Synthesis and biological evaluation of phosphonic and thiophosphoric acid derivatives of lysophosphatidic acid

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Abstract—Using an N-oleyl ethanolamide scaffold, a series of phosphate polar head group analogues of LPA comprised of various \( \alpha \)-substituted phosphonates and thiophosphates was prepared. In a broken cell GTP\(^{35}S \) binding assay, agonist activity was evaluated at the three LPA receptors of the endothelial differentiation gene (Edg) family. This study has resulted in the discovery of a nonhydrolyzable LPA \(_1\)-selective agonist (11). Additionally, thiophosphate 19 bears an isosteric phosphate mimetic that confers agonism at the LPA \(_1\) receptor but not LPA \(_2\).

Lysophosphatidic acid (LPA, 1- or 2-O-acyl-sn-glycero-3-phosphate) is an intercellular signaling phospholipid that induces an array of receptor-mediated biological effects. However, lipid phosphate phosphatase (LPP)-catalyzed hydrolysis of the phosphomonoester polar head group of LPA results in functional inactivation at LPA receptors. In recent years, progress has been made toward the development of phosphatase-resistant pharmacologic agents that modulate LPA signaling. The metabolically stabilized LPA analogue, 1-oleoyl-2S-O-methyl-glycerophosphothioate (2S-OMPT) is a potent and selective agonist for the LPA \(_3\) receptor. Further development of nonhydrolyzable molecular entities such as this may provide the necessary tools to define the receptor specific pathophysiological responses to LPA stimulation in intact animals (Fig. 1).

Replacement of the bridging oxygen of the phosphate group with carbon renders phosphonates hydrolytically stable. Not only does a phosphonate possess the same spatial distribution of functionality as a phosphate but it also allows similar conformations to be accessed. However, the strong electronegative property of the oxygen clearly affects the electronic attributes of a phosphate group. As a result, the second phosphate pK\(_a\) value is 1-log order more acidic than the corresponding \( \alpha \)-methylene phosphonate (6.4 compared to 7.6). This discrepancy may strongly affect receptor–ligand binding interactions wherein charge density on the polar head group is favorable. Electronegative substituents on the \( \alpha \)-carbon will inductively influence charge density thereby increasing acidity. Indeed, the introduction of \( \alpha, \alpha \)-difluoro decreases the second phosphonate pK\(_a\) by as much as 2-log orders. Alternatively, the substitution of sulfur for one of the nonbridging oxygen atoms of a phosphate yields the metabolically stable

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phosphothioate, which exhibits different steric and geometric properties as well as charge distribution in the respective dianions.\(^6\) Thiol phosphoric acids, in which the bridging oxygen of the phosphate is replaced by sulfur, have been explored to a lesser extent as biologically active thio-analogues. This isostere is similarly charged and has the same hydrogen-bonding capabilities as the phosphate ester, except for the bridging atom.

To explore the potential of phosphonates and thio-phosphates as potential nonhydrolyzable analogues of LPA, we now report the synthesis and biological evaluation of a series of phosphate-mimetic derivatives of the known LPA receptor agonist N-acyl ethanolamide phosphoric acid (NAEPA, 1).\(^7\)

Syntheses of all compounds listed in Table 1 except 20\(^8\) are described in Schemes 1–3. As outlined in Scheme 1, phosphite anion condensed with acrylonitrile in Micheal fashion to provide the resulting nitrile in good yield. Cobalt(II) chloride-catalyzed sodium borohydride reduction afforded the amine 3, which underwent routine acylation followed by transesterification of the phosphonodiester using bromotrimethylsilane (TMSBr) and subsequent desilylation with aqueous methanol to give \(\alpha\)-methylene phosphonate 5.

As outlined in Scheme 2, condensation of propanolamine 6 with oleyl chloride followed by pyridinium chlorochromate (PCC) oxidation generated the aldehyde 7. Phosphate addition to 7 afforded the racemic-hydroxy phosphonodiester 8, which is converted to the phosphonic acid by treatment with trifluoroacetic acid (TFA). Additionally, 8 is a common intermediate used to generate the \(\alpha\)-keto and \(\alpha\)-fluoro derivatives, 11 and 14, respectively. PCC oxidation of 8 followed by TFA deprotection afforded \(\alpha\)-keto phosphonate 11. For the latter, reaction of 8 with (diethylamino)sulfur trifluoride (DAST) at ice-bath temperature provided the racemic \(\alpha\)-fluorophosphonodiester in modest yield. Subsequent TFA deprotection gave 14.

