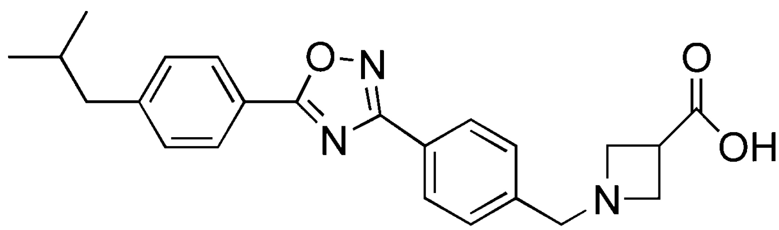
Journal of
Medicinal Chemistry

Letter

Discovery of Potent 3,5-Diphenyl-1,2,4-oxadiazole Sphingosine-1-phosphate-1 (S1P) Receptor Agonists with Exceptional Selectivity against S1P and S1P

Zhen Li, Weirong Chen, Jeffrey J. Hale, Christopher L. Lynch, Sander G. Mills, Richard Hajdu, Carol Ann Keohane, Mark J. Rosenbach, James A. Milligan, Gan-Ju Shei, Gary Chrebet, Stephen A. Parent, James Bergstrom, Deborah Card, Michael Forrest, Elizabeth J. Quackenbush, L. Alexandra Wickham, Hugo Vargas, Rose M. Evans, Hugh Rosen, and Suzanne Mandala

J. Med. Chem., **2005**, 48 (20), 6169-6173• DOI: 10.1021/jm0503244 • Publication Date (Web): 10 September 2005



Journal of
Medicinal
Chemistry

Volume 48, Number 20 October 6, 2005

Letters

Discovery of Potent 3,5-Diphenyl-1,2,4-oxadiazole Sphingosine-1-phosphate-1 ($S1P_1$) Receptor Agonists with Exceptional Selectivity against $S1P_2$ and $S1P_3$

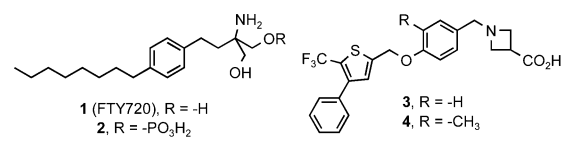
Zhen Li,*,† Weirong Chen,‡ Jeffrey J. Hale,† Christopher L. Lynch,⊥ Sander G. Mills,† Richard Hajdu,§ Carol Ann Keohane,§ Mark J. Rosenbach,§ James A. Milligan,§ Gan-Ju Shei,§ Gary Chrebet,§ Stephen A. Parent,§ James Bergstrom,§ Deborah Card,§ Michael Forrest,§ Elizabeth J. Quackenbush,§ L. Alexandra Wickham,§ Hugo Vargas,# Rose M. Evans,# Hugh Rosen,‖ and Suzanne Mandala§

Departments of Medicinal Chemistry and Immunology & Rheumatology Research, Merck Research Laboratories, Rahway, New Jersey 07065, and Department of Preclinical Safety Evaluation, Merck Research Laboratories, West Point, Pennsylvania 19486

Received April 8, 2005

Abstract: A class of 3,5-diphenyl-1,2,4-oxadiazole based compounds have been identified as potent sphingosine-1-phosphate-1 ($S1P_1$) receptor agonists with minimal affinity for the $S1P_2$ and $S1P_3$ receptor subtypes. Analogue **26** ($S1P_1$ IC_{50} = 0.6 nM) has an excellent pharmacokinetics profile in the rat and dog and is efficacious in a rat skin transplant model, indicating that $S1P_3$ receptor agonism is not a component of immunosuppressive efficacy.

The advent of the novel immunosuppressant 2-amino-2-(4-octylphenyl)ethylpropane-1,3-diol[1] (**1**, FTY720) may represent a new modality of immunosuppression and has inspired considerable interest in agonists and antagonists of sphingosine-1-phosphate (S1P) receptors.[2] S1P is a bioactive lysolipid with pleiotropic functions mediated via agonism of a family of G-protein-coupled receptors, $S1P_{1-5}$.[3] It has been demonstrated that **1** is


