Bioorganic & Medicinal Chemistry Letters 22 (2012) 1779–1783

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Isoform-selective thiazolo[5,4-*b*]pyridine $S1P_1$ agonists possessing acyclic amino carboxylate head-groups

Anthony B. Reed *, Brian A. Lanman, Susana Neira, Paul E. Harrington, Kelvin K.C. Sham, Mike Frohn, Alexander J. Pickrell, Andrew S. Tasker, Anu Gore, Mike Fiorino, Andrea Itano, Michele McElvain, Scot Middleton, Henry Morrison, Han Xu, Yang Xu, Min Wong, Victor J. Cee

Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, United States

ARTICLE INFO

Article history:
Received 18 November 2011
Revised 6 December 2011
Accepted 8 December 2011
Available online 21 December 2011

Keywords:
Sphingosine-1-phosphate receptor
$S1P_1$
Thiazolo[5,4-*b*]pyridine
Agonist
Immunosuppression

ABSTRACT

Replacement of the azetidine carboxylate of an $S1P_1$ agonist development candidate, AMG 369, with a range of acyclic head-groups led to the identification of a novel, $S1P_3$-sparing $S1P_1$ agonist, (−)-2-amino-4-(3-fluoro-4-(5-(1-phenylcyclopropyl)thiazolo[5,4-*b*]pyridin-2-yl)phenyl)-2-methylbutanoic acid (**8c**), which possessed good in vivo efficacy and pharmacokinetic properties. A 0.3 mg/kg oral dose of **8c** produced a statistically significant reduction in blood lymphocyte counts 24 h post-dosing in female Lewis rats.

The $S1P/S1P_1$ signaling axis is a key regulator of lymphocyte egress from lymphatic tissue.[1,2] In the past decade, synthetic $S1P_1$ agonists have been demonstrated to suppress normal lymphocyte recirculation, leading to peripheral lymphopenia and immunosuppression.[3,4] The first synthetic $S1P_1$ agonist, fingolimod, was recently approved in the US and EU for the treatment of relapsing–remitting multiple sclerosis.[5] However, clinical studies of fingolimod have associated use of this agent with transient, dose-dependent decreases in mean heart rate and asymptomatic atrioventricular blockade.[6] Since studies in rodents have indicated that lymphocyte sequestration and heart rate reduction are driven by distinct S1P receptor isoforms ($S1P_1$ and $S1P_3$, respectively),[7,8] considerable research has been directed toward the identification of $S1P_1$ agonists with minimal activity at the $S1P_3$ receptor.[9]

We have previously reported efforts toward the preparation of $S1P_3$-sparing $S1P_1$ agonists, ultimately leading to the discovery of the potent and highly isoform-selective $S1P_1$ agonist, AMG 369 (Fig. 1).[10] This work centered on the investigation of analogs possessing a shared azetidine carboxylate head-group.[10,11] As part of a back-up effort, we sought to identify structurally distinct analogs of AMG 369 which possessed similarly favorable pharmacokinetic and pharmacodynamic properties. Herein, we describe the investigation of a series of AMG 369 analogs wherein the azetidine carboxylate head-group is replaced with a range of acyclic head-groups. This work has culminated in the identification of a novel $S1P_3$-sparing $S1P_1$ agonist, (−)-2-amino-4-(3-fluoro-4-(5-(1-phenylcyclopropyl)thiazolo[5,4-*b*]pyridin-2-yl)phenyl)-2-methylbutanoic acid (**8c**), which possesses good in vivo efficacy and pharmacokinetic properties comparable to AMG 369.

Studies of the binding of S1P to $S1P_1$ and $S1P_1$ mutants support a model in which the amino phosphate head-group of S1P forms a set of polar interactions with side-chains of the residues R120, R292, and E121 of the $S1P_1$ receptor.[12] These interactions comprise three sets of hydrogen bonds: two between the phosphate group of S1P and a pair of receptor arginine residues (R120 and R292) and one between the primary amine of S1P and a glutamic acid residue (E121). In initial efforts leading to the identification of AMG 369, our decision to retain an azetidine carboxylate as a polar head-

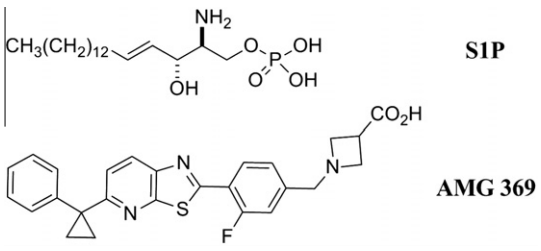


Figure 1. S1P and AMG 369.

