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Synthesis and pharmacological evaluation of second-generation phosphatidic acid derivatives as lysophosphatidic acid receptor ligands

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Abstract—Short-chain phosphatidic acid derivatives, dioctanoyl glycerol pyrophosphate (DGPP 8:0, **1**) and phosphatidic acid 8:0 (PA 8:0, **2**), were previously identified as subtype-selective LPA_1 and LPA_3 receptor antagonists. Recently, we reported that the replacement of the phosphate headgroup by thiophosphate in a series of fatty alcohol phosphates (FAP) improves agonist as well as antagonist activities at LPA GPCR. Here, we report the synthesis of stereoisomers of PA 8:0 analogs and their biological evaluation at LPA GPCR, PPARγ, and ATX. The results indicate that LPA receptors stereoselectively interact with glycerol backbone modified ligands. We observed entirely stereospecific responses by dioctyl PA 8:0 compounds, in which (*R*)-isomers were found to be agonists and (*S*)-isomers were antagonists of LPA GPCR. From this series, we identified compound **13b** as the most potent LPA_3 receptor subtype-selective agonist (EC_{50} = 3 nM), and **8b** as a potent and selective LPA_3 receptor antagonist (K_i = 5 nM) and inhibitor of ATX (IC_{50} = 600 nM). Serinediamide phosphate **19b** was identified as an LPA_3 receptor specific antagonist with no effect on LPA_1, LPA_2, and PPARγ.

Lysophosphatidic acid (LPA, 1-or 2-acyl-*sn*-glycero-3-phosphate) is a pleiotropic growth factor-like lipid mediator with diverse biological properties. LPA elicits numerous cellular responses including cell proliferation, cell survival, cell migration, platelet aggregation, smooth muscle contraction, cytokine and chemokine secretion, and neurite retraction in various cell types.[1,2] LPA mediates its effects through the activation of three G protein-coupled receptors (GPCR), LPA_1, LPA_2, and LPA_3, which belong to endothelial differentiation gene family.[3] LPA_4, formerly known as the P2Y9/GPR23 orphan GPCR, has recently been identified as a fourth plasma membrane LPA receptor.[4] LPA_4 is evolutionarily distant and shares only 20–24% homology with LPA_1, LPA_2, and LPA_3.[4] Recently, the nuclear transcription factor peroxisome proliferator-activator receptor-γ (PPARγ) has been identified as an intracellular receptor for LPA.[5]

LPA signaling pathways are involved in several physiological and pathological processes including cancer invasion,[2,6] atherogenesis,[7,8] inflammation,[9] angiogenesis,[10] neurogenesis,[11] wound healing,[12,13] protection against radiation- and chemotherapy-induced apoptosis,[14,15] and ischemia–reperfusion injury.[16] The autocrine motility factor autotaxin (ATX), originally isolated from invasive cancer cells, has recently been identified as the long elusive plasma lysophospholipase D activity[17,18] that is the major source of LPA production in serum[19,20] and tumor cells.[18] ATX expression increases motility and invasiveness of tumor cells in vitro via the production of LPA and subsequent activation of LPA_1.[21] Thus,

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LPA receptor agonists, antagonists, and compounds that block LPA production via ATX are likely to have great therapeutic potential. Despite the potential therapeutic applications of LPA receptor agonists and antagonists, a limited number of subtype-selective agents have been reported to date.[3,22] Furthermore, the agents that have been described in the literature have not been comprehensively characterized at each LPA target.

Several groups have reported that LPA receptors, unlike many other GPCR, lack stereospecificity or stereoselectivity in recognition of acyl- and alkyl-LPA analogs.[23–27] In contrast to the natural ligand LPA, LPA receptors have been shown to interact stereoselectively with backbone modified LPA analogs, in which the glycerol backbone has been replaced by serine or ethanolamine. Heise et al. showed that LPA receptors prefer the natural (*R*)-stereochemistry in a 2-substituted ethanolamide series of compounds.[28] Although both enantiomers were equally effective inhibitors of LPA-activated chloride currents in *Xenopus laevis* oocytes, (*S*)-*N*-palmitoyl serine phosphoric acid was more potent than the (*R*)-enantiomer in eliciting calcium mobilization in MDA MB231 cells.[29,30] Recently, (2*S*)-1-oleoyl-2-*O*-methyl-glycerophosphothionate (OMPT) was found to be more potent than (2*R*)-OMPT in calcium mobilization assays using transfected rat hepatoma RH7777 and insect Sf9 cells.[31] In contrast to the plasma membrane receptors, activation of the intracellular LPA receptor PPARγ shows a stereoselective preference for (*R*)-1-alkyl glycerophosphate 18:1 (AGP) over (*S*)-3AGP 18:1.[8] Thus, streochemically pure analogs are preferred over racemic mixtures as the enantiomers are likely to possess different pharmacological properties.

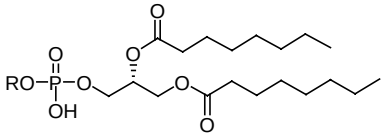
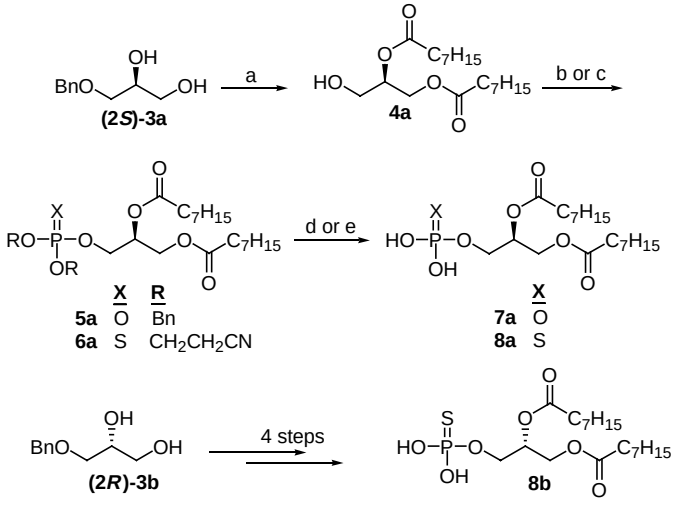


Figure 1. Structures of DGPP 8:0 (**1**) and PA 8:0 (**2**).

