CHAPTER THIRTEEN

Recent Advances in the Discovery and Development of Sphingosine-1-Phosphate-1 Receptor Agonists

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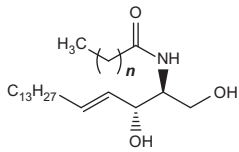
Contents

1. INTRODUCTION

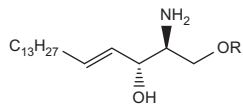
Sphingosine-1-phosphate (S1P, **3**) is a zwitterionic lysophospholipid metabolite of sphingosine (Sph, **2**), which in turn is derived from enzymatic cleavage of ceramides (**1**). Enzymatic phosphorylation of Sph by two kinases (SphK1 and SphK2) leads to the production of S1P largely from erythrocytes.[1] Originally thought to operate solely as an intracellular signaling molecule, S1P was subsequently determined to be a high-affinity ligand for five members of the endothelial differentiation gene class of G-protein-coupled receptors subsequently renamed S1P1–5.[2] The interaction of S1P with the S1P receptors plays a fundamental physiological role in a large number of processes including proliferation, vascular development, and lymphocyte trafficking.[3] Tissue levels of S1P are maintained lower than circulating levels through the action of degrading enzymes (S1P-phosphatases and S1P-lyase). The resulting gradient of S1P is sensed by lymphocytes through interaction with cell surface S1P1 receptors to promote their migration out of the lymphatic system.[4]

Annual Reports in Medicinal Chemistry, Volume 47
ISSN 0065-7743
http://dx.doi.org/10.1016/B978-0-12-396492-2.00013-8

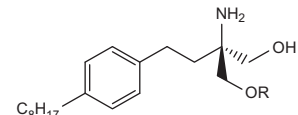
Research efforts around S1P receptors intensified after they were linked to the efficacy observed with the potent immunomodulatory agent fingolimod (**4**, FTY720). Administration of fingolimod was known to induce a significant, but reversible, reduction in circulating lymphocytes through a novel mechanism in which the affected cells were found to be trapped in the thymus and secondary lymphoid organs, thus preventing their access to sites of inflammation or tissue graft.[5] As such, fingolimod was found to be active in a wide array of animal models of inflammatory diseases and solid organ transplant.[6] The precise molecular mode of action of fingolimod was unknown until the discovery that it was stereospecifically monophosphorylated *in vivo* by SphK2, analogous to the conversion of Sph to S1P.[7,8] The resulting metabolite **5** (fingolimod-P) was found to act as a potent agonist of four of the five S1P receptors (S1P1, 3–5). Further genetic and pharmacological experiments linked the lymphocyte sequestration ability of fingolimod specifically to agonism of the S1P1 receptor by **5**. Agonism of S1P1 by **5** results in prolonged internalization and degradation of the receptor, in effect depleting it from the cell surface.[9,10] In this "functional antagonist" mode of operation, the affected lymphocytes can no longer sense the S1P gradient from tissue to blood, thereby losing their ability to exit into circulation. Consistent with this mechanistic hypothesis, recent disclosures have reported the ability of synthetic *antagonists* of S1P1 to also elicit lymphopenia.[11]



1 Ceramides



2 R = H **Sphingosine**
3 R = PO_3H_2 **Sphingosine-1-phosphate**



4 R = H
5 R = PO_3H_2

2. RECENT CLINICAL DEVELOPMENTS OF S1P1 AGONISTS

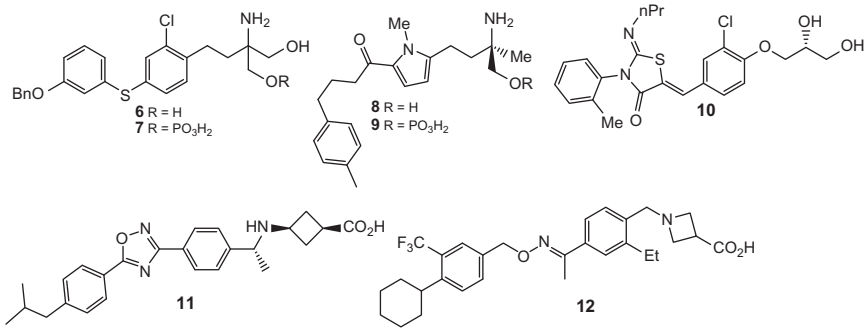
Fingolimod was initially advanced into the clinic for the prevention of graft rejection in renal transplant patients, and while mid-stage trials demonstrated proof of confidence, with results comparable to standard of care, the overall safety and efficacy profile that emerged from larger trials evaluating 2.5–5.0 mg QD fingolimod was not sufficient to continue development for this indication.[12–14] Concurrent trials initiated for the treatment of relapsing remitting multiple sclerosis (RRMS) provided more favorable results, with

efficacy improved over a standard of care that was maintained even with substantially lower doses than those required in the transplant trials (0.5 mg QD).[15] In 2010, fingolimod (Gilenya™) was approved in the United States by the FDA as the first oral disease-modifying treatment for RRMS. Concerns noted with fingolimod include cardiovascular effects (e.g., heart rate reduction, blood pressure elevation), macular edema, teratogenic effects, decline in pulmonary function, elevation of liver enzymes, and a long half-life.[16] Follow-on efforts have been directed toward improving upon this profile primarily in two ways. First, identification of compounds with a reduced pharmacokinetic (PK) half-life would mitigate concerns over the slow recovery from lymphocyte suppression observed upon drug withdrawal. Second, by improving receptor selectivity (eliminating interaction with S1P3), it was hoped to avoid the cardiovascular effects which were clearly tied to agonism of S1P3 in rodents.[17] Recent information from clinical trials of selective S1P1 agonists, however, indicates that the cardiovascular liabilities are not eliminated with S1P3-sparing agonists.[18] Nevertheless, as the activity on S1P3 does not appear to contribute to efficacy, the emphasis on identifying agonists with improved selectivity for S1P1 remains a consistent theme in nearly all recent reports.

