ACS Medicinal Chemistry Letters

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Featured Letter

Novel Potent Selective Orally Active S1P5 Receptor Antagonists

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Cite This: *ACS Med. Chem. Lett.* 2021, 12, 351−355

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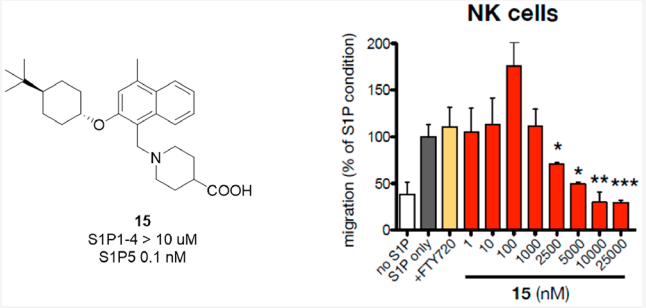
ABSTRACT: S1P5 is one of the five sphingosine-1-phosphate (S1P) receptors which play important roles in immune and CNS cell homeostasis, growth, and differentiation. Little is known about the effect of modulation of S1P5 due to the lack of S1P5 specific modulators with suitable druglike properties. Here we describe the discovery and optimization of a novel series of potent selective S1P5 antagonists and the identification of an orally active brain-penetrant tool compound **15**.



KEYWORDS: *S1P5, GPCR, antagonist, selectivity, brain penetration*

Sphingosine-1-phosphate (S1P, Figure 1) signals through cell surface GPCR receptors S1PRs (S1P1−5) and plays important roles in cell survival, trafficking, growth, and differentiation in many cell types, including cells of the immune, cardiovascular, and central nervous (CNS) systems.[1−4] S1P1 internalization has been well documented in the trafficking of B and T cells by preventing both from leaving the lymph nodes and entering the lymphatic circulation.[5] The U.S. Food and Drug Administration approved drug FTY720 induces S1P1 internalization, and S1P signaling has become an interesting therapeutic target.[6] However, the role and contribution of S1P5 to pharmacological effects of FTY720 or the next generation of S1PR signaling modulators such as Ozanimod remain unclear, despite reports suggesting the ability of these molecules to modulate oligodendrocyte biology via S1P5.[7,8]

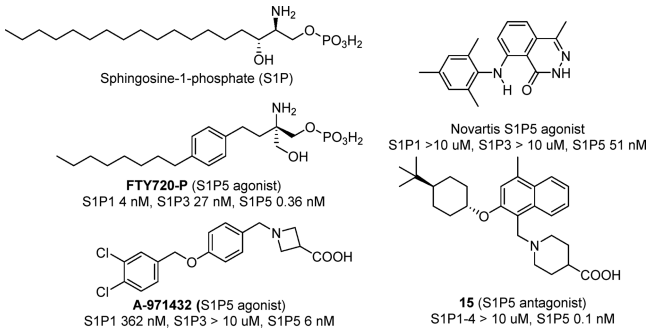


Figure 1. S1P5 agonists and antagonist.

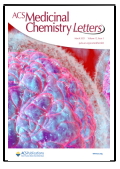
It has been reported that S1P5 is ubiquitously expressed in the cells of CNS and particularly highly expressed in the mature oligodendrocytes relative to other S1PRs and that it plays an important role in cell survival of mature oligodendrocytes.[9,10] It is also reported that S1P5 activation on brain endothelial cells enhances barrier integrity and reduces transendothelial migration of monocytes in vitro.[11] Nevertheless, little more is known about the effect of modulation of S1P5 due to the lack of suitable tool compounds.

There are several nonspecific S1P1 agonists that also bind to S1P5 including FTY720-P (Figure 1), BAF312, ONO-4641,[12] and AbbVie's A-971432.[13] Novartis reported a selective S1P5 agonist,[14] but it was proved to have insufficient pharmacodynamic properties for in vivo target validation.[13,15] So far, to the best of our knowledge, there are no S1P5 specific antagonists reported.