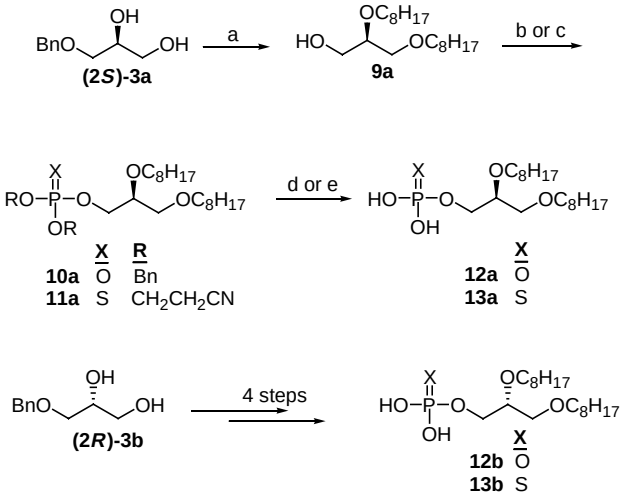
Based on the observation that shorter-chain LPA analogs exert little or no activity on LPA receptors,[24,32] we have identified previously short-chain phosphatidic acid derivatives, dioctanoyl glycerol pyrophosphate (DGPP 8:0, **1**) and phosphatidic acid 8:0 (PA 8:0, **2**), as subtype-selective LPA_1 and LPA_3 receptor antagonists (Fig. 1).[33] Recently, we reported that the replacement of the phosphate headgroup by thiophosphate in a series of fatty alcohol phosphates (FAP) improves agonist as well as antagonist activities at LPA GPCR.[34] Here, we report the synthesis of stereoisomers of PA 8:0 analogs and their biological evaluation at LPA GPCR, PPARγ, and ATX. Our present data extend previous reports,[28,30,31] in that LPA receptors stereoselectively interact with glycerol backbone modified ligands. With dioctyl PA 8:0 compounds, we observed entirely stereospecific responses, in which (*R*)-isomers were found to be agonists, whereas (*S*)-isomers were antagonists of LPA GPCR. From this series, we identified compound **13b** as the most potent LPA_3 receptor subtype-selective agonist (EC_{50} = 3 nM), and **8b** as a potent and selective LPA_3 receptor antagonist (K_i = 5 nM) and inhibitor of ATX (IC_{50} = 600 nM). Serinediamide phosphate **19b** was identified as an LPA_3 receptor specific antagonist with no effect on LPA_1, LPA_2, and PPARγ. These compounds therefore offer much improved selectivity and potency over the first generation of compounds for manipulating the biological responses mediated through the different targets of LPA.

Dioctanoyl PA analogs were synthesized as shown in Scheme 1. Commercially available (2*S*)-3-benzyloxy-1,2-propanediol (**3a**) was diacylated with octanoylchloride followed by debenzylation under catalytic



Scheme 1. Synthesis of dioctanoyl PA analogs. Reagents and conditions: (a) (i) $C_7H_{15}COCl$, pyridine, CH_2Cl_2, 70%; (ii) Pd–C/H_2, MeOH, 95%; (b) (i) dibenzyl-*N*,*N*-diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) 50% H_2O_2, 60%; (c) (i) bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) S, reflux, 72%; (d) Pd–C/H_2, MeOH, 80%; (e) bis(trimethylsilyl)trifluoro acetamide, DBU, pyridine, 40%.

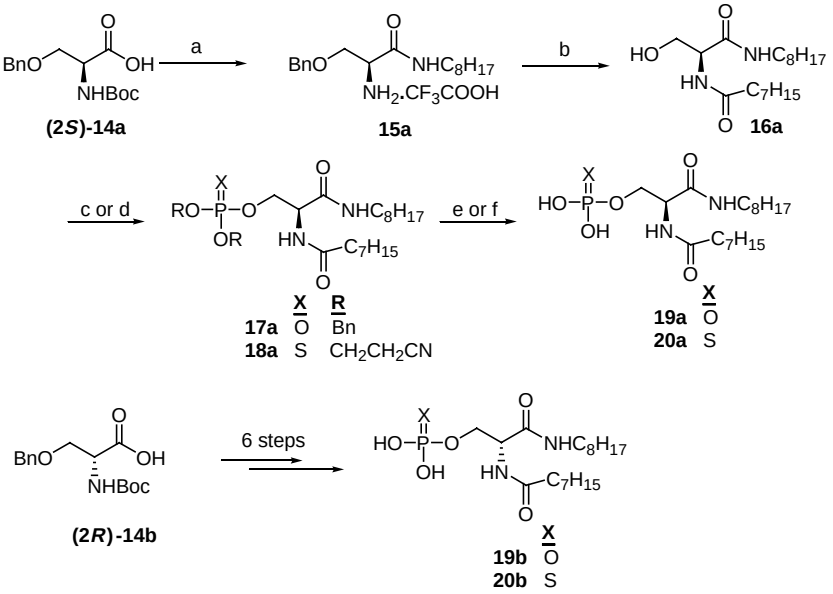
hydrogenation conditions to provide the alcohol (**4a**). Alcohol (**4a**) was then phosphorylated using dibenzyl-*N*,*N*-diisopropyl phosphoramidite to yield the dibenzyl protected phosphate after oxidation (**5a**),[35] which upon catalytic hydrogenation afforded the corresponding (2*S*)-dioctanoyl PA (**7a**). Treatment of **4a** with bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite followed by reflux in presence of elemental sulfur provided the dicyanoethyl protected thiophosphate (**6a**). The target thiophosphatidic acid 8:0 (TPA 8:0, **8a**) was obtained by removing the cyanoethyl groups using bis(trimethylsilyl)trifluoro acetamide and pyridine.[36] Similarly, the (2*R*)-thiophosphate analog **8b** was synthesized from (2*R*)-3-benzyloxy-1,2-propanediol (**3b**) in 4 steps. The (2*R*)-dioctanoyl PA compound (**2**) used in this study was purchased from commercial sources.