As outlined in Scheme 3, two thio-analogues were also prepared. Either ethanolamine or 2-aminoethane thiol condensed selectively with oleyl chloride to generate 15. Phosphitylation of the alcohol 15 followed by in situ sulfurization with elemental sulfur provided the phosphothioate triester 16 in good yield. Notably, TFA deprotection in this case required the addition of at least 20 equiv of the scavenger reagent triethylsilane so as to prevent intra- or intermolecular migration of the intermediate tert-butyl cation onto sulfur. An analogous alkyl migration has been noted elsewhere.\(^9\) Removal of volatiles under reduced pressure afforded the pure phosphothioate 17. The thiol phosphoric triester 18 was prepared by an Arbuzov-type redox reaction of trimethylphosphate with the thiol 15 in the presence of

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Table 1. Agonist activities of LPA analogues at individual LPA receptors using GTP\(^{[35]S}\) binding assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Y</th>
<th>LPA(_1)</th>
<th>LPA(_2)</th>
<th>LPA(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC(_{50}) (nM)</td>
<td>(E_{max})</td>
<td>EC(_{50}) (nM)</td>
</tr>
<tr>
<td>LPA</td>
<td>NA</td>
<td>NA</td>
<td>17</td>
<td>100</td>
<td>6.8</td>
</tr>
<tr>
<td>1</td>
<td>-O-</td>
<td>-PO(_2)H(_2)</td>
<td>197</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>-CH(_2)-</td>
<td>-PO(_2)H(_2)</td>
<td>1950</td>
<td>50</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>9</td>
<td>-CH(OH)-</td>
<td>-PO(_2)H(_2)</td>
<td>1225</td>
<td>80</td>
<td>2066</td>
</tr>
<tr>
<td>11</td>
<td>-C(O)-</td>
<td>-PO(_2)H(_2)</td>
<td>221</td>
<td>80</td>
<td>1089</td>
</tr>
<tr>
<td>14</td>
<td>-CH(F)-</td>
<td>-PO(_2)H(_2)</td>
<td>&gt;5000</td>
<td>NA</td>
<td>1960</td>
</tr>
<tr>
<td>20</td>
<td>-C(OH)-</td>
<td>-PO(_2)H(_2)</td>
<td>&gt;5000</td>
<td>NA</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>17</td>
<td>-O-</td>
<td>-P(S)O(_2)H(_2)</td>
<td>40</td>
<td>80</td>
<td>108</td>
</tr>
<tr>
<td>19</td>
<td>-S-</td>
<td>-PO(_2)H(_2)</td>
<td>318</td>
<td>50</td>
<td>&gt;5000</td>
</tr>
</tbody>
</table>

\(E_{max}\) = maximal efficacy of drug/maximal efficacy of 1-oleyl-LPA; expressed as the percentage. NA, not applicable.
TeCl₄ and 2,6-lutidine according to the method of Watanbe et al.¹⁰ TMSBr deprotection afforded the thiol phosphonic acid 19.

All compounds were characterized by ¹H and ¹³C NMR and mass spectroscopy.¹¹ A membrane-based GTP[³⁵S] binding assay was adapted to assess in vitro activity at the LPA₁, LPA₂, and LPA₃ receptors.¹² It has been demonstrated that NAEPA (1) is an excellent LPA mimic, indistinguishable from LPA in aggregating human platelets and mobilizing calcium in mammalian cells.¹³,¹⁴ However, this activity is primarily elicited by activation of the LPA₁ and LPA₂ receptors, but not LPA₃, where potency is markedly diminished.⁷ We conserved the ethanolamide scaffold of 1 and replaced the phosphate moiety with a series of phosphate isosteres. The resulting GTP[³⁵S] binding data for all compounds are presented in Table 1.

