138] A key feature of the ATP binding pocket that impacts inhibitor selectivity is the gatekeeper residue, which is a threonine residue at position 106 for p38 α and β.[139] The smaller size of the gatekeeper (as opposed to the glutamine and methionine side chains present in p38 γ and δ) allows for accommodation of larger compounds in the active site.[140] Consequently, Raf isoforms, due to their small gatekeeper side chains, can also interact with some p38 inhibitors.[141] BIRB 796, a diaryl urea compound that is structurally unrelated to SB 203580, inhibits all four p38 isoforms by indirectly competing with the binding of ATP.[142] There are several p38 pathway inhibitors being developed as therapeutics, including ralimetinib (LY 2228820), pexmetinib (ARRY 614) and losmapimod.[143-149]

Future prospects

The last several years have seen an increased awareness that protein kinases are more than phosphorylating enzymes; they are multifunctional molecules evolved to process critical biological information. Given the prominent roles of MAPK pathways in both normal signal transmission and a variety of disease states, examination of these core modules will continue to inform the general understanding of cellular signaling. MAPK networks rely on multiple kinases behaving in a multitude of ways, creating feedback loops and redundancies, switches and oscillators, and harnessing the activities of non-kinase proteins to give rise to robust signaling. The evolutionary success of these pathways is rooted in their adaptability and versatility, and while much has been learned about the roles of these pathways in specific biological processes, current research continues to focus on the spatio-temporal dynamics underlying context-dependent signaling. As microscopy, mass spectrometry, and deep sequencing techniques continue to improve, the scale of focus will continue to shift away from individual pathways onto entire signaling networks. In particular, pharmacological manipulation continues to be a crucial tool for assessing the functionality of MAPK signaling, paving the way for improved therapeutic strategies targeting numerous aspects of these multifaceted networks.

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Compounds Available from Tocris

Target	Cat No	Name	Description
MAP3K			
ASK	5641	MSC 2032964A	Potent and selective ASK1 inhibitor; orally bioavailable
	4429	NQDI 1	ASK1 inhibitor
	4825	TC ASK 10	Potent and selective ASK1 inhibitor; orally bioavailable
Raf	4836	AZ 628	Potent Raf inhibitor
	4453	GDC 0879	Potent B-Raf inhibitor
	1381	GW 5074	Potent, selective c-Raf1 inhibitor
	5260	KG 5	PDGFRβ, B-Raf, c-Raf, FLT3 and KIT inhibitor
	5475	Kobe 0065	H-Ras-cRaf1 interaction inhibitor; inhibits Raf signaling
	5036	ML 786	Potent Raf inhibitor; orally bioavailable
	1321	ZM 336372	Potent, selective c-Raf inhibitor
TAK	3604	(5*Z*)-7-Oxozeaenol	Potent and selective TAK1 MAPKKK inhibitor
	5429	NG 25	TGF-β-activated kinase (TAK1) inhibitor
MAP2K (MEK)			
	1777	Arctigenin	Potent MEK1 inhibitor; also inhibits IκBα phosphorylation
	4842	BIX 02189	Selective MEK5 and ERK5 inhibitor
	1532	10*Z*-Hymenialdisine	Pan kinase inhibitor; potently inhibits MEK1
	4192	PD 0325901	Potent inhibitor of MEK1/2
	4237	PD 184352	Selective MEK inhibitor
	2605	PD 198306	Selective inhibitor of MEK1/2
	4824	PD 334581	MEK1 inhibitor
	1213	PD 98059	MEK inhibitor
	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			
ERK	5843	AX 15836	Potent and selective ERK5 inhibitor
	4842	BIX 02189	Selective MEK5 and ERK5 inhibitor
	5774	DEL 22379	ERK dimerization inhibitor
	5393	ERK5-IN-1	Potent and selective ERK5 inhibitor
	3706	FR 180204	Selective ERK inhibitor
	4433	Pluripotin	Dual ERK1/RasGAP inhibitor; maintains ESC self-renewal
	4465	TCS ERK 11e	Potent and selective ERK2 inhibitor
	3675	TMCB	Dual-kinase inhibitor; inhibits CK2 and ERK8
	4132	XMD 8-92	ERK5/BMK1 inhibitor; also BRD4 inhibitor
JNK	2651	AEG 3482	Inhibitor of JNK signaling
	3314	BI 78D3	Selective, competitive JNK inhibitor
	4924	CEP 1347	Inhibitor of JNK signaling
	5575	IQ 1S	JNK inhibitor; anti-inflammatory
	4550	IQ 3	Selective JNK3 inhibitor
	1565	JIP-1 (153-163)	JNK-selective inhibitor peptide
	1496	SP 600125	Selective JNK inhibitor
	5044	SR 3576	Highly potent and selective JNK3 inhibitor
	3607	SU 3327	Selective JNK inhibitor
	2827	TCS JNK 5a	Selective inhibitor of JNK2 and JNK3
	3222	TCS JNK 6o	Selective JNK inhibitor
p38	4753	AL 8697	Potent and selective p38α inhibitor
	3920	AMG 548	Potent and selective p38α inhibitor
	2186	CMPD-1	Non-ATP-competitive p38α inhibitor; also tubulin polymerization inhibitor
	5095	DBM 1285	p38 MAPK inhibitor; anti-inflammatory

