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Review

Pharmacological tools for lysophospholipid GPCRs: development of agonists and antagonists for LPA and S1P receptors

Dong-Soon IM*

College of Pharmacy (BK21 Project) and Longevity Institute of Life Science and Technology, Pusan National University, Busan 609–735, Republic of Korea

Previous studies on lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) using various approaches have shown that both the molecules can act as intercellular signaling molecules. The discovery of the Edg subfamily of G-protein-coupled receptors (GPCRs) (later renamed LPA_{1-3} and $S1P_{1-5}$) for these molecules has opened up a new avenue for pathophysiological research on lysophospholipids. Genetic and molecular studies on lysophospholipid GPCRs have elucidated pathophysiological impacts and roles in cellular signaling pathways. Recently, lysophospholipid GPCR genes have been used to develop receptor subtype-selective agonists and antagonists. The discovery of FTY720, a novel immune modulator, along with other chemical tools, has provided a means of elucidating the functions of each lysophospholipid GPCR on an organ and the whole body level. This communication attempts to retrospectively review the development of agonists and antagonists for lysophospholipid GPCRs, provide integrated information on pharmacological tools for lysophospholipid GPCR signaling, and speculate on future drug development.

Keywords: lysophosphatidic acid; sphingosine 1-phosphate; agonist; antagonist; G-protein-coupled receptor; lysolipid

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Discovery of GPCRs for LPA and S1P

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are two representative lysophospholipid mediators acting on G-protein-coupled receptors (GPCRs). LPA was first identified three decades ago as a factor affecting blood pressure, platelet aggregation, and smooth muscle contraction[1–5] and rediscovered as a mitogenic serum lipid inducer of neurite retraction[6, 7]. The name 'lyso' originates from lysis of blood cells; thus, the non-specific detergent action of LPA has been doubted. In 1996, the high potency (nmol/L range) and GPCR implications of LPA were finally connected to the discovery of the first LPA receptor (LPA_1, formerly known as Edg-2)[8]. Subsequently, LPA_2 and LPA_3 were identified as members of the endothelial differentiation gene (Edg) subfamily of GPCRs[9–11]. These three LPA receptors (Edg family) share a high homology with each other[12].

Recently, the non-Edg family of LPA receptors, LPA_4 (GPR23, p2y9), LPA_5 (GPR92), and LPA_6 (p2y5), were reported as members of the purinergic GPCR cluster[13–19]. Using the GPCR-$G\alpha_{16}$ fusion expression system, Fujita's group reported GPR87 as another LPA receptor and $P2Y_{10}$ as a dual receptor for both LPA and S1P[20, 21]. These results have not yet been confirmed by other research groups[22]. Instead, lysophosphatidylserine has been suggested to act as a ligand for $P2Y_{10}$ without confirmation of LPA and S1P as ligands in NIH3T3 cells[23].

S1P was initially reported 15 years ago to be a second messenger, mediating an increase in calcium levels due to PDGF and IgE signaling[24, 25]. The molecular target of S1P in the cytosol has not yet been identified. The initial finding of an S1P-induced calcium increase stimulated research in S1P biology and was linked to its recognition as an intercellular first messenger. The involvement of trimeric G proteins in S1P-induced actions as well as pertussis toxin sensitivity strongly suggested the presence of S1P GPCRs in the plasma membrane[26–30]. The discovery of $S1P_1$ (formerly known as Edg-1) in 1998, along with four other receptors ($S1P_{2-5}$) of the Edg subfamily GPCRs, became a milestone in S1P biology[12, 31–36].

Development of agonists and antagonists for LPA receptors

Prior to receptor cloning, various aspects of LPA receptor

* To whom correspondence should be addressed.
E-mail imds@pusan.ac.kr
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structure-activity relationships had been studied: 1) fatty acid chain length and the presence of double bonds, 2) acyl and alkyl linkage, 3) stereo-selectivity on the *sn*-2 position, and 4) modification of phosphate groups[37]. Oleoyl LPA (18:1) was considered the optimum ratio to increase Ca^{2+} levels in A431 cells[38]. Alkyl LPA showed a better effect than acyl LPA in platelet aggregation assays and an equal effect in other responses[39, 40]. Several groups reported a lack of stereo-selectivity on the *sn*-2 position of LPA. Early studies showed that modification of the phosphate group resulted in an absence of LPA responses[38, 40].

