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Synthesis and evaluation of arylalkoxy- and biarylalkoxy-phenylamide and phenylimidazoles as potent and selective sphingosine-1-phosphate receptor subtype-1 agonists

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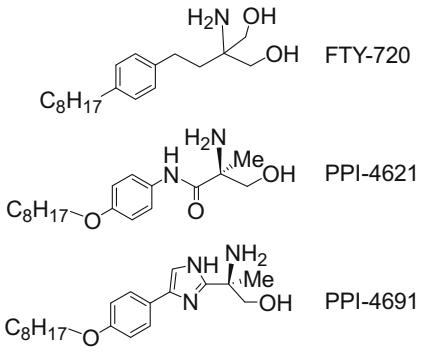
ABSTRACT

In pursuit of potent and selective sphingosine-1-phosphate receptor agonists, we have utilized previously reported phenylamide and phenylimidazole scaffolds to explore extensive side-chain modifications to generate new molecular entities. A number of designed molecules demonstrate good selectivity and excellent in vitro and in vivo potency in both mouse and rat models. Oral administration of the lead molecule **11c** (PPI-4667) demonstrated potent and dose-responsive lymphopenia.

Sphingosine-1-phosphate (S1P) receptors, a class of G-protein coupled receptors (GPCRs), are important molecular receptors for drug discovery and development because of the significant role they play in a diversity of physiological and pathophysiological processes.[1] Activation or antagonism of members of this cluster of five receptors ($S1P_{1-5}$) with the natural ligand S1P can induce various effects on cardiovascular and immune system function and other yet poorly defined effects on additional physiological systems including pulmonary function.[1]

FTY-720 phosphate, a potent non-selective S1P receptor agonist, has profound immunomodulatory activity through alteration of lymphocyte trafficking.[2] This activity is due to activation of S1P receptor subtype 1 ($S1P_1$) signaling cascades. In contrast, agonists of S1P receptor subtype 3 ($S1P_3$) have been associated with negative chronotropic effects in preclinical studies which may translate into clinical side effects including bradycardia.[3] We recently reported two classes of S1P receptor agonists with excellent potency at the $S1P_1$ receptor with good oral activity (Scheme 1).[4] In order to retain the positive therapeutic properties while further reducing the potential for side effects of non-selective S1P receptor agonists like FTY-720, we have explored structural modifications to



Scheme 1.

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these scaffolds to further improve the agonist selectivity for S1P receptor subtype 1 over 3. In the design of potent sphingosine-1-phosphate receptor agonists in the phenylamide and phenylimidazole scaffolds, we reported two potent lead molecules, PPI-4621 and PPI-4691 (Scheme 1), with moderate selectivity for $S1P_1$ versus $S1P_3$ and significant in vivo activity in mouse.[4]

To further improve agonist selectivity for $S1P_1$ over $S1P_3$, we explored extensive tail modifications and developed a robust structural–activity relationship (SAR) in the phenylamide scaffold series (Fig. 1). The initial effort was focused on carbocycle and heterocycle insertion (Q) in the tail region of PPI-4621, where X can be an inserted linker, and developed an early SAR to access potent orally active molecules using in vivo lymphopenia as the biological endpoint.

One general approach for the synthesis of the desired agonists is described in Scheme 2. Nucleophilic substitution of the desired alcohol[5] on 1-fluoro-4-nitrobenzene (**1**, Z = F) in the presence of base afforded nitrobenzene **2**. An alternative approach to synthesis of nitrobenzene **2** was through Mitsunobu reaction of 4-nitrophenol (**1**, Z = OH) with the desired alcohol.

Hydrogenation of the nitro group followed by peptidic coupling of the aniline with (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid using either *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIPEA) or *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and DIPEA afforded protected amino-alcohols **3**. An alternative approach to synthesis of **3a–3o** was through HATU coupling of (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid with 4-aminophenol followed by copper(II)-promoted coupling of various arylboronic acids with the phenol to generate biaryl-ether desired products. Removal of the Boc protecting group with trifluoroacetic acid (TFA) afforded final compound **4**.

