Celltransmissions

Newsletter for Cell Signaling and Neuroscience Research

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### Novel Research Tools from Sigma-RBI and GlaxoSmithKline

Sigma-RBI has signed an agreement with GlaxoSmithKline (GSK) to provide novel organic compounds for cell signaling and neuroscience research. These products, originally developed and patented by GSK, are being made available to life scientists in the pharmaceutical, biotechnology and academic sectors through a license agreement with Sigma-RBI.

This agreement underlines Sigma-RBI's commitment to providing life science researchers with a constant stream of innovative research tools. These products will prove invaluable to researchers worldwide investigating the biological mechanisms underlying cancer, pain, inflammation, neurodegeneration, psychiatric disorders and cardiovascular disease.

### GSK Compounds available from Sigma-RBI

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<td>AH 5183 (±)-Vesamicol</td>
<td>S 7936 SB-205384 GABA&lt;sub&gt;α&lt;/sub&gt; receptor modulator.</td>
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<td>AH 8809 DP/PR Prostanoid receptor antagonist.</td>
<td>S 180 SB-206553 5-HT&lt;sub&gt;2C&lt;/sub&gt; Serotonin receptor antagonist.</td>
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<tr>
<td>AH 21467 (S-Carboxamidotryptamine; 5-CT) S-HT&lt;sub&gt;1A&lt;/sub&gt;, S-HT&lt;sub&gt;1B&lt;/sub&gt;, S-HT&lt;sub&gt;1D&lt;/sub&gt;, S-HT&lt;sub&gt;5&lt;/sub&gt; and S-HT&lt;sub&gt;7&lt;/sub&gt; Serotonin receptor agonist.</td>
<td>S 3442 SB-216763 Glycogen synthase kinase-3 inhibitor.</td>
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<tr>
<td>B 9929 BRL-15572 S-HT&lt;sub&gt;1D&lt;/sub&gt; Serotonin receptor agonist.</td>
<td>S 8817 SB-218795 Non-peptide NK&lt;sub&gt;1&lt;/sub&gt; tachykinin receptor antagonist.</td>
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<td>B-169 BRL-37344 β&lt;sub&gt;1&lt;/sub&gt;-Adrenoceptor agonist.</td>
<td>S 5192 SB-222200 Non-peptide NK&lt;sub&gt;3&lt;/sub&gt; tachykinin receptor antagonist.</td>
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<td>B 5559 BRL-52537 κ&lt;sub&gt;o&lt;/sub&gt; Opioid receptor agonist.</td>
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<td>B-173 BRL-54443 S-HT&lt;sub&gt;1E&lt;/sub&gt; Serotonin receptor agonist.</td>
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<td>B 9305 BW245C DP Prostanoid receptor agonist.</td>
<td>S 7389 SB-269970 S-HT&lt;sub&gt;7&lt;/sub&gt; Serotonin receptor antagonist.</td>
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<td>A 9013 BW284c51 Acetylcholinesterase inhibitor.</td>
<td>S 0441 SB-366791 v&lt;sub&gt;R&lt;/sub&gt; Vaniloid receptor antagonist.</td>
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<td>B 9180 BW668c (GR 116117X) DP Prostanoid receptor antagonist.</td>
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<td>B-170 BW373U86 δ&lt;sub&gt;Opioid&lt;/sub&gt; receptor agonist.</td>
<td>F 102 SKF-7172-A2 (Fluphenazine N-Mustard DiHCl) Phosphodiesterase I (PDEI) inhibitor.</td>
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<td>B-175 BW723C86 S-HT&lt;sub&gt;2A&lt;/sub&gt; Serotonin receptor agonist.</td>
<td>A-114 SKF-10047 ((+)-N-allylnormetacocine HCl) α&lt;sub&gt;1&lt;/sub&gt; Sigma receptor antagonist.</td>
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<td>G 8543 GR 46611 S-HT&lt;sub&gt;1D&lt;/sub&gt; Serotonin receptor agonist.</td>
<td>S-101 R(+)-SKF-38393 D&lt;sub&gt;1&lt;/sub&gt; Dopamine receptor agonist.</td>
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<td>G-114 GR 73632 NK&lt;sub&gt;1&lt;/sub&gt; Tachykinin receptor agonist.</td>
<td>S-102 S(-)-SKF-38393 Less active enantiomer of (±)-SKF 38393.</td>
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<td>G-115 GR 82334 NK&lt;sub&gt;1&lt;/sub&gt; Tachykinin receptor antagonist.</td>
<td>S-168 (±)-SKF-77434 (N- Allyl-(±)-SKF 38393) D&lt;sub&gt;1&lt;/sub&gt; Dopamine receptor agonist.</td>
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<td>S-179 R(-)-SKF-81297 (R(-)-6-Chloro-PB HBr) D&lt;sub&gt;1&lt;/sub&gt; Dopamine receptor agonist.</td>
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<td>G 5918 GR 113808 S-HT&lt;sub&gt;5&lt;/sub&gt; Serotonin receptor antagonist.</td>
<td>F 6800 SKF-82526 (Fenoldopam bromide) D&lt;sub&gt;1&lt;/sub&gt; Dopamine receptor agonist.</td>
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<td>G 6043 GR 125487 S-HT&lt;sub&gt;2A&lt;/sub&gt; Serotonin receptor antagonist.</td>
<td>S 2317 SKF-94836 (Sigluazodan) Phosphodiesterase III (PDEIII) inhibitor.</td>
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<td>G 5793 GR 127935 S-HT&lt;sub&gt;1B&lt;/sub&gt; Serotonin receptor antagonist.</td>
<td>S-178 R (+)-SKF-82957 D&lt;sub&gt;1&lt;/sub&gt; Dopamine receptor agonist.</td>
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<tr>
<td>G 6418 GR 144053 Non-peptide GPIIb/IIIa fibrinogen receptor antagonist.</td>
<td>C-130 (±)-SKF-82958 (Chloro-APB HBr) D&lt;sub&gt;1&lt;/sub&gt; Dopamine receptor agonist.</td>
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<td>S 0568 SB-200646 S-HT&lt;sub&gt;2C&lt;/sub&gt; Serotonin receptor antagonist.</td>
<td>S 2809 SKF-96365 Inhibitor of receptor-mediated and voltage-gated Ca&lt;sup&gt;2+&lt;/sup&gt; entry.</td>
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<td>S 0443 SB-203186 S-HT&lt;sub&gt;5&lt;/sub&gt; Serotonin receptor antagonist.</td>
<td>A-196 SKF-97541 (3-APMPA) GABA&lt;sub&gt;γ&lt;/sub&gt; receptor agonist.</td>
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<td>S 8307 SB-203580 Inhibitor of p38 MAP kinase.</td>
<td>T 0318 Tranilast (BRL-9952) Inhibits LTC&lt;sub&gt;4&lt;/sub&gt; and PGE2 formation, mast cell degranulation and VEGF-induced angiogenesis in vivo.</td>
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<tr>
<td>S 3313 SB-204070 S-HT&lt;sub&gt;5&lt;/sub&gt; Serotonin receptor antagonist.</td>
<td>W 4262 1400W (GI 263735X) iNOS inhibitor.</td>
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Protein Tyrosine Phosphatases: Potential Roles in Disease

Stuart Kellie

The activation of protein tyrosine kinases (PTKs), followed by the reversible phosphorylation of tyrosyl residues in cellular proteins, accounts for the control of many fundamental cellular functions including proliferation, migration, morphogenesis, cytoskeletal changes and gene expression [1]. In recent years, protein tyrosine phosphatases (PTPs) have been recognized as a biochemical counterbalance to PTKs, and although much progress has been made in our understanding of the structure, function and regulation of PTKs, PTPs are less well characterized. PTPs are a large gene family of nearly 100 members and individual members exhibit substrate selectivity and control specific aspects of intracellular signaling [2]. Hence, cell function is regulated by the coordinated action of PTKs and PTPs.

PTPs can be divided into two structurally distinct subgroups: transmembrane (receptor-like) PTPs, referred to as RPTPs, and cytoplasmic PTPs. The cytoplasmic enzymes are further subdivided into tyrosine-specific PTPs and dual specificity PTPs (DSPs) which can dephosphorylate phosphoserine, phosphothreonine or phosphorylated lipids, in addition to phosphotyrosine (see Figure 1). Despite their marked diversity in overall structure, virtually all PTPs possess a core sequence (I/V)HC polarity and are commonly associated with other regulatory regions such as Src homology 2 (SH2) domains, ER targeting sequences, FERM domains or PEST sequence [3].

Intracellular Functions of Protein Tyrosine Phosphatases

Although initial observations of the effects of over-expression of PTKs suggested that these enzymes might simply counteract the signaling events elicited by PTKs, more recent genetic and biochemical evidence indicates that PTKs play more complex roles in a wide range of cellular activities [4,5]. For example, SHP-1 and SHP-2, two SH2 domain-containing cytoplasmic PTPs, play negative and positive regulatory roles, respectively, in PTK signaling [6]. Members of the band 4.1/FERM family of cytoplasmic PTPs, including PTPH1 and PTMPeg, are thought to regulate cytoskeletal reorganization [7,8]. The transmembrane enzyme PTPα affects cell-substratum adhesion and cellular transformation by regulating the activity of the PTK src [9]. In the immune system, the transmembrane PTP CD45 is essential for the activation of lymphocyte-specific kinase (Ick) and downstream signaling of the T cell receptor [10], and SHP-1 is a negative regulator of natural killer (NK) cell signaling [11]. Many receptor PTPs (RPTPs) are transmembrane molecules that possess both intracellular catalytic domains as well as defined extracellular motifs. RPTPs, such as leukocyte common antigen (LAR), PTPα and PTPβ influence neural development and synaptic plasticity [12-14]. An understanding of RPTP function has been hampered by the orphan status of most RPTPs, and, where ligands have been identified, their effects on RPTP activity are unclear. Recent studies have led to the hypothesis that ligands induce dimerization and suppress RPTP activity [15-17]. Due to their critical role in cell regulation, PTPs are implicated in many diseases. This review, in addition to surveying the major topics, will also highlight some less documented aspects of the role of PTPs in human disease.

Protein Tyrosine Phosphatases and Malignancy

PTKs have long been acknowledged to be potential oncogenes. However, there is now growing evidence that some PTPs can either induce neoplastic transformation or act as tumor suppressors. PTPα has been shown to dephosphorylate and activate c-src in breast cancer cell lines [18-20], potentially leading to unregulated proliferation. Indeed, increased PTPα levels have been detected in late stage colon carcinomas and squamous cell carcinomas [21,22]. However, there may also be some cell type specificity as it has been reported that PTPβ inhibits tumor cell growth and correlates with low grade tumor grade in breast carcinoma [23]. PTPα, another PTPα family member, has been reported to increase the risk of mammary hyperplasia in transgenic mice [24]. Several other PTPs, such as PTP1B and SHP-1, are thought to activate c-src by dephosphorylation of tyrosine 527 [25-28]. Although some progress has been made towards understanding the role of PTPs in c-src activation in neoplasia, there are still significant gaps in our knowledge of the basic cell biology of PTPα/β-induced oncogenesis, including its importance in epithelial cell neoplasia in breast, colon and prostate, the role of other PTPs in activating c-src, and the genetic changes induced by increases in PTP activity.

