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Review

Chemical modulation of glycerolipid signaling and metabolic pathways ☆

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ABSTRACT

Thirty years ago, glycerolipids captured the attention of biochemical researchers as novel cellular signaling entities. We now recognize that these biomolecules occupy signaling nodes critical to a number of physiological and pathological processes. Thus, glycerolipid-metabolizing enzymes present attractive targets for new therapies. A number of fields—ranging from neuroscience and cancer to diabetes and obesity—have elucidated the signaling properties of glycerolipids. The biochemical literature teems with newly emerging small molecule inhibitors capable of manipulating glycerolipid metabolism and signaling. This ever-expanding pool of chemical modulators appears daunting to those interested in exploiting glycerolipid-signaling pathways in their model system of choice. This review distills the current body of literature surrounding glycerolipid metabolism into a more approachable format, facilitating the application of small molecule inhibitors to novel systems. This article is part of a Special Issue entitled Tools to study lipid functions.

1. Introduction

With advances in mass spectrometry-based analysis of lipids the landscape of lipid biomolecule research has significantly broadened. Glycerol-based lipids are continually found playing prominent roles in human physiology and disease: from fat storage and metabolic disorders to survival pathways in cancer. As research efforts identified these core signaling nodes, chemical tools capable of interrupting lipid metabolism became essential tools for in depth characterization both *in vitro* and *in vivo*. Thus, the scientific literature is replete with studies on the medicinal chemistry and pharmacology associated with these inhibitors. This review focuses on the enzymes regulating the most critical biosynthetic steps of glycerolipids—for either signaling or fat storage—and distills the current body of literature to the most relevant and well-characterized inhibitors. Table 1 highlights these important tools and in this way serves as a summary of this review.

Several of the targets discussed herein, have a long history in the literature, stretching as far back as the early 1900s in some cases. This review does not detail their long, complex history of biochemical and molecular characterization and will direct the reader to more comprehensive reviews where necessary. Rather, we hope it serves as an accessible, practical body of information for those unfamiliar with the medicinal chemistry efforts undertaken over the years. It is with this audience in mind that we highlight not only the capabilities of these small molecule inhibitors, but their limitations as well. In this way, we envision this review serving as a resource for the design and implementation of novel experiments, regardless of one's specific field of study or technical expertise.

Abbreviations: PLC, phospholipase C; PLD, phospholipase D; PLA, phospholipase A; PtdBuOH, phosphatidylbutanol; PC, phosphatidylcholine; PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PS, phosphatidylserine; LPC, lysophosphatidylcholine; LPA, lysophosphatidic acid; ATX, autotaxin; PIP_2, phosphatidylinositol bisphosphate; DAG, diacylglycerol; AA, arachidonic acid; 2-AG, 2-arachidonoylglycerol; FA, fatty acid; FFA, free fatty acid; DGK, diacylglycerol kinase; PI3K, phosphatidylinositol 3-kinase; MGLL, monoacylglycerol lipase; DAGL, diacylglycerol lipase; TAG, triacylglycerol; HSL, hormone-sensitive lipase; ATGL, adipose triacylglycerol lipase; CGI-58, comparative gene identification 58; ADHD5, α/β hydrolase domain-containing protein 5; LPAAT, lysophosphatidic acid acyltransferase; PNPLA, patatin-like phospholipase domain-containing proteins; GPAT, glycerol-3-phosphate acyltransferase; DGAT, diacylglycerol acyltransferase

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2. Phospholipases

2.1. Phospholipase C

2.1.1. Enzyme activity and regulation

Phospholipase C (PLC) enzymes cleave phospholipids and produce diacylglycerol and the corresponding phospho-head group. Substrate specificity for either phosphatidylinositol-PLC (PI-PLC) or phosphatidylcholine-PLC (PC-PLC) defines the two main classes of PLCs. The cytosolic PI-PLC is the most well characterized class of PLC and localizes to the plasma membrane upon activation where it catalyzes the conversion of the minor membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2 or PIP_2) into the lipid second messengers inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (Fig. 1A). Both IP_3 and DAG serve as signaling molecules for Ca^{2+} mobilization or protein kinase activation, respectively. These two signaling molecules are exceptionally versatile and control distinct signaling pathways, making them responsible for dozens of cellular processes [1,2]. Cells tightly regulate PIP_2 depletion due to its role in protein activation at the membrane. One important example is a minor form of phosphorylated PIP_2, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which controls crucial signaling cascades via the phosphoinositide 3 kinase (PI3K) pathway [3].

Mammals have 13 different PI-PLC enzymes subdivided into six different enzyme families: β, γ, δ, ε, ζ and η; each family is characterized by its unique mechanism of regulation and localization. Most major signaling events sit upstream of distinct PI-PLC isozymes. Growth factors, antigens and other extracellular stimuli activate PLCγ; extracellular stimuli, hormones, neurotransmitters, and chemosensory molecules activate PLCβ via heterotrimeric G-proteins [4]. Additionally, PLCε is activated downstream of Ras signaling affording this family of enzymes a unique role in cellular communication and signal transduction [5].