A set of designed molecules are reported in Table 1. These amino-alcohols were orally administered to determine their in vivo activity by measuring redistribution of circulating lymphocytes in the mouse 6 h after administration of the compounds. When the tail was modified to a phenyl group or phenyl group substituted at positions 4- and/or 3- with an electron conductive halogen, an electron withdrawing group, or an electron rich alkyl or alkoxyl, no lymphopenia was observed (compounds **4a**–**4e** and **4g**–**4o**). However, the 4-biphenyl tail provided significant lymphopenia. When the X was changed from O to CH_2CH_2O little or no change in absolute lymphocyte count was observed regardless of the substitution on the phenyl group (Q) or when a naphthyl group was utilized (compounds **4p**–**4v**). Change of the R-Q group to a 4-biaryl system provided good to excellent activity especially when R

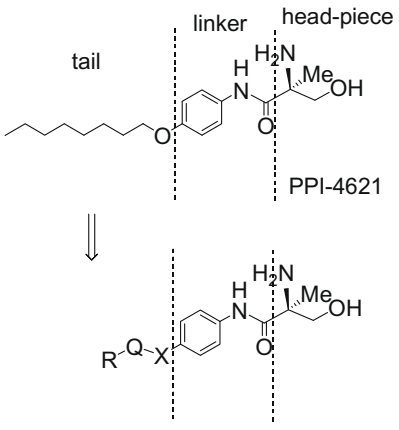
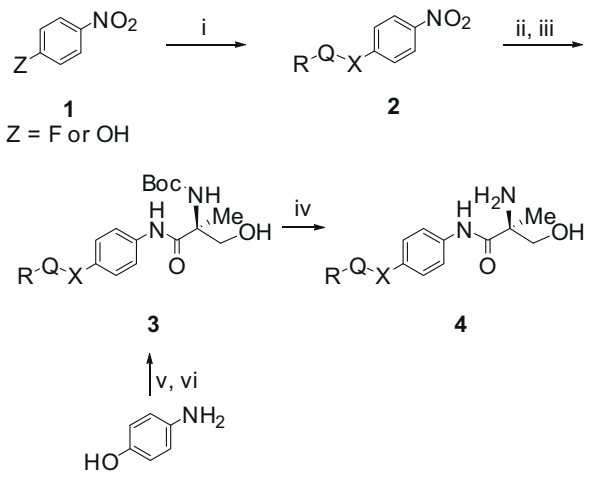


Figure 1. General approach for tail modification in PPI-4621.



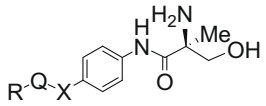
Scheme 2. Reagents and conditions: (i) alcohol, NaH or KO^{t}Bu, THF, 60–70 °C, or alcohol, PPh_3, diethylazodicarboxylate, CH_2Cl_2; (ii) H_2, 10% Pd/C, MeOH or N_2H_4, 10% Pd/C, EtOH, 80 °C; (iii) (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, EDC and HOBt or HATU, DIPEA, DMF or CH_2Cl_2; (iv) TFA, CH_2Cl_2; (v) (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, HATU, DIPEA, DMF; (vi) $ArB(OH)_2$, $Cu(OAc)_2$, molecular sieves, pyridine, CH_2Cl_2.

was unsubstituted phenyl or contained small-sized substitutions regardless of the electronic character (**4w**–**4ae**). When 3-biphenyl was used in place of 4-biphenyl the compound lost activity (**4af**). Heteroaromatic substitution at R was also well tolerated (**4ag**–**4ak**). When the X group was modified to C4 and C5 ethers, moderate to excellent in vivo activity was observed with R-Q being a simple aryl or cyclohexyl group.

In another series of tail modifications, we chose to explore the invention of a substituted phenyl system without the ether connection. The compounds were synthesized as described in Scheme 3. Suzuki cross-coupling of the boronic acid **6** with 4-bromoaniline **5** provided 4-biphenylamino **7**.[6] 4-Biphenylamino **7** was then coupled with (*R*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid treated with TFA to generate the final compound **9**. The in vivo biological activity is reported in Table 2. When R was a *para*-substituted Me or Et little or no reduction in circulating lymphocyte count was observed (**9a** and **9b**). *meta*-Substitution with an alkyloxy group gave a similar result to **9a** and **9b** regardless of the nature of the alkyl group. The 3,4-methylenedioxy group (**9g**) demonstrated the greatest lymphopenia, 40%, in this series. Therefore, regardless of the R substitution on the phenyl group, low to moderate lymphopenia was induced by this series of molecules.