Scheme 2. Synthesis of dioctyl PA analogs. Reagents and conditions: (a) (i) $C_8H_{17}Br$, KOH, toluene, reflux, Dean–Stark apparatus, 65%; (ii) Pd–C/H_2, MeOH 92%; (b) (i) di-*tert*-butyl-*N*,*N*-diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) 50% H_2O_2, 68%; (c) (i) bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) S, reflux, 73%; (d) TFA, CH_2Cl_2, 84%; (e) KOH/MeOH, rt, 3h, 46%.

The dialkyl PA 8:0 (APA 8:0) analogs were synthesized as shown in Scheme 2. Alkylation of commercially available (2*S*)-3-benzyloxy-1,2-propanediol (**3a**) with octylbromide followed by debenzylation provided the alcohol (**9a**). Compound **9a** was then phosphorylated using phosphoramidite chemistry to produce the di-*tert*-butyl protected phosphate (**10a**),[35] which upon treatment with TFA gave the corresponding (2*S*)-dioctyl PA compound (**12a**). Treatment of **9a** with bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite followed by reflux in presence of elemental sulfur provided the dicyanoethyl protected thiophosphate (**11a**). Removal of the cyanoethyl groups with treatment of KOH in methanol furnished the target dialkyl thiophosphatidic acid 8:0 (ATPA 8:0) compound **13a**. Similarly, the (2*R*)-analogs **12b** and **13b** were synthesized from (2*R*)-3-benzyloxy-1,2-propanediol (**3b**) in 4 steps.

The serinediamide phosphate/thiophosphate (SDP/SDTP) analogs were synthesized as outlined in Scheme 3. *O*-Benzyl-Boc-(L)-serine (**14a**) was coupled with octylamine using EDC and HOBt, and deprotection with TFA gave compound **15a**. Compound **15a** was acylated using octanoyl chloride followed by debenzylation to yield the key alcohol intermediate (**16a**). The alcohol (**16a**) was then phosphorylated to yield the target (2*S*)-compounds **19a** and **20a** via formation of intermediates **17a** and **18a**, using chemistry similar to that shown in Scheme 1. From *O*-benzyl-Boc-(D)-serine (**14b**) as the starting material, the (2*R*)-analogs **19b** and **20b** were



Scheme 3. Synthesis of serinediamide phosphate and thiophosphate analogs. Reagents: (a) (i) $C_8H_{17}NH_2$, EDC/HOBt, CH_2Cl_2, 92%; (ii) TFA, CH_2Cl_2, 90%; (b) (i) octanoyl chloride, NMM, THF, 82%; (ii) Pd–C/H_2, MeOH, 90%; (c) (i) dibenzyl-*N*,*N*-diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) 50% H_2O_2, 68%; (d) (i) bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; (ii) S, reflux, 66%; (e) Pd–C/H_2, MeOH, 80%; (f) bis(trimethylsilyl)trifluoro acetamide, DBU, pyridine, 38%.

synthesized. All compounds were characterized by ^{1}H NMR, mass spectroscopy and, in case of the final compounds, elemental analysis.[37]

Thiophosphates **8a–b**, **13a–b**, and **20a–b** were built with phosphate groups in the −2 ionization state in the MOE[38] molecular modeling software package. Partial charges were assigned and structures were geometry optimized using the MMFF94[39] forcefield. Docking studies with active and inactive models of the LPA receptors were performed using the Autodock 3.0 software[40] as previously described for DGPP 8:0.[41] Default parameters were used with the exception of the number of energy evaluations (9×10^{10}), generations (30,000), local search iterations (3000), and runs (15). Docking boxes included the extracellular loops and top of the transmembrane domains of the inactive receptor models and the transmembrane domains of the active receptor models. Complexes described in the Results section are those for each receptor:ligand pair with the lowest final docked energy. Residues are described using the helix indexing system of Ballesteros and Weinstein.[42] This system assigns numbers in the format of X.Y to residues in the transmembrane domains of GPCR using the transmembrane helix number (X) and an index relative to the most conserved residue in that helix assigned index position 50 (Y).

We reported earlier DGPP 8:0 (**1**) and PA 8:0 (**2**) as subtype selective antagonists of the LPA_1 and LPA_3 receptors with an order of magnitude preference for LPA_3.[33] However, these compounds were derived from the natural sources and were available only in (*R*)-enantiomeric form. The activities of the (*S*)-enantiomers have not previously been assessed at LPA GPCR. Since the discovery of PA 8:0 as an $LPA_{1/3}$ antagonist, there have been many advances in the LPA field. LPA receptors, which were previously reported to have no stereochemical preference for the natural ligand LPA,[23,24] have now been shown to interact stereoselectively with synthetic ligands, in which the glycerol backbone is either modified or has a substitution at the *sn*-2 position.[28,30,31,43] We hypothesized that the PA 8:0 scaffold would interact with LPA receptors in a stereoselective manner. To address this hypothesis, we synthesized and evaluated several PA 8:0 analogs. The effects of headgroup modification to a thiophosphate, glycerol backbone modification to serine, as well as the importance of the ester versus ether linkage of hydrophobic chain and stereochemistry are assessed in the current study using a constant octyl hydrophobic chain length that was previously found to be optimal.