* Corresponding author.
E-mail address: areed@amgen.com (A.B. Reed).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.12.073

group stemmed from the presumed ability of this moiety to preserve such polar interactions (as evinced by the high $S1P_1$ potency of these analogs). Following extensive optimization of the lipophilic 'tail' region of our agonists in work leading to AMG 369, we decided to examine whether alternative polar head-groups would afford similarly potent and isoform-selective $S1P_1$ agonists when appended to the optimized lipophilic 'tail' of AMG 369.

The past decade of research on synthetic $S1P_1$ agonists has seen considerable effort directed toward the investigation of polar mimics of the amino phosphate head-group found in S1P.[9] In contemplating our approach toward the identification of a suitable alternative to the azetidine carboxylate portion of AMG 369, we elected to initially investigate the relative contributions of the amine and carboxylic acid portions of the azetidine carboxylate head-group to $S1P_1$ receptor agonism through the preparation of acyclic analogs possessing only one of these two polar groups. Two assays were employed in determining the agonist activity of our compounds: $S1P_1$ activity was assessed by measuring receptor internalization (RI) of an $hS1P_1$-GFP fusion protein in U2OS cells, and $S1P_3$ activity was determined using a Ca^{2+} mobilization assay in $hS1P_3$- and $G_{q/i5}$-co-transfected CHO-K1 cells.[13]

We commenced our studies by preparing a series of homologous alkyl carboxylate analogs (**2a–d**) to probe the influence of carboxylate disposition on $S1P_1$ potency (Table 1). These analogs largely showed weak $S1P_1$ agonist activity, with the exception of butyric acid derivative **2c**, which, in addition to possessing significant $S1P_1$ activity ($hS1P_1$ EC_{50} = 62 nM), demonstrated no detectable activity at $hS1P_3$ (EC_{50} > 25 μM). Interestingly, however, **2c** proved no more potent toward $S1P_1$ than a truncated AMG 369 analog which completely lacked a head-group (cf., **1**).

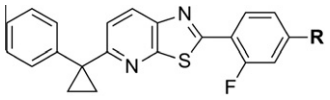
A similar investigation of homologous primary amine head-groups revealed such analogs to be uniformly more potent $S1P_1$ agonists than the corresponding carboxylic acids (Table 1). Aminomethyl (**3b**) and aminopropyl (**3d**) analogs proved exceptionally potent ($hS1P_1$ EC_{50} = 15 and 8 nM, respectively) and, like **2c**, displayed no detectible activity at $S1P_3$ (EC_{50} > 2.5 μM).

With lone amino or carboxylate groups unable to recapitulate the activity of the azetidine carboxylate head-group, we subsequently investigated the reintroduction of the carboxylic acid functionality into amine analogs **3a**, **3b**, and **3c**. In the aniline-derived series, propionate and butyrate derivatives **4a** and **4b** demonstrated threefold increases in activity at $S1P_1$. In the benzylamine series, appending an acetate unit to **3b** led to a fivefold increase in activity at $S1P_1$ (**5a**; EC_{50} = 3 nM), although such modification also led to measurable activity at $S1P_3$ ($hS1P_3$ EC_{50} = 1.56 μM).[14] The homologous propionate derivative **5b** proved similarly active ($hS1P_1$ EC_{50} = 3 nM), but interestingly, was devoid of activity at $S1P_3$ at 25 μM. Further homologation of **3b** (i.e., butyrate derivative **5c**) led to a dramatic drop-off in $S1P_1$ activity ($hS1P_1$ EC_{50} = 179 nM), consistent with the homologous carboxylic acid series (**2a–d**) where chain length demonstrated a profound influence on $S1P_1$ activity. N-methylation of propionate derivative **5b** led to a significant reduction in $S1P_1$ activity (**5d**; $hS1P_1$ EC_{50} = 196 nM), an interesting result given that **5d** is a simple ring-opened analog of AMG 369. Preparation of the isomeric homobenzylic amine derivative **6** (cf., **5b**) revealed that although alternative amine positioning was well-tolerated by $S1P_1$ (EC_{50} = 2 nM), placement of the amine closer to the carboxylate function also led to enhanced activity at $S1P_3$ (EC_{50} = 1.44 μM).

