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Volume 28, Number 1, 2002

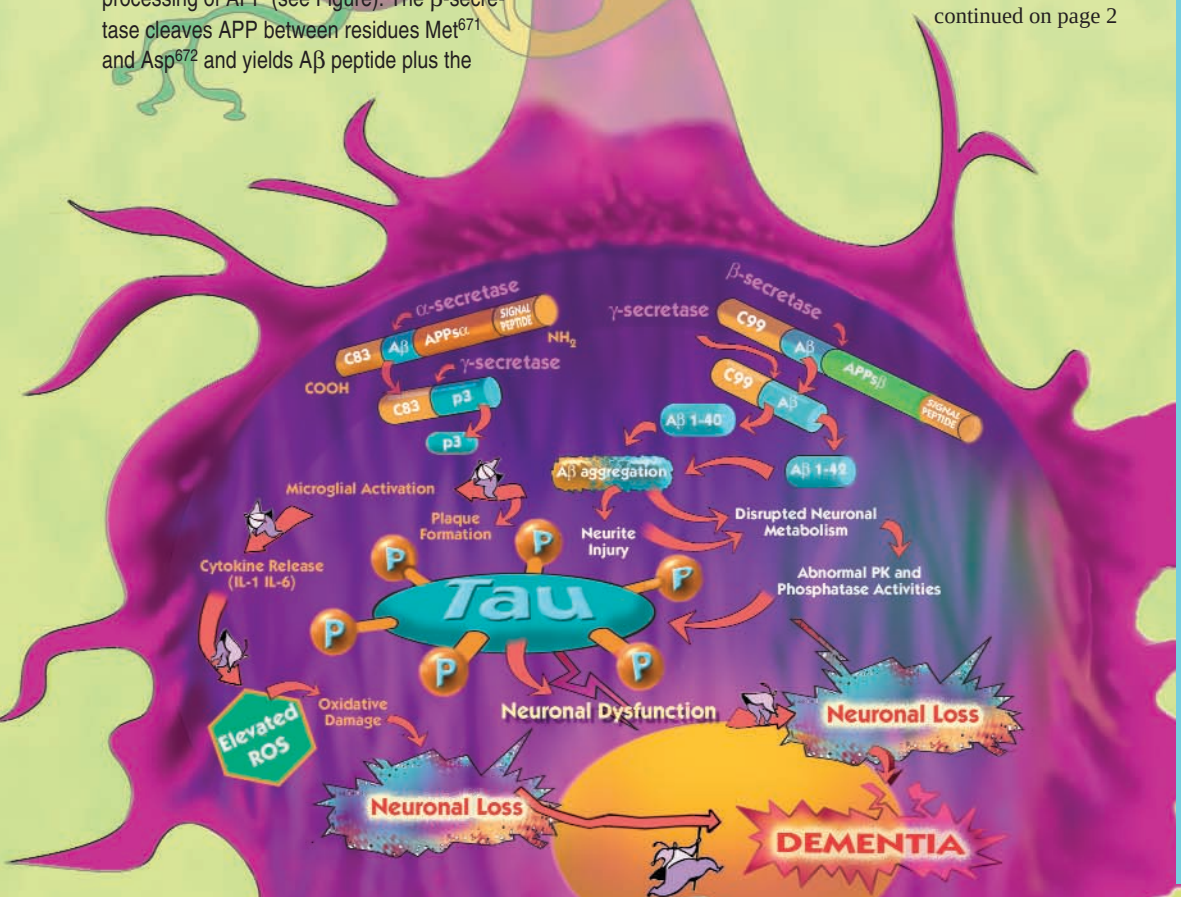
Alzheimer's Disease:

Misguided Slicing of Amyloid Precursor Protein by Secretases

Alzheimer's disease (AD) is characterized by a progressive deposition of the 4 kDa β-amyloid peptide (Aβ) in senile plaques and accumulation of Tau protein as neurofibrillary tangles. In normal healthy individuals, Aβ peptides are present only in small quantities as soluble monomers that circulate in cerebrospinal fluid and blood. However, in AD patients, their levels are significantly increased and they begin to accumulate as insoluble, fibrillar plaques. Aβ is a 40 to 43 amino acid peptide that originates from the proteolytic cleavage of the amyloid precursor protein (APP). APP is reported to occur in three common isoforms: APP695, APP751, and APP770. The APP695 is expressed exclusively in neurons, whereas APP751 and APP770 are present in both neural and non-neural cells. The primary structure of APP contains a small signal sequence, a large extramembranous N-terminal region, a single transmembrane domain, and cytoplasmic C-terminal tail. Processing of APP *in vivo* occurs by two major pathways. Cleavage of APP at the N-terminus of the Aβ region by β-secretase and at the C-terminus by γ-secretases represents the amyloidogenic pathway for processing of APP (see Figure). The β-secretase cleaves APP between residues Met^{671} and Asp^{672} and yields Aβ peptide plus the C99 fragment. The β-secretase has also been identified as an aspartyl protease (BACE or Asp-2) of unusual nature. It has a C-terminal transmembrane domain and two active site motifs located in the luminal domain. Newly synthesized BACE contains a propeptide domain, which is cleaved at residue E46 to produce the mature enzyme. The active site of BACE and the β-secretase cleavage site of APP are in precise topological orientation for endoproteinases.

Succeeding the β-secretase cleavage, a second cleavage occurs at the C-terminus of Aβ peptide that releases Aβ from C99. This cleavage occurs in the vicinity of residue 712 of the C-terminus. The γ-secretase can cleave the C-terminal region at either Val^{711} or Ile^{713} to produce a shorter Aβ peptide (Aβ1-40) or the longer Aβ peptide (Aβ1-42). The predominant form of Aβ found in the cerebrospinal fluid is the shorter Aβ40 peptide. Despite its lower rate of synthesis, Aβ42 is the peptide that is initially deposited within the extracellular plaques of AD patients. In addition, Aβ42 is shown to aggregate at a much lower concentration than the Aβ40 form.

continued on page 2



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Contents

...continued from page 1

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APP can also be processed by α-secretase, which cleaves within the Aβ domain between Lys[687] and Leu[688] and produces a large soluble α-APP domain and the C-terminal fragment containing P3 and C83 (see Figure). The latter can then be cleaved by γ-secretase at residue 711 or 713 to release P3 fragment. This pathway does not yield Aβ peptide. Hence, shunting APP towards the α-secretase pathway may have a beneficial effect in lowering Aβ peptide levels. It is reported that α-secretase shares many of its properties with the secretase that cleaves angiotensin-converting enzyme and is believed to be a zinc metalloproteinase of the ADAMs family. Muscarinic agonists (M1 and M3) and some PKC activators are reported to enhance α-secretase activity and are under consideration for their therapeutic value as AD treatment tools.

Another set of proteins, known as presenilins (PS1 and PS2), is reported to play an important role in APP processing. They are tightly linked to γ-secretase mediated cleavage. PS1 has been suggested to have either inherent γ-secretase activity or act as a co-factor for γ-secretase. More recent studies of Li et al. have indicated that the active site of γ-secretase is shared between the N- and C-terminal fragments of presenilin. Cells obtained from PS1/PS2 double knockout mice do not show any γ-secretase activity. Presenilins are also involved in the regulation of Notch signaling that is important in framing cell destiny during embryogenesis, hematopoiesis, and neural stem cell differentiation. PS1 also plays an important role in the formation of the axial skeleton and is important in neurogenesis and survival of progenitor cells and neurons in specific brain regions.

In AD patients all mutations in APP are shown to increase Aβ42 production. Most cases of familial AD are reported to result from mutations in one of the three genes, APP, PS1, and PS2. Any mutation in these genes results in elevated levels of Aβ peptide. The mutation in APP gene, located on chromosome 21, accounts for about 2% of all cases of familial AD (FAD) and approximately 5 - 20% of early-onset FAD. A substitution of Glu to Gln at codon 693 of APP is termed "Dutch mutation", which is responsible for hereditary cerebral hemorrhage with amyloidosis (Dutch type). Here amyloid deposits containing the Aβ peptide are found in cerebral vessel walls with diffuse plaques in the brain parenchyma. Another mutation known as "Flemish mutation" occurs at codon 692 that replaces Ala with Gly. It causes an intermediate phenotype between congophilic angiopathy and AD. A well-studied mutation, "Swedish mutation," results from the replacement of Lys and Met by Asp and Leu at codons 670 and 671. The "Swedish mutation" does not lie within Aβ peptide region but lies in the proximity of the secretase cleavage sites and produces mainly the soluble Aβ40 peptide. Fibroblast cell lines transfected with the "Swedish mutation" are shown to produce elevated levels of soluble form of Aβ peptide. Over 40 different mutations have been reported in PS1, which account for about 30 to 50% of all presenile FAD. The PS2 gene mutations are rather rare and account for less than 2% of all early-onset FAD. Mutations in both PS1 and PS2 are associated with an increased production of the Aβ42 peptide, the more amyloidogenic form of Aβ peptide. It has been suggested that mutant PS1 proteins alter the proteolytic processing of APP at the C-terminus of Aβ and favors the deposition of Aβ42 peptide.

