CALBIOCHEM®

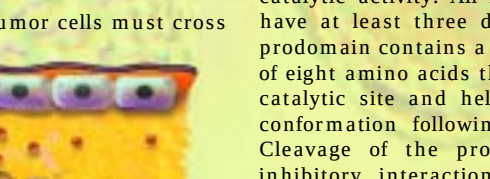
biologics

Volume 26 Number 1, Spring 2000

Tumor Metastasis:

Role of Cell Adhesion and Matrix Metalloproteases

Tumor invasion and metastasis largely depend on the interaction between the tumor cells, the extracellular matrix (ECM), and the blood vessels surrounding the tumor. The ECM, in addition to providing structural compartmentalization, is a dynamic matrix of structural proteins, growth factors, and latent enzymes that undergo constant remodeling. Matrix metalloproteases (MMPs) maintain the integrity of ECM by removing undesired proteins; however, overexpressed MMPs play a critical role in tumor invasion and metastasis. Tumor invasion requires a decreased affinity between tumor cells or between tumor cells and the ECM that facilitates their release from the primary tumor mass. Within a primary tumor mass, cell-cell adhesion is mediated by E-cadherin in combination with α- and β-catenin and other cytoplasmic components that link E-cadherin to the cytoskeleton. Aberrant N-glycosylation of E-cadherins can lead to increased motility and a reduction in adhesion between tumor cells resulting in release of these cells from the primary tumor mass that may ultimately lead to metastasis.



For metastasis to occur, tumor cells must cross the basal membrane and stroma to gain access to the blood stream. Those tumor cells that escape the immune system leave the blood vessels to form new colonies from the primary tumor. Invasion and metastasis are highly complex, yet well-coordinated, processes and can be summarized under the following scheme (Figure): (a) release of tumor cells from primary tumor mass; (b) adhesion of tumor cells to extracellular matrix and basement membrane; (c) hydrolytic activities (proteases and endoglycosidases) of tumor cells for destruction of extracellular matrix and basement membrane; (d) migration of tumor cells through the degraded matrix and into the blood circulation; (e) interaction of tumor cells with platelets and activation of platelets to express P-selectin, ICAM, and other adhesion proteins; (f) selectin and ICAM-dependent tumor cell adhesion to endothelial cells followed by extravasation; and (g) formation of metastatic deposit.

The proteolytic degradation of the ECM by tumor cells requires the action of highly specialized MMPs that are expressed in a cell- or tissue-specific pattern. They also play important roles in wound healing, angiogenesis, embryogenesis, and in pathological processes such as tumor invasion and metastasis. About 20 different types of MMPs have been identified and classified based on their protein domain structures. They are characterized by the presence of a zinc ion in the active site, which is required for their catalytic activity. All MMPs sequenced to date have at least three domains in common. The prodomain contains a highly conserved segment of eight amino acids that folds over to cover the catalytic site and helps maintain the inactive conformation following the release of MMPs. Cleavage of the prodomain destabilizes the inhibitory interaction between the unpaired cysteine in the sequence and the active site zinc atom. The catalytic domain contains the conserved structural metal-binding sites consisting of 106 to 119 residues. MMPs also contain a highly conserved zinc-binding active site domain containing 52 to 58 amino acids. The zinc-binding domain contains three histidine residues that occupy three of the

Look for...

Newly Updated Protein Kinases & Related Products Guide in this Issue.

In This Issue:

Cell Adhesion & MMPs 1
Inhibitors of Matrix Metalloproteases 2
Fluorogenic Substrates for MMP 2

Angiogenesis 3
Angiogenin & Angiostatic Proteins 3

Cytoskeletal Research Tools 3
NEW! Jasplakinolide, Monastrol, Indanocine and Ilimaquinone 3
NEW! Antibodies 3

Apoptosis 4
NEW! Caspase Assay Kits 4
Antibodies for Apoptosis Research 4
Triptolide and Pifithrin-α 4

Glycobiology 5
Antibodies to Cadherins 5
Antibodies to Catenins 5
NEW! NANA Quantitation Kit 5

Nitric Oxide & Oxid. Stress 6
Photolabile NO Donors 6
NEW! Cyclooxygenase Inhibitors 6

Neurochemicals 7
NEW! Anandamide Analogs 7
Antibodies to Metabotropic Glutamate Receptors 7

G-Proteins/Cell Adhesion 8
New Fusion Proteins 8
New Monoclonal Antibodies 8
GGTase & FTase Inhibitors 8

Antibodies to Signaling Molecules 9
Anti-EphB1, Vav2, and SLP-76 9

Biochemicals 9-10
NEW! Protein Phosphatase Inhibitors 9
NEW! ThioGlo™ Maleimide Reagents 10
Endotoxin-Free Proteases 10
TAFI 10

Meet Our Family! 11

Our Newest and Upcoming Publications! 12

Check our Web Site for the Latest Products & Information
www.calbiochem.com

...continued from page 1

coordination sites of the active site Zn^{2+}. Additional domains, such as transmembrane and gelatin-binding domains, have also been reported on some MMPs. In addition to these, the hemopexin like domain found in all MMPs (except MMP-7) plays a role in substrate specificity.

The activation of MMPs is dependent mainly on urokinase-type (uPA) and tissue-type plasminogen activators (tPA) that cleave plasminogen into active plasmin. The activity of these plasminogen activators is regulated by the levels of PAI-1 and PAI-2 that bind to the uPA receptor on the cell membrane and prevent the activation of uPA. Plasmin initiates an activation cascade by cleaving proMMP-1 and -3 into MMP-1 and -3, respectively. A major control point in the regulation of active enzyme is inhibition of the active form by the TIMP family of inhibitors (21 - 28 kDa). TIMPs regulate the function of MMPs either by inhibiting active MMPs or by controlling their activation process. They form tight, non-covalent inhibitory complexes with MMPs (K_d = 10 - 50 pM). During inhibition, the terminal amino group of the TIMP fills the fourth coordination site of the active site zinc.

Tumor growth beyond a few millimeters in diameter requires angiogenesis, the growth of new blood vessels, to bring oxygen and nutrients for tumor growth. ECM degradation by MMPs releases growth factors, such as VEGF and bFGF that stimulate endothelial cells to form new blood vessels. These growth factors also promote the synthesis and release of collagenases and plasminogen activators by the endothelial cells of blood vessels surrounding the tumor.