In separate efforts to further investigate the influence of altered amine and carboxylate disposition on $S1P_1$ activity, a series of branched amino acid head-groups related to succinamic acid **7a** were prepared (Table 2). As demonstrated by analog **7a**, introduction of an amide group into the alkyl carboxylate head-group of **4b** was well-tolerated, leading to a modest increase in both $S1P_1$ and $S1P_3$ activity. Further substitution of the succinamic acid head-group **7a** with a 2- or 3-amino group led to a series of potent $S1P_1$ agonists ($hS1P_1$ $EC_{50} \leqslant 2$ nM). Although the absolute configuration of the amine substituents had little impact on $S1P_1$ activity, it is interesting to note that 2-amino succinamic acid derivatives **7d** and **7e** were less active at $S1P_1$ and $S1P_3$ than the corresponding 3-amino analogs (**7b** and **7c**).[15]

The effect of α-amino substitution on shorter-chain carboxylate head-groups (i.e., butyric acid analog **2c**) was also examined through the preparation of glycine and alanine-derived analogs **8a**/**b** and **8c**/**d** (Table 3).[16] As anticipated given the potency and $S1P_1$-selectivity of **2c** and propylamine **3d**, these analogs proved exceptionally potent $S1P_1$ agonists (EC_{50} = 0.2–3 nM) with no detectible activity at $S1P_3$. It is again interesting to note that, just like analogs **7b**–**e**, the stereochemistry of **8a**/**b** and **8c**/**d** had only a moderate effect on $S1P_1$ activity (1.7–15×); this contrasts the more significant impact (~1000×) of head-group stereochemistry

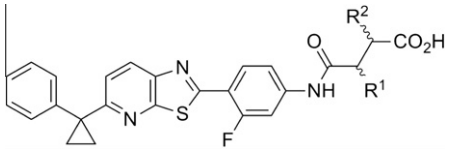
Table 1
$S1P_1$ and $S1P_3$ activity of agonists possessing acyclic head-groups[a]



Compound	R	$hS1P_1$ RI EC_{50} (μM) (% efficacy)	$hS1P_3$ Ca^{2+} EC_{50} (μM) (% efficacy)
AMG 369		0.002 (98)	0.888 (26)
1	–H	0.045 (98)	>25
2a	$-CO_2H$	2.82 (105)	>25
2b	$-(CH_2)_2CO_2H$	5.88 (81)	>25
2c	$-(CH_2)_3CO_2H$	0.062 (100)	>25
2d	$-(CH_2)_4CO_2H$	2.41 (103)	>25
3a	$-NH_2$	0.123 (92)	>25
3b	$-CH_2NH_2$	0.015 (113)	>2.5
3c	$-(CH_2)_2NH_2$	0.478 (155)	>25
3d	$-(CH_2)_3NH_2$	0.008 (121)	>2.5
4a	$-NH(CH_2)_2CO_2H$	0.042 (94)	>2.5
4b	$-NH(CH_2)_3CO_2H$	0.036 (113)	7.12 (18)
5a	$-CH_2NHCH_2CO_2H$	0.003 (103)	1.56 (17)
5b	$-CH_2NH(CH_2)_2CO_2H$	0.003 (96)	>25
5c	$-CH_2NH(CH_2)_3CO_2H$	0.179 (128)	>2.5
5d	$-CH_2NMe(CH_2)_2CO_2H$	0.196 (153)	>25
6	$-(CH_2)_2NHCH_2CO_2H$	0.002 (102)	1.44 (11)

[a] Data represent an average of at least two determinations. Percent efficacy is reported relative to 1.00 μM 1-(4-(6-benzylbenzofuran-2-yl)-3-fluorobenzyl)azetidine-3-carboxylic acid and 0.200 μM S1P for $hS1P_1$ RI and $hS1P_3$ Ca^{2+} assays, respectively. >[Highest concentration tested] is reported for compounds that do not achieve >10% of control activity.