About the Author

Stuart Kellie received his Ph.D. in 1980 from the University of St. Andrews in Scotland. Following postdoctoral appointments at the University of Leicester and the Imperial Cancer Research Fund Laboratories in London, during which time he worked on the identification of cellular substrates for the v-src oncogene, he took up a lectureship in 1986 in the Department of Biochemistry at the Royal College of Surgeons of England. In 1990 he was appointed Group Leader at the Yamanouchi Research Institute in Oxford where he led a team investigating the potential of tyrosine kinases and phosphatases as molecular targets for several human diseases. He currently holds a joint appointment as Senior Lecturer in Immunology and Group Leader at the Institute for Molecular Biosciences, University of Queensland, Brisbane, Australia, and is a project leader in the Cooperative Research Centre for Chronic Inflammatory Diseases.
A subfamily of RPTPs that contain a single catalytic domain in the cytoplasmic region, a single transmembrane domain and eight fibronectin type III-like domains in the extracellular region have also been implicated in the regulation of neoplastic cell growth. DEP-1 (Density Enhanced Phosphatase-1; CD148) is induced in breast carcinoma cells as they reach confluence, and its rat homolog has been reported to suppress the transformed phenotype of retrovirally transformed thyroid cells [37,38]. Further weight to the concept that DEP-1 may function as a tumor suppressor comes from the report that DEP-1 is the underlying gene in the mouse scc1 (susceptibility to colon cancer 1) locus and is frequently deleted in human cancer [39]. In contrast, another member of this family, Sap-1, has been reported to be abundant in a subset of pancreatic and colorectal cancer cell lines and tissues, but not in their normal counterparts [40,41].

PTPas (FAP-1) expression has also been shown to have a strong association with malignancy, however, this appears to occur by a mechanism distinct from that of other PTPases described previously. PTPbas is upregulated in hepatoblastomas and other carcinomas [42-44], and this leads to the induction of resistance to CD95-mediated apoptosis [45-47]. A dual specificity phosphatase, cyclin-dependent kinase-associated phosphatase (KAP), is overexpressed in breast and prostate cancer, and downregulation of this gene by antisense RNA leads to inhibition of the transformed phenotype [48]. PTPase domains have been identified by PCR amplification using degenerate primers. In mouse macrophages, for example, 10 PTPase family
Protein Tyrosine Phosphatases...(continued)

members were identified. One member, DEP-1, was identified as a macrophage-enriched PTPase that is regulated by colony stimulating factor (CSF-1) and lypopolysaccharide (LPS) [49]. A similar approach has identified PTPα and PTPε as genes that are down regulated in melanoma cells [50].

Protein Tyrosine Phosphatases and Inflammatory Disease

Macrophages are possibly the most important inflammatory cell type to be aberrantly activated in chronic inflammatory diseases such as rheumatoid arthritis, but little is known about intracellular regulation of PTPs in these cells. The best characterized PTP in macrophage-like cells is SHP-1, which is a negative regulator of CSF-1 receptor signaling. The activity of this protein is dramatically reduced in the allelic motheaten viable (Me'/Me') mouse and leads to constitutive activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway [51]. Although DEP-1 has been implicated in the control of epithelial cell proliferation (as discussed earlier), it also appears to regulate hematopoietic cell activation and proliferation. It is expressed throughout the hematopoietic system and in a wide variety of tissues including pancreas, thyroid, kidney, mammary gland and nervous system [52,53]. DEP-1 is expressed at low levels in resting T cells, but is upregulated in activated T cells. Ligation with anti-DEP-1 antibodies induced proliferation of anti-CD3 activated T cells, and gave a calcium flux response. Interestingly, in transient transfection experiments, DEP-1 has been reported to be a negative regulator of T cell activation, and also results in a reduction of both TCR-mediated extracellular signal-related kinase I (ERK-1) and ERK-2 activation and tyrosine phosphorylation [54-59]. Baker et al. [60] have shown that DEP-1 expression inhibits TCR-mediated activation of Ras and calcium release, with phospholipase C γ (PLCγ) and linker for activation of T cells (LAT) as potential targets.

Although the evidence is indirect, it is possible that PTPs may play a role in chronic inflammatory diseases and atherosclerosis. Midkine is a heparin-binding growth and differentiation factor that induces haptotaxis of macrophages, osteoclasts and smooth muscle cells [61]. One receptor for midkine is PTPζ, and antibodies against PTPζ enhance midkine-induced migration [62,63].

Protein Tyrosine Phosphatases and Type II Diabetes

Tyrosine phosphorylation is central to insulin-stimulated glucose uptake in adipose tissue and skeletal muscle. Several PTPs have been identified as negative regulators of the insulin signaling pathway, including PTPα and PTPε [64], PTP-LAR [65,66], PTP1B [67,68] and SHIP-2 [69]. Recently, a PTP1B knockout has been developed that has provided evidence that PTP1B is a key regulator of insulin signaling. Mice lacking PTP1B displayed both increased insulin sensitivity and obesity resistance [70]. Curiously, although PTP1B is expressed in many tissues, including adipose tissue and skeletal muscle, enhancement of glucose uptake in PTP1B knockout mice was restricted to skeletal muscle [71]. The reasons for this are unclear, but point to PTP1B having different functions in different cell types. The use of antisense oligonucleotides against PTP1B has confirmed its central role in insulin receptor signaling and glucose homeostasis [72], and highlighted the potential use of inhibitors of PTP1B activity as potential therapeutics for diabetes (see later).

Association of Protein Tyrosine Phosphatases with other Diseases

PTPs have been implicated in the etiology of a number of diseases, as summarized in Table 1. Recently, evidence has been published that suggests that mutations in the PTPN11 gene (SHP-2) leads to Noonan’s syndrome, a developmental disease characterized by craniofacial disorders [73]. Furthermore, knockout mouse studies have implicated many PTPs in neuronal cell function. For example, PTPs such as LAR, PTPβ, PTPζ and PTPε may play a role in neurite outgrowth and neuroregulation and are thus candidates for involvement in neurodegenerative diseases or possibly neuroprotection following trauma or stroke [74]. In addition, PTPβ regulates a voltage-gated sodium channel and may be central to long term potentiation in neurons [75].

The activation or suppression of PTP activity is also central to the etiology of a number of infectious diseases. As stated above, SHP-2 has been identified as a target of the CagA gene of H. pylori, potentially resulting in its deregulation and hyperproliferation [34]. Interestingly, both PTPβ and PTPα have been reported to be receptors for H. pylori, raising the possibility that several PTPs control H. pylori-mediated pathogenesis [76,77]. Other infectious agents also appear to regulate PTPs. For example, Vaccinia virus and other poxviruses can evade host defenses by a number of mechanisms. One of these is dependent on the expression of the VH1 gene, whose product is a dual specificity phosphatase essential for virus viability within cells [78,79]. More recent evidence indicates that the VH1 phosphatase may suppress host responses by inhibiting the activation of STAT-1 and therefore interferon γ signal transduction [80]. Another example of host suppression is the resistance to phagocytosis of Yersinia spp, occurs by the injection of a

Table 1. Summary of Protein Tyrosine Phosphatases and possible disease associations.

<table>
<thead>
<tr>
<th>PTP</th>
<th>Disease</th>
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<td>Diabetes</td>
<td>65-69</td>
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<td>PTPα, KAP, cdc25, FAP1, SHP-1, SHP-2, PTPCAAX</td>
<td>Cancer</td>
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<td>DEP-1, SAR-1, PTEN</td>
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<td>PRL3</td>
<td>Inflammation</td>
<td>54-60</td>
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<tr>
<td>TC-PTP, DEP-1, CD45, SHP-1</td>
<td>Neurodegeneration</td>
<td>12-14, 74,75</td>
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<tr>
<td>RPTPγ, RPTPβ, RPTPζ</td>
<td>Noonan’s syndrome</td>
<td>73</td>
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<tr>
<td>SHP-2</td>
<td>H. pylori ulceration</td>
<td>34, 76-77</td>
</tr>
<tr>
<td>RPTPζ</td>
<td>Atherosclerosis</td>
<td>61-63</td>
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Protein Tyrosine Phosphatases...(continued)

PTP, the YopH gene, into the phagocyte, thereby suppressing phagocyte function [81]. Mycobacteria and Salmonella spp. may also subvert host cell signaling in a similar manner [82,83]. There is also some evidence that Leishmania spp activates SHP-1 in macrophages, resulting in a downregulation of macrophage responses and intracellular survival of the pathogen [84].

Pharmacological Regulation of Protein Tyrosine Phosphatases

Due to its role in type II diabetes, PTP1B has potential as a therapeutic target. Many biotech and pharmaceutical companies and academic groups have developed selective chemical inhibitors of PTP1B that have shown efficacy in animal models and derivatives of these may lead to better therapies for diabetes. Several different classes of chemical inhibitors have been developed. Burke and his colleagues have pioneered the use of difluoromethylene phosphonates as phosphate mimetics [85-87]. Modification of oxalylaminobenzoic acid, another phosphotyrosine mimic, and the use of structure-based drug design have also generated a number of PTP1B inhibitors [88,89]. A number of other selective chemical inhibitors of PTP1B have been reported as potential leads for diabetes therapy [90-92]. Zhang and colleagues have also used structure-based design to develop selective inhibitors [93,94]. Further information on the therapeutic potential of PTP1B inhibitors is contained in three recent reviews [95-97]. Another possible approach to therapy in humans is the use of PTP1B antisense oligonucleotides which have been shown to normalize glucose levels in diabetic mice [72]. Derivatives of some of these compounds are now in clinical development and results are awaited with keen interest.