In order to determine the agonist binding activity for both $S1P_1$ and $S1P_3$, we selected several amino-alcohols based on the absolute in vivo lymphocyte reduction and synthesized the corresponding phosphates. These were synthesized through two different approaches as reported in Scheme 4. In the first approach, the reaction of free amine or Boc-protected amino-alcohol with excess diethyl chlorophosphate in the presence of triethylamine afforded phospho-triester **10**. The phopho-triester **10** was then treated with excess bromotrimethylsilane to give the final phosphate **11** after preparative HPLC purification. An alternative approach for phosphate synthesis was through synthesis of the intermediate di-*tert*-butyl phosphate using di-*tert*-butyl diisopropylphosphoramidite and 1*H*-tetrazole followed by oxidation to phosphate ester and removal of the *tert*-butyl groups by a method analogous to that reported by Clemens and co- workers.[7] S1P and synthetic phosphate agonist binding activities were measured using a [^{33}P]S1P receptor binding assay according to an earlier report (Table 3).[4] In the 4-biphenyl system when X was O, the agonist showed low

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

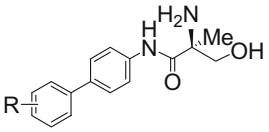


Agonist	R	Q	X	%L[a]
4a	H	Ph	O	N
4b	4-F	Ph	O	N
4c	4-Cl	Ph	O	N
4d	4-Et	Ph	O	N
4e	4-*n*Bu	Ph	O	10
4f	4-Ph	Ph	O	81
4g	4-O*n*Bu	Ph	O	N
4h	3-CF_3	Ph	O	N
4i	3-*i*Pr	Ph	O	N
4j	3-OMe	Ph	O	N
4k	3-OEt	Ph	O	N
4l	3-O*n*Pr	Ph	O	33
4m	3-O*i*Pr	Ph	O	N
4n	3-O*n*Bu	Ph	O	N
4o	3-OBn	Ph	O	10
4p	4-OMe	Ph	CH_2CH_2O	N
4q	4-OEt	Ph	CH_2CH_2O	N
4r	4-Cl	Ph	CH_2CH_2O	N
4s	3,4-diCl	Ph	CH_2CH_2O	10
4t	4-CF_3	Ph	CH_2CH_2O	N
4u	3-CF_3	Ph	CH_2CH_2O	N
4v	H	Naphthyl	CH_2CH_2O	N
4w	4-Ph	Ph	CH_2CH_2O	81
4x	4′-OEt-4-Ph	Ph	CH_2CH_2O	10
4y	4′-Et-4-Ph	Ph	CH_2CH_2O	N
4z	4′-*i*Pr-4-Ph	Ph	CH_2CH_2O	N
4aa	4′-Cl-4-Ph	Ph	CH_2CH_2O	N
4ab	3′-CF_3-4-Ph	Ph	CH_2CH_2O	51
4ac	2′-Me-4-Ph	Ph	CH_2CH_2O	79
4ad	2′-Cl-4-Ph	Ph	CH_2CH_2O	75
4ae	2′-CN-4-Ph	Ph	CH_2CH_2O	78
4af	3-Ph	Ph	CH_2CH_2O	N
4ag	4-(4-Pyridinyl)	Ph	CH_2CH_2O	78
4ah	4-(3-Pyridinyl)	Ph	CH_2CH_2O	60
4ai	4-(2-Thiophenyl)	Ph	CH_2CH_2O	78
4aj	4-(3-Furanyl)	Ph	CH_2CH_2O	68
4ak	4-(3,5-Dimethylisoxazol-4-yl)	Ph	CH_2CH_2O	62
4al	H	Ph	$CH_2(CH_2)_3O$	85
4am	H	Ph	$CH_2(CH_2)_4O$	64
4an	4-OMe	Ph	$CH_2(CH_2)_3O$	60
4ao	H	2-Thiophenyl	$CH_2(CH_2)_3O$	66
4ap	H	c-Hexyl	$CH_2(CH_2)_3O$	74

