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Lysophospholipid receptors in drug discovery

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Abstract

Lysophospholipids (LPs), including lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), lysophosphatidylinositol (LPI), and lysophosphatidylserine (LysoPS), are bioactive lipids that transduce signals through their specific cell-surface G protein-coupled receptors, LPA_{1–6}, S1P_{1–5}, LPI₁, and LysoPS_{1–3}, respectively. These LPs and their receptors have been implicated in both physiological and pathophysiological processes such as autoimmune diseases, neurodegenerative diseases, fibrosis, pain, cancer, inflammation, metabolic syndrome, bone formation, fertility, organismal development, and other effects on most organ systems. Advances in the LP receptor field have enabled the development of novel small molecules targeting LP receptors for several diseases. Most notably, fingolimod (FTY720, Gilenya, Novartis), an S1P receptor modulator, became the first FDA-approved medicine as an orally bioavailable drug for treating relapsing forms of multiple sclerosis. This success is currently being followed by multiple, mechanistically related compounds targeting S1P receptor subtypes, which are in various stages of clinical development. In addition, an LPA₁ antagonist, BMS-986020 (Bristol-Myers Squibb), is in Phase 2 clinical development for treating idiopathic pulmonary fibrosis, as is a distinct compound, SAR100842 (Sanofi) for the treatment of systemic sclerosis and related fibrotic diseases. This review summarizes the current state of drug discovery in the LP receptor field.

Keywords

Multiple sclerosis; idiopathic pulmonary fibrosis; FTY720; fingolimod; GPCR; LPA; S1P; LPS; cannabinoids

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Conflict of interest

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Introduction

Lysophospholipids (LPs) such as lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are a class of bioactive lipids [1, 2], which have a phosphate head group and a single fatty acyl chain attached to a 3-carbon backbone in their chemical structures. LPs have a fairly long history dating back to the early 20th century, but the field has shown markedly accelerated growth in recent decades (Figure 1A). LPA and S1P are the best studied LPs and play pivotal roles in physiological events including cell proliferation, survival, motility, cytoskeletal changes, and electrophysiological changes as well as pathophysiological processes that include autoimmune disease, fibrotic disease, cancer, inflammation, bone diseases, pain, metabolic syndrome, infertility, and hair loss. LPA is produced through several enzymatic pathways. Lysophospholipase D (known as autotaxin, ATX) liberates a choline group from lysophosphatidylcholine (LPC), whereas phospholipase A₁ and A₂ deacylate phosphatidic acid to produce 2-acyl and 1-acyl LPA, respectively [2]. Importantly, LPA is also *de novo* synthesized from glycerol-3-phosphate by the action of acyltransferases [3]. By comparison, S1P is produced by phosphorylation of sphingosine via the sphingosine kinases, Sphk1/2 [4].

LPs exert their effects by binding to specific G protein-coupled receptors (GPCRs) [5] that are the largest membrane receptor family in the human genome and contain nearly 800 receptors (including olfactory receptors) [6]. There are currently six LPA (LPA₁₋₆) and five S1P receptors (S1P₁₋₅) [2, 7, 8]. Recently, lysophosphatidylinositol (LPI) and lysophosphatidylserine (LysoPS) have been also shown to activate cognate GPCRs [9]. About 40 receptors within the 350 non-olfactory GPCRs have been identified as lipid GPCRs to date, of which ~40% are LP receptors (Figure 1B). It is likely that additional receptors for LPLs may be identified that interact with not only known receptor ligands but also other LPs such as lysophosphatidylethanolamine (LPE) and lysophosphatidylglycerol (LPG).

GPCRs remain a major target for drug discovery [10–12], and progress in the LP GPCR field has led to drug discovery efforts targeting LP receptors for a wide range of disorders, including the development of an actual medicine: fingolimod (FTY720, Gilenya) that was approved by the FDA for the treatment of multiple sclerosis (MS), and follow-up compounds targeting S1P receptors are being developed. Similarly, LPA signaling pathways are being explored, and LPA₁ antagonism is currently being evaluated clinically as a novel and effective drug for treating fibrotic disease. Here, we briefly review the LP receptors and recent advances of drug discovery efforts to target LP receptors; recent reviews on additional aspects of LP receptors have been published for LPA receptors [2, 7], S1P receptors [4, 13], and other LP receptors [9, 14].

