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Letter

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Discovery of Clinical Candidate GSK1842799 As a Selective $S1P_1$ Receptor Agonist (Prodrug) for Multiple Sclerosis

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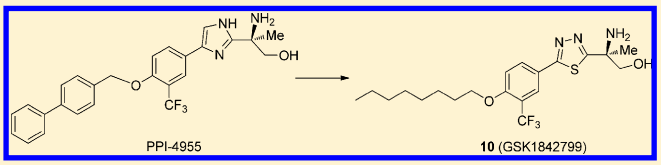
Supporting Information

ABSTRACT: To develop effective oral treatment for multiple sclerosis (MS), we discovered a series of alkyl-substituted biaryl amino alcohols as selective $S1P_1$ modulators. One exemplar is (*S*)-2-amino-2-(5-(4-(octyloxy)-3-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)propan-1-ol (**10**, GSK1842799). Upon phosphorylation, the compound (**10**-P) showed subnanomole $S1P_1$ agonist activity with >1000× selectivity over $S1P_3$. The alcohol **10** demonstrated good oral bioavailability and rapid in vivo conversion to **10**-P. Dosed orally at 0.1 mg/kg, **10** significantly reduced blood lymphocyte counts 6 h postdose, and at 3 mg/kg, **10** achieved efficacy equivalent to FTY720 in the mouse EAE model of MS. Further pharmacokinetic/pharmacodynamic (PK/PD) study with cynomolgus monkeys indicated that, after oral dosing of **10** at 3.8 mg/kg, the active phosphate reached plasma levels that are comparable to FTY-720 phosphate (FTY-P) revealed in human clinical pharmacokinetics studies. On the basis of the favorable in vitro ADME and in vivo PK/PD properties as well as broad toxicology evaluations, compound **10** (GSK1842799) was selected as a candidate for further clinical development.



KEYWORDS: *$S1P_1$ modulator, biaryl aminoalcohol, prodrug, multiple sclerosis, mouse EAE model*

Chronic demyelination and scarring of the neuronal cells in brain, spinal cord, and central nervous system lead to a debilitating neurodegenerative autoimmune disorder known as multiple sclerosis (MS). This disease has an onset usually in young adults and more commonly in females, affecting lives of hundreds and thousands of people across the globe.[1] This inflammatory disorder poses diverse neurological symptoms often leading to physical and cognitive disability.[2] The emergence of FTY-720 (Fingolimod) as a novel class of orally active immune-modulating agents for multiple sclerosis and solid organ transplant rejection prevention has established the sphingosine-1-phosphate (S1P) receptor class of G-protein coupled receptors (GPCRs) as a competitive and promising field of research and development. FTY-720 is a prodrug that, upon in vivo phosphorylation, activates the $S1P_1$ receptor and sequesters lymphocytes in lymph nodes, preventing them from trafficking to lymphoid tissues and contributing to autoimmune reactions.[3] FTY-720 reduces both relapses and disability progression in relapsing-remitting multiple sclerosis (RRMS) patients.[4−7] However, aside from the clinical benefits of FTY-720, a number of adverse side effects such as bradycardia, skin cancer, liver injury, infections, and increased blood pressure were attributed to activity on $S1P_3$, leaving a window of opportunity for exploration of alternative chemotypes with improved overall profiles.

Recently, we published a number of novel $S1P_1$ agonists, a few of which fall within the same class of prodrug as FTY-720 that require in vivo phosphorylation in order to trigger any effects on S1P receptors (Table 1).[8−11] The phosphorylation of prodrug FTY-720 to its active drug FTY-720-monophosphate is facilitated by sphingosine kinase 2 (SphK2).[12] One major challenge in the prodrug strategy in S1P activation is its in vivo phosphorylation of the prodrug to the desired phospho-drug since this process requires prodrug phosphorylation with either SphK1 or SphK2, while the phospho-drug could be converted back to the starting prodrug through the action of sphingosine-1-phosphate phosphatase 1 (S1PP1), sphingosine-1-phosphate phosphatase 2 (S1PP2), or a potential lipid phosphate phosphatase.[13] In our most recent prodrug publication,[10] we reported on preclinical candidate PPI-4955 (Table 1) and its phosphorylation outcome in rodent models. In an effort to establish a backup molecule to PPI-4955 with improved profile especially in in vivo phosphorylation, we carried out further structure−activity relationship (SAR) studies with a focus on sphingosine kinase activity and in vivo phosphorylation relationships. Since the PPI-4955 series are structurally homologous to FTY-720 and human SphK2 is known to phosphorylate FTY-720 30-fold better than SphK1,[14] our structural modifications were mainly driven by SphK2 activity. Herein, we report our compound design and synthesis as well as in vitro and in vivo activity data that led to the discovery of clinical development candidate GSK1842799.