The initial synthetic approach to LPA response was characterized using platelet aggregation assays. Replacement of the glycerol backbone with amino acids resulted in the production of *N*-palmitoyl serine phosphatidic acid (NPSPA) and *N*-palmitoyl tyrosine phosphatidic acid (NPTyrPA)[40]. These molecules were not active on platelet aggregation in humans, but did demonstrate activity as LPA receptor antagonists in *Xenopus*[41]. Later, NPSPA and NPTyrPA were reported as partial agonists of mammalian LPA receptors[42, 43]. Sugiura *et al* introduced the ethanolamine-based LPA mimetic, *N*-acyl aminoethanol phosphoric acid (NAEPA), as an equipotent agonist of platelet aggregation[40]. Later, research groups from the University of Virginia used NASPA and NAEPA as platforms to synthesize a series of VPC compounds[37, 44]. The phosphonate analogue of NAEPA lost its platelet aggregation properties[40], but methylene phosphonate LPA was equipotent to LPA as an inhibitor of forskolin-driven cAMP accumulation in rat C6 glioma cells[45]. Jalink *et al* synthesized various phosphonate analogues along with fatty alcohol phosphates and the methyl ester of LPA (lysophosphatidylmethanol, LPM), but could not show a significant impact of these compounds on Ca^{2+} increase in A431 cells[38]. Ironically, these chemicals turned out to be selective or non-selective agonists of cloned LPA receptors (see details below). In the early era of LPA biology, suramin and lysophosphatidylglycerol were used to demonstrate GPCR involvement in LPA responses[46] and as an antagonist of LPA-induced Ca^{2+} responses in Jurkat T cells[47], respectively.

LPA GPCR agonists

Since the discovery of the three-Edg family of LPA receptors, the development of selective receptor-subtype agonists and antagonists has accelerated. The optimal chain length and the presence of double bonds have been found to vary depending on receptor subtype. For example, LPA_3 showed a preference for unsaturated LPA similar to oleoyl LPA[48], whereas LPA_6 showed a preference for 2-acyl LPA[19]. Synthesis of LPA derivatives with phosphonate or thiophosphate groups instead of the phosphate group showed receptor-subtype selective activity similar to 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate (OMPT), 1-*O*-acyl-α-fluoromethylenephosphonate, and α-hydroxymethylenephosphonate LPA analogues as LPA_3 receptor selective agonists[49–52]. The phosphonate derivatives also provided a path toward the development of phosphatase-resistant long-lasting LPA derivatives[53]. A dialkyl phosphatidic acid (PA) 8:0 analog with a thiophosphate was reported as a potent and selective LPA_3 agonist[54], but later, agonistic activity on the LPA_5 receptor and antagonistic activity on the $LPA_{1,3}$ receptors was reported[55]. Based on computational modeling, dodecyl fatty alcohol phosphate was shown to be a specific LPA_2 receptor agonist[56], and later, oleoyl-thiophosphate was identified as a pan-agonist (LPA_{1-3})[43]. A methylene phosphonate LPA analogue was reported as a selective LPA_2 agonist, but it recently was shown to exert agonistic and antagonistic activities on LPA_5 and LPA_3, respectively[52, 57]. T-15 (LPA_1 agonist) and T-13 (LPA_3 agonist) were both synthesized using a carbohydrate scaffold[58]. Darmstoff analogues were introduced as novel scaffolds for subtype-selective LPA receptor ligands[59]. Finally, octadecenyl phosphate was shown to be a selective agonist for LPA_4 and LPA_5 receptors[55].