Few selective inhibitors for other PTPs have been published. However, the use of non-specific PTP inhibitors has indicated that more selective compounds may be useful for other diseases. The ability to express and purify PTPs in bacterial expression systems and subsequently perform structural studies has aided enormously the ability to both design potent and selective inhibitors and identify specific substrates. Active recombinant PTP1B (Prod. No. P 7365), LAR (Prod. No. L 0907), TC-PTP (Prod. No. T 1196) and YOP PTP (Prod. Nos. Y 4127 and Y 4252) are now available for routine laboratory use. Inhibitors with relative selectivity for the PTP family, such as sodium orthovanadate (Prod. No. S 6508), have been used extensively as research tools, and other, more stable vanadate derivatives are now available. One caveat for the use of such compounds is that they have effects on other enzymes, and vanadate can inhibit kinases and ATPases due its phosphate transition state mimicry. However, the IC50 values for most PTPs are in the micromolar range whereas ATP inhibition occurs in the millimolar range. Indeed, vanadium compounds have been used for over 100 years in the treatment of diabetes [98,99], and it is likely that the molecular target for vanadate in these studies was PTP1B or another PTP regulating insulin signaling. Recently, the antibiotic dephostatin (Prod. No. D 8065) has been reported to be a PTP inhibitor [100,101] and so may be useful in PTP functional studies, although its relative selectivity with respect to other enzymes has yet to be fully investigated. The utility of compounds in vitro is underscored by the extensive list of publications. The vast majority of these have reported that treatment of cells with vanadate has a similar effect to cell stimulation. Thus, it can be reasonably hypothesized that under normal conditions there is a basal PTP activity that suppresses cell activation. The logical extension of this is that cells can be stimulated either by activation of PTKs or by inhibition of PTPs, a possibility often overlooked in cell signaling experiments. Vanadate-containing compounds are competitive inhibitors probably by PTP transition state mimicry. On the other hand, pervanadate is an irreversible inhibitor due to its ability to oxidize the catalytic cysteine residue. Nitric oxide (NO) and reactive oxygen species (ROS) can also inactivate PTPs by oxidizing the active site cysteine. Thus NO donors, such as nitrosothiols, can lead to activation of cells by inactivation of PTPs [102]. There is growing evidence that some compounds and drugs which have been used historically to treat certain diseases, but whose mechanism of action is unknown, may function through regulation of PTPs. An example of this is sodium stibogluconate, an antimony-containing compound that has been used for the past 30 years as a treatment for leishmaniasis. This disease is characterized by the intracellular survival and proliferation of Leishmania spp. in macrophages. Stibogluconate appears to activate macrophages that subsequently destroy the intracellular organisms and there is growing evidence that this occurs via the inhibition of the PTP SHP-1. Cellular and in vitro assays have revealed that stibogluconate forms a stable complex with SHP-1, inhibiting its activity and resulting in augmentation of cytokine responses [103].
Several compounds have also been shown to activate PTPs. These include **phosphatidic acid** (Prod. No. P 4013), **phosphatidylserine** (Prod. No. P 7769), **cardiolipin** (Prod. No. C 0563) and other acidic phospholipids such as phosphatidylinositol 3,4,5 trisphosphate (PIP<sub>3</sub>) that activate SHP-1, possibly by binding to the carboxy-terminal domain [104,105]. Lipooarabinomannan, a derivative of *M. tuberculosis*, can also activate SHP-1, probably leading to suppression of macrophage activity and evasion of the host immune responses by the pathogen [106]. Similarly, phosphopeptides based on the tyrosine phosphorylation site of IRS-1 or PDGF receptor have been shown to activate SHP-2 [107]. While there has been intense effort directed at generating specific PTK inhibitors, the potential to selectively activate PTPs has been relatively ignored, although in principle this should lead to a similar suppression of cell activation. The therapeutic potential of PTP activators has yet to be explored, but would seem to be a worthwhile alternative approach.

Dual specificity PTPs (DSPs) such as cdc25 have been implicated in a number of proliferative diseases, particularly in cancer. There are three Cdc25 homologs: A, B and C [108], which are expressed and activated at different times during the cell cycle acting on different cyclin-Cdk complexes. Cdc25B and Cdc25C function primarily at the G2/M transition, while Cdc25A promotes S-phase entry. Microinjection of neutralizing Cdc25A antibodies prevented entry into S phase, demonstrating that Cdc25A is required for cellular progression through the G1/S checkpoint [109-111]. Overexpression of Cdc25A also induces premature activation of both cyclin E- and cyclin A-Cdk2 complexes. These cell cycle checkpoint genes are attractive targets for therapeutic agents in hyperproliferative diseases, such as cancer. In support of this Cdc25B mRNA was found to be expressed at high levels in almost one third of human breast cancers [112], in addition to being oncogenic when expressed as a transgene [113]. Cdc25A overexpression has also been associated with breast cancer [114]. Another DSP, KAP, is overexpressed in breast and prostate cancer and down regulation of this gene results in the loss of the transformed phenotype both in vitro and in vivo [48].

Several small molecular weight inhibitors of DSPs have been developed which are proving useful in dissecting out the relative roles of DSPs and other phosphatases [115,116]. One example is NSC 95397 (Prod. No. N 1786), a cdc25-selective inhibitor which has been shown to inhibit the proliferation of human and murine carcinoma cells by blocking the G2/M phase transition [117]. Another class of dual specificity phosphatase with relevance to human disease is PTEN, a phosphatase that possesses activity against both phosphotyrosine and inositol phospholipids [118]. Genetic studies have demonstrated that its absence leads to a number of human tumors and PTEN is now classed as a tumor suppressor [119,120]. Although originally described as a phosphotyrosine and phospholipid phosphatase, subsequent studies have demonstrated that it is the phospholipid phosphatase activity that is critical for its tumor suppression function [121]. While no small molecular weight molecules have been described which regulate the lipid phosphatase activity of PTEN, it remains an interesting target.

**Perspectives**

The success of the Abelson PTK inhibitor STI-157 (Glivec) as a therapy for chronic myeloid leukemia and other malignancies has provided 'proof of principle' that targeting molecules that regulate tyrosine phosphorylation can be clinically effective. Several phosphatase-selective inhibitors have now been developed that exhibit efficacy both *in vitro* and *in vivo*, and it seems likely that the number of such inhibitors entering clinical trials will increase in the next few years. The most likely therapeutic areas for the development of a clinically effective PTP inhibitor are non-insulin dependent (Type II) diabetes and/or obesity. However, we may also see inhibitors being used for the treatment of cancer in the near future. In the meantime, an increase in the availability of selective PTP inhibitors will undoubtedly generate a greater understanding of the role of these molecules in cell regulation.

**References**

47. Irie, S., DNA Seq., 11, 519-526 (2001).
GR 144053: Non-peptide antagonist of the platelet glycoprotein IIb/IIIa (GP IIb/IIIa) fibrinogen receptor

Following vascular injury, platelets become activated and adhere to damaged blood vessel walls and exposed subendothelial connective tissues thereby forming the initial platelet plug. Platelets play a central role in thrombus formation and are known to participate in many life-threatening thrombotic disorders such as acute myocardial infarction, stroke, and pulmonary embolism. One platelet receptor involved in this activation is the glycoprotein IIb/IIIa (GP IIb/IIIa) fibrinogen receptor (GP IIb/IIIa) on the platelet surface, providing a platform upon which the members of the coagulation cascade can assemble.

GR 144053 (Prod. No. G 6418) is a potent and selective, non-peptide antagonist at the glycoprotein IIb/IIIa (GP IIb/IIIa) fibrinogen receptor [1,2]. It acts as a mimetic of the peptide RGDS-sequence, a potent inhibitor of GP IIb/IIIa. Binding of GR 144053 to GP IIb/IIIa competitively blocks the binding of its normal ligand, fibrinogen (Prod. No. F 4883), and alters the signaling properties of the GP IIb/IIIa heterodimer. It attenuates platelet aggregation, activation and degranulation both in vivo and in vitro and inhibits ADP-induced platelet aggregation with an IC50 value of 17.7 nM [2].

GR 144053 also suppresses the activation of platelets by aurintricarbonylic acid (ATA, Prod. No. A 0885) [2]. The molecular mechanism of ATA action has not been completely elucidated. One possible mechanism is through its binding to GP IIb, thereby blocking binding of vWF. This observation suggests additional activities for GR 144053 that are not mediated by the GP IIb/IIIa receptor [2].

GR 144053 is a useful tool for studying the of mechanisms of platelet activation and degranulation events. Currently, anti-thrombotic therapy includes anti-platelet, anti-coagulant, pro-thrombolytic or fibrinolytic agents. GR 144053 may be potentially useful in achieving anti-thrombosis effects while maintaining the integrity of the vascular system.

References
New Product Highlights

VER-3323: A novel, orally active 5-HT$_{2C/2B}$ serotonin receptor agonist that reduces food intake

*Exclusively available from Sigma-RBI*

In view of the increasing prevalence of obesity in Western society, numerous pharmacological interventions are being investigated with a view to developing effective anti-obesity agents. One popular approach involves the development of selective 5-HT$_{2C}$ serotonin receptor agonists. In support of this strategy, the non-selective 5-HT$_{2C}$ serotonin receptor agonist 1-(3-chlorophenyl)piperazine (m-CPP; Prod. No. C 5554) has been shown to lower food intake, reduce body weight and accelerate the appearance of the behavioral satiety sequence in rats [1-3], in addition to promoting decreased food intake in both normal [4] and obese human volunteers [5]. Moreover, the anorectic effect of m-CPP is absent in mutant mice lacking the 5-HT$_{2C}$ serotonin receptor [6] and is attenuated by the selective 5-HT$_{2C}$ serotonin receptor antagonist SB-242084 (Prod. No. S 8061) in rats [7].

Recently, in an effort to develop compounds with improved 5-HT$_{2C}$ serotonin receptor selectivity and oral potency, researchers at Vernalis Group in the UK have developed a novel series of indoline alkylamine derivatives [8]. In radioligand binding studies performed on human serotonin receptors expressed in CHO-K1 cells, [3H]-5-HT was used to radiolabel 5-HT$_{2B}$ and 5-HT$_{2C}$ serotonin receptors, while [3H]-DOI (2,5-dimethoxy-4-iodoamphetamine) was used to label 5-HT$_{2A}$ serotonin receptors. VER-3323 (Prod. No. V 1889) displayed high affinity for 5-HT$_{2C}$ serotonin receptors (K$_i$ 17 nM) and 5-HT$_{2B}$ receptors (46 nM), but significantly lower affinity for 5-HT$_{2A}$ serotonin receptors (351 nM) [9]. VER 3323 bound poorly to other serotonin receptor subtypes as well as to a wide range of other neurotransmitter/neuropeptide receptors.

Of particular interest, VER-3323 (1, 3, 10 and 30 mg/kg s.c.) dose-dependently reduced food consumption in 23 hr food-deprived Lister-hooded rats over a 4 hr period, displaying a minimum effective dose (MED) of 3 mg/kg s.c. In a subsequent study, VER-3323 administered orally by gavage (10, 30 and 60 mg/kg p.o.) similarly reduced food consumption with a MED of 30 mg/kg p.o. In addition, the decrease in food intake induced by the acute administration of VER-3323 (10 mg/kg s.c.) was completely reversed by prior treatment with the selective 5-HT$_{2C}$ serotonin receptor antagonist SB-242084 [10].

These data suggest that VER-3323 is an orally active 5-HT$_{2C}$ serotonin receptor agonist that will provide a useful tool for studying the role of 5-HT$_{2C}$ serotonin receptors in food intake.

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References


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New Product Highlights

Secreted amyloid precursor proteins: Tools for Alzheimer’s disease research

Alzheimer’s disease (AD) is the most common dementia in Western societies with a gradual appearance of symptoms occurring between 60-70 years of age [1]. AD is characterized clinically by the progressive loss of cognitive function, and pathohistologically by the appearance in the brain of extracellular fibrillar amyloid deposits, referred to as plaques, and tau-rich neurofibrillary tangles.

Amyloid precursor protein (APP) is ubiquitously expressed in many tissues [2]. Following proteolytic cleavage by β- and γ-secretases, two fragments are formed: secreted amyloid precursor protein β (sAPPβ; Prod. No. S 4316) and β-amyloid peptide (Aβ; Prod. No. A 9810) [3]. Aβ is the major component of the amyloid plaques found in AD patients, while sAPPβ is thought to modulate neuronal function and cell survival [2].

Secreted amyloid precursor protein α (sAPPα; Prod. No. S 9564) is a 612 amino acid protein produced from the ubiquitously expressed APPβ, 695 isoform, following cleavage by α-secretase [2,4,5]. sAPPα is released from neurons in an activity-dependent manner and possesses neurotrophic activity that promotes long term neuronal survival [6]. sAPPα also promotes neurite outgrowth and synaptogenesis in addition to modulating neuronal excitability and synaptic plasticity [2,7]. In addition, it possesses pro-inflammatory activity by causing the release of excitotoxic levels of glutamate [7]. It potentially controls gene expression (i.e. NFκB expression) [2,8] and was recently identified as a ligand for the class A scavenger receptor [9].