Compared to NAEPA (1), α-methylene phosphonate 5 is less active by 1-log order at the LPA₁ receptor and inactive at LPA₂ and LPA₃. To modulate the second pKa value and thus more effectively mimic the electronics of the phosphate, electron-withdrawing heteroatom substituents were introduced on the α-carbon of phosphono-NAEPA derivatives 9, 11, 14, and 20. The phosphonate rank order potency at the LPA₁ receptor is α-keto > α-hydroxy > α-methylene > α-fluoro. Given the improved agonism of compounds 9 and 11, electron density and pKₐ of the polar head group does seem to impact activity at LPA receptors. However, the relative inactivity of α-fluoro phosphonate 14 is not consistent with this hypothesis; this moiety has been reported to most closely approximate the electronics, acidity, and conformation of phosphate,⁸ but results in a dramatic loss of LPA receptor activation. This unexpected result remains unresolved and will require additional studies. Notably, 14 is fully efficacious at the LPA₂ receptor albeit at a relatively high concentration. α-Hydroxy bisphosphonate 20 is inactive at all three receptors, perhaps due to steric and/or electrostatic factors. Thus, α-keto phosphonate 11 displays potency and efficacy at the LPA₁ receptor comparable to that of lead compound NAEPA. However, 11 is greater than 1-log order less potent than NAEPA at LPA₂ and therefore represents a nonhydrolyzable LPA₁-selective agonist.

Two thiophosphate analogues were analyzed for agonist activity at LPA receptors. Phosphothioate 17 displayed agonist potency comparable to that of lead compound NAEPA. Maximal efficacy of this derivative, however, is diminished by twofold at the LPA₂ receptor, compared to LPA or NAEPA. Hence 17 is a phosphatase-resistant dual LPA agonist, displaying EC₅₀ₙₜ at LPA₁, and LPA₂ of 40 and 108 nM, respectively. Additionally, thiol phosphonic acid 19, unlike dual-agonist NAEPA, is an LPA₁-selective agonist of relatively good potency and efficacy. This selectivity is presumably due to a lack of compatibility between this phosphate isostere, perhaps due to steric or geometry, and the LPA₂ receptor binding pocket. At least one group has reported on the chemical properties and enzymatic transformations of the phosphorylated thiol (Fig. 2).¹⁵

In summary, a series of polar head group analogues of LPA have been prepared and evaluated at three individual LPA receptors. This study has resulted in the discovery of a nonhydrolyzable LPA₁-selective agonist bearing an α-keto phosphonate head group as the phosphate mimic. The resistance of this new pharmacologic agent to phosphatase-catalyzed degradation renders 11 a useful tool to elucidate the functions of the LPA₁ receptor in vivo models. Additionally, thiol phosphonic acid 19 is a relatively potent and efficacious agonist at LPA₁ whose head group is not tolerated at the LPA₂ receptor. Finally, a phosphothioate analogue (17) of NAEPA is highly potent at the LPA₁ and LPA₂ receptors. The lack of activity at LPA₃ of the head group isosteres presented here is not surprising given the

Scheme 3. Synthesis of thiophosphoric acids 17, 19. Reagents and conditions: (a) di-tert-butyl diisopropyl phosphoramidite, tetrazole; (ii) sulfur, reflux, 86%; (b) trimethyl phosphite, TeCl₄ and 2,6-lutidine according to the method of Watanbe et al.¹⁰ TMSBr deprotection afforded the thiol phosphoric acid 19.

Figure 2. Agonist-induced GTP[³⁵S] binding¹¹ at the LPA₁ receptor. Points are in triplicate and are representative of at least two experiments.
inactivity of lead compound NAEPa itself at this receptor. Studies to determine binding affinity at additional LPA receptors are ongoing and will be published in due course.

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References and notes

8. In brief, Pamidronate (phosphoric acid (3-amino-1-hydroxypropylidene)bisdisodium salt) was condensed with oleoyl chloride in 2 M aqueous K2CO3. This afforded a mixture of mono- and di-acylated bisphosphonates, which were exposed to a solution of 1:1 2 M LiOH–THF. Upon addition of methanol to the reaction mixture, a precipitate formed which was isolated by filtration and washed with excess methanol. The white solid 20 was judged as pure by 1H and 13C NMR and mass spectroscopy analysis.

11. Thiophosphoric acid 2-((Z)-octadec-9-enoylamino)-ethyl ester (17): 1H NMR (300 MHz, CDCl3) δ 0.87 (t, 3H, J = 6.7 Hz), 1.22–1.39 (m, 11H), 1.60–1.70 (m, 2H), 1.95–2.02 (m, 4H), 2.33–2.46 (m, 2H), 3.54–3.64 (m, 2H), 4.08–4.19 (m, 2H), 5.34 (t, 2H, J = 6.0 Hz); 13C NMR (300 MHz, CDCl3) 14.13, 22.67, 25.88, 27.24, 29.16, 29.32, 29.54, 29.78, 31.92, 32.66, 36.03, 40.41, 40.81, 50.80, 65.14, 129.70, 130.02, 177.34; MS (ESI) m/z 419.8 [M–H]+, 100%.