

UC Irvine

UC Irvine Previously Published Works

Title

Design, Synthesis, and Antileukemic Activity of Stereochemically Defined Constrained Analogues of FTY720 (Gilenya)

Permalink

<https://escholarship.org/uc/item/7wr3164f>

Journal

ACS Medicinal Chemistry Letters, 4(10)

ISSN

1948-5875

Authors

Fransson, Rebecca
McCracken, Alison N
Chen, Bin
et al.

Publication Date

2013-10-10

DOI

10.1021/ml4002425

Peer reviewed

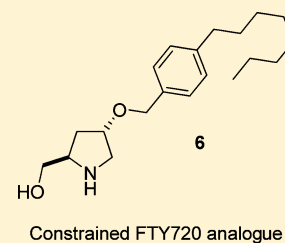
Design, Synthesis, and Antileukemic Activity of Stereochemically Defined Constrained Analogues of FTY720 (Gilenya)

Rebecca Fransson,^{†,§,||} Alison N. McCracken,^{‡,||} Bin Chen,[†] Ryan J. McMonigle,[‡] Aimee L. Edinger,^{*,‡} and Stephen Hanessian^{*,†}[†]Department of Chemistry, Université de Montréal, Station Centre-Ville, Montréal, Quebec H3C 3J7, Canada[‡]Department of Developmental and Cell Biology, University of California, Irvine, 2128 Natural Sciences 1, California 92697, United States

Supporting Information

ABSTRACT: FTY720 functions as an immunosuppressant due to its effect on sphingosine-1-phosphate receptors. At doses well above those needed for immunosuppression, FTY720 also has antineoplastic actions. Our published work suggests that at least some of FTY720's anticancer activity is independent of its effects on S1P receptors and due instead to its ability to induce nutrient transporter down-regulation. Compounds that trigger nutrient transporter loss but lack FTY720's S1P receptor-related, dose-limiting toxicity have the potential to be effective and selective antitumor agents. In this study, a series of enantiomerically pure and stereochemically diverse *O*-substituted benzyl ethers of pyrrolidines was generated and tested for the ability to kill human leukemia cells. The stereochemistry of the hydroxymethyl was found to be a key determinant of compound activity. Moreover, phosphorylation of this group was not required for antileukemic activity.

KEYWORDS: FTY720, leukemia, pyrrolidine, arylmethyl ether



Since its discovery as a synthetic immunomodulatory agent, FTY720 (**1**, also known as Fingolimod), has been the subject of intensive research efforts on many fronts (Figure 1).

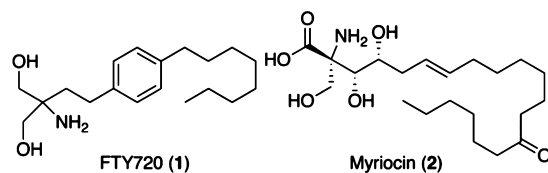


Figure 1. Structures of FTY720 and myriocin.

It is presently marketed as a drug under the trade name Gilenya for the treatment of relapsing–remitting multiple sclerosis. The conceptual basis for its structural derivation and deployment of an aminodiol functionality on an aromatic moiety bearing a hydrophobic aliphatic chain was inspired from the natural product myriocin (**2**). When employed as an immunosuppressant, FTY720 is a pro-drug as a result of an *in vivo* phosphorylation, leading specifically to the pro-*S*-phosphate ester isomer. Once phosphorylated, it acts as a functional antagonist for four of the five sphingosine-1-phosphate (S1P) receptors, reducing inflammation by preventing lymphocyte egress from secondary lymphoid organs.^{1,2} Significant research efforts have been directed toward developing S1P receptor selective agonists and antagonists. In an effort to study the stereochemistry of phosphorylation, a number of synthetic analogues of FTY720 that focus mainly on the polar subunit have been generated and the biological activities of the resulting phosphate esters reported.^{3–11}

Furthermore, the C₈H₁₇ lipophilic appendage on the phenyl ring of FTY720 was found to be optimal for activity at S1P receptors.

