Bioorganic & Medicinal Chemistry Letters 20 (2010) 2520–2524

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Exploration of amino alcohol derivatives as novel, potent, and highly selective sphingosine-1-phosphate receptor subtype-1 agonists

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ARTICLE INFO

Article history:
Received 23 November 2009
Revised 24 February 2010
Accepted 26 February 2010
Available online 3 March 2010

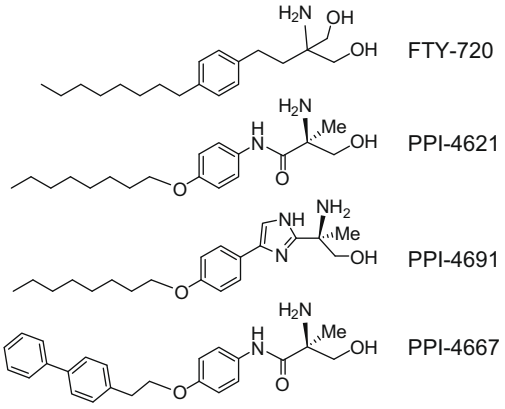
Keywords:
Sphingosine-1-phosphate (S1P) agonist
$S1P_1$ agonists
Phenylamide
Phenylimidazole
Immunosuppressants
Lymphopenia

ABSTRACT

In pursuit of a potent and highly selective sphingosine-1-phosphate receptor agonists with an improved in vivo conversion of the precursor to the active phospho-drug, we have utilized previously reported phenylamide and phenylimidazole scaffolds to identify a selectivity enhancing moiety (SEM) and selectivity enhancing orientation (SEO) within both pharmacophores. SEM and SEO have allowed for over 100 to 500-fold improvement in selectivity for S1P receptor subtype 1 over subtype 3. Utility of SEM and SEO and further SAR study allowed for discovery of a potent and selective preclinical candidate PPI-4955 (**21b**) with an excellent in vivo potency and dose responsiveness and markedly improved overall in vivo pharmacodynamic properties upon oral administration.

Emergence of FTY-720 as a potent and orally bioavailable immunomodulatory agent has unveiled novel therapeutic targets within the sphingosine-1-phosphate (S1P) pathway. Upon in vivo phosphorylation the pro-drug FTY-720 is converted to active FTY-720-phosphate, a potent and non-selective S1P receptor agonist ($S1P_{1,3-5}$). The phospho-FTY-720 induces immunomodulatory activity through direct alterations in lymphocyte trafficking[1] via triggering S1P receptor subtype 1 ($S1P_1$) signaling cascade.[2] This has created a stimulating and highly competitive area of research due to the fact that S1P receptors, a class of G-protein coupled receptors (GPCRs), play major roles in several biological processes[3] including cardiovascular and immune system function, and other yet poorly defined effects on additional physiological systems.[4]

We recently reported two classes of S1P receptor agonists with excellent potency at the $S1P_1$ receptor and a relative low potency on $S1P_3$.[5] In order to further improve the in vivo phosphorylation of the pro-drug to active agonist in rodents, as well as reducing the side effect profile of a non-selective S1P receptor agonist such as bradycardia,[6] while retaining the positive therapeutic properties, we explored medicinal chemistry optimization to further expand and improve our structure–activity relationship (SAR) while monitoring both in vivo phosphorylation and the agonist selectivity for S1P receptor subtype 1 over subtype 3. In the design of potent and selective sphingosine-1-phosphate receptor agonists in our phenylamide and phenylimidazole analogs, we reported three potent lead molecules, PPI-4621, PPI-4691 and PPI-4667 (Scheme 1) with moderate to good selectivity for S1P receptor subtype 1 over subtype 3 and in vivo activity in rodent.[5]



Scheme 1.