Although alkyl LPA has been shown to exert equipotent activity on each Edg-family LPA receptor compared to acyl LPA[60], alkyl LPA was found to be more potent than acyl LPA in platelet aggregation responses[3]. Therefore, the Edg-family LPA receptors were not able to account for the LPA response in platelets[61]. Recently, non-Edg (purinergic) LPA receptors have been identified. The LPA_5/GPR92 receptor has demonstrated alkyl preference and a presence in platelets[55]. Farnesyl diphosphate, *N*-arachidonyl glycine, and carba-cyclic phosphatidic acid were shown to be selective agonists for LPA_5[55, 62, 63]. LPA_6/p2y5 showed a preference for 2-acyl LPA over 1-acyl LPA[19]. Alkyl-OMPT, an LPA_3 agonist, and 2-linoleoyl LPA showed better agonistic effects on the LPA_6 receptor than 2-oleoyl LPA showed[19, 64]. In contrast, the methyl ester of LPA (LPM) was shown to be a pan-agonist for LPA_{1-5} although it was less potent than LPA[65].

LPA GPCR antagonists

The development of LPA derivatives with a bulky group on the *sn*-2 position has demonstrated the stereo-selectivity of LPA receptors and has led to the further development of the LPA_1/LPA_3 selective antagonists VPC12249 and VPC32183[66, 67]. 2-Pyridyl-containing phosphonate was developed as a non-hydrolyzable LPA_3 receptor antagonist[68]. Dioctylglyceropyrophosphate (DGPP) and Ki16425 were determined to be selective LPA_1/LPA_3 antagonists following screening of available lipid and non-lipid molecules[69, 70]. The thiophosphate version of PA 8:0 was shown to be the most potent LPA_3 antagonist, whereas the dialkyl PA 8:0 analog with a thiophosphate was shown to be a potent and selective $LPA_{3,5}$ agonist, as mentioned above[54, 55]. Tetradecyl-phosphonate was identified as a pan-antagonist (LPA_{1-3})[43]. Furthermore, farnesyl phosphate and farnesyl diphosphate potently and selectively blocked the LPA_3-mediated Ca^{2+} increase[71], although farnesyl diphosphate is a LPA_5 selective agonist, as mentioned above[55]. T-14, which used a carbohydrate scaffold, was shown to be an LPA_3 antagonist[58].

Recently, virtual screening identified non-lipid LPA_3 antagonist NSC161613, LPA_2 antagonist H2L5186303, $LPA_{4,5}$ antagonist 5987411, and $LPA_{1,6}$ antagonist 5765834[55, 72]. An LPA analogue of α-bromomethylene phosphonate was reported to act

as a pan-antagonist to LPA_{1-4} and an autotoxin inhibitor[52, 73].

Pharmacological tools for LPA GPCR signaling

Commercially available chemicals for studying LPA receptor subtypes are currently in development, although the effects of previously developed chemicals on recently identified non-Edg LPA receptors have not been completely verified (Figure 1). For LPA_1 or LPA_3 receptor signaling, a combined application of $LPA_{1,3}$ antagonists such as VPC12249, VPC32193, DGPP, and Ki16425 and LPA_3 agonists such as OMPT and α-fluoromethylene phosphonate would be more favorable. For LPA_2 receptor signaling, dodecyl phosphate is an adequate LPA_2 selective agonist. For LPA_5 receptor, farnesyl diphosphate could be used as a selective agonist. For LPA_6 receptor, alkyl OMPT, an $LPA_{3,6}$ agonist, could be used in combination with $LPA_{1,3}$ antagonists (Table 1).

Development of agonist and antagonist for S1P receptors

In contrast to LPA, the structure-activity relationship of S1P has a very short history. Using cloned S1P receptors, sphinganine-1-phosphate (dihydro-S1P) and sphingosylphosphorylcholine (SPC) were shown to be equipotent and far less potent on each S1P receptor[36, 74–76].

