Synthesis and Evaluation of Serinolamide Derivatives as Sphingosine-1-Phosphate-1 (S1P₁) Receptor Agonists

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Abstract: Sphingosine-1-phosphate-1 (S1P₁) receptor agonists are well-known drugs for treating multiple sclerosis (MS) caused by autoreactive lymphocytes that attack the myelin sheath. Therefore, an effective therapeutic strategy is to reduce the lymphocytes in the blood by inducing S1P₁ receptor internalization. We synthesized serinolamide A, a natural product of the sea, and performed S1P₁ receptor internalization assay to evaluate functionally antagonistic S1P₁ receptor agonist activity. In order to synthesize derivatives with better efficacy than serinolamide A and B, new derivatives were synthesized by introducing the phenyl ring moiety of fingolimod. Among them, compounds 19 and 21 had superior S1P₁ agonistic effects to serinolamide. We also confirmed that compound 19 effectively inhibited lymphocyte outflow in peripheral lymphocyte count (PLC) assay.

Keywords: serinolamide A; S1P₁ receptor; GPCR; multiple sclerosis; internalization

1. Introduction

Multiple sclerosis (MS) is a neuroinflammatory autoimmune disease [1]. Of the different types, relapsing–remitting multiple sclerosis (RRMS) is the most common disease course [2,3]. Multiple sclerosis is caused when autoreactive T cells migrate across the blood–brain barrier (BBB) and damage the myelin sheath in the central nervous system, leading to neurodegeneration and demyelination [4]. Sphingosine-1-phosphate (S1P) receptors are a class of G protein-coupled receptors (GPCR) with five subtypes, S1P₁–S5. Among them, sphingosine-1-phosphate-1 (S1P₁) plays a role in regulating the egress of lymphocytes from lymphoid tissue to the lymph [5]. Studies have shown that the S1P₁ receptor is internalized and degraded by functional antagonists, prompting lymphocyte sequestration in the lymph node and immunosuppression [6–8]. Therefore, developing functionally antagonistic S1P₁ receptor agonists is an effective strategy for overcoming autoimmune diseases. Natural marine products serinolamide A and serinolamide B have long lipophilic chains and polar substituents such as the well-known S1P₁ receptor agonist fingolimod (FTY720, Gilenya®), as shown in Figure 1. Serinolamide A synthesis methods have been reported in multiple papers [9–12]. In this study, we partially optimized the existing synthesis method for serinolamides A and B. Serinolamide derivatives were also synthesized by introducing the phenyl moiety of FTY720. S1P₁ receptor agonists bind to the S1P₁ receptor, which induces S1P₁ receptor internalization and, consequently, induces...
receptor degradation [5–8]. Therefore, synthesized compounds were evaluated as functional antagonists that effectively degrade S1P1 receptors by performing S1P1 receptor internalization analysis.

![Figure 1. Structures of fingolimod (a), serinolamide A (b), and serinolamide B (c).](image)

2. Results and Discussion

2.1. Chemical Synthesis of Serinolamide Derivatives

The synthesis of serinolamide A followed the procedures reported by Pandey [10] and Wang [11]. The amine part was synthesized following the amide coupling method reported by Wang. The O-methylation of commercially available methyl (tert-butoxycarbonyl)-L-serinate with commercially available methyl iodide yielded methyl N-(tert-butoxycarbonyl)-O-methyl-L-serinate 22. The reduction of the methyl ester with sodium borohydride yielded an alcohol derivative 23, and TBDMS protection of 23 with butyldimethylsilyl chloride (TBDMSCl) yielded 24. The methylation of 24 with commercially available methyl iodide yielded tert-butyl (S)-1-((tert-butyldimethylsilyl)oxy)-3-methoxypropan-2-yl)(methyl)carbamate 25. Boc and TBDMS deprotection of 25 with 4 M HCl yielded 26; Boc deprotection of 23 with 4 M HCl yielded 27 (Scheme S1 in the Supporting Information). The carboxylic-acid-containing counterpart was introduced following the metathesis method using the Grubbs catalyst reported by Pandey (Scheme 1) [10].

