ACS Medicinal Chemistry Letters

LETTER

pubs.acs.org/acsmedchemlett

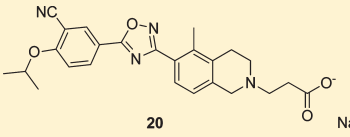
Discovery of a Selective $S1P_1$ Receptor Agonist Efficacious at Low Oral Dose and Devoid of Effects on Heart Rate

Emmanuel H. Demont,*[,†] Benjamin I. Andrews,[‡] Rino A. Bit,[†] Colin A. Campbell,[†] Jason W. B. Cooke,[‡] Nigel Deeks,[†] Sapna Desai,[‡] Simon J. Dowell,[‡] Pam Gaskin,[†] James R. J. Gray,[†] Andrea Haynes,[†] Duncan S. Holmes,[†] Umesh Kumar,[†] Mary A. Morse,[†] Greg J. Osborne,[‡] Terry Panchal,[†] Bela Patel,[‡] Alcide Perboni,[‡] Simon Taylor,[†] Robert Watson,[†] Jason Witherington,[†] and Robert Willis[†]

[†]Immuno Inflammation Center of Excellence for Drug Discovery and [‡]Platform Technology and Science, GlaxoSmithKline, Gunnels Wood Road, Stevenage, SG1 2NY, United Kingdom

Supporting Information

ABSTRACT: Gilenya (fingolimod, FTY720) was recently approved by the U.S. FDA for the treatment of patients with remitting relapsing multiple sclerosis (RRMS). It is a potent agonist of four of the five sphingosine 1-phosphate (S1P) G-protein-coupled receptors ($S1P_1$ and $S1P_{3-5}$). It has been postulated that fingolimod's efficacy is due to $S1P_1$ agonism, while its cardiovascular side effects (transient bradycardia and hypertension) are due to $S1P_3$ agonism. We have discovered a series of selective $S1P_1$ agonists, which includes 3-[6-(5-{3-cyano-4-[(1-methylethyl)oxy]phenyl}-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinyl]propanoate, **20**, a potent, $S1P_3$-sparing, orally active $S1P_1$ agonist. Compound **20** is as efficacious as fingolimod in a collagen-induced arthritis model and shows excellent pharmacokinetic properties preclinically. Importantly, the selectivity of **20** against $S1P_3$ is responsible for an absence of cardiovascular signal in telemetered rats, even at high dose levels.



20

$S1P_1$ pEC_{50} (β-arrestin) = 8.3
$S1P_3$ pEC_{50} ($GTP\gamma S$) < 4.4

Full lymphopoenia at 0.1 mg/kg p.o.

No bradycardia at 100 mg/kg p.o.

KEYWORDS: Fingolimod, S1P, agonism, bradycardia, lymphopenia, multiple sclerosis

Fingolimod (**1**)[1] was developed as an analogue of the natural product myriocin **2**, a potent inhibitor of palmitoyltransferase that demonstrates immunosuppressant activity (Figure 1). Compound **1** has displayed clinical efficacy in transplantation[2] and remitting relapsing multiple sclerosis[3] trials and is now marketed in the United States for the latter indication. Administration of **1** leads to the sequestration of lymphocytes in secondary lymphoid organs and consequently to a reduction of lymphocyte counts in the peripheral blood. The unique pharmacological profile of **1** is not due to any related myriocin-like activity. Indeed, **1** is enantioselectively phosphorylated in vivo[4,5] to give phosphate **3**, a potent agonist of four of the five G-protein-coupled receptors ($S1P_1$ and $S1P_{3-5}$) for sphingosine 1-phosphate (S1P) **4**. S1P has several physiological roles, but only agonism of $S1P_1$ is required to induce egress of T cells from lymphoid organs.[6,7] On the other hand, it has been demonstrated using selective tool compounds[8,9] or transgenic mice[10] that the unwanted cardiovascular effects seen with **1** are related to its agonism of the $S1P_3$ receptor, although selectivity against $S1P_3$ may not preclude bradycardia.[11]

Understanding of the unique mode of action of **1** has triggered intensive effort toward the discovery of $S1P_1$ agonists with an increased degree of selectivity versus $S1P_3$,[12,13] either as prodrugs such as KRP-203[14] **5** or as direct agonists such as ACT-128800[15] **6** (Figure 2). Zwitterions such as **7**[16] or propionic acids