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doi:10.1016/j.bmcl.2010.02.098

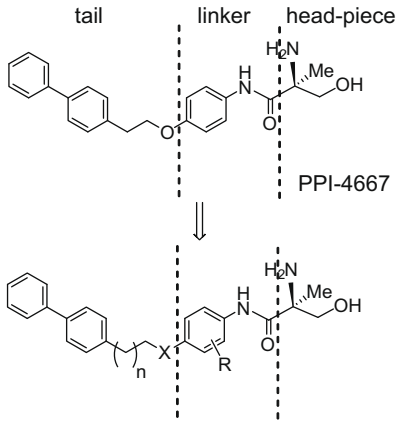


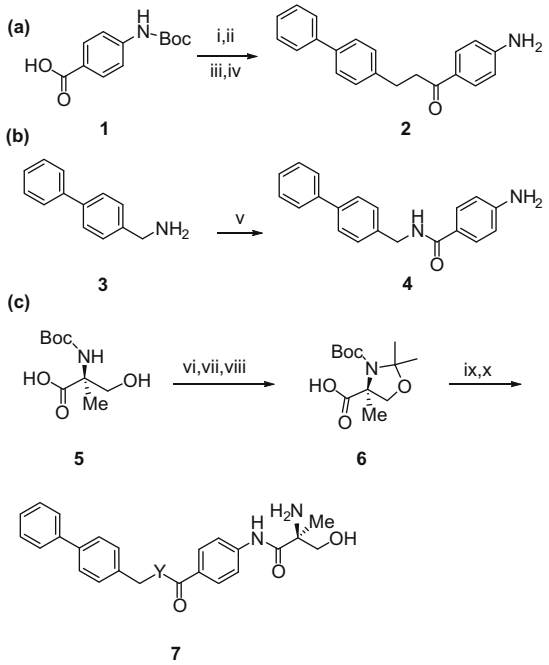
Figure 1. Lead optimization from PPI-4667.

In pursuit of further improving agonist selectivity for S1P receptor subtype 1 and to enhance in vivo phosphorylation of the pro-drug alcohol, we performed extensive modifications in the phenylamide scaffold (Fig. 1) through side-chain modifications and 4-aminophenol ring substitutions. Our earlier work on PPI-4621 indicated that substitutions on the linker 4-aminophenol provided an agonist with low to moderate in vivo activity.[5a] Furthermore, S1P1 selectivity was improved through incorporation of the biphenyl system in the lipophilic tail of the scaffold.[5b] The SAR study leading to PPI-4621 and PPI-4667 also revealed the tolerance of the receptor for the ether lipophilic tail section of the molecule. We predicted further lead optimization and structural modification between the biphenyl tail moiety and the amide section of the phenylamide linker of PPI-4667 with the goal of achieving a more selective S1P receptor subtype-1 agonist with a better in vivo phosphorylation in mouse than the 15% observed in the previous lead molecule PPI-4667 (Fig. 1).

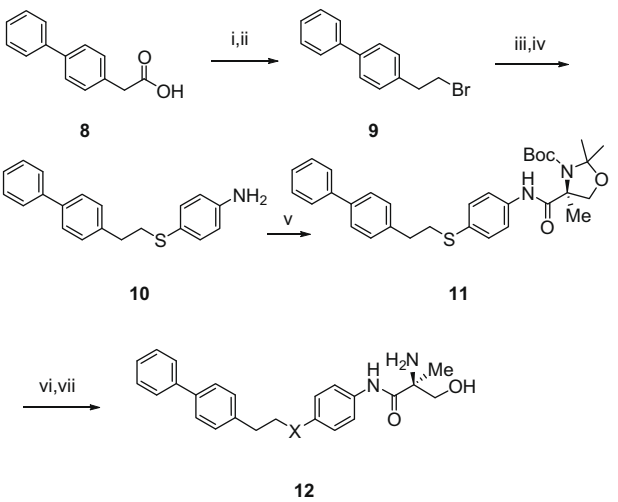
We envisioned further SAR exploration of structural modification and tolerability around the X section of the scaffold as a good starting point as described in Schemes 2 and 3. One attempt was to explore tolerability of a keto or an amide moiety replacing the ether oxygen while maintaining the same number of atoms between the aniline and the biphenyl group. Synthesis of the desired compounds is reported in Scheme 2. Reaction of Boc-protected 4-aminobenzoic acid with oxalyl chloride gave acid chloride which was then trapped with 4-ethynylbiphenyl through a cross-coupling with copper iodide (I). Hydrogenation of the alkyne group followed by removal of the Boc-protecting group afforded propanone **2**. Acylation of the amino moiety of propanone **2** with orthogonally protected amino acid **6**,[7] synthesized from the corresponding Boc-amino acid **5**, through an acid chloride intermediate followed by the removal of the protecting groups with *para*-toluenesulfonic acid afforded amino alcohol **7** (Y = CH_2). The corresponding amide was synthesized via coupling of 4-phenylbenzylamine **3** with 4-aminobenzoic acid followed by sequential manipulations thereafter analogously to compound **2** to afford amino alcohol **7** (Y = NH).