Here we report the discovery and optimization of a novel series of potent selective S1P5 antagonists without S1P1−4 activity, together with the identification of a brain-penetrable orally active tool compound **15**. In a recent publication, this compound was shown to demonstrate unique activity on blocking natural killer (NK) cell migration in vitro.[16]

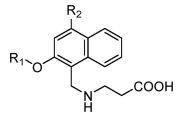
Screening an internal lipid mimetic library by using a calcium mobilization S1P1−5 assay,[17] we found a 4-methylnaphthalen-1-yl)methyl amino acid analogue **1** with moderate S1P5 antagonist activity (406 nM) without S1P1 activity (>5000 nM). A modification to the tail portion led to compound **3** with

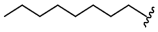
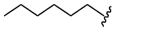
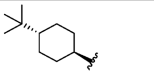
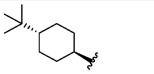
Received: November 29, 2020
Accepted: January 13, 2021
Published: January 15, 2021



https://dx.doi.org/10.1021/acsmedchemlett.0c00631
ACS Med. Chem. Lett. 2021, 12, 351−355

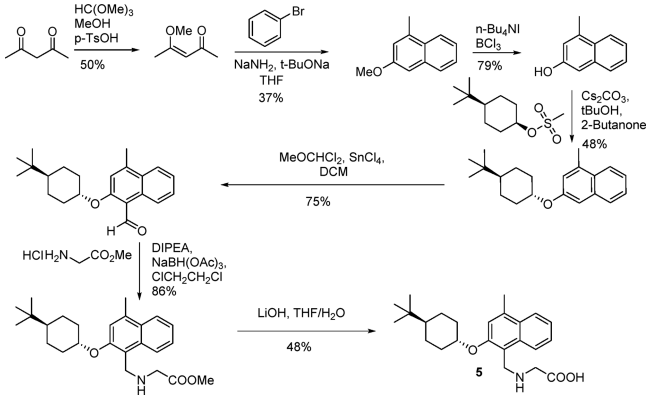
Table 1. S1P1-5 Activity of 3-Aminopropanoic Acid Analogues



Compound	R_1	R_2	S1P1 (nM)	S1P2 (nM)	S1P3 (nM)	S1P4 (nM)	S1P5 (nM) [a,b]
1		Me	>5000	>5000	2281	1526	406
2		Me	>5000	>5000	>5000	>5000	1.4
3		Me	>5000	NA	331	NA	0.1 [c]
4		I	>5000	NA	1664	NA	>5000

[a]Ca^{2+} mobilization assay EC_{50}, see ref 17. [b]Antagonist. [c]K_i = 1.4 nM in the S1P5 ^{33}P binding assay.

Scheme 1. General Synthetic Scheme of Amino Acid Analogues



much improved S1P5 activity (0.1 nM) while maintaining selectivity over other S1P receptor family members (Table 1). A ^{33}P -S1P radiolabel binding assay confirmed the observed S1P5 selectivity over S1P1 of compound **3** (K_i (S1P5) = 1.4 nM and K_i (S1P1) > 5 μM). Dosing of compound **3** (5 mg/kg) in mice led to good exposure (AUC = 2723 h·ng/mL, $T_{1/2}$ = 3h) and did not lead to any lymphopenia which is consistent with lack of S1P1 activity. Encouraged by this result, we decided to further explore the structure−activity relationship (SAR) based on this scaffold.

The chemistry was started by making the literature compound 4-methylnaphthalen-2-ol,[18] followed by alkylation and $SnCl_4$ mediated aldehyde formation, with subsequent reductive amination and hydrolysis as exemplified by the synthesis of compound **5** (Scheme 1).[19]

A small library based on varying the amino acid head portion of compound **3** was generated (Table 2). All analogues lack S1P1 and S1P2 activity. However, their activity on S1P3, S1P4, and S1P5 varies significantly depending on the secondary amine. Compounds **10**−**16** generally lack S1P3 and S1P4 activity, with the piperidine headgroup (**15**) being a clear standout as the most potent S1P5 antagonist (EC_{50} = 0.1 nM) with no activity on S1P1−4. The observed S1P5 selectivity over S1P1 of compound **15** was confirmed with a second S1P1/S1P5 binding assay (K_i (S1P5) = 4.4 nM, and K_i (S1P1) > 5 μM). Replacement of the core 4-Me group of **3** with an iodide led to the complete loss of S1P5 activity of compound **4**. Further

