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Highly selective and potent agonists of sphingosine-1-phosphate 1 ($S1P_1$) receptor

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Abstract—Novel series of sphingosine-1-phosphate (S1P) receptor agonists were developed through a systematic SAR aimed to achieve high selectivity for a single member of the S1P family of receptors, $S1P_1$. The optimized structure represents a highly $S1P_1$-selective and efficacious agonist: $S1P_1/S1P_2$, $S1P_1/S1P_3$, $S1P_1/S1P_4$ > 10,000-fold, $S1P_1/S1P_5$ > 600-fold, while EC_{50} ($S1P_1$) <0.2 nM. In vivo experiments are consistent with $S1P_1$ receptor agonism alone being sufficient for achieving desired lymphocyte-lowering effect.

Sphingosine-1-phosphate (S1P)[1] induces a range of cellular responses by its interaction with the S1P family of G-protein coupled receptors (GPCRs). A total of five S1P receptors are known: $S1P_1$–$S1P_5$. The in vivo immunosuppressive efficacy of a non-selective S1P receptor agonist has been evidenced by the numerous preclinical and clinical studies of FTY720[2,3] (Novartis), a prodrug for its monophosphate ester.[4] Recent studies have demonstrated that S1P receptor agonists affect lymphocyte trafficking[5] and that an interaction with $S1P_1$ receptors on lymphocytes drives the observed pharmacodynamics.[6] $S1P_3$ receptor activity has been linked to acute toxicity and bradycardia in rodents.[7] As no highly potent and selective agonists have been reported to this date,[8] definitive evidence has not yet been provided as to whether a selective interaction with $S1P_1$ receptors would be sufficient for immunosuppressive efficacy. We present here the design and SAR study leading to the preparation of highly $S1P_1$-selective agonists.

N-(4-(1,3,4-Oxadiazole)benzyl)azetidine-3-carboxylic and *N*-(4-(1,2,4-oxadiazole)benzyl)azetidine-3-carboxylic acids have been previously reported to be potent $S1P_1$ receptor agonists that select against $S1P_2$ and $S1P_3$ subtypes and have good rat and dog pharmacokinetic profiles.[5i,9] (4-(1,2,4-Oxadiazol-3-yl)-3-methyl)phenylpropionic acids **1a**–**1c** have been shown to be orally bioavailable potent $S1P_1$ receptor agonists that select against $S1P_2$ and $S1P_4$ subtypes.[5b,5e–5g] For the latter, the left-hand side aromatic substituent can influence selectivity against $S1P_5$ (Table 1).[10] Based on these observations, we proposed to test a new structural class of compounds with the SAR focused on both $S1P_1$ receptor efficacy and selectivity (Fig. 1).

In order to generate a diverse SAR dataset with respect to the central heterocycle X (Fig. 1), we reasoned that the core of the proposed series may be synthesized in a three-step synthetic sequence consisting of two palladium(0)-catalyzed cross-coupling reactions (Suzuki, Negishi, or Stille couplings) and a bromination. This hypothesis led to the development of a novel general methodology yielding a variety of 2,5-diarylheteropentalenes in a significantly more convergent manner compared to previously reported alternatives (Scheme 1). We believe that this synthetic approach represents the most efficient route to a diverse array of 2,5-disubstituted heterocycles yet reported.[11–13]

A direct comparison of several heterocyclic analogs sharing identical aromatic 2,5-disubstitution validated the basis of our proposal: $S1P_1/S1P_3$ selectivity varies profoundly as a function of the central heterocycle (Table 1). Superior selectivity against the $S1P_3$ receptor

Keywords: Sphingosine-1-phosphate; S1P; Immunosuppressant; Lymphocyte-lowering; Agonist; Heteropentalene.
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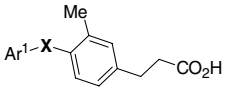
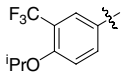
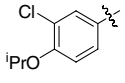
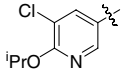
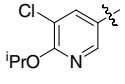
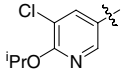
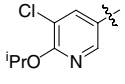
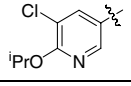
Table 1. S1P receptor agonism as a function of central heterocycle X and left-hand side aromatic substituent Ar^1 in 3-(4-heterocyclyl-3-methyl-phenyl)propionic acid series (**1c–g**)



Compound	X	Ar^1	EC_{50} (nM)[a,b] or % inhibition @ 10 μM		
			$S1P_1$	$S1P_3$	$S1P_5$
1a			0.10	110	13
1b			0.15	940	73
1c			0.73	950	950
1d			19	82%	44%
1e			2.1	36%	1300
1f			1000	8%	0%
1g			30%	0%	6%

[a] For a summary of biological assays used in this report, see Ref. 10; SD were generally ±20% of the average.

