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A novel series of 2-pyridyl-containing compounds as lysophosphatidic acid receptor antagonists: development of a nonhydrolyzable LPA_3 receptor-selective antagonist

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Abstract—A recently reported dual LPA_1/LPA_3 receptor antagonist (**1**) has been modified so as to modulate the basicity, sterics, and dipole moment of the 2-pyridyl moiety. Additionally, the implications of installing nonhydrolyzable phosphate head group isosteres with regard to antagonist potency and selectivity at LPA receptors is described. This study has resulted in the development of the first nonhydrolyzable and presumably phosphatase-resistant LPA_3-selective antagonist reported to date.

Lysophosphatidic acid (LPA 1- or 2-O-acyl-*sn*-glycero-3-phosphate) is one of several lipid mediators that evokes an array of biological responses by binding to extracellular and intracellular targets.[1] Subtype-selective/tissue-specific LPA receptor blockade has been proposed to have tremendous therapeutic potential.[2] However, the contribution of individual LPA receptors to various disease states is unknown due, in part, to a lack of highly potent and selective antagonist ligands.

Enzymatic hydrolysis of the phosphomonoester polar head group of phospholipid signaling molecules results in functional inactivation or conversion to different mediators. The degradation of LPA may be attributed to the activity of lipid phosphate phosphatases (LPPs), also designated type 2 phosphatidic acid phosphatases (PAP2),[3,4] a family of integral membrane glycoproteins that catalyze the dephosphorylation of a number of bioactive lipid mediators. LPPs exhibit broad specificity; K_m values for substrates such as LPA, phosphatidic acid, and *N*-oleoyl ethanolamine phosphoric acid are in the upper micromolar range for LPP1, 2, and 3.[5] In vitro enzyme assays show that LPPs can also dephosphorylate dual LPA_1/LPA_3 receptor antagonist diacyl-glycerol pyrophosphate (DGPP).[6] LPP overexpression can indeed functionally antagonize LPA signaling. Manipulation of LPP1 levels in fibroblasts suggested that this enzyme exerts a selective negative effect on LPA_1 receptor signaling.[7] Additionally, overexpression of LPP1 has been shown to attenuate the mitogenic response to LPA.[8] Finally, overexpression of each of the LPPs in HEK293 cells resulted in decreases in acute responsiveness of MAP kinase activation by LPA.[9]

The development of metabolically stable LPA receptor agonists as pharmacological tools has received considerable attention in recent years.[8,10] Strategies include replacement of the bridging oxygen of the phosphate group with carbon as well as substitution of sulfur for a bridging or nonbridging oxygen. However, to our knowledge, no phosphatase-resistant LPA receptor antagonists exist currently. It is therefore difficult to employ long-term in vivo models to study the physiological implications of LPA receptor blockade.

We recently reported a high-affinity LPA_1/LPA_3 receptor antagonist containing a 2-pyridyl moiety (**1**, Fig. 1).[11] We have investigated the importance of the Lewis-basicity and dipole moment of the *N*-heterocycle as well

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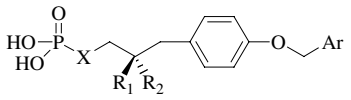


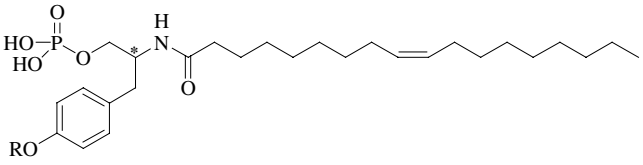
1: R_1 = NHC(O)$C_{17}H_{33}$; R_2 = H; Ar = 2-pyr; X = O
LPA_1: K_i = 18 nM; LPA_3: IC_{50}= 175 nM

Figure 1. Structure of dual LPA_1/LPA_3 antagonist (**1**).

as the steric constraints in this region of the antagonist binding pocket for the LPA receptors of the endothelial differentiation gene (Edg) family. Furthermore, we have elucidated the implications of installing nonhydrolyzable phosphate head group mimetics with regard to antagonist potency and selectivity at LPA receptors. These studies have resulted in a detailed structure–activity relationship (SAR) of LPA receptor antagonists described below.