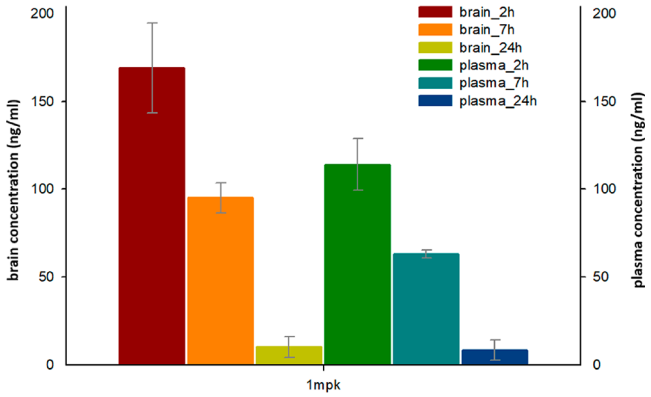


Figure 2. Plasma and total brain concentration after 1 mg/kg IV dosing of compound **15** in rats.

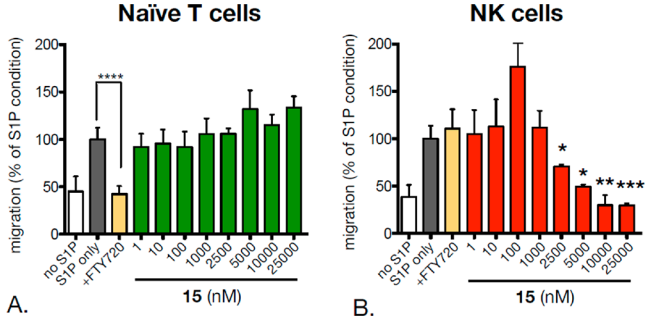


Figure 3. Chemotaxis assays of mouse spleen naive T cells (A) or NK cells (B) toward S1P. Cells were pretreated for 1 h with FTY720 or **15** at the indicated concentration in the top chamber. Results show the migration relative to control condition (no inhibitor). N = 3 experiments. $^{**}P < 0.01$; $^{***}P < 0.001$.

modification of the 4-Me position on compound **15** resulted in a sharp SAR with CF_3, I, Cl, and H replacement leading to significant loss of S1P5 activity without affecting S1P1−4 activity (compounds **17**−**20**).

With the optimized 4-carboxy piperidine of compound **15** in place, the cyclohexyl tail was further explored (Table 3). Replacing the 4-*trans*-*t*-butyl group of **15** with bulkier *trans*-*t*-amyl led to loss of activity, and further expansion to the *trans*-phenyl completely abolished the S1P5 activity. *cis*-4-Substituted

https://dx.doi.org/10.1021/acsmedchemlett.0c00631
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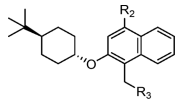
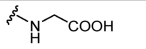
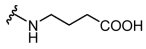
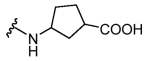
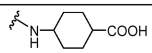
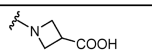
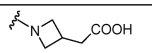
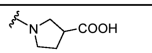
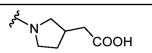
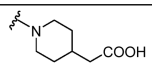
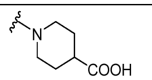
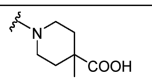
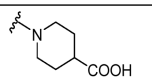
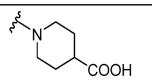
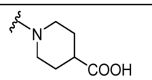
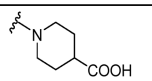
Table 2. S1P1−5 Activity of Different Amino Acid Analogues



Compound	R_3	R_2	S1P1 (nM)	S1P2 (nM)	S1P3 (nM)	S1P4 (nM)	S1P5 (nM) [a,b]
5		Me	>5000	>5000	>5000	>5000	267
6		Me	>5000	>5000	2660	>5000	1.4
7		Me	>5000	>5000	39	4360	0.063
8		Me	>5000	>5000	3675	2429	0.81
9		Me	>5000	>5000	>5000	>5000	0.27
10		Me	>5000	>5000	>5000	>5000	2.3
11		Me	>5000	>5000	>5000	>5000	>5000
12		Me	>5000	>5000	>5000	>5000	0.96
13		Me	>5000	>5000	>5000	>5000	3.7
14		Me	>5000	>5000	>5000	>5000	>5000
15		Me	>5000	>5000	>5000	>5000	0.1 [c]
16		Me	>5000	>5000	>5000	>5000	3812
17		CF_3	>5000	>5000	>5000	>5000	>5000
18		I	>5000	>5000	>5000	>5000	165
19		Cl	>5000	>5000	>5000	>5000	36
20		H	>5000	>5000	>5000	>5000	602