1 (FTY720), R = -H
2, R = $-PO_3H_2$
3, R = -H
4, R = $-CH_3$

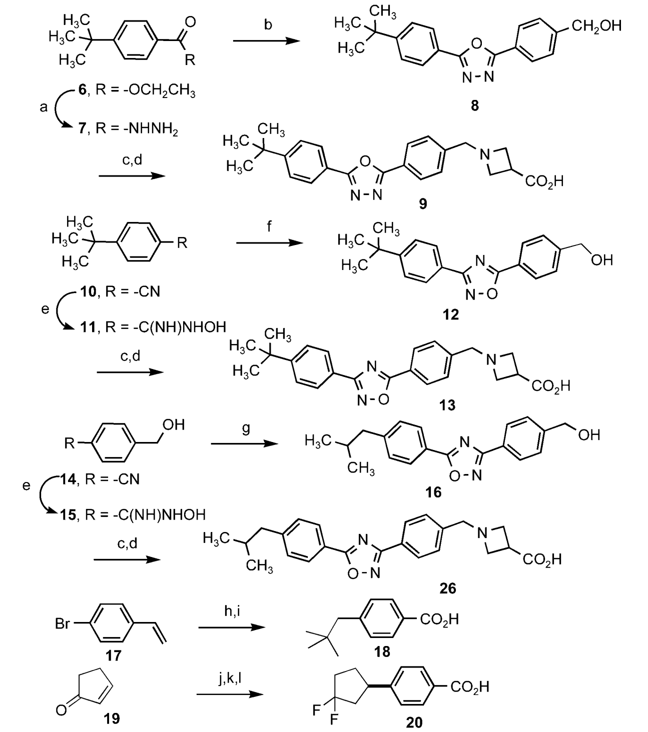
metabolized across species to a monophosphate ester (**2**), which is a high-affinity ligand for $S1P_{1,3-5}$ but not $S1P_2$.[4] Preclinical studies with **1** have revealed that its physiological effects include a redistribution of lymphocytes from blood to secondary lymphoid organs and regulation of cardiovascular function, with the former being a driver of immunosuppressive efficacy.[5] $S1P_1$ receptor agonism has been shown to correlate with lymphocyte recirculation,[6] while $S1P_3$ receptor agonism has been linked to acute toxicity and bradycardia in rodents.[7] A dose-dependent transient, asymptomic bradycardia has been seen in clinical studies with **1**,[8] suggesting that selecting against $S1P_3$ may be desirable with second-generation S1P receptor agonist immunosuppressants. A previous report from these laboratories disclosed a series of 1-benzyl-3-carboxyazetidine compounds (exemplified by **3** and **4)** as selective, orally bioavailable S1P receptor agonists.[9] While **3**, **4**, and many of their analogues were found to be about 500- to 1000-fold selective for $S1P_1$ over $S1P_3$, enhanced selectivity in this class of compounds was often accompanied by modest, yet significant, losses of $S1P_1$ receptor affinity.

Herein, we report the discovery of a class of potency-enhanced $S1P_1$ receptor agonists based on a 3,5-diphenyl-1,2,4-oxadiazole scaffold that exhibit exceptional selectivity against $S1P_2$ and $S1P_3$ receptor subtypes. Their syntheses, structure−activity relationships, and the significance of their enhanced potency and selectivity as it impacts immunosuppressive efficacy and pharmacology attributable to $S1P_3$ receptor agonism are the subjects of this communication.

* To whom correspondence should be addressed. Phone: (732) 594-8319. Fax: (732) 594-8080. E-mail: zhen_li@merck.com.
† Department of Medicinal Chemistry.
‡ Current address: Millennium Pharmaceuticals Inc., 35 Landsowne Street, Cambridge, MA 02139.
⊥ Current address: Abbott Laboratories, Building AP-10, 100 Abbott Park Road, Abbott Park, IL 60064.
§ Department of Immunology & Rheumatology.
# Department of Preclinical Safety Evaluation.
‖ Current address: The Scripps Research Institute, ICND-118, 10550 N. Torrey Pines Road, La Jolla, CA 92037.