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

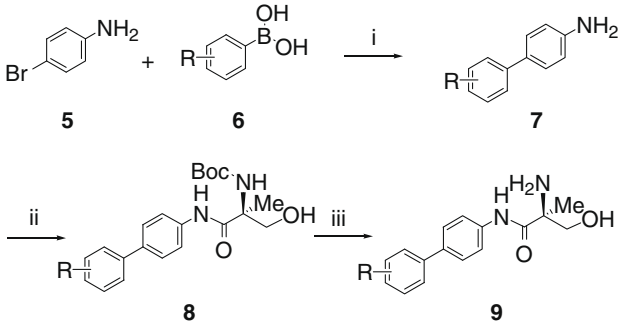
binding activity at both human receptors $S1P_1$ and $S1P_3$ with little selectivity (**11a**). The naphthyl group with X being an ethyl ether group also had low binding activity at both receptors (**11b**). However, the 4-biphenyl group with X being ethyl ether showed substantial improvement over the X = O counterpart in both $S1P_1$ binding activity and receptor selectivity (**11c**). Distortion of the coplanar structure of the two phenyl rings in the 4-biphenyl system with *ortho*-substitution was pursued (**11d**–**11f**). The 2-Cl (**11e**) substitution performed better than the 2-Me (**11d**) and 2-CN (**11f**) counterparts by increasing the binding activity at $S1P_1$ and maintaining similar selectivity as the corresponding 4-biphenyl analog (**11c**). Changing the phenyl position in the biphenyl system from 4- to 3- was detrimental to $S1P_1$ binding activity (**11g**). The 4-(2-thiophyl)phenyl analog showed relatively lower activity at $S1P_1$ with respect to the corresponding 4-biphenyl analog. Elongation of the X group to 5 and 6 atoms showed a good range of activities at $S1P_1$ except with lower selectivity for $S1P_1$ over $S1P_3$ (**11i**–**11m**). In general, the agonists evaluated (**11a**–**m**) demonstrated good to excellent binding activity at S1P receptor subtype 4 ($S1P_4$) with the exception of **11a**. Compound **11c** not only showed good selectivity for receptor subtype 1 over 3, it also showed similar selectivity for $S1P_1$ over $S1P_5$ as well.

Table 2
Percent lymphopenia obtained upon 10 mg/kg oral (PO) administration of the alcohol



Agonist	R	%L[a]
9a	H	N
9b	4-Me	18
9c	4-Et	29
9d	4-*n*Bu	N
9e	4-OMe	N
9f	4-O*n*Bu	N
9g	3,4-Methylenedioxy	40
9h	3-*i*Pr	22
9i	3-OMe	34
9j	3-OEt	15
9k	3-O*n*Pr	20
9l	3-O*i*Pr	23
9m	3-O*n*Bu	16
9n	3-OBn	31

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

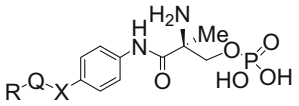


Scheme 3. Reagents and conditions: (i) 10% Pd/C, tetrabutyl ammonium chloride (TBAC), Na_2CO_3, DMF/H_2O, microwaved 10–60 min, 60–120 °C; (ii) (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, HATU, DIPEA, CH_2Cl_2; (iii) TFA, CH_2Cl_2.

The 4-biphenyl group appeared to be important in both receptor selectivity (**11c**) and in vivo bioactivity (**4w**). Further modifications were made to explore the linker length between the ether and 4-biphenyl group to potentially improve the agonist binding activity and selectivity for $S1P_1$ over $S1P_3$ (Table 4). When *n* was 2 (**11o**), the agonist maintained similar binding activity with reduced selectivity and in vivo activity with respect to the lead molecule **11c** (PPI-4667). However, when n was either 0 or 3, all three parameters (potency, selectivity, in vivo activity) were reduced significantly (**11n** and **11p**). All the agonists in this series (**11c** and **11n**, **11o**, and **11p**) had potent activity on $S1P_4$ with reduced activity at the $S1P_5$.