Synthesis of all the compounds listed in Tables 1 and 2 are described in Schemes 1 and 2. The synthetic route outlined in Scheme 1 has been described in detail elsewhere.[11] In brief, a one-pot procedure accomplished selective acylation of tyrosine methyl ester followed by *O*-silylation to provide **4** in acceptable yield. The phenol **6** was efficiently generated in three steps from **4**. This intermediate was *O*-alkylated by subjecting a series of benzylic alcohols to standard Mitsunobu conditions. The benzylic alcohols were either prepared in a straightforward manner from commercially available methyl esters or carboxylic acids (**7n**–**p**) or the synthetic methods have been described elsewhere (**7e**–**f**,[12] **7g**–**m**[13]). In most cases, the aryl ether Mitsunobu products were inseparable from the by-product tri-*n*-butyl phosphine-oxide by standard silica gel flash chromatography. This mixture was therefore subjected to trifluoracetic acid (TFA) deprotection of the phosphotriester and the pure

Table 1. Inhibitory evaluation of aryl ether derivatives **7a**–**n** as compared with lead compound **1**



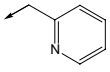
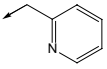
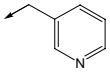
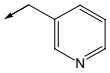
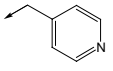
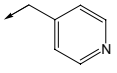
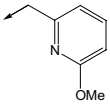
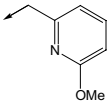
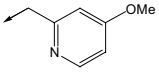
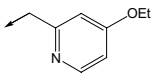
Compds	R	*S*/*R*	LPA_1		LPA_3 IC_{50}, nM
			IC_{50}, nM	K_i	
1[11]		*R*	109	18	175
14[11]		*S*	604	156	940
7a		*R*	992	N/D	1688
7b		*S*	4500	N/D	6236
7c		*R*	735	N/D	2075
7d		*S*	>10,000	N/D	6960
7e		*R*	2840	N/D	455
7f		*S*	>10,000	N/D	1093
7g		*R*	6250	N/D	102
7h		*R*	143	26	39

Table 1 (*continued*)

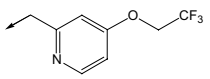
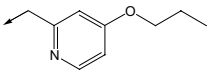
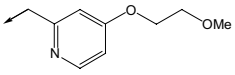
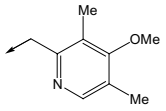
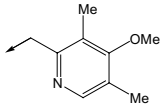
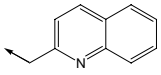
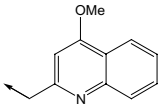
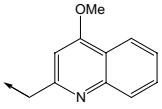
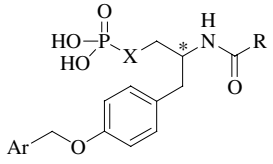
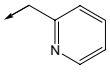
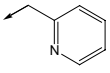
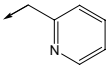
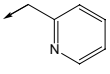
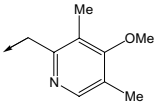
Compds	R	*S*/*R*	LPA_1		LPA_3 IC_{50}, nM
			IC_{50}, nM	K_i	
7i		*R*	84	19	48
7j		*R*	141	64	1660
7k		*R*	114	35	423
7l		*R*	62	28	39
7m		*S*	3690	N/D	561
7n		*R*	1430	N/D	533
7o		*R*	962	N/D	1156
7p		*S*	>10,000	N/D	2600

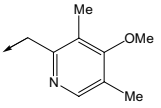
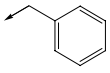
Table 2. Inhibitory evaluation of polar head group derivatives **13a**–**f** as compared with lead compound **1**