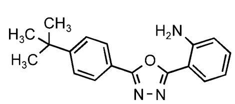
10.1021/jm0503244 CCC: $30.25
Published on Web 09/10/2005

Scheme 1[a]



[a] Reagents: (a) H_2NNH_2, EtOH, reflux (99%); (b) 4-(hydroxymethyl)benzoic acid, 2-chloro-1,3-dimethylimidazolinium chloride, TEA, CH_2Cl_2 (50%); (c) $(COCl)_2$, DMSO, DIEA, CH_2Cl_2, −78 °C to room temp; (d) 3-carboxyazetidine, $Na(CN)BH_3$, HOAc, MeOH (yields, two steps: **8**, **9**, 50%; **12**, **13**, 50%; **16**−**26**, 53%); (e) $HONH_2$·HCl $NaHCO_3$, MeOH, reflux (**10**, **11**, 95%; **14**, **15**, 99%); (f) 4-(hydroxymethyl)benzoic acid, EDC, HOBT, CH_3CN, reflux (85%); (g) 4-(2-methylpropyl)benzoic acid, EDC, HOBT, CH_3CN, reflux (85%); (h) 2,2-dimethylpropylmagnesium bromide, $Ni(dppf)Cl_2$, ether, reflux; (i) cat. RuO_2, $NaIO_4$, EtOAc/H_2O (52%, two steps); (j) 4-(bromo)phenylboronic acid, cat. (*R*)-BINAP, cat. $Rh(acac)(C_2H_4)_2$, 9:1 v/v dioxane/H_2O (54%, 91% ee); (k) [bis(2-methoxyethyl)amino]sulfur trifluoride, BF_3·Et_2O, toluene (65%); (l) *n*-BuLi, CO_2, THF/ether, −78 °C (75%).

After in-house leads identified in a high-throughput screen for $S1P_1$ agonists[10] led to the rational design of **3** and **4**, approximately 1000 commercially available compounds related to those leads were purchased and tested for S1P receptor agonism activity. Compound **5**,



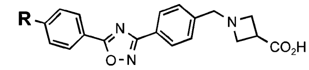
5

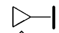
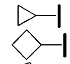
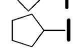
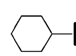
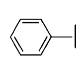
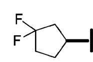
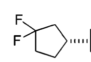
IC_{50}:
$S1P_1$ = 27 nm
$S1P_3$ >5000nm

with a 3,5-diphenyl substituted oxadiazole moiety, was identified in this set of analogues. Employing **5** as we had our previous leads, we started the investigation of 3,5-diphenyl substituted oxadiazole based compounds, which has led to the discovery of compound **26**.

Representative syntheses are outlined in Scheme 1. The key step in obtaining **9**, **13**, and **26** was the formation of their respective appropriately substituted oxadiazole heterocycles; this was efficiently realized using chemistry based on established literature procedures.[11] Syntheses of 4-(cyclopropyl)-,[12a] 4-(cyclobutyl)-,[12a] 4-(cyclopentyl)-,[12a] and 4-(4,4-difluorocyclohexyl)benzoic acid[12b] have appeared in the literature. The preparation of benzoic acid **18**, which featured a nickel-catalyzed cross-coupling,[13] is representative of the chemistry used to prepare the benzoic acids needed for targets **27** and **35**. The synthesis of benzoic acid **20** (required for target **36**) featured an asymmetric conjugate addition of 4-(bromo)phenylboronic acid to cyclopentenone;[14] analogous chemistry was used to prepare the benzoic acids needed for **37**.

Table 1. S1P Receptor Affinities (IC_{50}, nM)[a] and Rat Pharmacokinetic Data[b]