MMPs facilitate tumor cell invasion and metastasis by at least three distinct mechanisms: (a) by eradicating physical barriers to invasion through degradation of collagens, laminins, and proteoglycans in the ECM; (b) by modulating cell adhesion and enabling cells to form new cell-to-cell and cell-to-matrix attachments, while breaking the existing ones; and (c) by acting on ECM components and other proteins to expose hidden biological activities, such as release of angiostatin from plasminogen. In normal adults, MMP expression is very low except in rapidly remodeling tissue, such as wound healing and menstrual endometrium. Many control elements, such as secretion of MMPs in their latent form and the presence of TIMPs, tend to keep MMPs inactive in the ECM. MMP activity is also controlled by PAIs that diminish the activity of uPA and tPA. A discussion on the role of these various inhibitors in tumor progression is beyond the scope of this brief article.

From the foregoing account, it is evident that increased expression and activation of MMPs are necessary for ECM degradation. In tumor cell invasion and metastasis, a tightly controlled balance between the active proteases and their inhibitors appears to be essential for preventing invasion. A wide variety of MMP inhibitors have been designed and developed for use as cytostatic agents that show promise as chemotherapeutics in patients with malignant tumors. Monitoring changes in the activity of MMPs and TIMPs offer great prognostic value in determining the outcome of chemotherapy.

References:

Forget, M.A., et al. 1999. *Can. J. Physiol. Pharmacol.* **77,** 465.
Streuli, C. 1999. *Curr. Opin. Cell Biol.* **11,** 634.
Kleiner, D.E., and Stetler-Stevenson, W.G. 1999. *Cancer Chemother. Pharmacol.* **43** (suppl.), S42.
Kugler, A. 1999. *Anticancer Res.* **19,** 1589.
Murphy, G., and Gavrilovic, J. 1999. *Curr. Opin. Cell Biol.* **11,** 614.
Westermarck, J., and Kahari, V.M. 1999. *FASEB J.* **13,** 781.
Blavier, L., et al. 1999. *Ann. N.Y. Acad. Sci.* **878,** 108.
Chintala, S.K., and Rao, J.S. 1999. *Int. J. Dev. Neurosci.* **17,** 495.
Ellerbroek S.M., and Stack, M.S. 1999. *BioEssays* **21,** 940.
Glinsky, G.V. 1998. *Cancer Metast. Rev.* **17,** 177.
Toi, M., et al. 1998. *Breast Cancer Res. Treat.* **52,** 113.
Noël, A., et al. 1997. *Invasion Metastasis* **17,** 221.
Hakomori, S. 1996. *Cancer Res.* **56,** 5309.
Mannori, G., et al. 1995. *Cancer Res.* **55,** 4425.
Matrisian, L.M. 1992. *BioEssays* **14,** 455.
Springman, E.B., et al. 1990. *Proc. Natl. Acad. Sci. USA* **87,** 364.

NEW! Inhibitors of Matrix Metalloproteases

Product	Cat. No.	Comments	Size
GM 1489	364200	K_i = 200 pM for MMP-1, 500 nM for MMP-2, 20 µM for MMP-3, 100 nM for MMP-8, and 100 nM for MMP-9	1 mg 5 mg
GM 6001	364205	K_i = 400 pM for MMP-1, 500 pM for MMP-2, 27 nM for MMP-3, 100 pM for MMP-8, and 200 pM for MMP-9	1 mg 5 mg
GM 6001, Negative Control	364210	Negative control	1 mg 5 mg
MMP-2 Inhibitor I	444244	K_i = 1.7 µM	10 mg
MMP-2/MMP-3 Inhibitor I	444239	K_i = 17 µM for MMP-2 and 290 nM for MMP-3	5 mg
MMP-2/MMP-3 Inhibitor II	444240	K_i = 4.5 µM for MMP-2 and 520 nM for MMP-3	2 mg
MMP-2/MMP-9 Inhibitor I	444241	IC_{50} = 310 nM for MMP-2 and 240 nM for MMP-9	5 mg
MMP-3 Inhibitor II	444225	K_i = 130 nM	5 mg
MMP-3 Inhibitor III	444242	K_i = 3.2 µM	2 mg
MMP-3 Inhibitor IV	444243	K_i = 810 nM	2 mg
MMP-8 Inhibitor I	444237	IC_{50} = 4 nM	1 mg
MMP-8 Inhibitor I, Negative Control	444238	Negative control	1 mg

Fluorogenic Substrates For Matrix Metalloproteases

MMP Substrate, Fluorogenic	**444207**	1 mg	MMP-2/MMP-9 Substrate I, Fluorogenic	**444215**	5 mg
MMP-1 Substrate I, Fluorogenic	**444211**	5 mg	MMP-3 Substrate I, Fluorogenic	**444220**	5 mg
MMP-1 Substrate III, Fluorogenic	**444219**	1 mg	MMP-3 Substrate II, Fluorogenic	**444223**	500 µg
MMP-1/MMP-9 Substrate, Fluorogenic	**444221**	1 mg	MMP-7 Substrate, Fluorogenic	**444228**	1 mg
MMP-2 Substrate, Fluorogenic	**444212**	1 mg	MMP-8 Substrate, Fluorogenic	**444230**	5 mg
MMP-2/MMP-7 Substrate, Fluorogenic	**03-32-5032**	1 mg	MMP-13 Substrate, Fluorogenic	**444235**	1 mg

Introducing.....*New* Tools for Angiogenesis Research

Tumor angiogenesis is a multi-step process consisting of degradation of the basement membrane at the post-capillary venule, migration of endothelial cells to the tumor, proliferation of endothelial cells, canalization and branching, and formation of new basement membrane. Angiogenesis is required for proper nourishment and removal of metabolic wastes from tumor sites. Under physiological conditions, angiogenesis is a highly-regulated phenomenon under the control of angiogenic stimulators and inhibitors. Dormant tumors secrete inhibitory factors such as angiostatin, thrombospondins, and TIMPs that prevent tumors from switching to the angiogenic phenotype. Tumor-induced angiogenesis begins with MMP-induced dissolution of the basement membrane surrounding a pre-existing blood vessel. The non-mitotic endothelial cells then migrate towards the tumor through the disintegrated extracellular matrix. Anti-angiogenic therapy represents a promising approach to cancer treatment. The efficacy of endogenous angiogenesis inhibitors, including angiostatin and endostatin, has been demonstrated in many types of solid tumors in animal models.

Angiogenin, Human, Recombinant, *E. coli*

A plasma protein with angiogenic and ribonucleolytic activities. Binds specifically to aortic smooth muscle cells, activates second messenger pathways, and inhibits their proliferation. M.W. 14,000.

Cat. No. 175600 50 µg

Ref.: Hatzi, E., et al. 2000. *Biochem. Biophys. Res. Commun.* **267,** 719.