Our SAR study started with the modification of the head-piece amino-alcohol and the impact on both human SphK2

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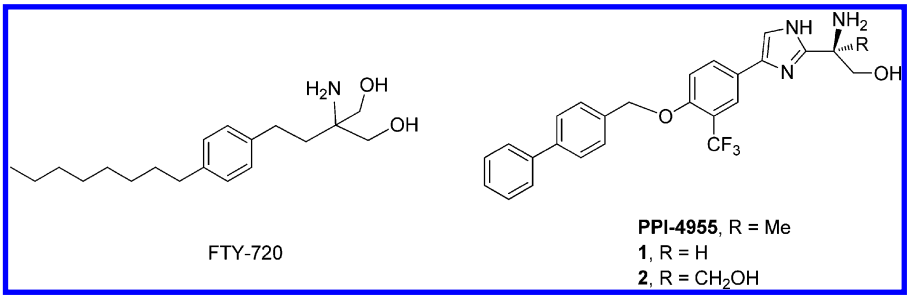
Table 1. Effects of Head-Piece Modification on SphK2 Activity, in Vivo Phosphorylation, and $S1P_1$ Activity and Selectivity



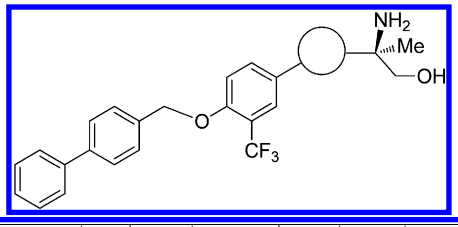
phosphate	R	$S1P_1$ (nM)[a]	$S1P_3$ (nM)[a]	selectivity ($S1P_1$/ $S1P_3$)	prodrug	mSphK2 (%FTY)	hSphK2 (%FTY)	% in vivo PO_4 (mice)[b]
FTY-720-P		1.20	13.3	11	FTY-720	100	100	70
PPI-4955-P	CH_3	0.83	7352	8862	PPI-4955	49.6	7.8	59
1-P	H	0.30	650	2166	**1**	5.2	2.8	3.0
2-P	CH_2OH	2.3	>10000	>4348	**2**	1.9	1.9	20

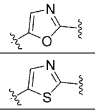
[a][^{33}P] binding activity on $S1P_1$ and $S1P_{3-5}$ receptor subtypes. [b]Percent conversion of alcohol (prodrug) to phosphate (drug) at 6 h dose administration upon 10 mg/kg oral (PO) administration of the alcohol.

(hSphK2) and mouse SphK2 (mSphK2) activity with respect to PPI-4955. The biological data for PPI-4955 and head-piece modified analogues **1** and **2** as well as FTY-720 (as control) are reported in Table 1. In pursuit of better understanding PPI-4955 and analogue activity as substrates of SphK2 and the correlation of this data with in vivo phosphorylation, all the compounds were tested for in vitro activity against mSphK2 and hSphK2 as well as in vivo conversion to the corresponding phosphates in mouse upon oral administration. In comparison to FTY-720 phosphate (FTY-720-P), PPI-4955 phosphate (PPI-4955-P) showed better potency and much improved selectivity for $S1P_1$ over $S1P_3$. Setting FTY-720 activity against mSphK2 and hSphK2 at 100%, PPI-4955 performed reasonably well in mSphK2 activity (about 50%) correlating to mouse in vivo phosphorylation of 59% compared to FTY-720 of 70%. However, activity of PPI-4955 against hSphK2 was low (7.8%) with respect to activity of FTY-720, indicating a difference between mouse and human SphK2 at the substrate binding site and a potential point for further improvement in SphK2 activity. The first attempt at modification of the head-piece, where the R group was changed either into a proton (**1**) or a hydroxymethyl (**2**), led to decreased SphK2 activities. However, phosphates of both compounds still showed potent $S1P_1$ binding activity and good selectivity against $S1P_3$.