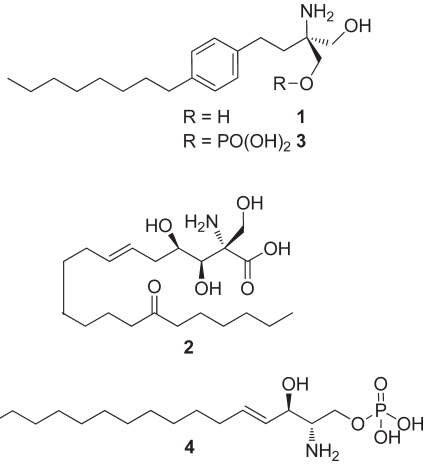


Figure 1. Structures of fingolimod **1** and its phosphate **3**, myriocin **2**, and S1P **4**.

such as **8**[17] are likely to interact with $S1P_1$ in a similar fashion to S1P itself.[18,19] Agonist **8** was of particular interest to us

Received: January 26, 2011
Accepted: March 24, 2011
Published: March 24, 2011

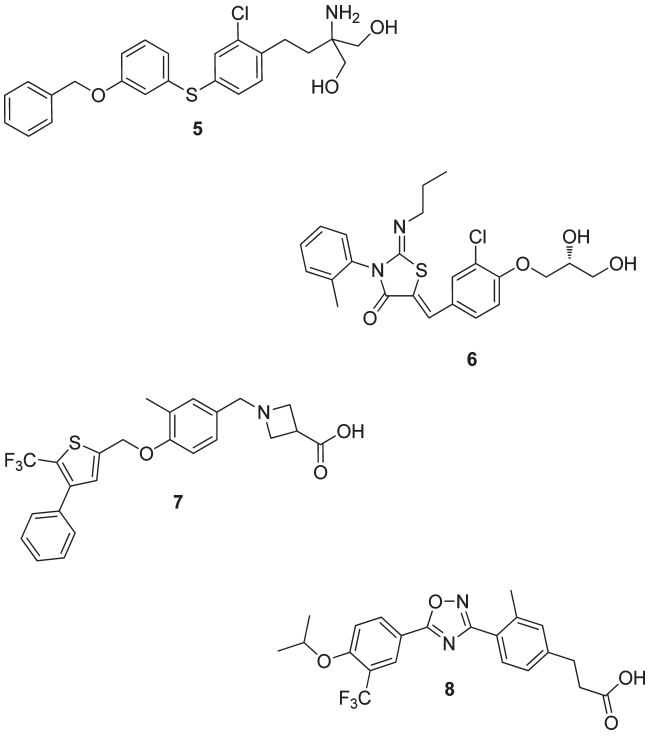


Figure 2. Structures of known $S1P_1$ agonists.

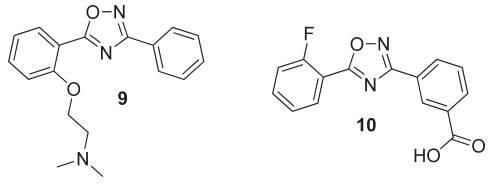
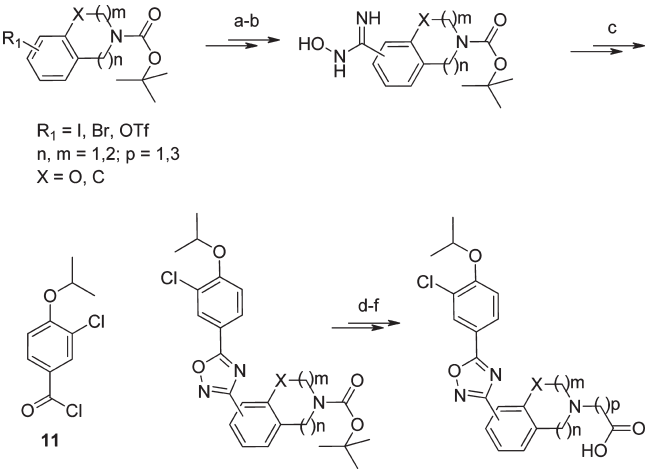


Figure 3. Structure of biaryl oxadiazoles.

because biaryl oxadiazoles **9**[20] and **10**[21] (Figure 3) are in clinical trials (not as $S1P_1$ agonists), suggesting good developability properties. Our strategy to develop novel orally active $S1P_1$ selective agonists focused on drugability:[22] Several reports[23,24] have highlighted that the attrition rate of oral compounds in clinical trials can be correlated with compound properties such as (high) molecular weight (MW),[25] (high) lipophilicity (cLogP, LogD), (low) polar surface area (PSA), or the number of rotable bounds.[26] Moreover, the odds of toxicity are minimal when compounds have cLogP < 3 and PSA > 75 Å^2.[27] We focused on these two parameters, in particular on lipophilicity, as the pharmacophore of S1P precludes the discovery of compounds with a MW < 350. Inspired by the structures of S1P agonists **7** and **8**, we pursued the design and synthesis of constrained triaryl scaffolds incorporating a zwitterionic moiety. The two charged residues should increase the PSA and hydrophilicity (Scheme 1).[28] The substitution pattern on the distal ring was influenced by the reported structure−activity relationship (SAR),[17] as it was likely to be optimal for in vitro potency.