cpd	R	$S1P_1$	SP_3	$S1P_4$	$S1P_5$	rat PK
9	--	100	>10000	4300	1000	*nd*
13	--	8.2	>10000	640	20	*nd*
21	$(CH_3)_3C$-	3.8	12000	370	5.2	*nd*
22	$CH_3CH_2(CH_3)_2C$-	1.3	3086	75	1.8	*nd*
23	$CH_3CH_2CH_2$-	1.3	58000	150	1.6	Cl_p = 32 Vd_{ss} = 1.7 $t_{1/2}$ = 0.8
24	$CH_3(CH_2)_2CH_2$-	2.9	>10000	350	2.4	*nd*
25	$CH_3(CH_2)_4CH_2$-	29	>10000	>10000	6.4	*nd*
26	$(CH_3)_2CHCH_2$-	0.6	12000	70	1.0	Cl_p = 4.1 Vd_{ss} = 2.8 $t_{1/2}$ = 8.5
27	$(CH_3)_3CCH_2$-	3.8	~10000	90	6.6	*nd*
28	CH_3CH_2O-	5.1	>1000	>1000	48	*nd*
29	$(CH_3)_2CHO$-	1.8	>10000	370	12	Cl_p = 4.1 Vd_{ss} = 2.2 $t_{1/2}$ = 7.1
30		4.5	>10000	>1000	12	*nd*
31		2.2	>10000	520	4.0	*nd*
32		0.4	14000	49	0.5	Cl_p = 70 Vd_{ss} = 5.6 $t_{1/2}$ = 1.2
33		1.4	12000	40	0.8	Cl_p = 51 Vd_{ss} = 4.6 $t_{1/2}$ = 1.2
34		1.7	>10000	240	11	Cl_p = 2.9 Vd_{ss} = 2.8 $t_{1/2}$ = 11
35	$CF_3CH_2CH_2$-	0.4	13000	430	0.3	Cl_p = 5.3 Vd_{ss} = 2.3 $t_{1/2}$ = 5.8
36		0.9	4282	68	0.5	Cl_p = 10 Vd_{ss} = 3.0 $t_{1/2}$ = 4.1
37		0.8	4018	89	0.4	Cl_p = 5.8 Vd_{ss} = 3.0 $t_{1/2}$ = 6.6

[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as the mean of n = 3 determinations. All compounds had $S1P_2$ IC_{50} > 10 000 nM. SD were generally ±20% of the average. See ref 7a for assay protocol. [b] 1 mg/kg iv. Units: Cl_p, mL min^{-1} kg^{-1}, Vd_{ss}, L/kg, $t_{1/2}$, h. Data are reported as the average for n = 2 iv. Compound plasma levels for individual animals used to calculate PK parameters were with ±25% of average. See ref 19.

Ligand competition studies between [^{33}P]S1P and all new compounds were carried out for each of the five human S1P receptors stably expressed in Chinese hamster ovary (CHO) cell membranes.[4a] S1P receptor agonism by the test compounds was determined by measurement of ligand-induced [^{35}S]-5′-*O*-3-thiotriphosphate (GTPγS) binding.[15] Some general features about the data generated for the new compounds are noteworthy (Table 1). While the $S1P_1$ IC_{50} values for the new compounds (**9**, **13**, **21**−**37**) spanned almost 100-fold, all of them had minimal affinity for $S1P_2$ and $S1P_3$ receptors. This absolute disconnection of $S1P_1$ and $S1P_3$ SARs is in sharp contrast to what was seen previously for other classes of S1P receptor agonists where these receptor affinities were separable but effected by changes in structure in similar ways.[9,16] Most of the new compounds showed a more modest 100-fold selectivity against $S1P_4$ while being nonselective in regard to $S1P_5$. Interestingly, compounds such as **26** were found to be inactive in the GTPγS binding and ERK activation[17] assays used to assess $S1P_4$ functional activity which indicates that the new compounds differ from compounds such as **3** and **4** in that they are weak antagonists of the $S1P_4$ receptor.

The $S1P_1$ receptor data (Table 1) for the three 4-(*tert*-butyl)phenyloxadiazole analogues **9**, **13**, and **21** indicated a preference for the heterocycle of the last isomer. There appears to be an optimal length and/or size of the substituent of analogues of **21** at which $S1P_1$ affinity is maximized. Extending the carbon chain on the pendent phenyl ring of analogues of **21** was found to be $S1P_1$ affinity-enhancing with isobutyl analogue (**26**) having subnanomolar $S1P_1$ IC_{50} values, but this had limits because *n*-hexyl analogue **25** was significantly less potent. The $S1P_1$ data for cycloalkyl analogues **30**−**33** and phenyl analogue **34** also support the notion that an appropriately sized alkyl substituent affords maximal binding to $S1P_1$. Fluorination of some selected alkyl groups was found to be tolerated and in one case $S1P_1$-potency-enhancing (compare **35** vs **23**). As previously noted, none of these structural changes significantly altered selection against $S1P_4$ or $S1P_5$ compared to that seen for lead analogue **21**.