Chemical modification of the PPI-4955 imidazole ring was then explored. Similarly, all of the corresponding compounds were tested for in vitro activity as potential substrates for mSphK2 and hSphK2 as well as in vivo phosphorylation in mouse upon oral administration. A quick profiling of the azole compounds generated provided insight and guidance in further SAR exploration (Table 2). As S1P receptor subtype binding data indicated, all the corresponding phosphates for compounds **3**–**5** and **6**–**8** maintained excellent $S1P_1$ binding activity and analogous selectivity for $S1P_1$ over $S1P_3$ as PPI-4995-phosphate. For sphingosine kinase activity, while the added 4-methyl group on the imidazole ring (**3**) was detrimental for both mouse and human SphK2 enzymes, the corresponding 5-methyl oxazole analogue (**4**) gave a similar profile as that for PPI-4955. The 2,4-disubstituted thiazole (**5**) and 2,5-disubsituted oxazole (**6**) analogues were less active than imidazole-containing PPI-4955. Interestingly, the 2,5-substituted thiazole (**7**) and 1,3,4-thiadiazole (**8**) showed much improved activity against both mSphK2 and hSphK2 enzymes,

Table 2. Structural Modification of PPI-4955 Imidazole Ring Effects on Agonist Profile



phosphate	R	$S1P_1$ (nM)[a]	$S1P_3$ (nM)[a]	Selectivity ($S1P_1$/ $S1P_3$)	Prodrug	mSphK2 (%FTY)	hSphK2 (%FTY)	% *in vivo* PO_4 (mice)[b]
3-P		0.65	7667	11856	**3**	21.7	4.1	40
4-P		2.10	>10 000	>5000	**4**	65	6.0	65
5-P		1.60	>10 000	>6250	**5**	8.7	2.1	5
6-P		1.1	>10 000	>8000	**6**	17.3	4.9	37
7-P		0.73	867	1192	**7**	140	36	89
8-P		1.77	4500	2542	**8**	192	30	90

[a][^{33}P] binding activity on $S1P_1$ and $S1P_{3-5}$ receptor subtypes. [b]Percent conversion of alcohol (prodrug) to phosphate (drug) at 6 h dose administration upon 10 mg/kg oral (PO) administration of the alcohol.

suggesting the SphK2 enzyme activity is very sensitive to the azole moiety modifications where a 2,5-disubstituted thiazole (**7**) and thiadiazole (**8**) orientations are analogous to a para-subtituted phenyl moiety, while the 2,4-disubstituted thiazole (**5**) and 2,5-disubsituted oxazole (**6**) analogues are closer to a meta-substituted phenyl group. Accordingly, the corresponding in vivo phosphorylation levels were also significantly increased (89% and 90%, respectively), a demonstration of the correlation between SphK2 enzyme activity and the in vivo phosphorylation.

The findings in 2,5-disubstituted thiazole **7** and disubstituted-1,3,4-thiadiazole **8** provided the basis and rationale for the next generation agonist design. Previously, it was observed that both FTY-720 and a number of prodrugs with the alkyl tail group,

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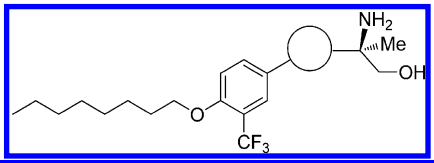
analogous to FTY-720, have demonstrated good mSphK2 and in vivo lymphopenia activity. It was hypothesized that replacement of the tail group in both **7** and **8** with an alkyl group might lead to further improvement in SphK2 activity. As per earlier reports,[8] the best alkyl tail group with improved lead prodrug profile was octane ether; thus, a series of azole analogues with the *n*-octyl tail group were synthesized as shown in Table 3. Investigation of SphK2 activity of the prodrugs with

Table 3. mSphK2 and hSphK2 Activity of Prodrug Analogues Containing Octyl Ether Tail