Among several bicyclic rings/bqn containing a basic nitrogen that we explored, the C-5 substituted tetrahydroisoquinoline (THIQ) appeared most promising and was used for further optimization. A representative synthesis of these agonists is

Scheme 1. Lead Generation Strategy Toward Druglike $S1P_1$ Agonist Leads[a]



[a] Reagents and conditions: (a) Palladium-mediated nitrile formation. (b) Addition of hydroxylamine. (c) Compound **11**, base, heat. (d) HCl. (e) Alkylation. (f) Saponification.

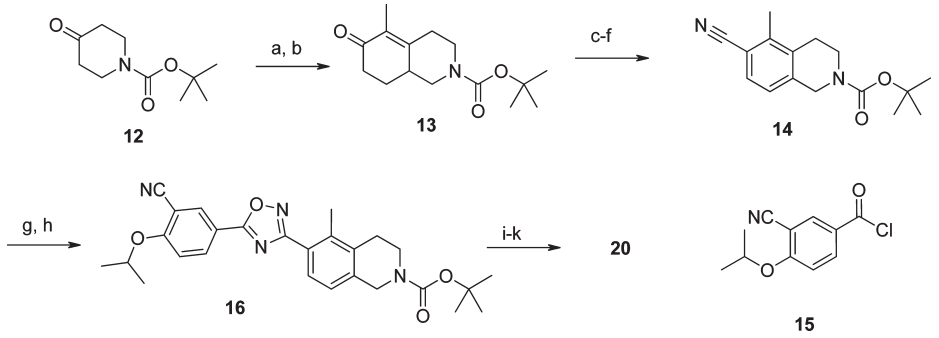
depicted in Scheme 2: The α,β-unsaturated ketone **13** was accessed via reaction of an enamine derived from ketone **12**. Its palladium-mediated oxidation led to the corresponding phenol, which was transformed into **14** via triflation followed by cyanation. The addition of hydroxylamine to **14** and reaction of the corresponding hydroxy-amidine with **15** followed by dehydration of the noncyclized intermediate gave oxadiazole **16**. Deprotection in acidic media of the secondary amine and Michael addition to ethyl acrylate followed by saponification led to agonist **20**.

The key SAR findings are summarized in Table 1 and show (1) the need for a meta-substituent on the distal aromatic ring (cf. **18** vs **19** and **20**); lipophilic functionalities (**19**) and substituents with some polarity (**20**) are tolerated in this position, but the nitrile group proved optimal in terms of potency, selectivity, and lipophilicity (cf. **20** vs **19**). (2) The isopropoxy para-substituent on this ring was optimal for potency (cf. **21** vs **20**)[29] and $S1P_3$ selectivity (cf. **22**, **23** vs **20**). (3) Replacement of the phenyl ring with electron-poor aromatic leads to a significant loss of potency (**24** and **19** as representative examples).[30] (4) Introduction of a C-4 substituent on the THIQ aromatic ring is beneficial for $S1P_3$ selectivity without compromising $S1P_1$ activity (cf. **25** vs **20**). (5) Introduction of an acid group as a phosphate mimetic[31] on the N-substituent is beneficial for activity and selectivity (cf. **26** vs **20**, **27**, and **28**), but the length of the chain (1−3 carbons) has no impact on these parameters (cf. **20** vs **27** and **28**).

With these data in hand, the most potent and selective compounds were screened in our pharmacodynamic (PD) lymphocyte reduction model in rats following oral administration. Compound **20** proved to deliver full lymphopenia at the lowest oral dose (0.1 mg/kg po, Figure 4). As opposed to **1**, this reduction of lymphocyte count is reversible within 24 h. Our pharmacokinetic (PK)/PD modeling shows that this differentiation is due to the much shorter half-life of agonist **20** in rats (vide infra). A head-to-head comparison with **1** in a collagen-induced arthritis model was performed (Figure 5). At a dose of 3 mg/kg po, agonist **20** shows a clear dose-dependent reduction of paw

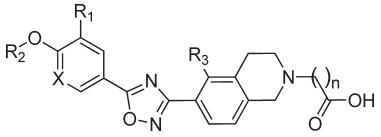
dx.doi.org/10.1021/ml2000214 |ACS Med. Chem. Lett. 2011, 2, 444–449

