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Exploring amino acids derivatives as potent, selective, and direct agonists of sphingosine-1-phosphate receptor subtype-1

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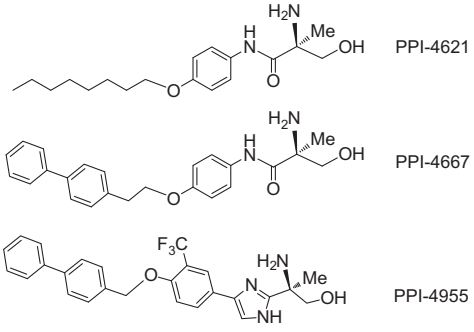
ABSTRACT

In the quest to discover a potent and selective class of direct agonists to the sphingosine-1-phosphate receptor, we explored the carboxylate functional group as a replacement to previously reported lead phosphates. This has led to the discovery of potent and selective direct agonists with moderate to substantial in vivo lymphopenia. The previously reported selectivity enhancing moiety (SEM) and selectivity enhancing orientation (SEO) in the phenylamide and phenylimidazole scaffolds were crucial to obtaining selectivity for S1P receptor subtype 1 over 3.

Exploration of sphingosine-1-phosphate (S1P) receptors, a class of G-protein coupled receptors (GPCRs), as a potential therapeutic target in various physiological and pathophysiological processes[1] has generated a highly competitive area of research and development. S1P, the endogenous natural ligand, effects activation and inhibition of this cluster of five receptors ($S1P_{1-5}$) inducing multiple effects on cardiovascular and immune system function.[2]

FTY-720 (Fingolimod), a novel molecular entity, approved under the trade name of Gilenya for treatment of relapsing-remitting multiple sclerosis,[3] has allowed for better understanding of this class of GPCR.[4] FTY-720 needs to be phosphorylated to the desired phosphate drug, FTY-720-phosphate, to interact with S1P receptor subtypes. Recently, we published several novel classes of S1P receptor subtype-1 agonists that fall within the same class of pro-drug approach as FTY-720 that require in vivo phosphorylation to influence S1P receptors.[5] One major challenge in a pro-drug strategy in S1P activation is in vivo phosphorylation of the pro-drug to the desired phospho-drug. The challenges of low phosphorylation and variability observed in the in vivo phosphorylation of the lead molecules in the pro-drug approach led to consideration of exploring the potential for a direct agonist approach. The use of a carboxylate as direct S1P receptor subtype-1 agonist was recently reported by Mandala and co-workers.[6] Utility of a carboxylate as a potential phosphate mimic led to reinvestigation of the previously reported pro-drug chemotypes and reconsideration of the corresponding carboxylate analogs as potential novel therapeutic agents. In an earlier report, detailed biological data on lead alcohol PPI-4621 (Scheme 1) was disclosed.[5] The low in vivo conversion of the pro-drug PPI-4621 to the corresponding potent phosphate (Table 1) and poor selectivity of PPI-4621-phosphate for S1P subtype-1 over 3 were the drawback to further progress of this promising lead. Thus, further SAR exploration of the lipophilic tail moiety of PPI-4621-phosphate led to discovery of potent



Scheme 1.