[b] EC_{50} and IC_{50} values for $S1P_2$ and $S1P_4$ receptors, respectively, exceeded the $S1P_1$ receptor EC_{50} by more than 1000-fold.

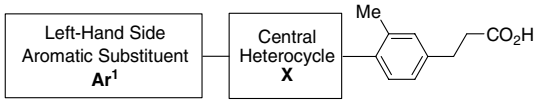
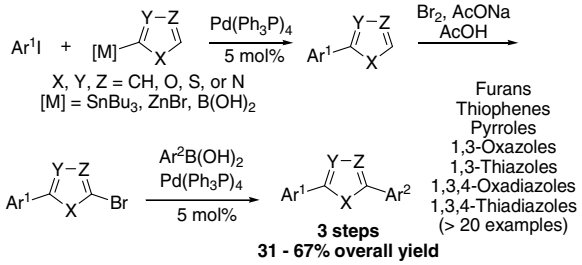


Figure 1. Design of the structural series reported in this disclosure.

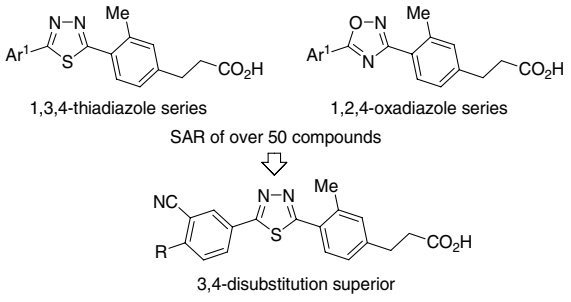


Scheme 1. General synthesis of 2,5-diarylheteropentalenes served for the preparation of many of the compounds reported in this disclosure.[11]

observed for 1,3,4-thiadiazole **1e** relative to its derivatives is particularly important given the association of $S1P_3$ agonism and acute toxicity and bradycardia in rodents.[7]

As previously disclosed series of S1P agonists have generally not selected against $S1P_5$ subtype, we focused on achieving a good $S1P_1/S1P_5$ selectivity, while maintaining strong affinity for $S1P_1$ receptor by optimizing lead **1e**. In due course, we benefited from the parallel agreement of trends in $S1P_1$ receptor efficacy invoked by corresponding members of two simultaneously optimized series: 1,2,4-oxadiazoles (represented by **1c**) and 1,3,4-thiadiazoles (represented by **1e**).[5d–g,14] The SAR of the two series suggested that the superior substitution pattern for Ar^1 was 3,4-disubstitution.[15] $S1P_1$ efficacy in 1,3,4-thiadiazole series benefited from the strongly electron-withdrawing nature of the 3-substituent, $S1P_1/S1P_5$ selectivity was mostly dependent on the nature of the 4-substituent. Generally, an electron-donating ether or alkyl substituent in the 4-position provided the highest selectivity against $S1P_5$ receptor. A detailed SAR of

Table 2. Parallel of $S1P_1$ receptor agonism in 1,3,4-thiadiazole and 1,2,4-oxadiazole series expedited identification of the optimal substitution pattern of the 1,3,4-thiadiazole series; SAR optimization of the 4-substituent in 3-cyanophenyl-1,3,4-thiadiazole series (**2a-v**) for $S1P_1$ receptor efficacy and $S1P_5/S1P_1$ selectivity



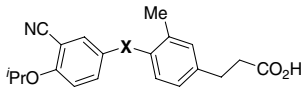
Compound, R	EC_{50}[a]	Selectivity $S1P_5/S1P_1$[b]
2a, H	>1000	N/A[c]
2b, MeO	97	>100
2c, EtO	2.1	480
2d, *n*-PrO	3.4	110
2e, *i*-PrO	0.20	210
2f, *i*-Bu	0.16	320
2g, *i*-BuO	2.8	200
2h, *cyc*-$PrCH_2O$	0.61	230
2i, *sec*-BuO	0.28	120
2j, *cyc*-BuO	0.79	170
2k, F	570	18
2l, FCH_2CH_2O	9.5	>100
2m, CF_2HCH_2O	1.1	230
2n, CF_3CH_2O	0.6	400
2o, $CF_3(CH_2)_2O$	7.7	30
2p, $(CF_3)_2CHO$	38	2
2q, HO_2CCH_2O	360	28
2r, $NCCH_2O$	45	220
2s, $F_3CH(Me)O$	0.19	93
2t, $(FCH_2)_2CHO$	0.23	390
2u, AcO	94	110
2v, 2-thienyl	1.9	110

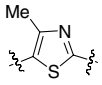
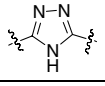
[a] EC_{50} (nM) for $S1P_1$ receptor; for a summary of biological assays used in this report, see Ref. 10; SD were generally ±20% of the average.