Angiostatin® Protein, Human

A proteolytic fragment of plasminogen containing kringles 1-4. Inhibits bFGF-induced endothelial cell proliferation. M.W. 50,000.

Cat. No. 176700 500 µg

Ref.: Sten-Linder M., et al. 1999. *Anticancer Res.* **19**, 3409.

Angiostatin K1-3, Human

Exhibits higher anti-angiogenic activity (ED_{50} = 70 nM) than to the kringles 1-4 (ED_{50} = 135 nM). M.W. 38,000.

Cat. No. 176705 250 µg

Ref.: Cao, Y., et al. 1997. *J. Biol. Chem.* **272,** 22924.

Angiostatin K1-5, Human

Inhibits endothelial cell proliferation with over 50-fold greater inhibitory activity (IC_{50} = 50 pM) as compared to angiostatin. M.W. 57,000.

Cat. No. 176706 250 µg

Ref.: Cao, R., et al. 1999. *Proc. Natl. Acad. Sci. USA* **96,** 5728.

Endostatin™, Human, Recombinant, *Pichia pastoris*

A C-terminal fragment of collagen XVIII that acts as a specific inhibitor of endothelial cell proliferation, migration, and angiogenesis. M.W. 20,000.

Cat. No. 324746 250 µg
1 mg

Ref.: Cirri, L., et al. 1999. *Int. J. Biol. Markers* **14,** 263.

NEW.....Cytoskeletal Research Tools

Jasplakinolide, *Jaspis johnstoni*

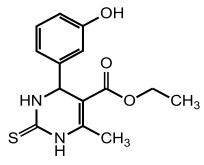
A cyclodepsipeptide with antifungal and anti-tumor properties. Stabilizes F-actin and reorganizes actin filaments into a tight cortical layer adjacent to the plasma membrane. Prevents activation of store-mediated Ca^{2+} entry into cells. Reported to inhibit the growth of Lewis lung carcinoma and prostate carcinoma cell lines (IC_{50} = 65 - 170 nM). M.W. 709.7.

Cat. No. 420107 100 µg

Ref.: Rosado, J.A., et al. 2000. *J. Biol. Chem.* **275,** 7527; Tilney, L.G., et al. 2000. *J. Cell Sci.* **113,** 1255; Bubb, M.R., et al. 2000. *J. Biol. Chem.* **275,** 5163; Takeuchi, H., et al. 1998. *Cancer Chemother. Pharmacol.* **42,** 491; Senderowicz, A.M., et al. 1995. *J. Natl. Cancer Inst.* **87,** 46.

Monastrol

A potent, cell-permeable, nontubulin-interacting mitosis inhibitor. Arrests mammalian cells in mitosis with monopolar spindles. *In vitro*, it specifically inhibits the motility of the mitotic kinesin Eg5, a motor protein involved in the assembly and maintenance of the mitotic spindle (IC_{50} = 14 µM). M.W. 292.4.



Cat. No. 475879 1 mg
5 mg

Ref.: Mayer, T.U., et al.1999. *Science* **286,** 971.

Indanocine

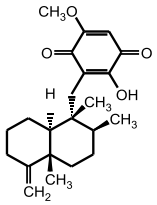
(NSC 698666). A potent cytostatic and cytotoxic agent that blocks tubulin polymerization. Reported to induce apoptosis in stationary-phase multi-drug resistant cells. M.W. 339.4.

Cat. No. 402080 1 mg

Ref.: Leoni, L.M., et al. 2000. *J. Natl. Cancer Inst.* **92**, 217.

Ilimaquinone

An anti-microbial, anti-HIV, anti-inflammatory, and anti-mitotic agent that is reported to cause the vesiculation of Golgi membranes through activation of βγ-subunits of G-protein and depolymerization of cytoplasmic microtubules. Acts as an inhibitor of RNase H activity of the reverse transcriptase of HIV-1. Also causes the disruption of AMF-R tubules in MDCK kidney cells. M.W. 358.5.



Cat. No. 401250 100 µg

Ref.: Radeke, H.S., et al. 1999. *Chem. Biol.* **6,** 639; Feldman, P.A., et al. 1997. *J. Membr. Biol.* **155,** 275; Jamora, C., et al. 1997. *Cell* **91,** 617; Loya, S., and Hizi, A. 1993. *J. Biol. Chem.* **268,** 9323; Wang, H.-J., et al. 1997. *J. Cell Sci.* **110,** 3043.

Caspases: A Group of Novel Therapeutic Targets

Caspases are a group of cysteine proteases that play a crucial role in apoptotic pathways induced by a variety of stimuli. Caspases are activated either by ligand binding to a death receptor that leads to rapid induction of initiator caspases or by mild cytotoxic stimuli, which stimulates the release of cytochrome c and apoptosis-activating factor from mitochondria in a protracted manner. However, it is not clear if the activation of caspases by either of these mechanisms represents a "point of no return" in the life of a cell. Activated caspases cleave a variety of intracellular proteins including major structural elements, a number of protein kinases, and the DNA repair machinery, which disrupts cell survival pathways.

Deregulation of apoptosis pathways and of caspase activity contributes to a large number of pathological conditions, including neurodegenerative disorders, autoimmune disease, and cancer. Hence, caspases have become the prime targets for therapeutic interventions in these diseases. Several reversible (aldehyde-based) and irreversible (fluoromethyl or chloromethyl ketone-based) peptide inhibitors have been designed and developed; however, the peptide nature of these inhibitors may limit their therapeutic potential.

For Convenience, Consistency, and Reliability . . .

Try CALBIOCHEM's New Caspase Assay Kits...

Product	Cat. No.	Substrate Sequence	Price / Kit
Caspase-1 Assay Kit, Colorimetric	**218790**	YVAD-*p*NA	
Caspase-1 Assay Kit, Fluorometric	**218791**	YVAD-AFC	
Caspase-2 Assay Kit, Colorimetric	**218792**	VDVAD-*p*NA	
Caspase-2 Assay Kit, Fluorometric	**218793**	VDVAD-AFC	
Caspase-5 Assay Kit, Colorimetric	**218804**	WEHD-*p*NA	

Product	Cat. No.	Substrate Sequence	Price / Kit
Caspase-5 Assay Kit, Fluorometric	**218801**	WEHD-AFC	
Caspase-6 Assay Kit, Colorimetric	**218802**	VEID-*p*NA	
Caspase-6 Assay Kit, Fluorometric	**218803**	VEID-AFC	
Caspase-10 Assay Kit, Colorimetric	**218810**	AEVD-*p*NA	
Caspase-10 Assay Kit, Fluorometric	**218811**	AEVD-AFC	

Each kit is provided with a detailed protocol. Each kit is suitable for up to 100 assays.