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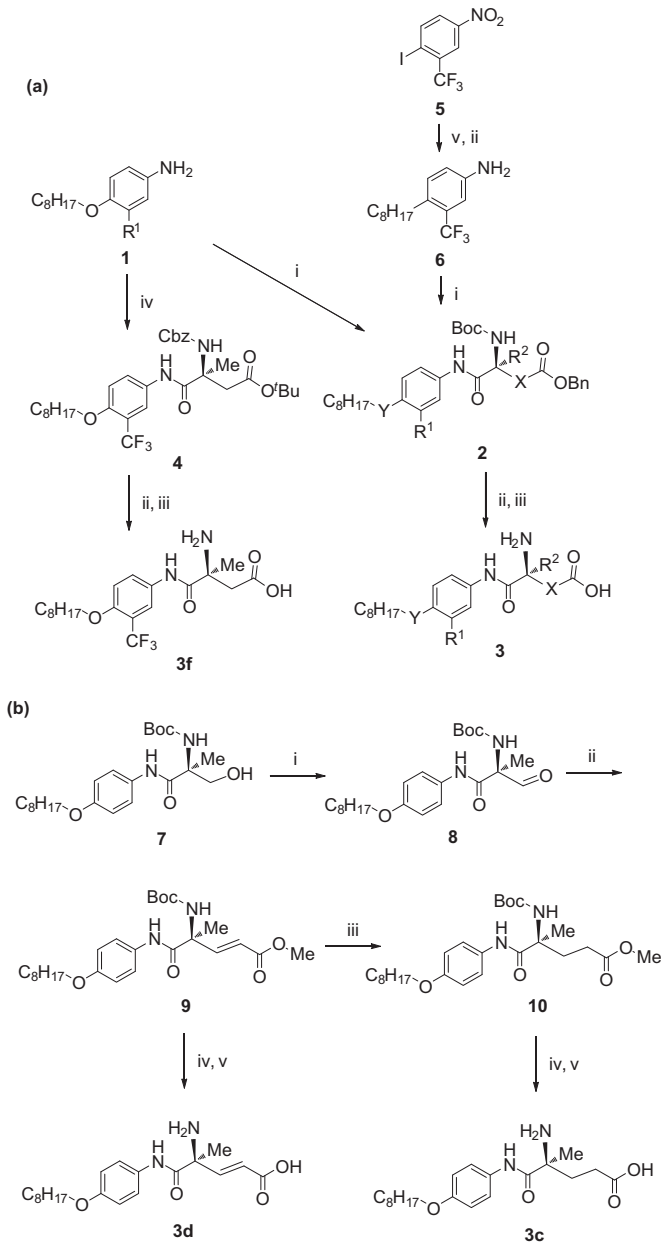
and selective PPI-4667-phophate with a better selectivity profile for S1P subtype-1 over 3. However, the drawback to PPI-4667 alcohol came with its poor in vivo conversion to the corresponding phosphate in a rat model. Further structure–activity relationship studies resulted in discovery of PPI-4955 with efficient rodent in vivo phosphorylation and excellent potency and selectivity profiles. Unfortunately, although PPI-4955 was shown to have a high degree of phosphorylation in vivo in rodents, it demonstrated poor in vivo phosphorylation in higher species. Therefore, two routes were considered to overcome the phosphorylation predicament; (a) direct agonist approach, (b) extensive exploration in the pro-drug approach to further improve in vivo phosphorylation of the desired alcohol to phosphate in higher species. In this Letter, we describe the direct agonist approach.

Table 1
Percent in vivo pro-drug coversion to the corresponding phosphate upon 10 mg/kg oral (PO) administration of the pro-drug alcohol

Pro-drug	% Phosphate in mice	% Phosphate in rats
PPI-4621	10	20
PPI-4667	70	14
PPI-4955	60	49

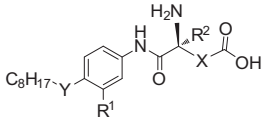


Scheme 2. Reagents and conditions: (a) (i) *N*-Boc-amino acid benzyl ester, HATU, DIPEA, DMF; (ii) 10% Pd on carbon, H_2 (gas), MeOH; (iii) TFA, CH_2Cl_2; (iv) (*S*)-2-(benzyloxycarbonylamino)-4-*tert*-butoxy-2-methyl-4-oxobutanoic acid,[9] HATU, DIPEA, DMF; (v) 1-octyne, $Pd(dba)_2$, PPh_3, CuI, DIEA, MeCN. (b) (i) DMSO, oxalyl chloride, Et_3N, CH_2Cl_2; (ii) (methoxycarbonylmethyl)triphenyl-phosphonium chloride; (iii) 10% Pd on carbon, H_2 (gas), MeOH; (iv) LiOH, MeOH, H_2O, THF; (v) TFA, CH_2Cl_2.

In pursuit of a carboxylate as a direct S1P receptor subtype-1 agonist, we initiated synthesis of the carboxylate corresponding to the previously reported phosphate lead molecules as shown in Scheme 2.[7] Acylation of the amino group of the substituted aniline **1** with the desired orthogonally protected amino acid using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) afforded esters **2** and **4** (Scheme 2a).[8] The corresponding des-oxy analog of **1** was synthesized from Sonogashira cross-coupling of aryl iodide **5** with 1-octyne followed by hydrogenation of both the nitro and the alkyne in one step to afford compound **6**. Coupling of the aniline **6** to the desired orthogonally protected amino acids was achieved analogous to aniline **1** to afford compound **2**. Two step deprotection of the intermediates **2** and **4** gave the desired final compounds **3** and **3f**, respectively. Additional carboxylate analogs were synthesized as described in Scheme 2b. Swern oxidation of alcohol **7** provided aldehyde **8** which upon a Horner–Wadsworth–Emmons reaction afforded olefin **9**. Hydrogenation of the olefin residue produced compound **10**. Two step deprotection of the functional groups in **9** and **10** gave acids **3d** and **3c**, respectively.