The characterization of the APP secretases during the past few years has provided significant advancement in therapeutic strategies that may lead to limiting the build up of Aβ peptide in the brain and eliminate or delay the pathological effects of AD. Recent characterization of secretases has uncovered several common features, particularly their sensitivity to certain metalloprotease inhibitors and up-regulation of their activity by phorbol esters. Presenilins and γ-secretases are considered to be the best molecular targets for developing therapeutic agents that may minimize the debilitating effects of AD. Major targets in AD research are identifying the genetic and environmental factors responsible for Aβ build-up in nerve cells.

References:

Esler, W.P., and Wolfe, M.S. 2001. *Science* **293,** 1449.

Li, Y-M. 2001. *Mol. Interv.* **1,** 198.

Selkoe, D.J. 2001. *Physiol. Rev.* **81,** 741.

Li, Y-M., et al. 2000. *Nature* **405,** 689.

Nunan, J., and Small, D.H. 2000. *FEBS Lett.* **483,** 6.

Vassar, R., and Citron, M. 2000. *Neuron* **27,** 419.

Zhang, Z., et al. 2000. *Nat. Cell Biol.* **2,** 463.

Haass, C., and De Stooper, B. 1999. *Science* **286,** 916.

Sinha, S., and Lieberburg, I. 1999. *Proc. Natl. Acad. Sci. USA* **96,** 11049.

Chen, M. 1997. *FEBS Lett.* **417,** 163.

Hardy, J. 1997. *Trends Neurosci.* **20,** 154.

Selkoe, D.J. 1997. *Science* **275,** 630.

Hutton, M., et al. 1996. *NeuroReport* **7,** 801.

Lemere, C.A., et al. 1996. *Nat. Med.* **2,** 1146.

Schweuner, D., et al. 1996. *Nat. Med.* **8,** 864.

Citron, M., et al. 1994. *Proc. Natl. Acad. Sci. USA* **91,** 11993.

Tanzi, R.E., et al. 1992. *Am. J. Hum. Genet.* **51,** 273.

Bakker, E., et al. 1991. *Am. J. Hum. Genet.* **49,** 518.

Levy, E., et al. 1990. *Science* **248,** 1124.

Siman, R., et al. 1990. *J. Neurosci* **10,** 2400.

New! Neuroprotective Agents

Kaempferol

A phytoestrogen that offers protection against $A\beta_{5-35}$ induced cell death in neonatal cortical neurons. Blocks the activation of Aβ-induced activity of caspase-2, -3, -8, and -9, and reduces neuronal apoptosis. Also acts as an inhibitor of COX-1 (IC_{50} = 180 μM).

Cat. No. 420345 25 mg

Clioquinol

A neurotoxic antibiotic that is reported to dissolve senile plaques and reduce amyloid's ability to clump together, apparently by trapping the Cu^{2+} and Zn^{2+} that stud these deposits.

Cat. No. 233165 1 g

NEW! Antibodies for Alzheimer's Research

Product	Cat. No.	Comments	Applications*	Size	Price
Anti-Amyloid Precursor Protein, C-Terminal (751-770), Human (Rabbit)	171610	Detects C-terminal soluble products CTFγ (~6 kDa), CTFα (~9 kDa), CTFβ ~11 kDa) and full-length APP.	IB, IF, IH, IP	50 µl	
Anti-BACE (26-45), Human (Rabbit)	195100	Recognizes bands of ~65 and ~75 kDa in CHO cells transfected with BACE cDNA corresponding to BACE with an intact prodomain.	IB, IP	100 µl	
Anti-BACE (46-65), Human (Rabbit)	195101	Recognizes bands of ~65 and ~75 kDa in CHO cells transfected with BACE.	IB, IP	100 µl	
Anti-BACE (487-501), Human (Rabbit)	195102	Recognizes bands of ~65 and ~75 kDa in CHO cells transfected with BACE cDNA.	IB, IP	100 µl	
Anti-BACE2 (131-142), Human (Rabbit)	195103	Recognizes the expected ~47 kDa band in guinea pig brain lysate. Also detects a ~28 kDa band not detected by the preimmune serum.	IB	100 µl	
Anti-BACE2 (504-518), Human (Rabbit)	195104	Recognizes the expected ~47 kDa band in guinea pig brain lysate. Also detects a ~62 kDa band not detected by the preimmune serum.	IB	100 µl	
Anti-ERAB (101-119), Mouse (Rabbit)	324918	Detects the predicted ~27 kDa band in SK-N-SH cell lysates.	IB	100 µl	
Anti-KUZ (240-259), Mouse (Rabbit)	422750	KUZ is a disintegrin metalloprotease (ADAM) encoded by the kuzbanian (kuz) gene.	IB	100 µl	
Anti-KUZ (735-749), Mouse (Rabbit)	422751	Detects ~98 and ~105 kDa bands that are augmented in transfected CHO-K1 cells. ~60 kDa and ~30 kDa bands representing the cleaved mature protein and a degradation product are also observed.	IB	100 µl	
Anti-Tau, Phospho-Specific (Ser^{199}), Human (Rabbit)	577807	Reacts with Ser^{199} phosphorylated Tau in human, mouse, and rat.	EIA, IB, IH	10 T	
Anti-Tau, Phospho-Specific (Ser^{202}), Human (Rabbit)	577808	Reacts with Ser^{202} phosphorylated Tau in human, mouse, and rat.	EIA, IB, IH	10 T	
Anti-Tau, Phospho-Specific (Thr^{205}), Human (Rabbit)	577809	Reacts with Thr^{205} phosphorylated Tau in human, mouse, and rat.	IB, IH	10 T	
Anti-Tau, Phospho-Specific (Thr^{212}), Human (Rabbit)	577810	Reacts with Thr^{212} phosphorylated Tau in human, mouse, and rat.	EIA, IB	10 T	
Anti-Tau, Phospho-Specific (Ser^{214}), Human (Rabbit)	577811	Reacts with Ser^{214} phosphorylated Tau in human, mouse, and rat.	EIA, IB, IH	10 T	
Anti-Tau, Phospho-Specific (Thr^{217}), Human (Rabbit)	577812	Reacts with Thr^{217} phosphorylated Tau in human, mouse, and rat.	IB	10 T	
Anti-Tau, Phospho-Specific (Thr^{231}), Human (Rabbit)	577813	Reacts with Thr^{231} phosphorylated Tau in human, mouse, and rat.	EIA, IB	10 T	
Anti-Tau, Phospho-Specific (Ser^{262}), Human (Rabbit)	577814	Reacts with Ser^{262} phosphorylated Tau in human, mouse, and rat.	IB	10 T	
Anti-Tau, Phospho-Specific (Ser^{396}), Human (Rabbit)	577815	Reacts with Ser^{396} phosphorylated Tau in human, mouse, and rat.	EIA, IB	10 T	
Anti-Tau, Phospho-Specific (Ser^{409}), Human (Rabbit)	577816	Reacts with Ser^{409} phosphorylated Tau in human, mouse, and rat.	IB	10 T	
Anti-Tau, Phospho-Specific (Ser^{422}), Human (Rabbit)	577817	Reacts with Ser^{422} phosphorylated Tau in human, mouse, and rat.	IB	10 T	

*** EIA:** enzyme immunoassay; **IB:** immunoblotting. **IF:** immunofluorescence; **IH:** immunohistochemistry; **IP:** immunoprecipitation **Note:** 1 T = 1 test

NEW! Inhibitors for β and γ-Secretases

OM99-2

(Glu-Val-Asn-Leu-ΨAla-Ala-Glu-Phe)

A peptidomimetic, highly potent, tight–binding transition-state analog inhibitor of human brain β-secretase (K_i = 1.6 nM for recombinant memapsin-2 and 9.58 nM for recombinant pro-memapsin 2). Designed from the template of the β-secretase site of Swedish β-amyloid precursor protein (APP) with Asp to Ala replacement.

Cat. No. 496000 250 µg

γ-Secretase Inhibitor VIII (DFK 11)

A substrate–based difluoro ketone peptidomimetic that acts as a reversible inhibitor of γ-secretase (IC_{50} = 10 – 25 µM for both $Aβ_{40}$ and $Aβ_{42}$).

Cat. No. 565769 1 mg

$γ_{-40}$-Secretase Inhibitor II

(Boc-Gly-Val-Val-CHO)

A cell-permeable substrate-based (γ-40-site) γ-secretase inhibitor that preferentially (≥90%) inhibits Aβ cleavage at site 40 rather than 42 in transiently transfected 293 T cells overexpressing APP695NL.

Cat. No. 565766 1 mg
5 mg

γ-Secretase Inhibitor IX

(N-N[-3,5-Difluorophenacetyl-L-alanyl]-S-phenylglycine-*t*-butyl ester)

A cell-permeable dipeptide that reduces Aβ production by inhibiting γ -secretase activity (Aβ total IC_{50} = 115 nM, $Aβ_{42}$ IC_{50} = 200 nM). Does not affect the secretion of APP.

Cat. No. 565770 5 mg

γ-Secretase Inhibitor X (L-685,458)

A cell-permeable, specific, potent inhibitor of γ-secretase ($Aβ_{total}$ IC_{50} = 17 nM, $Aβ_{40}$ IC_{50} = 48 nM, and $Aβ_{42}$ IC_{50} = 67 nM in SHSY5Y cells overexpressing spbA4CTF). Binds to presenilin 1 and presenilin 2 (PS1 and PS2) and blocks Notch intracellular domain production in neuronal cells.