The olefin metathesis reaction of commercially available pentadec-1-ene with commercially available pent-4-enoic acid yielded (E)-octadec-4-enoic acid 3. Amide coupling of secondary amine derivative 26 and primary amine derivative 27 with the carboxylic acid derivative 3 yielded serinolamide A and serinolamide B (Scheme 2) [12]. Amide coupling of 3 with commercially available serinol yielded 4. The olefin metathesis reaction of commercially available pentadec-1-ene with commercially available pent-4-enal yielded (E)-octadec-4-enal (5). The reductive amination of 5 with commercially available serinol yielded 6 (Scheme 2).
Scheme 1. Synthesis routes for serinolamides A and B.

Scheme 2. Synthesis of 1, 2, 4, 6.
2.2. Chemical Synthesis of Fingolimod Analogues

The nucleophilic substitution reaction of commercially available 1-(bromomethyl)-4-nitrobenzene with commercially available diethyl 2-acetamidomalonate yielded diethyl 2-acetamido-2-(4-nitrobenzyl)malonate 7. The reduction of the nitro group with iron yielded an amine derivative 8, and amide coupling of 8 with carboxylic acid derivative 3 yielded 9. Reducing the ethyl ester derivative 9 with lithium aluminum hydride (LAH) yielded compounds 10–12, and the hydrolysis of 12 with sodium hydroxide yielded an undesired product (Scheme 3). The reductive amination of 8 with aldehyde derivative 5 yielded 13.

![Scheme 3. Synthesis of 10–13.](image)


![Scheme 4. Synthesis of 19–21.](image)
2.3. Evaluation of the Synthesized Serinolamide Derivatives as S1P1 Receptor Agonists

The ability of the compounds to internalize the S1P1 receptor from the cell surface was evaluated using a commercially available in vitro assay system to test the functionally antagonistic S1P1 receptor agonist activity of the synthesized serinolamide derivatives [13–15]. The efficacy of the synthetic compounds was expressed as a percentage of maximal efficacy at 1 μM of FTY720, a highly potent S1P1 agonist. In the S1P1 receptor internalization assay, compounds 12, 19, and 21 showed more than 80% efficacies at 30 μM. Notably, compound 19 showed good efficacy of 147%. In addition, compounds 4, 6, and 13 showed lower S1P1 receptor internalization efficacies than other derivatives (Table 1). The efficacy of compounds 19 and 21, in which a phenyl ring was introduced, was significantly improved compared to those of compounds 4 and 6, in which a phenyl ring was not introduced.

Table 1. Effects of Serinolamide A derivatives on ligand binding to G protein-coupled receptor.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S1P1 Receptor Internalization</th>
<th>30 μM (% Efficacy)</th>
<th>20 μM (% Efficacy)</th>
<th>10 μM (% Efficacy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Serinolamide A)</td>
<td></td>
<td>60.4</td>
<td>36.2</td>
<td>30.1</td>
</tr>
<tr>
<td>2 (Serinolamide B)</td>
<td></td>
<td>53.5</td>
<td>46.4</td>
<td>31.7</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>27.8</td>
<td>22.5</td>
<td>15.8</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0.98</td>
<td>13.7</td>
<td>nd</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>44.4</td>
<td>40.1</td>
<td>nd</td>
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<tr>
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<td></td>
<td>84.4</td>
<td>77.8</td>
<td>43.1</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>22.2</td>
<td>16.7</td>
<td>nd</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>147</td>
<td>107.3</td>
<td>37.8</td>
</tr>
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<td>21</td>
<td></td>
<td>94.2</td>
<td>97.5</td>
<td>34.2</td>
</tr>
</tbody>
</table>

1 The activity of S1P1 receptor internalization was determined based on HEK293-S1P1 expressing cells. 2 % efficacy was calculated compared to 1 μM of FTY720-phosphate, the active pharmacological species. 3 nd = not determined.