Additional S1P1 agonists that have entered clinical trials and for which a structure has been disclosed include two prodrug compounds KRP-203 (**6**) and CS-0777 (**8**).[19,20] Their corresponding phosphates demonstrated improved S1P1 versus S1P3 selectivity relative to fingolimod-P (P3/P1 = 10 for **5** vs. >300 for **9** and >1000 for **7**).[21,22] KRP-203 is under evaluation for the treatment of ulcerative colitis as well as subacute cutaneous lupus erythematosus.[23] CS-0777 was evaluated in a single ascending dose study at 0.1–2.5 mg, with dose-related lymphocyte reductions ranging from 7% to 85%.[24] CS-0777 was found to have a long human half-life of 171–211 h, similar to that of fingolimod. Repeat dosing of CS-0777 (0.1–0.6 mg QW or Q2W) in patients with MS revealed a dose-dependent reduction in absolute lymphocyte counts and reduction in heart rate.[25]

Diol **10** (ponesimod) is a direct-acting agonist of S1P1; unlike the previously described compounds, it does not require phosphorylation for its pharmacological activity. Ponesimod displays slightly improved receptor selectivity over fingolimod (P3/P1 = 18) and a shorter human half-life of 22–33 h.[26] In addition to completing a Phase 2b trial for RRMS, ponesimod is under evaluation in separate trials for psoriasis.[27] Additional direct-acting agonists include amino-cyclobutane carboxylic acid **11** (PF-991) and

azetidine carboxylic acid **12**. Advanced into early clinical trials as a potential treatment for rheumatoid arthritis, selective direct-acting agonist **11** (P3/P1 > 3000) demonstrated 50% or 60% reduction of lymphocytes at 24 h from doses of 1 and 3 mg, respectively.[28] Compound **12** was administered to healthy subjects in a 1-month repeat-dose study across a dose range from 0.3 to 20 mg QD.[29] The mean change in absolute lymphocyte counts ranged from 42% to 85% with reductions in heart rate also being observed within this dose range. These data have been separately associated with BAF312 (structure not disclosed), a compound reported to demonstrate excellent receptor selectivity (P3/P1 > 3000) and a human half-life of approximately 30 h.[30] An initial trial in RRMS patients was recently completed for BAF312, and a separate ongoing trial is evaluating its efficacy in patients with the chronic inflammatory diseases, polymyositis or dermatomyositis.[31]

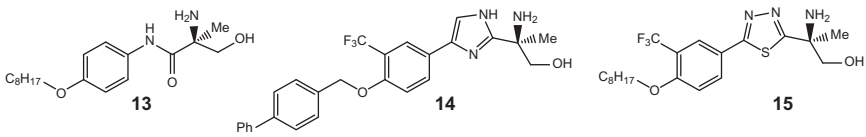


3. RECENT PRECLINICAL DEVELOPMENTS OF S1P1 AGONISTS

The medicinal chemistry efforts that were initiated shortly after the identification of S1P1 as the molecular target of fingolimod-P emphasized two main branches. In one, the focus turned to modifications of the amino diol prodrug compounds, optimizing for potency, selectivity, and *in vivo* properties, such as reduced half-life and increased formation of circulating phosphate metabolite. In the other area, direct-acting agonists were explored, with early examples focusing on ionic phosphate mimetics. Early design efforts relied on homology models, refined by mutagenesis, which defined a lipophilic-binding pocket and pointed to key ion-pair interactions of the ligand amino with specific residues (including Arg120 and Glu121) within S1P1.[32] A recently disclosed cocrystal structure of an antagonist

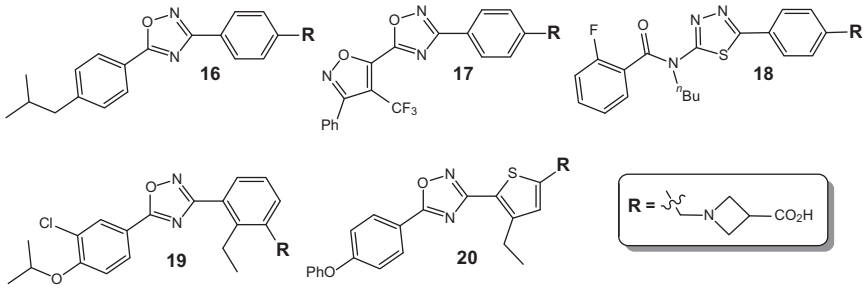
bound to S1P1 is a major advance for the field and will undoubtedly provide the basis for further structure-based design.[33] Stabilized phosphate surrogates such as phosphonates, thiophosphates, and carboxylic acids were designed to retain the putative interaction of the phosphate group with Arg120 of S1P1.[34] This was met with the greatest success in the case of the amino acid analogs, in particular, when azetidine-3-carboxylate was utilized in conjunction with a 3,5-diaryl-1,2,4-oxadiazole scaffold, first identified from high-throughput screening (HTS).[35] The resulting compounds (e.g., **16**) were potent agonists of S1P1, possessed good PK properties, and were able to induce lymphopenia in multiple species upon oral dosing.[36] Variations around this triaryl amino acid template have been explored by numerous research groups over the past few years, with modifications to the aryl core, the amino acid, and the lipophilic region of the molecules.[37,38] This is evidenced directly in **12**, and the influence is also apparent in the amino-cyclobutane of **11**.