All synthesized analogs were tested for agonist and antagonist activities at LPA GPCR, as activators of PPARγ and as inhibitors of ATX. RH7777 cells, which are devoid of LPA GPCR, were stably transfected with individual LPA_1, LPA_2, and LPA_3 receptors and used for in vitro screening.[33,44] PPARγ activation was assayed in CV1 cells transfected with PPARγ and an acyl-coenzyme A oxidase-luciferase (PPRE-Acox-Rluc) reporter gene.[8] ATX inhibition was determined using bis(*para*-nitrophenyl) phosphate hydrolysis by recombinant ATX in conditioned medium from transiently transfected HEK293 cells as described previously.[45] These results are shown in Table 1.

(2*S*)-PA 8:0 (**7a**) and its enantiomer (2*R*)-PA 8:0 (**2**) showed subtype selective antagonism at the LPA_1 and LPA_3 receptors with no effect on the LPA_2 receptor. PA analogs enantioselectively antagonized both LPA_1 and LPA_3 receptors with a moderate preference for the *S*-isomer at LPA_1. The antagonist selectivity is reversed at the LPA_3 receptor, which showed a preference for (2*R*)-PA 8:0 over the (2*S*)-isomer. In accordance with previous reports,[46] thiophosphate headgroup modification improved the activity of PA 8:0 analogs. (2*R*)-TPA 8:0 (**8b**) was a more potent antagonist than the phosphate analog (2*R*)-PA 8:0 (**2**) at both the LPA_1 and LPA_3 receptors, and was a partial agonist of LPA_2. Compound **8b** was identified as the most potent and selective LPA_3 receptor antagonist reported to date with a K_i value of 5 nM with a 75-fold selectivity over LPA_1.

The modeled complex of **8b** with the inactive model of the previously validated LPA_3 receptor[47] is shown in Figure 2A. This model shows significantly stronger interactions between **8b** and the receptor than did the

Table 1. Effects of PA 8:0 analogs on LPA_{1-3} transfected RH7777 cells, activation of PPARγ, and ATX inhibition

Compound	*R*/*S*	X	LPA_1		LPA_2		LPA_3		ATX
			EC_{50} (E_{max})[a] (nM)	IC_{50} (K_i) (nM)	EC_{50} (E_{max}) (nM)	IC_{50} (K_i) (nM)	EC_{50} (E_{max}) (nM)	IC_{50} (K_i) (nM)	IC_{50} (nM) (Max. inhib. %)
7a	*S*	O	NE[b]	433 (221)	NE	NE	NE	207 (119)	NE
2	*R*	O	NE	692 (407)	NE	NE	NE	85 (39)	1644 (49.9)
8a	*S*	S	NE	NE	7170 (17)	NE	115 (30)	NE	727 (52.3)
8b	*R*	S	NE	686 (360)	6330 (58)	NE	NE	11 (5)	597 (72.5)
12a	*S*	O	NE	1580 (486)	NE	NE	NE	143 (50)	NE
12b	*R*	O	3260 (57)	NE	NE	NE	164 (109)	NE	NE
13a	*S*	S	NE	328 (139)	NE	NE	NE	184 (67)	NE
13b	*R*	S	695 (30)	NE	5720 (27)	NE	3 (109)	NE	NE
19a	*S*	O	NE	NE	NE	NE	NE	414 (196)	NE
19b	*R*	O	NE	NE	NE	NE	NE	935 (489)	NE
20a	*S*	S	NE	476 (152)	NE	NE	NE	251 (117)	NE
20b	*R*	S	NE	7390 (2850)	NE	NE	NE	302 (118)	NE

[a] E_{max} = maximal efficacy of the drug/maximal efficacy of LPA 18:1, expressed as the percentage.
[b] NE = no effect.

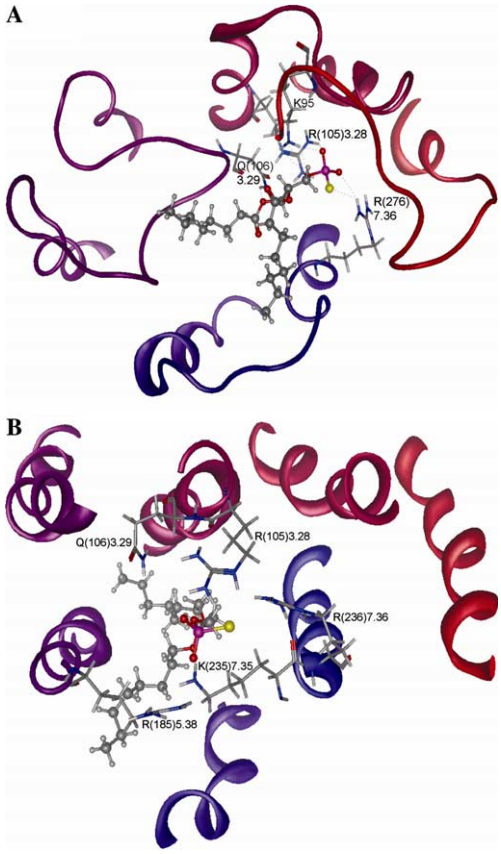


Figure 2. Computational models of LPA_3 complexes. Ribbons represent portions of the LPA_3 receptor colored from red at the amino terminal end to blue at the carboxy terminal end. Synthetic ligands are shown as ball and stick models. Key residues of LPA_3 interacting with synthetic ligands are shown as stick models and labeled. (A) Best docked position of **8b** in the inactive model of LPA_3. (B) Best docked position of **13b** in the active model of LPA_3.