The corresponding thio-ether based modifications were feasible through Scheme 3. Reduction of the biphenyl acetic acid **8** with BH_3 and bromination of the alcohol product gave biphenyl ethyl bromide **9**. Biphenyl ethyl bromide **9** was then reacted with 4-nitrobenzenethiol. Reduction of the nitro group with $SnCl_2$ gave the thio-aniline **10**. One pot acid chloride formation of carboxylic acid **6** and acylation of the thio-aniline **10** afforded thio-ether amide **11**. The thio-ether **11** then was selectively oxidized to the corresponding sulfoxide and sulfone using *meta*-chloroperbenzoic acid (mCPBA). Removal of the protecting groups gave the final compound **12**.

The lymphopenia observed for a set of designed molecules is reported in Table 1. The amino alcohol in vivo activity was determined by measuring redistribution of circulating lymphocytes in mouse, 6 h after single oral administration of the pro-drug alcohol. When the ether oxygen was replaced with a keto moiety, excellent in vivo activity was observed analogous to the lead molecule PPI-4667. However, the corresponding amide and the thio-ether

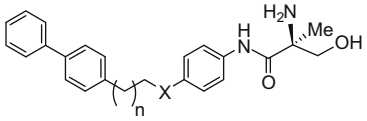


Scheme 2. Reagents and conditions: (i) $(COCl)_2$, DMF, THF, room temperature, 20 min; (ii) 4-ethynylbiphenyl, CuI, Et_3N; (iii) H_2, 10 Pd/C, MeOH; (iv) TFA, CH_2Cl_2; (v) 4-aminobenzoic acid, HATU, DIPEA, DMF; (vi)TMS/CHN_2, MeOH, 0 °C; (vii) 2,2-dimethoxypropane, $BF_3{\cdot}Et_2O$, acetone, room temperature, 4–12 h; (viii) LiOH, THF/H_2O/MeOH (2:1:1), reflux, 1–3 h; (ix) $(COCl)_2$, DMF (catalytic), THF, room temperature, 20 min then added two and stirred overnight; (x) *para*-toluenesulfonic acid, MeOH, reflux, 3–12 h.



Scheme 3. Reagents and conditions: (i) BH_3, THF, 0 °C, 2–3 h; (ii) CBr_4, PPh_3, CH_2Cl_2; (iii) 4-nitrobenzenethiol, Et_3N, THF; (iv) $SnCl_2$, EtOH, reflux, 2–4 h; (v) (*R*)-3-(*tert*-butoxycarbonyl)-2,2,4-trimethyloxazolidine-4-carboxylic acid, $(COCl)_2$, DMF (catalytic), THF, room temperature, 20 min then added **10** and stirred overnight; (vi) used mCPBA to oxidize thio-ether, (1.0 equiv for sulfoxide and 2.00 equiv for sulfone), CH_2Cl_2, room temperature, 30 min; (vii) *para*-toluenesulfonic acid, MeOH, reflux, 3–12 h.

Table 1
Percent lymphopenia obtained upon 10 mg/kg oral (PO) administration of the alcohol



Agonist	*n*	X	%Lymphopenia	%In vivo phosphorylation
PPI-4667	1	O	81	14
7a	1	CO	84	40
7b	0	NHCO	32	—
12a	1	S	21	—
12b	1	SO	N	—
12c	1	SO_2	N	—

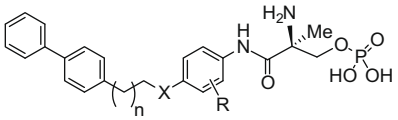
[a]N = negligible.