S1P GPCR agonists

Following the cloning of the S1P receptors, the development of S1P agonists and antagonists began. The importance of the D-*erythro* configuration of S1P was demonstrated using the cloned receptors[77]. The linkage of the immune modulator FTY720 to S1P receptors, however, boosted this area of research and opened a new direction for S1P biology[78–80]. Lymphopenia induction by inhibiting lymphocyte egress from lymphoid organs was shown to be mediated through the $S1P_1$ receptor[81]. High-throughput screening (HTS) of an available chemical library showed that SEW2871 acted as an *in vivo* active heterocyclic $S1P_1$ selective agonist[81, 82] and compound 26 was synthesized as a potent 3,5-diphenyl-12,4-oxadiazole $S1P_1$ agonist[83]. Later, using ultra-HTS, 3,5-diaryloxadiaxole (CYM5181) and dicyclohexylamide were found to be selective agonists for $S1P_1$ and $S1P_3$, respectively[84]. Using computational modeling, CYM-5442 was developed as an $S1P_1$ selective agonist that was more potent than CYM5181[85]. AUY954, an aminocarboxylate analogue of FTY720, was also introduced as an $S1P_1$ selective agonist[86]. VPC01091, a cyclized analogue of FTY720, was shown to act as an orally active $S1P_1$ agonist and an $S1P_3$ antagonist[87]. KRP-203 is a pro-drug immune modulator similar to FTY720; the phosphorylated form of KRP-203 was shown to be a selective $S1P_1$ agonist[88, 89]. Constrained azacyclic analogues of FTY720 showed selective agonist activities on $S1P_4$ and $S1P_5$ receptors[90]. Finally, phytosphingosine-1-phosphate was shown to act as a potent and selective agonist on the $S1P_4$ receptor[76].

S1P GPCR antagonists

Suramin was temporarily used as an $S1P_3$ antagonist[75, 91]. Human $S1P_5$ was also reported to be sensitive to suramin and its analogue NF023[92].

Following screening of an available chemical library, JTE-013, a pyrazopyridine derivative, was identified as an $S1P_2$ antagonist[93, 94]. Modification of the FTY720-phosphate structure led to the development of VPC23019 and VPC25239 as selective $S1P_1/S1P_3$ antagonists[95]. As mentioned above, VPC01091 is an orally active $S1P_1$ agonist and $S1P_3$ antagonist[87]. W146, hexyl phenyl amide phosphonate, was found to