complex obtained using the active model of the LPA_3 receptor. Figure 2A shows ionic interactions between the thiophosphate group of **8b** and R(105)3.28, R(276)7.36, and K95. A hydrogen bond involving Q(106)3.29 and **8b** is also observed. The ionic interactions are consistent with our previously reported model of DGPP 8:0 with the inactive model of LPA_3.[41] The hydrogen bonding interaction with Q(106)3.29, however, is unique to compound **8b** and may explain its exceptional potency. The compound (2*S*)-TPA 8:0 (**8a**) was devoid of $LPA_{1/3}$ antagonism, but was a partial agonist at LPA_2 and LPA_3. The models of **8a** with both the inactive and active models of LPA_3 (data not shown) showed relatively poor ionic interactions with the receptor, indicating that the stereochemical difference prevents the thiophosphate group from interacting with the key residues required for either full agonist or antagonist activity. These results, in accordance with the previously published reports,[31] show that LPA receptors exhibit stereoselectivity in interacting with *sn*-2 substituted glycerol analogs.

To increase the stability of the acyl-PA 8:0 analogs against chemical as well as phospholipase A (PLA) degradation, we synthesized alkyl derivatives of PA 8:0 (**12a–b** and **13a–b**) and evaluated their agonist and antagonist properties at LPA GPCR. None of the compounds evoked Ca^{2+} transients in wild-type RH7777 cells. In general, thiophosphates were more potent than the corresponding phosphates regardless of agonist/antagonist activity. We observed enantiospecific activation of the $LPA_{1/3}$ receptors by (2*R*)-APA 8:0 (**12b**) and (2*R*)-ATPA 8:0 (**13b**). Compound **13b**, which has the identical (*R*)-configuration as natural LPA, was the most potent and LPA_3-selective agonist in this series (EC_{50} = 3 nM, E_{max} = 109%) and was ~230- and ~1900-fold selective for LPA_3 over LPA_1 and LPA_2, respectively. The high potency of **13b** is evident from the molecular model shown in Figure 2B. The thiophosphate headgroup forms ion pairs with R(105)3.28, R(236)7.36, K(235) 7.35, and R(185)5.38. The ion pair with R(236)7.36 is unique to this thiophosphate due to the greater phosphorus–sulfur bond length compared to that of phosphorus–oxygen. In fact, mutagenesis of LPA_3 demonstrates that the R(236)7.36A mutant maintains activation by LPA, demonstrating that the phosphate of LPA does not form the same ion pair observed for the thiophosphate.[47] The additional ion pair explains the enhanced potency of this and other thiophosphate agonists of LPA_3. At the LPA_3 receptor, dioctyl thiophosphate analog **13b** was a more potent agonist than the corresponding phosphate (**12b**) and LPA 18:1 (Fig. 3). In contrast to the (2*R*)-alkyl analogs, the (2*S*)-enantiomers were antagonists at the $LPA_{1/3}$ receptors. Although compounds (*R*)-VPC12204 and (*S*)-VPC12249 were the first to demonstrate enantiospecific agonist and antagonist responses, respectively, at LPA_1, both enantiomers were antagonists at LPA_3.[28] The enantiosepecific activation of the $LPA_{1/3}$ receptors by APA analogs is presumably due to the favorable orientation of the conformationally flexible alkyl side chains of the (*R*)-isomers within the ligand binding pocket of the receptors. The side chains of acyl PA analogs are relatively constrained due to their ester linkage to glycerol, which may prevent these favorable ligand–receptor interactions. With the exception of 2(*R*)-ATPA 8:0 (**13b**), which was a weak partial agonist of LPA_2, all alkyl PA analogs tested had no effect on LPA_2 receptor.

Replacement of the glycerol backbone by serine is well tolerated at LPA GPCR.[29,30,48] Surprisingly, SDP 8:0 analogs (**19a–b**) were identified as LPA_3 receptor subtype-specific antagonists that had no effect on LPA_1 and LPA_2. As expected, SDP 8:0 isomers also demonstrated enantioselective LPA_3 antagonism. The (*S*)-isomer (**19a**) was a better antagonist than was the (*R*)-isomer (**19b**). Thiophosphate headgroup modification in serinediamides (**20a–b**) not only improved the LPA_3 antagonist activity but also resulted in loss of LPA_3 subtype-specificity by conferring added LPA_1 antagonism.

Results of in vitro PPARγ activation by these compounds are shown in Figure 4. Zhang et al. reported that PPARγ showed a preference for alkyl-LPA over acyl-LPA and the *R*-isomer over the *S*-isomer in the AGP 18:1 enantiomeric pair.[8] In our series, only alkyl-PA analogs elicited PPARγ activation, while acyl-PA and serinediamide analogs had no activity (Fig. 4). Unlike