Cat. No. 565771 250 µg

β-Secretase Inhibitor II

(Z-Val-Leu-Leu-CHO)

A potent, cell-permeable, reversible β-secretase inhibitor that corresponds to the β-secretase cleavage site (VNL-DA) of the Swedish mutant APP. Inhibits the formation of both $Aβ_{total}$(IC_{50} = 700 nM) and $Aβ_{1-42}$(IC_{50} = 2.5 µM) in Chinese hamster ovary (CHO) cells stably transfected with wild-type APP751.

Cat. No. 565749 1 mg
5 mg

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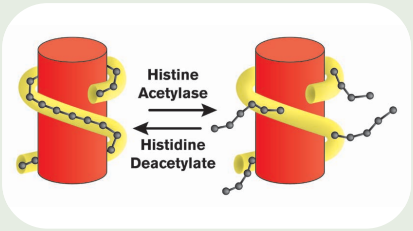
Histone Acetylation and Deacetylation: The Wrapping and Unwrapping of DNA into Nucleosome

Gene expression, to a large extent, is controlled by a host of protein complexes that continuously pack and unpack the chromosomal DNA from the inaccessible, tightly coiled nucleosomal particles to the accessible, unwound nucleosomal particles. This packing and unpacking is achieved by the acetylation and deacetylation of the histones in the nucleosomal core. Acetylated histone proteins confer accessibility of the DNA template to the transcriptional machinery for expression. Histone acetylation has been linked to gene-specific activation by transcription factors. It plays an important role in cell cycle control and has been linked to the uncontrolled cell proliferation. Histone deacetylases (HDAC), on the other hand, are chromatin remodeling factors that deacetylate histones and act as transcriptional repressors or silencers of genes. They regulate histone acetylation by catalyzing the removal of acetyl groups on the amino terminal lysine residues of the core nucleosomal histones.

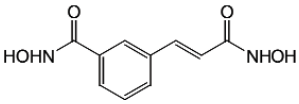


Studies have shown that certain oncogenes repress transcription by recruitment of HDACs. This has lead to the interest in small molecules that act as inhibitors of HDAC and have potential for the treatment of cancer. They act as potent inducers of growth arrest, differentiation, and apoptotic cell death in a variety of transformed cells in culture and in tumor–bearing animals. They are shown to increase the DNA-binding activities of AP1, CREB, and NF-κB transcription factors. The best studied inhibitors of HDAC is Trichostatin A, a hydroxamic acid that complexes with zinc at the catalytic site and mediates the acetamide cleavage. HDAC inhibitors are also reported to down-regulate telomerase activity via suppression of hTERT mRNA expression.

Ref.: Suenaga, M., et al. 2002. *Int. J. Cancer* **97,** 621; Jung. M., et al. 2001. *Curr. Med. Chem.* **8,** 1505; Marks, P.A., et al. 2001. *Curr. Opin. Oncol.* **13,** 477; Munster, P.N., et al. 2001. *Cancer Res.* **61,** 8492; Pandolfi, P.P. 2001. *Cancer Chemother. Pharmacol.* **48** (Suppl 1), S17; Yoshida, M., et al. 2001. *Cancer Chemother. Pharmacol.* **48** (Suppl 1):S20.

Histone Deacetylase Inhibitor II

A second generation hybrid polar agent that inhibits HDAC by binding of the hydroxamic moiety to the active site zinc. Shown to be a potent inducer of transformed cell growth arrest and terminal differentiation (~4 μM). *Purity: ≥95% by HPLC.* M.W. 222.2.

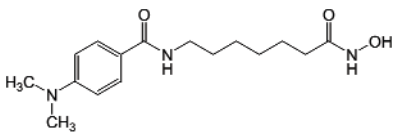


Cat. No. 382148 5 mg

Ref.: Coffey, D.C., et al. 2001. *Cancer Res.* **61,** 3591; Marks, P.A., et al. 2000. *J. Natl. Cancer Inst.* **92,** 1210.

Histone Deacetylase Inhibitor III

An amide analog of Trichostatin A (Cat. No. 647925) that potently inhibits histone deacetylases (IC_{50} = 40 nM for rat liver HDAC and IC_{50} = 100 nM for maize HDAC). Induces differentiation and inhibits proliferation (~2 μM) of murine erythroleukemia cells. *Purity: ≥95% by HPLC.* M.W. 307.4.



Cat. No. 382149 1 mg
5 mg

Ref.: Jung, M., et al. 1999. *J. Med. Chem.* **42,** 4669.

Scriptaid

A relatively non-toxic hydroxamic acid containing HDAC inhibitor that is reported to facilitate transcriptional activation (TGFβ/Smad4) in both stable and transient receptor assays in a concentration-dependent manner. At 6 - 8 μM concentrations, results in a greater than 100-fold increase in histone acetylation in PANC-1 cells. *Purity: ≥95% by HPLC.* M.W. 326.4.

Cat. No. 565730 5 mg

Ref.: Su, G.H., et al. 2000. *Cancer Res.* **60,** 3137.

Sirtinol

A cell-permeable 2-hydroxy-1-naphthaldehyde derivative that acts as a specific and direct inhibitor of the sirtuin class of deacetylase activity. Does not affect human HDAC1. Blocks Sir2p transcriptional silencing activity *in vivo* (IC_{50} = 25 μM) and NAD-dependent HDAC activity in purified recombinant yeast Sir2p and human SIRT2 *in vitro* (IC_{50} = 70 and 40 μM, respectively). *Purity: ≥97% by HPLC.* M.W. 394.5.

Cat. No. 566320 5 mg

Ref.: Grozinger, C.M., et al. 2001. *J. Biol. Chem.* **276,** 38837.

SBHA

A bishydroxamic acid inhibitor of HDAC that inhibits HDAC1 and HDAC3 with equal potency (ID_{50} = 250 – 300 nM). A hybrid polar class of compounds that acts as an inducer of differentiation as well as of apoptosis. *Purity: ≥98% by TLC.* M.W. 204.2.

Cat. No. 559418 100 mg

Ref.: Brinkman, H., et al. 2001. *J. Biol. Chem.* **276,** 22491; Richon, U.M., et al. 1998. *Proc. Natl. Acad. Sci. USA* **92,** 3003.

NEW! Antibodies to Histone Deacetylases

Product	Cat. No.	Comments	Applications	Size	Price
Anti-Histone Deacetylase 1 (Ab-1) (Rabbit)	**PC544**	Immunogen used was a peptide corresponding to amino acids 467 - 482 of human HDAC1.	**IB, IF**	50 μg	
Anti-Histone Deacetylase 2 (475-488), Human (Rabbit)	**382153**	Reacts with canine, hamster, human, mouse, and rat. Recognizes the ~55 kDa HDAC2.	**IB, IC**	100 μg	
Anti-Histone Deacetylase 3 (415-428), Human (Rabbit)	**382154**	Reacts with canine, hamster, human, and rat. Recognizes the ~48 kDa HDAC3.	**IB, IC**	100 μg	
Anti-Histone Deacetylase 4 (129-137), Human (Rabbit)	**382161**	Reacts with human and mouse. Recognizes the ~119 kDa HDAC4.	**IB**	50 μg	
Anti-Histone Deacetylase 5 (194-206 and 536-545), Human (Rabbit)	**382162**	Reacts with human and rat and weakly with mouse. Recognizes the ~160 kDa HDAC5.	**IB**	50 μg	

IB: immunoblotting, **IF;** immunofluorescence; **IC:** immunocytochemistry.

New! Protein Kinase Inhibitors

Now Available...

Protein Tyrosine Phosphatase Assay Kit, Fluorometric

A convenient, highly sensitive assay kit for the measurement of Protein Tyrosine Phosphatase (PTP) activity of purified enzyme. The assay uses a fluorogenic phosphopeptide substrate quenched internally by resonance energy transfer. The kit contains the PTP substrate, assay buffer, chymotrypsin solution, control peptide, microtiter plate, stop solution, and a directional insert. Each kit is suitable for up to 100 assays.

Cat. No. 539742 1 kit

Akt Inhibitor

A phosphatidylinositol ether analog that potently and selectively inhibits Akt (PKB; IC_{50} = 5.0 µM). Acts only as a weak inhibitor of phosphatidylinositol 3-kinase (IC_{50} = 83 µM). Reported to inhibit the growth of HT-29 (IC_{50} = 10 µM), MCF-7 (IC_{50} = 1.2 µM), HeLa (IC_{50} = 2.5 µM), and PC-3 (IC_{50} = 2.0 µM) cancer cell lines. *Purity: ≥95% by NMR.* M.W. 578.8.

Cat. No. 124005 1 mg

Ref.: Hu, Y., et al. 2000. *J. Med. Chem.* **43,** 3045.

Bohemine

A synthetic, cell-permeable, cyclin-dependent kinase (Cdk) inhibitor (IC_{50} = 1 µM) that is structurally similar to Olomoucine (Cat. No. 495620) and Roscovitine (Cat. No. 495620). Arrests cell cycle in the G_1/S boundary. *Purity: ≥95% by HPLC.* M.W. 340.4.