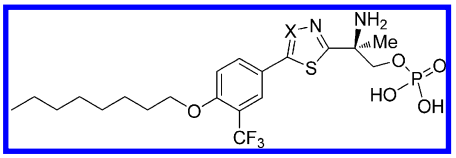
phosphate	R	$S1P_1$ (nM)[a]	$S1P_3$ (nM)[a]	Selectivity ($S1P_1$/$S1P_3$)	Prodrug	mSphK2 (%FTY)	hSphK2 (%FTY)	% Lymph.[b] (10 mpk, PO)	% *in vivo* PO_4 (mice)[c]
9-P		0.18	300	1667	**9**	228	155	98	98
10-P		0.52	1898	3287	**10**	339	142	119	99
11-P		27.8	>10 000	>359	**11**	8.7	49	-	-
12-P		0.83	>10 000	>12048	**12**	90	37	-	-
13-P		0.14	170	1214	**13**	91	84	-	-
14-P		1.9	>10 000	>5263	**14**	90	69	-	-
15-P		1.46	4867	3341	**15**	14	14	-	-

[a][^{33}P] binding activity on $S1P_1$ and $S1P_{3-5}$ receptor subtypes. [b]Percent lymphopenia observed in mouse 6 h postdose upon 10 mg/kg oral (PO) administration of the corresponding alcohol in comparison to oral administration of 1 mg/kg of FTY-720. [c]Percent conversion of alcohol (prodrug) to phosphate (drug) at 6 h dose administration upon 10 mg/kg oral (PO) administration of the alcohol.

tail group and azole ring modifications proved to be instrumental in directing the research effort toward the next generation of the lead molecules. As demonstrated in Table 3, replacement of the [1,1′-biphenyl]-4-ylmethyl ether group with an octyl ether in both **7**-P and **8**-P gave compounds **9**-P and **10**-P, which showed a good improvement in $S1P_1$ binding activity, while still maintaining excellent selectivity for $S1P_1$ over $S1P_3$. At the same time, both prodrugs **9** and **10** showed a substantial improvement in both mSphK2 (2-fold) and hSphK2 (5-fold) over the predecessors **7** and **8**. Both prodrugs **9** and **10** gave analogous lymphopenia profile as FTY720 with quantitative in vivo phosphoryaltion when administered orally in mouse. Further modification of the azole system to oxadiazole **11**-P, pyrazole **12**-P, isoxazole **13**-P, oxadiazole **14**-P, and imidazole **15**-P provided comparable $S1P_1$ binding profile as the compounds **9**-P and **10**-P, while no further improvement was observed in SphK2 activity, especially hSphK2 activity. It was interesting to see that, while the 1,3-thiazole (**9**) and 1,3,4-thiadiazole (**10**) analogues gave the best SphK2 activity profile, the 1,3,4-oxodiazole (**11**) and imidazole (**15**) analogues were poor substrates for SphK2 in the series, suggesting that the insertion of a sulfur atom in the azole ring might have changed the geometry of the molecule significantly.

In order to confirm agonist activity and selectivity of the corresponding phosphates, compounds **9-P** and **10-P** were further investigated in a [γ-^{35}S]GTP functional assay[15] as reported in Table 4. The two compounds showed analogous

Table 4. [γ-^{35}S]GTP Functional Activity of 9-P and 10-P on all S1P Receptor Subtypes



agonist	X	$hS1P_1$ EC_{50} (nM)	$hS1P_2$ EC_{50} (nM)	$hS1P_3$ EC_{50} (nM)	$hS1P_4$ EC_{50} (nM)	$hS1P_5$ EC_{50} (nM)	$S1P_3/S1P_1$
S1P		4.25	1.3	3.94	13	1.8	0.9
9-P	CH	0.44		>3000	3.60	2.00	>6800
10-P	N	0.26		>3000	4.57	0.66	>11500

activity profiles toward all of the S1P receptor subtypes with subnanomolar agonist affinity on $S1P_1$. Neither of the agonists had any binding effect on $S1P_3$, demonstrating an excellent selectivity for $S1P_1$ over $S1P_3$. Notably, both compounds also had potent agonist activity on $S1P_4$ and $S1P_5$.