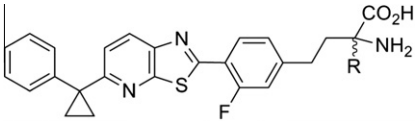
Table 2
$S1P_1$ and $S1P_3$ activity of agonists possessing succinamic acid head-groups[a]



Compound	R^1	R^2	$hS1P_1$ RI EC_{50} (μM) (% efficacy)	$hS1P_3$ Ca^{2+} EC_{50} (μM) (% efficacy)
7a	H	H	0.019 (80)	5.56 (39)
7b	(*R*)-NH_2	H	0.0006 (100)	0.834 (31)
7c	(*S*)-NH_2	H	0.0005 (134)	1.13 (35)
7d	H	(*R*)-NH_2	0.002 (110)	>2.5
7e	H	(*S*)-NH_2	0.002 (102)	>2.5

[a] Data represent an average of at least two determinations. Percent efficacy is reported as in Table 1. >[Highest concentration tested] is reported for compounds that do not achieve >10% of control activity.

Table 3
$S1P_1$ and $S1P_3$ activity of agonists possessing 2-aminobutyrate head-groups[a]



Compound	R	Enantiomer	$hS1P_1$ RI EC_{50} (μM) (% efficacy)	$hS1P_3$ Ca^{2+} EC_{50} (μM) (% efficacy)
2c			0.062 (100)	>25
8a	H	1	0.0003 (128)	>2.5
8b	H	2	0.0005 (115)	>2.5
8c	Me	(−)-1[b]	0.0002 (114)	>2.5
8d	Me	(+)-2[b]	0.003 (93)	>2.5

[a] Data represent an average of at least two determinations. Percent efficacy is reported as in Table 1. >[Highest concentration tested] is reported for compounds that do not achieve >10% of control activity.
[b] See Supplementary data for specific rotations.

on $S1P_1$ activity reported with the aminophosphate agonist FTY720-P.[17]

To assess the physiological effects of agonists identified from these studies, compounds demonstrating $S1P_1$ activities equal to or greater than that of AMG 369 were dosed orally at 1 mg/kg in female Lewis rats to determine their impact on circulating lymphocyte counts 24 h post-dose (Table 4). Compounds exhibiting the maximum physiologically achievable peripheral lymphocyte reduction (PLR) of ~70%[18] were further profiled in dose–response studies.

Table 4
Peripheral lymphocyte reduction (PLR) following oral dosing of potent $S1P_1$ agonists in female Lewis rats[a]

Compd	% PLR ± SE @ 1 mg/kg	Plasma concentration ± SE (ng/mL)
AMG 369	71 ± 2*	116 ± 12
5a	37 ± 17*	7 ± 3
5b	27 ± 17	12 ± 6
6	15 ± 10	1 ± 0.5
7b	0 ± 16	2 ± 0.3
7c	0 ± 15	1 ± 0.3[b]
7d	0 ± 27	4 ± 0.5
7e	4 ± 4	8 ± 1
8a	55 ± 5*	5 ± 0.8
8b	22 ± 4*	2 ± 0.4
8c	71 ± 3*	10 ± 1
8d	15 ± 11	6 ± 1

[a] Lymphocyte counts and compound plasma concentrations measured 24 h post-dosing. % PLR and plasma concentrations reported as average values. ($N = 5$/group; *$P < 0.05$ versus vehicle by ANOVA/Dunnett's Multiple Comparison Test; vehicle = 20% Captisol, 1% HPMC, 1% Pluronic F68, pH = 2).
[b] Plasma concentration ± SD ($N = 2$/group, as plasma concentrations for three rats were below the quantifiable limit).

Of the eleven compounds tested at 1 mg/kg, α-methyl-α-aminobutyrate **8c** demonstrated the best efficacy in the PLR assay.[19] The dose response profile of **8c** was therefore determined at 0.1, 0.3, and 1 mg/kg (Fig. 2). Although **8c** demonstrated no detectable PLR at a dose of 0.1 mg/kg due to extremely low plasma exposure, an oral dose of 0.3 mg/kg was sufficient to produce statistically significant lymphocyte depletion (29 ± 6% vs vehicle) 24 h post-dosing, with a near maximal lymphocyte depletion (56 ± 11% vs vehicle), at 1 mg/kg (Fig. 2).[20]

Pharmacokinetic profiling of **8c** in male Sprague-Dawley rats (1 mg/kg i.v.; 3 mg/kg p.o.) revealed **8c** to possess acceptable characteristics for further pre-clinical investigation: clearance was low