It was possible to differentiate among the new compounds based on their rat pharmacokinetics (PK) and their ability to drive a pharmacodynamic (PD) response characteristic of $S1P_1$ receptor agonists, i.e., the dose response of lowering peripheral blood lymphocyte counts.[4a,6,18] While all of the compounds for which rat pharmacokinetics were determined[19] were highly orally bioavailable (>50%), many of the more interesting simple alkyl and cycloalkyl analogues (e.g., **23**, **32**, **33**) were found to have high rates of plasma clearance in relation to relatively modest steady-state volumes of distribution. Isobutyl analogue **26** and phenyl analogue **34** were outliers in this respect with significantly longer half-lives in the rat (**26** $t_{1/2} = 8.5$ h, **34** $t_{1/2} = 11$ h). It was proposed that metabolism of the terminal alkyl groups of the higher clearance compounds might be responsible in part for their poorer pharmacokinetics; a subsequent metabolism and disposition experiment in bile duct cannulated Sprague-Dawley rats with [^{14}C]-**26** revealed that oxidation of the isobutyl group, the phenyl ring to which it is attached, and the azetidine nitrogen were major phase I metabolic processes for that compound.[20] The problem of high clearance could be remedied with fluorination of the pendent alkyl groups; analogues **35**−**37** were all found to have extended half-lives compared to their *des*-fluoro counterparts. While it is not clear why isobutyl analogue **26** is a better pharmacokinetics player than many of its close analogues, it is interesting to note that isopropoxy analogue **29** (which is isosteric to **26**) also had an extended rat half-life ($t_{1/2} = 7.1$ h). Beagle dog pharmacokinetics[9] for selected compounds (**23**, **26**, **29**, **32**, **33**) were found to parallel those in the rat, with **26** having a superior profile ($Cl_p = 3.3$ mL min^{-1}kg^{-1}, $Vd_{ss} = 2.5$ L/kg, $t_{1/2} =$ 10 h, % $F = 66$ after 1.0 mpk po and 0.5 mpk iv doses).

Since the immunosuppressive efficacy of **1** has been proposed to arise from its ability to promote the sequestration of peripheral blood lymphocytes (PBLs), measurement of PBL levels can be used as a surrogate marker for efficacy amenable to the screening of multiple analogues in vivo.[18] The ability of these new compounds to lower PBL counts after oral administration[9] provided another basis for analogue differentiation. While many of the compounds in Table 1 were capable of eliciting a maximal PBL lowering response 3 h after a sufficient oral dose, only **26**, **29**, **32**, **36**, and **37** were capable of doing so after a dose as low as 0.3 mpk po. While murine pharmacokinetics were not determined as part of these screening experiments, it is noteworthy that the most potent of the new compounds in this assay were also the ones with the best rat pharmacokinetics profiles. The pharmacodynamic ED_{50} for **26** in the PBL lowering screening assay was determined to be 0.03 mpk po, making it comparable to **1** and approximately 10-fold more potent than **3** or **4**. The oral doses of **1**, **3**, and **26** required to elicit a maximum PBL lowering response 24 h postdose in the mouse, rat, and dog were also determined (Table 2); PBL counts appeared to rebound in all three species when plasma concentrations of **26** reached 25−50 nM, indicating that this compound was at least 3- to 4-fold more potent than **3** in its ability to drive the lowering of PBLs.[21]

Table 2. Minimum Single Dose (mg/kg, po) Required for Maximal PBL Lowering Response 24 h Postdose

compd	mouse	rat	dog
1	1.0[a]	0.2[a]	0.1[c]
3	10[a,b]	3.0[c]	1.0[c]
26	10[a]	0.5[a]	0.5[c]

[a] Data generated 24 h after compound challenge in $n = 3$ animals according to protocol described in ref 4a. [b] b.i.d. [c] Data generated from PK/PD time-course studies in $n = 3$ animals as described in ref 9.

On the basis of its $S1P_1$ receptor agonist potency, selectivity, pharmacokinetics/pharmacodynamic properties, and the ready availability of the intermediates, **26** was subjected to more in-depth characterizations.