NEW! Antibodies for Apoptosis Research

Anti-ARC, C-Terminal (191-208), Human (Rabbit).
Apoptosis Repressor with Caspase recruitment domain (ARC) interacts with caspase-2 and caspase-8. Inhibits the enzymatic activity of caspase-8 and blocks apoptosis. A synthetic peptide corresponding to amino acids 191 - 208 of human ARC protein [(C)PDPEPEPDFEERDESEDS], coupled to KLH, was used as an immunogen.

Cat. No. 181320 100 µg

Anti-Bim (22-40), Human (Rabbit). Bim is a BH3 domain-containing protein that induces apoptosis. Acts as a death signal that neutralizes the action of selected members of the Bcl-2 sub-family. A synthetic peptide corresponding to amino acids 22-40 of human Bim protein [(C)AERPPQLRPGAPTSLQTEP], coupled to KLH, was used as an immunogen.

Cat. No. 202000 100 µg

Anti-CAD (205-221), Mouse (Rabbit). CAD (Caspase-Activated Deoxyribonuclease) causes DNA fragmentation. A synthetic peptide corresponding to amino acids 205 - 221 of murine CAD protein (YNGSYFDRGAEASSRLC), coupled to KLH, was used as an immunogen.

Cat. No. 205543 100 µg

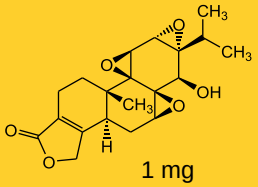
Anti-CD95L, Human (Mouse), Fluorescein Conjugate.
Soluble human Fas Ligand was used as an immunogen. Recognizes membrane bound and soluble human Fas ligand. Sufficient for 120 tests at 80 µl /test.

Cat. No. 217952 120 T

Introducing.....

Triptolide, *Tripterygium wilfordii*

An immunosuppressive agent with anti-inflammatory and anti-tumor properties. Blocks TNF-α-mediated induction of c-IAP1 and c-IAP2 and NF-κB activation. Reported to induce apoptosis in T cells by activating DEVD-cleaving caspases.



Cat. No. 645900 1 mg
5 mg

Ref.: Lee, K.Y., et al. 1999. *J. Biol. Chem.* **274,** 13451; Yang, Y., et al. 1998. *Immunopharmacology* **40,** 139.

Now Available.....

p53 Inhibitor *Pifithrin-α*

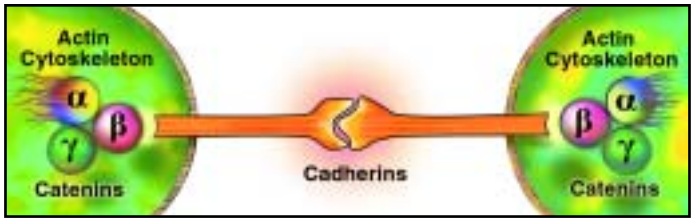
A new reversible inhibitor of p53 that inhibits p53-mediated apoptosis and protects normal tissues from deleterious side-effects of chemotherapeutic agents. Does not exhibit any effects on p53-deficient animals and is not reported to induce any visible tumors.

Cat. No. 506132 5 mg 10 mg

Ref.: Komarova, E.A., and Gudkov, A.V. 2000. *Biochemistry (Mosc.)* **65**, 41; Komarov, P.G., et al. 1999. *Science* **285**, 1733.

Cadherins: Transmembrane Adherins Regulating Tumor Invasion

Cadherins, a family of Ca^{2+}-dependent transmembrane glycoproteins, function in mediating cell-cell adhesion in virtually all multicellular organisms by homotypic interaction, although heterotypic binding between different cadherin molecules has also been reported. They are subdivided into several subclasses. E (epithelial)- and P (placental)-cadherins are involved in the selective adhesion of epidermal cells. E-cadherin is expressed on the cell surfaces of all epidermal layers and P-cadherin is expressed only on the surfaces of basal cells. At the cellular level, E-cadherin is concentrated at the adherens junction and interacts with E-cadherin molecules of adjacent cells. According to the crystal structure, individual cadherin molecules cooperate to form a linear cell adhesion zipper. The dynamic association with another group of proteins, termed catenins, regulate the intracellular anchorage of cadherins. This complex formation regulates the adhesive function of cadherins, most likely by connecting cadherins with actin microfilaments.



Reduced expression of E- and P-cadherins on invasive neoplastic cells has been demonstrated in a variety of tumors and is considered to be an important prognostic factor in disease progression. A diminution in cell adhesion contributes to the release of cancer cells from the primary tumor mass that may ultimately result in metastasis.

Ref.: Vleminckx, K., and Kemler, R. 1999. *BioEssays* **21,** 211; Furukawa, F., et al. 1997. *Microsc. Res. Tech.* **38,** 343; Paul, R., et al. 1997. *Br. J. Urol.* **79** (Suppl. 1), 37; Aberle, H., et al. 1996. *J. Cell. Biochem.* **61,** 514; Shiozaki, H., et al. 1996. *Cancer* **77** (Suppl.),1605; Kemler, R. 1992. *Semin. Cell Biol.* **3,** 149.

New Antibodies to Cadherins

Anti-E-Cadherin, Human (Mouse MoAb)
Cat. No. 205601 100 µg

Anti-E-Cadherin, Human Placenta (Mouse MoAb)
Cat. No. 205602 100 µg

Anti-E-Cadherin, Mouse (Rat)
Cat. No. 205603 100 µg

Anti-E-Cadherin, Mouse Liver (Rat)
Cat. No. 205604 100 µg

Anti-N-Cadherin, Chick Embryo (Rat)
Cat. No. 205605 100 µg

Anti-N-Cadherin (808-827), Human (Rabbit)
Cat. No. 205606 100 µg

Anti-P-Cadherin, Human (Mouse MoAb)
Cat. No. 205607 100 µg

Anti-P-Cadherin, Mouse (Rat)
Cat. No. 205608 100 µg

Anti-P,E,N-Cadherin, Human (Rabbit)
Cat. No. 205609 100 µg

New Antibodies to Catenins

Anti-α-Catenin, Mouse (Mouse MoAb)
Cat. No. 219351 100 µg

Anti-β-Catenin (Core) Mouse (Mouse MoAb)
Cat. No. 219353 100 µg

Anti-β-Catenin (Exon 3), Mouse (Mouse MoAb)
Cat. No. 219354 100 µg

Anti-β-Catenin C-Terminal (Exon 14), Mouse (Mouse MoAb)
Cat. No. 219355 100 µg

Anti-β-Catenin C-Terminal (769-781), Mouse (Mouse MoAb)
Cat. No. 219356 100 µg

Anti-β-Catenin N-Terminal, Mouse (Mouse MoAb)
Cat. No. 219357 100 µg

N-Acetylneuraminic Acid (NANA) Quantitation Kit

A kit for rapid and accurate assay of NANA (sialic acid). Contains all reagents required for quantitation of free NANA as well as that found in glycoproteins. *Sensitivity: 1 nmol. Suitable for up to 25 assays.*

Cat. No. 110192 1 kit

NEW!.... Fast, Simple, and Accurate

NEW! High Titer Antibody to Human P-Glycoprotein

Recognizes the NH_2-terminal sequence of P-glycoprotein. Detects nanogram quantities of P-glycoprotein in Western blots.