Lysophospholipid receptors and signaling

The International Union of Basic and Clinical Pharmacology (IUPHAR) receptor nomenclature currently lists two LP receptor groups as class A GPCRs that are the LPA receptors (LPA₁₋₆) and the S1P receptors (S1P₁₋₅) [8]. Recent studies identifying other LP receptors for LPI (LPI₁) and LysoPS (LysoPS₁₋₃) [8] will add to the LP receptor family

(provisional IUPHAR names are noted). Figure 1C summarizes the G proteins that couple with LP GPCRs.

LPA receptors (LPA₁₋₆)

A first LP receptor that recognizes LPA was reported in 1996 and is now called LPA₁ [15], and subsequently LPA₂ and LPA₃ were found by homology as reported in 1998 by Goetzl's group and in 1999 by Aoki's group, respectively (Figure 1A) [16, 17]. In 2003, Shimizu and colleagues discovered a fourth LPA receptor, LPA₄, from an orphan GPCR, GPR23/p2y9, that showed less than 20% amino acid sequence identity with LPA₁₋₃ [18]. In 2006, we and others identified GPR92, which was another orphan GPCR closely related to LPA₄, as a fifth LPA receptor, LPA₅ [19, 20]. In 2008, a P2Y family orphan GPCR, p2y5, was deorphanized as the latest LPA receptor, LPA₆ [21].

LPA₁ couples to G_{ai/o}, G_{aq/11}, and G_{α12/13}, resulting in LPA₁-dependent cellular responses such as cytoskeletal change, cell migration, adhesion, and Ca²⁺ mobilization [2, 7, 8]. Studies of mice lacking LPA₁ showed pathophysiological roles of LPA-LPA₁ axis in neural development, bone homeostasis, pain, hydrocephalus, and autoimmune disorders [2, 5, 7, 22]. LPA₁ antagonists have been developed and are entering Phase 2 clinical trials to treat idiopathic pulmonary fibrosis and fibrotic diseases (discussed below). LPA₂ couples to multiple G-proteins including G_{ai/o}, G_{aq/11}, and G_{α12/13} and is associated with cell migration and survival, cancer metastasis, neural development, and immune function [2, 7, 8]. LPA₃ transduces signaling through G_{ai/o} and G_{aq/11} and prefers 2-acyl-LPA containing unsaturated fatty acids. LPA₃ knockout mice showed a reproductive defect whereby embryo implantation was abnormal [23]. In addition, LPA₃ also regulates chemotaxis of immature dendritic cells and levels of pain [2, 7, 8]. LPA₄ couples to G_{as}, G_{asi/o}, G_{aq/11} and G_{α12/13} and is involved in neurite retraction, cell motility, cell aggregation and adhesion, osteoblast differentiation, and blood and lymphatic vessel formation [2, 7, 8]. A Japanese patent (2012-239450) indicates that LPA₄ mediates adipocyte differentiation and its removal improves insulin resistance, based upon LPA₄ knockout mice. LPA₅ can couple to G_{aq/11} and G_{α12/13}, and increases cAMP accumulation via a non-G_{as} mechanism [20]. LPA₅ regulates neuropathic pain [24] and immune function [25, 26]. LPA₆ utilizes G_{α12/13} for transducing signals through the Rho pathway, and it has some preference for 2-acyl-LPA rather than 1-acyl-LPA [27]. LPA₆ was discovered as an autosomal dominant genetic factor from patients with hypotrichosis simplex that is characterized by familial hair loss [21]. Genetics and *in vitro* studies support the idea that PA-PLA_{1α} was required to activate LPA₆ by generating 2-acyl-LPA [28].

S1P receptors (S1P₁₋₅)

Hla *et al.* discovered an orphan GPCR, endothelial differentiation gene-1 (Edg-1), by a differential display method in 1990 [29], and Hla, Spiegel, and colleagues identified it as an S1P receptor in 1998 based on the sequence homology with LPA₁/Edg-2/Vzg-1 [30], which is now designated as S1P₁. In 1997, two orphan GPCRs (Edg-5 and Edg-3, now called S1P₂ and S1P₃, respectively) were deorphanized as S1P receptors by Goetzl, Coughlin, and colleagues [31]. In 2000, the other two orphan GPCRs (Edg-6 and Edg-8), which show a

highly conserved amino acid sequence with S1P_{1/2/3}, were reported as S1P receptors S1P₄ and S1P₅, respectively [32, 33].