2.4. In Vivo Reduction of Peripheral Blood Lymphocyte Count by Treatment of Compound 19 and 21 in Mice

S1P1 agonists, such as fingolimod, are known to induce peripheral lymphopenia by inhibiting S1P1-mediated lymphocyte outflow from lymphoid tissues. Lymphopenia caused by these drugs contributes to the therapeutic effect of autoimmune diseases such as multiple sclerosis [6,16]. Therefore, we investigated the effect of compounds to induce lymphopenia in blood by peripheral lymphocyte count (PLC) analysis (Figure 2). After intravenous administration of compounds 19, 21 (15 mg/kg, 30 mg/kg, maximum solubility concentration) and fingolimod (3 mg/kg) to mice, blood samples were collected by orbital bleed. At this time, there was no visual change compared to the vehicle treatment group containing the same amount of DMSO. As a result of measuring the number of lymphocytes in the blood, the number of lymphocytes started to decrease within 2.5 h after administration. In particular, the number of lymphocytes in the blood of mice administered 19 was significantly decreased during the first 5 h. In contrast to fingolimod, mice treated with 19 and 21 began to recover lymphocyte counts after 5 h. As a result of a single dose administration, peripheral lymphocyte counts in fingolimod-treated mice continued to decrease for 24 h post-dose. In contrast, lymphocyte counts in mice treated with compound 19 or 21 returned to near baseline levels, suggesting that the cardiac toxicity of
fingolimod due to its long-term efficacy on lymphocyte reduction can be overcome (Figure 2) [17]. Collectively, these results suggest that administration of 19 and 21 can inhibit the egress of lymphocytes from lymphoid tissues to peripheral blood, and that lymphopenia can be reversed within 24 h.

![Figure 2](image)

**Figure 2.** Reduction of the blood lymphocyte count by the treatment of 19 and 21 in mice. Mice were intravenously administered with the vehicle (n = 8), 19 (15 mg/kg, n = 7), 21 (30 mg/kg, n = 10), or positive control fingolimod (3 mg/kg, n = 8). Blood lymphocyte counts were measured before (0 h) and after the administration (2.5 and 5, 24 h). Percentage of the lymphocyte count at the time before administration (0 h) was considered as a baseline (100%). * p < 0.05, *** p < 0.001, **** p < 0.0001 compared to vehicle-treated group (one-way ANOVA with Dunnett’s test). Data are presented as mean ± SEM.

3. Experimental Section

3.1. General Methods

All chemicals, reagents, and solvents were obtained from commercially available sources as reagent grades without further purification. The yields reported are for purified products and were not optimized. Synthesized compounds were checked by thin-layer chromatography (TLC) and 1H and 13C nuclear magnetic resonance (NMR), melting point (MP), high-resolution mass spectrometry (HRMS), and high-performance liquid chromatography (HPLC) analyses. Analytical thin-layer chromatography plates monitored reactions (Merck, Cat No. 1.05715, Darmstadt, Germany) and analyzed by ultraviolet light at 254 nm and 280 nm. The reactions were purified by MPLC (Biotage®, Isolera™ one, Uppsala, Sweden). The NMR spectra were recorded at 400 MHz (1H)/100 MHz (13C) using Bruker spectrometers (Billerica, USA.). Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane (TMS). HPLC analysis was performed using a Waters E2695 system (Milford, USA.) equipped with a YMC-Triart C18 /S-5 μm /12 nm/ Lot No. 17452 (150 mm × 4.6 mm diameter). The HPLC data were recorded using the following parameters: DW (0.1% AcOH)/acetonitrile. Method A: 10/90 → 100/0 in 15 min, +5 min isocratic, flow rate of 0.5 mL/min to 1.0 mL/min, λ = 254 and 280 nm. HRMS was performed with electrospray ionization on a Q-Exactive (Thermo Fisher Scientific, Waltham, USA.) instrument. Specific rotation was measured with the autopol® III polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA).

3.2. General Procedure for Amide Coupling Reaction (Method A)

A mixture of carboxylic acid derivatives, EDC, HOBt, and DIPEA, was dissolved in dichloromethane ([C] ~ 0.1 M) and stirred for 20 min at room temperature. Amine derivatives were added into the reaction mixture and stirred overnight to afford serinolamide A. The reaction mixture was diluted with distilled water and extracted with ethyl acetate. The combined organic layer was dried with Na2SO4 and evaporated in vacuo. The obtained residue was purified by column chromatography on SiO2.
3.3. General Procedure for Boc Deprotection and Deacetylation Reaction (Method B)

To a mixture of NHBoc derivatives in dichloromethane or ethanol ([C] ~ 0.1 M), 4 M HCl was added, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was evaporated in vacuo.