The lead prodrugs **9** and **10** were evaluated in an in vivo dose–response analysis when orally administered in mice (Figure 1). At the lowest dose of 0.1 mg/kg, both prodrugs

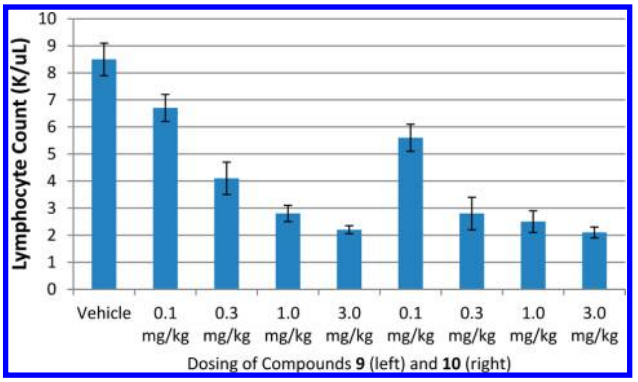


Figure 1. Dose response lymphopenia of lead compounds **9** and **10** relative to the vehicle.

showed a mild reduction in lymphocyte counts, while a significant lymphopenia was observed in all three higher doses. Both compounds showed excellent dose responsiveness when administered orally at doses of 0.1, 0.3, 1.0, and 3.0 mg/kg. The prodrugs **9** and **10** were further evaluated in the MOG peptide experimental autoimmune encephalitis (EAE) mouse multiple sclerosis disease model (Figure 2).[16] In this experiment, both compounds demonstrated efficacy equivalent to FTY-720 when administered orally once a day at 3 mg/kg, nearly completely reversing the signs and symptoms of disease (Figure 2).

In vitro ADME profiles of **9** and **10** met general criteria for a development candidate with reasonable aqueous solubility and protein binding, high permeability in Caco-2 with no efflux, no CYP450 inhibition, and good metabolic stability (Table S1, Supporting Information). An *in vivo* dog PK study suggested phosphate drug exposure, half-life, and oral bioavailability (Table S2, Supporting Information) that support once a day dosing. When dosed orally (single-dose) in multiple species

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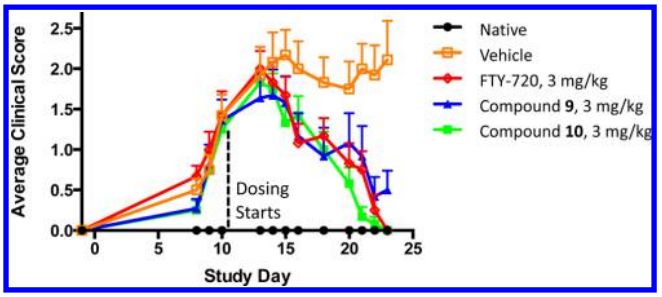
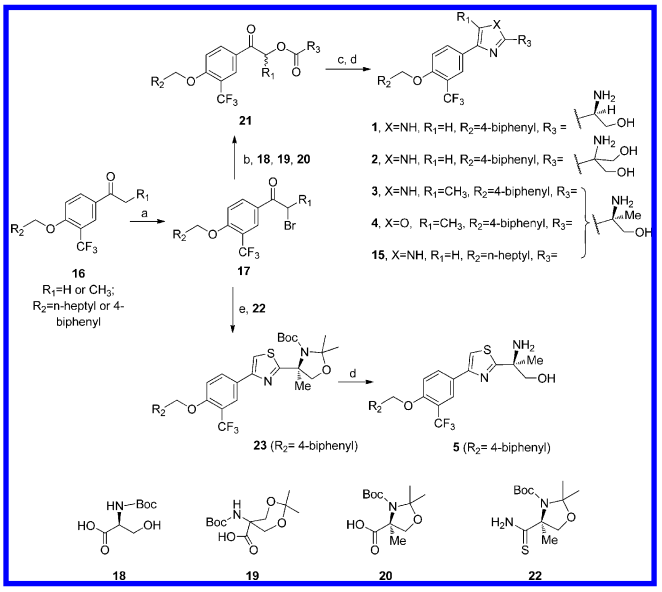


Figure 2. Effect of lead $S1P_1$ agonists **9** and **10** in the MOG peptide EAE model of multiple sclerosis.

(Table S3, Supporting Information), the active phosphate of **10-P** reached plasma levels that ranged ~10−50 ng/mL. In cynomolgus monkeys administered a dose of 3.8 mg/kg, a plasma C_{max} = 50.9 ng/mL was achieved and a lymphocytosis EC_{90} = 16.2 ng/mL, which is comparable to FTY-720 phosphate levels revealed in human clinical pharmacokinetics studies.[17,18] After broad toxicology evaluations, compound **10** (GSK1842799) was selected as a candidate for further development.