Several research groups have demonstrated that, at higher doses than are required for its immunosuppressive effect, FTY720 is an effective and selective anticancer agent.^{12–16} Although FTY720 is very effective in diverse animal cancer models, it cannot be used in human patients due to dose-limiting bradycardia that occurs secondary to the activation of S1P1 and S1P3.^{17,18} Until recently, it was thought that the anticancer activities of FTY720 were intimately associated with its effects on S1P receptors. Indeed, FTY720's antiangiogenic properties depend on S1P1 receptor effects.¹⁹ However, the dose of FTY720 required for anticancer activity is well above that required for immunosuppression due to S1P receptor effects suggesting an alternate mode of action in cancer. Recent studies from our group demonstrated that the anticancer and S1P receptor effects of FTY720 are clearly separable and provided a novel mechanism of action for the drug at the elevated anticancer dose: nutrient transporter down-regulation.²⁰ These studies suggest that an FTY720 analogue that does not activate S1P receptors has the potential to be an effective and selective anticancer agent that could be safely used in humans.

In a previous study, we had prepared two enantiomeric 2,3,5-trisubstituted pyrrolidines, compounds **3** and **4** as constrained

Received: June 26, 2013

Accepted: August 3, 2013

Published: August 21, 2013

azacyclic analogues of FTY720 (Figure 2).⁴ Remarkable selectivity was observed for S1P4 and S1P5 over S1P1 and

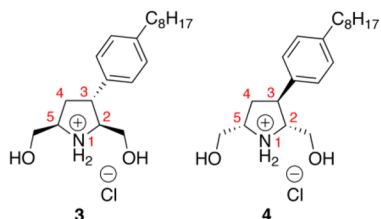


Figure 2. Structures of enantiomeric pyrrolidine analogues 3 and 4.

S1P3, demonstrating that chemical modification of the conformationally flexible aminodiol portion of FTY720 could lead to selective affinities toward the S1P receptors. Furthermore, one of the diastereomers, compound 4, was not susceptible to phosphorylation by sphingosine kinase 1 or 2, and when chemically rather than enzymatically phosphorylated, the individual (*2R*) and (*5S*) phosphate esters of 4 had no activity at S1P1 or S1P3. In an effort to simplify the synthetic protocol and incorporate a conformationally more flexible aryl appendage, we synthesized a series of enantiomerically pure and stereochemically diverse *O*-substituted benzyl ethers of pyrrolidines starting with appropriately substituted 4-hydroxy *D*- or *L*-prolines (Figure 3). Herein, we describe the anticancer effects of these analogues in multiple human cancer cell lines using FTY720 as a control.

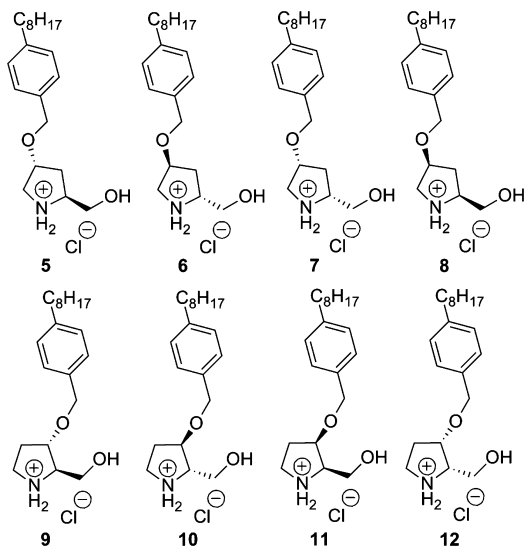


Figure 3. Structures of diastereomeric 2-hydroxymethyl pyrrolidine 3- and 4-ether analogues, 5–12.