In addition to the above secreted amyloid precursor proteins, Sigma-RBI is pleased to offer two sAPPα fragments. Specifically, sAPPα (304-612) (Prod. No. S 8065) is a fragment of sAPPα that lacks the N-terminal domain of the full length protein. The sAPPα (304-612) sequence comprises several functional domains. The RERMS peptide, APP (328-332), found in the region corresponding to sAPPα (319-335), is active in inducing fibroblast proliferation and neurite outgrowth in neuroblastoma cells [2,10]. The sequence corresponding to sAPPα (444-592) is linked to attenuation of glutamate excitotoxicity and to promotion of neurite outgrowth [2,11]. The C-terminal domain corresponding to sAPPα (597-612) acts as a heparin-binding domain [2]. Of particular interest, sAPPα (304-612) will enable the study of the biological effects of these domains without interference from the sAPPα N-terminal cysteine rich domain.

sAPPα (444-612) (Prod. No. S 8190) is a fragment of sAPPα that has been shown to attenuate glutamate excitotoxicity and to promote neurite outgrowth [2,11]. In addition, this fragment contains the sAPPα C-terminal domain (597-612) that acts as a heparin-binding domain [2], sAPPα (444-612) enables the study of the biological effects of these domains without interference of the sAPPα N-terminal, cysteine-rich domain and the RERMS peptide domain.

These proteins and protein fragments will assist researchers in understanding the functional role of APP and its effects on neurodegeneration. They will be of particular interest to researchers studying the etiology of AD.

References
The advent of relatively specific cell-permeable inhibitors of protein kinases in the mid 1990s has had a major impact on the study of signal transduction. The ability to rapidly suppress the cellular activity of a particular protein kinase has proved to be a powerful method for identifying the physiological substrates of these enzymes and the roles of the signaling pathways in which they participate. These compounds enter cells within minutes, so that indirect effects caused, for example, by changes in gene expression (a potential hazard when using cells deficient in a particular protein kinase), are excluded. Moreover, the use of protein kinase inhibitors avoids the need for transfection-based approaches, that have the potential to give misleading results since the fidelity of signaling can break down when components are overexpressed. Nevertheless, in order to use protein kinase inhibitors effectively it is important to realize their limitations, as well as their strengths.

An appreciation of the degree of specificity of any particular inhibitor is clearly a critical issue. There are just over 500 protein kinases encoded by the human genome, most of which belong to the same superfamily. It is therefore a challenging and difficult task to develop compounds that inhibit one particular protein kinase, without inhibiting several related enzymes. Table 1 provides current information about the specificities of 41 protein kinase inhibitors, many of which are available from Sigma-RBI, on a wide range of protein kinases.

Most inhibitors of protein kinases target more than one enzyme. There is, therefore, a danger that, in cell-based assays, the observed effects do not result from inhibition of the kinase of interest, but rather from inhibition of another protein kinase. In order to exclude this possibility, it is necessary to show that the effects of an inhibitor disappear in cells that express an inhibitor-resistant mutant of the kinase of interest. However, at present, the availability of such cells is very limited [1,2]. In order to reduce this risk, it is important to examine, wherever possible, the effects of at least two structurally unrelated inhibitors of the same protein kinase. For example, kenpaullone (Prod. No. K 3888) and roscovitine (Prod. No. R 7772), which are relatively specific inhibitors of cyclin-dependent protein kinases (CDKs), also inhibit a few other protein kinases. However, the other enzymes inhibited by roscovitine are not the same as those inhibited by kenpaullone (see Table 1). Thus, if identical effects are observed with roscovitine or kenpaullone, one can have greater confidence that the effects are mediated by a CDK. For similar reasons, it is advisable to use both LiCl (Prod. No. L 0505) and kenpaullone to study glycogen synthase kinase-3 (GSK-3), wortmannin (W 1628) and LY 294002 (Prod. No. L 9908) to identify potential roles of phosphoinositide 3-kinase (PI3K), PP1 or PP2 and SU6656 (Prod. No. S 9692) for Src family kinases, and Y 27632 and HA1077 (Prod. No. H-139) for Rho kinase (ROCK) and protein kinase C-related kinase 2 (PRK2) (Tables 1 and 2) [3,4].

Even compounds that inhibit a number of protein kinases can sometimes be useful in excluding the involvement of one or more protein kinases in the control of a particular process. For example H89 (Prod. No. B 1427), which inhibits isoforms of mitogen- and stress-activated kinase (MSK), but not the structurally related isoforms of ribosomal S6 kinase (RSK), has been used to provide evidence that RSKs do not mediate the growth factor-induced phosphorylation of the transcription factor cAMP-response-element-binding protein (CREB) [5]. MSK isoforms were later shown to be the physiologically relevant protein kinases using cells deficient in these kinases [6]. Similarly, UCN01, which inhibits checkpoint kinase 1 (CHK1), but not CHK2, can be used to exclude the involvement of CHK2 in the control of responses to DNA damage or cell cycle checkpoints (Tables 1 and 2) [3].

It is also possible to vary the concentrations of inhibitors in the culture medium to differentially inhibit particular protein kinases. For example, at low concentrations PD184352 inhibits the classical mitogen-activated protein kinase (MAPK) cascade specifically, but at higher concentrations it also blocks the mitogen-activated protein kinase 5 (MKK5/ERK5) pathway [7]. However, the precise concentrations needed can vary from cell to cell. For this reason, it is essential to define the minimum concentration of an inhibitor required to suppress activity by 80-90% by examining the phosphorylation of a validated substrate of the protein kinase that is under investigation.

The vast majority of protein kinase inhibitors target the adenosine 5′-triphosphate (ATP)-binding site of a protein kinase. For this reason, much higher concentrations of

**About the Author**

Philip Cohen received his Ph.D. in Biochemistry from University College, London. Following a period of postdoctoral research working in the laboratory of Edmond Fischer at the University of Washington, Seattle, USA, he was appointed to a Lectureship in Biochemistry at the University of Dundee, Scotland in 1971. Having been promoted to Reader in 1977 and to Professor in 1981, he became a Royal Society Research Professor in 1984, the position that he currently holds. He is also Director of the Medical Research Council Protein Phosphorylation Unit and Director of Research in the School of Life Sciences at Dundee. In a long and illustrious career, in which he has published over 440 peer reviewed research papers, he has made extensive contributions to understanding the role of protein phosphorylation in the regulation of cellular function.
Table 1. Inhibition of protein kinases by various inhibitors. Results indicate percent activity observed in the presence of inhibitor expressed as a percentage of control incubations in the absence of inhibitor. Data are the means of duplicate determinations. Data highlighted in boxes indicate instances when a given inhibitor reduced kinase activity to ≤ 25% of control values. Assays were carried out at a magnesium ion concentration of 10 mM and an ATP concentration of 0.1 mM. Column headers indicate the protein kinase inhibitors tested, together with the concentrations at which they were used and their Sigma-RBI product numbers in red.

<table>
<thead>
<tr>
<th>Protein kinase</th>
<th>(A) Core panel</th>
<th>(B) Other kinases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M KK1</strong></td>
<td>90 103 89 106 101 56</td>
<td>16 72 37 79 89 88 86 93 83 95 92 70</td>
</tr>
<tr>
<td><strong>MAPK2/ERK2</strong></td>
<td>87 94 94 139 92 92 107 85 85 89 90 114 113 90 107 92 97 98 107</td>
<td></td>
</tr>
<tr>
<td><strong>JNK1α1/SAPK1c</strong></td>
<td>97 98 96 49</td>
<td>0 92 89 95 91 100</td>
</tr>
<tr>
<td><strong>JNK/SAPK1c</strong></td>
<td>99 94 93 111 95 75 100 85</td>
<td>2 0 86 98 138 93 108 102 84 88 104</td>
</tr>
<tr>
<td><strong>SAPK2a/p38</strong></td>
<td>97 107 97 127 98 90 119 95</td>
<td>5 3 74 98 150 88 96 104 97 92 115</td>
</tr>
<tr>
<td><strong>SAPK2b/p38i</strong></td>
<td>97 107 97 127 98 90 119 95</td>
<td>5 3 74 98 150 88 96 104 97 92 115</td>
</tr>
<tr>
<td><strong>SAPK3/p38y</strong></td>
<td>106 100 87 146 95</td>
<td>100 100 96 96 80 75 97 132 100 99 82 89 116</td>
</tr>
<tr>
<td><strong>SAPK4/p38i</strong></td>
<td>105 95 103 130 110 111 98 94 93 87 87</td>
<td>103 82 84 99 104 113 133</td>
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<tr>
<td><strong>MAPKAP-K1a</strong></td>
<td>30 35</td>
<td>10 3 6 5</td>
</tr>
<tr>
<td><strong>MAPKAP-K1b</strong></td>
<td>16 72 37 79 89 88 86 93 83 95 92 70</td>
<td>20 95 95 78 2 9 2</td>
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<td><strong>MASK</strong></td>
<td>3 57 19</td>
<td>10 0 86 98 138 93 108 102 84 88 104</td>
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<td><strong>PRK1</strong></td>
<td>91 35 17</td>
<td>10 0 86 98 138 93 108 102 84 88 104</td>
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<td><strong>PRK2</strong></td>
<td>91 35 17</td>
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<tr>
<td><strong>GSK-3</strong></td>
<td>92 99 98 107 99 93 97</td>
<td>102 74 90 125 72 98 103 90 97</td>
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<tr>
<td><strong>SAPK1c</strong></td>
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<td>102 74 90 125 72 98 103 90 97</td>
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<tr>
<td><strong>SAPK2b</strong></td>
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<tr>
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<td>92 99 98 107 99 93 97</td>
<td>102 74 90 125 72 98 103 90 97</td>
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<tr>
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<td>92 99 98 107 99 93 97</td>
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<td><strong>AMPK</strong></td>
<td>19 95 77 98 97 85 89</td>
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<td><strong>CK1</strong></td>
<td>104 98 102 103 103 107 96 87 97 93 98</td>
<td>18 19 104 73 112 104 101 106</td>
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<tr>
<td><strong>CK2</strong></td>
<td>104 98 102 103 103 107 96 87 97 93 98</td>
<td>18 19 104 73 112 104 101 106</td>
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<tr>
<td><strong>PKH</strong></td>
<td>51 81 58 63 106 101 117 87 104 91 100 44</td>
<td>32 103 96 93 57 54</td>
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<tr>
<td><strong>LCK</strong></td>
<td>76 109 94 70 92 87 99 85</td>
<td>32 37 95 85 83 102 99 105 79 86 86</td>
</tr>
<tr>
<td><strong>CHK1</strong></td>
<td>21 99 82 107 104 95 104 99 95 99 90 56 102 96 97 42 60 40</td>
<td></td>
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<tr>
<td><strong>SAPK3</strong></td>
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<td>94 109</td>
</tr>
<tr>
<td><strong>M KK3</strong></td>
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<td>87 94</td>
</tr>
<tr>
<td><strong>M KK4</strong></td>
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<td>108 113</td>
</tr>
<tr>
<td><strong>M KK6</strong></td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td><strong>M KK7</strong></td>
<td>91</td>
<td>89</td>
</tr>
<tr>
<td><strong>PI3K</strong></td>
<td>0</td>
<td>13 18</td>
</tr>
<tr>
<td><strong>PRK2</strong></td>
<td>6 15</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations:
AMPK: AMP-activated protein kinase  
CaM-KII: Calmodulin-dependent protein kinase II  
CDK2/Cyclin A: Cyclin-dependent kinase 2/Cyclin A complex  
CK1α1: Casein kinase 1  
CK2α: Casein kinase 2  
CSK: C-terminal Src kinase  
DYRK1A: Dual-specificity tyrosine phosphorylation-regulated kinase 1A  
GSK3β: Glycogen synthase kinase-3β  
JNK/SAPK1cα: c-Jun N-terminal kinase  
JNK1α1/SAPK1cα: c-Jun N-terminal kinase  
LCK: T-cell specific kinase  
MAPKAP-K1α: Mitogen-activated protein kinase-activated protein kinase-1α  
MAPKAP-K1β: Mitogen-activated protein kinase-activated protein kinase-1β  
MAPKAP-K2α: Mitogen-activated protein kinase-activated protein kinase-2α  
MAPKAP-K2β: Mitogen-activated protein kinase-activated protein kinase-2β  
M KK1: Mitogen-activated protein kinase kinase 1  
M KK3: Mitogen-activated protein kinase kinase 3  
M KK4: Mitogen-activated protein kinase kinase 4  
M KK5: Mitogen-activated protein kinase kinase 5  