Modification of the amide linker region to a rigid imidazole ring is reported in Scheme 5 and Table 5. Nucleophilic substitution of the desired alcohol on 4-fluoroacetophenone (**13**) afforded the ether-acetophenone **14**. The ether-acetophenone **14** was then converted to the bromo-acetophenone using $CuBr_2$. Reaction of the bromo-acetophenone with (*S*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid gave the intermediate ester which

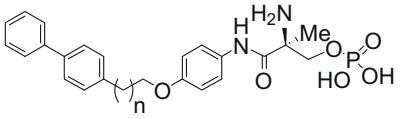
Table 3
[^{33}P]S1P binding activity on human $S1P_1$ and $S1P_{3-5}$ receptor subtypes



Agonist	R	Q	X	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	—
11a	4-Ph	Ph	O	186	628	123	455	3.3
11b	H	Naphthyl	C_2H_4O	220	2500	nd	nd	11
11c	4-Ph	Ph	C_2H_4O	1.05	200	4.17	122	190
11d	2′-Me-4-Ph	Ph	C_2H_4O	0.78	50	nd	nd	64
11e	2′-Cl-4-Ph	Ph	C_2H_4O	0.84	160	nd	nd	190
11f	2′-CN-4-Ph	Ph	C_2H_4O	12	1040	nd	nd	87
11g	3-Ph	Ph	C_2H_4O	2000	10000	nd	nd	5
11h	4-(2-Thiophenyl)	Ph	C_2H_4O	21	3750	nd	nd	178
11i	H	Ph	C_4H_8O	2.1	100	10.3	43.6	48
11j	H	Ph	$C_5H_{10}O$	0.21	0.36	3.6	2.9	1.7
11k	4-OMe	Ph	C_4H_8O	4.75	100	nd	nd	21
11l	H	2-Thiophenyl	C_4H_8O	30	120	nd	nd	4
11m	H	c-Hexyl	C_4H_8O	1.2	31.3	8.3	26.2	25

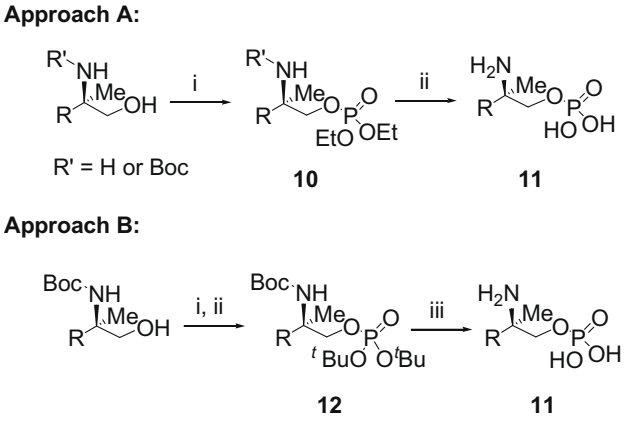
nd = not determined.

Table 4
[^{33}P]S1P binding activity on human $S1P_1$ and $S1P_{3-5}$ receptor subtypes



Agonist	*n*	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]
11c	1	1.05	200	4.17	122	190	81
11n	0	2.5	21.1	6.15	104	8.4	28
11o	2	0.9	27.1	5.24	70.4	61	61
11p	3	6.9	10.2	24.8	99.8	1.5	39

[a] %L = Lymphopenia (percent decrease in circulating lymphocytes compared to time-matched vehicle control after 6 h upon 10 mg/kg oral (PO) administration of the corresponding alcohol.



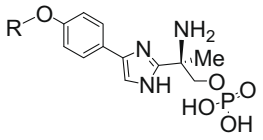
Scheme 4. Reagents and conditions: *Appraoch A*: (i) excess diethyl phosphorochloridate, Et_3N, CH_2Cl_2, rt, 12–18 h; (ii) excess TMSBr, CH_2Cl_2, rt, 4–10 h; *Approach B*: (i) di-*tert*-butyl diisopropylphosphoramidite, 1*H*-tetrazole, THF or THF/CH_2Cl_2 (1:1), rt, 2–12 h; (ii) excess H_2O_2, rt, 1–6 h; (iii) TFA/CH_2Cl_2 (1:3 v/v), rt, 1–3 h.

was then converted to the imidazole precursor **16** using $AcONH_4$. Deprotection of the Boc group provided the amino-alcohol **17** (see Scheme 5).