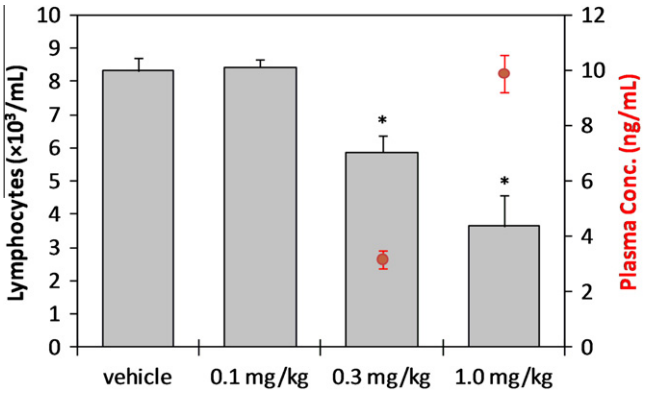
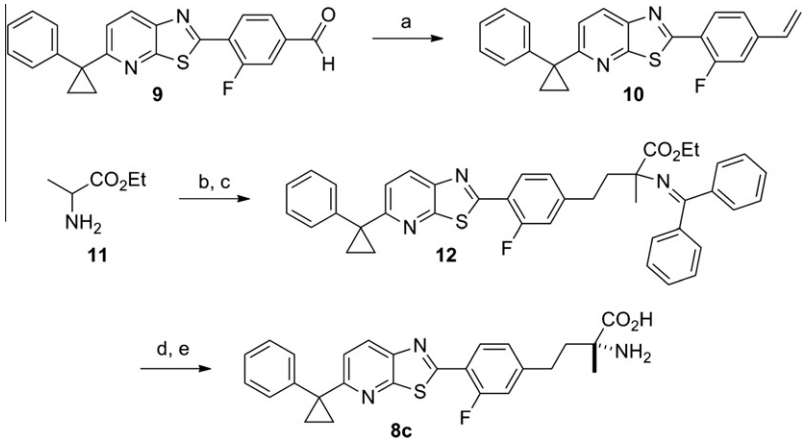


Figure 2. Compound **8c** dosed orally reduces blood lymphocyte counts in female Lewis rats 24 h post-dose. ($N = 5$/group; bars represent average blood lymphocyte counts + SE; circles represent average plasma concentration ± SE; *$P < 0.05$ versus vehicle by ANOVA/Dunnett's Multiple Comparison Test; vehicle = 20% Captisol, 1% HPMC, 1% Pluronic F68, pH = 2).



Scheme 1. Synthesis of **8c**. (a) $TMSCH_2MgCl$, KO^tBu, THF, 0 °C, 48%; (b) benzophenone imine, DCM, rt, 85%; (c) Cs_2CO_3, DMF, 35 °C; then **10**, 25%; (d) chiral separation (SFC); (e) 5 N HCl, THF, 80 °C, 68%.

(0.36 L/h/kg), with a moderate steady-state volume of distribution (V_{ss} = 4.1 L/kg) and acceptable mean residence time (11.4 h). Oral bioavailability was low (16%)—likely accounting for the low plasma concentrations 24 h post oral dosing.[21] In vitro studies established that **8c** had moderate protein binding in human and rat plasma (fraction unbound = 3.7% and 3.9%, respectively), neither induced nor inhibited CYP3A4 or CYP2D6 nor inhibited the hERG channel in either binding or electrophysiology[22] assays (>30 μM and >10 μM, respectively).

The synthesis of **8c** is shown in Scheme 1. Synthesis commenced with the Peterson olefination of benzaldehyde **9**, which has been previously described as an intermediate in the preparation of AMG 369.[10] The derivatization of the resulting styrene (**10**) was inspired by earlier work establishing that styrenes react at the terminal carbon with diethyl acetamidomalonate in the presence of cesium carbonate.[23] We found that this procedure could also be extended to benzophenone imines of amino acid esters: combination of ethyl alanine benzophenone imine, **10**, and cesium carbonate in warm DMF was found to provide racemic iminoester **12**. The racemate was resolved by chiral supercritical-fluid chromatography, and the separated enantiomers were then deprotected in the presence of HCl to afford **8c** (and **8d**).