We first compared the activity of a diastereomeric series of compounds comprising two enantiomeric pairs (5, 6 and 7, 8) in cell viability assays using the BCR-ABL positive human acute lymphoblastic leukemia (ALL) cell line, Sup-B15. All compounds in this series killed leukemia cells as efficiently as FTY720 (Figure 4 and Supporting Information). Interestingly, the (*2R,4S*)-isomer 6 showed a 4-fold increase in activity compared to its enantiomer 5. Moreover, compound 8, the syn-diastereomer in this series, exhibited a dramatic 8-fold decrease in activity compared to compound 6 and was 2-fold weaker than its enantiomer 7. Together, these results demonstrate that the 3-dimensional orientation of the ether appendage relative to

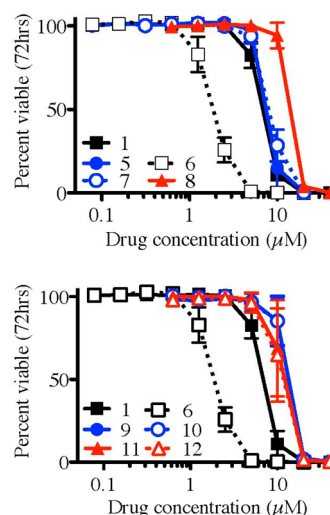


Figure 4. Cytotoxic action of diastereomeric 2-hydroxymethyl pyrrolidine 3- or 4-arylmethyl ethers on Sup-B15 leukemia cells. Cell viability was measured by vital dye exclusion and flow cytometry at 72 h.

the hydroxymethyl group in this series has a strong influence over the ability of these compounds to kill these leukemia cells. Furthermore, it is clear that the novel, constrained diastereoisomers represented by compounds 5–8 trigger cell death with similar potency to the more flexible parent FTY720, with a distinct preference for compound 6.

Given the observed influence of stereochemistry over activity, we next evaluated the antileukemic activity of compounds 9–12 in which the position of the ether appendage was changed to be spatially proximal to the hydroxymethyl group (Figure 3).

Compound 10 was 6-fold less active than compound 6, similar to the other members of this series of analogues (Figure 4). Thus, it appears that the stereochemistry and particular orientation of the ether appendage in compound 6 is unique in conferring enhanced anticancer activity relative to its diastereomeric congeners and to FTY720 in this leukemic cell line.

While the ability to interfere with S1P1 receptor signaling is critical for its immunosuppressive activity at low nanomolar doses, activation of S1P1 and S1P3 by FTY720 prevents it from being used in cancer therapy. Since the constrained analogues might also be subject to phosphorylation in the cells, we synthesized a series of compounds in which the hydroxymethyl group was modified or entirely removed. For example, in compounds 13 and 14, the hydroxymethyl group present in 5 and 6, respectively, was replaced with a methyl group (Figure 5). Loss of this potential phosphorylation site had no detectable effect on the potency of 13 relative to 5 but decreased the activity of 14 relative to 6 (Figure 6). Similarly, protecting the hydroxyl group as the *O*-methyl ether as in compound 15 reduced the potency relative to 6. Removing the hydroxymethyl group from 5 to give the pyrrolidine 16 had only a marginal effect on activity in cell viability assays. However, the enantiomeric pyrrolidine analogue 17 exhibited a 6-fold reduction in activity relative to 6. As compounds 13–17 cannot be phosphorylated but retain the ability to kill leukemia cells, the results are consistent with our model where phosphorylation is not important for the ability of FTY720 to kill cancer cells.²⁰ Although phosphorylation is not required

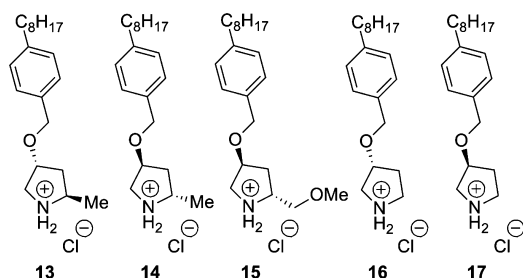


Figure 5. Structures of 2-substituted pyrrolidine 4-ethers (13–17).

