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BIOACTIVE LIPIDS

Bioactive lipids, generated during the remodeling of membrane lipids by activated lipases, serve as intra- and extracellular mediators in cell signaling. They play important roles in a variety of processes involving cell-cell communication, inflammation, host-defense mechanisms, and ischemia-reperfusion. Lipid mediators such as eicosanoids exhibit cell type specificity; however, others such as diacylglycerol (DAG) and ceramide are ubiquitous in their distribution. Ceramide shows a high degree of specificity when compared to some of the other closely-related lipids.

There are three major pathways involved in lipid signaling (see figure below). Ceramide has emerged as a potentially important pleiotropic signal transducer in apoptosis as well as in cell proliferation and differentiation. Ceramide is generated from sphingomyelin by the action of sphingomyelinase. All sphingolipids contain ceramide as the basic hydrophilic component that is released by activated sphingomyelinase. Sphingomyelinase can be activated by a variety of extracellular signals including tumor necrosis factor-α (TNF-α), γ-interferon (γ-IFN), interleukin-1 (IL-1), and ionizing radiation. The development of exogenous cell-permeable analogs of ceramide has facilitated the understanding of intracellular effects of ceramide. Ceramide is reported to play an important role in the activation of NF-κB, protein kinase C_ζ, phospholipase A_2 (PLA_2), JNK/SAPK, and p42 MAP kinase. Recently, a ceramide-activated protein phosphatase (CAPP), belonging to the PP2A serine/threonine phosphatase family, has been reported and linked directly to the role of ceramide in apoptosis. Ceramide is also involved in the action of another major pathway in lipid signaling – the synthesis of eicosanoids via the activation of cyclooxygenase (also known as COX or prostaglandin endoperoxidase H synthase) and PLA_2.

Activation of PLA_2 and generation of arachidonic acid is a major step in the downstream synthesis of prostaglandins, thromboxanes, and leukotrienes. PLA_2 is found in a wide variety of cells and its expression is stimulated by the action of a number of extracellular signals including the TNF, IL-2, IL-6, and several inflammatory signals. The secreted PLA_2 requires millimolar levels of calcium for its activation. The activated PLA_2 then translocates to the membrane where it

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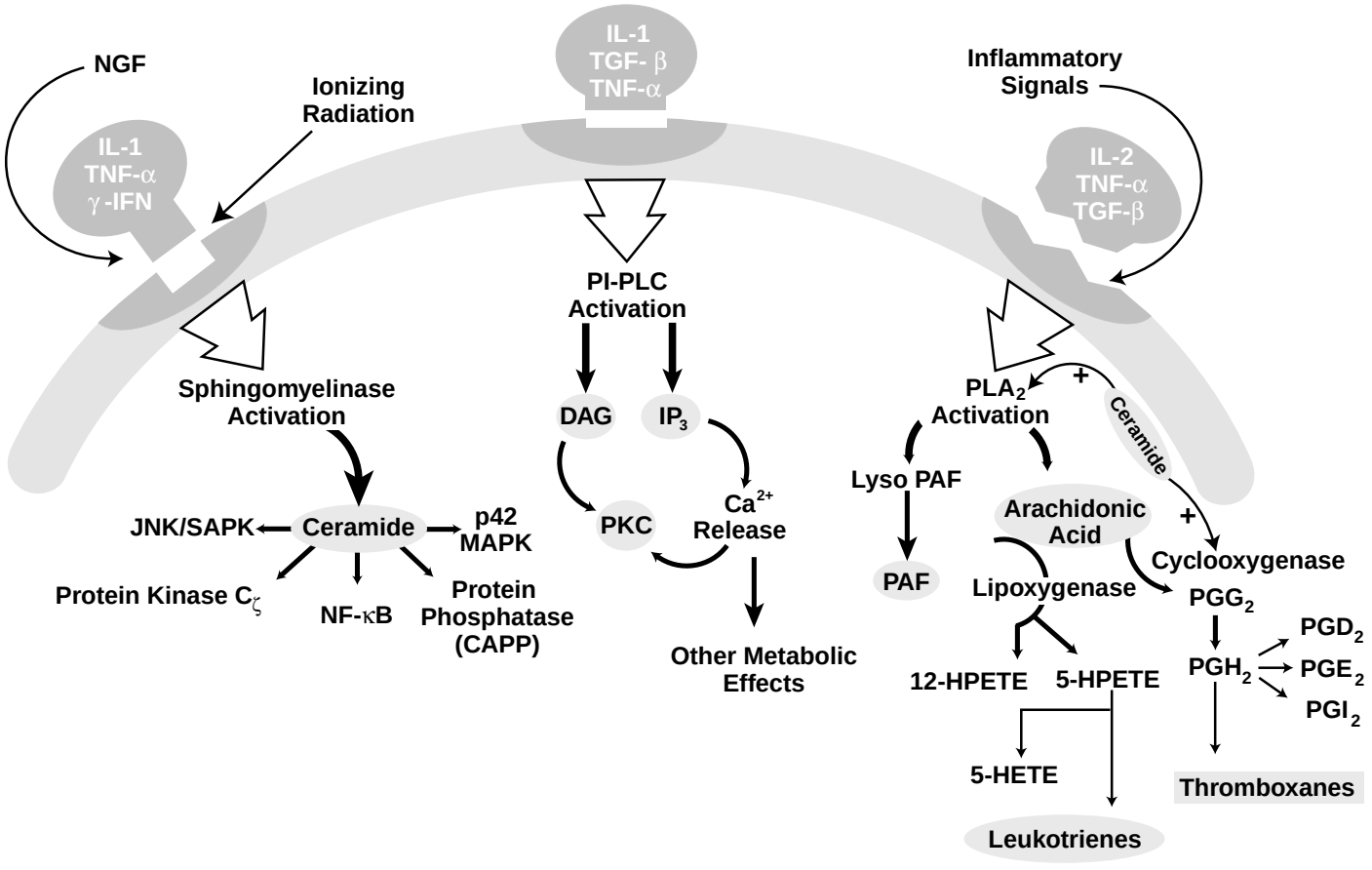