S1P₁ couples to G_{ai/o} and induces intracellular signaling such as inhibition of cyclic AMP accumulation, intracellular Ca²⁺ increase, MAPK activation, small GTPase Rac activation, and likely other signals. S1P₁ knockout mice [34] identified important roles of S1P₁ in vascular and cardiac morphogenesis. Studies using genetic and pharmacological approaches have established that S1P₁ regulates cell migration and lymphocyte trafficking in both homeostatic and disease settings [35], and is a major target of drug discovery as evidenced by fingolimod, which is discussed below. S1P₂ preferably utilizes G_{α12/13} for activating Rho signaling, while coupling to multiple G-proteins including G_{ai/o} and G_{αq/11}. S1P₂ abrogates motility by inhibiting Rac activity. Indeed, Cyster and colleagues demonstrated that B cells are retained in the germinal centers of lymphoid follicles through the S1P-S1P₂ axis [36]. S1P₂ is reported to play important roles in vascular development and remodeling, cardiovascular function, bone maintenance, inner ear function, and metastasis [4, 7, 8, 13]. S1P₃ couples to G_{ai/o}, G_{αq/11}, and G_{α12/13}. No obvious phenotype or developmental defects were observed in S1P₃ knockout mice [37]. S1P_{2/3} double knockout mice have reduced fertility [38], indicating the existence of redundancy among S1P receptors. S1P-S1P₃ signaling may be relevant to several diseases including breast cancer, sepsis, and liver fibrosis [4, 7, 8, 13]. S1P₄ transduces signaling through G_{ai/o} and G_{α12/13}. This receptor is most prominent in adult hematopoietic tissues and is thought to promote cell migration. Studies in S1P₄ knockout mice showed pivotal roles in thrombopoiesis, neutrophil migration, and T_H17 differentiation [4, 7, 8, 13]. S1P₅ can couple to G_{ai/o} and G_{α12/13} and considering its common roles in inhibition of cell migration and promotion of cell process retraction, it may preferentially couple to G_{α12/13}. S1P₅ plays important roles in lymphocyte trafficking and NK cell differentiation [4, 7, 8, 13]. S1P₅ has yet-to-be determined functions in the central nervous system (CNS) in view of its expression in oligodendrocytes [39, 40].

Other lysophospholipid receptors (LPI₁ and LysoPS₁₋₃)

GPR55, was first reported as an LPI receptor in 2007 [41], although it was thought at the time to be a cannabinoid receptor. Other studies support its identity as an LPI receptor [14], leading to its provisional IUPHAR name [8]. LPI₁ couples with G_{α13} and transduces signals to the RhoA-ROCK pathway [9, 14]. Studies of mice lacking *Lpir1* gene identified effects on bone homeostasis, pain, and autoimmune disorders [9, 14].

The first LysoPS receptor, GPR34, was reported in 2006 [42] and confirmed by Aoki and colleagues who identified 2-acyl-LysoPS as a preferred ligand over 1-acyl-LysoPS [43]. They developed a novel TGFα shedding assay that detects GPCR signaling [44]. This assay validated the first LysoPS receptor and identified additional members, leading to the proposed IUPHAR names designed to avoid confusion between the well-known abbreviation LPS for lipopolysaccharides [8]: GPR34 as LysoPS₁, P2Y₁₀ as LysoPS₂, A630033H20 as LysoPS_{2L}, and GPR174 as LysoPS₃. Further studies are necessary for understanding the roles of LysoPS-LysoPS_x axis.