Cat. No. 203600 1 mg
5 mg

Ref.: Chmela, Z., et al. 2001. *Drug Metab. Dispos.* **29,** 326; Alberio, R., et al. 2000. *Mol. Reprod. Dev.* **55,** 422.

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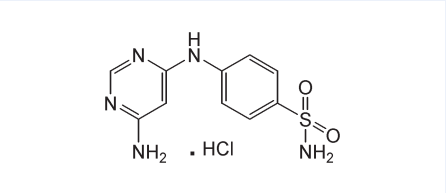
Cdk2/5 Inhibitor

An aminopyrimidine derivative that acts as a selective, ATP-competitive inhibitor of Cdk2 and Cdk5 (K_i = 2 µM for Cdk2/GST-cyclin E and Cdk5/GST-p25). Binds to the ATP-binding pocket of Cdk2. Does not affect the activities of c-met, IGF-1 receptor tyrosine kinase, cAMP-dependent kinase, and ERK2 even at 100 µM concentrations. *Purity: ≥95% by HPLC. Soluble in DMSO.* M.W. 301.8.



Cat. No. 219448 5 mg

Ref.: Clare, P.M., et al. 2001. *J. Biol. Chem.* **276,** 48292.

GSK-3 Inhibitor

{3-(3-Carboxy-4-chloroanilino)-4-(3-nitrophenyl)maleimide}

A potent glycogen synthase kinase-3 (GSK-3) inhibitor (IC_{50} = 26 nM for GSK-3α; 100% inhibition of GSK-3β at 10 µM). Does not affect the activities of over 20 other kinases studied, including Cdk2. *Purity: ≥97% by HPLC.* M.W. 387.7.

Cat. No. 361535 1 mg

Ref.: Smith, D.G., et al. 2001. *Bioorg. Med. Chem. Lett.* **11,** 635.

Y-27632

A cell-permeable, selective inhibitor of Rho-associated protein kinase [K_i = 140 nM for $p160^{ROCK}$ (ROCK-I)]. Also inhibits ROCK-II with almost equal potency. Inhibition is achieved by competing with ATP for binding to the catalytic site. *Purity: ≥95% by HPLC.* M.W. 338.3

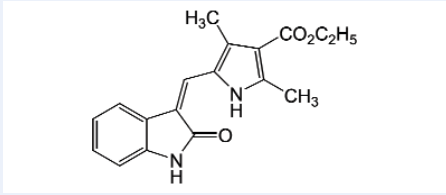
Cat. No. 688000 1 mg
5 mg

Ref.: Chitaley, K., et al. 2001. *Nat. Med.* **7,** 119; Davies, S.P., et al. 2000. *Biochem. J.* **351,** 95; Narumiya, S., et al. 2000. *Methods Enzymol.* **325,** 273.

Sold under license of U.S. Patent 4,997,834 and PCT Patent WO98/06433A1 and under license from Mitsubishi Pharma Corporation.

VEGF Receptor 2 Inhibitor I

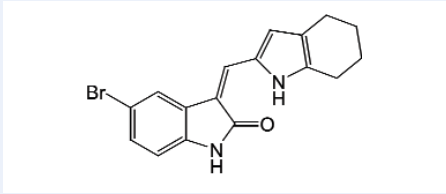
A highly selective, cell-permeable indolin-2-one class of receptor tyrosine kinase (RTK) inhibitor (IC_{50} = 70 nM) for murine vascular endothelial growth factor receptor 2 (VEGF-R2; KDR/Flk-1). The inhibition is suggested to be competitive with respect to ATP. Does not inhibit PDGF, EGF, and IGF-1 RTK activities (IC_{50} ≥100 µM). *Purity: ≥95% by HPLC.* M.W. 310.4.



Cat. No. 676480 1 mg

VEGF Receptor 2 Inhibitor II

A cell-permeable indolin-2-one class of receptor tyrosine kinase (RTK) inhibitor [IC_{50} = 70 nM for VEGF-R2 (KDR/Flk-1), 920 nM for PDGF-Rb, 4.92 µM for $p60^{c\text{-}src}$, and 13.3 µM for FGF-R1]. The inhibition is suggested to be competitive with respective to ATP. *Purity: ≥95% by HPLC.* M.W. 343.2.



Cat. No. 676485 1 mg

Ref.: Sun, L., et al. 2000. *J. Med. Chem.* **43,** 2655.

Debromohymenialdisine, *Stylotella aurantium*

An alkaloid from marine sponge that acts as a highly selective inhibitor of G_2 phase DNA damage checkpoint (IC_{50} = 8 µM). Acts by blocking the activities of check point kinases Chk1 (IC_{50} = 3 µM) and Chk2 (IC_{50} = 3.5 µM). The inhibition is competitive with respect to ATP. *Purity: ≥95% by HPLC.*

Cat. No. 252010 100 µg

Ref.: Curman, D., et al. 2001. *J. Biol. Chem.* **276,** 17914.

Also Available... for Histone Deacetylase Research...

Oxamflatin

An aromatic sulfonamide derivative that inhibits mammalian histone deacetylase (IC_{50} = 15.7 nM). Acts as a ligand for the enzyme active site metal ion. Induces a transcriptional activation of Jun-D and causes a morphological reversion in various NIH3T3-derived cell lines transformed with several oncogenes (MIC ~40 nM). *Purity: ≥95% by HPLC.* M.W. 343.4.

Cat. No. 499700 1 mg
5 mg

Ref.: Dear, A.E., and Medcalf, R.L. 2000. *Biochim. Biophys. Acta.* **1492,** 15; Kim, Y.B., et al. 1999. *Oncogene* **18,** 2461; Ohtani, M., et al. 1996. *J. Med. Chem.* **39,** 2871.

Trichostatin A, *Streptomyces* sp.

A potent and reversible inhibitor of histone deacetylase. Blocks cell cycle progression at the G_1 phase in HeLa cells and induces a 12–fold increase in intracellular levels of gelsolin. Induces reversion of oncogenic *ras*–transformed NIH/3T3 cells to a normal morphology. *Purity: ≥98% by HPLC.* M.W. 302.4.

Cat. No. 647925 1 mg

Ref.: Gray, S.G., and Ekstrom, T.J. 1998. *Biochem. Biophys. Res. Commun.* **245,** 423; Takahashi, I., et al. 1996. *J. Antibiot.* **49,** 453; Taunton, J., et al. 1996. *Science* **272,** 408; Futamura, M., et al. 1995. *Oncogene* **10,** 1119; Hoshikawa, Y., et al. 1994. *Exp. Cell Res.* **214,** 189.

NF-κB, NEMO, and Inflammation

Activation of NF-κB is involved in immune and inflammatory responses in animals. In resting cells, NF-κB remains attached to inhibitory proteins known as IκBs. In response to an inflammatory signal, the IκB-kinase (IKK) complex consisting of a pair of catalytic enzymes (IKKα and IKKβ), is activated and phosphorylates IκB proteins. The phosphorylated IκB is then rapidly degraded and frees NF-κB to trigger the inflammatory response. The IKKs require a regulatory subunit, NEMO (NF-κB essential modifier), for activation. NEMO is also reported to suppress the intrinsic basal activity of the IKK complex. An amino-terminal α-helical region of NEMO associated with a carboxyl-terminal segment of IKKα and IKKβ, is known as the NEMO-binding domain (NBD). The cell-permeable NBD peptide blocks association of NEMO with the IKK complex and blocks NF-κB activation. Hence, the NBD is considered as a target for the development of drugs to block proinflammatory activation of the IKK complex without inhibiting basal NF-κB activity.

NEMO-Binding Domain Binding Peptide, Cell-Permeable

Cat. No. 480025 500 µg

NEMO-Binding Domain Binding Peptide, Cell-Permeable, Negative Control

Cat. No. 480030 500 µg

NF-κB Antisense Oligonucleotide, Sodium Salt

(5′-TGGATCATCTTCTGCCATTCT-3′)

Cat. No. 481400 50 nmol

NF-κB Antisense Oligonucleotide, Fluorescein-Labeled, Sodium Salt

(5′-TGGATCATCTTCTGCCATTCT-3′, FAM)

Cat. No. 481405 10 nmol

I-κB Antisense Oligonucleotide, Sodium Salt

(5′-GCGCTCGGCCGCCTGGAACATGGC-3′)

Cat. No. 401475 50 nmol

I-κB Antisense Oligonucleotide, Fluorescein-Labeled, Sodium Salt

(5′-GCGCTCGGCCGCCTGGAACATGGC-3′, FAM)

Cat. No. 401476 10 nmol

Chemokines:
The Pro-inflammatory Chemoattractants

Chemokines belong to a superfamily of small (8-10 kDa) pro-inflammatory cytokines involved in several immune and inflammatory responses. They act as chemoattractants and activate specific types of leukocytes. Based on the arrangement of the conserved cysteine residues, they are classified as CXC (one amino acid separating the conserved 2 cysteine residues); the CC or β chemokines (the first two conserved cysteine residues are adjacent); and the C or γ chemokines (lack two, the first and third, of the four conserved cysteine residues). Chemokines act by binding to G-protein coupled chemokine receptors. The chemokine receptors that bind CXC chemokines are designated CXCRs and those binding to the CC chemokines are known as CCRs. Various CXCRs and CCRs are reported to exhibit overlapping ligand specificities.