In designing analogs of fingolimod, the only conserved element required for bioactivation has been the aminoethanol fragment. An amide was introduced as a rigidifying element in **13**, which was selective (phosphate: P3/P1 = 230) and active in eliciting lymphopenia in mice, but exhibited low conversion to the phosphate *in vivo*.[39] Elaboration of the amides to heterocycles was first realized in imidazole analogs such as **14** where ortho-CF3 substitution enhanced selectivity (phosphate: P3/P1 > 1800). Oral administration of **14** to mice led to maximal lymphopenia at doses as low as 1 mg/kg, with improved phosphorylation.[40,41] The compound had good PK in mouse ($t_{1/2}$ = 20 h) and was active in models of transplant and multiple sclerosis.[42] A similar heterocyclic constraint and ortho-substitution were retained in the structure of development candidate **15** (GSK1842799).[43]



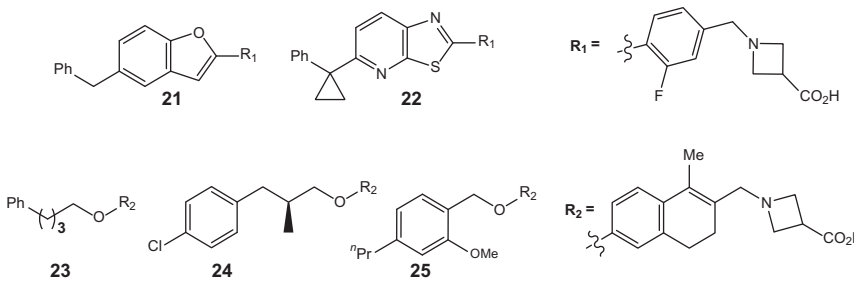
Among direct-acting agonists, the azetidine carboxylic acid originally described for **16** has been by far the most replicated surrogate for the amino phosphates. While in **17** an isoxazole was found to replace the phenyl ring of **16**, compound **18** utilizes a benzamido-thiadiazole to presumably occupy the same lipophilic pocket.[44,45] In **19**, the orientation of the azetidine and oxadiazole was changed from *para* to *meta*, yet good potency and selectivity were maintained (S1P1 pEC_{50} = 9.5; S1P3 pEC_{50} < 5.5).[46]

Alternatively, in CS-2100 (**20**), an ethyl-thiophene was found to be an effective phenyl replacement (S1P1 $EC_{50} = 4.0$ nM; S1P3 $EC_{50} > 20{,}000$ nM) leading to its selection as a clinical candidate.[47] However, development of CS-2100 was discontinued, reportedly due to concerns over the formation of 4-phenoxy benzoic acid via enterobacterial intestinal metabolism of the oxadiazole.[48] Efforts to identify metabolically stable replacements for the oxadiazole have been described.[49]



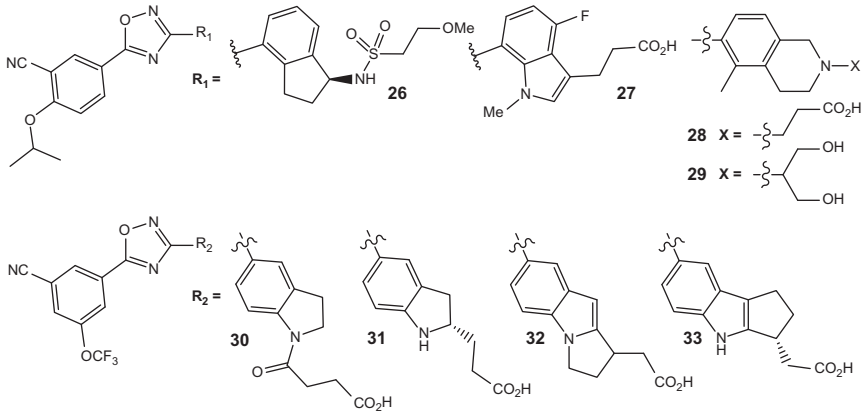
The azetidine carboxylate appears intact in several other noteworthy reports.[50–53] Toxicological observations of an undisclosed nature were noted after 1-month evaluation of benzofuran **21** as well as a close benzothiazole analog. Modifications aimed at increasing polarity were introduced to avoid off-target toxicity. Ultimately, an aza-benzothiazole core along with incorporation of a cyclopropyl constraint in AMG369 (**22**) led to an increase in potency of more than 10-fold (S1P1 $EC_{50} = 2$ nM; P3/P1 = 444; rat lymphopenia $EC_{50} = 1.6$ ng/mL). After demonstrating an adequate safety margin in 1-month toxicological evaluations, AMG369 was selected for further development.