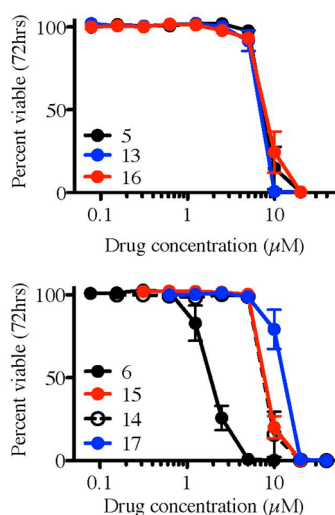


Figure 6. Cytotoxic action of 2-substituted pyrrolidine 4-arylmethyl ethers and 3-pyrrolidine arylmethyl ethers on Sup-B15 leukemia cells.

for activity, compound **6** appears to dominate the stereochemical spectrum in these enantiomerically pure 2-hydroxymethyl pyrrolidines given its enhanced activity relative to the other stereoisomers and to FTY720. Finally, we also noted that the nature and length of the aliphatic chain was critical to the activity. Analogues of compound **5** with shorter and longer chains or with a simple heptyl chain were less active, as was a MOM ether (see Supporting Information).

Having observed an increased potency of **6** relative to its diastereoisomers **5**, **7**, and **8**, we wished to determine whether this differential activity was also seen in other cancer cell lines. Cell viability assays were used to compare the activity of the constrained analogues and FTY720 in an additional BCR-ABL positive ALL cell line, BV173. In this cell line, compound **6** was again more active than its stereoisomers **5**, **7**, and **8** (Table 1). Interestingly, compound **6** was also 10-fold more active than compound **5** in murine bone marrow transformed by

introduction of the BCR-ABL fusion protein p190. Nalm-6, Blin-1, and CCRF-CEM are also ALL cell lines but do not express the oncogenic BCR-ABL fusion protein. In these three human leukemia cell lines, **6** no longer exhibited increased potency relative to other compounds in the series. The effect of compound **6** and its diastereoisomeric congeners on the prostate cancer cell lines PC3 and DU145 was also determined. Compounds **5** and **6** induced cell death to a similar extent, and the potency of the constrained analogues was slightly reduced relative to FTY720. From these findings, we conclude that the enhanced potency of **6** over its diastereoisomers is a characteristic associated with hematologic but not prostate cancers and may be linked to expression of the BCR-ABL fusion protein. BCR-ABL-dependent signaling drives the survival and proliferation of chronic myelogenous leukemias and a subset of ALLs. The discovery of the Abl kinase inhibitor imatinib (Gleevec) and development of more potent second generation drugs has allowed for the long-term survival of patients with chronic myelogenous leukemia. However, imatinib and its analogues do not lead to a cure, and drug resistance often develops over time. Thus, novel agents with activity against BCR-ABL positive leukemias could have clinical utility.

Finally, to test whether the constrained analogues were working through the antineoplastic mechanism we previously identified for FTY720, we compared the effect of FTY720 and compounds **5**, **6**, **13**, and **14** on surface expression of the amino acid transporter-associated protein, 4F2hc. All five compounds triggered the rapid down-regulation of 4F2hc suggesting that FTY720 and the conformationally constrained analogues described herein kill cancer cells through the same mechanism (Figure 7).²⁰

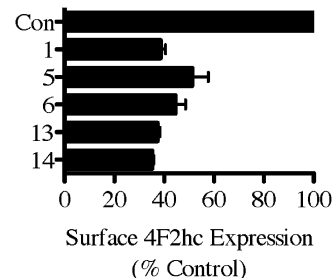


Figure 7. Diastereomeric 2-hydroxymethyl pyrrolidine 4-arylmethyl ethers (10 μ M) trigger nutrient transporter loss in Sup-B15 leukemia cells.