The ability of **1** to prolong allograft survival in a variety of preclinical species and to synergize with agents that effect lymphocyte proliferation and IL-2 production (such as CsA and FK-506) is well-established.[22] To demonstrate that an $S1P_1$ agonist with attenuated affinity for $S1P_3$ receptors could be expected to maintain those properties of **1**, a rat skin allograft experiment based on a previously published protocol[23] was conducted. Compounds **26** (5.0 mpk po/day, 28 days, $n = 14$ rats) and **1** (1 mpk po/day, 28 days, $n = 14$ rats) were independently combined with a subtherapeutic dose of CsA (1 mpk/day, delivered intraperitoneally till graft death) and tested in Lewis rats that received skin grafts from DA rats. The median graft survival times were 22 days in the **26** + CsA arm (100% of grafts were rejected by day 43) and 13 days in the **1** + CsA arm (100% of grafts were rejected by day 34). Rats treated with vehicle or CsA alone had 100% graft death between days 10 and 12, respectively. Exposure to CsA was comparable in all of the cohorts to which it was administered. These results indicate that S1P receptor agonists that select against the $S1P_3$ subtype should be expected to be efficacious as immunosuppressive agents. The consequences for efficacy of selecting against the $S1P_4$ and/or $S1P_5$ receptor subtypes remain to be established.

Cardiovascular evaluations of the new compounds in the previously described[7a] conscious rat model were hindered by our inability to administer compounds at doses sufficient to elicit responses attributable $S1P_3$ agonism.[24] Fortunately, a determination of compound effects on airways resistance in the rat appears to provide an attractive means for demonstrating the undesirability of $S1P_3$ agonism. In addition to modulating lymphocyte trafficking, it was recently demonstrated that S1P can stimulate the contraction of human airway smooth muscle in vitro.[25] While this finding implies that S1P has a role in airway tone, it was uncertain whether S1P receptor activation would augment airway resistance in a whole animal model. To assess whether S1P receptors can alter airway resistance, the respiratory effects of **1**, **3**, and **26** were evaluated in anesthetized rats. In this study, airway resistance was measured directly using a computer-controlled small animal ventilator to measure drug-induced changes in respiratory mechanics.[26] In this model, intravenous infusion of the muscarinic agonist methacholine (30 μg kg^{-1} min^{-1} $\times$ 30 min), a known bronchconstrictor, caused a significant increase in airway resistance (152 $\pm$ 6%) in anesthetized rats. Administration of **1** and **3** at 10 mpk iv infused over 30 min induced bronchoconstriction and elevated airway resistance by 344 $\pm$ 33% and 140 $\pm$ 5% compared to baseline. Infusion of **26** was devoid of any effect on airway resistance and was similar to vehicle treatment (104 $\pm$ 1%). These findings suggest that agonists with high to moderate affinity for $S1P_3$ receptors, e.g, **1** and **3**, can produce bronchoconstriction in the rat and suggest that airways smooth muscle contraction can be mediated by $S1P_3$ receptors. This is supported by the observation that a highly selective $S1P_1$ agonist with minimal affinity for $S1P_3$ receptors, e.g, **26**, lacked the ability to stimulate airway smooth muscle contraction in vivo.

In conclusion, a series of potent $S1P_1$ receptor agonists exemplified by **26** with high selectivity against the $S1P_2$ and $S1P_3$ receptor subtypes have been discovered. While it has been previously shown that lymphocyte trafficking is not influenced by $S1P_3$ agonism, the ability of **26** to prolong allograft survival in a rat skin transplant model serves as an indication that $S1P_3$ agonism is not required for immunosuppressive efficacy. Moreover, our initial findings regarding the relative respiratory effects of **1**, **3**, and **26** further support the notion that agonism of $S1P_3$ may be associated with undesirable pharmacologies.[7] Details of the further evaluation of **26** and our investigations of other S1P receptor agonists as a novel immunosuppressive agents will be reported in due course.

Acknowledgment. We are grateful to Drs. Larry Colwell and William Feeney for coordinating the rat and dog pharmacokinetics studies, Dr. Bernard Choi and Ms. Falguni Patel for HRMS determinations, and Ms. Alysia Markey for identifying and arranging for the purchase compounds for S1P receptor agonist screening.

Supporting Information Available: Experimental details and characterization data for final in vivo tested compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Aki, F. T.; Kahan, B. D. FTY720: A New Kid on the Block for Transplant Immunosuppression. *Expert Opin. Biol. Ther.* **2003**, *3*, 665−681.

(2) Parrill, A. L.; Sardar, V. M.; Yuan H. Sphingosine 1-Phosphate and Lysophosphatidic Acid Receptors: Agonist and Antagonist Binding and Progress toward Development of Receptor-Specific Ligands. *Semin. Cell Dev. Biol.* **2004**, *15*, 467−476.