Product	Cat. No.	Comments	Size	Price
I-TAC/CXCL11, Human, Synthetic	**419850**	A synthetic chemokine with a peptide sequence representing amino acid residues 22 – 94 of human CXCL11. Known receptor is CXCR3.	10 µg	
MCP-1/CCL2, Human, Synthetic	**443900**	A synthetic chemokine with peptide sequence representing amino acid residues 24 – 99 of human CCL2. Known receptor is CCR2.	10 µg	
MDC/CCL22, Human, Synthetic	**443950**	A synthetic chemokine with peptide sequence representing amino acid residues 25 – 93 of human CCL22. Known receptor is CCR4.	10 µg	
MIP-3α/CCL20, Murine, Synthetic	**475853**	A synthetic chemokine with peptide sequence representing amino acid residues 28 – 97 of murine CCL20. Known receptor is CCR6.	10 µg	
MIP-3β/CCL19, Human, Synthetic	**475854**	A synthetic chemokine with peptide sequence representing amino acid residues 22 – 98 of human CCL19. Known receptor is CCR7.	10 µg	
SDF1-α/CXCL12, Human, Synthetic	**565609**	A synthetic chemokine with a peptide sequence representing amino acid residues 22 – 88 of human CXCL12. Known receptor is CXCR4.	10 µg	

Introducing...NEW!

TNF-α Convertase (TACE) Inhibitors

TAPI-0

A hydroxamate-based inhibitor of TACE, collagenase, gelatinase, and TACE (TNF-α convertase, ADAM17; IC_{50} = 100 nM). *Purity: ≥98% by HPLC.* M.W. 456.5.

Cat. No. 579050 1 mg

TAPI-1

A structural analog of TAPI-0 (Cat. No. 579050) with similar *in vitro* efficacy for the inhibition of MMPs and TACE. However, TAPI-1 is more stable in serum than TAPI-0. Also blocks the shedding of several cell surface proteins such as IL-6 receptor, p60 TNF receptor, and 80 kDa TNF receptor. *Purity: ≥98% by HPLC.* M.W. 499.6.

Cat. No. 579051 1 mg

TAPI-2

A hydroxamate-based inhibitor of MMPs and TACE. Inhibits the activation-induced shedding of L-selectin from neutrophils, eosinophils, and lymphocytes. Also acts as an inhibitor of ACE secretase (IC_{50} = 18 µM). *Purity: ≥98% by HPLC.* M.W. 415.5.

Cat. No. 579052 1 mg

NEW! Mitochondrial Research Tools

Bcl-x_L $BH4_{4-23}$, Human, Cell-Permeable

A cell-permeable peptide that prevents apoptotic cell death by directly binding to the voltage-dependent anion channel (VDAC) to block its activity. Inhibits the release of cytochrome *c* and prevents the loss of mitochondrial membrane potential ($\Delta\Psi_m$). Following its uptake, it is mainly localized in the mitochondria. *Purity: ≥95% by HPLC.* M.W. 3825.4.

Cat. No. 197217 1 mg

Helenalin, *A chamissonis* ssp. *foliosa*

A naturally-occurring, cell-permeable pseudoguaianolide sesquiterpenoid lactone that inhibits NF-κB-DNA binding activity by selectively alkylating the p65 subunit of NF-κB. Induces apoptosis (10 - 50 µM) in Jurkat T cells by releasing cytochrome *c* from mitochondria, leading to the loss of mitochondrial transmembrane potential ($\Delta\Psi_m$). M.W. 262.3.

Cat. No. 374000 500 µg

Ref.: Dirch, V.M., et al. 2001. *Cancer Res.* **61,** 5817; Lyss, G., et al. 1998. *J. Biol. Chem.* **273,** 33508

Stolonoxide A, Methyl Ester

A cell-permeable derivative of the naturally–occurring endoperoxide Stolonoxide A isolated from Mediterranean tunicate *Stolonica socialis.* A potent and specific inhibitor of mitochondrial respiratory chain at the ubiquinone junction by affecting both complex II and complex III (IC_{50} < 1 µM). *Purity: ≥95% by NMR.* M.W. 420.6.

Cat. No. 569410 100 µg

Ref.: Fontana, A., et al. 2001. *J. Med. Chem.* **44,** 2362

TMRM (Tetramethylrhodamine Methyl Ester Perchlorate)

A lipophilic, cationic fluorophore analog of Rhodamine 123 (Cat. No. 555505) that can be used to detect changes in membrane potential ($\Delta\Psi_m$) in isolated mitochondria or in intact cells. At low concentrations (~500 nM), it does not exhibit any inhibitory effect on mitochondrial respiration. TMRM does not accumulate in depolarized mitochondria. *Purity: ≥95% by HPLC. Excitation max. : ~550 nm; emission max.: ~585 nm.* M.W. 500.9.

Cat. No. 605100 10 mg

Ref.: Elmore, S.P., et al. 2001. *FASEB J.* **15,** 2286; Petronilli, V., et al. 2001. *J. Biol. Chem.* **276,** 12030; Rasola, A., and Geuna, M. 2001. *Cytometry* **45,** 151.

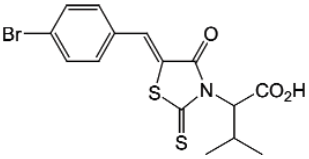
NEW! Substrates for Apoptosis Research

Product	Cat. No.	Comments	Size	Price
Caspase–1/Caspase–4 Substrate I, Fluorogenic	**400005**	A fluorogenic substrate for caspase-1 (k_{cat}/K_m = 7.55 x 10^5 $M^{-1}s^{-1}$) and caspase-4. Also displays greater sensitivity than Ac-YVAD-AMC towards caspase-1. (Ac-Trp-Glu-Ala-Asp-AMC)	1 mg 5 mg	
Caspase–1/Caspase–4 Substrate II, Fluorogenic	**400006**	A fluorogenic substrate for caspase-1 (k_{cat}/K_m = 2.41 x 10^5 $M^{-1}s^{-1}$) and caspase-4. Also displays nearly 4–fold greater sensitivity than Ac-YVAD-AMC towards caspase-1. (Ac-Trp-Val-Ala-Asp-AMC)	1 mg 5 mg	
Caspase–1/Caspase–4 Substrate III, Colorimetric	**400007**	A colorimetric substrate for caspase-1 and caspase-4. Exhibits higher affinity towards caspase-1 than caspase-4. Also displays greater sensitivity than Ac-YVAD-*p*NA towards caspase-1. (Ac-Trp-Glu-Ala-Asp-*p*NA)	5 mg	
Caspase–1/Caspase–4 Substrate IV, Colorimetric	**400008**	A colorimetric substrate for caspase-1 and caspase-4. Exhibits higher affinity towards caspase-1 than caspase-4. Also displays greater sensitivity than Ac-YVAD-*p*NA towards caspase-1. (Ac-Trp-Val-Ala-Asp-*p*NA)	5 mg	
Granzyme B Substrate VI	**368065**	A granzyme B substrate (k_{cat}/K_m = 52.7 $M^{-1}s^{-1}$). The cleavage (Asp-Trp) is monitored at 220 nm by reverse phase HPLC using a C18 column. (Ac-Ile-Glu-Pro-Asp-Trp-Gly-Ala-NH_2)	5 mg	
Granzyme B Substrate VII	**368066**	A granzyme B substrate (k_{cat}/K_m = 9.4 $M^{-1}s^{-1}$). The cleavage (Asp-Trp) is monitored at 220 nm by reverse phase HPLC using a C18 column. (Ac-Ile-Glu-Pro-Asp-Trp-Asn-Ala-NH_2)	5 mg	
Granzyme B Substrate VIII, Colorimetric	**368067**	A colorimetric substrate for granzyme B (k_{cat}/K_m = 6.6 x 10^4 $M^{-1}s^{-1}$). (Ac-Ile-Glu-Pro-Asp-*p*NA)	5 mg	
Granzyme B Substrate IX, Fluorogenic	**368068**	A fluorogenic substrate for granzyme B (k_{cat}/K_m = 3,330 $M^{-1}s^{-1}$) that also recognizes granzyme B variants (k_{cat}/K_m = 55 $M^{-1}s^{-1}$ for R192A and k_{cat}/K_m = 8.8 $M^{-1}s^{-1}$ for R192E). (Ac-Ile-Glu-Pro-Asp-AMC)	1 mg 5 mg	

NEW! Apoptosis Inducers

BH3I-1

A cell-permeable BH3 mimetic that induces apoptosis by specifically preventing BH3 domain-mediated interaction between pro-apoptotic and anti-apoptotic members of the Bcl-2 family (K_i = 2.4 - 7.8 µM). Inhibits the binding of Bak BH3 peptide to Bcl-x_L by targeting the BH3 binding pocket of Bcl-x_L. *Purity: ≥95% by HPLC.* M.W. 400.3



Cat. No. 286890 5 mg

Ref.: Degterev, A., et al. 2001. *Nat. Cell Biol.* **3,** 173.