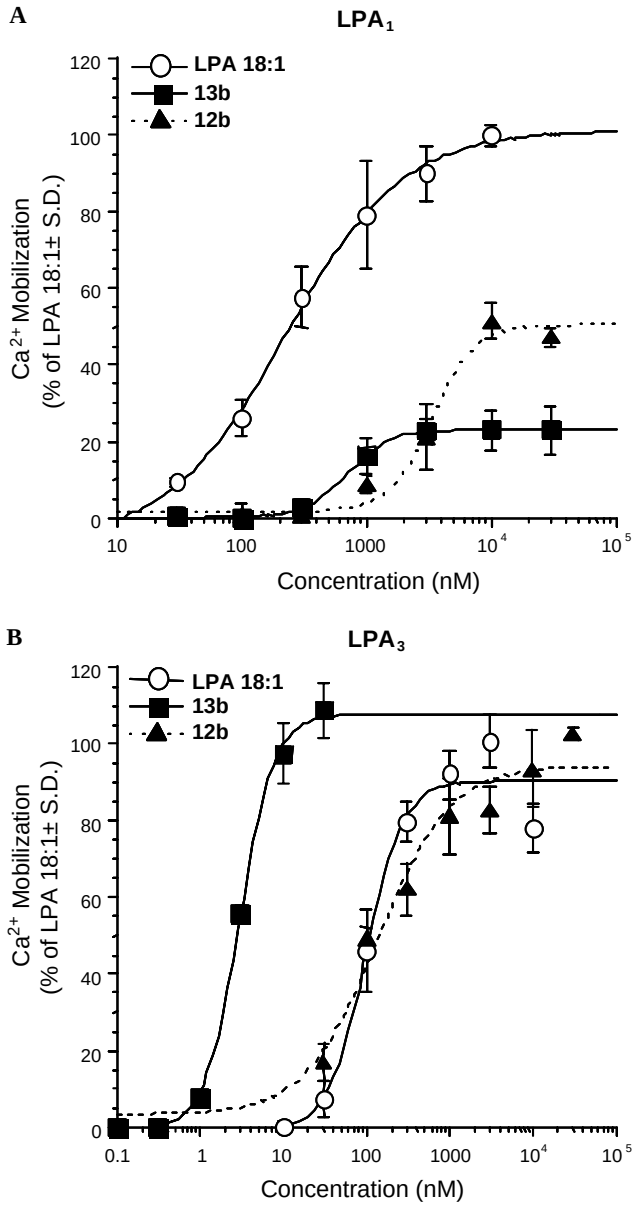


Figure 3. (2*R*) Alkyl PA analogs (**12b** and **13b**) are agonists at LPA_1 and LPA_3 receptors expressed in RH7777 cells. Dose–response relationships for LPA 18:1, **12b** and **13b** in RH7777 cells expressing LPA_1 (A) and LPA_3 (B). Intracellular Ca^{2+} transients were measured in response to the application of increasing concentrations of compounds **12b** and **13b**, and compared to transients elicited by LPA 18:1. Data points represent the average of four measurements.

the enantiospecific responses at LPA GPCR, there was no stereoselectivity observed in PPARγ activation by these analogs. Compound **19a**, which is a selective LPA_3 antagonist with no effect on $LPA_{1/2}$, also showed PPARγ activation. In contrast, **19b** retains LPA_3 receptor selectivity and had no effect on PPARγ making it a true LPA_3 receptor specific antagonist.

ATX inhibition was noted for only acyl thiophosphates **8a** and **8b** (Table 1). The (*R*)-isomer (**8b**) was slightly more potent and effective than its corresponding (*S*)-isomer (**8a**). Modification of the thiophosphate headgroup by phosphate (**2** and **7a**) significantly decreased ATX

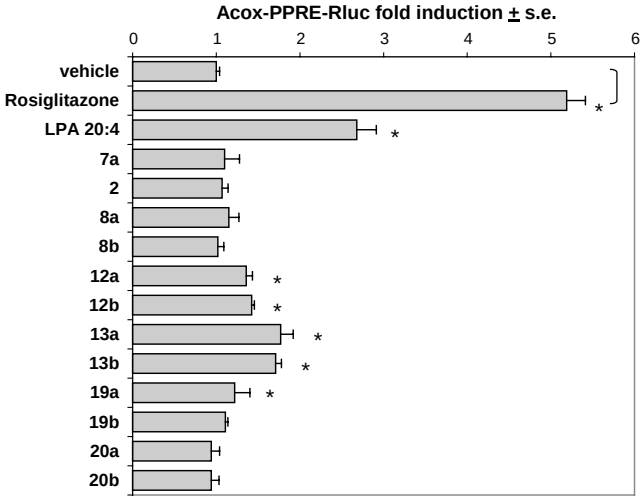


Figure 4. In vitro PPARγ activation by PA analogs in CV1 cells transfected with PPARγ and PPRE-Acox-Rluc reporter genes. Effects are compared with rosiglitazone, a known PPARγ agonist. CV1 cells were treated with vehicle or 10 μM test compound dissolved in DMSO for 20 h. Luciferase and β-galactosidase activities (mean ± SEM) were measured in the cell lysate ($n = 4$). $^*P < 0.05$, significant differences over vehicle control.

inhibition. Likewise, dialkyl substitution (**13a–b**) in the thiophosphate series has resulted in loss of ATX inhibitory activity. All four compounds containing the serine backbone showed no inhibition of ATX.

In summary, a series of PA analogs has been synthesized and evaluated at LPA GPCR, PPARγ, and ATX. Our results substantiate previously published reports that LPA receptors show marked enantioselectivity with phospholipid analogs having *sn*-2 substituted glycerol or serine backbones. Enantioselectivity is determined, not only by the backbone but also by the nature of the hydrocarbon chain linkage and stereochemistry of the ligands. These results indicate that fine-tuning the structural features of the available lead structures will aid in development of LPA receptor type- and subtype-specific ligands. This study has resulted in identification of compound **13b** as a potent LPA_3 receptor subtype-selective agonist and compound **8b** ($K_i = 5$ nM) as the most potent subtype-selective LPA_3 receptor antagonist reported to date. In addition, compound **8b** blocks LPA_1 and ATX, two proteins linked to cancer cell invasion and metastasis. Finally, using serine as a glycerol backbone substitute, an LPA_3 receptor-specific antagonist **19b** was discovered that has no effect on LPA_1, LPA_2, and PPARγ.

Acknowledgment

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References and notes

1. Tigyi, G.; Parrill, A. L. *Prog Lipid Res.* **2003**, *42*, 498.
2. Mills, G. B.; Moolenaar, W. H. *Nat. Rev. Cancer* **2003**, *3*, 582.