Cat. No. 517309 50 µl

Ref.: Nuti, S. L., et al. 2000. *Biochemistry* **39,** 3424.

Western blot analysis of Sf9 cell membrane fraction. The antibody was used at 1:40,000 dilution. Lanes 1 and 5 were loaded with 5 µg of control membrane fraction. Lanes 2, 3, and 4 were loaded with 5, 2.5, and 1 µg of cell membrane fraction expressing P-glycoprotein. For immunofluorescence, use 1:20,000 dilution.

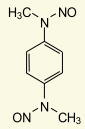
Bis-N-Nitroso Caged Nitric Oxide: Photolabile Nitric Oxide Donors

NEW!

CALBIOCHEM® introduces three novel caged nitric oxide compounds that act as photolabile NO donors and are suitable for precise delivery of nitric oxide into intracellular compartments in microsecond time scale without any cytotoxic effects. Upon irradiation with a xenon lamp or laser flashlight (300 - 360 nm), these donors produce two NO molecules per molecule of BNN. Thiols present in biological systems do not interfere with the release of NO from these caged compounds. They exhibit stability over a wide physiological range of pH.

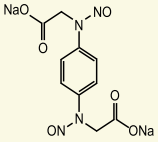
Ref: Namiki, S., et al. 1999. *Bioorg. Med. Chem.* **7,** 1695; Namiki, S., et al. 1997. *J. Am. Chem. Soc.* **119,** 3840.

BNN3 (Cell-permeable) (N,N´-Dimethyl-N,N´-dinitroso-*p*-phenylenediamine).



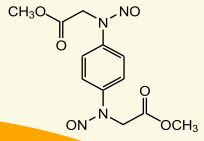
Cat. No. 203420 1 mg

BNN5Na (N,N-Dicarboxymethyl-N,N´-dinitroso-*p*-phenylenediamine, disodium)



Cat. No. 203421 1 mg

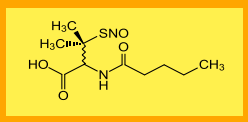
BNN5 Methyl Ester (Cell-permeable) (N,N-Dinitroso-*p*-phenylenediamine-N,N´-diacetic acid dimethyl ester)



Cat. No. 203422 1 mg

SNVP

(S-Nitroso-N-valeryl-D,L-penicillamine). Exhibits higher lipophilicity and greater stability in solution than SNAP (Cat. No. 487910). Its decompostion is accelerated by the presence of Cu(II) and cysteine. M.W. 262.3.



Cat. No. 487921 10 mg

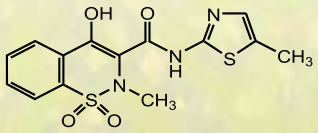
Ref.: Megson, I.L., et al. 1999. *Br. J. Pharmacol.* **126,** 639.

New! Cyclooxygenase Inhibitors

Cyclooxygenases (COX) act on arachidonic acid to generate prostaglandins. They exist either as COX-1 or COX-2. COX-1 is a constitutive enzyme that is associated with the endoplasmic reticulum and is responsible for maintaining normal physiologic function. It is found throughout the body, especially in the GI tract, kidneys, and platelets. Inhibition of COX-1 by NSAID is reported to cause GI damage, renal dysfunction, and platelet abnormalities. COX-2 is an inducible enzyme that is mainly associated with the nuclear envelope. Its activity is induced by several growth factors, cytokines, and inflammatory signals. COX-2 expression is markedly increased in 85 - 90% of human colorectal adenocarcinomas, whereas COX-1 levels remain unchanged. Based on structural differences in the active sites of these isozymes, new drugs have been developed that specifically inhibit only the COX-2 activity. COX-2 inhibitors have the potential to provide the traditional benefits of NSAID with significantly reduced incidence of endoscopic ulcers. Hence, they offer great therapeutic promise in the treatment of inflammation and cancer.

Ref.: Fournier, D.B., and Gordon, G.B.. 2000. *J. Cell Biochem.* **77,** 97; Crofford, L.J., et al. 2000. *Arthritis Rheum.* **43,** 4; Masferrer, J.L., et al. 1999. *Ann. N. Y. Acad. Sci.* **889,** 84; Williams, C., et al. 1999. *Ann. N. Y. Acad. Sci.* **889**, 72.

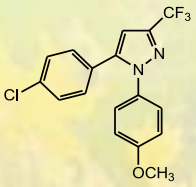
Meloxicam. A non-steroidal, anti-inflammatory agent that preferentially inhibits COX-2 (IC_{50} = 4.7 µM) relative to COX-1 (IC_{50} = 36.6 µM). Also inhibits the growth of colorectal cancer cells *in vitro*.



Cat. No. 444800 100 mg

Ref.: Blanco, F.J., et al. 1999. *J. Rheumatol.* **26**, 1366; Davies, N.M., and Skjodt, N.M. 1999. *Clin. Pharmacokinet.* **36**, 115; Goldman, A.P., et al. 1998. *Carcinogenesis* **19**, 2195; Engelhardt, G., et al. 1996. *Biochem. Pharmacol.* **51**. 29.

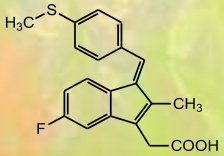
SC-560. A highly potent and selective inhibitor of COX-1 (IC_{50} = 9 nM). Inhibits COX-2 only at higher concentrations (IC_{50} = 6.3 µM).



Cat. No. 565610 5 mg

Ref.: Smith, C.J., et al.1998. *Proc. Natl. Acad. Sci. USA* **95,** 13313.

Sulindac Sulfide. Selectively inhibits COX-1 (ID_{50} = 0.5 µM) relative to COX-2 (ID_{50} = 14 µM).



Cat. No. 574102 5 mg

Ref.: Meade, E.A., et al. 1993. *J. Biol. Chem.* **268,** 6610.