showed low to negligible in vivo activity. Oxidation of the thio-ether to either sulfoxide or sulfone ablated the modest in vivo activity observed in the thio-ether **12a**. The corresponding phosphate for amino alcohol **7a** was synthesized from salt free amino alcohol **7a** in two steps utilizing diethyl chlorophosphate and bromotrimethylsilane, as reported by Evindar et al.[5], to determine the agonist receptor binding activity and selectivity for S1P receptor subtype 1 over subtype 3 (Table 2). The **7a**-phosphate (**7a-P**) showed analogous binding activity to PPI-4667 phosphate (PPI-4667-P) with fivefold improvement in selectivity. More importantly, the in vivo phosphorylation analysis for amino alcohol **7a** showed 40% conversion to **7a-P**, a threefold improvement over PPI-4667. We speculated that the keto oxygen in amino alcohol **7a-P** interacts with a specific region of the S1P receptor subtype 3 that lowers its affinity for the receptor subtype with minimal changes in the agonist binding activity at receptor subtype 1, therefore increasing the agonist selectivity for S1P receptor subtype 1 over 3. With the identification of this specific region within the S1P receptor, we envisaged utilization of an *ortho*-substitution adjacent to the oxygen ether in PPI-4667 to potentially induce an analogous selectivity effect. Therefore, we initiated an exploration with an extensive substitution and modification on the aminophenol (the linker) region of the pharmacophore.

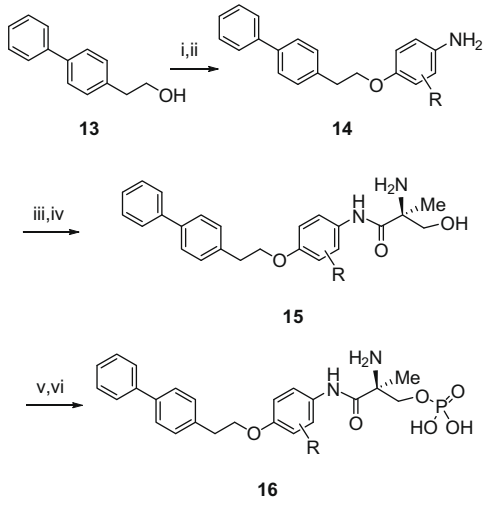
In pursuit of linker modification in the phenylamide pharmacophore a number of targets were designed and synthesized as reported in Scheme 4. Nucleophilic aromatic substitution of the 2-(biphenyl-4-yl)ethanol (**13**) on substituted 1-fluoro-4-nitrobenzene in presence of base followed by hydrogenation of the nitro group afforded substituted aniline **14**. Coupling of the substituted aniline **14** with (*S*)-2-(*tert*-butoxycarbonylamino)-3-hydroxy-2-methylpropanoic acid using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) followed by Boc removal afforded amino alcohol **15**. In cases where the R group was an *ortho* substitution to the amine group in compound **14**, coupling of the Boc-amino acid with HATU generally afforded poor yield, therefore the acid chloride approach was used analogous to Scheme 2 to generate the final alcohol **15**. All the corresponding phosphates were synthesized from amino alcohol **15** or the Boc-protected precursor analogous to that reported by Evindar et al.[5] The binding activity of the designed agonists was measured using a [^{33}P] binding assay similarly to our earlier report[5] (Table 3) and compared to S1P and lead molecule PPI-4667-P. When R was CO_2Me (**16a**), the agonist showed relatively lower binding activity at receptor subtype 1 but with improved selectivity with respect to the lead molecule PPI-4667-P. The hydrophilic carboxylic acid functional group (**16b**) abolished the binding activity at both receptor subtypes but the amide and the methyl amide (**16c** and **16d**) analogs maintained a weak binding activity at $S1P_1$. The corresponding nitrile (**16e**) showed excellent binding activity on $S1P_1$ with a threefold improvement in selectivity over PPI-4667-P. When R was a halogen (**16f** and **16g**) the agonists maintained similar binding activity to the lead molecule PPI-4667-P with a 2- to 4-fold improvement in selectivity. The corresponding Me (**16h**) gave analogous result as the halogen counterpart while the CF_3 analog (**16i**) further improved the agonist binding activity with respect to PPI-4667-P from 1.05 to 0.24 nM. More significantly, the CF_3 analog improved the agonist selectivity for S1P receptor subtype 1 over 3 by 100-fold. However, the corresponding 2-CF_3 (**16j**) showed no improvement over the lead molecule PPI-4667-P. Therefore, this observation demonstrated a selectivity enhancing orientation (SEO) for the substitution position with respect to the agonist side-chain (tail) component. The exploration also allowed for discovery of several selectivity enhancing groups, especially CF_3, as a specific selectivity enhancing moiety (SEM).