In conclusion, an examination of acyclic replacements for the amino carboxylate head-group of AMG 369 lead to the identification of α-methyl-α-aminobutyrate **8c**, which demonstrated exceptional activity at $hS1P_1$ (EC_{50} = 0.2 nM) and no measurable activity at $hS1P_3$ (EC_{50} > 2.5 μM). Oral dosing (1 mg/kg) of **8c** in female Lewis rats produced a durable depletion of circulating lymphocytes 24 h post-dose (71% reduction vs vehicle); moderate but statistically significant lymphocyte depletion could be observed even at 0.3 mg/kg. The positive pharmacodynamic and acceptable pharmacokinetic profiles of **8c**, as well as its selectivity over $S1P_3$, reveal this compound to be a suitable non-azetidine carboxylate derivative of AMG 369 for further preclinical evaluation.

Acknowledgments

We thank Jim Meyer, Ronya Shatila, Beth Tominey, and Philip Wong for in vivo PKDM studies and Wesley Barnhart for chiral separation support.

Supplementary data

Supplementary data (statistical analysis of data presented in Tables 1–3, synthetic routes for the preparation of **1–8d** (including analogs in endnotes 14 and 15), full experimental details for the synthesis of **8c**, and details of in vitro and in vivo assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.073.

References and notes

1. Brinkmann, V. *Pharmacol. Ther.* **2007**, *115*, 84.
2. Gardell, S. E.; Dubin, A. E.; Chun, J. *Trends Mol. Med.* **2006**, *12*, 65.
3. Marsolais, D.; Rosen, H. *Nat. Rev. Drug Disc.* **2009**, *8*, 297.
4. Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. *Science* **2002**, *296*, 346.
5. Brinkmann, V.; Billich, A.; Baumruker, T.; Heining, P.; Schmouder, R.; Francis, G.; Aradhye, S.; Burtin, P. *Nat. Rev. Drug Disc.* **2010**, *9*, 883.
6. (a) Kappos, L.; Radue, E.-W.; O'Connor, P.; Polman, C.; Hohlfeld, R.; Calabresi, P.; Selmaj, K.; Agoropoulou, C.; Leyk, M.; Zhang-Auberson, L.; Burtin, P. *N. Engl. J. Med.* **2010**, *362*, 387; (b) Cohen, J. A.; Barkhof, F.; Comi, G.; Hartung, H.-P.; Khatri, B. O.; Montalban, X.; Pelletier, J.; Capra, R.; Gallo, P.; Izquierdo, G.; Tiel-Wilck, K.; de Vera, A.; Jin, J.; Stites, T.; Wu, S.; Aradhye, S.; Kappos, L. *N. Engl. J. Med.* **2010**, *362*, 402; (c) Schmouder, R.; Serra, D.; Wang, Y.; Kovarik, J. M.; DiMarco, J.; Hunt, T. L.; Bastien, M.-C. *J. Clin. Pharmacol.* **2006**, *46*, 895.
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. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 758; (b) 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. *J. Biol. Chem.* **2004**, *279*, 13839.
8. Recent research has suggested that $S1P_1$ rather than $S1P_3$ mediates heart rate reduction in humans: (a) Gergely, P.; Wallström, E.; Nuesslein-Hildesheim, B.; Bruns, C.; Zécri, F.; Cooke, N.; Traebert, M.; Tuntland, T.; Rosenberg, M.; Saltzman, M. *Mult. Scler.* **2009**, *15*, S125; (b) Brossard, P.; Hofmann, S.; Cavallaro, M.; Halabi, A.; Dingemanse, J. *Clin. Pharmacol. Ther.* **2009**, *85*, S63.
9. (a) Bolli, M. H.; Lescop, C.; Nayler, O. *Curr. Top. Med. Chem.* **2011**, *11*, 726; (b) Buzard, D. J.; Thatte, J.; Lerner, M.; Edwards, J.; Jones, R. M. *Expert Opin. Ther. Patents* **2008**, *18*, 1141–1159; (c) Cooke, N.; Zécri, F. *Ann. Rep. Med. Chem.* **2007**, *42*, 245.
10. Cee, V. J.; Frohn, M.; Lanman, B. A.; Golden, J.; Muller, K.; Neira, S.; Pickrell, A.; Arnett, H.; Buys, J.; Gore, A.; Fiorino, M.; Horner, M.; Itano, A.; Lee, M. R.; McElvain, M.; Middleton, S.; Schrag, M.; Rivenzon-Segal, D.; Vargas, H. M.; Xu, H.; Xu, Y.; Zhang, X.; Siu, J.; Wong, M.; Bürli, R. W. *ACS Med. Chem. Lett.* **2011**, *2*, 107.
11. (a) 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.; Rivenson-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. *ACS Med. Chem. Lett.* **2011**, *2*, 102; (b) 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. *ACS Med. Chem. Lett.* **2011**, *2*, 97–101.
12. Parrill, A. L.; Wang, D.; Bautista, D. L.; Van Brocklyn, J. R.; Lorincz, Z.; Fischer, D. J.; Baker, D. L.; Liliom, K.; Spiegeli, S.; Tigyi, G. *J. Biol. Chem.* **2000**, *275*, 39379.
13. See Supplementary data for details.
14. The corresponding glycolic acid derivative demonstrated dramatically reduced activity at $S1P_1$ (EC_{50} = 1.27 μM).
15. The corresponding 2- and 3-hydroxy succinamic acid analogs were also prepared and gave $S1P_n$ activities similar to those of the amino succinamic acids **7b–e** (data not shown).