3.4. General Procedure for the Reductive Amination with Aldehyde (Method C)

A mixture of aldehyde derivatives in methanol and tetrahydrofuran in a ratio of 1:1 ([C] ~ 0.1 M) was added a mixture of amine derivatives with triethylamine. The resulting suspension was stirred at room temperature (0.5 h). Then, sodium cyanoborohydride was added and stirred at room temperature. The reaction mixture was evaporated in vacuo. The product residue was washed with ethyl acetate and distilled water. The combined organic layer was dried with anhydrous NaSO4 and evaporated in vacuo. The obtained residue was purified by column chromatography on SiO2.

3.4.1. Synthesis of Serinolamide A (1)

Using Method A, 3 (50 mg, 0.17 mmol), EDC (68 mg, 0.44 mmol), HOObt (85 mg, 0.63 mmol) and DIPEA (0.23 mL, 1.33 mmol) gave 80 mg (33%) of serinolamide A as clear oil; Rf = 0.34 (n-hexane/ethyl acetate 1:2); [α]c28 = +2.78 (c = 0.18, CHCl3); IR (KBr): ν 3619 (CDCl3). 2917, 2848, 1732, 1616, 1469 cm−1; 1H NMR (CDCl3, 400 MHz) δ 5.43–5.47 (m, trans–2H), 4.37–4.40 (m, 1H), 3.54–3.78 (m, 5H), 3.01–2.83 (m, 3H), 2.30–2.43 (m, 4H), 1.95–1.98 (m, 2H), 1.24–1.32 (m, 22H), 0.88 (t, J = 7.0 Hz, CH3); 13C NMR (CDCl3, 100 MHz) δ 174.3, 131.6, 128.4, 70.9, 62.0, 58.9, 57.4, 34.2, 33.5, 32.5, 31.9, 29.6, 29.6, 29.5, 29.3, 29.2, 28.0, 22.6, 14.1; HRMS (M + H)+ (ESI+) 384.3478 [M + H]+ (calcd for C29H41NO6H+ 384.3477).

3.4.2. Synthesis of Serinolamide B (2)

Using Method A, 3 (120 mg, 0.42 mmol), EDC (169 mg, 1.09 mmol), HOObt (212 mg, 1.15 mmol) and DIPEA (0.6 mL, 3.29 mmol), 27 (96 mg, 0.68 mmol) gave 45 mg (49%) of serinolamide B as clear oil; Rf = 0.13 (n-hexane/ethyl acetate 1:1); mp: 84–86 °C; [α]c29 = −17.22 (c = 0.18, CHCl3); IR (KBr): ν 3619, 2917, 2849, 1639, 1543, 1466 cm−1; 1H NMR (CDCl3, 400 MHz) δ 6.19–6.21 (m, NH), 5.37–5.48 (m, trans–2H), 4.02–4.07 (m, 1H), 3.75–3.78 (m, 1H), 3.61–3.65 (m, 1H), 3.54–3.58 (m, 1H), 3.47–3.51 (m, 1H), 3.34 (s, CH3), 3.16–3.19 (m, 1H), 2.24–2.31 (m, 4H), 1.92–1.97 (m, 2H), 1.23–1.30 (m, 22H), 0.86 (t, J = 7.0 Hz, CH3); 13C NMR (CDCl3, 100 MHz) δ 173.1, 132.1, 127.9, 72.9, 59.2, 50.5, 36.6, 32.5, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 22.6, 14.1; HRMS (M + H)+ (ESI+) 370.3321 [M + H]+ (calcd for C28H39NO5H+ 370.3321).