Drug discovery targeting S1P receptors

The most important success of drug discovery in the LP receptor field has been fingolimod (also known as FTY720) (Figure 2A) [45]. In 1995, fingolimod was first synthesized from an immunosuppressive natural product, myriocin (ISP-I), that was isolated from culture broths of *Isaria sinclairii* [45]. As expected, oral administration of fingolimod prevents rodents from exhibiting symptoms in the allograft model [45]. Additionally, fingolimod sequesters circulating lymphocytes, resulting in decreased peripheral blood lymphocyte count and increased numbers of lymphocytes in the secondary lymphoid organs [45]. However, the molecular basis of fingolimod activities was not demonstrated until 2002, when two groups independently proposed that fingolimod interacts with S1P receptors [46, 47] through a phosphorylated metabolite of FTY720, FTY720-P, which binds to S1P₁, S1P₃, S1P₄, and S1P₅ as a high affinity agonist. Fingolimod was reported to be phosphorylated *in vivo* by S1P-generating enzyme, Sphk1 or particularly Sphk2 [48]. Paradoxically, fingolimod activity on lymphocytes involves not agonist but rather functional antagonist activity against S1P₁, revealed by phenocopying S1P₁ loss in lymphocytes [49]. FTY720-P downregulates S1P₁ on the cell surface by sustained internalization and subsequent degradation of the receptor [50, 51], explaining reductions of lymphocyte egress from secondary lymphoid organs, thymus, and bone marrow [45]. In 2002, Brinkmann and colleagues first reported the prophylactic effects of fingolimod on an animal model of MS, experimental autoimmune encephalomyelitis (EAE) [46]. Moreover, Webb *et al.* demonstrated the therapeutic use of fingolimod on relapsing–remitting EAE in SJL mice [52]. These studies indicated that fingolimod had the potential for treating autoimmune diseases like MS. Figure 2B illustrates the possible actions of fingolimod in MS.

Because of its hoped-for immunosuppressive effects, fingolimod was first tested in humans as an agent to improve renal transplantation [53]. However, it failed to reach its clinical endpoints even as an adjunctive agent at high dosage combined with cyclosporine for preventing renal allograft rejection in two Phase 3 studies, and was accompanied by adverse events [53], resulting in termination of the renal transplantation program. However, evidence from animal models of MS suggested fingolimod utility in MS, and in 2006, Phase 2 clinical data supported fingolimod use in MS [54]. In 2010, fingolimod (Gilenya, Novartis) received Food and Drug Administration (FDA) approval in the U.S. as a first-line agent for the treatment of relapsing forms of MS, followed by European Medicines Agency (EMA) approval in 2011 [55]. A 2014 phase 3 extension study (FREEDOMS II) report confirmed the beneficial effects of fingolimod on relapse rates in MS patients and showed a consistent safety profile [56]. Fingolimod was ranked in the top 100 prescribed medicines by U.S. National Sales in the 4th quarter 2013 [57]. On the other side of the coin, fingolimod has potential side effects [58] that will need to be assessed, as well as in other agents targeting S1P receptors.

S1P receptor-targeted drugs, including fingolimod, are in clinical trials for treatment of several diseases including MS (Figure 2A), ulcerative colitis, dermatomyositis, along with proof-of-concept evaluations in other disorders like amyotrophic lateral sclerosis (ALS) and stroke. Newer compounds are designed to have receptor subtype selectivity, such as S1P₁/S1P₅ specificity (siponimod, ponesimod, and RPC1063) and S1P₁ specificity

(KRP-203 and GSK2018682). Considering the success of fingolimod, these next-generation compounds may exhibit improved efficacy and/or fewer side effects for a range of possible therapeutic indications.

Drug discovery targeting LPA receptors

Currently, no drugs targeting LPA receptors have been approved by any regulatory agency, although advances in the LP field support therapeutic relevance for a range of diseases including fibrotic diseases of the skin, lung, kidney, and liver (Figure 2C). One disease that may benefit from LPA receptor modulation is idiopathic pulmonary fibrosis (IPF) which is characterized by interstitial infiltrates in lung bases, progressive dyspnea, and worsening of pulmonary function [59], and is associated with high mortality rates and thus represents an unmet medical need. In 2008, Tager *et al.* identified receptor-mediated LPA signaling as an important mechanism in lung fibrosis [60]. In a mouse model of bleomycin-induced lung fibrosis, LPA concentrations in bronchoalveolar lavage rose to 400 nM at 14 days after challenge. LPA₁ knockout mice were significantly protected from bleomycin-induced fibrosis based upon reduced mortality, fibroblast and leukocyte recruitment, and vascular leakage. In 2010, Amira Pharmaceuticals reported that a potent and orally bioavailable LPA₁ antagonist, AM966, was beneficial in treating bleomycin-induced lung fibrosis [61]. In 2011, Bristol-Myers Squibb acquired Amira Pharmaceuticals, and had completed Phase 1 clinical trials of an LPA₁ antagonist, BMS-986202 (previously AM152). In 2014, another LPA₁ antagonist, BMS-986020, began Phase 2 clinical trials to test its efficacy on IPF (NLM ID: NCT01766817).