A dihydronaphthalene core provided the basis for another series of azetidine carboxylate S1P1 agonists (**23**–**25**).[54–56] Compound **23** bearing a 4-phenylbutoxy side chain was potent (S1P1 $EC_{50} = 2.9$ nM) and selective (S1P3 $EC_{50} > 10{,}000$ nM) with a long half-life in rodent (rat $t_{1/2} = 16.7$ h) resulting in a low efficacious dose that maintained lymphocyte suppression through 24 h (mouse $ED_{50} = 1.9$ mg/kg). Truncated analog **24** provided a 20-fold improvement to *in vivo* potency (mouse 24 h lymphopenia $ED_{50} = 0.095$ mg/kg) while maintaining good selectivity (P3/P1 = 15,000). Further truncation afforded benzylic ether **25**.



A large number of recent examples utilize indane or indole frameworks, the genesis of which traces back to early examples of S1P1 direct-acting agonists.[57] Indane **26** maintained lymphopenia in rat at a low oral dose (24 h $ED_{50} = 0.25$ mg/kg).[58] Demonstrating good receptor selectivity (S1P1 $pEC_{50} > 11$; S1P3 $pEC_{50} < 5$), **27** was central nervous system (CNS) penetrant and efficacious in a rodent model of multiple sclerosis despite a relatively short half-life (mouse $t_{1/2} = 3.8$ h).[59] Substituted tetrahydroisoquinolines (THIQs) have also provided effective S1P1 agonists including advanced compounds **28** and **29**.[60,61] Carboxylic acid-substituted THIQ **28** was able to induce maximal lymphopenia in rat through 12 h with doses as low as 0.1 mg/kg. Partial recovery from lymphopenia was evident by 24 h with doses up to 1.0 mg/kg, consistent with the compound's short half-life (rat $t_{1/2} = 3.0$ h). Significant efficacy in the rat adjuvant arthritis model was demonstrated with a 3 mg/kg once-daily oral dose of **28**, and PK/PD modeling suggested a human dose of < 10 mg would provide the desired target of 60% reduction in circulating lymphocytes.[62] As **28** was both zwitterionic and a substrate for P-glycoprotein, it was expected to have low CNS penetration, a factor believed to limit its utility in multiple sclerosis for which additional beneficial effects of S1P agonism in the CNS have been postulated.[63,64] Efforts to identify analogs of **28** with improved CNS penetration culminated in the discovery of **29**, which provided a steady-state brain-to-blood concentration ratio of 1.85:1. The diol motif of **29** is reminiscent of **10** and is likewise not phosphorylated *in vivo*. This potent and selective direct-acting agonist had an estimated *in vivo* EC_{50} of less than 0.1 nM representing an unexpectedly significant improvement over the *in vivo* potency of **28** ($EC_{50} = 9$ nM), a difference that was attributed to a more favorable tissue distribution.

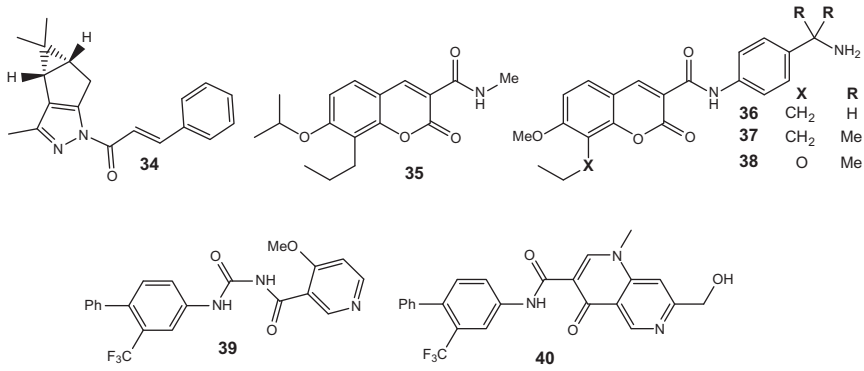
A series of disclosures illustrate the evolution of an indoline framework.[65–69] *N*-acylated indoline **30**, derived from lead optimization efforts around an indazole–oxadiazole template, was able to drive significant lymphopenia in mouse with an oral dose of 1.0 mg/kg, although the effects were not sustained through 24 h. Side chain migration led to C-linked carboxylate **31** (S1P1 $EC_{50} = 8$ nM), which was then used in the design of constrained tricyclic indole analogs through annulation resulting in **32** or **33**. Although **33** was nearly fivefold more potent than **32** *in vitro* (mouse S1P1 $EC_{50} = 0.52$ and 2.6 nM, respectively), their *in vivo* potencies were nearly identical (mouse lymphopenia $EC_{50} = 750–760$ ng/mL).