Scheme 2. Synthesis of 20[a]



[a] Reagents and conditions: (a) Pyrrolidine, toluene, Dean−Stark, reflux. (b) Pent-1-en-3-one, hydroquinone, 59% (2 steps). (c) Lithium bis-(trimethylsilyl)amide (LiHMDS), THF, −63 °C and then trimethylsilyl chloride (TMSCl). (d) $Pd(OAc)_2$, CH_3CN, T < 35 °C, and then tetra-*n*-butylammonium fluoride (TBAF), 55% (2 steps). (e) Tf_2O, pyridine, CH_2Cl_2, −30 °C. (f) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 100 °C, 92% (2 steps). (g) Aqueous NH_2OH, EtOH, 80 °C, 86%. (h) Compound **15**, pyridine, toluene, 0−110 °C, 51% (2 steps). (i) HCl, dioxane, room temperature, 98%. (j) Ethyl acrylate, diaza(1,3)bicycle[5.4.0]undecane (DBU), CH_3CN, room temperature, 94%. (k) NaOH, EtOH/water, room temperature, 91%.

Table 1. SAR Data in the Triaryl THIQ $S1P_1$ Agonist Series



						pEC$_{50}$ (n)			
compd	R_1	R_2	R_3	X	n	$S1P_1$ GTPγS	$S1P_1$ β-arrestin	$S1P_3$ GTPγS	CHROM LogD[a] at pH 7.4
3						8.4 ± 0.31 (130)	7.7 ± 0.17 (44)	8.3 ± 0.31 (38)	
18	H	$-CH(CH_3)_2$	CH_3	CH	2	6.5 ± 0.25 (7)	6.5 ± 0.30 (10)	4.9 (1)	3.81
19	Cl	$-CH(CH_3)_2$	CH_3	CH	2	7.9 ± 0.24 (13)	8.5 ± 0.12 (11)	5.2 ± 0.27 (5)	4.35
20	CN	$-CH(CH_3)_2$	CH_3	CH	2	7.5 ± 0.24 (8)	8.3 ± 0.11 (11)	<4.4 (8)	3.41
21	CN	$-n$-C_2H_5	CH_3	CH	2	7.3 ± 0.31 (7)	7.5 ± 0.14 (10)	<4 (9)	3.01
22	CN	$-n$-C_3H_7	CH_3	CH	2	7.5 ± 0.22 (7)	7.8 ± 0.15 (10)	5.1 ± 0.21 (4)	3.54
23	CN	$-n$-C_4H_9	CH_3	CH	2	7.2 ± 0.30 (7)	7.7 ± 0.15 (9)	5 ± 0.34 (8)	4.06
24	Cl	$-CH(CH_3)_2$	CH_3	N	2	7.1 ± 0.17 (5)	7.6 ± 0.49 (5)	4.7 ± 0.04 (2)	4.46
25	CN	$-CH(CH_3)_2$	H	CH	2	8.2 ± 0.23 (11)	8.5 ± 0.11 (8)	5.4 ± 0.14 (11)	3.30
26	CN	$-CH(CH_3)_2$	CH_3	CH	0 (NH)	7.6 ± 0.3 (10)	7.2 ± 0.89 (27)	4.9 ± 0.43 (4)	4.21
27	CN	$-CH(CH_3)_2$	CH_3	CH	1	7.8 ± 0.3 (9)	8.3 ± 0.1 (8)	<4 (7)	3.32
28	CN	$-CH(CH_3)_2$	CH_3	CH	3	7.7 ± 0.27 (9)	8.2 ± 0.09 (8)	<4 (11)	3.51

[a] CHROM LogD = chromatographic hydrophobicity index [CHI] × 0.0857 − 2; for comparison, CHI LogD = CHI × 0.0525 − 1.467. See Valko, K. ; Bevan, C. ; Reynolds, D. *Anal. Chem.* **1997**, *69*, 2022−2029.

volume and similar efficacy to **1**.[32] We next turned our attention to the effect on heart rate of our compounds following oral administration. To our delight, **20** did not show any statistically significant effect on heart rate at doses as high as 100 mg/kg po[33] and therefore clearly differentiates it from **1** (Figure 6).

Because of these promising data, further profiling of **20** was implemented. This compound has excellent intrinsic properties (Table 2), and its poor solubility is compensated by excellent permeability, allowing linear PK in rats up to 300 mg/kg po. No significant CYP inhibition was observed with this molecule, and no covalent adducts were detected in glutathione trapping experiments (nor time-dependent inhibition of CYP 3A4 and 2D6). Low in vitro hepatocyte clearance also translated into low in vivo clearance in three preclinical species (Table 3). Agonist **20** showed good distribution in tissues translating into moderate half-life; oral bioavailability was excellent in all species tested (mouse, rat, and dog).