(3) Anliker, B.; Chun, J. Cell Surface Receptors in Lysophospholipid Signaling. *Semin. Cell Dev. Biol.* **2004**, *15*, 457.

(4) (a) Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. Alteration of Lymphocyte Trafficking by Sphingosine-1-Phosphate Receptor Agonists. *Science* **2002**, *296*, 346−349. (b) Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R., Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. The Immune Modulator FTY720 Targets Sphingosine-1-Phosphate Receptors. *J. Biol. Chem.* **2002**, *277*, 21453-21457.

(5) Chiba, K.; Yanagawa, Y.; Masubuchi, Y.; Kataoka, H.; Kawaguchi, T.; Ohtsuki, M.; Hoshino, Y. FTY720, a Novel Immunosuppressant, Induces Sequestration of Circulating Mature Lymphocytes by Acceleration of Lymphocytes Homing in Rats. I. FTY720 Selectively Decreases the Number of Circulating Mature Lymphocytes by Acceleration of Lymphocytes Homing. *J. Immunol.* **1998**, *160*, 5037−5044.

(6) (a) Matloubian, M.; Lo, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Proia, R. L.; Cyster, J. G. Lymphocyte Egress from Thymus and Peripheral Lymphoid Organs Is Dependent on S1P Receptor 1. *Nature* **2004**, *427*, 355−360. (b) Allende, M. L.; Dreier, J. L.; Mandala, S.; Proia, R. L. Expression of the Sphingosine-1-Phosphate, $S1P_1$, on T-Cells Controls Thymic Emigration. *J. Biol. Chem.* **2004**, *279*, 15396−15401.

(7) (a) Forrest, M.; Sun, S.-Y.; Hajdu, R.; Bergstrom, J.; Card, D.; Doherty, G.; Hale, J.; Keohane, C.; Meyers, C.; Milligan, J.; Mills, S.; Nomura, N.; Rosen, H.; Rosenbach, M.; Shei, G.-J.; Singer, I. I.; Tian, M.; West, S.; White, V.; Xie, J.; Proia, R. L.; Mandala, S. Immune Cell Regulation and Cardiovascular Effects of Sphingosine-1-Phosphate Receptor Agonists in Rodents Are Mediated via Distinct Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 758−768. (b) Sanna, M. G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M.-Y.; Peterson, M. S.; Webb, B.; Lefebvre, S.; Chun, J.; Rosen, H. Sphingosine-1-Phosphate (S1P) Receptor Subtypes $S1P_1$ and $S1P_3$, Respectively, Regulate Lymphocyte Recirculation and Heart Rate. *J. Biol. Chem.* **2004**, *279*, 13839−13848.

(8) Ferguson, R. FTY720 Immunomodulation: Optimism for Improved Transplant Regimens. *Transplant. Proc.* **2004**, *36*, 549S−553S.

(9) Hale, J. J.; Lynch, C. L.; Neway, W. E.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.-J.; Parent, S. A.; Chrebet, G.; Bergstrom, J.; Card, D.; Rosen, H.; Mandala, S.; Ferrer, M.; Hodder, P.; Strulovivi, B.; Rosen, H.; Mandala, S. A Unique Utilization of High Throughput Screening Leads To Afford Selective, Orally Bioavailable 1-Benzyl-3-carboxyazetidine $S1P_1$ Receptor Agonists. *J. Med. Chem.* **2004**, *47*, 6662−6665.

(10) Ferrer, M.; Kolodin, G. D.; Zuck, P.; Peltier, R.; Berry, K.; Mandala, S. M.; Rosen, H.; Ota, H.; Ozaki, S.; Inglese, J.; Strulovici, B. A Fully Automated [^{35}S]GTPγS Scintillation Proximity Assay for the High-Throughput Screening of G_i-Linked G Protein-Coupled Receptors. *Assay Drug Dev. Technol.* **2003**, *1*, 261−273.

(11) (a) Isobe, T.; Ishikawa, T, 2-Chloro-1,3-dimethylimidazolinium Chloride. 2. Its Application to the Construction of Heterocycles through Dehydration Reactions. *J. Org. Chem.* **1999**, *64*, 6989−6992. (b) Pipik, B.; Ho, G.-J.; Williams, J. M.; Conlon, D. A. A Preferred Synthesis of 1,2,4-Oxadiazole. *Synth. Commun.* **2004**, *34* (10), 1863−1870.