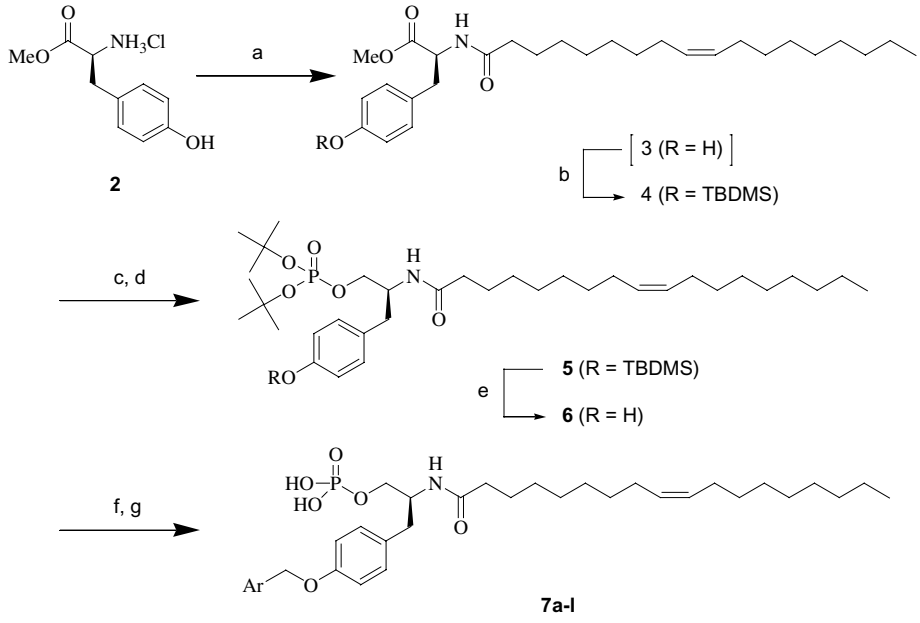
Compds	X	Ar	R	*S*/*R*	LPA_1		LPA_3, IC_{50} nM
					IC_{50}, nM	K_i, nM	
1	–O–		Oleoyl, 17:1[b]	*R*	109	18	175
13a	$–CH_2–$		Oleoyl, 17:1[b]	*R*	>10,000	N/D	>10,000
13b	$–CH_2–$		Oleoyl, 17:1[b]	*S*	>10,000	N/D	>10,000
13c	$–CH_2–$		n-$C_{15}H_{31}$	*S*	>10,000	N/D	1050
13d	$–CH_2–$		Oleoyl, 17:1[b]	*R*	>10,000	N/D	150

(*continued on next page*)

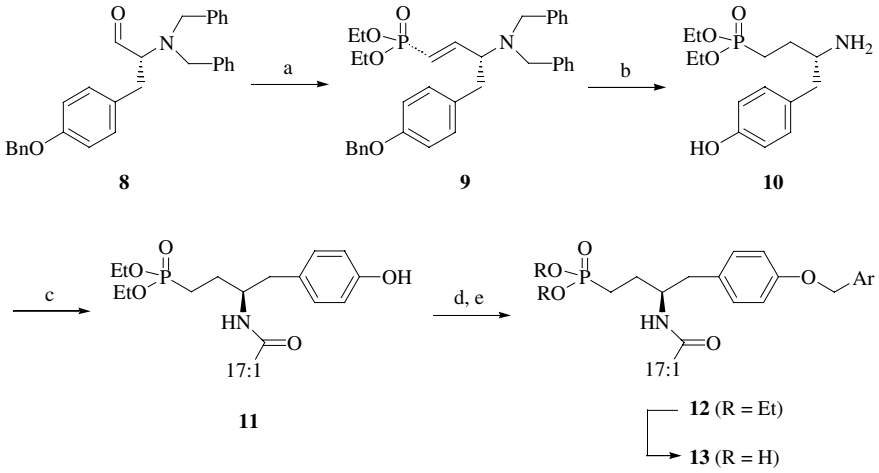
Table 2 (*continued*)

Compds	X	Ar	R	*S*/*R*	LPA_1		LPA_3, IC_{50} nM
					IC_{50}, nM	K_i, nM	
13e	$–CH_2–$		Oleoyl, 17:1[b]	*S*	>10,000	N/D	4000
13f	–CH(OH)–[a]		Oleoyl, 17:1[b]	*S*	4230	N/D	232

[a] To the best of our knowledge, the additional stereocenter (α to P) is racemic. **13f** is therefore evaluated as the unseparated mixture of diastereomers.
[b] 18 carbon chain; *cis* double bond located between C-9 and C-10 from the carbonyl.



Scheme 1. Synthesis of compounds **7a**–**l**. Reagents and conditions: (a) oleoyl chloride, DIEA, CH_2Cl_2, 4 h; (b) TBDMSCl, DIEA, DMAP, 2 h; (c) $NaBH_4$, $CaCl_2$, EtOH–THF (2:1), 18 h, 50% over three steps; (d) di-*tert*-butyldiisopropyl phosphoramidite, tetrazole, 4 h, then 30% H_2O_2, 4 h, 83%; (e) TBAF·$3H_2O$, THF, 1 h, 95%; (f) ROH, PBu_3, DIAD, CH_2Cl_2, 18 h, 73–88%; (g) TFA–CH_2Cl_2 (1:2), 1 h, 80–90% after crystallization from MeOH/Et_2O.