The imidazole analog of **11c** (PPI-4667) is **11q** which showed a good overall profile but with relatively reduced binding activity and selectivity in vitro and less potent in vivo activity on lymphopenia when the corresponding alcohol was administered orally. However, decreasing the length of the ethylene section of R to a methylene generated a more potent agonist **11r** with similar selectivity to **11q**. Two other analogs in which R was either phenylbutylene (**11s**) or phenyl-pentylene (**11t**) showed excellent lymphocyte reduction, with **11t** demonstrating a relative improvement in binding activity with respect to lead molecule **11c** (PPI-4667) and its corresponding imidazole analog **11q**. The imidazole series did not generate any agonists with overall improvement in profile over **11c** (PPI-4667), but it demonstrated once again the potential for transferring the SAR information from the phenylamide series to the phenylimidazole series with relatively reduced selectivity for $S1P_1$ over $S1P_3$.

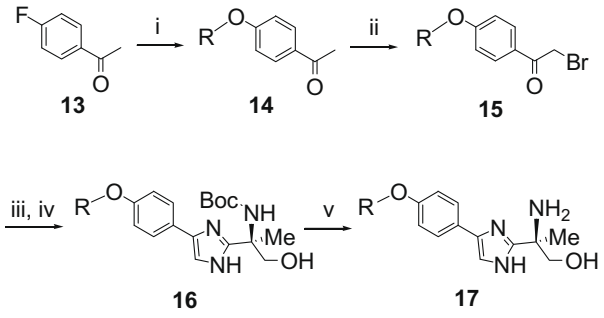
A [^{35}S]GTPγS functional assay[8] was used to further characterize the agonist potency and selectivity for $S1P_1$ over $S1P_3$ (Table 6). The lead molecule **11c** showed excellent potency at $S1P_1$ and a high degree of selectivity for $S1P_1$ over $S1P_3$. The biphenyl ether analog **11a** had marginal potency at $S1P_1$. Elongation of X from C2 to C3 and C4 decreased the receptor subtype 1 potency as well as agonist selectivity. Increased flexibility in the tail region either decreased the agonist potency (**11i**) or the selectivity (**11j** and **11m**). The imidaz-

Table 5
[^{33}P]S1P binding activity on human $S1P_1$ and $S1P_{3-5}$ receptor subtypes



Agonist	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]
11q	4-Biphenyl-CH_2CH_2	7.7	210	26.9	207	27	60
11r	4-Biphenyl-CH_2	1.03	20	4.9	9.6	19	84
11s	$PhCH_2CH_2CH_2CH_2$	0.9	20	11.7	12.8	22	82
11t	$PhCH_2CH_2CH_2CH_2CH_2$	0.2	6	1.23	3.5	30	81

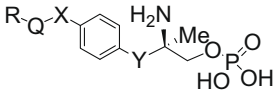
[a] %L = Lymphopenia (percent decrease in circulating lymphocytes compared to time-matched vehicle control after 6 h upon 10 mg/kg oral (PO) administration of the corresponding alcohol.



Scheme 5. Reagents and conditions: (i) R-OH, KO^{t}Bu, THF, 80 °C; (ii) $CuBr_2$, ethyl acetate/chloroform, reflux; (iii) (*R*)-2-*tert*-butoxycarbonylamino-3-hydroxy-2-methyl-propionic acid, Cs_2CO_3, DMF; (iv) $AcONH_4$, toluene, 120 °C; (v) TFA, CH_2Cl_2.

ole analog of lead molecule **11c** (**11q**) resulted in a major reduction in potency while its C1 analog (**11r**) possessed excellent potency at $S1P_1$ with reduced selectivity with respect to **11c**. The less rigid compounds **11s** and **11t** had good $S1P_1$ activity at the expense of selectivity. The data in Table 6 confirmed the previous observation based on receptor binding data, that compound **11c** (PPI-4667) had the best potency for $S1P_1$ and widest selectivity profile in both the phenylamide and phenylimidazole series.