NEW! Anandamide Analogs

Anandamide, a neuromodulator, functions as an endogenous ligand of the cannabinoid receptor. Several synthetic analogs of anandamide have been developed that possess markedly improved cannabinoid receptor affinity and metabolic stability compared to those of the parent ligand. At least three cannabinoid receptor subtypes, CB1, CB2, and CB1A, have been cloned, all of which belong to the superfamily of G protein-coupled receptors. Anandamide and its analogs exhibit greater selectivity for the CB1 receptor and modest selectivity for the CB2 receptor. The CB1 receptor mediates inhibition of adenylate cyclase, inhibition of N, P, and Q-type calcium channels, stimulation of potassium channels, and activation of MAP kinase. The CB2 receptor mediates inhibition of adenylate cyclase and activation of MAP kinase.

Ref.: Ameri, A. 1999. *Prog. Neurobiol.* **58,** 315; Khanolkar, A.D., and Makriyannis, A. 1999. *Life Sci.* **65,** 607; Axelrod, J., and Felder, C.C. 1998. *Neurochem. Res.* **23,** 575; Hillard, C.J., and Campbell, W.B. 1997. *J. Lipid Res.* **12,** 2383.

Product	Cat. No.	Comments	Size
AM404	**127670**	A competitive inhibitor of anandamide uptake in neurons (IC_{50} = 1 μM).	5 mg
Arachidonyl-2-chloroethylamide	**181100**	Binds selectively to the CB1 receptor (K_i = 1.4 nM) relative to the CB2 receptor (K_i = 3.1 μM).	5 mg
Arachidonylcyclopropylamide	**181105**	Binds selectively to the CB1 receptor (K_i = 2.2 nM) relative to the CB2 receptor (K_i = 700 μM).	5 mg
Linoleoylamide	**436620**	A linoleoyl derivative of anandamide that acts as a reversible and competitive inhibitor of brain fatty acid amide hydrolase (K_i = 14.4 μM).	10 mg
Linoleoylethanolamide	**436630**	A linoleoyl derivative of anandamide that acts as a reversible and competitive inhibitor of brain fatty acid amide hydrolase (K_i = 9.0 μM).	10 mg
S(-)-Methanandamide	**444198**	Offers significantly greater metabolic stability than anandamide (Cat. No. 172100).	5 mg

NEW! Antibodies to Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) comprise a family of eight members grouped into three classes according to their amino acid sequence identity and pharmacological profile. mGluRs are coupled to G-proteins, either positively linked to phospholipase C (class I) or negatively linked to adenylate cyclase (class II and III). mGluRs are known to increase intracellular Ca^{2+} concentration via IP_3 and ryanodine-sensitive Ca^{2+} stores in neurons. Activation of mGluRs is reported to block the slow Ca^{2+}-dependent K^+ conductance and increase the membrane excitability of neurons. CALBIOCHEM® introduces four new antibodies to mGluRs *that are suitable for immunoblotting, immunohistochemistry, immunoprecipitation, and immunofluorescence assays.*

Anti-Metabotropic Glutamate Receptor 1α, Rat (Rabbit)
Cat. No. 445870 100 μl

Anti-Metabotropic Glutamate Receptor 2/3, Rat (Rabbit)
Cat. No. 445871 100 μl

Anti-Metabotropic Glutamate Receptor 5, Rat (Rabbit)
Cat. No. 445872 100 μl

Anti-Metabotropic Glutamate Receptor 5/1, Rat (Rabbit)
Cat. No. 445873 100 μl

NEW! Tools for Mitochondrial Metabolism Research

Bongkrekic Acid, Triammonium Salt

Cat. No. 203671 500 μg

Carboxyatractyloside, *Atractylis gummifera*

Cat. No. 216200 10 mg

Ru360

Cat. No. 557440 1 set (10 x 100 μg) NEW!
500 μg
1 mg

NEW! Proteasome Inhibitors

Epoxomicin, Synthetic. A potent, highly specific, and irreversible inhibitor of chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide hydrolyzing activities of the proteasome. Modifies the proteasomal catalytic subunits LMP-7, MECL1, and Z.

Cat. No. 324800 100 μg

YU101. A potent, highly specific, and irreversible inhibitor of chymotrypsin-like activity of the proteasome. A weak inhibitor of trypsin-like and peptidylglutamyl peptide hydrolyzing activities of the proteasome.

Cat. No. 688500 100 μg

NEW! Fusion Proteins

CD40-muIg Fusion Protein, Human Recombinant, CHO Cell Line. A member of the TNF receptor family present on all B cells except plasma cells. CD40 plays an important role in B cell activation and the interaction with its ligand, CD154, is essential for isotype switching. Consists of extracellular domain of CD40 (193 amino acids) fused to murine IgG_{2a} Fc (223 amino acids). M.W. 110,000.

Cat. No. 217588 25 µg

Ref.: Ozaki, M.E., et al. 1997. *J. Immunol.* **159,** 214; Foy T.M., et al. 1996. *Annu. Rev. Immunol.* **14,** 591.

CD152-muIg Fusion Protein, Human, Recombinant, BHK Cell Line. A dimeric fusion protein consisting of the extracellular domain of human CD152 fused with murine IgG_{2a} Fc. Specifically binds to receptors on Raji tumor cells. May function as a negative regulator of T cell activation. M.W. 110,000.

Cat. No. 217690 25 µg

Ref.: Karandikar, N.J., et al. 1996. *J. Exp. Med.* **184,** 783; Morton, P.A., et al. 1996. *J. Immunol.* **156**, 1047.

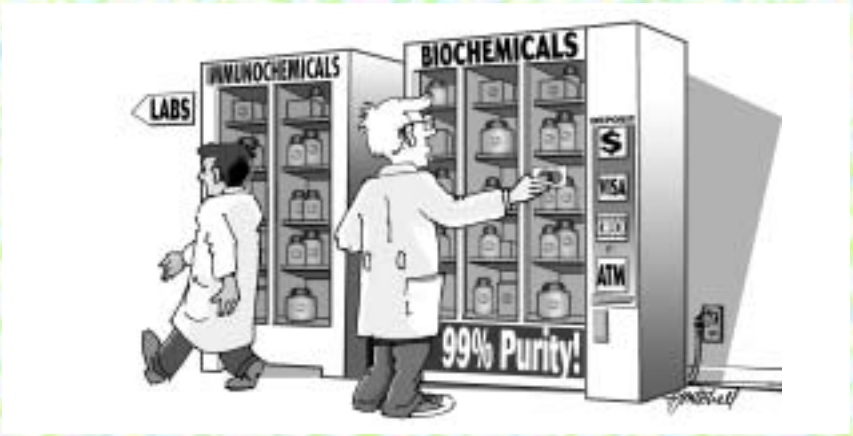
Mouse Monoclonal Antibodies For Cell Adhesion Research