| 84 | 99 | 99 | 99 | 64 | 59 | 20 | 90 | 93 | 80 | 94 | 103 | 96 | 52 | 55 | 89 | 82 | 89 | 99 | 94 | 102 | 95 |
| 86 | 101 | 105 | 106 | 34 | 37 | 57 | 87 | 81 | 26 | 104 | 70 | 64 | 75 | 61 | 92 | 102 | 55 | 106 | 53 | 85 | 101 |
| 95 | 100 | 95 | 103 | 88 | 82 | 81 | 98 | 91 | 84 | 21 | 128 | 89 | 98 | 98 | 99 | 103 | 38 | 99 | 76 | 97 | 102 |
| 90 | 101 | 115 | 117 | 92 | 100 | 103 | 92 | 96 | 101 | 83 | 84 | 79 | 13 | 21 | 100 | 70 | 86 | 102 | 105 | 100 | 94 |
| 88 | 104 | 102 | 102 | 81 | 88 | 98 | 91 | 104 | 117 | 45 | 108 | 64 | 22 | 26 | 84 | 90 | 75 | 100 | 87 | 89 | 98 |
| 83 | 99 | 107 | 116 | 72 | 64 | 95 | 76 | 94 | 105 | 74 | 87 | 55 | 92 | 90 | 83 | 75 | 82 | 103 | 85 | 95 | 98 |
| 90 | 88 | 129 | 112 | 96 | 93 | 95 | 91 | 106 | 98 | 65 | 104 | 73 | 102 | 96 | 95 | 78 | 98 | 105 | 60 | 75 | 86 |

For more information on Sigma-RBI’s extensive range of protein kinase inhibitors, visit our website at sigma-aldrich.com/cellsignaling.
inhibitors are generally needed to suppress the activity of a protein kinase in cells (where the ATP concentration is in the millimolar range) compared to the amounts required for inhibition in vitro (where assays are performed at much lower ATP concentrations, typically 0.01-0.1 mM).

There are, however, a few inhibitors that are actually more potent in cell-based assays than they are in vitro. For example, PD 98059 (Prod. No. P-215) and U0126 (Prod. No. U-120), which are non-competitive inhibitors of mitogen-activated protein kinase kinase 1 (MKK1), bind much more strongly to the dephosphorylated, inactive form of this protein kinase than the phosphorylated, active enzyme. These compounds prevent the conformational change required for activation of MKK1 and therefore suppress the classical MAPK cascade at much lower concentrations than those needed to inhibit activated MKK1 in vitro [3,8]. Similarly, lithium ions, a relatively specific inhibitor of GSK-3, compete for binding with magnesium ions. The free concentration of magnesium ions in cells is less than 0.5 mM, much lower than the concentration used to assay GSK-3 routinely (10 mM, see Table 1). For this reason, lithium ions inhibit GSK-3 more potently in cells than in vitro [3].

In recent years, potent and highly specific inhibitors of a variety of protein kinases have been developed by several pharmaceutical companies. Many have entered human clinical trials and, in two cases (Glivec and Iressa) have been approved for the treatment of different types of cancer [9]. Over the next ten years, one can therefore expect that many more protein kinase inhibitors will become available to the scientific community, which should advance at an even faster pace our understanding of the function of these enzymes.

References.

Table 2. How to use the more specific inhibitors of protein kinases in cell-based assays

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Specificity</th>
<th>Target Kinase(s)***</th>
<th>Concentration to use in Culture Medium (µM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin</td>
<td>Very high</td>
<td>mTOR</td>
<td>0.1</td>
</tr>
<tr>
<td>PD 98059</td>
<td>High</td>
<td>MKK1</td>
<td>50</td>
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<tr>
<td>PD 184352**</td>
<td>High</td>
<td>MKK1</td>
<td>1-2</td>
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<tr>
<td>PD 184352**</td>
<td>High</td>
<td>MKK1, MKK5</td>
<td>10-20</td>
</tr>
<tr>
<td>U0126 (U-120)</td>
<td>High</td>
<td>MKK1, MKK5</td>
<td>5-10</td>
</tr>
<tr>
<td>SB-203580 (S 8307)</td>
<td>High</td>
<td>SAPK2a/p38α, SAPK2b/p38β2</td>
<td>1-10</td>
</tr>
<tr>
<td>SB-202190 (S 7067)</td>
<td>High</td>
<td>SAPK2a/p38α, SAPK2b/p38β2</td>
<td>1-5</td>
</tr>
<tr>
<td>KN62 (I 2142)</td>
<td>High</td>
<td>CaM-KII, other CaM-Ks</td>
<td>10</td>
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<tr>
<td>Wortmannin (W 1628)</td>
<td>High</td>
<td>PI3K</td>
<td>0.1</td>
</tr>
<tr>
<td>LY 294002 (L 9908)</td>
<td>Quite high</td>
<td>PI3K</td>
<td>50-100</td>
</tr>
<tr>
<td>Y27632</td>
<td>Quite high</td>
<td>ROCK, PRK2</td>
<td>10-20</td>
</tr>
<tr>
<td>HA1077 (H-139)</td>
<td>Medium</td>
<td>ROCK, PRK2</td>
<td>10-100</td>
</tr>
<tr>
<td>LiCl (L 0505)</td>
<td>Quite high</td>
<td>GSK-3</td>
<td>10</td>
</tr>
<tr>
<td>Kenpaullone (K 3888)</td>
<td>Quite high</td>
<td>GSK-3, CDKs</td>
<td>10</td>
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<tr>
<td>Roscovitine (R 7772)</td>
<td>High</td>
<td>CDKs</td>
<td>10-100</td>
</tr>
<tr>
<td>PP1</td>
<td>Quite high</td>
<td>Src, Fyn, Lck</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>PP2</td>
<td>Quite high</td>
<td>Src, Fyn, Lck</td>
<td>0.1-1.0</td>
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<tr>
<td>SU66565 (S 9692)</td>
<td>Medium</td>
<td>Src, Fyn, Lck</td>
<td>10-50</td>
</tr>
<tr>
<td>ML7 (L 2764)</td>
<td>Quite high</td>
<td>Sm-MLCK</td>
<td>50-100</td>
</tr>
<tr>
<td>H89** (B 1427)</td>
<td>Medium</td>
<td>PKA</td>
<td>5-10</td>
</tr>
<tr>
<td>H89** (B 1427)</td>
<td>Medium</td>
<td>PKA, MSKs but not RSKs</td>
<td>10-25</td>
</tr>
<tr>
<td>Ro 31-8220** (R-136)</td>
<td>Medium</td>
<td>Conventional PKCs</td>
<td>1</td>
</tr>
<tr>
<td>Ro 31-8220** (R-136)</td>
<td>Medium</td>
<td>PKCs, MSKs, RSKs, etc</td>
<td>5</td>
</tr>
<tr>
<td>UCN01</td>
<td>Quite low</td>
<td>PDK1 and CHK1, but not CHK2</td>
<td>0.3-1</td>
</tr>
</tbody>
</table>

* The suggested concentrations are only guidelines. The optimal concentrations can vary and need to be defined for each cell used, as discussed in the text. Sigma-RBI product numbers are shown in red.
** Depending on the concentration range at which they are used, these kinase inhibitors can be used to target different groups of protein kinases.
***Kinase names are provided in the Table 1 legend.
New Product Highlights

Phosphospecific monoclonal antibodies to β-Catenin: transmembrane signaling and gene expression markers

Cell adhesion is important during development, as well as in cell sorting, induction of cellular morphogenesis and maintenance of tissue integrity [1-3]. Calcium-dependent cell adhesion is mediated by the cadherins, a family of transmembrane glycoproteins that regulate homophilic interactions in cells. These interactions initiate a cascade of events that lead to the structural and functional reorganization of cells. Cadherin function involves both specific binding of extracellular domains at the cell surface and interaction with components of the cytoplasm. These components include α-, β- and γ-catenin (also called plakoglobin), all of which bind to the cytoplasmic domain of cadherins [4]. β-catenin (92-97 kDa) shares 70% sequence identity to a protein encoded by armadillo, a Drosophila segment polarity gene [5-8].

β-catenin binds to a diverse set of proteins including the presenilins, epidermal growth factor receptor (EGF-R, Prod. No. E 2645), the actin-binding protein fascin and the transcription factor Teashirt [9,10]. β-catenin is composed of a series of protein-protein interaction motifs that allow it to function as a scaffold. The amino terminal domain, containing the binding site for α-catenin and its phosphorylation sites, is recognized by glycogen synthase kinase-3β (GSK-3β). The carboxyl terminal region contains the transcriptional activation domain and the binding site for Teashirt [9,10]. β-catenin translocates into the nucleus, where it complexes with transcription factors of the LEF-1 family and thus regulates the expression of specific genes. By playing a dual function, i.e. a structural role in cell-cell junctions and a regulatory role in the nucleus, β-catenin can transduce changes in cell adhesion and junction formation to control transmembrane signaling and gene expression [1,11].

Sigma-RBI is pleased to offer two new phosphospecific monoclonal antibodies to β-catenin that will be of interest to researchers studying cell adhesion and junction function. Monoclonal anti-phospho-β-catenin (pSer33), Clone BC-76 (Prod. No. C 2363) was developed using a synthetic phosphorylated peptide corresponding to amino acids 32-45 (pSer33) of human β-catenin. The antibody does not detect the nonphosphorylated or the Ser33 monophosphorylated protein. Monoclonal anti-phospho-β-catenin (pSer33) recognizes human β-catenin phosphorylated at Ser33 (approximately 94 kDa). The antibody may be used in ELISA and immunoblotting applications.