The number of examples appearing in recent literature that offer structural diversity significantly different than the initially described S1P1 agonists is quite limited. Pyrazole **34**, identified through HTS, is notable for its lack of polar head group, and it served as the basis for the proposal of a pharmacophore model for S1P1 agonism.[70,71] Also identified through HTS were coumarin-based agonists such as **35** (S1P1 $EC_{50} = 3$ nM; S1P3 $EC_{50} = 790$ nM).[72,73] The potency of the series was enhanced through modification of the carboxamide as in **36** (S1P1 $EC_{50} = 0.3$ nM), however, the compound suffered from poor aqueous solubility and the formation of a long-lived *N*-acylated metabolite. Installation of gem-dimethyl (**37**) prevented the formation of the metabolite, while aqueous solubility was then improved through conversion of the C8 propyl group to ethoxy (**38**). Coumarin **38** demonstrated a long half-life in rat (34.8 h), was efficacious in a rat model of multiple sclerosis, and was selective

across the S1P receptor family (S1P1 $EC_{50} = 2$ nM; S1P5 $EC_{50} = 560$ nM; S1P3,4 > 10,000 nM).

An additional series of structurally atypical agonists was generated from an HTS lead, optimization of which gave acylurea **39** as a monoselective agonist (S1P1 $EC_{50} = 35$ nM; >100-fold vs. S1P2–5) with an extended pharmacodynamic effect in rat (78% lymphopenia at 24 h after 1 mg/kg oral dose) consistent with its long half-life (19 h).[74] The optimized trifluoromethyl-substituted biphenyl of **39** is reminiscent of the lipophilic groups in previously described S1P1 agonists, including **12** above, suggesting similar orientation of these compounds in the ligand-binding pocket of S1P1. Subsequent production of conformationally constrained analogs followed by a systematic N-scan SAR strategy and installation of a hydroxymethyl group afforded compound **40**.[75,76] In a rat lymphopenia assay, oral administration of **40** afforded 60% reduction at 24 h (corresponding to 84 ng/mL plasma concentration).



4. CONCLUSIONS

Following the identification of its molecular target, the impressive *in vivo* biological activity and continued clinical success of fingolimod have spurred tremendous and sustained efforts in the identification of novel agonists of S1P1 with improved profiles. The focus of optimization has been the identification of compounds with reduced half-life to permit a more rapid restoration of normal lymphocyte trafficking as well as improving upon the receptor selectivity profile of fingolimod-P. Although initial modifications retained the prodrug nature of fingolimod, direct-acting S1P1 agonists that do not require bioactivation were identified and have since come to form the majority of new disclosures. While this review

focused only on the past few years of literature, it is clear that the foundational studies to define stable phosphate mimics of fingolimod such as **16** have had a lasting impact on the field, with elements of those early agonists being readily identifiable in the great majority of recently disclosed compounds described above. The continued evaluation of fingolimod in patients with RRMS will define the long-term significance of the identified liabilities. Multiple examples of both classes of S1P1 agonists (direct-acting and prodrug) have now entered human clinical trials, and it remains to be determined if they can provide a similar therapeutic benefit to fingolimod with an improved safety profile.

REFERENCES

(1) Pappu, R.; Schwab, S.R.; Cornelissen, I. *Science* **2007**, *316*, 295.
(2) Chun, J.; Hla, T.; Lynch, K.R.; Spiegel, S.; Moolenaar, W.H. *Pharmacol. Rev.* **2010**, *62*, 579.
(3) Olivera, A.; Rivera, J. *Adv. Exp. Med. Biol.* **2011**, *716*, 123.
(4) Matloubian, M.; Lo, C.G.; Cinamon, G.; Lesneski, M.J.; Xu, Y.; Brinkmann, V. *Nature* **2004**, *427*, 355.
(5) Chiba, K.; Yanagawa, Y.; Masubuchi, Y.; Kataoka, H.; Kawaguchi, T.; Ohtsuki, M. *J. Immunol.* **1998**, *160*, 5037.
(6) Dumont, F.J. *IDrugs* **2005**, *8*, 236.
(7) 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.
(8) 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. *J. Biol. Chem.* **2002**, *277*, 21453.
(9) Billich, A.; Bornancin, F.; Devay, P.; Mechtcheriakova, D.; Urtz, N.; Baumruker, T. *J. Biol. Chem.* **2003**, *278*, 47408.
(10) Paugh, S.W.; Payne, S.G.; Barbour, S.E.; Milstien, S.; Spiegel, S. *FEBS Lett.* **2003**, *554*, 189.
(11) Fujii, Y.; Hirayama, T.; Ohtake, H.; Ono, N.; Inoue, T.; Sakurai, T.; Takayama, T.; Matsumoto, K.; Tsukahara, N.; Hidano, S. *J. Immunol.* **2012**, *188*, 206.
(12) Mulgaonkar, S.; Tedesco, H.; Oppenheimer, F.; Walker, R.; Kunzendorf, U.; Russ, G.; Knoflach, A.; Patel, Y.; Ferguson, R. *Am. J. Transplant.* **1848**, *2006*, 6.
(13) Tedesco-Silva, H.; Pescovitz, M.D.; Cibrik, D.; Rees, M.A.; Mulgaonkar, S.; Kahan, B.D.; Gugliuzza, K.K.; Rajagopalan, P.R.; de M. Esmeraldo, R.; Lord, H.; Salvadori, M.; Slade, J.M. *Transplantation* **2006**, *82*, 1689.
(14) Salvadori, M.; Budde, K.; Charpentier, B.; Klempnauer, J.; Nashan, B.; Pallardo, L.M.; Eris, J.; Schena, F.P.; Eisenberger, U.; Rostaing, L.; Hmissi, A.; Aradhye, S. *Am. J. Transplant.* **2006**, *6*, 2912.
(15) Warnke, C.; Stüve, O.; Hartung, H.-P.; Fogdell-Hahn, A.; Kieseier, B.C. *Neuropsychiatr. Dis. Treat.* **2011**, *7*, 519.
(16) Pelletier, D.; Hafler, D.A. *N. Engl. J. Med.* **2012**, *366*, 339.
(17) 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. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3501.