16. **8a/b** and **8c/d** were prepared as racemates and separated by chiral chromatography (see Supplementary data).
17. Ma, B.; Guckian, K. M.; Lin, E. Y.-S.; Lee, W.-C.; Scott, D.; Kumaravel, G.; Macdonald, T. L.; Lynch, K. R.; Black, C.; Chollate, S.; Hahm, K.; Hetu, G.; Jin, P.; Luo, Y.; Rohde, E.; Rossomando, A.; Scannevin, R.; Wang, J.; Yang, C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2264.
18. 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. *J. Med. Chem.* **2004**, *47*, 6662.
19. For previous reports of acyclic aminocarboxylates as aminophosphate surrogates in $S1P_1$ agonists, see: (a) Kurata, H.; Kusumi, K.; Otsuki, K.; Suzuki, R.; Kurono, M.; Takada, Y.; Shioya, H.; Komiya, T.; Mizuno, H.; Ono, T.; Hagiya, H.; Minami, M.; Nakade, S.; Habashita, H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3885; (b) Kurata, H.; Otsuki, K.; Kusumi, K.; Kurono, M.; Terakado, M.; Seko, T.; Mizuno, H.; Ono, T.; Hagiya, H.; Minami, M.; Nakade, S.; Habashita, H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1390; (c) Högenauer, K.; Hinterding, K.; Nussbaumer, P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1485; (d) Pan, S.; Mi, Y.; Pally, C.; Beerli, C.; Chen, A.; Guerini, D.; Hinterding, K.; Nuesslein-Hildesheim, B.; Tuntland, T.; Lefebvre, S.; Liu, Y.; Gao, W.; Chu, A.; Brinkmann, V.; Bruns, C.; Streiff, M.; Cannet, C.; Cooke, N.; Gray, N. *Chem. Biol.* **2006**, *13*, 1227; (e) 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. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3351–3355.
20. By comparison, AMG 369 demonstrated a % PLR = 65 ± 2* (plasma concentration = 10 ± 0.4 ng/mL) 24 h post-oral dosing at 0.1 mg/kg.
21. Reduced apical-to-basolateral permeability for **8c** (4 μcm/s) relative to AMG 369 (14.6 μcm/s) may account for the reduced oral bioavailability of **8c**, as **8c** demonstrated enhanced solubility (0.01 N HCl: 6 ng/mL; PBS: <1 ng/mL; SIF: 108 ng/mL) relative to AMG 369 (0.01 N HCl: 3 ng/mL; PBS: 7 ng/mL; SIF: 65 ng/mL) in multiple media and was not a Pgp substrate (efflux ratio = 1.8; rat MDR1-transfected LLC-PK1 cells). Simulated intestinal fluid: 5 mM sodium taurochol and 1.5 mM lecithin in 0.029 M KH_2PO_4, 0.22 M KCl at pH 6.8. Others have similarly reported an azetidine carboxylate-containing $S1P_1$ agonist to possess higher oral exposure relative to a related acyclic amino carboxylate analog (cf., Ref. 19a).
22. PatchXpress® 7000A Automated Parallel Patch-Clamp System, Molecular Devices, 1311 Orleans Drive, Sunnyvale, CA 94089.
23. Chino, M.; Kiuchi, M.; Adachi, K. *Tetrahedron* **2008**, *64*, 3859.