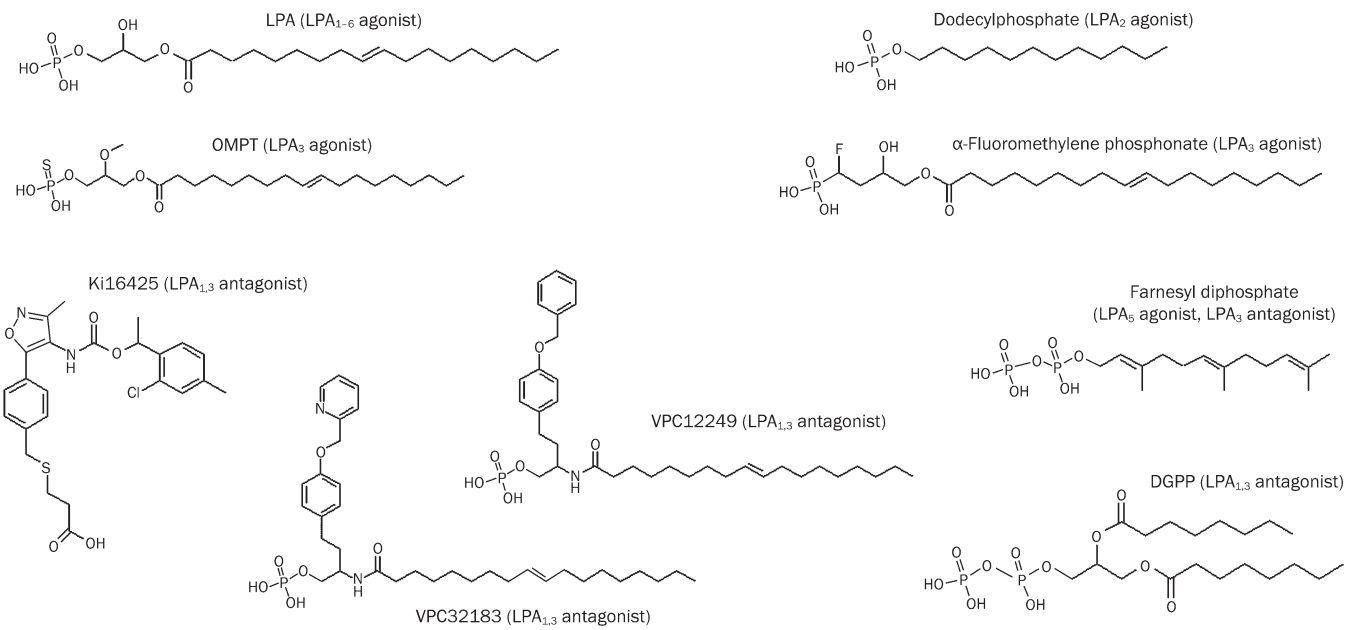

Figure 1. Structures of commercially available agonists and antagonists for LPA GPCR signaling. Sources are Avanti polar lipid, Biomol international, Echelon bioscience, Enzo Life sciences, and Sigma-Aldrich.

Table 1. Agonistic and antagonistic characters of each compound on each LPA receptor. Numbers (nmol/L) mean EC_{50} or K_D values for agonists and IC_{50} or K_I values for antagonists.

Name	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	LPA_6
Ki16425[70]	Antagonist (250 nmol/L)	Antagonist (5600 nmol/L)	Antagonist (360 nmol/L)			
DGPP[69]	Antagonist (6600 nmol/L)		Antagonis (106 nmol/L)			
VPC32183[67]	Antagonist (109 nmol/L)		Antagonist (175 nmol/L)			
VPC12249[66]	Antagonist (137 nmol/L)		Antagonist (428 nmol/L)			
2-Pyridyl phosphonate[68]			Antagonist			
Thiophosphatidic acid 8:0[54]	Antagonist (360 nmol/L)		Antagonist (5 nmol/L)			
T14[58]			Antagonist			
NSC161613[72]			Antagonist (24 nmol/L)			
Compound 12[120]	Antagonist (48 nmol/L)		Antagonist (230 nmol/L)			
H2L5186303[72]		Antagonist (7.2 nmol/L)	Antagonist (310 nmol/L)			
5987411[55]				Antagonist (741 nmol/L)	Antagonist (1300 nmol/L)	
5765834[55]	Antagonist (48 nmol/L)				Antagonist (292 nmol/L)	
α-bromomethylene phosphonate[52,55,73,117]	Antagonist (751 nmol/L)	Antagonist (304 nmol/L)	Antagonist (380 nmol/L)	Antagonist (167 nmol/L)	Weak agonist	
Tetradecyl-phosphonate[43]	Antagonist (10000 nmol/L)	Antagonist (5500 nmol/L)	Antagonist (3100 nmol/L)			
Farnesyl diphosphate[55,71]		Antagonist (2100 nmol/L)	Antagonist (155 nmol/L, 4600 nmol/L)	Antagonist (1980 nmol/L)	Agonist (40 nmol/L)	
Carba cyclic PA[55]					Agonist	
OMPT[49]			Agonist (68 nmol/L)			
Alkyl OMPT[19,64]	Agonist (790 nmol/L)		Agonist (62 nmol/L)			Agonist
α-fluoromethylene phosphonate[51]	Weak agonist	Weak agonist	Agonist (0.5 nmol/L)			
α-hydroxymethylene-phosphonate[52]			Agonist (393 nmol/L)			
Compound 8bo[121]	Agonist (9.1 nmol/L)		Agonist (123 nmol/L)			
Dialkyl thiophosphatidic acid 8:0[54, 55]	Agonist (695 nmol/L) Antagonist (382 nmol/L)	Agonist (5720 nmol/L)	Agonist (3 nmol/L) Antagonist (184 nmol/L)		Agonist	
Dodecylphosphate[56]		Agonist (700 nmol/L)	Antagonist (90 nmol/L)			
α-methylene phosphonate[52,55]		Agonist (>281 nmol/L) Antagonist (1420 nmol/L)		Weak agonist (3900 nmol/L)	Agonist	
T15[58]	Agonist (5 nmol/L)		Agonist (50 nmol/L)			

(Continued)