(18) Gergely, P.; Wallstroüm, E.; Nuesslein-Hildesheim, B.; Bruns, C.; Zécri, F.; Cooke, N.; Traebert, M.; Tuntland, T.; Rosenberg, M.; Saltzman, M. *Mult. Scler.* **2009**, *15*, S125.
(19) 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. *Circulation* **2005**, *111*, 222.
(20) Nishi, T.; Miyazaki, S.; Takemoto, T.; Suzuki, K.; Iio, Y.; Nakajima, K.; Ohnuki, T.; Kawase, Y.; Nara, F.; Inaba, S.; Izumi, T.; Yuita, H.; Oshima, K.; Doi, H.; Inoue, R.; Tomisato, W.; Kagari, T.; Shimozato, T. *ACS Med. Chem. Lett.* **2011**, *2*, 368.
(21) Song, J.; Matsuda, C.; Kai, Y.; Nishida, T.; Nakajima, K.; Mizushima, T.; Kinoshita, M.; Yasue, T.; Sawa, Y.; Ito, T. *J. Pharmacol. Exp. Ther.* **2007**, *324*, 276.
(22) Zahir, H.; Moberly, J. B.; Ford, D.; Truitt, K. E.; Bar-Or, A.; Vollmer, T. L. AAN Annual Meeting, **2010**; P04.196.
(23) http://www.clinicaltrials.gov/ct2/results?term=KRP203.
(24) Moberly, J.B.; Rohatagi, S.; Zahir, H.; Hsu, C.; Noveck, R.J.; Kenneth, E. *J. Clin. Pharmacol.* **2012**, *52*, 996.
(25) Moberly, J.B.; Ford, D.M.; Zahir, H.; Chen, S.; Mochizuki, T.; Truitt, K.E.; Vollmer, T.L. *J. Neuroimmunol.* **2012**, *246*, 100.
(26) Brossard, P.; Hofmann, S.; Cavallaro, M.; Halabi, A.; Dingemanse, J. *Clin. Pharmacol. Ther.* **2009**, *85*, S63 PII-87.
(27) http://www.clinicaltrials.gov/ct2/show/NCT00852670?term=ACT-128800&rank=3.
(28) Walker, J. K.; Huff, R. M.; Cornicelli, J.; Mickelson, J. W.; Brown, M. F.; Wendling, J. M.; Funckes-Shippy, C. L.; Hiebsch, R. R.; Radi, Z.; Lawson, J. P.; Nagiec, M. M.; Strohbach, J. W.; Beebe, J. S.; Bradley, J.; Blinn, J. R. 239th ACS National Meeting, San Francisco, CA, March 21-25, **2010**; Abstracts of Papers, MEDI-39.
(29) Legangneux, E. US20110039818, **2011**.
(30) Nuesslein-Hildesheim, B.; Gergely, P.; Wallström, E.; Luttringer, O.; Groenewegen, A.; Howard, L.; Pan, S.; Gray, N.; Chen, Y. A.; Bruns, C.; Zécri, F. Abstr ECTRIMS, **2009**; P438.
(31) http://www.clinicaltrials.gov/ct2/results?term=baf312.
(32) 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.
(33) Hanson, M.A.; Roth, C.B.; Jo, E.; Griffith, M.T.; Scott, F.L.; Reinhart, G.; Desale, H.; Clemons, B.; Cahalan, S.M.; Schuerer, S.C.; Sanna, M.G.; Han, G.W.; Kuhn, P.; Rosen, H.; Stevens, R.C. *Science* **2012**, *335*, 851.
(34) Fujiwara, Y.; Osborne, D.A.; Walker, M.D.; Wang, D.; Bautista, D.A.; Liliom, K.; Van Brocklyn, J.R.; Parrill, A.L.; Tigyi, G. *J. Biol. Chem.* **2007**, *282*, 2374.
(35) 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.
(36) Li, Z.; Chen, W.; Hale, J.J.; Lynch, C.L.; Mills, S.G.; Hajdu, R.; Keohane, C.A.; Rosenbach, M.J.; Milligan, J.A.; Shei, G.-J.; Chrebet, G.; Parent, S.A.; Bergstrom, J.; Card, D.; Forrest, M.; Quackenbush, E.J.; Wickham, L.A.; Vargas, H.; Evans, R.M.; Rosen, H.; Mandala, S. *J. Med. Chem.* **2005**, *48*, 6169.
(37) 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.; Muller, C.; Nayler, O.; Scherz, M.; Schmidt, G.; Sippel, V.; Steiner, B.; Strasser, D.; Treiber, A.; Weller, T. *J. Med. Chem.* **2010**, *53*, 4198.
(38) Schmidt, G.; Reber, S.; Bolli, M.H.; Abele, S. *Org. Process Res. Dev.* **2012**, *16*, 595–604.
(39) Evindar, G.; Bernier, S.G.; Kavarana, M.J.; Doyle, E.; Lorusso, J.; Kelley, M.S.; Halley, K.; Hutchings, A.; Wright, A.D.; Saha, A.K.; Hannig, G.; Morgan, B.A.; Westlin, W.F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 369.