BH3I-2'

A cell-permeable BH3 mimetic that induces apoptosis by preventing BH3 domain-mediated interaction between pro-apoptotic and anti-apoptotic members of the Bcl-2 family (K_i = 3.3 µM). *Purity: ≥95% by HPLC.*

Cat. No. 286891 1 mg
5 mg

Ref.: Degterev, A., et al. 2001. *Nature Cell Biol.* **3,** 173.

3-BAABE

A benzoic acid analog with strong anticancer activity on human leukemia and lymphoma cells (IC_{50} < 200 ng/ml) and on cell lines of prostate, colon, ductal, and kidney cancer (IC_{50} = 800 - 880 ng/ml). Elicits apoptosis through a pathway that is limited to the specific activation of apical caspase-9. *Purity: ≥95% by HPLC.* M.W. 286.1.

Cat. No. 195000 1 mg

Ref.: Schlesinger, M., et al. 2000. *Biochem. Pharmacol.* **60,** 1693.

Smad Proteins: The Transducers of TGF-β Signaling

Smad proteins are important transducers of signals from TGF-β superfamily ligands during cell proliferation, differentiation, and death. Binding of TGF-β to the cell surface receptors results in activation of receptor kinases that phosphorylate and activate downstream Smad and Smad proteins. Activated Smad proteins translocate into the nucleus and associate with specific DNA-binding proteins and activate gene expression. Most Smads consist of two conserved domains - Mad homology (MH) domains I and 2 that are separated by a non-conserved linker region. These domains lack enzymatic activity and, instead, Smads mediate their effects through protein-protein and protein-DNA interactions. CALBIOCHEM® introduces four new antibodies for your TGF-β-Smad pathway research.

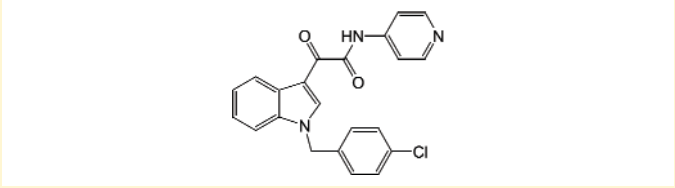
Product	Cat. No.	Comments	Application*	Size	Price
Anti-Smad1, Human (Rabbit)	**566410**	Immunogen used was a fusion protein corresponding to amino acid residues 147 – 258 of human Smad1. Recognizes the 55 - 60 kDa Smad1. Also detects Smad5, but does not cross-react with Smad2.	IB, IP	50 µg	
Anti-Smad1, Phospho-Specific (Ser^{463}/Ser^{465}), Human (Rabbit)	**566411**	Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues 455 – 465 of human Smad1. Recognizes the 65 kDa phosphorylated Smad1.	IB	50 µg	
Anti-Smad2/3, Human (Rabbit)	**566412**	Immunogen used was a fusion protein corresponding to amino acid residues 186 – 273 of human Smad2. Recognizes the 55 - 60 kDa Smad2, as well as Smad3. Does not cross-react with Smad1.	IB, IP	50 µg	
Anti-Smad2, Phospho-Specific, (Ser^{465}/Ser^{467}), Human (Rabbit)	**566413**	Immunogen used was a synthetic phosphopeptide corresponding to amino acid residues 457 – 467 of human Smad2. Recognizes the 55 - 60 kDa phosphorylated Smad2.	IB	50 µg	
Anti-Smad3 (206-220), Human (Rabbit)	**566414**	Immunogen used was a synthetic peptide corresponding to amino acid residues 206 – 220 of human Smad3. Recognizes the ~50 kDa Smad3.	IB	50 µg	

***IB:** immunoblotting; **IP:** immunoprecipitation

NEW! Cytoskeletal Research Tools

D-24851

A potent anticancer agent that displays antitumor activity both *in vitro* and *in vivo*. It destabilizes microtubules in tumor cells as well as in a cell-free system. Binds to tubulin directly and inhibits polymerization (IC_{50} = 300 nM). Also blocks cell cycle at G_2/M phase. Effective towards multidrug-resistant (MDR) tumor cells.
Purity: ≥98% by HPLC. M.W. 398.8.



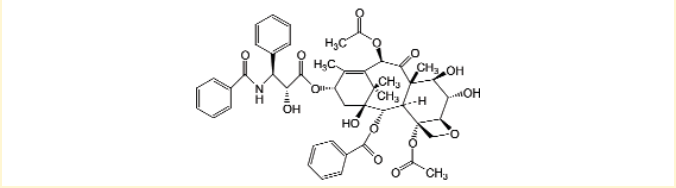
Cat. No. 251405 1 mg

Ref.: Bacher, G., et al. 2001. *Cancer Res.* **61,** 392.

Also Available...

Paclitaxel, 6α-Hydroxy

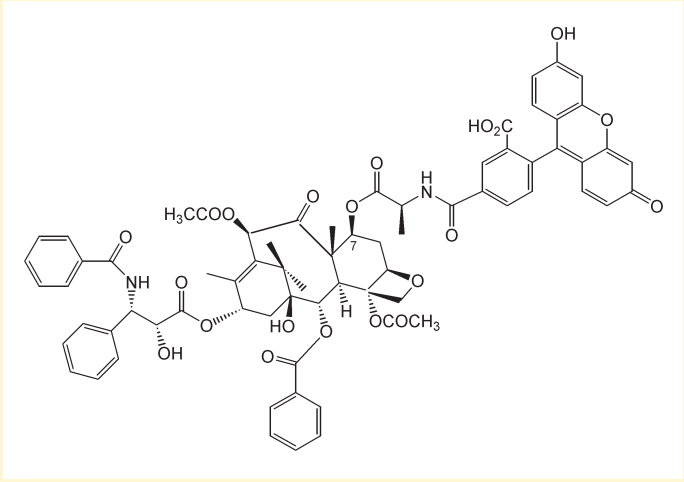
A metabolite of the anticancer agent Paclitaxel (Cat. No. 580555).
Purity: ≥98% by HPLC. M.W. 869.9. Note: 20 nmol = 17.4 µg



Cat. No. 580559 20 nmol

Flutax-1

A fluorescent taxoid that reversibly interacts with the paclitaxel binding sites of microtubules with high affinity ($K_a = 10^7\ M^{-1}$). A useful probe for developing screening assays for new drugs binding to the paclitaxel site.
Purity: ≥95% by HPLC. M.W. 1283.3.



Cat. No. 344082 10 µg
50 µg

Ref.: Abal, M., et al. 2001. *Cell Motil. Cytoskeleton* **49,** 1; Diaz, J.F., et al. 2001. *J. Biol. Chem.* **275,** 26265; Evangelio, J.A., et al. 1998. *Cell Motil. Cytoskeleton* **39,** 73

New! Antibodies to Cathepsins

Product	Cat. No.	Comments	Application*	Size	Price
Anti-Cathepsin K, Human (Rabbit)	**219386**	Recognizes both the proenzyme form and the mature form of cathepsin K.	IB	100 µl	
Anti-Cathepsin L, Human (Rabbit)	**219387**	Recognizes both the proenzyme form and the mature form of cathepsin L.	IB	100 µl	
Anti-Cathepsin S, Bovine (Rabbit)	**219389**	Recognizes both the proenzyme form and the mature form of cathepsin S. Reacts with cathepsin S from both human and bovine.	IB	100 µl	
Anti-Cathepsin S, Human (Rabbit)	**219384**	Recognizes both the proenzyme form and the mature form of cathepsin S.	IB	100 µl	

***IB:** immunoblotting

New! Tools for Nitric Oxide and Oxidative Stress Research

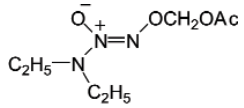
Cepharanthine
Stephania cepharantha Hayata

A biscoclaurine alkaloid with anti-inflammatory, anti-allergic, and immunomodulatory properties. More potent than α-tocopherol in inhibiting mitochondrial lipid peroxidation (IC_{50} = 23 µM). Induces apoptosis in doxorubicin-sensitive (P388/S) and resistant (P388/DOX) cells. Inhibits both the α- and the β-form of phospholipase B. *Purity: ≥95% by TLC.* M.W. 606.7.

Cat. No. 219500 1 g

Diethylamine NONOate/AM

An esterase-sensitive, O2-acetoxymethylated diazeniumdiolate that acts as an intracellular nitric oxide (NO) donor (1.83 mol of NO/mol). Acts as an intiproliferative agent and induces apoptosis in NO-sensitive human leukemia HL-60 (IC_{50} = 8.3 mM) and U937 (IC_{50} = 53 mM) cell lines. Also reported to suppress oxidized LDL-induced caspase-3 activation in bovine aortic endothelial cells. Exhibits enhanced stability in physiological buffers. Useful for temporal and spatial release of NO. *Purity: ≥95% by NMR.* M.W. 205.2.



Cat. No. 292505 1 mg
5 mg

Ref.: Kotamraju, S., et al. 2001. *J. Biol. Chem.* **276,** 17316; Saavedra, J.E., et al. 2000. *J. Med. Chem.* **43,** 261.