Target	Cat No	Name	Description
	2908	EO 1428	Selective inhibitor of p38α and p38β2
	2657	JX 401	Potent, reversible p38α inhibitor
	4586	ML 3403	p38 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
	5040	SB 706504	p38 MAPK inhibitor
	3528	SCIO 469	Selective p38 MAPK inhibitor
	2008	SKF 86002	p38 MAPK inhibitor; anti-inflammatory agent
	3639	SX 011	p38 MAPK inhibitor
	4254	TAK 715	Potent p38 MAPK inhibitor; anti-inflammatory
	3916	VX 702	Orally active p38α and p38β inhibitor
	3915	VX 745	Potent and selective p38α inhibitor
Upstream and Receptor Signaling			
EGFR	5318	AEE 788	Potent EGFR and VEGFR inhibitor
	1276	AG 1478	Highly potent EGFR-kinase inhibitor
	0493	AG 18	EGFR/PDGFR-kinase inhibitor
	0414	AG 490	EGFR-kinase inhibitor; also JAK2, JAK3 inhibitor
	0618	AG 555	Potent EGFR-kinase inhibitor
	0616	AG 556	EGFR-kinase inhibitor
	1555	AG 825	Selective ErbB2 inhibitor
	2617	AG 879	ErbB2 inhibitor; also inhibits TrkA and VEGFR-2
	0497	AG 99	EGFR-kinase inhibitor
	2417	BIBU 1361	Selective inhibitor of EGFR-kinase
	2416	BIBX 1382	Highly selective EGFR-kinase inhibitor
	5022	BMS 599626	Potent, selective EGFR and ErbB2 inhibitor
	3360	CGP 52411	EGFR inhibitor; also inhibits Aβ42 fibril formation
	4502	DIM	Inhibits phosphorylation of EGFR and downstream activation of ERK; also activates Chk2
	1110	Genistein	EGFR kinase inhibitor; also estrogen and PPARγ ligand
	2239	GW 583340	Potent dual EGFR/ErbB2 inhibitor; orally active
	2646	HDS 029	Potent inhibitor of the ErbB receptor family
	3580	HKI 357	Dual irreversible inhibitor of ErbB2 and EGFR
	3000	Iressa	Orally active, selective EGFR inhibitor
	3352	JNJ 28871063	Potent ErbB receptor family inhibitor
	1331	Lavendustin A	EGFR, p60c-src inhibitor
	1037	PD 153035	EGFR kinase inhibitor
	2615	PD 158780	Potent ErbB receptor family inhibitor
	4941	PKI 166	Potent EGFR-kinase inhibitor
	3599	TAK 165	Potent and selective ErbB2 inhibitor
	3115	WHI-P 154	JAK3 kinase inhibitor; also inhibits EGFR
FAK	3414	FAK Inhibitor 14	Selective FAK inhibitor
	4278	PF 431396	Dual FAK/PYK2 inhibitor
	3239	PF 573228	Potent and selective FAK inhibitor
	4498	Y 11	Potent and selective FAK inhibitor
FTI (Ras)	5424	Deltarasin	High affinity PDEδ-KRAS interaction inhibitor
	2407	FTI 277	Inhibits H-Ras and K-Ras processing; prodrug form of FTI 276 (Cat. No. 2406)
	5607	GNF 7	Ras signaling inhibitor; inhibits Ack1 and GCK
	4989	Salirasib	Ras inhibitor; also induces autophagy
	3101	XRP44X	Ras-Net (Elk-3) pathway inhibitor