Monoclonal anti-phospho-β-catenin (pSer13,37), Clone BC-22 (Prod. No. C 4231) was developed using a synthetic peptide corresponding to amino acids 32-45 (pSer13,37) of human β-catenin. Monoclonal anti-phospho-β-catenin (pSer13,37) reacts specifically with β-catenin dually phosphorylated at (pSer13,37) [12]. The antibody does not detect the nonphosphorylated or the (pSer13) monophosphorylated protein. Anti-phospho-β-catenin (pSer33,37) recognizes human, rat, mouse and chicken β-catenin phosphorylated at (pSer33,37). It does not recognize phosphorylated plakoglobin despite the high homology in the phosphorylation site with β-catenin [12]. The product may be used in ELISA, immunofluorescence [12] and immunoblotting applications [12,13].

Monoclonal antibodies reacting specifically with β-catenin phosphorylated at Ser33 and Ser37 should prove to be extremely useful tools for determining the distribution and interactions of phosphorylated β-catenin and for defining the role of β-catenin phosphorylation in signal transduction.

Related Antibodies

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
<th>Species</th>
<th>Product No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 2081</td>
<td>Anti-Catenin α (rabbit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 7082</td>
<td>Monoclonal Anti-Catenin β, Clone 6F9 (mouse)</td>
<td></td>
<td></td>
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<tr>
<td>C 7207</td>
<td>Monoclonal Anti-Catenin β, Clone 15B8 (mouse)</td>
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<td>C 2206</td>
<td>Anti-Catenin β (rabbit)</td>
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<tr>
<td>G 6414</td>
<td>Monoclonal Anti-Glycogen Synthase Kinase-3β (GSK-3β), Clone GSK-4B (mouse)</td>
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<tr>
<td>G 7914</td>
<td>Anti-Glycogen Synthase Kinase-3β (GSK-3β) (rabbit)</td>
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<tr>
<td>G 6542</td>
<td>Anti-phospho-GSK-3β (pSer9) (rabbit)</td>
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<tr>
<td>L 3275</td>
<td>Monoclonal Anti-LEF-1 (All isoforms), Clone 1C3.1D10 (mouse)</td>
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<tr>
<td>L 4020</td>
<td>Monoclonal Anti-LEF-1 (β-Catenin Binding Domain), Clone REMB1 (mouse)</td>
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<tr>
<td>L 7901</td>
<td>Monoclonal Anti-LEF-1 (HMG DNA Binding Domain), Clone REMB6 (mouse)</td>
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<td>L 4270</td>
<td>Anti-LEF-1/TCF (sheep)</td>
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<td>L 3150</td>
<td>Monoclonal Anti-LEF-1 (Transactivation Domain 236-242), Clone 3A12 (mouse)</td>
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<td>L 3276</td>
<td>Monoclonal Anti-LEF-1 (Transactivation Domain 256-276), Clone 1C3 (mouse)</td>
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<tr>
<td>P 8087</td>
<td>Monoclonal Anti-Plakoglobin (Catenin γ), Clone 15F11 (mouse)</td>
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<td>P 2732</td>
<td>Monoclonal Anti-p120γ (Catenin-related), Clone 6H11 (mouse)</td>
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<tr>
<td>T 5567</td>
<td>Monoclonal Anti-TCF-1 (T-Cell Factor-1), Clone 7H3 (mouse)</td>
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<tr>
<td>T 5692</td>
<td>Monoclonal Anti-TCF-3/4 (T-Cell Factor-3/4), Clone 6F12-3 (mouse)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References

**New Products for Cell Signaling & Neuroscience**

**ALZHEIMER’S DISEASE AND NEURODEGENERATIVE DISEASE RESEARCH**

| S 5566 | Monoclonal Anti-α Synuclein, Clone SYN11 (mouse) |

**APOPTOSIS**

| A 1351 | Anti-Acinus (rabbit) |
| A 1601 | Anti-ASC/TMS1 (rabbit) |
| B 0559 | Monoclonal Anti-BAD, mouse, Clone 64103 (rat) |
| B 0684 | Anti-BAD (rabbit) |
| B 0809 | Monoclonal Anti-Bcl-w (Minus C-Terminus), Clone 9031611 (mouse) |
| B 1059 | Bcl-w (Minus C-Terminus), human, recombinant |
| B 9934 | Bcl-xL, human, recombinant |
| B 1595 | Anti-Bmf (Bcl-2 modifying factor), C-Terminal (rabbit) |
| B 1684 | Anti-Bmf (Bcl-2 modifying factor), N-Terminal (rabbit) |
| C 7862 | Anti-CARD9 (caspase recruitment domain) (rabbit) |
| C 5737 | Monoclonal Anti-Caspase-3, Clone 84803111 (mouse) |
| C 7987 | Anti-CIDE-A (cell death-inducing DFF-like effector A), C-Terminal (rabbit) |
| D 3191 | Anti-DAP Kinase-2 (DAPK2) (rabbit) |
| D 3441 | Anti-DDC (Deleted in Colorectal Cancer), mouse (goat) |
| D 3566 | Anti-DcR1 (Decoy Receptor 1)/TRAIL-R3 (rabbit) |
| D 3316 | Anti-DEDAF (death effector domain-associated factor) (rabbit) |
| E 5654 | Anti-Endonuclease G (EndoG) (rabbit) |
| F 4428 | Anti-F1A67 (rabbit) |
| F 2928 | Monoclonal Anti-Fas Ligand, Clone 101624 (mouse) |
| F 3553 | Anti-FLASH (FLICE-associated huge protein), C-Terminal (rabbit) |
| L 1040 | Livin/ML-IAP (melanoma inhibitor of apoptosis protein), human, recombinant |
| P 4618 | Anti-PUMA/bbc3 (p53 upregulated modulator of apoptosis), C-Terminal (rabbit) |
| P 4743 | Anti-PUMA/bbc3 (p53 upregulated modulator of apoptosis), N-Terminal (rabbit) |
| R 4277 | Anti-RIP3 (receptor interacting protein) (rabbit) |
| S 5941 | SMAC/Diablo (second-mitochondria-derived activator of caspase), human, recombinant |
| S 8191 | Anti-Survivin (KD-21) (rabbit) |
| X 3378 | XIAP (X-Linked Inhibitor of Apoptosis Protein), human, recombinant |

**CELL CYCLE**

| A 6093 | Monoclonal Anti-ATM, Clone SYR604 (mouse) |
| A 6218 | Monoclonal Anti-ATM, Clone SYM16A10 (mouse) |
| M 3566 | Anti-MTBP (MDM2 binding protein), Intracellular Domain (rabbit) |
| P 4868 | Anti-p53DINP1/SIP (rabbit) |
| P 4993 | Anti-p53R2, N-Terminal (rabbit) |
| P 5243 | Anti-PERP (rabbit) |
| P 5118 | Anti-PID/MTA2 (rabbit) |
| T 2948 | Monoclonal Anti-Tob (Transducer of ErbB2), Clone 481B1 (mouse) |

**CYTOKINES, GROWTH FACTORS AND HORMONES**

| A 1726 | Anti-APRIL, Extracellular Domain (rabbit) |
| A 1851 | Anti-APRIL, Extracellular Domain 2 (rabbit) |
| B 1434 | Bone Morphogenetic Protein-7 (BMP-7), human, recombinant |
| D 2066 | DAN (Differential Screening-selected Gene Aberrative in Neuroblastoma), mouse, recombinant Antigen for the TGF-β superfamily. |
| D 2316 | DAN (Differential Screening-selected Gene Aberrative in Neuroblastoma), human, recombinant Antigen for the TGF-β superfamily. |
| E 3654 | Anti-Ephrin A, rat (goat) |
| E 4279 | Anti-Ephrin A6, mouse (goat) |
| E 4529 | Anti-Ephrin A7, mouse (goat) |
| F 4404 | Anti-Ephrin A8, mouse (goat) |
| F 5029 | Anti-Ephrin B6, mouse (goat) |
| F 5154 | Anti-Ephrin A2, mouse (goat) |
| F 5279 | Anti-Ephrin A3 (goat) |
| F 3301 | Anti-Fractalkine, mouse (goat) |
| F 2803 | Fit-3/Fc Chimera, mouse, recombinant |
| I 8657 | Interferon α, rat, recombinant |
| I 8907 | Interferon β, rat, recombinant |
| I 8782 | Interferon αA, mouse, recombinant |
| I 9032 | Interferon β, mouse, recombinant |
| I 2407 | Anti-Interleukin-3 (IL-3) Receptor (CD123) |
| I 2657 | Anti-Interleukin-5 (IL-5) Receptor (CD125) |
| I 2532 | Monoclonal Anti-Interleukin 7 (IL-7), Clone 7417.111 (mouse) |
| I 1278 | Anti-Interleukin-7 (IL-7), mouse (goat) |
| I 2907 | Monoclonal Anti-Interleukin-7 (IL-7) Receptor α, Clone 40131 (mouse) |
| I 3157 | Monoclonal Anti-Interleukin-9 (IL-9) Receptor (CD129) |
| I 3907 | Interleukin 21 (IL-21), human, recombinant |
| I 4032 | Interleukin-21 (IL-21), mouse, recombinant |
| I 5282 | Anti-Interleukin-22 Receptor (IL-22R), Intracellular Domain (rabbit) |
| I 4157 | Interleukin-21 Receptor (IL-21R)/Fc Chimera, mouse, recombinant |
| I 5407 | Anti-Interleukin-21 Receptor (IL-21R), Extracellular Domain (rabbit) |
| I 5532 | Anti-Interleukin-21 Receptor (IL-21R), N-Terminal (rabbit) |
| I 4282 | Interleukin-22 (IL-22), human, recombinant |
| I 4407 | Interleukin-22 (IL-22), mouse, recombinant |
| I 5782 | Anti-Interleukin-22 Receptor (IL-22R), C-Terminal (rabbit) |
| I 5657 | Anti-Interleukin-22 Receptor (IL-22R), N-Terminal (rabbit) |
| L 2540 | L-368,899 Non-peptide oxytocin receptor antagonist. |
| L 0915 | Leukemia Inhibitory Factor (LIF) Soluble Receptor α, human, recombinant |
| L 0790 | Anti-Lymphotactin, human (rabbit) |
| L 1169 | Anti-Lymphotactin, mouse (rabbit) |
| L 3573 | Rank Ligand/TRANCE, human, recombinant |
| T 3823 | TROY (Toxicity and JNK inducer), mouse, recombinant |
| T 3327 | Anti-TROY (Toxicity and JNK inducer) (goat) |