Name	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	LPA_6
T13[58]	Week agonist (500 nmol/L)		Agonist (0.5 nmol/L)			
Octadecenyl phosphate[55]				Agonist (608 nmol/L)	Agonist	
NPSPA[11,43,122]	Weak agonist (1850 nmol/L)	Weak agonist	Weak agonist (1600 nmol/L)			
NPTyrPA[43,55,122]	Antagonist (3450 nmol/L)	Weak agonist (11000 nmol/L)	Antagonist (5570 nmol/L)			Agonist
NAEPA[11]	Agonist	Agonist	Weak agonist			
Oleoyl-thiophosphate[43]	Agonist (193 nmol/L)	Agonist (244 nmol/L)	Agonist (546 nmol/L)			
LPM[65]	Agonist	Agonist	Agonist	Agonist	Agonist	

be a selective $S1P_1$ antagonist[96]. VPC44116, an octyl analogue of W146 and γ-aminophosphonate analogue of VPC23019, antagonized lymphopenia and lung permeability via the $S1P_1$ receptor[97]. SB64146 was reported to act as an inverse agonist on the $S1P_1$ receptor[98]. Ascotricins A and B were isolated from a cultured broth of a fungus identified as *Ascotricha chartarum* and shown to inhibit the $S1P_1$ receptor and S1P-mediated HUVEC migration[99]. Sankyo Co synthesized compound lead 2 (CL2), 2-(4-ethoxyphenoxy)-5-(3-octadecyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonate, which antagonized the $S1P_1$>$S1P_3$>$S1P_2$ receptors[100]. Human $S1P_1$ receptor-selective antagonist and agonist effects of a rat monoclonal antibody (4B5.2) *in vivo* were also reported[101]. Using a 3D database search, BML-241, 2-alkylthiazolidine-4-carboxylic acid, was found to act as an $S1P_3$ antagonist, but its selectivity and potency were not recapitulated in CHO-K1 cells expressing the $S1P_3$ receptor[102, 103]. A pharmacophore-based design of an $S1P_3$ antagonist with a 3,4-dialkyoxybenzophenone scaffold was suggested[104].

Pharmacological tools for S1P GPCR signaling

Commercially available tools for studying S1P receptor subtypes are highlighted in Figure 2. For $S1P_1$ receptor signaling, CYM-5442 or SEW2871, both potent selective $S1P_1$ agonists, and W146, a selective $S1P_1$ antagonist, should be sufficient to elucidate $S1P_1$ receptor involvement. $S1P_2$ receptor signaling could be dissected using JTE-013, an $S1P_2$ selective antagonist. For $S1P_3$ GPCR signaling, a combined application of an $S1P_{1,3}$ antagonist (VPC23019) and $S1P_1$ antagonist (W146) or $S1P_1$ agonist (CYM-5442) could be useful. Phytosphingosine 1-phosphate, an $S1P_4$ selective agonist, could be used to study $S1P_4$-mediated signaling. $S1P_{1,3}$ antagonist (VPC23019)-insensitive, $S1P_2$ antagonist (JTE-013)-insensitive, $S1P_4$ agonist-nonresponsive, and S1P- or FTY720-phosphate-sensitive signaling might be interpreted as $S1P_5$ receptor or unidentified S1P receptor signaling (Table 2).

Development of drugs acting on lysophospholipid GPCRs

Prior to the molecular cloning of GPCRs for LPA and S1P, a number of studies were conducted with the primary goal of determining the functions of lipid mediators at both the cellular and the organ level. These functions included platelet aggregation, smooth muscle contraction, and cell proliferation, among others[1–6, 105]. The discovery of GPCRs allowed signal