In conclusion, we have identified a druglike $S1P_3$-sparing $S1P_1$ agonist[34] showing similar efficacy to **1** at low doses in a model of arthritis. This compound, unlike **1**, does not cause bradycardia in rats even at high oral doses. Its excellent PK suggest low human therapeutic doses (<10 mg/kg po once daily).

dx.doi.org/10.1021/ml2000214 |*ACS Med. Chem. Lett.* 2011, 2, 444–449

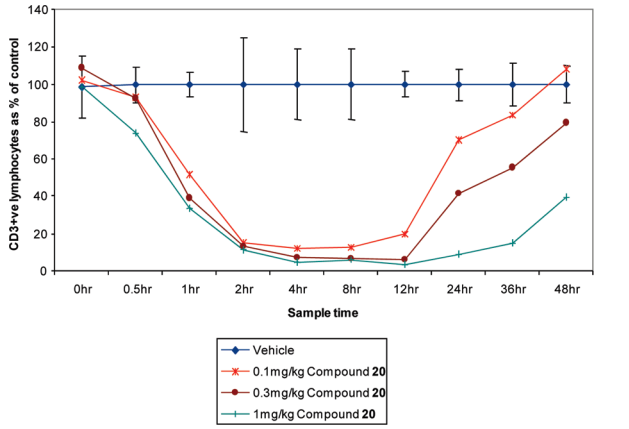


Figure 4. Lymphocyte reduction over time following oral administration of **20** (as sodium salt) in rats.

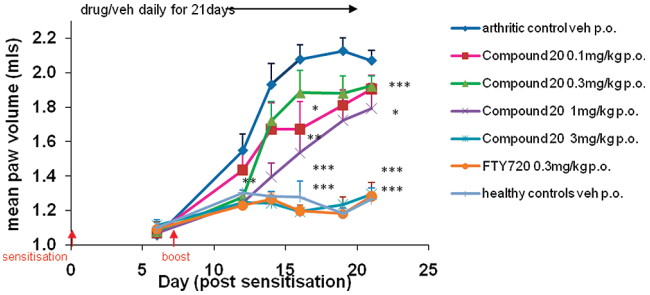


Figure 5. Effect of once daily dosing of **20** (as free base) on paw volume in rat collagen-induced arthritis model. *$p < 0.05$, **<0.01, and ***<0.001 vs arthritic control ANOVA, posthoc LS means.

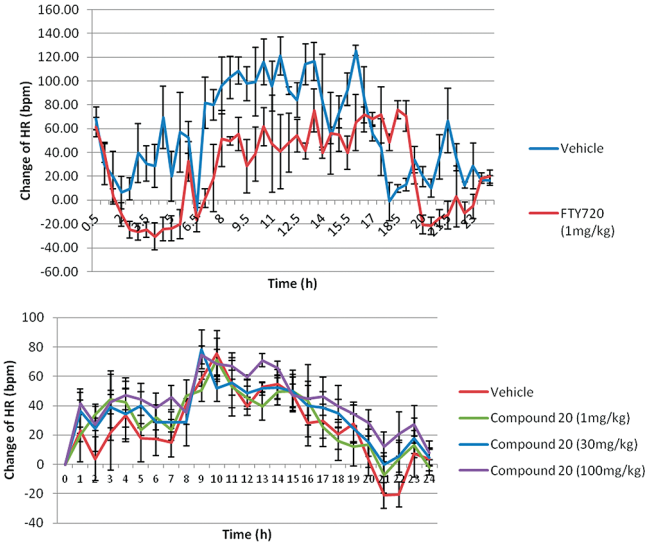


Figure 6. Comparison of the effect of **20** (sodium salt) and **1** on heart rate over time in rats following oral administration.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for the synthesis of compounds **12**−**28**, in vitro assay protocols for the determination of EC_{50}, and protocols for in vivo studies (lymphocyte reduction and CIA model). This material is available free of charge via the Internet at http://pubs.acs.org.