**CYTOSKELTON AND EXTRACELLULAR MATRIX**

| C 2362 | Monoclonal Anti-phospho-β-Catenin (pSer3), Clone BC-76 (mouse) |
| C 2361 | Monoclonal Anti-phospho-β-Catenin [pSer33,37], Clone BC-22 (mouse) |
| D 3816 | Anti-Drebrin (rabbit) |
| M 5566 | Anti-Myosin IX (Mry5) (rabbit) |
| T 0198 | Monoclonal Anti-β-Tubulin, Clone D66 (mouse) |
| A 2476 | Anti-ADAM-8, Catalytic Domain (rabbit) |
| A 2226 | Anti-ADAM-8, C-Terminal (rabbit) |
| A 2351 | Anti-ADAM-8, Propeptide Domain (rabbit) |
| A 3537 | Anti-ADAM-9, Cytoplasmic Domain (rabbit) |
| A 3101 | Anti-ADAM-9, Propeptide Domain (rabbit) |
| A 2726 | Anti-ADAM-10, Cytoplasmic Domain (rabbit) |
| A 3226 | Anti-ADAM-10, N-Terminal (rabbit) |
| A 2851 | Anti-ADAM-10, Prohormone Convertase (rabbit) |
| A 3101 | Anti-ADAM-10, Propeptide Domain (rabbit) |
| A 2601 | Anti-ADAM-12, Cytoplasmic Domain (rabbit) |
| A 3202 | Anti-ADAM-12, N-Terminal (rabbit) |
| A 3851 | Anti-ADAM-15, Cytoplasmic Domain (rabbit) |
| A 3726 | Anti-ADAM-15, Propeptide Domain (rabbit) |
| A 3976 | Anti-ADAM-17, Propeptide Domain (rabbit) |
A 4351  Anti-ADAM-17, Cytoplasmic Domain  (rabbit)
A 4226  Anti-ADAM-17, Propeptide Domain  (rabbit)
A 4101  Anti-ADAM-19, Cytoplasmic Domain  (rabbit)
A 3601  Anti-ADAM-19, N-Terminal  (rabbit)
A 3476  Anti-ADAM-19, Propeptide Domain  (rabbit)
A 4476  Anti-ADAMTS-1, C-Terminal  (rabbit)
A 4851  Anti-ADAMTS-1, Propeptide Region  (rabbit)
A 4726  Anti-ADAMTS-4, C-Terminal  (rabbit)
E 4029  Anti-Emmprin (Extracellular Matrix Metalloproteinase Inhibitor), human  (goat)
E 4154  Anti-Emmprin (Extracellular Matrix Metalloproteinase Inhibitor, mouse  (goat)
P 6243  Anti-Procollagen C-Proteinase Enhancer Protein-1  (rabbit)
P 6118  Anti-Procollagen C-Proteinase Enhancer Protein-2  (rabbit)

G PROTEINS AND CYCLIC NUCLEOTIDES
A 4102  Anti-ASAP1/Centaurin  (mouse)
A 4227  Anti-ASAP1/Centaurin  (mouse)
B 5559  B581  Farnesyltransferase (FTase) inhibitor.
S 3317  SKF-94396  Phosphodiesterase III (PDEIII) inhibitor.

GENE REGULATION AND EXPRESSION
C 8112  Anti-CIKS/Act1, N-Terminal  (rabbit)
D 5816  Depudecin  Inhibitor of histone deacetylase (HDAC) both in vivo and in vitro; exhibits antiangiogenic activity
H 2662  Anti-Histone Deacetylase 7 (HDAC7)  (rabbit)
H 2537  Anti-Histone Deacetylase 7 (HDAC7)  (rabbit)
H 5912  Anti-phospho Histone H2AX [pSer139]  (rabbit)
I 4907  Anti-IKKz-IKK-4, C-Terminal  (rabbit)
I 5032  Anti-IKKz/NEMO, N-Terminal  (rabbit)
1906  Import lipid Signaling  Conjugated with rhodamine B and nuclear localization signaling peptide (NLS).
I 9781  Importin  β1, human, recombinant
I 9157  Anti-IRAK-M (interleukin-1 receptor-associated kinase)  (rabbit)
K 4263  Anti-KAIOS  (rabbit)
L 2167  L-165,041  PPAR β (PPAR δ) selective agonist.
N 4160  Nuclear Transport Factor 2 (NTF2), human, recombinant  Mediates the nuclear import of RanGDP.
T 8573  Monoclonal Anti-Topoisomerase I, Clone mAb1  (mouse)
T 2573  Trogotizone  PPARγ agonist; anti-diabetic thiazolidinedione with anti-inflammatory and antitumor activity; induces apoptosis via p53 pathway.

IMMUNE CELL SIGNALING
B 1184  BCMA (B Cell Maturation)/Fc Chimera, human, recombinant
B 1309  BCMA (B-Cell Maturation)/Fc Chimera, mouse, recombinant
A 6851  Monoclonal Anti-Bovine IgG-Agarose, Clone BG-18  (mouse)
C 5862  CD30/Fc Chimera, human, recombinant
C 6112  CD30 Ligand, human, recombinant
C 6237  CD30 Ligand, mouse, recombinant
C 5612  Anti-CD30 Ligand, mouse  (goat)
C 5987  Monoclonal Anti-CD40, Clone 82105  (mouse)
C 6362  CD40 Ligand, human, recombinant
C 6487  Anti-CD40 Ligand  (goat)
C 6612  Anti-CTLA-4, mouse  (goat)
D 2191  Monoclonal Anti-DC-SIGN1/CD209, Clone 120507  (mouse)
D 2566  Monoclonal Anti-DC-SIGN2/CD209L, Clone 120604  (mouse)
D 2691  Monoclonal Anti-DC-SIGN1/DC SIGN2, Clone 120612  (mouse)
G 6418  GR 144053 hydrochloride  Non-peptide glycoprotein IIb/IIa fibrinogen receptor antagonist. Sold for research purposes under agreement from GlaxoSmithKline.
H 2412  Monoclonal Anti-Hamster IgG, Clone MAH1.12  (mouse)
T 5698  Anti-TCCR (T-Cell Cytokine Receptor), N-Terminal  (rabbit)
T 5823  Anti-TCCR (T-Cell Cytokine Receptor), C-Terminal  (rabbit)

INTRACELLULAR CALCIUM SIGNALING
A 3168  W-12 hydrochloride  Calmodulin antagonist; inhibits Ca2+/calmodulin activated phosphodiesterase and myosin light chain kinase.
A 0791  W-5 hydrochloride  Used as a control against W-7 HCl (Prod. No. A 3281).

ION CHANNELS
A 5476  Agitoxin-3, Originally isolated from the venom of the scorpion L. quinquestriatus hebraeus, recombinant, expressed in E. coli. Potent blocker of Shaker voltage-gated K+ channels as well as the mammalian homologs of Shaker 1.
C 5488  Anti-Calcium Channel (α1a Subunit)  (rabbit)
C 5613  Anti-Calcium Channel (α1a Subunit)  (rabbit)
C 5728  Anti-Calcium Channel (β1 Subunit)  (rabbit)
C 5863  Anti-Calcium Channel (β1 Subunit)  (rabbit)
C 6238  Anti-Calcium Channel (γ1 Subunit)  (rabbit)
C 6363  Anti-Calcium Channel (γ2 Subunit)  (rabbit)
H 2287  Hongotoxin-1, Originally isolated from the venom of scorpion Centruroides limbatus, recombinant, expressed in E. coli. Blocks cloned and heterologously expressed KV1.1, KV1.2 and KV1.3 channels (in HEK 293 cells) with high affinity and blocks KV1.6 channels with low affinity.
L 0540  Levcromakalim (BRL 38227)  More active enantiomer of cromakalim; potent K+ATP channel opener; relaxes vascular smooth muscle in vivo and in vitro.
M 1692  MRS 1845  Store-operated calcium (SOC) channel blocker.

LIPID SIGNALING
B 9305  BW245C  Potent and selective DP prostanoid receptor agonist. Sold for research purposes under agreement from GlaxoSmithKline.
L 1167  Latanoprost  Potent, selective FP prostanoid receptor agonist.
M 2692  MK-886  Potent and specific leukotriene biosynthesis inhibitor.
P 3244  Phospholipase A2 Group XII, mouse, recombinant
G 6910  Ginkgolide B, ginkgo leaves (BN-52021)  Platelet-activating factor (PAF) receptor antagonist.
**New Products for Cell Signaling & Neuroscience**

### MULTI-DRUG RESISTANCE

**M 8316** Anti-MRP2 (Multi-Drug Resistance-Associated Protein-2) (rabbit)

### NEUROTRANSMISSION

**A 5475**
- ALX-1393
  - GlyT-2 glycine transporter inhibitor.

**A 5352**
- L-[(+)-2-Amino-6-phosphonohexanoic acid (L-AP6) Selective AMPA receptor agonist.

**A 1977** N-Arachidonoylglucine
  - Endogenous anandamide analog with analgesic activity.

**A 5227**
- Assrettin-B
  - Peptide CRF₂ corticotropin releasing-factor receptor antagonist.

**B 5559**
- BRL 52537 hydrochloride
  - κ/µ Opioid receptor agonist. Sold for research purposes under agreement from GlaxoSmithKline.

**D 8941**
- 2,6-Difluoro-4-[2-(phenylsulfonylamino)ethylthio]phenoxyacetamide (PEPA)
  - Novel, selective allosteric modulator of AMPA glutamate receptors. Sold under exclusive license from Nippon Suisan Kaisha, Ltd.

**D 8816**
- N-(3,3-Diphenylpropyl)glycinamide (N20C)
  - NMDA glutamate receptor open channel blocker.

**G 5918**
- GR 113808
  - 5-HT₁ Serotonin receptor antagonist. Sold for research purposes under agreement from GlaxoSmithKline.

**G 6043**
- GR 125487 sulfamate
  - 5-HT₂ Serotonin receptor antagonist. Sold for research purposes under agreement from GlaxoSmithKline.

**G 5792**
- GR 127935 hydrochloride
  - Potent, selective and orally active 5-HT₁B/1D serotonin receptor antagonist. Sold for research purposes under agreement from GlaxoSmithKline.

**G 8543**
- GR 46611
  - 5-HT₁D Serotonin receptor agonist. Sold for research purposes under agreement from GlaxoSmithKline.

**H 1162**
- Hemokinin-1 (HEK-1)
  - Mammalian tachykinin that acts as a full agonist at NK₁, NK₂ and NK₃ tachykinin receptors with selectivity towards the NK₁ tachykinin receptor.

**I 0783**
- Monoclonal Anti-ILK (integrin-linked kinase), Clone 65.1 (mouse)

**J 2270**
- JIP-1 Fragment 153-163 Amide
  - c-Jun N-terminal kinase (JNK) inhibitor.

**N 2661**
- Monoclonal Anti-NAK (NF-κB Activating Kinase), Clone NAK369 (mouse)

**N 2911**
- Monoclonal Anti-NCK-2, Clone 8.8 (mouse)

**S 0193**
- SKF-86002
  - p38 MAP kinase inhibitor.

**S 3442**
- SB-216763
  - Glycogen synthase kinase-3 (GSK-3) inhibitor. Sold for research purposes under agreement from GlaxoSmithKline.

**S 3562**
- SB-415286
  - Glycogen synthase kinase-3 (GSK-3) inhibitor. Sold for research purposes under agreement from GlaxoSmithKline.

**S 7062**
- SB-202190
  - Potent, selective, cell permeable p38 MAP kinase inhibitor. Sold for research purposes under agreement from GlaxoSmithKline.

**T 2949**
- Anti-mTOR (FRAP) (mammalian target of rapamycin) (rabbit)

**Z 4626**
- 2M 39923
  - Selective janus kinase 3 (JAK3) inhibitor.

### NITRIC OXIDE AND CELL STRESS

**A 6093**
- Monoclonal Anti-ATM, Clone SYR6D4 (mouse)

**A 6216**
- Monoclonal Anti-ATM, Clone SYML6A10 (mouse)

**E 5654**
- Anti-Endonuclease G (EndoG) (rabbit)

**F 5303**
- Monoclonal Anti-Falkor/PHD1, Clone FLK-49 (mouse)

**N 2786**
- Anti-Nedd8 (rabbit)

**S 4441**
- D,L Sulforaphane
  - Phase II enzyme inducer.

**S 6317**
- L-Sulforaphane
  - Phase II enzyme inducer.

### PHOSPHORYLATION

**C 8863**
- 7-Cyclopentyl-5-(4-phenoxy)phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamine
  - Potent and selective inhibitor of Ick, a src family tyrosine kinase.