Product	Cat. No.	Comments & Applications*	Size
Anti-CD28, Human	**217669**	Recognizes CD28 expressed on T cells and also on BHK cells transfected with human CD28. Applications: FC, IH, IP	100 µg
Anti-CD56, Human	**217671**	Recognizes the CD56 molecule found on NK cells. Applications: FC, IB, IH, IP	100 µg
Anti-CD152, Human	**218120**	Blocks the binding of CD152 (CTLA-4) Ig fusion protein to its CD80/CD86 receptor. Applications: FC, IH, IP	100 µg
Anti-CD162, Human	**218130**	Blocks the binding of CD162 to CD62P. Applications: FC, IB, IH, IP	100 µg
Anti-CD162, Human, R-Phycoerythrin Conjugate	**218131**	Blocks the binding of CD162 to CD62P. Applications: FC, IH, IP	120 T

***FC: Flow cytometry; IB: Immunoblotting; IH: Immunohistochemistry; IP: Immunoprecipitation.**

New Inhibitors of Geranylgeranyl- and Farnesyltransferases

Product	Cat. No.	Comments	Size
GGTI-297	**345882**	A potent, selective peptidomimetic inhibitor of GGTase I (IC_{50} = 50 nM) relative to FTase (IC_{50} = 200 nM).	250 µg
GGTI-298	**345883**	A cell-permeable, prodrug form of GGTI-297. Inhibits the processing of Rap 1A (IC_{50} = 3 µM) but has no effect on the processing of H-Ras even at concentrations of 15 µM.	250 µg
GGTI-2133	**345884**	A potent, selective non-thiol inhibitor of GGTase I (IC_{50} = 38 nM) with a 140-fold greater selectivity over FTase (IC_{50} = 5.4 µM).	250 µg
GGTI-2147	**345885**	Methyl ester derivative of GGTI-2133 that blocks the geranylgeranylation of Rap1A with an IC_{50} value about 60-fold lower (IC_{50} = 500 nM) than that required to disrupt the farnesylation of H-Ras (IC_{50} >30 µM).	250 µg



New! Zwitterionic Amidosulfobetaine Detergents

ASB-14
Cat. No. 182750 5 g
25 g

ASB-16
Cat. No. 182755 5 g
25 g

New! Antibodies to Signaling Molecules

New! Antibodies to EphB1 (Elk Receptor)

Eph family receptor tyrosine kinases signal axonal guidance, neuronal bundling, and angiogenesis. EphB1 functions as a ligand density sensor to signal integrin-mediated cell-matrix attachment.

Ref.: Huynh-Do, U., et al. 1999. *EMBO J.* **18,** 2165; Stein, E., et al. 1998. *J. Biol. Chem.* **273,** 1303.

Anti-EphB1, Cytoplasmic Tyrosine Kinase Domain, Human (Sheep). GST·Tag™-cytoplasmic domain fusion protein corresponding to amino acid residues 586 - 984 of the human EphB1 (Elk receptor) protein was used as an immunogen.

Cat. No. 324828 250 µg

Anti-EphB1, Extracellular Domain, Human (Sheep). GST·Tag™-extracellular domain fusion protein corresponding to amino acid residues 16 - 351 of the human EphB1 (Elk receptor) protein was used as an immunogen.

Cat. No. 324829 250 µg

New! Antibodies to Vav2

Vav2 is a member of the Vav family of oncoproteins that acts as a guanosine nucleotide exchange factor (GEF) for RhoG and RhoA-like GTPases in a phospho-tyrosine-dependent manner. Displays a complex array of structural motifs, including calponin-homology, acidic, dbl-homology, pleckstrin-homology, cysteine-rich, SH3, and SH2 domains. It is activated by tyrosine kinases *in vivo* and couples tyrosine kinase signals with the activation of distinct subsets of the Rho/Rac family of GTPases.

Ref.: Schuebel, K.E., et al. 1998. *EMBO J.* **17,** 6608; Bustelo, X.R. 1996. *Crit. Rev. Oncog.* **7,** 65.

Anti-Vav2, DPH Domain, Human (Sheep). His·Tag®-Vav2 fusion protein corresponding to amino acid residues 287 - 578 of human Vav2 was used as an immunogen.

Cat. No. 676650 250 µg

Anti-Vav2, SH2 Domain, Human (Sheep). His·Tag®-Vav2 fusion protein corresponding to amino acid residues 578 - 878 of human Vav2 was used as an immunogen.

Cat. No. 676652 250 µg

NEW!

Antibody to SLP-76

Anti-SLP-76, Human (Mouse MoAb). GST·Tag™ SLP-76 fusion protein was used as an immunogen. SLP-76 [Src homology 2 (SH2) domain-containing leukocyte protein of 76 kDa] is a complex adapter protein that serves as a substrate for ZAP-70 and Syk and has the capacity to bind to smaller adapter proteins, such as Grb2, which subsequently binds the nucleotide exchange protein SOS in the transmission of intracellular signals leading to the activation of Ras.

Cat. No. 567350 50 µg

Ref.: Pivniouk, V.I., et al. 1999. *J. Clin. Invest.* **103,** 1737; Chu, J., et al. 1998. *Blood* **92,** 1697.

New! Photo-Reversible Protein Tyrosine Phosphatase Inhibitors

Product	Cat. No.	Comments	Size
PTP Inhibitor I	**540200**	A potent, cell-permeable, photo-reversible PTP inhibitor that inhibits SHP-1 (K_i = 43 µM) and PTP1B (K_i = 42 µM).	10 mg
PTP Inhibitor II	**540205**	Exhibits lower potency (K_i =128 µM) but higher K_{inact} (2.4 min^{-1}) than PTP Inhibitor I.	25 mg
PTP Inhibitor III	**540210**	Exhibits lower potency (K_i =193 µM) but higher K_{inact} (1.8 min^{-1}) than PTP Inhibitor I.	10 mg

Introducing.... Convenience and Reliability

Phosphatase Inhibitor Cocktail Set I

A ready-to-use cocktail containing 2.5 mM (-)*p*-Bromotetramisole Oxalate, 500 mM Cantharidin, and 500 nM Microcystin-LR in DMSO.