In conclusion, we have reported on the anticancer activity of a series of novel constrained analogues of FTY720, including a stereochemically distinct isomer (**6**) with enhanced activity. A

Table 1. Mean IC_{50} (in μ M \pm SEM) of Analogues in Cell Viability Assays in a Range of Human Cancer Cell Lines and BCR-ABL-Expressing Murine Bone Marrow (BM); Viability Was Measured by Vital Dye Exclusion and Flow Cytometry at 72 h; When Compared to FTY720 Using a *t*-test (two-tailed): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

	SupB15		BV173		CCRF-CEM	Nalm-6	Blin-1	PC3	DU145
	Ph + ALL	BM-p190	Ph + ALL	Ph - ALL	Ph - ALL	Ph - ALL	Ph - ALL	prostate	prostate
1	6.8 \pm 0.7	3.3 \pm 0.2	6.3 \pm 0.4	6.8 \pm 0.3	9.6 \pm 1.9	5.5 \pm 0.1	9.8 \pm 0.9	6.5 \pm 0.9	
5	7.7 \pm 0.8	5.7 \pm 1.1*	10.4 \pm 0.7***	11.0 \pm 1.3***	15.0 \pm 2.1	7.5 \pm 0.1***	14.3 \pm 1.2*	10.8 \pm 0.4*	
6	2.0 \pm 0.2***	0.5 \pm 0.1***	3.8 \pm 0.4**	8.2 \pm 0.7	13.5 \pm 2.4	6.9 \pm 0.3**	13.5 \pm 2.6	15.1 \pm 1.0**	
7	8.3 \pm 0.8	4.0 \pm 0.4	9.7 \pm 1.0**	8.1 \pm 1.4					
8	16.7 \pm 2.4***	8.4 \pm 1.5**	13.8 \pm 0.8***	11.6 \pm 1.1***					

subset of these compounds, and particularly those lacking the hydroxymethyl group, may lack the dose-limiting toxicities of the parent compound yet kill leukemia cells with similar potency.²¹

■ ASSOCIATED CONTENT

● Supporting Information

Experimental procedures, biological procedures, ¹H and ¹³C NMR spectra, HPLC analysis, and references. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Authors

*(A.L.E.) aedinger@uci.edu

*(S.H.) E-mail: stephen.hanessian@umontreal.ca

Present Address

§(R.F.) Department of Medicinal (Organic Pharmaceutical) Chemistry, Uppsala University, Uppsala, Sweden.

Author Contributions

The manuscript was written by A.L.E. and S.H. and edited by A.N.M. All authors have given approval to the final version of the manuscript.

Author Contributions

||R.F. and A.N.M. contributed equally to this work.

Funding

A.N.M. is supported by Grant Number T32CA009054 from the National Cancer Institute (NCI). A.L.E. is supported by grants from the NIH (R01 GM089919), the American Cancer Society (RSG-11-111-01-CDD), the Department of Defense (W81XWH-11-1-0535), University of California, Irvine Council on Research, Computing, and Libraries (MIIG-2011-12-24 with S.H.), and the William Lawrence and Blanche Hughes Foundation. S.H. is supported by grants from Isis Pharmaceuticals, Sigma Tau USA, NSERC Canada, Servier Canada, and Servier France. S.H. is the Isis Pharmaceuticals Chairholder at the Université de Montréal.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Jordan Thompson, Saurabh Ghosh Roy, Gang Liu, Tiffany Nguyen, and Jessica Kemp for their contributions to IC₅₀ assays.