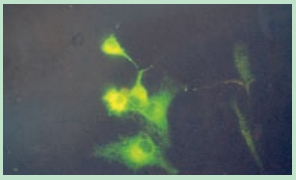
BEC, Hydrochloride

A boronic acid-based arginine analog that acts as a slow-binding competitive inhibitor of the binuclear manganese metalloenzyme arginase (Ki = 0.4 - 0.6 mM). BEC does not inhibit nitric oxide synthase (NOS). Causes significant enhancement of NO-dependent smooth muscle relaxation in human penile corpus cavernosum tissue. *Purity: ≥95% by TLC.* M.W. 229.5.

Cat. No. 197900 5 mg

Ref.: Kim, N.N., et al. 2001. *Biochemistry* **40,** 2678.

Anti-Nitric Oxide Synthase, Inducible, Human, C-Terminal (Rabbit)



Raised against a synthetic peptide (SLEMSAL) from the C-terminal region of human iNOS plus 4 additional residues. Recognizes iNOS protein (130 kDa) in cell lysates from cytokine-stimulated human chondrocytes. Does not cross–react with eNOS or bNOS. Supplied with 200 µl of human chondrocyte lysate as a positive control.

Cat. No. 482755 1 set

Ref.: Wang, M.X., et al. 2001. *Nitric Oxide* **5,** 219; Nicholson, S., et al. 1996. *J. Exp. Med.* **183,** 2293.

HCMV Inhibitor

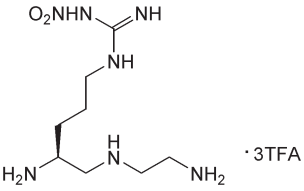
A potent inhibitor of human cytomegalovirus (HCMV; IC_{50} = 19 nM); only weakly inhibits PKC (IC_{50} = 10 µM) and is not cytotoxic in cell culture. *Purity: ≥95% by HPLC.* M.W. 353.4.

Cat. No. 373200 1 mg

Ref.: Slater, M.J., et al. 2001. *Bioorg. Med. Chem. Lett.* **11,** 1993.

nNOS Inhibitor I

A potent and highly selective inhibitor of neuronal nitric oxide synthase (nNOS; K_i = 120 nM). Displays >2500-fold and 320-fold selectivity over eNOS and iNOS, respectively. *Purity: ≥95% by TLC.* M.W. 625.4.

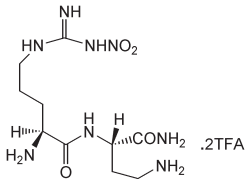


Cat. No. 490070 1 mg
5 mg

Ref.: Hah, J.M., et al. 2001. *J. Med. Chem.* **44,** 2667.

nNOS Inhibitor II

A potent and highly selective inhibitor of neuronal nitric oxide synthase nNOS; (K_i = 130 nM). Displays >1500-fold and 192-fold selectivity over eNOS and iNOS, respectively. *Purity: ≥95% by TLC.* M.W. 564.4.

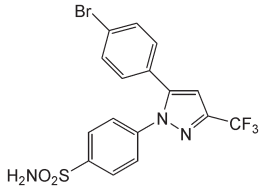


Cat. No. 490071 1 mg
5 mg

Ref.: Huang, H., et al. 1999. *J. Med. Chem.* **42,** 3147.

SC 558

A potent and selective COX-2 inhibitor that displays about 1900-fold greater selectivity for COX-2 over COX-1 (IC_{50} = 9.3 nM for hCOX-2 versus 17.7 µM for hCOX-1). *Purity: ≥95% by HPLC.* M.W. 446.2.



Cat. No. 565608 1 mg

SC-58125

A non-steroidal anti–inflammatory agent that acts as a potent and selective inhibitor of COX-2 (IC_{50} = 50 nM) compared to COX-1 (IC_{50} ≥ 10 µM). *Purity: ≥98% by HPLC.* M.W. 384.3.

Cat. No. 565620 5 mg

Ref.: Williams, C.S., et al. 2001. *Neoplasia* **3,** 428; Nakayama, M., et al. 1998. *Proc. Natl. Acad. Sci. USA* **95,** 10954.

Look for this symbol in the 2002/03 General Catalog for our complete line of Nitric Oxide and Oxidative Stress Research Tools

NEW! Tools for Angiogenesis Research

Angiogenesis is a multi-step process that permits solid tumor growth by generating new vasculature. It consists of the degradation of basement membrane at the post-capillary venule, migration of endothelial cells to the tumor, proliferation of endothelial cells, canalization and branching, and the formation of new basement membrane. Angiogenic growth factors such as FGF, TNF-α, VEGF, and angiogenin promote the process of angiogenesis by acting as autocrine or paracrine agents. Dormant tumors secrete inhibitory factors such as angiostatin, thrombospondins, and tissue inhibitors of metalloproteinases that prevent tumors from switching to the angiogenic phenotype and arrest the tumor growth. Most tumors can persist for years without any angiogenic activity and may remain 2 to 3 mm in size. In this dormant stage, the rate of tumor cell proliferation is balanced by apoptosis of tumor cells. However, when they switch to the angiogenic phenotype they grow rapidly. Neovascularization is a rather uncommon process under normal conditions. However, tumor growth and metastasis involve neovascularization, and, angiogenesis is a prominent target for therapeutic intervention. Most of the commonly used antitumor agents are cytotoxic in nature to all cells, whereas angiogenesis inhibitors are more selective and affect only the vasculature, thereby reducing toxicity.

Ref.: Fox, S.B., et al. 2001. *Lancet Oncol.* **2,** 278; Ribatti, D, et al. 2001. *Acta Haematol.* **106,** 157; Beck, L., and D'Amore, P.A. 1997. *FASEB J.* **11,** 365; Gastl, G., et al. 1997. *Oncology* **54,** 177 ; Uhr, J.W., et al. 1997. *Nature Med.* **3,** 505.

Look for this symbol in the 2002/03 General Catalog for our complete line of Angiogenesis Related Research Tools

BTB09702

Acts as a dose-dependent and selective Mesangial Cell (MC) Proliferation inhibitor. Has a fit value of 4.67 with the catalyst-generated pharmacophore model. *Purity: ≥95% by HPLC.* M.W. 447.5.

Cat. No. 203880 5 mg

Ref.: Kurogi, Y., et al. 2001. *J. Med. Chem* **44,** 2304.

DL-Thiorphan

A thiol-containing amido acid that selectively binds to the active site zinc (Zn^{2+}) of MMPs and blocks their activity (IC_{50} = 2.1 nM for neutral endopeptidase). Also reported to inhibit the activity of angiotensin-converting enzyme (ACE) at much higher concentrations (IC_{50} = 14 µM). M.W. 253.3.

Cat. No. 598510 10 mg

PR-39, Porcine

A member of the proline/arginine-rich group of cathelicidin peptides that acts as a regulator of angiogenesis. Inhibits the ubiquitin-proteasome-dependent degradation of hypoxia-inducible factor-1α, resulting in accelerated formation of vascular structures. Reported to attenuate myocardial ischemia-hyperfusion injury in mice and has been shown to induce the synthesis of syndecan-1, a proteoglycan involved in cell-to-matrix interactions and wound healing. *Purity: ≥98% by HPLC.* M.W. 4719.7.

Cat. No. 529645 100 µg

Ref.: Li, J., et al. 2000. *Nat. Med.* **6,** 49.

SMC Proliferation Inhibitor-2w

A diaryl amide analog that exhibits about 15 times greater selectivity in inhibiting the proliferation of human coronary artery smooth muscle cells (IC_{50} = 310 nM) over the endothelial cells (IC_{50} = 4.6 µM). Exhibits over 80-fold greater potency than Tranilast (Cat. No. 616400). *Purity: ≥95% by HPLC.* M.W. 419.4.

Cat. No. 573117 5 mg

VEGF Inhibitor, Flt_{2-11}

(NITVTLKKFPL)

A peptide derived from the second Ig-like domain of VEGFR-1 that inhibits angiogenesis in chick chorioallantoic membrane and inhibits VEGF-induced vascular permeability. Neither binds to vascular endothelial growth factor (VEGF) nor inhibits its binding to VEGFR. *Purity: ≥95% by HPLC.* M.W. 1273.6.

Cat. No. 676493 1 mg

Ref.: Tan, D.C.W., et al. 2001. *FEBS Lett.* **494,** 150.

VEGF Inhibitor, *Je*-11

[$(RTELNVGIDFNWEYPAS)_2$K-NH_2]

A dimerized peptide derived from the third Ig-like domain of the human VEGFR-2 comprising residues 247-261. Inhibits VEGF-stimulated autophosphorylation of VEGFR-2 and blocks the proliferation and migration of cultured microvascular endothelial cells. Binds to VEGF and blocks its interaction with VEGFR-2 (IC_{50} = 500 nM on extracellular VEGFR-2 fragments). *Purity: ≥95% by HPLC.*

Cat. No. 676494 500 µg
1 mg

Ref.: Piossek, C., et al. 1999. *J. Biol. Chem.* **274,** 5612.

VEGF Inhibitor, V1

(ATWLPPR)

A heptapeptide that binds to the VEGFR-2 with high affinity and effectively blocks the interaction of VEGF with VEGFR-2 (IC_{50} = 80 µM). Inhibits the proliferation of human endothelial cells *in vitro* and abolishes VEGF-induced angiogenesis *in vivo*. *Purity: ≥95% by HPLC.* M.W. 840.0.