Target	Cat No	Name	Description
IRAK	5665	IRAK 1/4 inhibitor I	IRAK4 and IRAK1 inhibitor
PI3-K	1983	740 Y-P	Cell-permeable PI 3-kinase activator
	4839	AZD 6482	Potent and selective PI 3-Kβ inhibitor
	4674	CZC 24832	Selective inhibitor of PI 3-kinase γ
	4840	KU 0060648	Dual DNA-PK and PI 3-K inhibitor
	1130	LY 294002	Prototypical PI 3-kinase inhibitor; also inhibits other kinases
	2418	LY 303511	Negative control of LY 294002 (Cat. No. 1130)
	3977	3-Methyladenine	Class III PI 3-kinase inhibitor; also inhibits autophagy
	4820	PF 04691502	Potent and selective dual PI 3-K/mTOR inhibitor
	2930	PI 103 hydrochloride	Inhibitor of PI 3-kinase, mTOR and DNA-PK
	1125	Quercetin	Non-selective PI 3-kinase inhibitor
	1232	Wortmannin	Potent, irreversible inhibitor of PI 3-kinase; also inhibitor of PLK1
VEGFR/PDGFR	5013	AC 710	Potent and selective PDGFR family inhibitor
	4274	AP 24534	Potent VEGFR, PDGFR and FGFR inhibitor; also pan-Bcr-Abl inhibitor
	4350	Axitinib	Potent VEGFR-1, -2 and -3 inhibitor
	4471	DMH4	Selective VEGFR-2 inhibitor
	1222	DMPQ	Potent, selective inhibitor of PDGFRβ
	4931	EG 00229	Neuropilin 1 (NRP1) receptor antagonist; inhibits VEGFA binding to NRP1
	5260	KG 5	PDGFRβ, B-Raf, c-Raf, FLT3 and KIT inhibitor
	2542	Ki 8751	Potent, selective VEGFR-2 inhibitor
	3785	PD 166285	Potent Src inhibitor; also inhibits FGFR1, PDGFRβ and Wee1
	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 FLT3
	3335	SU 6668	PDGFR, VEGFR and FGFR inhibitor
	3768	Sunitinib	Potent VEGFR, PDGFRβ and KIT inhibitor
	3909	Toceranib	Potent PDGFR and VEGFR inhibitor
	5680	Vatalanib	Potent VEGFR inhibitor; also aromatase inhibitor
	5422	XL 184	Potent VEGFR inhibitor; also inhibits other RTKs
	2499	ZM 306416	Inhibitor of VEGF receptor tyrosine kinase
	2475	ZM 323881	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
Akt	3897	API-1	Selective Akt/PKB inhibitor; also antitumor
	2151	API-2	Selective inhibitor of Akt/PKB signaling; also antitumor and antiviral
	2558	10-DEBC	Selective Akt/PKB inhibitor
	2926	FPA 124	Akt/PKB inhibitor
	4144	GSK 690693	Akt kinase inhibitor; also antitumor
	5682	OSU 03012	PDK1 inhibitor; inhibits Akt signaling
	4598	PHT 427	Dual Akt and PDK1 inhibitor; antitumor
	4398	SC 66	Allosteric Akt inhibitor
	4635	SC 79	Akt activator
Hsp90	1515	17-AAG	Selective Hsp90 inhibitor
	4608	BIIB 021	Selective Hsp90 inhibitor
	2435	CCT 018159	Hsp90 inhibitor
	2610	17-DMAG	Water-soluble Hsp90 inhibitor
	4701	EC 144	High affinity, potent and selective Hsp90 inhibitor
	3387	Gedunin	Hsp90 inhibitor; exhibits anticancer and antimalarial activity
	1368	Geldanamycin	Selective Hsp90 inhibitor