■ ABBREVIATIONS

ALL, acute lymphoblastic leukemia; BCR-ABL, breakpoint cluster region-Abelson tyrosine kinase fusion protein; Ph, Philadelphia chromosomal translocation t(9;22)(q34;q11); S1P, sphingosine-1-phosphate; S1P1-5, sphingosine-1-phosphate receptor 1-5; 4F2hc, 4F2 heavy chain amino acid transporter-associated protein

■ REFERENCES

- (1) Brinkmann, V. FTY720 (fingolimod) in Multiple Sclerosis: therapeutic effects in the immune and the central nervous system. *Br. J. Pharmacol.* **2009**, *158*, 1173–1182.
- (2) See for example: Tigyi, G.; Valentine, W. J.; Kiss, G. N.; Liu, J.; Lu, X.; Sun, C.; Bittman, R. FTY720 S-ene-phosphonate is a novel panantagonist of the S₁P receptors that inhibits lymphocyte egress. *FASEB J.* **2010**, *24*, lb100.
- (3) Clemens, J. J.; Davis, M. D.; Lynch, K. R.; Macdonald, T. L. Synthesis of 4(5)-phenylimidazole-based analogues of sphingosine-1-

phosphate and FTY720: Discovery of potent S1P₁ receptor agonist. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3568–3572.

- (4) Hanessian, S.; Charron, G.; Billich, A.; Guerini, D. Constrained azacyclic analogues of the immunomodulatory agent FTY720 as molecular probes for sphingosine 1-phosphate receptors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 491–494.

- (5) Davis, M. D.; Clemens, J. J.; Macdonald, T. L.; Lynch, K. R. Sphingosine 1-phosphate analogs as receptor antagonists. *J. Biol. Chem.* **2005**, *280*, 9833–9841.

- (6) Zhu, R.; Snyder, A. H.; Kharel, Y.; Schaffter, L.; Sun, Q.; Kennedy, P. C.; Lynch, K. R.; Macdonald, T. L. Asymmetric synthesis of conformationally constrained fingolimod analogues: discovery of an orally active sphingosine 1-phosphate receptor type-1 agonist and receptor type-3 antagonist. *J. Med. Chem.* **2007**, *50*, 6428–6435.

- (7) 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.

- (8) Valentine, W. J.; Kiss, G. N.; Liu, J.; Shuyu, E.; Gotoh, M.; Murakami-Murofushi, K.; Pham, T. C.; Baker, D. I.; Parrill, A. L.; Li, X.; Sun, C.; Bittman, R.; Pyne, N. J.; Tigyi, G. (S)-FTY720-Vinylphosphonate, an analogue of the immunosuppressive agent FTY, is a pan-antagonist of sphingosine 1-phosphate GPCR signaling and inhibits autotaxin activity. *Cell. Signalling* **2010**, *22*, 1543–1553.

- (9) Lim, K. G.; Sun, C.; Bittman, R.; Pyne, N. J.; Pyne, S. (R)-FTY20 methyl ether is a specific sphingosine kinase 2 inhibitor: effect on sphingosine kinase 2 expression in HEK 293 cells and active rearrangement and survival of MCF-7 breast cancer cells. *Cell Signalling* **2011**, *23*, 1590–1595.

- (10) Sun, C.; Bittman, R. A Photoreactive Analogue of the Immunosuppressant FTY720. *J. Org. Chem.* **2006**, *71*, 2200–2202.

- (11) Kiuchi, M.; Adachi, K.; Kohara, T.; Minoguchi, M.; Hanano, T.; Aoki, Y.; Mishina, T.; Arita, M.; Nakao, N.; Ohtsuki, M.; Hoshino, Y.; Teshima, K.; Chiba, K.; Sasaki, S.; Fujita, T. Synthesis and immunosuppressive activity of 2-substituted 2-aminopropane-1,3-diols and 2-aminoethanols. *J. Med. Chem.* **2000**, *43*, 2946–2961.

- (12) Lee, T. K.; Man, K.; Ho, J. W.; Wang, X. H.; Poon, R. T.; Xu, Y.; Ng, K. T.; Chu, A. C.; Sun, C. K.; Ng, I. O.; Sun, H. C.; Tang, Z. Y.; Xu, R.; Fan, S. T. FTY720: A promising agent for treatment of metastatic hepatocellular carcinoma. *Clin. Cancer Res.* **2005**, *11*, 8458–8466.