**F 4425**
- Fostriein sodium (PD 110,161; CI-920) from Streptomyces pulveraceus
  - Potent PP2A inhibitor.

**J 2270**
- JIP-1 Fragment 153-163 Amide
  - c-Jun N-terminal kinase (JNK) inhibitor.

**N 2661**
- Monoclonal Anti-NAK (NF-κB Activating Kinase), Clone NAK369 (mouse)

**N 2911**
- Monoclonal Anti-NCK-2, Clone 8.8 (mouse)

**10783**
- Monoclonal Anti-ILK (integrin-linked kinase), Clone 65.1 (mouse)

**S 0193**
- SKF-86002
  - p38 MAP kinase inhibitor.

**S 3442**
- SB-216763
  - Glycogen synthase kinase-3 (GSK-3) inhibitor. Sold for research purposes under agreement from GlaxoSmithKline.

**S 3562**
- SB-415286
  - Glycogen synthase kinase-3 (GSK-3) inhibitor. Sold for research purposes under agreement from GlaxoSmithKline.

**S 7062**
- SB-202190
  - Potent, selective, cell permeable p38 MAP kinase inhibitor. Sold for research purposes under agreement from GlaxoSmithKline.

**T 2949**
- Anti-mTOR (FRAP) (mammalian target of rapamycin) (rabbit)

**Z 4626**
- 2M 39923
  - Selective janus kinase 3 (JAK3) inhibitor.
New Product Highlights

**Troglitazone: A potent and selective PPARγ agonist**

Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors that belong to the nuclear hormone receptor superfamily. These receptors play an important role in many cellular functions including lipid metabolism, cell proliferation, cell differentiation, adipogenesis and inflammatory signaling [1,2]. Currently, three PPAR subtypes have been identified and are referred to as PPARα, PPARβ (also known as PPARδ) and PPARγ. PPARγ is the most studied of the three subtypes on account of its role in adipocyte differentiation as well as its involvement in glucose and lipid metabolism [2]. Thus, this receptor has become an important drug target for the treatment of various diseases including diabetes, cancer, atherosclerosis and hypertension [2-6].

Troglitazone was approved for the treatment of insulin resistance and hyperglycemia in Type II diabetes, but was removed from the market due to its liver toxicity. However, the preclinical data suggest that troglitazone should serve as an important research tool for elucidating the role of PPARγ in various metabolic diseases.

In addition to troglitazone, Sigma-RBI is pleased to provide several other PPAR research tools, specifically GW9662 (Prod. No. M 6191), a selective PPARγ antagonist, GW1929 (Prod. No. G 5668), a PPARα agonist and GW7647 (Prod. No. G 6793), a PPARγ agonist. These products are sold for research purposes only, pursuant to an agreement from GlaxoSmithKline.

Sigma-RBI is pleased to offer Troglitazone (Prod. No. T 2573) a member of the thiazolidinedione (TZD) class of anti-diabetic agents commonly referred to as “glitazones”. These compounds were the first agents to be identified as high affinity PPARγ agonists and include ciglitizone (Prod No. C 3974), rosiglitazone and pioglitzone. Using a cell-based PPAR-GAL4 transactivation assay, troglitazone was shown to be a selective PPARγ agonist displaying EC_{50} values of 780 nM and 550 nM for murine and human receptors, respectively [2]. In this same assay, troglitazone was inactive against both mouse and human PPARα and PPARβ receptors at concentrations up to 10 µM [2]. In a separate study, troglitazone exhibited a dose-dependent effect on cell cycle arrest as well as apoptosis in several hepatocarcinoma cell lines with an EC_{50} value of 10 µM [6].

**NBI 27914: A potent, selective, non-peptide CRF₁, corticotropin-releasing factor receptor antagonist**

Corticotropin-releasing factor (CRF) plays an important role in the regulation of the hypothalamic-pituitary-adrenal axis. In response to a variety of stressors, CRF causes the release of hormones such as adrenocorticotropic hormone (ACTH, Prod. No. O 2275) and hydrocortisone (Cortisol, Prod. No. H 5885). In addition, clinical findings support the hypothesis that dysfunction of the CRF system is implicated in certain stress-related neuropsychiatric disorders such as anxiety and depression [1]. The effects of CRF are mediated through two receptor types referred to as CRF₁ and CRF₂ (CRF₂α and CRF₂β). CRF₁ receptors, unlike CRF₂ receptors, are widely distributed throughout the central nervous system [2]. Recently, emphasis has been placed on developing non-peptide CRF₁ receptor antagonists as potential therapeutic agents.

Sigma-RBI is pleased to introduce NBI 27914 (Prod. No. N 3911), a potent and selective non-peptide CRF₁ receptor antagonist [3]. NBI 27914 binds to the CRF₁ receptor with high affinity with a Kᵢ value of 1.7 nM and appears to be devoid of activity at the human CRF₂ receptor [4]. It inhibits CRF-mediated increases in adenylyl cyclase activity and ACTH release from rat anterior pituitary cells with EC_{50} values of 150 nM and 70 nM, respectively [5]. In addition, when administered centrally in rats, NBI 27914 increases the latency and decreases the duration of CRF-induced seizures [6].

NBI 27914 is therefore a selective tool with which to study the function of CRF₁ receptors and should prove useful in elucidating the contribution of CRF to the genesis of neuropsychiatric disorders.

**References**

New Product Highlights

MRS 1845: A blocker of store-operated calcium (SOC) entry that does not activate intracellular calcium release

Cytosolic calcium acts as a ubiquitous second messenger and is involved in the regulation of a myriad of cellular processes ranging from growth and differentiation to cell death and apoptosis. Calcium signals are generated by both the release of stored calcium from the endoplasmic reticulum and the influx of extracellular calcium across the plasma membrane. While the release of stored calcium is generally mediated by inositol 1,4,5-trisphosphate (IP₃, Prod. No. I 7012) and cyclic ADP ribose (cADPR, Prod. No. C 7344), influx of extracellular calcium can be directed through various mechanisms [1,2]. The entry of extracellular calcium generally results from IP₃-related depletion of intracellular stores in a process referred to as capacitative calcium entry or store-operated calcium (SOC) entry. The mechanisms underlying SOC entry are poorly understood but may involve members of the transient receptor potential (TRP) family of channel proteins [3,4].

Efforts to study the phenomenon of SOC entry have been hampered by the lack of blockers that effectively abolish SOC entry without activating intracellular calcium release. Sigma-RBI has recently introduced MRS 1845 (N-propar-glynitrendipine, Prod. No. M 1692), an N-substituted dihydropyridine that possesses micromolar potency at SOC channels in HL-60 cells [5]. Unlike currently used SOC blockers, such as clotrimazole (Prod. No. C 6019) and SKF 96365 (Prod. No. S 7809), MRS 1845 does not activate intracellular calcium release at concentrations required to block SOC entry. MRS 1845 is therefore a valuable tool with which to further investigate the physiology and pharmacology of SOC entry.

Interleukins 21 and 22: Two new cytokines available from Sigma-RBI

Interleukin-21 (IL-21) is a novel cytokine expressed in activated T cells that is most closely related to IL-2, IL-4 and IL-15. The receptor for IL-21 (IL-21R), also called NLR for novel interleukin receptor [1,2] forms a complex with IL-2 Rγc) and mediates IL-21 signaling [3,4]. Together, IL-21 and its receptor (IL-21R) appear to play important roles in the regulation of the immune system. This complex regulates the proliferation and maturation of NK (natural killer), B and T cell populations. IL-21 and its receptor activate the JAK-STAT signaling pathway.

Interleukin-22 (IL-22), also known as IL-10-related T cell-derived inducible factor (IL-TIF), was originally identified as a derived inducible factor (IL-TIF), was originally identified as a product of IL-9 in mouse T cells and mast cells [5]. The IL-22 receptor complex consists of two receptor subunits belonging to the class II cytokine receptor family, IL-22R (formerly an orphan receptor named CRF2-9) and IL-10Rβ (formerly known as CRF2-4) [6,7]. In humans, IL-22 is produced by normal T cells upon anti-CD3 stimulation. IL-22 activates STAT1 and STAT3 in several hepatoma cell lines and upregulates the production of acute phase proteins.

Sigma-RBI is pleased to introduce both human and mouse recombinant interleukin-21 and interleukin-22 together with a series of related antibodies that will be of interest to researchers investigating the role of these important cytokines in cell signaling mechanisms.

References
New Product Highlights

SB-216763 and SB-415286: Novel, potent and selective glycogen synthase kinase-3 (GSK-3) inhibitors

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase that exists as two isozymes referred to as α and β (Prod. No. G 1663). Their catalytic domains (ATP-binding site) have about 90% identity, but the amino terminal region of GSK-3α has approximately 60 additional amino acid residues. The differences in function between the isozymes have yet to be established [1]. GSK-3 is a principal physiological substrate of protein kinase B (PKB; also known as Akt) and the activity of GSK-3 is inhibited by PKB/Akt-mediated phosphorylation in response to growth factor stimulation [1-3]. Insulin (Prod. No. I 5500) and certain growth factors, such as nerve growth factor (NGF, Prod. No. N 1408) and glial-derived neurotrophic factor (GDNF, Prod. No. G 1777), activate phosphatidylinositol 3-kinase (PI3K, Prod. No. P 8615) and its downstream effector PKB, which in turn phosphorylates and inactivates GSK-3. Inhibition of GSK-3 leads to the modulation of multiple GSK-3 regulated cellular processes including glycogen synthesis in skeletal muscle [4], neuronal cell survival [2] and alleviation of hyperglycemia via increased glycogen synthesis, even in insulin-resistant cells [3,4].

Sigma-RBI is pleased to offer two novel, potent and selective cell-permeable inhibitors of GSK-3, SB-216763 (Prod. No. S 3442) and SB-415286 (Prod. No. S 3567). Both compounds inhibit GSK-3α activity with IC_{50} values of 34 and 78 nM, respectively, and show similar potency towards purified GSK-3β [1-3]. Each compound specifically inhibits GSK-3 with no significant activity towards a panel of 24 other protein kinases, including PKB, PDK1 and CDK-2 [1-3]. In addition, both compounds potently promote survival of central and peripheral neurons in culture following treatment with the PI3K inhibitor LY-294,002 (Prod. No. L 9908) or potassium withdrawal, as measured by thiazolyl blue tetrazolium bromide (MTT, Prod. No. M 2128) assay. Maximal neuroprotection was observed with 3 µM SB-216763 or 30 µM SB-415286 [2]. Furthermore, both compounds inhibit expression of the glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) genes, which leads to increased glycogen synthesis [3,4].

SB-216763 and SB-415286 will prove to be useful tools in studying both the PKB/Akt pathway and GSK-3 regulated processes.

References

NHN O

O2N

HO

Cl

SB-415286
(Prod. No. S 3567)

SB-216763
(Prod. No. S 3442)

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