3.4.3. Synthesis of (E)-N-(1,3-dihydroxypropan-2-yl)octadec-4-enamide (4)

Using Method A, 3 (180 mg, 0.63 mmol), EDC (254 mg, 1.64 mmol), HOObt (122 mg, 0.90 mmol) and DIPEA (0.86 mL, 4.93 mmol), commercially available serinol (158 mg, 1.01 mmol) gave 80 mg (33%) of 4 as a white solid; Rf = 0.1 (n-hexane/ethyl acetate 1:1); mp: 119–121 °C; IR (KBr): ν 3285, 2955, 2917, 2849, 1637, 1545, 1465 cm−1; 1H NMR (CDCl3, 400 MHz) δ 6.19 (br, 1H), 5.36–5.53 (m, trans–2H), 3.88–3.97 (m, 1H), 3.83–3.86 (m, 2H), 3.76–3.81 (m, 2H), 2.40–2.42 (m, 2H), 2.27–2.34 (m, 4H), 1.94–1.99 (m, 2H), 1.23–1.33 (m, 22H), 0.87 (t, J = 7.0 Hz, CH3); 13C NMR (DMSO-d6, 100 MHz) δ 171.9, 130.7, 129.4, 60.6, 53.2, 35.8, 32.4, 31.7, 29.5, 29.4, 29.4, 29.3, 29.1, 29.0, 28.7, 22.5, 14.4; HRMS (M + H)+ (ESI+) 356.3165 [M + H]+ (calcd for C27H39NO3H+ 356.3164).
3.4.4. Synthesis of (E)-2-(octadec-4-en-1-ylamino)propane-1,3-diol (6)

Using Method C, 5 (100 mg, 0.37 mmol), commercially available serinol (38 mg, 0.41 mmol), triethylamine (0.15 mL, 1.11 mmol), and sodium cyanoborohydride (46.5 mg, 0.74 mmol) gave 18 mg (14%) of 6 as yellow oil; Rf = 0.1 (n-hexane/EtOAc 1:1); IR (KBr): ν 3291, 2918, 2850, 1636, 1389, 1358 cm⁻¹; 1H NMR (CD₃OD, 400 MHz) δ 5.41–5.59 (m, trans–2H), 3.74–3.86 (m, 4H), 3.25–3.29 (m, 1H), 3.09–3.13 (m, 2H), 2.12–2.17 (m, 2H), 1.98–2.06 (m, 2H), 1.78–1.84 (m, 2H), 1.24–1.32 (m, 2H), 0.88 (t, J = 7.0 Hz, CH₃); 13C NMR (CD₃OD, 100 MHz) δ 132.1, 127.7, 60.3, 57.5, 44.6, 32.1, 31.6, 29.3, 29.3, 29.2, 29.1, 29.0, 28.8, 25.6; HRMS (M + H)⁺ (ESI) 342.3372 [M + H]⁺ (calcd for C₁₂H₂₁NO₂H 342.3372).

3.4.5. Synthesis of diethyl (E)-2-acetamido-2-(4-(octadec-4-enamido)benzyl)malonate (9)

Using Method A, 3 (105 mg, 0.37 mmol), EDC (77 mg, 0.49 mmol), HOBt (38 mg, 0.27 mmol) and DIPEA (0.26 mL, 1.5 mmol) gave 150 mg (82%) of 9 as yellow oil; Rf = 0.53 (n-hexane/EtOAc 1:1); IR (KBr): ν 3235, 2848, 1742, 1707, 1512 cm⁻¹; 1H NMR (CDCl₃, 400 MHz) δ 7.40 (d, J = 8.2 Hz, 2 ArH), 7.22 (s, CONH), 6.94 (d, J = 8.3 Hz, 2 ArH), 6.52 (s, NH), 5.42–5.57 (m, trans–2H), 4.22–4.29 (m, 1H), 3.60 (s, CH₃), 2.40 (br, 4H), 2.02 (s, COCH₃), 1.96–1.99 (m, 2H), 1.25–1.30 (m, 22H), 0.87 (t, J = 7.0 Hz, CH₃); 13C NMR (CDCl₃, 100 MHz) δ 169.0, 167.4, 137.0, 135.2, 130.9, 130.4, 128.0, 119.5, 67.2, 62.6, 37.6, 37.2, 32.5, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 29.1, 28.3, 22.6, 14.1, 14.0; HPLC purity: 15.2 min, 100%; HRMS (M + H)⁺ (ESI) 587.4060 [M + H]⁺ (calcd for C₃₅H₄₅N₂O₇ 587.4060).