hydrolyzes glycerophospholipids at the *sn*-2 position yielding free fatty acid (arachidonic acid) and lysophospholipid. The latter can yield platelet activating factor (PAF) , another important second messenger, by the action of an acetyltransferase. Arachidonic acid undergoes a stepwise catalysis to yield reactive intermediates, PGG_2 and PGH_2, that serve as precursors of prostaglandins, prostacyclins, and thromboxanes. Prostaglandin, the 20-carbon polyunsaturated molecule generated by the action of prostaglandin endoperoxidase H synthase (PGHS), functions in an autocrine or paracrine manner. Prostaglandin action is mediated by a series of G-protein coupled cell surface receptors. PGHS can exist as either PGHS-1 or PGHS-2. PGHS-1 is a constitutive enzyme and is associated with the endoplasmic reticulum. On the other hand, PGHS-2 is an inducible enzyme that is mainly associated with the nuclear envelope. Its activity is induced by the action of several growth factors, cytokines, and inflammatory signals.

The third major pathway in lipid-signaling involves phosphoinositide-specific phospholipase C (PLC) that generates two ubiquitous second messengers – diacyglycerol (DAG) and inositol trisphosphate (IP_3). PLC is reported to exist in three major forms – β, γ, and δ. The PLC_γ is activated by tyrosine kinases, while the PLC_β is regulated by the α-subunit of the Gq family of G-proteins as well as through the βγ-subunits of the pertussis toxin-sensitive G-protein. Binding of a hormone or other affector molecule to the membrane receptor results in the activation of PLC via a G-protein-dependent phenomenon. The activated PLC hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce DAG and IP_3. The IP_3 binds to the IP_3 receptor on the endoplasmic reticulum and causes the release of endogenous Ca^{2+} that binds to the cytosolic PKC and exposes the phospholipid binding site. The regulatory domain of PKC contains a Ca^{2+} binding site, designated the C2 region, that is found only on α, β, and γ-isozymes. The PKC isozymes δ, ε, η, θ, μ, and ζ lack the C2 region and do not require Ca^{2+} for their activation. The binding of Ca^{2+} translocates PKC to the membrane where it interacts with DAG to transform into a fully active enzyme. All PKC isoenzymes, with the exception of ζ and λ, are activated by diacylglycerol (DAG) that acts by increasing the affinity of PKC for Ca^{2+} and helps in full activation of PKC without a net increase in Ca^{2+} concentration.