Scheme 2. Synthesis of compounds **13a**–**f**. Reagents and conditions: (a) tetraethyl methylenediphosphonate, *n*-BuLi, −78 to 0 °C, 3 h, 90%; (b) H_2 (100 psi), $Pd(OH)_2$, EtOH, 18 h, 95%; (c) oleoyl chloride, DIEA, CH_2Cl_2, 4 h, 65%; (d) ROH, PBu_3, DIAD, CH_2Cl_2, 18 h, 73–88%; (e) TMSBr, CH_2Cl_2, 4 h, then 95% MeOH, 1 h, 80–90% after crystallization from MeOH/Et_2O.

phosphoric acids were obtained by recrystallization from methanol/diethyl ether.

α-Methylene phosphonates **13a**–**e** were realized from the *N*,*N*-dibenzylamino aldehyde **8**, which was easily prepared from tyrosine following the procedure reported by Reetz et al.[14] Next, treatment of tetraethyl methylenediphosphonate with *n*-butyllithium at −78 °C generated the lithiated carbanion, which condensed with **8** to afford the vinylphosphonate **9** in good yield.[15] Catalytic dual-hydrogenation/hydrogenolysis using Pearlmann's catalyst provided the amino-phenol **10**, which was converted to the phosphonodiester **12** using the above-mentioned procedures. Finally, transesterification of each ester **12** using bromotrimethylsilane (TMSBr) and subsequent desilylation with aqueous methanol provided the pure α-methylene phosphonates **13a**–**e** after crystallization. The detailed synthesis of the diastereomeric α-hydroxy phosphonate **13f** will be reported in a manuscript which is in preparation.

All compounds were characterized by ^{1}H and ^{13}C NMR, mass spectroscopy and, in most cases, C, H, N analyses.[16] A membrane-based GTP[$\gamma^{35}S$] binding assay was adapted to assess in vitro activity at the LPA/Edg receptors.[11,17] As in the case of **1**, all compounds presented herein are devoid of any significant activity at the LPA_2 receptor at concentrations below 30 μM. Antagonist potency data for 2-pyridyl aryl analogues of **1** (**7a**–**p**) are presented in Table 1 in a manner analogous to previously published LPA receptor antagonist data.[11,18]

It is evident that the serendipitously discovered[11] 2-pyridyl regioisomer in the *R* configuration exhibits superior dual antagonism (**1** as compared with **7a**–**d**). Additionally, the net dipole moment of the aromatic system may play a role in LPA receptor antagonist binding, as compounds **7e**, **7g**, and **7i** exhibit varying inhibitory potencies at each receptor. Lewis-basicity of the *N*-heterocycle moiety could also potentially account for this phenomenon. Electron-rich compounds **7g**–**i**, **l** are more potent at the LPA_3 receptor than the unsubstituted lead compound **1** (Fig. 2). Indeed, the 4-methoxy-3,5-dimethyl-pyridine system of **7l** has been reported to exhibit basicity approximately one pK_a unit greater than that of pyridine[13,19] (pK_a of pyridine in water 5.17[20]). Of this series, compound **7g** (1.71 pK_a units higher than pyridine in acetonitrile[21]) is particularly potent and LPA_3-selective. Alternatively, for compounds **7j**–**k**, increased steric hindrance of the 4-alkoxy pyridyl substituent improves selectivity at the LPA_1 receptor. *n*-Propyloxy derivative **7j** displays ~10-fold LPA_1 to LPA_3 receptor selectivity. The quinoline series **7n**–**p** exhibits a marked dual reduction in potency compared to **1** and therefore may be too sterically demanding for either antagonist binding pocket. Notably, trifluoroethoxy derivative **7i** is equipotent to **1** at the LPA_1 receptor and ~threefold more potent at LPA_3. Indeed, this compound is the most potent LPA_1/LPA_3 dual antagonist reported to date. Thus, we have shown that by altering the sterics and electronics of the outermost aromatic system, highly potent and somewhat selective phosphate-bearing antagonists of LPA receptors may be realized.