In order to determine the pro-drug alcohol conversion to the active phosphate drug and in vivo activity of the phosphate through measuring redistribution of circulating lymphocytes in the mouse 6 h after oral administration of the compounds, the corresponding alcohol to **16i** was orally administered to the mouse at 10 mg/kg.

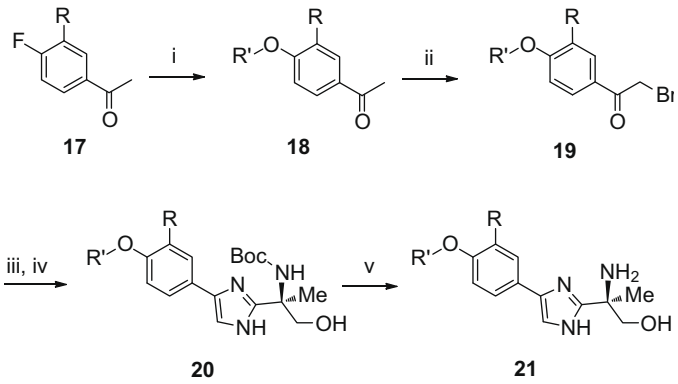
Table 2
[^{33}P] binding activity on $S1P_1$ and $S1P_{3-5}$ receptor subtypes



Agonist	*n*	X	R	$hS1P_1$ IC_{50} (nM)	$hS1P_3$ IC_{50} (nM)	$hS1P_4$ IC_{50} (nM)	$hS1P_5$ IC_{50} (nM)	$S1P_3/S1P_1$
S1P	—	—	—	0.78	0.92	1.04	2.0	—
7a-P	1	CO	H	4.14	3800	13.1	1360	917
PPI-4667-P	1	O	H	1.05	200	10.3	43.6	190
16a	1	O	3-CO_2Me	21	>10,000	—	—	>476
16b	1	O	3-CO_2H	>10,000	>10,000	—	—	—
16c	1	O	3-$CONH_2$	150	>1000	—	—	—
16d	1	O	3-CONHMe	500	>1000	—	—	—
16e	1	O	3-CN	3.4	2000	—	—	588
16f	1	O	3-Cl	0.8	285	—	—	356
16g	1	O	3-Br	1.3	1000	—	—	769
16h	1	O	3-Me	6.0	3250	—	—	542
16i	1	O	3-CF_3	0.24	4500	3.4	87.2	18,750
16j	1	O	2-CF_3	6.4	700	—	—	109



Scheme 4. Reagents and conditions: (i) 2-(biphenyl-4-yl)ethanol, substituted 1-fluoro-4-nitrobenzene , KO^tBu, THF, 65 °C; (ii) H_2, 10% Pd/C, MeOH/EtOAc (4:1 v/v); (iii) (*S*)-2-(*tert*-butoxycarbonylamino)-3-hydroxy-2-methylpropanoic acid, HATU, DIPEA, DMF; (iv) TFA, CH_2Cl_2; (v) excess diethyl phosphorochloridate, Et_3N, CH_2Cl_2, rt, 12–18 h; (vi) excess TMSBr, CH_2Cl_2, rt, 4–10 h.

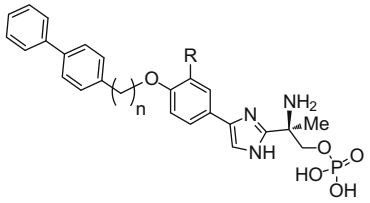


Scheme 5. Reagents and conditions: (i) Alcohol, KO^tBu, THF, 65 °C; (ii) Bu_4NBr_3, CH_2Cl_2/MeOH (9:1 v/v), rt, 1–3 h; (iii) (*R*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, Cs_2CO_3, DMF; (iv) $AcONH_4$, toluene, 100 °C (v) TFA, CH_2Cl_2.