(40) Evindar, G.; Satz, A.L.; Bernier, S.G.; Kavarana, M.J.; Doyle, E.; Lorusso, J.; Taghizadeh, N.; Halley, K.; Hutchings, A.; Kelley, M.S.; Wright, A.D.; Saha, A.K.; Hannig, G.; Morgan, B.A.; Westlin, W.F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2315.
(41) Evindar, G.; Bernier, S.G.; Doyle, E.; Kavarana, M.J.; Satz, A.L.; Lorusso, J.; Blanchette, H.S.; Saha, A.K.; Hannig, G.; Morgan, B.A.; Westlin, W.F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2520.
(42) Evindar, G.; Bernier, S. G.; Satz, A. L.; Kavarana, M. J.; Doyle, E.; Lorusso, J.; Taghizadeh, N.; Blanchette, H. S.; Halley, K.; Hutchings, A. 238th ACS National Meeting, Washington, DC, August 16–20, **2009**; Abstracts of Papers, MEDI-002.
(43) Anson, M.S.; Graham, J.P.; Roberts, A.J. *Org. Process Res. Dev.* **2011**, *15*, 649.
(44) Watterson, S. H.; Dyckman, A. J.; Pitts, W. J.; Spergel, S. PCT International Application WO201008558, **2010**.
(45) Aguilar, N.; Mir, M.; Grima, P. M.; Lopez, M.; Godessart, N.; Tarrason, G.; Domenech, T.; Vilella, D.; Armengol, C.; Cordoba, M.; Sabate, M.; Casals, D.; Dominguez, M. 240th ACS National Meeting, Boston, MA, August 22–26, **2010**; Abstracts of Papers, MEDI-164.
(46) Deng, G.; Lin, X.; Ren, F.; Zhao, B. PCT International Application WO2011134280, **2011**.
(47) Nakamura, T.; Asano, M.; Sekiguchi, Y.; Mizuno, Y.; Tamaki, K.; Kimura, T.; Nara, F.; Kawase, Y.; Shimozato, T.; Doi, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1788.
(48) Nakamura, T.; Asano, M.; Sekiguchi, Y.; Mizuno, Y.; Tamaki, K.; Nara, F.; Kawase, Y.; Yabe, Y.; Nakai, D.; Kamiyama, E.; Urasaki-Kaneno, Y.; Shimozato, T.; Doi-Komuro, H.; Kagari, T.; Tomisato, W.; Inoue, R.; Nagasaki, M.; Yuita, H.; Oguchi-Oshima, K.; Kaneko, R.; Nishi, T. *Eur. J. Med. Chem.* **2012**, *51*, 92.
(49) Asano, M.; Nakamura, T.; Sekiguchi, Y.; Mizuno, Y.; Yamaguchi, T.; Tamaki, K.; Shimozato, T.; Doi-Komuro, H.; Kagari, T.; Tomisato, W.; Inoue, R.; Yuita, H.; Oguchi-Oshima, K.; Kaneko, R.; Nara, F.; Kawase, Y.; Masubuchi, N.; Nakayama, S.; Koga, T.; Namba, E.; Nasu, H.; Nishi, T. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3083.
(50) 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.; Burli, 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.
(51) Cee, V. J. 241st ACS National Meeting, Anaheim, CA, March 27–31, **2011**; Abstracts of Papers, MEDI-25.
(52) 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. *ACS Med. Chem. Lett.* **2011**, *2*, 102.
(53) Cee, V.J.; Frohn, M.; Lanman, B.A.; Golden, J.; Muller, K.; Neira, S.; Pickrell, A.; Arnett, H.; Buys, J.; Gore, A. *ACS Med. Chem. Lett.* **2011**, *2*, 107.
(54) Kurata, H.; Kusumi, K.; Otsuki, K.; Suzuki, R.; Kurono, M.; Takada, Y.; Shioya, H.; Komiya, T.; Mizuno, H.; Ono, T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3885.
(55) Kurata, H.; Kusumi, K.; Otsuki, K.; Suzuki, R.; Kurono, M.; Tokuda, N.; Takada, Y.; Shioya, H.; Mizuno, H.; Komiya, T. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 144.
(56) Habashita, H.; Kurata, H.; Nakade, S. PCT International Application WO2006064757, **2006**.
(57) Colandrea, V. J.; Doherty, G. A.; Hale, J. J.; Huo, P.; Legiec, I. E.; Toth, L.; Vachal, P.; Yan, L. PCT International Application WO2005058848, **2005**.
(58) Martinborough, E.; Boehm, M. F.; Yeager, A. R.; Tamiya, J.; Huang, L.; Brahmachary, E.; Moorjani, M.; Timony, G. A.; Brooks, J. L.; Peach, R. PCT International Application WO2011060392, **2011**.