[a] Ca^{2+} mobilization assay EC_{50}. [b] Antagonist. [c] K_i = 4.4 nM in the S1P5 ^{33}P binding assay.

analogues (compound **23**−**25**) did not show much S1P5 activity, with the unsubstituted cyclohexyl analogue **26** giving only moderate activity (EC_{50} (S1P5) = 236 nM). Quite interestingly, the spiro analogues (**28**) showed the best potency on S1P5 (EC_{50} (S1P5) = 0.03 nM), which was confirmed with the S1P5 binding assay (K_i = 0.3 nM). However, **28** showed high clearance in rat PK.

Compound **15** was further profiled and showed high plasma protein binding with 0.60% free fraction in human. It had decent permeability with P_{app} (A-B, 10^{-6} cm/s) = 9.1 and efflux ratio = 1.84 in Caco-2 cells. IC_{50}'s at CYP isoforms (3A4, 1A2, 2C19, 2C9, 2D6) were all greater than 10 μM. The in vitro hepatocyte stability was favorable with Qh% = 30% in rat and 49% in human; therefore, **15** was tested in rat PK. At 5 mg/kg oral dosing, **15** demonstrated good exposure (AUC = 6510 ng/mL·h) and excellent oral bioavailability (F = 92%), with a half-life of 4.5 h (Table 4). And in IV dosing, it showed CL = 12.2 mL/min/kg (%Qh = 22%). Compound **15** also showed good brain penetration with a plasma/brain ratio of 0.67 at 2 h, 0.66 at 7 h, and 0.83 at 24 h (Figure 2).

Compound **15** was further tested on the migratory response of mouse spleen lymphocytes toward S1P. Compound **15**

https://dx.doi.org/10.1021/acsmedchemlett.0c00631
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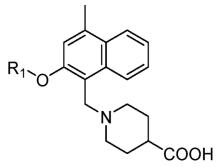
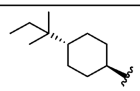
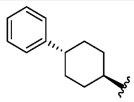
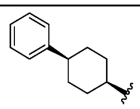
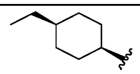
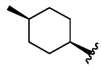
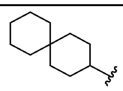
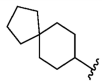
Table 3. S1P1-5 Activity of Different Tail Analogues



Compound	R_1	S1P1 (nM)	S1P2 (nM)	S1P3 (nM)	S1P4 (nM)	S1P5 (nM) [a,b]
21		>5000	>5000	>5000	>5000	67
22		>5000	>5000	>5000	>5000	>5000
23		>5000	>5000	>5000	>5000	1535
24		>5000	>5000	>5000	>5000	1244
25		>5000	>5000	>5000	>5000	>5000
26		>5000	>5000	>5000	>5000	236
27		>5000	>5000	>5000	>5000	1.1
28 [d]		>5000	>5000	>5000	2890	0.03 [c]

[a] Ca^{2+} mobilization assay EC_{50}. [b] Antagonist. [c] K_i = 0.3 nM in the S1P5 ^{33}P binding assay. [d] High clearance in rat PK.