Lymphocyte count analysis revealed less than a 20% decrease in absolute lymphocyte counts. Further investigation revealed a marginal 2% in vivo conversion of the pro-drug alcohol to the corresponding phosphate **16i** leading to only modest reduction in circulating lymphocytes. Since our earlier SAR and S1P agonist receptor profile was transferable between phenylamide and phenylimidazole scaffolds, we envisioned further investigation of the SEM and in vivo phosphorylation of the pro-drug in the more rigid phenylimidazole pharmacophore. The corresponding phenylimidazole to phenylamide **16i** and a number of designed analogs were prepared as described in Scheme 5. A nucleophilic substitution reaction by an alcohol on 4-fluoroacetophenone **17** afforded the ether-acetophenone **18**. The ether-acetophenone **14** was then converted to the bromo-acetophenone **19** using Bu_4NBr_3 at ambient temperature. The bromo-acetophenone **19** was then trapped with (*R*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid affording an intermediate ester which upon heating in presence of $AcONH_4$ gave phenylimidazole **20**. Removal of the Boc protecting group provided the amino alcohol **21**. The corresponding phosphates to the alcohols were synthesized from alcohol **20** analogous to those reported by Evindar et al.[5]

A side by side comparison of the phenylamide **16i** and the corresponding phenylimidazole **21a-P** revealed a relative lower binding affinity to both S1P receptor subtypes 1 and 3 by the phenylimidazole analog while maintaining an excellent selectivity for the $S1P_1$ subtype (Table 3). Further investigation of the corresponding prodrug **21a**, upon oral administration in mouse at 10 mg/kg, demonstrated a significant redistribution in circulating lymphocytes resulting in marked in vivo activity. Determination of the in vivo conversion of the alcohol **21a** to phosphate **21a-P** was found to be 6.8% at 6 h after oral administration in mice. Despite the low conversion of the alcohol to phosphate, a sufficient amount of active phosphate was generated to induce a significant lymphopenia. In order to further improve the in vivo conversion of the alcohol to phosphate in the phenylimidazole pharmacophore, we decided to examine structural changes from amide **16i** to phenylimidazole **21a-P**. Our earlier SAR revealed that the tail section of the S1P receptor is relatively forgiving to structural and conformational changes,[5] however the overall pharmacophore length is important in the agonist binding activity. Changing the pharmacophore from phenylamide to phenylimidazole adds about one bond length to the distance between the phosphate group and the SEM. In order to account for this change, we designed a reduction of a methylene unit in the tail region of **21a-P** generating **21b-P** (PPI-4955-P) (Table 3). Regardless of the conformational changes from **21a-P** to **21b-P**, the tail modification resulted in potent and highly selective **21b-P** with significant improvement over **21a-P** in its binding activity. Oral administration of the corresponding alcohol to **21b-P** (**21b** or PPI-4955) in mouse at 10 mg/kg induced a marked drop in absolute lymphocyte counts at 6 h after administration. Furthermore, PPI-4995 in vivo conversion to the phosphate **21b-P** showed 10-fold improvement over the phenylimidazole **21a**. Removal of the SEM CF_3 in **21b-P** demonstrated the importance of the group in improving the agonist selectivity for the S1P receptor subtype 1 over 3 in phenylimidazole

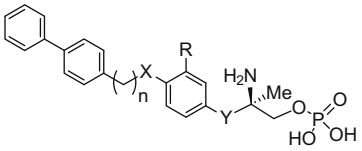
Table 3
[^{33}P] binding activity on $S1P_1$ and $S1P_{3-5}$ receptor subtypes



Agonist	*n*	R	hS1P$_1$ IC$_{50}$ (nM)	hS1P$_3$ IC$_{50}$ (nM)	hS1P$_4$ IC$_{50}$ (nM)	hS1P$_5$ IC$_{50}$ (nM)	S1P$_3$/S1P$_1$	%L[a]	%C[b]
21a-P	2	CF_3	9.4	>100,000	6.5	184	>10,000	64	6.8
21b-P	1	CF_3	0.23	2000	4.4	9.6	8695	85	70.1
21c-P	1	H	1.03	20	4.87	9.6	19	–	–

[a] Percent lymphopenia observed in mouse 6 h post dose upon 10 mg/kg oral (PO) administration of the corresponding alcohol.
[b] Percent conversion of alcohol (pro-drug) to phosphate (drug) at 6 h dose administration upon 10 mg/kg oral (PO) administration of the alcohol.