(12) (a) Hahn, R. C.; Corbin, T. F.; Shechter, H. Electrical Effects of Cycloalkyl Groups. *J. Am. Chem. Soc.* **1968**, *90*, 3404−3415. (b) Kawada, M.; Uesugi, Y.; Yamashita, T.; Matsumoto, S.; Pponda, H. JP Patent 06239775 A2, 1994.

(13) Hayashi, T.; Konishi, M.; Yokota, K.; Kumada, M. Cross Coupling of Tertiary Alkyl Grignard Reagents with β-Bromostyrene Catalyzed by Dichloro[1,1′-bis(diphenylphosphino)ferrocene] Nickel(II). *Chem. Lett.* **1980**, 767−768.

(14) Hayashi, T.; Takahashi, M.; Takaya, Y.; Ogasawara, M. Catalytic Cycle of Rhodium-Catalyzed Asymmetric 1,4-Addition of Organoboronic Acids. Arylrhodium Oxa-π-allyrhodium, and Hydroxorhodium Intermediates. *J. Am. Chem. Soc.* **2002**, *124*, 5052−5058.

(15) All of the test compounds were found to be agonists for $S1P_1$ and $S1P_5$ receptors; most of their EC_{50} agreed with the corresponding IC_{50} within 5-fold. For **13**, **25**, **27**, **33** the ranges were within 10-fold. The test compounds had minimal affinity for $S1P_2$ and $S1P_3$.

(16) Hale, J. J.; Doherty, G.; Toth, L.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.-J.; Chrebet, G.; Bergstrom, J.; Card, D.; Forrest, M.; Sun, S.-Y.; West, S.; Xie, H.; Nomura, N.; Rosen, H.; Mandala, S. Selecting against $S1P_3$ Enhances the Acute Cardiovascular Tolerability of S1P Receptor Agonists. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 3501−3505.

(17) Hale, J. J.; Yan, L.; Neway, W. E.; Hajdu, R.; Bergstrom, J. D.; Milligan, J. A.; Shei, G.-J.; Chrebet, G. L.; Thornton, R. A.; Card, D.; Rosenbach, M.; Rosen, H.; Mandala, S. Synthesis, Stereochemical Determination and Biochemical Characterization of the Enantiomeric Phosphate Esters of the Novel Immunosuppressive Agent FTY720. *Bioorg. Med. Chem.* **2004**, *12*, 4803−4807.

(18) Hale, J. J.; Neway, W.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.-J.; Chrebet, G.; Bergstrom, J.; Card, D.; Koo, G. C.; Koprak, S. L.; Jackson, J. J.; Rosen, H.; Mandala, S. Potent S1P Receptor Agonists Replicate the Pharmacologic Actions of the Novel Immune Modulator FTY720. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3351−3355.

(19) Compound plasma levels for individual animals used to calculate PK parameters were within ±25% of average. See also Supporting Information.

(20) Tschirret-Guth, R.; Tang, W.; Evans, D. C. Unpublished results. See Supporting Information for details.

(21) Previously described experiments. See ref 9 and Supporting Information.

(22) Wang, M.-E.; Tejpal, N.; Qu, X.; Yu, J.; Okamoto, M.; Stepkowski, S.; Kahan, B. Immunosuppressive Effects of FTY720 Alone or in Combination/with Cyclosporine and/or Sirolimus. *Transplantation* **1998**, *65*, 899−905.

(23) Chiba, K.; Hoshino, Y.; Suzuki, C.; Masubuchi, Y.; Yanagawa, Y.; Ohtsuki, M.; Sasaki, S.; Fujita, T. FTY720, a novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rats. *Transplant. Proc.* **1996**, *28*, 1056−1059.

(24) Sun, S.-Y.; Forrest, M. Unpublished results. See Supporting Information for details.

(25) Rosenfeldt, H. M.; Amrani, Y.; Watterson, K. R.; Murthy, K. S.; Panettieri, R. A.; Speigel, S. Sphingosine-1-phosphate stimulates contraction of human airway smooth muscle cells. *FASEB J*. **2003**, *17*, 1789−1799.

(26) Schuessler, T. F.; Bates, J. H. A Computer-Controlled Research Ventilator for Small Animals: Design and Evaluation. *IEEE Trans. Biomed. Eng*. **1995**, *42*, 860−866.

JM0503244