References:
Malisan, F., and Testi, R. 1999. *FEBS Lett.* **452,** 100.
Jaffrezou, J.P., et al. 1998. *FASEB J.* **12,** 999.
Martin, T.F.J. 1998. *Annu. Rev. Cell Dev. Biol.* **14,** 231.
Serhan, C.N., et al. 1996. *FASEB J.* **10,** 1147.
Roberts, M. 1996. *FASEB J.* **10,** 1159.
Spiegel, S., and Milstein, S. 1995. *J. Membr. Biol.* **146,** 225.
Obeid, L.M., and Hannun, Y.A. 1995. *J. Cell. Biochem.* **58,** 191.
Banno, Y., et al. 1994. *FEBS Lett.* **340,** 185.
Hannun, Y.A. 1994. *J. Biol. Chem.* **269,** 3125.
Piomelli, D. 1993. *Curr. Opin. Cell Biol.* **5,** 274.
Bielawska, A., et al. 1993. *J. Biol. Chem.* **268,** 26226.
Dobrowsky, R.T., and Hannun, Y.A. 1992. *J. Biol. Chem.* **267,** 5048.
Nishizuka, Y. 1992. *Science* **258,** 607.
Whatley, R.E., et al. 1990. *Prog. Lipid Res.* **29,** 45.
Mene, P., et al. 1989. *Am. J. Physiol.* **256,** F375.

Platelet-Activating Factors, Analogs, and Antagonists

Platelet activating factor (PAF), one of the most potent phospholipid agonists, exerts its action by stimulating G-protein-linked cell surface receptors. High affinity binding sites for PAF have been detected in the plasma membrane of a number of cell types. Stimulation of these receptors causes the activation of PLC and PLA_2 with resultant formation of IP_3 and DAG that activate protein kinase C and mobilize calcium from intracellular stores. Second messengers generated by these multiple signalling pathways also regulate the PAF-induced expression of primary response genes. The major metabolic effects of PAF are: vasodilation, stimulation of platelet aggregation, increased amplitude of smooth muscle contraction, increased renal blood flow, and bronchial hyper-responsiveness.

Product	Cat. No.	Comments
Platelet Activating Factor-16	511075	Potent mediator of inflammation. Activates MAP kinase and MAP kinase kinase (MEK).
lyso-Platelet Activating Factor-16	511077	Synthetic analog that serves as a precursor of PAF.
Platelet Activating Factor-16 Antagonist	511082	PAF analog that inhibits phospholipid turnover and PAF-induced platelet aggregation.
Platelet Activating Factor-16, Propionyl-	511096	A more potent analog of PAF.
Platelet Activating Factor-16, 2-Thioacetyl-	511074	A thiolated analog of PAF.
Platelet Activating Factor-18	511078	Synthetic, water-soluble PAF.
lyso-Platelet Activating Factor-18	511079	Synthetic, water-soluble PAF.