Table 2. In Vitro Profile of 20

MW, PSA, cLogP	446, 112, 1.94
CHI LogD at pH 2.0, 7.4, and 10.5	1.25, 1.72, 1.41
solubility (FeSSIF, mg/mL)	0.16
permeability (MDCK type 2, nm/s)	200
hepatocyte CLi (mg/min/g liver; rat, dog, mouse, human)	<0.85, <1.70, <0.85, <0.85
CYP IC_{50} (μM, 1A2, 2C9, 2C19, 2D6, 3A4VG, 3A4 VR, n = 4)	>50, >50, >50, >50, >50, 29 ± 15

Table 3. In Vivo PK of 20 (Sodium Salt)

species	mouse	rat	dog
strain	CD-1	CD	beagle
dose iv,[a] po (mg/kg)	1, 3	1, 3	1, 2
CLb[b] (mL/min/kg), % liver blood flow	18 ± 4,[c] 15%	5 ± 1, 6%	10 ± 3, 25%
Vss (L/kg)	1.9 ± 0.6[c]	1.1 ± 0.1	2.2 ± 0.6
$t_{1/2}$ (iv, h)	1.6 ± 0.7[c]	3.0 ± 0.1	4.8 ± 3.0
F, po %[d]	64 ± 17	98 ± 13	53 ± 11

[a] iv dose was 1 h of infusion in DMSO:10% (w/v) Kleptose HPB (2:98). [b] Values are means (n = 3) ± SD unless otherwise noted. [c] iv value for n = 4. [d] Dose vehicle: 1% (w/v) methylcellulose (400 cps) (aq).

■ AUTHOR INFORMATION

Corresponding Author

*Tel: + 44 1438 764319. Fax: + 44 1438 768302. E-mail: emmanuel.h.demont@gsk.com.

■ ACKNOWLEDGMENT

We thank Dr. Richard Upton and Nick Waite for NMR support, Dr. Bill Leavens for recording HRMS spectra, and Dr. Eric Rossman, Jason Payseur, and Xuejun Wu for supporting the cardiovascular studies. Dr. Tom Heightman and Dr. Karen Philpott and their co-workers are gratefully acknowledged for their contribution to the early phase of this effort.

■ REFERENCES

(1) Martini, S.; Peters, H.; Böhler, T.; Budde, K. Current Perspectives on FTY720. *Expert Opin. Invest. Drugs* **2007**, *16*, 505–518.

(2) Budde, K.; Schütz, M.; Glander, P.; Peters, H.; Waiser, J.; Liefeldt, L.; Neumayer, H-H; Böhler, T. FTY720 (fingolimod) in renal transplantation. *Clin. Transplant.* **2006**, *20* (Suppl. 17), 17–24.

(3) Kappos, L.; Antel, J.; Comi, G.; Montalban, X.; O'Connor, P.; Polman, C. H.; Haas, T.; Korn, A. A.; Karlsson, G.; Radue, E. W. Oral Fingolimod (FTY720) for Relapsing Multiple Sclerosis. *N. Engl. J. Med.* **2006**, *355*, 1124–1139.

(4) Billich, A.; Bornancin, F.; Dévay, P.; Mechtcheriakova, D.; Urtz, N.; Baumruker, T. Phosphorylation of the Immunomodulatory Drug FTY720 by Sphingosine Kinases. *J. Biol. Chem.* **2003**, *278*, 47408–47415.

(5) Albert, R.; Hinterding, K.; Brinkmann, V.; Guerini, D.; Müller-Hartwieg, C.; Knecht, H.; Simeon, C.; Streiff, M.; Wagner, T.; Welzenbach, K.; Zécri, F.; Zollinger, M.; Cooke, N.; Francotte, E. Novel Immunomodulator FTY720 Is Phosphorylated in Rats and Humans to Form

a Single Stereoisomer. Identification, Chemical Proof, and Biological Characterisation of the Biologically Active Species and Its Enantiomer. *J. Med. Chem.* **2005**, *48*, 5373–5377.

(6) 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.

(7) Wei, S. H.; Rosen, H.; Matheu, M. P.; Sanna, M. G.; Wang, S.-K.; Jo, E.; Wong, C.-H.; Parker, I.; Cahalan, M. D. Sphingosine 1-Phosphate Type 1 receptor Agonism Inhibits Transendothelial Migration of Medullary T Cells to Lymphatic Sinuses. *Nature Immunol.* **2005**, *6*, 1228–1235.

(8) 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 3-(N-benzyl)aminopropylphosphonic acid S1P receptor agonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3501–3505.

(9) 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.

(10) Sanna, M. G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M.-Y.; Peterson, M. S.; Webb, B.; Lefebvre, S.; Chun, J.; Gray, N.; 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.