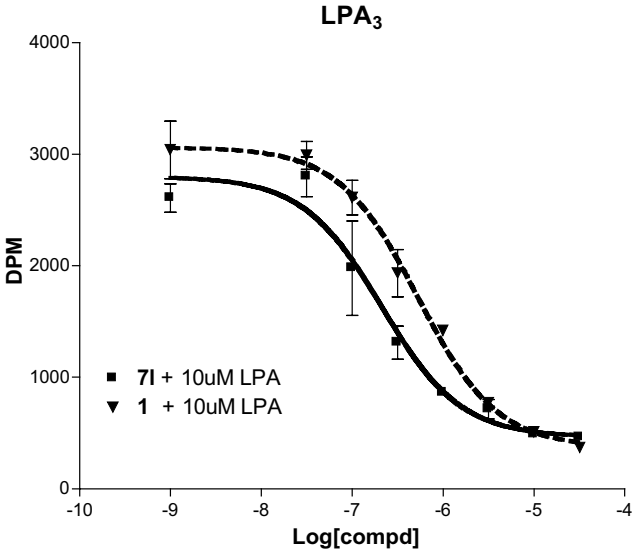


Figure 2. Effect of aryl ether analogues on LPA_3 receptor GTP[$\gamma^{35}S$] binding.[11,17]

Antagonist potency data for phosphonate derivatives **13a**–**f** are presented in Table 2. α-Methylene phosphonate compounds **13a**–**e** do not retain the inhibitory activity of their phosphate analogues at the LPA_1 receptor (Table 2). Hence, by applying this strategy for selectivity to compound **7l**, a truly subtype-selective LPA_3 receptor antagonist was developed (**13d**). Compound **13d** shows improved potency over lead compound **1** at the LPA_3 receptor (Fig. 3) and is the first nonhydrolyzable and presumably phosphatase-resistant LPA antagonist reported to date. Compound **13d** displays no antagonism at the LPA_1 receptor (Fig. 4). Only **13f**, an α-hydroxy phosphonate derivative of an early lead antagonist, retained modest inhibitory activity at the LPA_1 receptor. The divergent properties of phosphonic acid antagonist head group analogues (**13a**–**e**) at

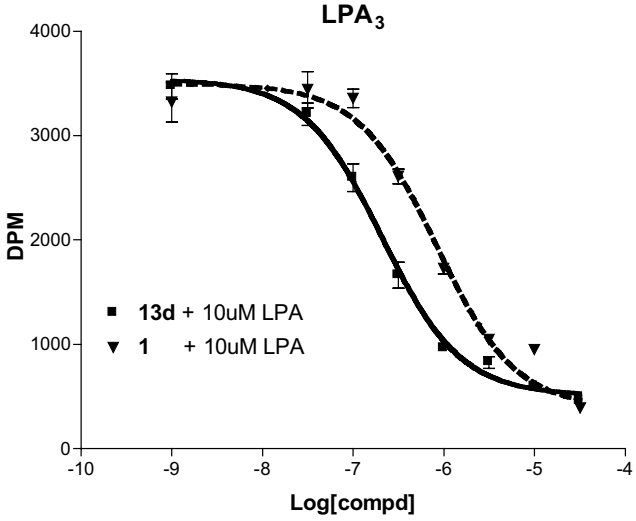


Figure 3. Effect of LPA antagonists on LPA_3 receptor GTP[$\gamma^{35}S$] binding.[11,17] A comparison of α-methylene phosphonate **13d** with lead antagonist **1**.