Sphingolipid-Related Products

Product	Cat. No.	Comments	Ref.
Lactosyl Ceramide, Bovine	427572	A common precursor of the ganglio-, globo-, globoiso-, lacto-, neolacto-, and muco-series of glycosphingolipids. Stimulates Ras-GTP loading, MEK, Raf kinase, and p44 MAP kinase, and c-*fos* expression in human aortic smooth muscle cells.	1, 2
NBD-Ceramide, D-*erythro*-C_6-	219545	Used to selectively stain the *trans*-Golgi in living and fixed cells. Useful for studying the polarization of terminally differentiated epithelial cells.	3
NBD-C_6-Sphingomyelin	479824	A metabolic product of NBD-C_6-Ceramide (Cat. No. 219545). Useful as a probe for endocytosis, translocation, and intracellular distribution of sphingolipids.	4
Safingol	559300	A lysosphingolipid that acts as a protein kinase C (PKC) inhibitor. Competitively interacts at the regulatory phorbol binding domain of PKC (IC_{50} = 37.5 µM).	5, 6
Sphingomyelin, Bovine Brain	567705	Contains primarily nervonic and stearic acids. Precursor to ceramide second messenger via the action of sphingomyelinase. Zwitterionic at pH 7.0.	7
D-*erythro*-Sphingosine, Free Base, Bovine Brain	567725	A potent and selective inhibitor of protein kinase C (PKC; IC_{50} = 2.8 µM) and insulin receptor tyrosine kinase. PKC inhibition is competitive with respect to diacylglycerol, phorbol dibutyrate, and Ca^{2+}. Induces apoptosis in human leukemia HL-60 cells.	8
D-*erythro*-Sphingosine, Free Base, Bovine Brain, High Purity	567726	Highly purified preparation of Cat. No. 567725 containing >99% of the *erythro* isomer.	
D-*erythro*-Sphingosine, N-Acetyl- (C_2 Ceramide)	110145	Biologically active, cell-permeable, non-physiological ceramide analog. Induces intranucleosomal DNA fragmentation. Inhibits cell growth and induces apoptosis in HL-60 cells. Stimulates a cytosolic serine/threonine protein phosphatase in T9 cells and stress activated protein kinase in HL-60 human promyelocytic cells.	9, 10
D-*erythro*-Sphingosine, Dihydro-	300230	Biosynthetic precursor of sphingosine. Inhibits protein kinase C in Chinese hamster ovary cells (IC_{50} = 2.8 µM) and in HL-60 cells. Also directly inhibits phospholipase A_2 and phospholipase D.	11, 12
D-*erythro*-Sphingosine, Dihydro-, N-Acetyl-	219537	May be used as a negative control in studies of C_2 ceramide (Cat. No. 110145). Its initial uptake appears to be slower than that of active C_2 ceramide.	13
D-*erythro*-Sphingosine, N,N-Dimethyl-	310500	Inhibits protein kinase C (IC_{50} = 12 µM) and stimulates Src kinase activity. Useful for inhibiting cell surface expression of selectins that promote adhesion of leukocytes and tumor cells to platelets and endothelial cells. Induces apoptosis in human leukemia HL-60 cells. An inhibitor of sphingosine kinase.	14, 15
D-*erythro*-Sphingosine, N-Hexanoyl- (C_6 Ceramide)	376650	Biologically active, cell-permeable, non-physiological ceramide analog. Arrests in the G_0/G_1 phase of the cell cycle. Activates MAP kinase and protein phosphatase 2A (PP2A). Induces apoptosis in Molt-4 leukemia cells. Inhibits Ca^{2+} increase caused by Bay K 8644. Induces enlargement of late endosomes and lysosomes.	16 - 19
D-*erythro*-Sphingosine, N-Octanoyl- (C_8 Ceramide)	219540	A short chain cell-permeable analog of endogenous ceramide. Shown to inhibit cell proliferation and induce apoptosis in a variety of cell lines. Activates MAP kinase in HL-60 cells.	20
D-*erythro*-Sphingosine, N-Palmitoyl-	506420	Plays an important role in the mitochondrial (effector) phase of apoptosis.	21, 22
D-*erythro*-Sphingosine, N-Thioacetyl-	567730	A cell-permeable thioacetyl derivative of sphingosine. Induces apoptosis in Molt 4 and HaCaT keratinocyte cell lines.	23, 24
D-*erythro*-Sphingosine, N,N,N-Trimethyl-	567740	A stable N-methylated derivative of sphingosine with antitumor and anti-inflammatory properties. Inhibits PAF activation and PKC activity. Also reported to inhibit IL-1β-induced NF-κB activation and cell surface expression of crucial selectins which promote adhesion of Le^x- or sialyl-Le^x-expressing cells with platelets and endothelial cells.	25, 26
D-*erythro*-Sphingosine-1-phosphate	567727	A putative second messenger that mobilizes calcium from intracellular stores via an IP_3-independent pathway. Activates phospholipase D and inhibits chemotactic motility and invasiveness of tumor cells. Induces apoptosis in cultured hippocampal neurons.	27, 28
D-*erythro*-Sphingosine-1-phosphate, N-Octanoyl-	219535	Phosphorylated analog of N-Octanoyl-D-*erythro*-sphingosine (Cat. No. 219540). Useful tool for studying the sphingomyelin cycle. Stimulates DNA synthesis and cell division.	29
Sphingosylphosphorylcholine	567735	A deacylated derivative of sphingomyelin that acts as a potent mitogen and increases intracellular free Ca^{2+} and free arachidonate through pathways that are only partially PKC-dependent. Stimulates DNA-binding activity of the transcription activator protein AP-1. Rapidly induces tyrosine phosphorylation of focal adhesion kinase ($p125^{FAK}$) and paxillin and causes a rapid and transient activation of MAP kinase in Swiss 3T3 cells.	30, 31

References:

1. Bhunia, A.K., et al. 1997. *J. Biol. Chem.* **272,** 15642.
2. Bhunia, A.K., et al. 1996. *J. Biol. Chem.* **271,** 10660.
3. Putz, U., and Schwartzmann, G. 1995. *Eur. J. Cell Biol.* **68,** 113.
4. Babia, T., et al. 1993. *Int. J. Cancer* **54,** 839.
5. Kedderis, L.B., et al. 1995. *Fundam. Appl. Toxicol.* **25,** 201.
6. Sachs, C.W., et al. 1995. *J. Biol. Chem.* **270,** 26639.
7. Slife, C.W., et al. 1989. *J. Biol. Chem.* **264,** 10371.
8. Arnold, R.S., and Newton, A.C. 1991. *Biochemistry* **30,** 7747.
9. Westwick, J.K., et al. 1995. *J. Biol. Chem.* **270,** 22689.
10. Zhang, Q.H., et al. 1999. *Life Sci.* **65,** 1715.
11. Yung, B.Y., et al. 1994. *Biochem. Biophys. Res. Commun.* **199,** 888.
12. Franson, R.C., et al. 1992. *Biochim. Biophys. Acta* **1136,** 169.
13. Hannun, Y.A. 1994. *J. Biol. Chem.* **269,** 3125.
14. Ohta, H., et al. 1995. *Cancer Res.* **55,** 691.
15. Sakakura, C., et al. 1996. *FEBS Lett.* **379,** 177.
16. Chik, C.L., et al. 1999. *Endocrinology* **140,** 5682.
17. Li, R., et al. 1999. *J. Biol. Chem.* **274,** 21121.
18. Jayadev, S., et al. 1995. *J. Biol. Chem.* **270,** 2047.
19. Raines, M.A., et al. 1993. *J. Biol. Chem.* **268,** 14572.
20. Condorelli, F., et al. 1999. *Br. J. Pharmacol.* **127,** 75.
21. Thomas, R.L., et al. 1999. *J. Biol. Chem.* **274,** 30580.
22. Payne, J.G., et al. 1999. *J. Cell Physiol.* **180,** 263.
23. Wieder, T., et al. 1997. *FEBS Lett.* **411,** 260.
24. Bektas, M., et al. 1998. *Exp. Dermatol.* **7,** 342.
25. Murohara, T., et al. 1996. *Circ. Res.* **78,** 780.
26. Masamune, A., et al. 1995. *FEBS Lett.* **367,** 205.
27. Moore, A.N., et al. 1999. *Neuroscience* **94,** 405.
28. Mattie, M., et al. 1994. *J. Biol. Chem.* **269,** 3181.
29. Gomez-Munoz, A., et al. 1995. *Mol. Pharmacol.* **47,** 833.
30. Berger, A., et al. 1995. *Proc. Natl. Acad. Sci. USA* **92,** 5885.
31. Dettbarn, C., et al. 1995. *Brain Res.* **669,** 79.