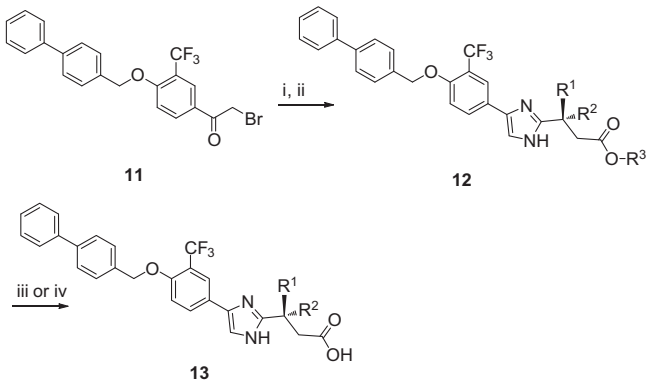
The synthesized acids where then evaluated for S1P receptor binding activity and in vivo activity (Table 2). The binding activity of the designed agonists was measured using a [^{33}P] binding assay as described in an earlier report.[5a] The carboxylate corresponding to the lead molecule PPI-4621 (**3a**) demonstrated excellent binding activity at S1P receptor subtype-1 and 73-fold selectivity for receptor subtype-1 over 3. Compound **3a** also provided potent in vivo activity with a 57% decrease in circulating lymphocytes following an oral dose of 10 mg/kg. Changing the amino acid aspartate (**3a**) to glutamate (**3b**) decreased S1P receptor subtype-1 binding activity by sixfold and decreased in vivo lymphopenia activity. Modification of the glutamic acid **3b** to α-methyl-glutamate (**3c**) maintained similar receptor potency and selectivity, however, these compounds lacked any an effect on circulating lymphocytes upon oral administration. The corresponding olefin **3d** also gave analogous binding activity and lymphopenia.

Table 2
[^{33}P] Binding activity on $S1P_1$ and $S1P_3$ receptor subtypes and percent lymphopenia obtained upon 10 mg/kg oral (PO) administration of the agonist at 6 h post-dosing in mice[10]



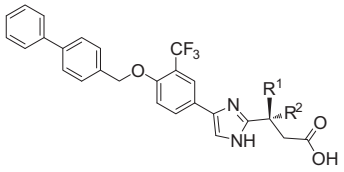
A	R^1	R^2	Y	X	hS1P$_1$ IC_{50} (nM)	hS1P$_3$ IC_{50} (nM)	%L[a]
3a	H	H	O	CH_2	14.4	1050	57
3b	H	H	O	CH_2CH_2	92	10,000	44
3c	H	Me	O	CH_2CH_2	77	10,000	N
3d	H	Me	O	CH=CH (*E*)	74	10,000	25
3e	CF_3	H	O	CH_2	53	>10,000	33
3f	CF_3	Me	O	CH_2	95	>10,000	24
3g	CF_3	Me	—	CH_2	370	>10,000	—

[a] %L = Lymphopenia (percent decrease in circulating lymphocytes compared to time-matched vehicle control).
[b] N = negligible.



Scheme 3. Reagents and conditions: (i) butanoic acid or protected 2-amino-butanoic acid as mono-ester, Cs_2CO_3, DMF; (ii) $AcONH_4$, toluene, 100 °C (iii) 10% Pd on carbon, H_2 (gas), MeOH (where R^1 = NHCbz or R^3 = Bn); (iv) TFA, CH_2Cl_2 (where R^1 = NHBoc or R^3 = ^{t}Bu).

Table 3
[^{33}P] Binding activity on $S1P_1$ and $S1P_3$ receptor subtypes and percent lymphopenia obtained upon 10 mg/kg oral (PO) administration of the agonist at 6 h post-dosing in mice[10]



Carboxylate	R^1	R^2	hS1P$_1$ IC_{50} (nM)	hS1P$_3$ IC_{50} (nM)	%L[a]
13a	NH_2	Me	82	>10,000	N
13b	NH_2	H	17	>10,000	N
13c	H	H	3000	>10,000	N

[a] %L = Lymphopenia (percent decrease in circulating lymphocytes compared to time-matched vehicle control).
[b]N = negligible.

Incorporation of the selectivity enhancing moiety (SEM), with previously reported selectivity enhancing orientation (SEO), in **3a** gave compound **3e** with improved agonist selectivity. Compound **3e** provided moderate lymphopenia (60%) upon 20 mg/kg intravenous (iv) administration at 6 h post-dosing in mice, indicating the necessity for further improvement in agonist potency at S1P receptor subtype-1. Use of an unusual α-methyl amino acid in **3e** provided **3f**, which demonstrated similar binding activity, receptor selectivity, and lymphopenia profile as **3e**. However, the des-oxy analog (**3g**) demonstrated poor binding activity, indicating the importance of the oxygen group in the tail portion of the agonist.