(11) Hamada, M.; Nakamura, M.; Kiuchi, M.; Marukawa, K.; Tomatsu, A.; Shimano, K.; Sato, N.; Sugahara, K.; Asayama, M.; Takagi, K.; Adachi, K. Removal of Sphingosine 1-Phosphate receptor-3 ($S1P_3$) Agonism is Essential, But Inadequate to Obtain Immunomodulating 2-Aminopropane-1,3-diol $S1P_1$ Agonists with Reduced Effect on Heart Rate. *J. Med. Chem.* **2010**, *53*, 3154–3168.

(12) Buzard, D. J.; Thatte, J.; Lerner, M.; Edwards, J.; Jones, R. M. Recent Progress in the Development of Selective $S1P_1$ Receptor Agonists for the Treatment of Inflammatory and Autoimmune disorders. *Expert Opin. Ther. Patents* **2008**, *18*, 1141–1159.

(13) Crosignani, S.; Bombrun, A.; Covini, D.; Maio, M.; Marin, D.; Quattropani, A.; Swinnen, D.; Simpson, D.; Sauer, W.; Françon, B.; Martin, T.; Cambet, Y.; Nichols, A.; Martinou, I.; Burgat-Charvillon, F.; Rivron, D.; Donini, C.; Schott, O.; Eligert, V.; Novo-Perez, L.; Vitte, P.-A.; Arrighi, J.-F. Discovery of a Novel Series of Potent $S1P_1$ Agonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1516–1519.

(14) Shimizu, H.; Takahashi, M.; Kaneko, T.; Murakami, T.; Hakamata, Y.; Kudou, S.; Kishi, T.; Fukuchi, K.; Iwanami, S.; Kuriyama, K.; Yasue, T.; Enosawa, S.; Matsumoto, K.; Takeyoshi, I.; Morishita, Y.; Kobayashi, E. KRP-203, a Novel Synthetic Immunosuppressant, Prolongs Graft Survival and Attenuates Chronic Rejection in Rat Skin and Heart Allografts. *Circulation* **2005**, *111*, 222–229.

(15) Bolli, M. H.; Abele, S.; Binkert, C.; Bravo, R.; Buchmann, S.; Bur, D.; Gatfield, J.; Hess, P.; Kohl, C.; Mangold, C.; Mathys, B.; Menyhart, K.; Müller, C.; Nayler, O.; Scherz, M.; Schmidt, G.; Sippel, V.; Steiner, B.; Strasser, D.; Treiber, A.; Weller, T. 2-Imino-thiazolidin-4-one Derivatives as Potent, Orally Active $S1P_1$ Receptor Agonists. *J. Med. Chem.* **2010**, *53*, 4198–4211.

(16) Hale, J. J.; Lynch, C. L.; Neway, W.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.-J.; Parent, S. A.; Chrebet, G.; Bergstrom, J.; Card, D.; Ferrer, M.; Hodder, P.; Strulovici, B.; Rosen, H.; Mandala, S. A Rational Utilisation of High-Throughput Screening Affords Selective, Orally Bioavailable 1-Benzyl-3-carboxyazetidine Sphingosine-1-phosphate-1 Receptor Agonists. *J. Med. Chem.* **2004**, *47*, 6662–6665.

(17) Yan, L.; Huo, P.; Hale, J. J.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M. J.; Milligan, J. A.; Shei, G.-J.; Chrebet, G.; Bergstrom, J.; Card, D.; Mandala, S. M. SAR studies of 3-Arylpropionic Acids as Potent and Selective Agonists of Sphingosine-1-phosphate Receptor-1 ($S1P_1$) with Enhanced Pharmacokinetics Properties. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 828–831.

(18) During the preparation of this manuscript, $S1P_1$ agonists analogous to **7** have been reported as follows:Lanman, B. A.; Cee, V. J.; Cheruku, S. R.; Frohn, M.; Golden, J.; Lin, J.; Lobera, M.; Marantz, Y.; Muller, K. M.; Neira, S. C.; Pickrell, A. J.; Rivenzon-Segal, D.; Schutz, N.; Sharadendu, A.; Yu, X.; Zhang, Z.; Buys, J.; Fiorino, M.; Gore, A.; Horner, M.; Itano, A.; McElvain, M.; Middleton, S.; Schrag, M.; Vargas, H. M.; Xu, H.; Xu, Y.; Zhang, X.; Siu, J.; Bürli, R. W. Discovery of a Potent $S1P_3$-Sparing Benzothiazole Agonist of Sphingosine-1 Phosphate Receptor 1 ($S1P_1$). *ACS Med. Chem. Lett.* **2011**, *2*, 102–106.