Target	Cat No	Name	Description
	1629	Herbimycin A	Hsp90 inhibitor; also Src family kinase inhibitor
	5480	KRIBB11	HSF1 inhibitor
	3061	Macbecin I	Hsp90 inhibitor
	3104	PU H71	Potent Hsp90 inhibitor
	1589	Radicicol	Hsp90 inhibitor; antifungal antibiotic
PAK	5190	FRAX 486	Potent p21-activated kinase (PAK) inhibitor; brain penetrant and orally bioavailable
	3622	IPA 3	Group I p21-activated kinase (PAK) inhibitor
	4212	PIR 3.5	Negative control of IPA 3 (Cat. No. 3622)
PKC	4128	Bisindolylmaleimide II	Potent PKC inhibitor and nicotinic receptor antagonist
	2383	Bryostatin 1	Protein kinase C activator
	5237	Bryostatin 2	Protein kinase C activator
	1626	Calphostin C	Potent, selective and photo-dependent PKC inhibitor
	1330	Chelerythrine	Cell-permeable 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
	4738	LY 333531	Protein kinase C inhibitor; selective for β isozymes
	1193	Melittin	PKC and cAMP-dependent protein kinases inhibitor; also inhibits G_s and stimulates G_i activity
	4054	PEP 005	Protein kinase C activator
	4153	Phorbol 12,13-dibutyrate	Protein kinase C activator
	1201	Phorbol 12-myristate 13-acetate	Protein kinase C activator
	2992	PKC 412	Protein kinase C inhibitor
	1791	PKC ζ pseudosubstrate	PKC ζ inhibitor peptide (attached to cell-permeable vector)
	5739	Prostratin	PKC activator; also NF-κB activator
	1790	Pseudo RACK1	Protein kinase C activator peptide (attached to cell permeable vector)
	1610	Rottlerin	Reported PKCδ inhibitor
	2549	ZIP	Cell-permeable inhibitor of atypical PKC isozyme PKMζ
PP2A	1336	Calyculin A	Protein phosphatase 1 and 2A inhibitor
	1548	Cantharidin	Protein phosphatase 1 and 2A inhibitor
	1840	Fostriecin sodium salt	Potent PP2A and PP4 inhibitor
	1136	Okadaic acid	Protein phosphatase 1 and 2A inhibitor
SGK	3572	GSK 650394	Serum- and glucocorticoid-regulated kinase (SGK) inhibitor
Src	3914	A 419259	Inhibitor of Src family kinases
	3963	AZM 475271	Src tyrosine kinase inhibitor
	4361	Bosutinib	Dual Src-Abl inhibitor; antiproliferative
	4660	KB SRC 4	Potent and selective c-Src inhibitor
	2265	Lyn peptide inhibitor	Inhibits Lyn-dependent activities of IL-5 receptor; cell-permeable
	4582	MLR 1023	Selective allosteric activator of Lyn kinase
	2877	MNS	Selective inhibitor of Src and Syk
	3063	1-Naphthyl PP1	Src family kinase inhibitor; also inhibits c-Abl
	3785	PD 166285	Potent Src inhibitor; also inhibits FGFR1, PDGFRβ and Wee1
	1554	Piceatannol	$p56^{lck}$ and Syk kinase inhibitor; inhibits TNF-induced NF-κB activation
	1397	PP 1	Potent, selective Src family kinase inhibitor
	1407	PP 2	Potent, selective Src family kinase inhibitor
	2794	PP 3	Negative control for PP 2 (Cat. No. 1407)
	4763	Pyridostatin	Targets the proto-oncogene SRC; stabilizes G-quadruplexes
	3642	Src I1	Dual site Src kinase inhibitor
	3567	TC-S 7003	Potent Lck inhibitor
	5413	WH-4-023	Potent and selective Lck and Src inhibitor; also inhibits SIK