Syntheses of the above compounds are outlined in Schemes 1−3. In Scheme 1, analogously to the synthesis of PPI-4955,[10]

Scheme 1. Synthesis of Imidazole, Oxazole, and Thiazole Analogues (1−5 and 15)[a]

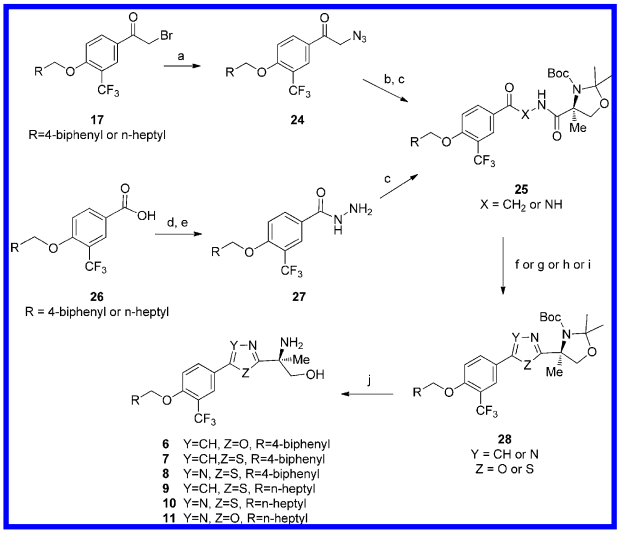


[a]Reagents and conditions: (a) $CuBr_2$, EtOAc/$CHCl_3$ (1:1), reflux; (b) Cs_2CO_3, DMF, **18**, **19**, or **20**; (c) NH_4OAc, toluene, reflux; (d) 6 N HCl, THF or TFA, DCM; or TsOH, MeOH, reflux; (e) **22**, THF, reflux.

the substituted bromo-acetophenones (**17**) were converted to corresponding esters (**21**) by reacting with different head-piece moieties including Boc-serine−OH, 5-(*tert*-butoxycarbonylamino)-2,2-dimethyl-1,3-dioxane-5-carboxylic acid (**18**), 5-((*tert*-butoxycarbonyl)amino)-2,2-dimethyl-1,3-dioxane-5-carboxylic acid (**19**), and (*S*)-3-(*tert*-butoxycarbonyl)-2,2,4-trimethyloxazolidine-4-carboxylic acid (**20**). Using NH_4OAc under toluene refluxing conditions, these esters were cyclized to substituted imidazole or oxazole intermediates, which upon acidic treatment provided final compounds **1**, **2**, **3**, **4**, and **15**. Meanwhile, the head-piece (**20**) was first converted to amide then to thioamide (**23**) by Lawesson's reagent under THF refluxing condition. The thioamide further reacted with bromo-acetophenone (**17**, R_1 = H) to form 2,4-disubstituted thiazole (**23**), which afforded the desired compound **5** after removal of the protecting group.

In Scheme 2, the intermediate **25**, prepared from bromo-acetophenone (**17**) through azide and amine intermediates

Scheme 2. Synthesis of Oxazole, Thiazole, Thiadiazole, and 1,3,4-Oxadiazole Analogues 6−11[a]



[a]Reagents and conditions: (a) (i) $CuBr_2$, EtOAc/$CHCl_3$ (1:1), reflux; (ii) NaN_3, DMF; (b) H_2 (gas), 5% Pd−C, MeOH, HCl; (c) **20**, HATU, DIPEA, DCM/DMF; (d) R^2CH_2OH, KO^{t}Bu, THF, 70 °C; (e) hydrazine, HATU, DIPEA, DCM/DMF; (f) Lawesson's reagent, toluene, 120 °C; (g) Lawesson's reagent, DCM; (h) PPh_3 (5 equiv), hexachloroethane (C_2Cl_6, 2.5 equiv), TEA (10 equiv); (i) PPh_3, CCl_3CN, CH_3CN, MW, 120 °C, 20 min; (j) 6 N HCl, dioxane or TFA, DCM; or TsOH, MeOH, reflux.

followed by coupling with the orthogonally protected α-methyl serine (**20**) or from 3-trifluoromethyl-4-fluorobenzoic acid (**26**) via hydramide formation and coupling reaction with **20**, was cyclized using Lawesson's reagent either in toluene or dichloromethane to afford thiazole and thiadiazole analogues **7**, **8**, **9**, and **10** after deprotection with TFA. This synthetic route was developed into a fully telescoped process synthesis for compound **10** at multikilogram scale.[19] Alternatively, the intermediates **25** were converted to oxazole **6** or 1,3,4-oxadiazole **11** using triphenylphosphine and hexachloroethane or triphenylphosphine and trichloroacetonitrile correspondingly after deprotection with TFA.