[b] EC_{50} and IC_{50} values for $S1P_2$ and $S1P_4$ receptors, respectively, exceeded the $S1P_1$ receptor EC_{50} by more than 1000-fold. $S1P_5/S1P_1$ is defined as the ratio of the respective EC_{50} values.

[c] $S1P_5/S1P_1$ not calculated due to low $S1P_1$ receptor efficacy.

Table 3. S1P receptor agonism as function of central heterocycle X in 3-cyano-4-isopropyloxyphenyl left-hand side series (**1a**, **2e**, and **3a**–**n**)



Compound	X	EC_{50} (nM)[a,b]		
		$S1P_1$	$S1P_3$	$S1P_5$
1a		0.08	1144	6.5
2e		0.20	>10,000	42
3a		1.1	>10,000	460
3b		0.93	>10,000	220
3c		>10,000	>10,000	>10,000
3d		>10,000	>10,000	>10,000
3e		14	>10,000	200
3f		0.18	>10,000	120
3g		>3600	>10,000	>10,000
3h		1.6	>10,000	43
3i		0.98	>10,000	150
3j		5.1	>10,000	>10,000
3k		290	>10,000	3200
3l		1200	>10,000	3200
3m		>10,000	>10,000	>10,000
3n		380	>10,000	>10,000

[a] For a summary of biological assays used in this report, see Ref. 10; SD were generally ±20% of the average.

[b] EC_{50} and IC_{50} values for $S1P_2$ and $S1P_4$ receptors, respectively, exceeded the $S1P_1$ receptor EC_{50} by more then 1000-fold.

2-((3-cyano-4-substituted)-phenyl)-1,3,4-thiadiazoles optimizing the 4-position for the highest $S1P_1$ receptor efficacy and $S1P_1/S1P_5$ selectivity is summarized in Table 2.[16] The most $S1P_1/S1P_5$ selective (>200-fold) as well as highly $S1P_1$ efficacious (EC_{50} < 1 nM) derivatives include **2e**, **2f**, **2h**, **2n**, and **2t**.

Having identified several desirable left-hand side aromatic substituents, we conducted a detailed SAR of the central heterocycle, while maintaining both the left- and right-hand side aromatic substituents unchanged.[17] Our three-step synthetic sequence (Scheme 1) provided facile access to the core of many compounds listed in Table 3.[18] While many of the heterocyclic derivatives proved to be efficacious $S1P_1$ receptor agonists, only a handful of these concurrently exhibited a high degree of selectivity for $S1P_1$ receptor against all other members of the S1P family: thiadiazole **2e**, oxadiazole **3a**, thiophene **3b**, thiazole **3f**, and tetrazole **3i** (Table 3). Among these, thiazole **3f** was found to be the most effective to maximally drive the lymphocyte-lowering response (MOSL) inducing the strongest lymphopenia after oral administration to mice.[10,19]

In conclusion, a systematic SAR analysis has identified selective, highly potent, small molecule $S1P_1$ receptor agonists (Table 3). Optimized structures are exemplified by compound **3f** with an in vitro $S1P_1$ receptor efficacy EC_{50} = 0.18 nM, greater than 10,000-fold selectivity against $S1P_2$, $S1P_3$, and $S1P_4$ receptors and greater than 600-fold selectivity against $S1P_5$ receptor. After oral administration of a 0.3 mpk dose of **3f** to mice, it was found to maximally drive the lymphocyte-lowering response previously seen with other S1P receptor agonists.[10] These results are consistent with the notion that selective agonism of $S1P_1$ receptor is sufficient for achieving the desired degree of lymphocyte-lowering. While agonist **3f** exhibited good oral bioavailability in the rat (F = 39%), its pharmacokinetic properties in that species remained suboptimal ($t_{1/2}$ < 1 h). The optimization of **3f** toward analogs with more desirable pharmacokinetic profiles will be a subject of our future disclosures.