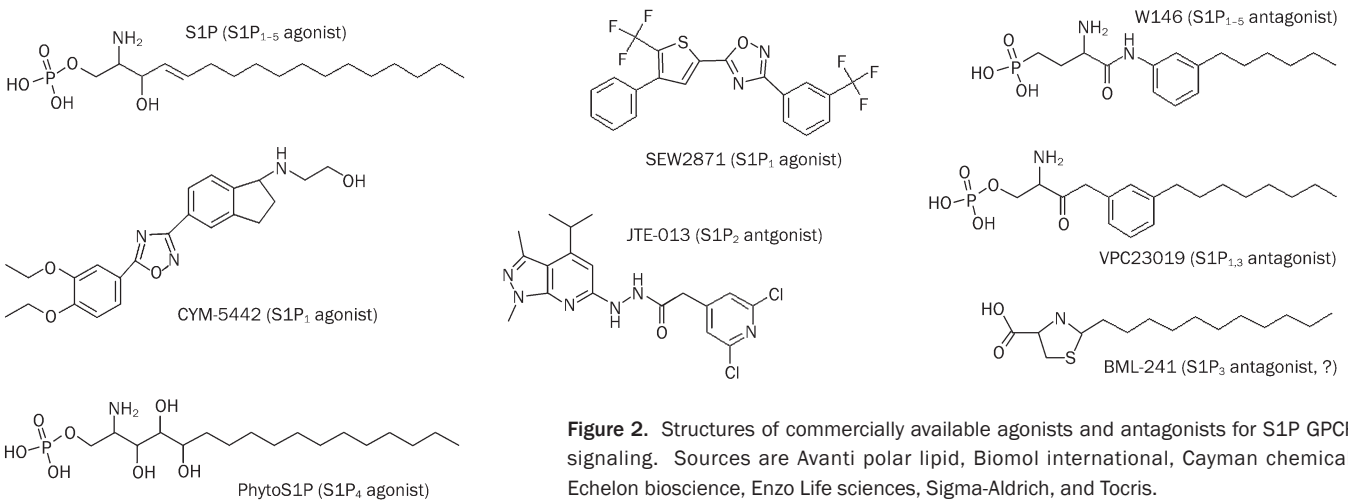

Figure 2. Structures of commercially available agonists and antagonists for S1P GPCR signaling. Sources are Avanti polar lipid, Biomol international, Cayman chemical, Echelon bioscience, Enzo Life sciences, Sigma-Aldrich, and Tocris.

Table 2. Agonistic and antagonistic characters of each compound on each S1P receptor. Numbers (nmol/L) mean EC_{50} or K_D values for agonists and IC_{50} or K_I values for antagonists.

Name	SIP_1	SIP_2	SIP_3	SIP_4	SIP_5
AUY954[86]	Agonist (1.2 nmol/L)		Agonist (1210 nmol/L)		Agonist (340 nmol/L)
CYM-5442[85]	Agonist (1.2 nmol/L)				
CYM-5181[84]	Agonist (3.4 nmol/L)				
SEW2871[81]	Agonist (13 nmol/L)				
Compound 26[83]	Agonist (0.6 nmol/L)		Agonist (12000 nmol/L)	Agonist (70 nmol/L)	Agonist (1.0 nmol/L)
Compound 12[90]				Agonist (7.4 nmol/L)	Agonist (10.2 nmol/L)
Compound 18[90]				Agonist (16.8 nmol/L)	Agonist (5.8 nmol/L)
VPC44116[97]	Antagonist (30 nmol/L)		Antagonist (300 nmol/L)		Partial agonist (33 nmol/L)
W146[96,106]	Antagonist (36 nmol/L)				
SB649146[98]	Antagonist (300 nmol/L)				
VPC23019[95]	Antagonist (13.8 nmol/L)		Antagonist (1175 nmol/L)	Agonist (263 nmol/L)	Partial agonist (85.1 nmol/L)
VPC25239[95]	Antagonist (13.4 nmol/L)		Antagonist (97.7 nmol/L)	Agonist (166 nmol/L)	Partial agonist (11.5 nmol/L)
CL2[100]	Antagonist (4400 nmol/L)	Antagonist (37000 nmol/L)	Antagonist (6700 nmol/L)		
VPC01091-P[87]	Agonist (6.6 nmol/L)		Antagonist	Agonist	Partial agonist
Ascotricins A and B[99]	Antagonist (8200 nmol/L, 1800 nmol/L)				
JTE-013[93]		Antagonist (17 nmol/L)			
BML-241[102, 103]			Antagonist (?)		
TY-52156[123]			Antagonist (110 nmol/L)		
DS-SG-44[124]	Agonist	Agonist	Agonist		
KRP-203-P[88,89]	Agonist (0.8 nmol/L)			Agonist (9.6 nmol/L)	
PhytoS1P[76]				Agonist (1.6 nmol/L)	
DihydroS1P[36,75,76]	Agonist	Agonist	Agonist	Agonist (8.6 nmol/L)	Agonist
SPC[34,36,75]	Partial agonist	Partial agonist	Partial agonist	Partial agonist	Partial agonist
FTY720-P[78,79]	Agonist (6.3 nmol/L), functional antagonist		Agonist (4.0 nmol/L)	Agonist (6.3 nmol/L)	Agonist (6.3 nmol/L)
AFD-R[79]	Agonist (2.5 nmol/L)		Agonist (4.0 nmol/L)	Agonist (4.0 nmol/L)	Agonist (1.3 nmol/L)