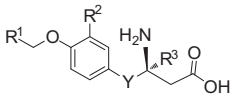
In order to improve binding activity and in vivo lymphopenia profile of the agonist while maintaining S1P receptor subtype selectivity, we utilized our previous learning of the pro-drug approach and decided to modify the amide component to a more rigid imidazole in our exploration. Therefore the corresponding imidazole analogs of PPI-4955 were synthesized as described in Scheme 3. Reaction of the bromoacetophenone **11** with orthogonally protected amino acid or mono-protected dicarboxylate provided the ester intermediate which upon intramolecular cyclization in the presence of excess ammonium acetate, afforded the desired phenylimidazole **12**. Removal of the protecting group(s) in phenylimidazole **12** afforded the desired final product **13**.

The in vitro and in vivo evaluation of the synthesized phenylimidazole carboxylates is reported in Table 3. The corresponding phenylimidazole analog of PPI-4955 (**13a**) showed an S1P receptor subtype-1 binding activity of 82 nM with no detectable activity at S1P receptor subtype-3. However, compound **13a** also gave no detectable lymphopenia upon 10 mg/kg oral administration. Changing R^2 from a methyl to an H improved the S1P receptor subtype-1 binding activity to 17 nM, with no binding activity at S1P receptor subtype-3, but no lymphopenia was observed upon oral administration of **13b** either. Further modification of the amino acid portion of the agonist by replacement of the amino group (R^1 = NH_2) with an H, diminished the agonist activity, indicating that the imidazole NH could not replace the required interaction observed with the primary amine required to obtain the potent agonist activity. The overall observation of lack of lymphopenia with the imidazole carboxylate was suggestive of the need for further SAR exploration of the amide carboxylate series.

In order to reconfirm the agonist potency and selectivity, a number of designed carboxylates were further investigated in [γ-^{35}S]GTP functional assays as reported in Table 4.[11] The amide **3a**, analogous to binding activity data, showed a relatively lower in vitro activity and selectivity for S1P subtype-1 over 3. However, insertion of the SEM group, with required SEO, in **3e** and **3f** diminished S1P receptor subtype-3 activity, providing agonist selectivity while maintaining potent activity at receptor subtype-1. The corresponding imidazole to PPI-4955 (**13a**) showed fivefold weaker activity at S1P receptor subtype-1 in comparison to any of the amide analogs. Replacement of the R^2 methyl with a H, improved the S1P receptor subtype 1 activity in the imidazole scaffold similar to the amide scaffold level. However, overall, the data in the functional assay and the lymphopenia indicated the necessity for further improvement in direct agonist activity at S1P receptor subtype-1.

In summary, in an attempt to develop the corresponding carboxylates to our earlier reported pro-drug lead molecules and to overcome the in vivo phosphorylation issue in the pro-drug strategy, we discovered a few carboxylates with excellent activity at

Table 4
[γ-^{35}S]GTP functional activity on $S1P_1$ and $S1P_3$ receptor subtypes



Agonist	R^1	R^2	R^3	Y	hS1P$_1$ EC_{50} (nM)	hS1P$_3$ EC_{50} (nM)	S1P$_3$/S1P$_1$
S1P	—	—	—	—	5.6	2.4	—
3a	C_7H_{13}	H	H	Amide	164	1800	11
3e	C_7H_{13}	CF_3	H	Amide	231	>3000	>13
3f	C_7H_{13}	CF_3	Me	Amide	135	>3000	>22
13a		CF_3	Me	Imidazole	700	>3000	>4
13b		CF_3	H	Imidazole	161	>3000	>18.6

S1P receptor subtype-1. The concept of the SEM group with appropriate SEO that was established in an earlier report[5c] was once again demonstrated to improve agonist selectivity for receptor subtype-1 over 3. The amide direct agonists demonstrated moderate to substantial lymphopenia upon oral administration. However, further lead optimization is necessary to improve the binding activity and in vivo activity (lymphopenia) of the current carboxylate lead molecules.

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7. For synthesis of compounds **1** and **5** refer to Ref. 4a.
8. For synthesis of aniline **1** refer to Ref. 4a.
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10. FTY-720 was used as the control in these experiments. Upon 1.0 mg/kg oral (PO) administration of FTY-720 generally 80% lymphopenia was observed.
11. For [γ-^{35}S]GTP functional assay protocol refer to Ref. 5.