3.4.6. Synthesis of 10, 11, 12

A mixture of 9 (110 mg, 0.19 mmol) in tetrahydrofuran (5 mL) was added lithium aluminum hydride (36 mg, 0.94 mmol) at 0 °C for 5 min. The resulting suspension was stirred at room temperature for 12 h. The mixture was filtered through a pad of celite and the solvent was evaporated. The residue was purified by column chromatography to give 10 (30 mg, 29%, white solid, Rf = 0.5); mp: 69–71 °C, 11 (15 mg, 16%, ivory solid, Rf = 0.33); mp: 84–86 °C, 12 (8 mg, 8%, ivory solid, Rf = 0.16) (n-hexane/EtOAc 1:5); mp: 130–132 °C; 10 IR (KBr): ν 3158, 2845, 2765, 1731, 1698, 1478 cm⁻¹; 1H NMR (CDCl₃, 400 MHz) δ 7.42 (d, J = 8.4 Hz, 2 ArH), 7.13 (s, CONH), 7.05 (d, J = 8.4 Hz, 2 ArH), 5.86–5.88 (m, NH), 5.43–5.58 (m, trans–2H), 4.81–4.86 (m, 1H), 4.14–4.20 (m, CH₃), 3.05–3.14 (m, 2H), 2.41 (m, 4H), 1.98–1.99 (m, 5H), 1.24–1.31 (m, 28H), 0.87 (t, J = 7.0 Hz, CH₃); 13C NMR (CDCl₃, 100 MHz) δ 171.5, 170.7, 169.5, 136.9, 132.5, 131.6, 129.8, 128.0, 119.8, 61.5, 53.1, 37.6, 32.5, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 29.1, 28.3, 22.6, 14.1, 14.0; HPLC purity: 15.2 min, 95%; HRMS (M + H)⁺ (ESI) 545.3954 [M + H]⁺ (calcd for C₃₅H₄₃N₃O₇ 545.3954). 11 IR (KBr): ν 2324, 2954, 2914, 2848, 1742, 1707, 1511, 1470 cm⁻¹; 1H NMR (CDCl₃, 400 MHz) δ 9.62 (s, CHO), 7.43 (d, J = 8.2 Hz, 2 ArH), 7.17 (s, CONH), 7.09 (d, J = 8.3 Hz, 2 ArH), 5.94–5.96 (m, NH), 5.43–5.56 (m, trans–2H), 4.67–4.72 (m, 1H), 3.11–3.15 (m, 2H), 2.40–2.41 (m, 4H), 1.96–2.01 (m, 5H), 1.25–1.31 (m, 25H), 0.87 (t, J = 7.0 Hz, CH₃); 13C NMR (CDCl₃, 100 MHz) δ 198.7, 170.8, 170.0, 136.9, 132.6, 131.2, 129.8, 127.9, 120.1, 59.8, 37.6, 34.4, 32.5, 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 23.0, 22.6, 14.1; HPLC purity: 7.7 min, 99%; HRMS (M + H)⁺ (ESI) 501.3692 [M + H]⁺ (calcd for C₇₁H₁₀₅N₄O₂ 501.3692).

12 IR (KBr): ν 3342, 2953, 2914, 2849, 1686, 1515, 1470 cm⁻¹; 1H NMR (CD₃OD, 400 MHz) δ 7.45–7.47 (m, 2 ArH), 7.17–7.19 (m, 2 ArH), 5.44–5.55 (m, 3H, CHO), 3.58–3.69 (m, 3H, CH₃), 3.02 (s, CH₂), 2.37–2.41 (m, 4H), 1.99–2.00 (m, 2H), 1.96 (s, COCH₃), 1.26–1.36 (m, 2H), 0.91 (t, J = 7.0 Hz, CH₃); 13C NMR (CD₃OD, 100 MHz) δ 172.7, 172.5, 136.8, 132.5, 131.5, 130.5, 130.0, 128.0, 119.8, 119.8, 61.8, 61.7, 36.6, 36.0, 34.2, 32.1, 31.6, 29.3, 29.3, 29.2, 29.0, 28.7, 28.4, 22.3, 13.0; HPLC purity: 14.0 min, 98.6%; HRMS (M + H)⁺ (ESI) 503.3849 [M + H]⁺ (calcd for C₇₁H₁₀₅N₄O₂H 503.3848).
3.4.7. Synthesis of diethyl (E)-2-acetamido-2-(4-(octadec-4-en-1-ylamino)benzyl)malonate (13)