Leukotrienes, Prostaglandins, and other Eicosanoids

Product	Cat. No.	M.W.	Comments	Ref.
Carbacyclin	212402	350.5	Stable, synthetic carbacyclic analog of prostacyclin. Mimics the effects of naturally occurring prostacyclin, but is only 10% as potent. Activates adenylate cyclase via G-protein-coupled PGI_2 receptors.	1,2
Hepoxilin A_3	375425	336.5	An endogenous lipid mediator that increases intracellular Ca^{2+} levels in human neutrophils and activates the release of arachidonic acid and diacylglycerol.	3
Leukotriene B_4	434625	336.5	Potent inflammatory agent. Stimulates c-*fos* and c-*jun* proto-oncogene transcription in human monocytes.	4,5
Leukotriene C_4	434692	625.8	Sulfidopeptide implicated in renal vasoconstriction associated with sepsis. Also plays a role in bronchioconstriction. Produced by conjugation of LTA_4 with reduced glutathione. Has been implicated in the pathophysiology of asthma.	6
Leukotriene D_4	434694	496.7	Implicated in renal and bronchial vasoconstriction. Significant mediator in asthmatic reactions. Reported to increase intracellular Ca^{2+} and activate MAP kinase in monospecific leukemic THP-1 cells.	7,8
Leukotriene E_4	434696	439.6	A potent mediator of anaphylaxis and inflammation. May play a role in bronchial asthma by participating in edema formation, mucous secretion, or muscle contraction.	9
Lipoxin A_4	437720	352.5	Potent inhibitor of cytotoxic activity of human natural killer cells. Shown to be as potent as LTB_4 in stimulating human neutrophils to generate superoxides. Acts as a potent activator of human protein kinase C.	10,11
15d-PGJ_2	538927	316.4	A metabolite of PGD_2 that has recently been identified as a natural ligand for PPARγ-dependent adipogenesis in transfected 3T3 cells and for PPARγ response element (PPRE) in CV-1 cells.	12,13
Prostaglandin B_2	538932	334.5	Most abundant prostaglandin released from osteoblasts. Induces pulmonary hypotension.	14,15
Prostaglandin D_2	538909	352.5	Major prostaglandin metabolite and an important inflammatory mediator in mast cells. Neuromodulator that evokes nerve-mediated Cl^- secretion. Stimulates adenylate cyclase.	16,17
Prostaglandin E_1	538903	354.5	Activates adenylate cyclase activity via a G-protein coupled receptor.	18
Prostaglandin $F_{2\alpha}$	538907	354.5	Major uterine luteolytic prostaglandin. A pulmonary vasoconstrictor. Inhibits nitric oxide production by the uterine tissue and increases uterine contractility.	19,20
Prostaglandin H_2	434729	352.5	Endoperoxidase involved in contraction of vascular and bronchial smooth muscles, secretion and aggregation of platelets, and lysis of cellular membranes.	21
Prostaglandin I_2, Sodium Salt	538925	374.5	Potent vasodilatory prostaglandin produced by the endothelium. Inhibits platelet aggregation by receptor-mediated stimulation of adenylate cyclase.	22
Thromboxane B_2	538911	350.4	Stable metabolite of thromboxane A_2. Often used to measure arachidonic acid metabolism.	23
U-46619	538944	350.4	Stable thromboxane A_2 mimetic and a vasoconstrictor.	24

References:

1. Sawai, T., et al. 1993. *J. Biol. Chem.* **268,** 1995.
2. Turner, J.T., et al. 1992. *J. Pharmacol. Exp. Ther.* **263,** 708.
3. Reynaud, D., et al. 1995. *Biochem. Biophys. Res. Commun.* **207,** 191.
4. Kendall, A.P., et al. 1995. *Anesthesia* **50,** 590.
5. Dokter, W.H., et al. 1994. *Leukemia* **8,** 1181.
6. Shimizu, T., et al. 1994. *Pediatr. Pulmonol.* **18,** 129.
7. Iwamoto, I., et al. 1995. *Int. Arch. Allergy Immunol.* **108,** 68.
8. Jedlitschky, G., et al. 1991. *J. Biol. Chem.* **266,** 24763.
9. Henderson, W.R., Jr. 1994. *Ann. Int. Med.* **121,** 684.
10. Flore, S., et al. 1992. *J. Biol. Chem.* **267,** 16168.
11. Badr, K.F., et al. 1989. *Proc. Natl. Acad. Sci. USA* **86,** 3438.
12. Forman, B.M., et al. 1995. *Cell* **83,** 803.
13. Ricote, M., et al. 1998. *Nature* **391,** 79.
14. Liu, F., et al. 1994. *Am. J. Physiol.* **267,** L602.
15. Feyen, J.H.M., et al. 1984. *Prostaglandins* **28,** 769.
16. Frieling, T., et al. 1994. *Am. J. Physiol.* **266,** G132.
17. Huttemeier, P.C., et al. 1993. *Prostaglandins* **45,** 177.
18. Ammer, H., and Schultz, R. 1993. *Mol. Pharmacol.* **43,** 556.
19. Vincent, D.L., et al. 1986. *Prostaglandins* **31,** 715.
20. Dong, Y.L., et al. 1997. *Am. J. Obstet. Gynecol.* **177,** 907.
21. Bowling, N., et al. 1994. *J. Mol. Cell. Cardiol.* **26,** 915.
22. Vane, J.R. 1985. *Adv. Prostaglandin Thromboxane Leukot. Res.* **15,** 11.
23. Hardie, W.D., et al. 1993. *Prostaglandins* **45,** 47.
24. Kaye, D., et al. 1997. *Eur. J. Pharmacol.* **340,** 187.

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