References and notes

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2. FTY720 is currently in Phase III clinical trials for organ transplantation and in Phase II for the treatment of multiple sclerosis; Novartis Press Release Jun-21-2005, Basel, Switzerland.
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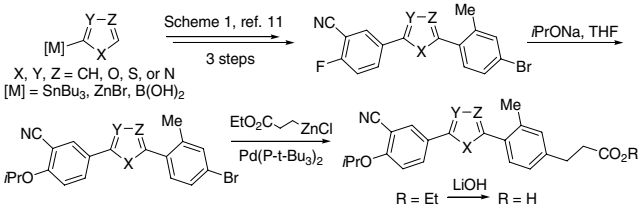
8. For the most active and selective $S1P_1$ receptor agonist reported thus far, see Ref. 7 and footnote 2 therein.
9. A limited prior SAR of a few additional *N*-(4-(heterocyclyl)benzyl)azetidine-3-carboxylic acids have suggested that variation of the central heterocycle may be tolerated without compromising the affinity for $S1P_1$ receptors: Hale, J. J.; Bugianesi, R.; Neway, W. Unpublished results.
10. Summary of the primary biological assay used in this report: In vitro EC_{50}: agonism of GPCR receptors: induced [^{35}S]GTPγS binding by test compounds (see Ref. 4 for details); In vivo Pharmacokinetics in the rat. Pharmacodynamics: induction of peripheral lymphocyte lowering (MOSL; see Ref. 5a for details).
11. This synthetic strategy was previously communicated: Vachal, P.; Toth, L. *Tet. Lett.* **2004**, *45*, 7157.
12. A representative procedure for the Suzuki coupling: a heterogeneous mixture of 5-bromo-2-arylheteropentalane (1.0 mmol), DMF (20 mL), 1 M aqueous solution of sodium carbonate (5 mL), and boronic acid (1.1 mmol) was degassed with a steady stream of argon for 10 min. Solid $Pd(Ph_3P)_4$ (0.1 mmol) was added and the mixture was degassed with argon for 2 min after which it was heated under argon to 85 °C for 3 h. The reaction mixture was combined with 1 M HCl (100 mL) and ethyl acetate (200 mL). The organic layer was separated, washed sequentially with 1 M HCl (50 mL) and brine (50 mL), and dried over sodium sulfate. Desired product was obtained by column chromatography. See Ref. 11 for details.
13. A representative procedure for selective bromination of 2-arylheteropentalenes: to a stirred homogeneous solution of 2-arylheteropentalene (5.0 mmol) and sodium acetate (10 mmol) in acetic acid (25 mL), bromine (5.0 mmol) was added dropwise via syringe at room temperature over 30 min. The reaction progress was monitored by TLC or LC–MS analyses: upon completion, the reaction mixture was combined with 1 M NaOH (250 mL) and ethyl acetate (250 mL), the organic layer separated, washed sequentially with 1 M NaOH (100 mL) and brine (100 mL), and dried over sodium sulfate. Desired product was obtained by column chromatography. See Ref.11 for details.
14. Parallel nature of the two series is exemplified in Table 1 (**1c** vs. **1e**) and Table 3 (**1a** vs. **2e**) of this report. Additional examples of similarities between corresponding 1,2,4-oxadiazole and 1,3,4-thiadiazole efficacy for $S1P_1$ receptor include (left-hand side aryl residues listed): 4-isopropyloxy-3-(trifluoromethyl)phenyl (EC_{50} = 0.09 vs. 0.13 nM) and 4-cyclohexylphenyl (EC_{50} = 7.1 vs. 11 nM), respectively. For a full scope of the analogy, see Ref. 5g.
15. Key role of 3,4-disubstitution for $S1P_1$ receptor efficacy is in a good agreement with trends observed in several previously reported series of S1P agonists; see Ref. 5 for details.
16. Only EC_{50} values for $S1P_1$ receptor are listed in Table 3 as the efficacy for this receptor and selectivity against $S1P_5$ receptor were the primary objectives of the SAR described therein. The EC_{50} values for $S1P_2$–$S1P_4$ receptors generally exceeded the $S1P_1$ EC_{50} by more than 1000-fold.
17. The primary reason for selecting **2e** among the potential candidates for further optimization was the in vivo efficacy after oral administration to mice: **2e** was found to be most effective agonist to maximally drive the lymphocyte-lowering response at 0.3 mpk dose; see Ref. 10 for details.
18. The following scheme represents a typical overall synthetic route to compounds in Table 3:



19. While 3 mpk of each of **2e**, **3a**, **3b**, and **3i** was needed to maximally drive the lymphocyte-lowering after oral administration to mice, 0.3 mpk dose of **3f** was sufficient to achieve the same effect; see Ref. 10 for details.