(59) Meng, Q.; Zhao, B.; Xu, Q.; Xu, X.; Deng, G.; Li, C.; Luan, L.; Ren, F.; Wang, H.; Xu, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2794.
(60) Demont, E.H.; Andrews, B.I.; Bit, R.A.; Campbell, C.A.; Cooke, J.W.B.; Deeks, N.; Desai, S.; Dowell, S.J.; Gaskin, P.; Gray, J.R.J. *ACS Med. Chem. Lett.* **2011**, *2*, 444.
(61) Demont, E.H.; Arpino, S.; Bit, R.A.; Campbell, C.A.; Deeks, N.; Desai, S.; Dowell, S.J.; Gaskin, P.; Gray, J.R.J.; Harrison, L.A. *J. Med. Chem.* **2011**, *54*, 6724.
(62) Taylor, S.; Gray, J.R.J.; Willis, R.; Deeks, N.; Haynes, A.; Campbell, C.; Gaskin, P.; Leavens, K.; Demont, E.; Dowell, S.; Cryan, J.; Morse, M.; Patel, A.; Garden, H.; Witherington, J. *Xenobiotica* **2012**, *16*, 671.
(63) Brinkmann, V. *Br. J. Pharmacol.* **2009**, *158*, 1173.
(64) Noguchi, K.; Chun, J. *Crit. Rev. Biochem. Mol. Biol.* **2011**, *46*, 2.
(65) Buzard, D.J.; Han, S.; Thoresen, L.; Moody, J.; Lopez, L.; Kawasaki, A.; Schrader, T.; Sage, C.; Gao, Y.; Edwards, J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6013.
(66) Kawasaki, A.; Thoresen, L.; Buzard, D. J.; Moody, J.; Lopez, L.; Ullman, B.; Lehmann, J.; Zhu, X.; Edwards, J.; Barden, J. 241st ACS National Meeting, Anaheim, CA, March 27–31, **2011**; Abstracts of Papers, MEDI-254.
(67) Han, S.; Thoresen, L.; Moody, J.; Buzard, D. J.; Lopez, L.; Sage, C.; Edwards, J.; Barden, J.; Kawasaki, A.; Ullman, B. 241st ACS National Meeting, Anaheim, CA, March 27–31, **2011**; Abstracts of Papers, MEDI-98.
(68) Jones, R. M.; Buzard, D. J.; Han, S.; Kim, S. H.; Lehmann, J.; Ullman, B.; Moody, J. V.; Zhu, X.; Stirn, S. PCT International Application WO2010011316, **2010**.
(69) Montalban, A. G.; Buzard, D. J.; Demattei, J. A.; Gharbaoui, T.; Johannsen, S. R.; Krishnan, A. M.; Kuhlman, Y. M.; Ma, Y. -A.; Martinelli, M. J.; Sato, S. M. PCT International Application WO2011094008, **2011**.
(70) Baenteli, R.; Cooke, N. G.; Zecri, F.; Alexander, B. PCT International Application WO2008092930, **2008**.
(71) Zecri, F.J.; Albert, R.; Landrum, G.; Hinterding, K.; Cooke, N.G.; Guerini, D.; Streiff, M.; Bruns, C.; Nuesslein-Hildesheim, B. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 35.
(72) Zecri, F. J.; Albert, R.; Baenteli, R.; Landrum, G.; Cooke, N. G.; Beerli, C.; Bruns, C.; Guerini, D.; Streiff, M.; Nusslein-Hildesheim, B. 239th ACS National Meeting, San Francisco, CA, March 21–25, **2010**; Abstracts of Papers, MEDI-31.
(73) Baenteli, R.; Cooke, N. G.; Weiler, S.; Zecri, F. PCT International Application WO2007115820, **2007**.
(74) Pennington, L.D.; Sham, K.K.C.; Pickrell, A.J.; Harrington, P.E.; Frohn, M.J.; Lanman, B.A.; Reed, A.B.; Croghan, M.D.; Lee, M.R.; Xu, H.; Zhang, X.; McElvain, M.; Xu, Y.; Zhang, X.; Fiorino, M.; Horner, M.; Morrison, H.G.; Arnett, H.A.; Fotsch, C.; Wong, M; Cee, V.J. *ACS Med. Chem. Lett.* **2011**, *2*, 752.
(75) Pennington, L.D.; Croghan, M.D.; Sham, K.K.C.; Pickrell, A.J.; Harrington, P.E.; Frohn, M.J.; Lanman, B.A.; Reed, A.B.; Lee, M.R.; Xu, H.; McElvain, M.; Xu, Y.; Zhang, X.; Fiorino, M.; Horner, M.; Morrison, H.G.; Arnett, H.A.; Fotsch, C.; Tasker, A.S.; Wong, M.; Cee, V.J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 527.
(76) Harrington, P.E.; Croghan, M.D.; Fotsch, C.; Frohn, M.; Lanman, B.A.; Pennington, L.D.; Pickrell, A.J.; Reed, A.B.; Sham, K.K.C.; Tasker, A.S.; Arnett, H.A.; Fiorino, M.; Lee, M.R.; McElvain, M.; Morrison, H.G.; Xu, H.; Xu, Y.; Zhang, X.; Wong, M.; Cee, V.J. *ACS Med. Chem. Lett.* **2012**, *3*, 74.