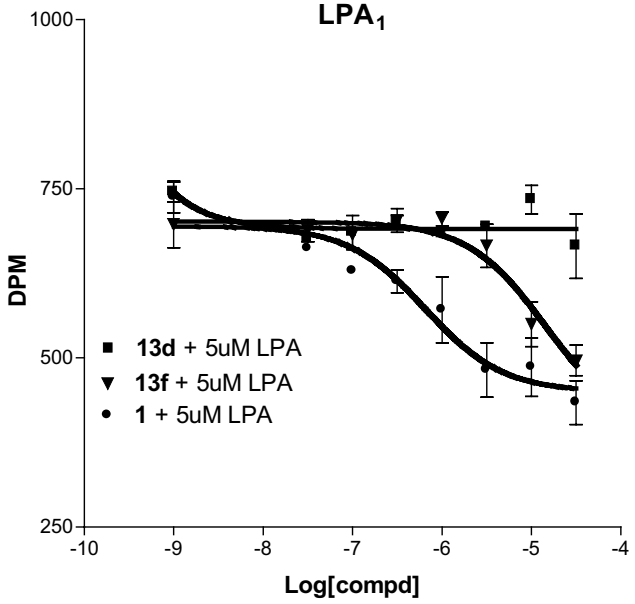


Figure 4. Effect of LPA antagonists on LPA_1 receptor GTP[$\gamma^{35}S$] binding.[11,17] A comparison of **13d** and α-hydroxy phosphonate **13f** with lead antagonist **1**.

LPA_1 may be due to a discrepancy between the second pK_a value of unsubstituted phosphonic acids versus the relatively more acidic phosphoric acids.[22–24] Hence, the substitution of an electronegative heteroatom onto the methylene group α to phosphorous (**13f**) increases acidity and regains LPA_1 antagonism. α-Substituted phosphonates (Fig. 1, 'X' = CH(OH or F)) will therefore provide a useful platform for the realization of metabolically stable LPA_1/LPA_3 dual antagonists. Progress toward this end will be reported in due course.

In summary, a detailed SAR of LPA receptor antagonists has been described. A nonhydrolyzable and highly potent LPA_3 receptor-selective antagonist has been developed. Compound **13d** represents a pharmacologic agent with which to manipulate LPA signaling in a number of experimental systems so as to define the LPA_3 receptor-specific pathophysiological responses to LPA stimulation in intact animals.

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16. (*R*)-Phosphoric acid mono-{2-octadec-9-enoylamino-3-[4-(pyridin-3-ylmethoxy)-phenyl]-propyl} ester (**7a**): ^1H NMR (300 MHz, CD_3OD) δ 0.83 (t, 3H, $J = 6.9$ Hz), 1.14–1.29 (m, 11H), 1.39–1.49 (m, 2H), 1.90–1.99 (m, 4H), 2.07 (t, 2H, $J = 7.7$ Hz), 2.66 (dd, 1H, $J = 8.5$, 14.2 Hz), 2.85 (dd, 1H, $J = 8.5$, 14.2 Hz), 3.82–3.90 (m, 2H), 4.14–4.23 (m, 1H), 5.23–5.29 (m, 4H), 6.92 (d, 2H, $J = 8.5$ Hz), 7.16 (d, 2H, $J = 8.5$ Hz), 7.99 (t, 1H, $J = 6.5$ Hz), 8.57 (d, 1H, $J = 8.1$ Hz), 8.74 (d, 1H, $J = 5.8$ Hz), 8.88 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 13.51, 21.27, 22.78, 26.05, 27.19, 29.09, 29.25, 29.40, 29.48, 29.66, 29.89, 32.10, 36.00, 36.18, 51.32, 66.15, 67.06, 114.79, 114.96, 127.12, 129.83, 129.91, 130.67, 131.81, 138.61, 141.29, 142.04, 144.28, 157.01, 175.11; MS (ESI) m/z 603.3 [$M+H^+$, 100%]. Anal. Calcd for $C_{33}H_{51}N_2O_6P$: C, 65.76; H, 8.53; N, 4.65. Found: C, 64.63; H, 8.53; N, 4.58. (*S*)-Phosphoric acid mono-{2-octadec-9-enoylamino-3-[4-(pyridin-3-ylmethoxy)-phenyl]-propyl} ester (**7b**): this product had spectroscopic properties identical to its enantiomer.
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18. DNAs encoding LPA_1, LPA_2, or LPA_3 receptors were transfected into HEK293T cells along with DNAs encoding $G\alpha_{i2}$, Gβ, and $G\gamma_2$ proteins. After 48 h, microsomes were prepared and used in a GTPγ[^{35}S] binding assay, as described.[14] For determination of IC_{50} values, a concentration of 1-oleoyl-LPA equal to the EC_{80} value (LPA_1: 1 μM; LPA_3: 10 μM) was collided with antagonists at concentrations ranging from 0.1 to 30,000 nM. Points are in triplicate and are representative of at least two experiments.
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