Target	Cat No	Name	Description
Downstream Effectors/Targets			
AP-1	5309	SP 100030	NF-κB and AP-1 dual inhibitor
	2476	SR 11302	AP-1 inhibitor; antitumor agent
JNK/c-Jun	1290	Anisomycin	JNK, SAPK and p38 activator
	1989	c-JUN peptide	Peptide inhibitor of JNK/c-Jun interaction
MK2	4279	PF 3644022	Potent MK2 inhibitor
	3140	PHA 767491	MK2 inhibitor; also inhibits cdc7/cdk9
Mnk	4500	Cercosporamide	Potent Mnk2 inhibitor
	2731	CGP 57380	Selective inhibitor of Mnk1
	5183	ETP 45835	Mnk1 and Mnk2 inhibitor
MSK1	4630	SB 747651A	Potent MSK1 inhibitor; also inhibits other AGC group kinases
NFAT	3930	NFAT Inhibitor	Inhibitor of calcineurin-mediated NFAT activation
	5710	NFAT inhibitor, Cell Permeable	Cell permeable NFAT inhibitor
RSK	4037	BRD 7389	Ribosomal S6 kinase inhibitor
	3603	Kaempferol	RSK2 inhibitor; also downregulates PLK1 expression and activates mitochondrial Ca^{2+} uniporter
	4032	PF 4708671	S6K1 inhibitor
	1292	Rapamycin	Inhibits p70 S6K activation; mTOR inhibitor
	2250	SL 0101-1	Selective ribosomal S6 kinase (RSK) inhibitor

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- Angiogenesis
- Epigenetics in Cancer
- Cell Cycle and DNA Damage Repair
- Invasion and Metastasis

Immunology Product Listing
A collection of over 190 products for immunology research, the guide includes research tools for the study of:

- Chemokine and Cytokine Signaling
- Chemotaxis
- Complement System
- Immune Cell Signaling
- Inflammation

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You may also be interested in:

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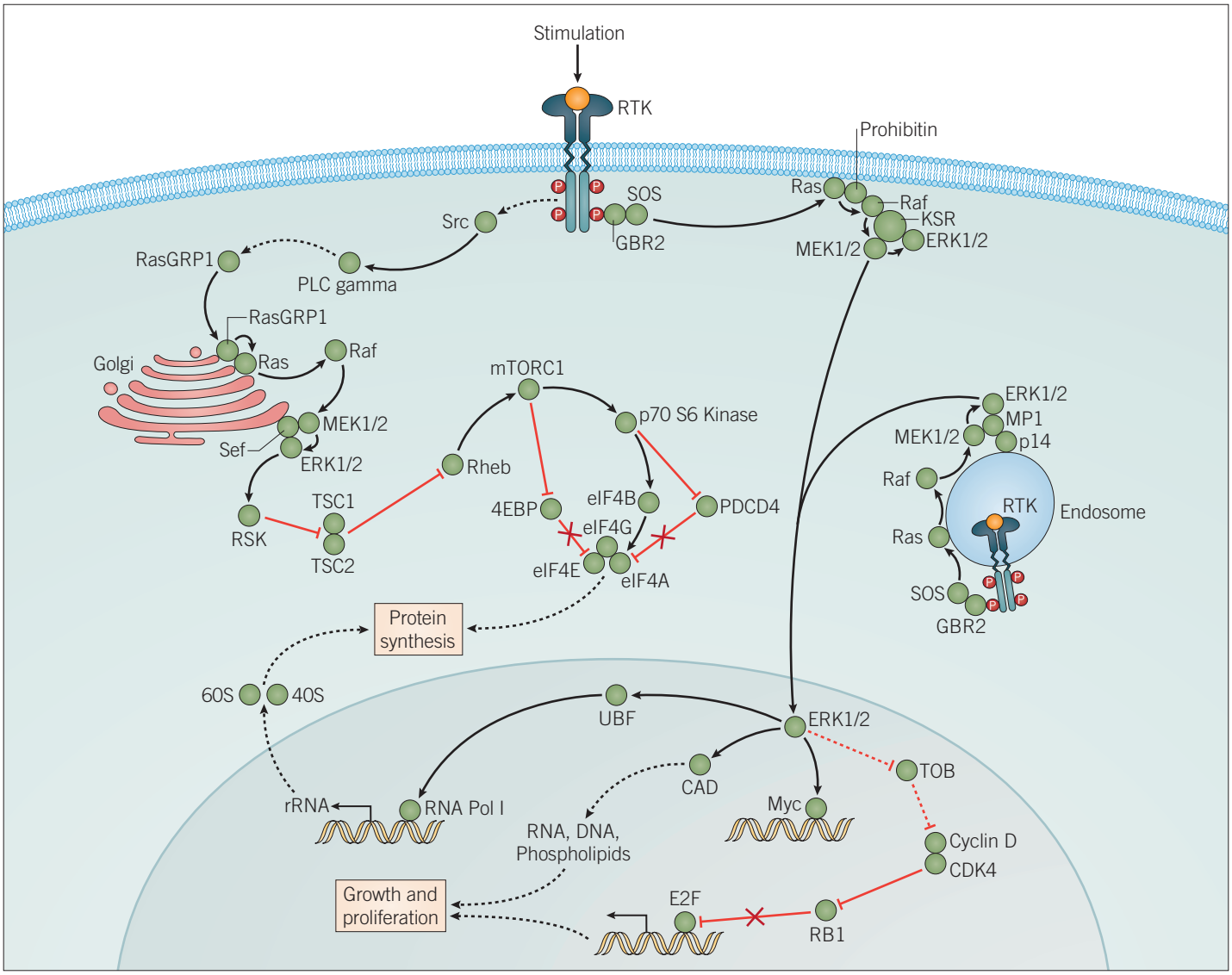
MAPK Signaling Pathway: Mitogen Stimulation Pathway