Using Method C, 5 (165 mg, 0.62 mmol), 8 (200 mg, 0.62 mmol), triethylamine (0.26 mL, 1.86 mmol) and sodium cyanoborohydride (78 mg, 1.24 mmol) gave 150 mg (70%) of 13 as a white solid; Rf = 0.3 (n-hexane/EtOAc 1/2); mp: 67–69 °C; IR (KBr): ν 3205, 2946, 2814, 1673, 1480 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.57 (d, J = 8.3 Hz, 2 ArH), 6.52 (s, CONH), 6.47 (d, J = 8.4 Hz, 2 ArH), 5.41–5.43 (m, trans–2H), 4.22–4.28 (m, 4H), 3.56–3.63 (m, 1H), 3.51 (s, 2H), 3.05–3.08 (m, 2H), 1.96–2.09 (m, 7H), 1.63–1.67 (m, 2H), 1.25–1.30 (m, 28H), 0.87 (t, J = 7.0 Hz, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 168.9, 167.7, 147.5, 131.4, 130.6, 129.1, 123.2, 122.5, 67.4, 62.4, 43.4, 37.0, 32.5, 31.9, 30.1, 29.6, 29.6, 29.5, 29.5, 29.3, 29.2, 23.0, 22.6, 14.1, 14.0; HPLC purity: 17.5 min, 95.5%; HRMS (M + H)+ (ESI) 447.3951 [M + H]+ (calcd for CaH₂N₃O₂H⁺ 447.3950).

3.4.8. Synthesis of (E)-2-amino-2-(4-(octadec-4-en-1-ylamino)benzyl)propane-1,3-diol (19)

Using Method B, 18 (70 mg, 0.12 mmol) and 4 M HCl in dioxane (0.16 mL, 0.64 mmol) gave 37 mg (65%) of 19 as a brown solid; mp: 135–137 °C (decomp.); [α]D²⁵ = +1.11 (c = 0.18, EtOH); IR (KBr): ν 3301, 2919, 2850, 1664, 1513, 1412, 1163 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 6.57 (br, 4 ArH), 3.53–3.55 (m, trans–2H), 3.51–3.58 (m, 4H), 3.32–3.39 (m, 2H), 3.13 (s, CH₃), 2.13–2.18 (m, 2H), 1.98–2.03 (m, 2H), 1.86 (br, 2H), 1.26–1.37 (m, 22H), 0.91 (t, J = 6.9 Hz, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 136.4, 134.5, 132.3, 132.1, 127.6, 122.8, 61.0, 60.2, 51.7, 35.3, 32.2, 31.6, 29.4, 29.3, 29.2, 29.1, 28.9, 28.9, 25.5, 22.3, 13.1; HPLC purity: 7.8 min, 96.1%; HRMS (M + H)+ (ESI) 447.3951 [M + H]+ (calcd for CaH₂N₃O₂H⁺ 447.3950).