Table 4. Single Dose Pharmacokinetic Parameters of 15 in Rats[a]

compd	route	dose (mg/kg)	Cl (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	AUC_{0-24h} (ng/mL·h)	C_{max} (ng/mL)	T_{max} (h)	F (%)
15[d]	IV[b]	1	12.2 ± 2.7	4.2 ± 0.5	4.6 ± 1.6	1416 ± 356			
	PO[c]	5			4.5 ± 0.5	6515 ± 23	947 ± 44	0.75 ± 0	92

[a] Mean ± SD (n = 3). [b] Formulation: solution in 1:1:1:7 EtOH:PEG400:Solutol:water. [c] Formulation: Suspension in 15% HPCD. [d] Plasma/brain ratio at 2/7/24 h = 0.67/0.66/0.83.

prevented NK cell migration toward S1P, with an IC_{50} of 553 nM, while it had no effect on T cell migration in contrast to FTY720 which blocked T cell but not NK cell migration (Figure 3). Of note, at the 100 nM concentration, compound **15** tended to increase NK cell migration to S1P, an effect that was not seen with higher doses. Further studies will be needed to understand what causes this biphasic response to the compound in the experimental setting. Nevertheless, as FTY720-P is an agonist on S1P1,3,4,5 (EC_{50} = 4, 27, 22, 0.36 nM) except S1P2 (EC_{50} > 5 μM), the S1P5 specific antagonist effect of compound **15** is remarkable.

In summary, we have discovered a series of S1P5 specific antagonists with excellent potency and identified a brain-penetrable tool, compound **15**, with good oral bioavailability. Compound **15** demonstrated an inhibitory effect on NK cell migration toward S1P but not on T cells. This is in agreement with the phenotype of the mice harboring genetic deficiency in S1P5.[20]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00631.

Experimental details for synthetic procedures and analytical data for key compounds and assay conditions (PDF)

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https://dx.doi.org/10.1021/acsmedchemlett.0c00631
ACS Med. Chem. Lett. 2021, 12, 351−355

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Biography

Bin Ma is senior scientist of medicinal chemistry at Biogen. Bin received BS and MS degrees from Lanzhou University and a PhD degree in organic chemistry from Boston University. Bin gained his postdoc training at Harvard University in Professor Kishi's labs. In 2007, Bin moved to Biogen and started his industrial career. Bin contributed multiple development candidates in multiple therapeutic areas at Biogen and has extensive experience on target validation, hit ID, lead optimization, candidate selection, and preclinical development and has served as leader for chemistry teams and project teams. Bin's interests span the broad scope of drug discovery for neurodegenerative diseases.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the DMPK group for the support on compound profiling and PK studies.

■ ABBREVIATIONS

CL, clearance; CNS, central nervous system; HPCD, hydroxypropyl-beta-cyclodextrin; IV, intravenous; NK, natural killer; PK, pharmacokinetics; Qh, normalized clearance based on hepatic blood flow; S1PR, sphingosine 1-phosphate receptor; SAR, structure−activity relationship.

■ REFERENCES

(1) Van Brocklyn, J. R.; Lee, M.; Menzeleev, R.; Olivera, A.; Edsall, L.; Cuvillier, O.; Thomas, D. M.; Coopman, P. J. P.; Thangada, S.; Liu, C. H.; Hla, T.; Spiegel, S. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor Edg-1 and intracellular to regulate proliferation and survival. *J. Cell Biol.* **1998**, *142* (1), 229−240.

(2) Toman, R. E.; Spiegel, S. Lysophospholipid receptors in the nervous system. *Neurochem. Res.* **2002**, *27* (7−8), 619−627.

(3) Terada, N.; Banno, Y.; Ohno, N.; Fujii, Y.; Murate, T.; Sarna, J. R.; Hawkes, R.; Zea, Z.; Baba, T.; Ohno, S. Compartmentation of the mouse cerebellar cortex by sphingosine kinase. *J. Comp. Neurol.* **2004**, *469* (1), 119−127.

(4) Kono, M.; Allende, M. L.; Proia, R. L. Sphingosine-1-phosphate regulation of mammalian development. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2008**, *1781* (9), 435−441.

(5) Hla, T.; Brinkmann, V. Sphingosine 1-phosphate (S1P): physiology and the effects of S1P receptor modulation. *Neurology* **2011**, *76* (8 Suppl 3), S3−S8.

(6) David, O. J.; Kovarik, J. M.; Schmouder, R. L. Clinical pharmacokinetics of fingolimod. *Clin. Pharmacokinet.* **2012**, *51*, 15−28.

(7) Miron, V. E.; Jung, C. G.; Kim, H. J.; Kennedy, T. E.; Soliven, B.; Antel, J. P. FTY720 modulates human oligodendrocyte progenitor process extension and survival. *Ann. Neurol.* **2008**, *63* (1), 61−71.