3. Anliker, B.; Chun, J. *J. Biol. Chem.* **2004**.
4. Noguchi, K.; Ishii, S.; Shimizu, T. *J. Biol. Chem.* **2003**, *278*, 25600.
5. McIntyre, T. M.; Pontsler, A. V.; Silva, A. R.; St. Hilaire, A.; Xu, Y.; Hinshaw, J. C.; Zimmerman, G. A.; Hama, K.; Aoki, J.; Arai, H.; Prestwich, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 131.
6. Fang, X.; Gaudette, D.; Furui, T.; Mao, M.; Estrella, V.; Eder, A.; Pustilnik, T.; Sasagawa, T.; Lapushin, R.; Yu, S.; Jaffe, R. B.; Wiener, J. R.; Erickson, J. R.; Mills, G. B. *Ann. N.Y. Acad. Sci.* **2000**, *905*, 188.
7. Siess, W.; Zangl, K. J.; Essler, M.; Bauer, M.; Brandl, R.; Corrinth, C.; Bittman, R.; Tigyi, G.; Aepfelbacher, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6931.
8. Zhang, C.; Baker, D. L.; Yasuda, S.; Makarova, N.; Balazs, L.; Johnson, L. R.; Marathe, G. K.; McIntyre, T. M.; Xu, Y.; Prestwich, G. D.; Byun, H. S.; Bittman, R.; Tigyi, G. *J. Exp. Med.* **2004**, *199*, 763.
9. Rizza, C.; Leitinger, N.; Yue, J.; Fischer, D. J.; Wang, D. A.; Shih, P. T.; Lee, H.; Tigyi, G.; Berliner, J. A. *Lab. Invest.* **1999**, *79*, 1227.
10. Sugiura, T.; Nakane, S.; Kishimoto, S.; Waku, K.; Yoshioka, Y.; Tokumura, A. *J. Lipid Res.* **2002**, *43*, 2049.
11. Chun, J. *Crit. Rev. Neurobiol.* **1999**, *13*, 151.
12. Sturm, A.; Sudermann, T.; Schulte, K. M.; Goebell, H.; Dignass, A. U. *Gastroenterology* **1999**, *117*, 368.
13. Sturm, A.; Dignass, A. U. *Biochim. Biophys. Acta* **2002**, *1582*, 282.
14. Deng, W.; Balazs, L.; Wang, D. A.; Van Middlesworth, L.; Tigyi, G.; Johnson, L. R. *Gastroenterology* **2002**, *123*, 206.
15. Deng, W.; Wang, D. A.; Gosmanova, E.; Johnson, L. R.; Tigyi, G. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2003**, *284*, G821.
16. Okusa, M. D.; Ye, H.; Huang, L.; Sigismund, L.; Macdonald, T.; Lynch, K. R. *Am. J. Physiol. Renal Physiol.* **2003**, *285*, F565.
17. Tokumura, A.; Majima, E.; Kariya, Y.; Tominaga, K.; Kogure, K.; Yasuda, K.; Fukuzawa, K. *J. Biol. Chem.* **2002**, *277*, 39436.
18. Umezu-Goto, M.; Kishi, Y.; Taira, A.; Hama, K.; Dohmae, N.; Takio, K.; Yamori, T.; Mills, G. B.; Inoue, K.; Aoki, J.; Arai, H. *J. Cell Biol.* **2002**, *158*, 227.
19. Sano, T.; Baker, D.; Virag, T.; Wada, A.; Yatomi, Y.; Kobayashi, T.; Igarashi, Y.; Tigyi, G. *J. Biol. Chem.* **2002**, *277*, 21197.
20. Aoki, J.; Taira, A.; Takanezawa, Y.; Kishi, Y.; Hama, K.; Kishimoto, T.; Mizuno, K.; Saku, K.; Taguchi, R.; Arai, H. *J. Biol. Chem.* **2002**, *277*, 48737.
21. Hama, K.; Aoki, J.; Fukaya, M.; Kishi, Y.; Sakai, T.; Suzuki, R.; Ohta, H.; Yamori, T.; Watanabe, M.; Chun, J.; Arai, H. *J. Biol. Chem.* **2004**, *279*, 17634.
22. Parrill, A. L.; Sardar, V. M.; Yuan, H. *Semin. Cell Dev. Biol.* **2004**, *15*, 467.
23. Yokoyama, K.; Baker, D. L.; Virag, T.; Liliom, K.; Byun, H. S.; Tigyi, G.; Bittman, R. *Biochim. Biophys. Acta* **2002**, *1582*, 295.
24. Jalink, K.; Hengeveld, T.; Mulder, S.; Postma, F. R.; Simon, M. F.; Chap, H.; van der Marel, G. A.; van Boom, J. H.; van Blitterswijk, W. J.; Moolenaar, W. H. *Biochem. J.* **1995**, *307*, 609.
25. Nilsson, U. K.; Andersson, R. G.; Ekeroth, J.; Hallin, E. C.; Konradsson, P.; Lindberg, J.; Svensson, S. P. *Lipids* **2003**, *38*, 1057.
26. Gueguen, G.; Gaige, B.; Grevy, J. M.; Rogalle, P.; Bellan, J.; Wilson, M.; Klaebe, A.; Pont, F.; Simon, M. F.; Chap, H. *Biochemistry* **1999**, *38*, 8440.
27. Simon, M. F.; Chap, H.; Douste-Blazy, L. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1743.
28. Heise, C. E.; Santos, W. L.; Schreihofer, A. M.; Heasley, B. H.; Mukhin, Y. V.; Macdonald, T. L.; Lynch, K. R. *Mol. Pharmacol.* **2001**, *60*, 1173.