Cat. No. 524624 1 set (5 x 1 ml)

Phosphatase Inhibitor Cocktail Set II

An aqueous solution of five phosphatase inhibitors containing 200 mM Imidazole, 100 mM Sodium Fluoride, 115 mM Sodium Molybdate, 100 mM Sodium Orthovanadate, and 400 mM Sodium Tartrate Dihydrate.

Cat. No. 524625 1 set (5 x 1 ml)

Introducing.....

ThioGlo™ Maleimide Reagents

For detection, determination, and derivatization of -SH groups in proteins and peptides.

- *High fluorescence quantum yields with large Stokes Shifts*
- *Fast acting*
- *Resistant to hydrolysis*
- *Minimal photobleaching*
- *Greater sensitivity*

ThioGlo™ 1 Fluorescent Thiol Reagent
Cat. No. 595501 5 mg

ThioGlo™ 3 Fluorescent Thiol Reagent
Cat. No. 595503 5 mg

ThioGlo™ 5 Fluorescent Thiol Reagent
Cat. No. 595505 5 mg

New! New!

c-Raf Kinase Inhibitor ZM 336372

A potent, specific, competitive inhibitor of the kinase activity of c-Raf (IC_{50} = 70 nM).

Cat. No. 692000 1 mg

NEW! Endotoxin-Free Proteases and Protease Inhibitor

Elastase, Porcine, High Purity, Endotoxin-Free
Specific activity: ≥25 units/mg protein. Purity: ≥98% by SDS-PAGE.
Cat. No. 324723 5 mg, 15 mg

Trypsin, Bovine Pancreas, High Purity, Endotoxin-Free.
Specific activity: ≥3000 units/mg protein. Purity: ≥90% by HPLC.
Cat. No. 650200 100 mg, 300 mg

Chymotrypsin, Bovine Pancreas, High Purity, Endotoxin-Free.
Specific activity: ≥1000 units/mg protein. Purity: ≥90% by HPLC.
Cat. No. 230834 200 mg, 500 mg

Trypsin Inhibitor, Soybean, High Purity, Endotoxin-Free
Specific activity: ≥10,000 units/mg protein. Purity: ≥90% by SDS-PAGE.
Cat. No. 650358 25 mg, 100 mg

Introducing.....

Histone Deacetylase Substrate, Fluorogenic [Boc-Lys(Ac)-AMC]

(Excitation max: 335 nm; emission max: 395 nm).
Cat. No. 382155 1 mg

Histone Deacetylase Inhibitor

SAHA (Suberoylanilide Hydroxamic Acid)
Cat. No. 559301 5 mg

Thrombin Activatable Fibrinolysis Inhibitor

NEW! **Thrombin Activatable Fibrinolysis Inhibitor (TAFI)**, a 60-kDa glycoprotein related to pancreatic carboxypeptidase B, is activated by the thrombin/thrombomodulin complex by proteolytic cleavage at Arg^{92}. The active form of TAFI (TAFIα) inhibits fibrinolysis by removal of the carboxyl-terminal lysine from partially degraded fibrin which stimulates plasminogen activation. TAFIα increases the rate constant for plasminogen activation that occurs following modification of fibrin by plasmin in the early stages of fibrin degradation. Levels of thrombin required for TAFI activation far exceed the thrombin concentration required for fibrin formation. M.W. 60,000.

Cat. No. 605197 50 µg

Ref.: Boffa, M.B., et al. 1999. *Biochemistry* **38**, 6547; Mosnier, L.O., et al. 1998. *Thromb. Haemost.* **80**, 829; Wang, W., et al. 1998. *J. Biol. Chem.* **273**, 27176; Bajzar, L., et al. 1995. *J. Biol. Chem.* **270**, 14477.

NEW! **Anti-Thrombin-Activatable Fibrinolysis Inhibitor, Human (Mouse)**
Cat. No. 605198 100 µg

NEW! **Anti-Thrombin-Activatable Fibrinolysis Inhibitor, Human (Sheep)**
Cat. No. 605199 1 mg

Meet Our CN biosciences Family

As part of CN Biosciences, Calbiochem joins with Novagen, Oncogene Research Products, and Novabiochem to provide an ever-growing family of innovative products to the Life Science Community. Our family partnerships enable us to further broaden our product offerings to provide you with more of the reagents you need for your research from one convenient source.

CALBIOCHEM® has been providing high quality biochemicals and immunochemicals to life science researchers for over 45 years. Calbiochem provides industry-leading product lines in glycobiology, apoptosis, and signal transduction.

oncogene™ RESEARCH PRODUCTS is a provider of high quality, applications-tested antibodies, assay kits, and related products for the detection and quantitation of apoptosis, cancer, and cell cycle/proliferation.

Novagen® is the gold standard for protein expression, offering an extensive range of innovative products for gene cloning, gene expression, and gene analysis.

novabiochem™ is the industry leader in innovative reagents for peptide synthesis and for combinatorial chemistry applications, including resins, and linkers as well as the latest in solid supports, condensation reagents, and building blocks for Solid and Solution Phase Organic Chemistry (SPOC).

CALBIOCHEM® A brand of CN biosciences, inc.
An Affiliate of Merck KGaA, Darmstadt, Germany

Printed in U.S.A. on recycled paper.

VWR Scientific Products
Customer Service
(800) 932-5000

VWR Canlab
A VWR COMPANY
Technical Service
(800) 628-8470

Newly Updated

otein Kinases and Related Products Guide Inside.

6th Edition 2000 Signal Transduction Catalog

CALBIOCHEM® introduces its new 900+ page, 3500 products, 6th edition, Year 2000 Signal Transduction Catalog. Included are over 800 new products including assay kits, sets, antibodies, and reagents for the study of all major signaling pathways. Also featured are dozens of new Caspases, telomerase, and MMP-related products, several new antibodies for ST research, and an expanded section on useful protocols.

COMING... AUTUMN 2000!

2000 CALBIOCHEM® General Catalog

This millennium edition Catalog contains over 6,000 products, including over 1500 new products for the study of Cell Biology, Signal Transduction, Glycobiology, Apoptosis, Protein Kinases, G-Proteins, and much more. Also included is a new *Molecular Biology Tools* section.

2000 ONCOGENE™ Apoptosis Catalog

A guide to over 1500 monoclonal and polyclonal antibodies, peptides, probes, ELISAs, and assay kits for Apoptosis, Cancer, and Cell Cycle / Proliferation. Contains a wide selection of products for Signal Transduction and Neuroscience Research. Includes alphabetical, numerical, and clone number indices. Complete with applications information, detailed graphics, and a comprehensive Materials and Methods Section.

Your Research is Complicated. Getting the Right Reagents Shouldn't Be!

For the latest in new products, visit www.calbiochem.com