Table 6
[^{35}S]GTPγS functional activity on human $S1P_1$ and $S1P_3$ receptor subtypes



Agonist	Y	R	Q	X	hS1P$_1$ EC$_{50}$ (nM)	hS1P$_3$ EC$_{50}$ (nM)	S1P$_3$/ S1P$_1$
S1P	—	—	—	—	5.6	2.4	—
11a	Amide	4-Ph	Ph	O	247	>3000	>12
11n	Amide	4-Ph	Ph	CH_2O	56.1	47.3	0.8
11c	Amide	4-Ph	Ph	C_2H_4O	0.52	120	231
11o	Amide	4-Ph	Ph	C_3H_6O	2.9	63.3	21.8
11p	Amide	4-Ph	Ph	C_4H_8O	37	42.2	1.1
11i	Amide	H	Ph	C_4H_8O	44.1	>3000	>68
11j	Amide	H	Ph	$C_5H_{10}O$	1.1	1.38	1.3
11m	Amide	H	c-Hexyl	C_4H_8O	2.67	28.8	10.8
11r	Imidazole	4-Ph	Ph	CH_2O	2.2	54.8	24.9
11q	Imidazole	4-Ph	Ph	C_2H_4O	37.2	>3000	80.7
11s	Imidazole	H	Ph	C_4H_8O	7.3	117	16
11t	Imidazole	H	Ph	$C_5H_{10}O$	10.2	285	27.9

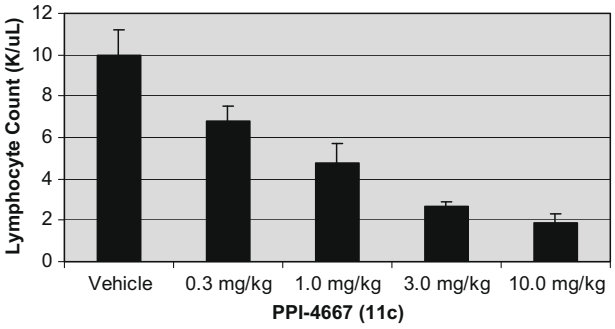


Figure 2. Dose-responsive lymphopenia for lead compound **11c** relative to the vehicle in mice 6 h after oral administration.

The lead molecule **11c** (PPI-4667) was further investigated for potency in vivo when orally administered. 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, the lead molecule **11c** demonstrated excellent dose responsiveness when administered orally at doses between 0.3 and 10 mg/kg with maximal activity observed at doses of 3 mg/kg and above. Following oral dosing of the lead molecule **11c** (PPI-4667) in mice at 1 mg/kg, plasma concentrations of phosphorylated active drug (0.4–1.0 ng/mL) were detected for 48 h after dosing that correlated with a duration of lymphopenia of greater than 48 h.

In summary, we have generated a detailed SAR in both the phenylamide and phenylimidazole scaffolds. We have obtained potent and selective molecules in both scaffolds. The lead molecule **11c** (PPI-4667) was chosen based on its potency and selectivity for further in vivo investigation. PPI-4667 was found to be potent and selective with an excellent in vivo dose response and good pharmacodynamic properties when orally administered. Further optimization of this scaffold to increase in vivo conversion of prodrug to active phosphate is warranted.

References and notes

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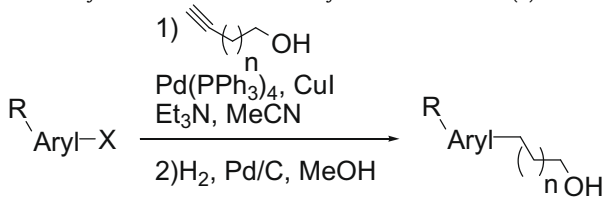
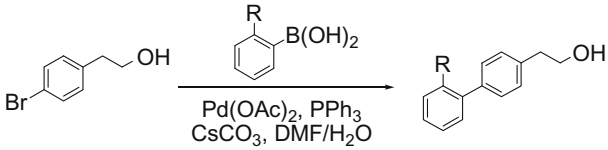
5. Commercially unavailable alcohols were synthesized as follows: (a)



(b) 2-(biphenyl-4-yl)ethanol was synthesized from 2-(biphenyl-4-yl)acetic acid via BH_3 reduction at 0 °C



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