Cat. No. 676495 1 mg

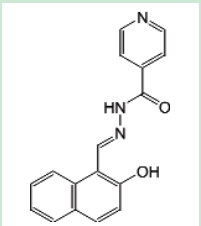
Ref.: Binetruy-Tournaire, R., et al. 2000. *EMBO J.* **19,** 1525.

Introducing New Iron Chelators...

Highly potent, cell-permeable, iron (Fe^{3+}) chelators that mobilize iron from cells and prevent iron uptake from transferrin, even at low concentrations. Reported to prevent iron-mediated oxyradical formation and minimize tissue damage.

NIH

(2-Hydroxy-1-naphthaldehyde isonicotinoyl hyrazone)



Cat. No. 481910 50 mg

Ref.: Gao, J. and Richardson, D.R. 2001. *Blood* **98,** 842; Darnell, G., and Richardson, D.R. 1999. *Blood* **94,** 781.

Look for this symbol in the 2002/03 General Catalog for our complete line of Apoptosis Related Research Tools

PIH

(Pyridoxal isonicotinoyl hydrazone)

Cat. No. 528110 50 mg

Ref.: Thomas, S.R., et al. 2001. *J. Immunol.* **166,** 6332; Hermes-Linda, M., et al. 2000. *Biochim. Biophys. Acta* **1523,** 154.

TOPOISOMERASE INHIBITORS: DNA Zipper "Snagged" in Cancer Chemotherapy

DNA topoisomerases are nuclear enzymes that regulate the conformational changes in DNA topology by catalyzing the breakage and rejoining of DNA strands during the normal cell cycle. They relieve torsional stress during replication and transcription. Three different types of topoisomerases, type I (91 kDa monomer), IIα (170 kDa dimer), and IIβ (180 kDa dimer) have been reported. Simpler organisms possess only topoisomerase I; however, higher organisms have all three types of topoisomerases. While topoisomerase IIα is present in all eukaryotes, IIβ is present only in vertebrates and appears to be more closely associated cell differentiation than proliferation. Topoisomerases act by catalyzing the breakdown and rejoining reactions in the phosphodiester backbone of the DNA molecules. Topoisomerase I reversibly cleaves a single strand in duplex DNA molecule, whereas topoisomerase II breaks and rejoins both DNA strands.

During the past decade topoisomerases have become important chemotherapeutic targets for cancer treatment. Several novel compounds have been developed that can target either topoisomerase I or topoisomerase 2α-/2β- isoforms, or all three types of topoisomerases.

Ref.: Bakshi, R.P., et al. 2001. *Crit. Rev. Biochem. Mol. Biol.* **36,** 1; Felix, C.A. 2001. *Med. Pediatr. Oncol.* **36,** 525; Topcu, Z. 2001. *J. Clin. Pharm. Ther.* **26,** 405; Champoux, J.J. 2000. *Ann. N. Y. Acad. Sci.* **922,** 56; Fortune, J.M., and Osheroff, N. 2000. *Prog. Nucleic Acid Res. Mol. Biol.* **64,** 221.

Metastin: A Novel Endogenous Ligand for a G-Protein Coupled Receptor that Suppresses Metastasis

CALBIOCHEM® introduces the 54-amino acid Metastin, as well as two derivative peptides. Metastin (45-54) is found to be about ten times more potent than the parent peptide. Metastin is reported to inhibit chemotaxis and invasion of hOT7T175-transfected cells *in vitro* (IC_{50} = 50 nM), and attenuate pulmonary metastasis of hOT7T175-transfected B16-BL6 melanomas *in vivo*. Metastin 40 - 54 exhibits a 3-fold higher receptor binding affinity (K_i = 100 pM), while Metastin 45 - 54 exhibits the highest receptor binding affinity (K_i = 42 pM). The C-terminally amidated LRF-motif of these peptides is considered to be important for receptor binding, while the N-terminal portion is critical for stabilization and protection from proteolytic digestion.

Product	Cat. No.	Size	Price
Metastin, Synthetic	**445885**	250 µg	
Metastin (40-54)	**445887**	1 mg	
Metastin (45-54)	**445888**	1 mg	

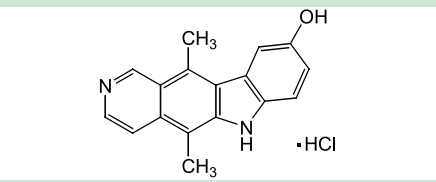
NEW! Topoisomerase Inhibitors

Product	Cat. No.	Comments	Size	Price
Merbarone	**445800**	Inhibits the catalytic activity of topoisomerase II (topo II) without damaging DNA or stabilizing DNA-topo II cleavable complexes (IC_{50} = 20 µM).	25 mg	
Netropsin, DiHCl, *Streptomyces netropsis*	**480676**	An A-T specific minor groove-binding antibiotic that acts as a weak topoisomerase I inhibitor.	10 mg	
Topotecan, HCl	**614800**	A synthetic derivative of Camptothecin (Cat. No. 208925) that acts as a potent inhibitor of topoisomerase I. Exhibits strong anti-proliferative effects on HT-29 cells (IC_{50} = 40 nM). Arrests cell cycle in S and G_2/M phases.	1 mg	

NEW! Topoisomerase II Inhibitor

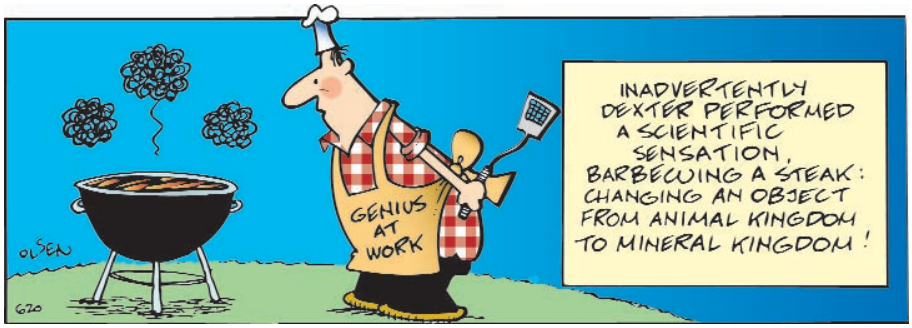
(Ellipticine, 9-Hydroxy, Hydrochloride)

An antitumor alkaloid that acts as a potent inhibitor of topoisomerase II (IC_{50} = 3.3 µM). Restores functional wild-type p53 activity via inhibition of mutant p53 protein phosphorylation. *Purity: ≥95% by HPLC.* M.W. 298.8.



Cat. No. 324680 10 mg

Ref.: Mizumoto, K., et al. 2000. *Cancer Lett.* **149,** 85; Perry, P.J., et al. 1999. *Anticancer Drug Des.* **14,** 373.



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JNK Inhibitor I, (L)–Form, Cell–Permeable

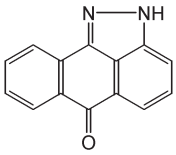
A cell-permeable, biologically–active peptide that blocks the activation domain of c-Jun NH_2-terminal kinase (JNK) and prevents the activation of the transcription factor c-Jun (IC_{50} ~1 µM). Contains the minimal 20-amino acid inhibitory domain of islet-brain (IB), which is critical for interaction with JNK. The peptide is covalently linked to the 10-amino acid HIV-TAT_{48-57} sequence that acts as a carrier peptide and two proline residue spacers. *Purity: ≥97% by HPLC.* M.W. 3923.6.

Cat. No. 420116 1 mg

Ref.: Bonny, C., et al. 2001. *Diabetes* **50,** 77.

JNK Inhibitor II

A potent, cell-permeable, selective, and reversible inhibitor of c-Jun N-terminal kinase (JNK) (IC_{50} = 40 nM for JNK-1 and JNK-2 and 90 nM for JNK-3). The inhibition is competitive with respect to ATP. *Purity: ≥ 98% by HPLC.* M.W. 220.2.



Cat. No. 420119 5 mg

Ref.: Bennett, B.L., et al. 2001. *Proc. Natl. Acad. Sci. USA* **98,** 13681; Han, Z., et al. 2001. *J. Clin. Invest.* **108,** 73.

Also Available...

JNK Inhibitor Negative Control

JNK Inhibitor I, (L)–Form Cell–Permeable, Negative Control	**Cat. No. 420118**	1 mg

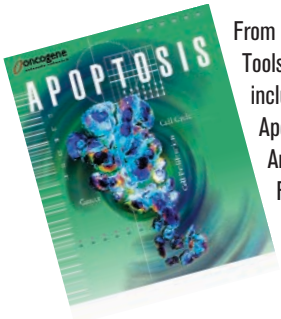
JNK Substrate and Assay Kit

JNK Substrate, c-Jun 1-79	**Cat. No. 420108**	100 µg
JNK Activity Immunoassay Kit	**420115**	1 Kit

JNK Antibodies

Anti-SAPK/JNK (Rabbit)	**Cat. No. 559304**	200 µl
Anti-SAPK/JNK, Phospho-Specific (Thr^{183}, Thr^{185}), Human (Rabbit)	**559309**	10 Tests
Anti-JNK, Phospho-Specific (Ab-1) (Rabbit)	**PC452**	25 µl

NEW! Apoptosis IV Catalog & Technical Resource



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