(19) Saha, A. K.; Yu, X.; Lin, J.; Lobera, M.; Sharadendu, A.; Chereku, S.; Schutz, N.; Segal, D.; Marantz, Y.; McCauley, D.; Middleton, S.; Siu, J.; Bürli, R. W.; Buys, J.; Horner, M.; Salyers, K.; Schrag, M.; Vargas, H. M.; Xu, Y.; McElvain, M.; Xu, H. Benzofuran Derivatives as Potent, Orally Active $S1P_1$ Receptor Agonists: A Preclinical Lead Molecule for MS. *ACS Med. Chem. Lett.* **2011**, *2*, 97–101.

(20) Feigin, V. Irampanel. *Curr. Opin. Invest. Drugs.* **2002**, *3*, 908–910.

(21) Hirawat, S.; Welch, E. M.; Elfring, G. L.; Northcutt, V. J.; Paushkin, S.; Hwang, S.; Leonard, E. M.; Almstead, N. G.; Ju, W.; Peltz, S. W.; Miller, L. L. Safety, Tolerability, and Pharmacokinetics of PCT124, a Nonaminoglycoside Nonsense Mutation Suppressor, Following Single- and Multiple-Dose Administration to Healthy Male and Female Adult Volunteers. *J. Clin. Pharmacol.* **2007**, *47*, 430–444.

(22) Leeson, P. D.; Springthorpe, B. The Influence of Drug-like Concepts on Decision-making in Medicinal Chemistry. *Nat. Rev. Drug Discovery* **2007**, *6*, 881–890.

(23) See, for example:Lipinski, C. A. Lead- and Drug-like Compounds: The Rule-of-five Revolution. *Drug Discovery Today Technol.* **2004**, *1*, 337–341.

(24) See also:Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.

(25) Wenlock, M. C.; Austin, R. P.; Barton, P.; Davis, A. M.; Leeson, P. D. A Comparison of Physiochemical Property Profiles of Development and Marketed Oral Drugs. *J. Med. Chem.* **2003**, *46*, 1250–1256.

(26) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence The Oral Bioavailability of Drug Candidates. *J. Med. Chem.* **2002**, *45*, 2615–2623.

(27) Hughes, J. D.; Blagg, J.; Price, D. A.; Bailey, S.; DeCrescenzo, G. A.; Devraj, R. V.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Greene, N.; Huang, E.; Krieger-Burke, T.; Loesel, J.; Wager, T.; Whiteley, L.; Zhang, Y. Physiochemical Drug Properties Associated With In Vivo Toxicological Outcomes. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4872–4875.

(28) Reported SAR suggests that the three aryl rings present in agonist **8** are the frame critical to place the substituent in the right position to see agonism; therefore, no attempt to modify these vectors was initiated. See ref 17 and references cited therein.

(29) $S1P_1$ is coupled to Gi so the GTPγS assay was used to confirm agonist function and reflects proximal signaling events induced by agonist engagement. Loss of $S1P_1$ function following agonist-induced receptor internalization is the proposed mechanism of action of these synthetic $S1P_1$ ligands, so we also confirmed $S1P_1$ receptor internalisation using a β-arrestin assay (a surrogate of $S1P_1$ internalization). For $S1P_3$, an absence of activity was required for our compounds. In this case, a GTPγS assay was used.

(30) A few examples with substituted 5-membered ring heterocycles in this position were made and were all very significantly less active than the phenyl derivative.

(31) It has not been unambiguously proven that the acid functionality in this template interacts with the same residues as do the phosphates of **3** and S1P. See the following:Gonzalez-Cabrera, P. J.; Jo, E.; Sanna, M. G.; Brown, S.; Leaf, N.; Marsolais, D.; Schaeffer, M.-T.; Chapman, J.; Cameron, M.; Guerrero, M.; Roberts, E.; Rosen, H. Full

Pharmacological Efficacy of a Novel $S1P_1$ Agonist That Does Not Require S1P-Like Headgroup Interactions. *Mol. Pharmacol.* **2008**, *74*, 1308–1318.

(32) In this experiment, as the free base of **20** was used rather than the sodium salt, maximal lymphopenia was observed only at the 3 mg/kg po dose (in sharp contrast with what was observed in the lymphocyte count experiment using the sodium salt; Figure 4). See the experimental part for details.

(33) The exposure of **20** depends linearly of the dose given between 0.1 and 300 mg/kg po in rats.

(34) Compound **20** was inactive at $S1P_2$ ($pEC_{50} < 4.48$, $n = 6$) and $S1P_4$ ($pEC_{50} < 4.38$, $n = 4$) and a partial agonist at $S1P_5$ ($pEC_{50} = 6.8 \pm 0.16$, 77% of maximum response, $n = 6$). See the experimental part for assay protocols.