(8) Scott, F. L.; Clemons, B.; Brooks, J.; Brahmachary, E.; Powell, R.; Dedman, H.; Desale, H. G.; Timony, G. A.; Martinborough, E.; Rosen, H.; Roberts, E.; Boehm, M. F.; Peach, R. J. Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity. *Br. J. Pharmacol.* **2016**, *173* (11), 1778−1792.

(9) Snaidero, N.; Simons, M. Myelination at a glance. *J. Cell Sci.* **2014**, *127* (14), 2999−3004.

(10) Kim, H. J.; Miron, V. E.; Dukala, D.; Proia, R. L.; Ludwin, S. K.; Traka, M.; Antel, J. P.; Soliven, B. Neurobiological effects of sphingosine 1-phosphate receptor modulation in the cuprizone model. *FASEB J.* **2011**, *25* (5), 1509−1518.

(11) Van Doorn, R.; Lopes Pinheiro, M. A.; Kooij, G.; Lakeman, K.; van het Hof, B.; van der Pol, S. M. A.; Geerts, D.; van Horssen, J.; van der Valk, P.; van der Kam, E.; Ronken, E.; Reijerkerk, A.; de Vries, H. E. Sphingosine 1-phosphate receptor 5 mediates the immune quiescence of the human brain endothelial barrier. *J. Neuroinflammation* **2012**, *9*, 133.

(12) O'Sullivan, S.; Dev, K. K. Sphingosine-1-phosphate receptor therapies: Advances in clinical trials for CNS related diseases. *Neuropharmacology* **2017**, *113* (Pt B), 597−607.

(13) Hobson, A. D.; Harris, C.; van der Kam, E.; et al. Discovery of A-971432, an orally bioavailable selective sphingosine-1-phosphate receptor 5 (S1P5) agonist for the potential treatment of neurodegenerative disorders. *J. Med. Chem.* **2015**, *58*, 9154−9170.

(14) Mattes, H.; Dev, K. K.; Bouhelal, R.; Barske, C.; Gasparini, F.; Guerini, D.; Mir, A. K.; Orain, D.; Osinde, M.; Picard, A.; Dubois, C.; Tasdelen, E.; Haessig, S. Design and synthesis of selective and potent orally active S1P5 agonists. *ChemMedChem* **2010**, *5*, 1693−1696.

(15) Miron, V. E.; Hall, J. A.; Kennedy, T. E.; Soliven, B.; Antel, J. P. Cyclical and dose-dependent responses of adult human mature oligodendrocytes to Fingolimod. *Am. J. Pathol.* **2008**, *173*, 1143−1152.

(16) Drouillard, A.; Mathieu, A. L.; Marçais, A.; Belot, A.; Viel, S.; Mingueneau, M.; Guckian, K.; Walzer, T. S1PR5 is essential for human NK cell migration towards sphingosine-1 phosphate. *J. Allergy Clin. Immunol.* **2018**, *141* (6), 2265−2268.

(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. Stereochemistry activity relationship of orally active tetralin S1P agonist prodrugs. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2264−2269.

(18) Essiz, M.; Guillaumet, G.; Brunet, J. J.; Caubere, P. Arynic condensations of ketone enolates. 15. New synthetic applications of the condensation of α,β-unsaturated ketone enolates on benzyne. *J. Org. Chem.* **1980**, *45* (2), 240−246.

(19) Guckian, K.; Kumaravel, G.; Liu, X.; Ma, B.; Peng, H. Preparation of naphthalene compounds as S1P receptor modulating agents for therapy. PCT/WO 2014/120764, 1−96.

(20) Jenne, C. N.; Enders, A.; Rivera, R.; Watson, S. R.; Bankovich, A. J.; Pereira, J. P.; Xu, Y.; Roots, C. M.; Beilke, J. N.; Banerjee, A.; Reiner, S. L.; Miller, S. A.; Weinmann, A. S.; Goodnow, C. C.; Lanier, L. L.; Cyster, J. G.; Chun, J. T-bet-dependent S1P5 expression in NK cells promotes egress from lymph nodes and bone marrow. *J. Exp. Med.* **2009**, *206* (11), 2469−2481.