PI-PLC enzymes are highly conserved across phyla—bacteria, flies, and mammals all express PI-PLC isozymes. While the overall core structure of the PI-PLCs shows little variance between families, they share very little sequence homology. All family members contain pleckstrin homology domains (PH) (except PLCζ), EF hand motifs, X and Y domains, and a C2 domain [6]. Each of these core domains has important regulatory and catalytic functions for PLC [6]. PH domains mediate membrane recruitment and facilitate binding to both PI and PIP_2. EF hand motifs bind Ca^{2+} ions, required for enzyme activity. X and Y domains dimerize, forming a triosephosphate isomerase (TIM) barrel, with the catalytic residues on the X portion of the TIM barrel. Finally, C2 domains, also essential for Ca^{2+} activation and anionic lipid binding, are found in repeating units of either 2 or 4 on the PI-PLCs, depending on the isoform. Other PI-PLC isoforms may contain more specialized regions, such as a Ras-GEF in PI-PLCε and PDZ-motifs found in β and η isoforms, believed to scaffold large protein complexes following G-protein coupled receptor (GPCR) activation [6].

Each isozyme class has unique signaling roles and tissue distribution. The β isoforms rely on Ca^{2+} release downstream of GPCR signaling for activation. Certain β isozymes actually serve as GTPase-activating-proteins, or GAPs, for Gα, which in turn activates other PLC isozymes [7]. PLCβ isoforms often localize to the nucleus but are also found in the cytosol. PLCβ1$^{-/-}$ animals have ocular and central nervous system (CNS) developmental deficiencies suggesting a critical role for PLCβ in the CNS [6]. Members of the PLCγ family are activated by receptor tyrosine kinases and cellular tyrosine kinases making them critical players in cell proliferation, and their expression in T and B lymphocytes suggests a role in immunity [8]. Ca^{2+} or $G\alpha_{i/o}$ and $G\alpha_q$ activate PLCδs and play roles in Alzheimer's disease [9], fertilization [10], and balding [11]. Mammalian expression of PLCζ in sperm heads facilitates fertilization [2]. PLCη is expressed in the brain and neurons, resembles PLCδ in

Table 1
Tools for lipid signaling modulation.

Target	Compound name	Isoform specificity	Mode of inhibition	Shown *in vivo* efficacy	References
Phospholipase C	U73122	Unknown	Indirect	[21]	[19,20]
	3013	PI-PLC-β3,γ1,δ1	Direct	ND	[24]
	3017	PI-PLC-β3,γ1,δ1	Direct	ND	[24]
Phospholipase D	Raloxifene	PLD1/2	Direct	Yes—for ER	[77]
	FIPI	PLD1/2	Direct	[83]	[78]
	VU0359595	PLD1	Direct	ND	[85]
	VU0364739	PLD2	Direct	ND	[86]
Phospholipase A	Varespladib methyl	sPLA2	Direct active site	Yes [102]	[101]
	Ecopladib	cPLA2	Direct	Yes [100]	[103]
	Giripladib	cPLA2	Direct	Yes [100]	[105]
	FKGK11	iPLA2			[107]
	Darapladib	Lp-PLA1	Direct	Yes [111]	[110]
Autotaxin	S32826	pan	Direct	ND	[125]
	PF-8380			[128]	[128]
	HA130		Direct: active site	ND	[129]
DAG kinase	R59022	Type I	Allosteric	ND	[153]
PI3 kinase	LY294002	Pan	Direct: ATP competitive	ND	[163]
	Wortmannin	Pan	Covalent irreversible	ND	[164]
MAG lipase	JZL-184		Irreversible catalytic site inhibitor	Yes [182]	[182]
	JJKK048		Irreversible catalytic site inhibitor	ND	[184]
DAG lipase	KT172	DAGLβ	Irreversible catalytic site inhibitor	Yes [189]	[189]
	KT109	DAGLβ		Yes [189]	[189]
Hormone-sensitive lipase	Aventis 7600		Reversible; transient inhibitor	ND	[225]
	CAY 10499				[231]
ATG lipase	Atglistatin		Direct	Yes [249]	[249]
GPAT	FSG67	1 and 2	Substrate mimic	Yes [304]	[303]
LPAAT	CT-32228	LPAAT-β	Direct binding	Yes [314]	[314]
DGAT	Niacin	DGAT2	Noncompetitive		[341]
	AZD7687	DGAT1	Direct	Yes [347]	[347]
	LCQ-908	DGAT1	Direct	Yes [359]	[359]

ND: not determined, ER: estrogen receptor.