- (13) Azuma, H.; Takahara, S.; Ichimaru, N.; Wang, J. D.; Itoh, Y.; Otsuki, Y.; Morimoto, J.; Fukui, R.; Hoshiga, M.; Ishihara, T.; Nonomura, N.; Suzuki, S.; Okuyama, A.; Katsuoka, Y. Marked prevention of tumor growth and metastasis by a novel immunosuppressive agent, FTY720, in mouse breast cancer models. *Cancer Res.* **2002**, *62*, 1410–1419.

- (14) Chua, C. W.; Lee, D. T.; Ling, M. T.; Zhou, C.; Man, K.; Ho, J.; Chan, F. L.; Wang, X.; Wong, Y. C. FTY720, a fungus metabolite, inhibits in vivo growth of androgen-independent prostate cancer. *Int. J. Cancer* **2005**, *117*, 1039–1048.

- (15) Azuma, H.; Takahara, S.; Horie, S.; Muto, S.; Otsuki, Y.; Katsuoka, Y. Induction of apoptosis in human bladder cancer cells in vitro and in vivo caused by FTY720 treatment. *J. Urol.* **2003**, *169*, 2372–2377.

- (16) Neviani, P.; Santhanam, R.; Oaks, J. J.; Eiring, A. M.; Notari, M.; Blaser, B. W.; Liu, S.; Trotta, R.; Muthusamy, N.; Gambacorti-Passerini, C.; Druker, B. J.; Cortes, J.; Marcucci, G.; Chen, C. S.; Verrills, N. M.; Roy, D. C.; Caligiuri, M. A.; Bloomfield, C. D.; Byrd, J. C.; Perrotti, D. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J. Clin. Invest.* **2007**, *117*, 2408–2421.

- (17) 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 S1P₁ and S1P₃,

respectively, regulate lymphocyte recirculation and heart rate. *J. Biol. Chem.* **2004**, *279*, 13839–13848.

(18) Koyrakh, L.; Roman, M. I.; Brinkmann, V.; Wickman, K. The heart rate decrease caused by acute FTY720 administration is mediated by the G protein-gated potassium channel I_{KACH} . *Am. J. Transplant.* **2005**, *5*, 529–536.

(19) LaMontagne, K.; Littlewood-Evans, A.; Schnell, C.; O'Reilly, T.; Wyder, L.; Sanchez, T.; Probst, B.; Butler, J.; Wood, A.; Liao, G.; Billy, E.; Theuer, A.; Hla, T.; Wood, J. Antagonism of sphingosine-1-phosphate receptors by FTY720 inhibits angiogenesis and tumor vascularization. *Cancer Res.* **2006**, *66*, 221–231.

(20) Romero Rosales, K.; Singh, G.; Wu, K.; Chen, J.; Janes, M. R.; Lilly, M. B.; Peralta, E. R.; Siskind, L. J.; Bennett, M. J.; Fruman, D. A.; Edinger, A. L. Sphingolipid-based drugs selectively kill cancer cells by down-regulating nutrient transporter proteins. *Biochem. J.* **2011**, *439*, 299–311.

(21) For a recent synthesis of FTY720, see Kandagatla, B.; Raju, V. V. V. K. V. P.; Kumar, N. S.; Reddy, G. M.; Srinivas, K.; Iqbal, J.; Bandichhor, R.; Orugantio, S. Practical synthesis of fingolimod from diethyl acetamidomalonate. *RSC Adv.* **2013**, *3*, 9687–9689 and references cited therein.

■ NOTE ADDED AFTER ASAP PUBLICATION

This manuscript was published on the Web on August 26, 2013. A citation number was corrected in the text and the revised version was reposted on August 28, 2013.