3.4.9. Synthesis of (E)-N-(4-(2-amino-3-hydroxy-2-(hydroxymethyl)propyl)phenyl)octadec-4-enamide (21)

Using Method B, 20 (12 mg, 0.02 mmol) and 4 M HCl in dioxane (0.02 mL, 0.08 mmol) gave 10 mg (96%) of 21 as an ivory solid; mp: 156–158 °C (decomp.); [α]D²⁵ = +7.22 (c = 0.18, EtOH); IR (KBr): ν 3288, 2916, 2849, 1655, 1598, 1511, 1414, 1119, 1050 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.54 (d, J = 8.3 Hz, 2 ArH), 7.27 (d, J = 8.3 Hz, 2 ArH), 5.43–5.56 (m, trans–2H), 3.55 (s, 4H), 2.99 (s, CH₂), 2.38–2.43 (m, 4H), 1.98–2.03 (m, 2H), 1.27–1.34 (m, 22H), 0.92 (t, J = 7.0 Hz, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 131.5, 130.5, 129.6, 128.0, 120.1, 61.0, 60.4, 36.6, 35.4, 32.1, 31.6, 29.3, 29.3, 29.1, 28.9, 28.8, 28.4, 22.3, 13.0; HPLC purity: 8.8 min, 99.1%; HRMS (M + H)+ (ESI) 461.3743 [M + H]+ (calcd for CaH₂N₃O₂H⁺ 461.3743).

3.5. Cell Culture

For S1P receptor internalization assay, PathHunter® EDG1 HEK 293 cells (93-0784C1; DiscoverX, Fremont, CA, USA) were cultured in DMEM containing 10% (v/v) fetal bovine serum (Biowest), 100 U/mL penicillin-streptomycin (Gibco), 0.25 μg/mL puromycin (Invivogen), and 200 μg/mL hygromycin B (Invitrogen). Cells were incubated at 5% CO₂ in a 37 °C humidified atmosphere.

3.6. S1P Receptor Internalization Assay

The S1P receptor internalization activity of synthesized compounds was evaluated using PathHunter® EDG1 total GPCR internalization HEK293 cell line (93-0784C1; DiscoverX). The cell lines are engineered to co-express two fragments of β-galactosidase at S1P receptor and endosome, respectively. The endocytosis of receptor leads β-galactosidase to complemented form, and the internalization activity was measured by chemiluminescent signal. The HEK293 EDG1 cells (1 × 10⁴ cells/well) in cell plating 28 reagent (DiscoverX) were seeded in 96-well white plates and incubated overnight at 37 °C. The test compounds were prepared in cell plating 28 Reagent (DiscoverX) and treated for 3 h at 37 °C. Then, 50 μL of detection reagent (PathHunter® Detection Kit, 93-0001L; DiscoverX) was added to the wells and incubated for 1 h at room temperature in the dark. The
chemiluminescent signals were measured at all wavelengths using a microplate reader (SpectraMax®i3; Molecular Devices).

3.7. Measurement of Peripheral Lymphocyte Count

Compound 19 and fingolimod were dissolved in 5% DMSO and distilled water, and 21 was dissolved in 50% DMSO and distilled water (final volume was 20 μL). All test compounds were intravenously administered to B6C3H mice (10 wks, 20 g). Blood samples were obtained from retro-orbital sinus of the mice under anesthesia (4% isoflurane) at different time points and were collected into a K2-EDTA-coated tube. Blood lymphocyte counts were measured using an automatic blood cell counter (Horiba).

4. Conclusions

In this study, we optimized previous synthetic methods for serinolamides A and B. Structural similarities with FTY720 indicated that serinolamides may act as S1P1 receptor agonists. We synthesized a series of derivatives and evaluated their efficacy in S1P1 receptor internalization. Compounds 19 and 21 were rationally designed by hybridization of serinolamide A with the FTY720 scaffold and exhibited favorable efficacies in S1P1 receptor internalization. Finally, we confirmed that compound 19 in vivo activity by effectively reducing the number of blood lymphocytes in mice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27092818/s1, Scheme S1: Synthesis of 26 and 27.

Author Contributions: S.J.P. and J.K. (Jushin Kim) carried out the experimental work and wrote the paper; J.K. (Jushin Kim), H.J.K. and R.K. participated in the discussion of biological activities; J.W.C., S.J.P. and Y.K. constructed the target compound structure; S.J.P., E.H.L. and B.K. synthesized the compounds; J.K. (Jaehwan Kim), S.K. and J.K. (Jushin Kim) conducted PLC experiment and discussed the biological activities; J.-H.P. and K.D.P. directed and supervised the whole experimentation. All authors have read and agreed to the published version of the manuscript.

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Sample Availability: Samples of the compounds are available from the authors upon the reasonable request.
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