In Scheme 3, the head piece building block **20** was converted to methylketone **29** through Weinreb ketone synthesis. The methylketone **29** was then reacted with acid **26** through acid chloride intermediate to give diketone **30**, which further condensed with either hydrazine or hydroxylamine followed by deprotection with TFA affording the desired pyrazole and isoxazole analogues **12** and **13**. Meanwhile, using 4-fluoro-3-(trifluoromethyl)benzonitrile (**32**) as starting material, the 1,2,4-oxadiazole analogue **14** was prepared through the formation of hydroxylamidine intermediates **33** and **34** followed by cyclization and Boc deprotection. All of the phosphates were synthesized from corresponding alcohols analogously to the synthesis of PPI-4955-P.[10]

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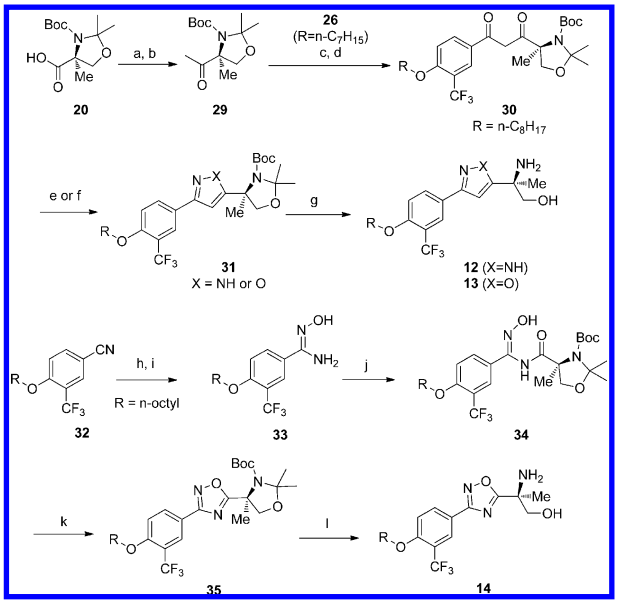
Scheme 3. Synthesis of Pyrazole, Isoxazole, and 1,2,4-Oxadiazole Analogues 12−14[a]



[a]Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, HATU, DIPEA, DMF; (b) MeLi, THF, −78 °C; (c) **26** (*R* = *n*-heptyl), $(COCl)_2$, DCM; (d) LiHMDS, THF, −78−0 °C; (e) hydrazine, MeOH; (f) hydroxylamine hydrochloride, pyridine; (g) TFA, DCM; (h) *n*-octyl−OH, KO^{t}Bu, THF, 70 °C; (i) hydroxylamine hydrochloride, EtOH, Na_2CO_3, H_2O; (j) **20**, HATU, DIPEA, DCM/DMF; (k) DMF, 120 °C; (l) TFA, DCM.

In summary, replacement of the imidazole ring of the novel $S1P_1$ prodrug PPI-4955 with a series of bioisosteric heterocycles (azoles) and switching the biphenyl moiety with an *n*-octyl tail led to the identification of several potent and selective prodrugs for modulating $S1P_1$ receptors. Among them, compound **10** demonstrated good in vitro ADME and in vivo PK properties. Upon phosphorylation, it showed subnanomole $S1P_1$ agonist activity with >1000× selectivity over $S1P_3$. Dosed orally at 0.1 mg/kg, **10** significantly reduced blood lymphocyte counts 6 h postdose and achieved in vivo efficacy analogous to FTY720 in the mouse EAE model of MS. Further pharmacokinetic/pharmacodynamic (PK/PD) study with monkeys indicated that oral dosing of **10** at 3.8 mg/kg led to active phosphate species that reached plasma levels comparable to FTY-720 phosphate revealed in human clinical pharmacokinetics studies. Compound **10** (GSK1842799) was selected as a candidate for clinical development.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the synthesis of all the compounds and in vitro ADME and in vivo PK/PD data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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