29. Liliom, K.; Bittman, R.; Swords, B.; Tigyi, G. *Mol. Pharmacol.* **1996**, *50*, 616.
30. Hooks, S. B.; Ragan, S. P.; Hopper, D. W.; Honemann, C. W.; Durieux, M. E.; Macdonald, T. L.; Lynch, K. R. *Mol. Pharmacol.* **1998**, *53*, 188.
31. Qian, L.; Xu, Y.; Hasegawa, Y.; Aoki, J.; Mills, G. B.; Prestwich, G. D. *J. Med. Chem.* **2003**, *46*, 5575.
32. Bandoh, K.; Aoki, J.; Taira, A.; Tsujimoto, M.; Arai, H.; Inoue, K. *FEBS Lett.* **2000**, *478*, 159.
33. Fischer, D. J.; Nusser, N.; Virag, T.; Yokoyama, K.; Wang, D.; Baker, D. L.; Bautista, D.; Parrill, A. L.; Tigyi, G. *Mol. Pharmacol.* **2001**, *60*, 776.
34. Durgam, G. G.; Virag, T.; Walker, M. D.; Tsukahara, R.; Yasuda, S.; Liliom, K.; van Meeteren, L. A.; Moolenaar, W. H.; Wilke, N.; Siess, W.; Tigyi, G.; Miller, D. D. *J. Med. Chem.* **2005**, *48*, 4919.
35. Lindberg, J.; Ekeroth, J.; Konradsson, P. *J. Org. Chem.* **2002**, *67*, 194.
36. Tamaruya, Y.; Suzuki, M.; Kamura, G.; Kanai, M.; Hama, K.; Shimizu, K.; Aoki, J.; Arai, H.; Shibasaki, M. *Angew Chem. Int. Ed. Engl.* **2004**, *43*, 2834.
37. Characteristic data for some compounds: Compound **8b**: ^{1}H NMR ($CD_3OD/CDCl_3$): δ 5.22 (m, 1H), 3.98–4.04 (m, 2H), 3.56–3.61 (m, 2H), 2.23–2.38 (m, 4H), 1.58 (m, 4H), 1.26 (br s, 16H), 0.84 (t, J = 6.8 Hz, 6H); ^{31}P NMR ($CD_3OD/CDCl_3$): δ 81.1; MS: [M−H]- at *m/z* 439.1. Anal. ($C_{19}H_{37}O_7PS$) calcd: C, 51.80; H, 8.47; S, 7.28. Found: C, 51.95; H, 8.43; S, 7.56. Compound **13b**: ^{1}H NMR (DMSO-d_6): δ 3.78–3.94 (m, 2H), 3.30–3.64 (m, 7H), 1.45 (m, 4H), 1.25 (s, 20H), 0.85 (t, J = 6.6 Hz, 6H); ^{31}P NMR ($CD_3OD/CDCl_3$): δ 80.1; MS: [M−H]- at *m/z* 411.5. Anal. ($C_{19}H_{41}O_5PS$) calcd: C, 55.31; H, 10.02; S, 7.77. Found: C, 55.05; H, 10.02; S, 7.40. Compound **19b**: ^{1}H NMR ($CDCl_3$): δ 7.68 (s, 1H), 7.53 (s, 1H), 4.77 (m, 1H), 4.24 (m, 2H), 3.22 (t, J = 5.6 Hz, 2H), 2.33 (t, J = 6.5 Hz, 2H), 1.61 (m, 2H), 1.50 (m, 2H), 1.28 (br s, 18H), 0.87 (t, J = 6.2 Hz, 6H); ^{31}P NMR ($CDCl_3$): δ 17.8; MS: [M−H]- at *m/z* 421.3. Anal. ($C_{19}H_{39}N_2O_6P$) calcd: C, 54.01; H, 9.30. Found: C, 54.39; H, 9.21.
38. Dillon, D. A.; Chen, X.; Zeimetz, G. M.; Wu, W. I.; Waggoner, D. W.; Dewald, J.; Brindley, D. N.; Carman, G. M. *J. Biol. Chem.* **1997**, *272*, 10361.
39. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
40. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
41. Sardar, V. M.; Bautista, D. L.; Fischer, D. J.; Yokoyama, K.; Nusser, N.; Virag, T.; Wang, D. A.; Baker, D. L.; Tigyi, G.; Parrill, A. L. *Biochim. Biophys. Acta* **2002**, *1582*, 309.
42. Ballesteros, J. A.; Weinstein, H. *Methods in Neurosciences*; Academic Press: San Diego, **1995**; Chapter 19, pp. 366.;
43. Hooks, S. B.; Santos, W. L.; Im, D. S.; Heise, C. E.; Macdonald, T. L.; Lynch, K. R. *J. Biol. Chem.* **2001**, *276*, 4611.
44. Virag, T.; Elrod, D. B.; Liliom, K.; Sardar, V. M.; Parrill, A. L.; Yokoyama, K.; Durgam, G.; Deng, W.; Miller, D. D.; Tigyi, G. *Mol. Pharmacol.* **2003**, *63*, 1032.
45. van Meeteren, L. A.; Ruurs, P.; Christodoulou, E.; Goding, J. W.; Takakusa, H.; Kikuchi, K.; Perrakis, A.; Nagano, T.; Moolenaar, W. H. *J. Biol. Chem.* **2005**.
46. Santos, W. L.; Heasley, B. H.; Jarosz, R.; Carter, K. M.; Lynch, K. R.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3473.

47. Fujiwara, Y.; Sardar, V.; Tokumura, A.; Baker, D. L.; Murakami-Murofushi, K.; Parrill, A. L.; Tigyi, G. *J. Biol. Chem.* **2005**, *280*, 35038.

48. Sugiura, T.; Tokumura, A.; Gregory, L.; Nouchi, T.; Weintraub, S. T.; Hanahan, D. J. *Arch. Biochem. Biophys.* **1994**, *311*, 358.