Another fibrotic disease is systemic sclerosis (scleroderma), which is a chronic connective tissue disease characterized by vasculopathy, autoimmunity, and extensive fibrosis [62]. Tokumura *et al.* reported elevated levels of 2-arachidonoyl-LPA (as well as S1P) in sera of systemic sclerosis patients [63]. In 2011, Tager and colleagues revealed that LPA₁ knockout mice were protected in a mouse model of scleroderma [64], suggesting that LPA₁ antagonists may be effective for treating systemic sclerosis. Sanofi has entered Phase 2 clinical trials of an LPA_{1/3} inhibitor, SAR100842, for systemic sclerosis (NLM ID, NCT01651143) with results suggesting efficacy but that require validation in larger clinical trials [65].

Conclusions

The LP field has advanced with an expanding repertoire of both receptors and ligands. Fingolimod's entry into the treatment of MS has established lysophospholipid signaling as a clinically validated molecular pathway, raising prospects for novel therapeutics in a number of medically important therapeutic areas. Basic studies on LP receptor signaling mechanisms and their relationship to normal physiological and pathophysiological processes should continue to expand the development of novel therapeutics.

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Abbreviations

LP	Lysophospholipid
LPA	lysophosphatidic acid
S1P	sphingosine 1-phosphate
LPI	lysophosphatidylinositol
LysoPS	lysophosphatidylserine
LPC	lysophosphatidylcholine
LPE	lysophosphatidylethanolamine
LPG	lysophosphatidylglycerol
ATX	autotaxin
Sphk	sphingosine kinases
GPCR	G protein-coupled receptor
MS	multiple sclerosis
EAE	experimental autoimmune encephalomyelitis
NMO	neuromyelitis optica
IPF	idiopathic pulmonary fibrosis
FDA	Food and Drug Administration
EMA	European Medicines Agency
NLM ID	National Laboratory of Medicine Identifier

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Figure 1. Chronology of the LP field, LP and other lipid receptors, and overview of proximal LP signaling features
(A) Chronology of the LP receptor field. Vertical bars indicate the number of publications within 5-year bins, which were searched in PubMed with unabbreviated names of the indicated keywords. (B) Chronological table for identification of lipid GPCRs. LP receptors are noted in red. (C) The chemical structures of LPs, GPCR names, and associated heterotrimeric G-proteins as defined by their Gα subunits.

Figure 2. Disease mechanisms being accessed by LP-based drug discovery and compounds in clinical development

(A) LP receptor-based compounds currently in clinical trials. NLM, National Laboratory of Medicine; IPF, idiopathic pulmonary fibrosis; CIDP, chronic inflammatory demyelinating polyneuropathy. (B) Multiple sclerosis pathology and actions of FTY720. Lymphocytes expressing S1P₁ migrate from lymph nodes to blood based on S1P concentration differences. FTY720 redistributes these lymphocytes into lymph nodes (*i.e.*, secondary lymphoid organs), resulting in reduced entry of pathogenic lymphocytes into the CNS. FTY720 also inhibits astrocyte function, may alter oligodendrocyte function via S1P₅ and results in reduced inflammation, demyelination, with possibly other beneficial functions. As, astrocytes; BBB, blood brain barrier; CNS, central nervous system; Neu, neuron; OL, oligodendrocyte. (C) IPF pathology and accessed mechanisms for an LPA₁ antagonist. Circulating and resident fibroblasts (Fb) are recruited to the injured site, which may be interrupted by LPA₁ antagonists. Epithelial cells differentiate into fibroblasts by epithelial mesenchymal transition (EMT). These fibroblasts differentiate into myofibroblasts (MyoFb), which accelerate fibrosis and may also be inhibited by LPA₁ antagonists.