transduction studies to proceed in cells over-expressing the receptors and in receptor knock-down transgenic mouse models[106–109]. The discovery of the pathophysiological significance of LPA and S1P, particularly on each receptor subtype, would contribute favorably to new drug development. In developing new medications acting on LPA or S1P receptors, subtype selectivity would be a major issue along with potency and efficacy to avoid side effects and ensure drug safety.

FTY720 was initially developed as an immune modulator for organ transplant patients[110]. At present, this compound is under clinical study for the treatment of multiple sclerosis[111]. Amira Pharmaceuticals reported LPA_1 selective antagonist (4'-{4-[(R)-1-(2-chloro-phenyl)-ethoxycarbonylamino]-3-methyl-isoxazol-5-yl}-biphnyl-4-yl)-acetic acid, AP2966, which showed good therapeutic potential in idiopathic pulmonary fibrosis and good pharmacokinetic profiles, including oral bioavailability[112]. Pfizer global research and development introduced the $S1P_1$ selective agonists PF-A and PF-B. These agonists resulted in lymphopenia in rats and monkeys similar to FTY720 and reduced collagen-induced arthritis[113]. Using a different approach, Lpath Inc. recently introduced monoclonal antibodies against S1P and LPA. Humanized anti-S1P monoclonal antibody (mAb) sonepcizumab blocked the tumorigenic effect of S1P produced by cancer cells as well as the angiogenic effect induced during pathological angiogenesis[114, 115]. The LPA receptor pan-antagonist (LPA_{1-4}) and autotoxin inhibitor, α-bromomethylene phosphonate LPA analogue, was shown to be an excellent anti-cancer agent[116, 117].

Closing remarks

Following the initial discoveries of LPA activity 30 years ago and S1P activity 15 years ago, a dark dawn era in lysophospholipid biology occurred due to the lack of identifiable targets. The discovery of the Edg-family GPCRs for LPA and S1P shined light on this field. The first meeting that focused on lysophospholipid biology was a New York Academy of Science meeting conducted in 1999 at Rockefeller University[118]. Since 2001, FASEB summer research conferences on lysophospholipids have been held biannually. Lysophospholipid receptor nomenclature has been systematically assigned[119], and more LPA receptors (purinergic or non-Edg LPA recep-

tors) are being discovered each year. Every two years, we have had exciting findings including the linkage of FTY720 to the S1P receptor, discovery of an autotoxin as a LPA-producing lysoPLD, lysolipid-sensitive proton-sensing GPCRs (OGR1 subfamily), and finally, the development of new chemicals. This review integrates accumulated information regarding pharmacological tools for lysophospholipid GPCR signaling to compare their characteristics and provides valuable information such as available chemical sources. Using a combination of receptor expression patterns in each organ or cell, these pharmacological tools might prove useful in defining the pathophysiological impact and significance of lysophospholipids. As mentioned above, the control of lysophopholipid functions using specific agonists or antagonists will contribute toward novel drug development. At a 2009 FASEB meeting in Carefree (Arizona, USA), we began to see several of the chemicals described here, in addition to FTY720, being used in clinical applications[112–114, 116]. We now know more about many things than ever before. It is very likely that in the near future, the agonists/antagonists for LPA or S1P receptors will be on the market commercially and that there will be a section on lysophospholipid GPCRs in every basic pharmacology textbook.

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