Molecule	Catalog Number	Species	Clonality	Antibody Applications
4EBP1	AF3227	Human/Mouse	Polyclonal Antibody	SW, WB
c-Myc	MAB3696	Human	Monoclonal Antibody	FC, ICC/IF, IHC, IP, WB
eIF4E	MAB3228	Human/Mouse/Rat	Monoclonal Antibody	ICC/IF, WB
ERK1/ERK2 (T202/Y204, T185/Y187)	MAB1018	Human/Mouse/Rat	Monoclonal Antibody	ICC/IF, FC, SW, WB
p70 S6 Kinase	AF8962	Human/Mouse/Rat	Polyclonal Antibody	IHC, FC, SW, WB
p70 S6 Kinase (T389)	MAB8963	Human	Recombinant Monoclonal Antibody	ICC/IF, WB
PLC-gamma 1 (Y783)	MAB74541	Human/Mouse/Rat	Recombinant Monoclonal Antibody	WB
PLC-gamma 2	MAB3716	Human/Mouse	Monoclonal Antibody	ICC/IF, WB
PLC-gamma 2(Y759)	MAB7377	Human	Monoclonal Antibody	ICC/IF, WB
Rheb	MAB3426	Human/Mouse/Rat	Monoclonal Antibody	IHC, WB
Ribosomal Protein S6/RPS6 (S235/S236)	AF3918	Human/Mouse/Rat	Polyclonal Antibody	IHC, SW, WB
RSK (Pan)	MAB2056	Human/Mouse/Rat	Monoclonal Antibody	SW, WB
RSK1/RSK2 (S221, S227)	MAB892	Human/Mouse	Recombinant Monoclonal Antibody	SW, WB
Src (Y416)	MAB2685	Human	Recombinant Monoclonal Antibody	ICC/IF, SW, WB
TOR	AF15371	Human/Mouse/Rat	Polyclonal Antibody	IP, WB

Application Key: FC FC, ICC/IF ICC/IF/Immunofluorescence, IHC IHC, IP IP, SW Simple Western, WB Western Blot

View Interactive Pathway: rndsystems.com/pathways_mapkmitogen

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