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



Agonist	*n*	X	R	Y	hS1P$_1$ EC$_{50}$ (nM)	hS1P$_3$ EC$_{50}$ (nM)	S1P$_3$/S1P$_1$
S1P	—	—	—	—	5.6	2.4	—
PPI-4667-P	2	O	H	Amide	0.52	120	231
7a-P	2	CO	H	Amide	18.3	>3000	>164
16i	2	O	CF_3	Amide	1.44	>3000	>2083
21a-P	2	O	CF_3	Imidazole	22.4	>3000	>134
21b-P	1	O	CF_3	Imidazole	1.62	>3000	>1852
21c-P	1	O	H	Imidazole	2.2	54.8	25

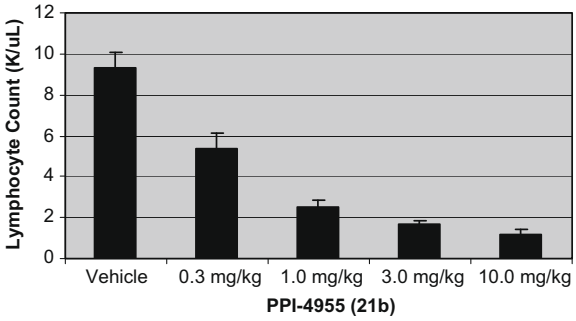


Figure 2. Dose–response lymphopenia for lead compound PPI-4955 (**21b**) relative to the vehicle.

pharmacophore (**21c-P**). All the phenylimidazole analogs also showed a significant potency at both S1P receptor subtypes 4 and 5.

In order to confirm the agonist potency and selectivity, a number of designed phosphates were further investigated in [γ-^{35}S]GTP functional assay[8] as reported in Table 4. The keto-compound **7a-P**, analogous to binding data, once again showed a relatively lower in vitro activity with improved selectivity for S1P subtype 1 over 3. The agonist **16i** activity and selectivity were confirmed in the assay and demonstrated the importance of the SEM group in the phenylamide scaffold. In the phenylimidazole analogs, **21a-P** was relatively less potent with respect to the corresponding phenylamide analog **16i**, but showed an improved selectivity for $S1P_1$ over $S1P_3$. The phenylimidazole analog with a short tail region (**21a-P**, PPI-4955-P) demonstrated over 10-fold improvement in agonist potency while maintaining the high degree of selectivity. The des-CF_3 analog abolished selectivity and again confirmed the importance of the SEM in both phenylamide and phenyimidazole pharmacophore.

Due to high in vivo conversion of PPI-4955 (**21b**) to PPI-4955-P (**21b-P**) and the phosphate receptor profile, the alcohol was chosen as a preclinical lead molecule for further evaluation. PPI-4955 was investigated in an in vivo dose–response when orally administered in mice. The agonist showed significant lymphopenia at all doses between 0.3 mg/kg and 10 mg/kg at 6 h post-dose in mice (Fig. 2). Overall, PPI-4955 demonstrated excellent dose responsiveness when administered orally at doses between 0.3 and 10 mg/kg with maximal activity observed at doses of 1 mg/kg and above.

In summary, in pursuit of improving the agonist selectivity for S1P receptor subtype 1 over subtype 3, we have identified a selectivity enhancing moiety (SEM) in both phenylamide and phenylimidazole series. The *ortho*-CF_3 substitution adjacent to the tail section of the pharmacophore was found to be an excellent SEM with a general improvement of 100- to 500-fold in agonist selectivity for S1P receptor 1 over 3. We demonstrated the importance of the SEM orientation leading to discovery of the selectivity enhancing orientation (SEO) for the CF_3 group. In pursuit of improving in vivo conversion of the pro-drug alcohol to phosphate drug, we have discovered a potent and selective lead molecule, PPI-4955 (**21b**) with significant improvement in both receptor profile and in vivo phosphorylation. PPI-4955 was selected as a preclinical candidate for further evaluation and was found to be potent with excellent dose responsiveness and overall pharmacodynamic properties upon oral administration. Further SAR studies will be reported in due course.

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