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Identification of potent tricyclic prodrug S1P₁ receptor modulators†‡

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Recently, our research group reported the identification of prodrug amino-alcohol **2** as a potent and efficacious S1P₁ receptor modulator. This molecule is differentiated preclinically over the marketed drug fingolimod (Gilenya **1**), whose active phosphate metabolite is an S1P₁ full agonist, in terms of pulmonary and cardiovascular safety. S1P₁ partial agonist **2**, however, has a long half-life in rodents and was projected to have a long half-life in humans. The purpose of this communication is to disclose highly potent partial agonists of S1P₁ with shorter half-lives relative to the clinical compound **2**. PK/PD relationships as well as their preclinical pulmonary and cardiovascular safety assessment are discussed.

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Introduction

Autoimmune disorders are characterized by an abnormal immune response targeting different tissues. Immune cell trafficking from circulation to the affected tissue is a critical process tied to the progression of autoimmune disease.¹ The family of sphingosine-1-phosphate receptors (S1P₁₋₅) have been shown to be regulators of a variety of processes, including angiogenesis, differentiation, cell migration and cell trafficking.² In particular, it has been demonstrated that S1P₁ is essential for lymphocyte recirculation and regulation of lymphocyte egress from secondary lymphoid organs.³

Although activation of S1P₁ receptor by S1P initially increases cell trafficking, overstimulation promotes receptor desensitization, endocytosis and recycling.⁴ Fingolimod-P (**1-P**) (Fig. 1) was shown to induce internalization and degradation with no or slow recycling.⁵ The net effect of this action is functional antagonism of S1P₁ leading to robust lymphopenia.⁶ In 2010, fingolimod (**1**), was approved by the FDA for the treatment of relapsing-remitting multiple sclerosis. It has been clinically associated with decreased pulmonary function, elevation of blood pressure as well as transient

bradycardia.⁷ These effects may be associated with the non-selective nature of the active phosphate metabolite **1-P** of fingolimod (which binds to and activates S1P_{1,3,4,5}), or that **1-P** is a full agonist at S1P₁ (eliciting an agonist response comparable to S1P in magnitude). In addition, the associated long human *T*_{1/2} (100–200 h) is of concern due to its robust immunosuppressive nature and the potential susceptibility for infection.

We recently reported the identification of S1P₃ sparing, S1P₁ partial agonist prodrug **2/2-P** (BMS-986104) with an improved preclinical pulmonary and cardiovascular safety profile compared to **1/1-P** (Fig. 1).⁸ These safety improvements have been hypothesized to be attributed not only to its selectivity for S1P₁ over S1P₃ ref. 9 but also to ligand-biased signaling of **2-P** at S1P₁ (including the observed partial agonism in GTPγS and internalization assays).¹⁰ However, the *in vivo* half-life (*T*_{1/2}) of amino alcohol **2** was found to be long in rat (162 h) and was predicted to be long in human as well.

Fig. 1 Structures of fingolimod (Gilenya, **1**) and partial agonist **2** and their corresponding active metabolite.

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Fig. 2 Merging the direct acting and pro-drug programs.

Concurrent to this work, we have identified tricyclic azetidine carboxylic acids **3** as potent full and direct acting agonists of S1P₁ with shorter *in vivo* $T_{1/2}$ (<20 h) (Fig. 2).¹¹ This report details our efforts to combine the desired *in vivo* $T_{1/2}$ profile of the direct agonist framework **3** with the potent, S1P₃ sparing, S1P₁ partial agonist prodrug chemotype **2**.

Results and discussion

Scheme 1 summarizes the general synthesis of these tricyclic S1P₁ modulators which commences from 2-tetralone **5**.¹² Condensation with thiourea under oxidative conditions allowed the formation of aminothiazole **6** in 79% yield.¹³ Sandmeyer reaction then gave rise to chloro intermediate **7** or

Scheme 1 (a) Thiourea, I₂, EtOH, reflux, 79% (b) *t*-BuONO, CuX, MeCN, 0 °C, X = Cl; 35%, I; 55% (c) Fe(acac)₃, NMP, THF, RMgCl, 23 °C 65–88% or R = ROH or RSH, *t*BuOK, dioxane, reflux, 45–75% or RB(OH)₂, Pdppf, Na₂CO₃, dioxane, reflux, 75–89% or RRNH, Pd₂dba₃, Xphos, PhMe, 23 °C 44% (d) LiOH, dioxane, reflux, 65–89%.

iodo intermediate **8** in 35% and 55% yield, respectively. Intermediate **7** underwent Fe-catalyzed coupling with alkyl Grignards¹⁴ giving access to amino alcohols **10–14** after hydrolysis. Alternatively, iodo intermediate **8** can be treated with alcohols or thiols providing thiazole **15–20**. Buchwald–Hartwig or Suzuki cross coupling also allowed access to analogues **22–24**.

All compounds were then evaluated in rats (2 mg kg^{−1}, po) to assess the effects on circulating lymphocytes and generate

Table 1 Rat PK/PD relationship of various tricyclic S1P₁ modulators

4 h			24 h			48 h			Parent <i>in vivo</i> $T_{1/2}$ ^a (h)
Lymp. red.	Parent (nM)	Phos. (nM)	Lymp. red.	Parent (nM)	Phos. (nM)	Lymp. red.	Parent (nM)	Phos. (nM)	
10	41%	110	79	0%	<19	<10	—	—	<2
11	66%	77	97	48%	9.4	8.2	12%	<5	<4
12	33%	100	91	83%	55	56	56%	25	16
13	59%	110	140	82%	66	160	74%	<10	50
14	40%	66	63	77%	110	300	80%	43	150
15	74%	35	34	68%	7	20	26%	<5	4.0
16	67%	81	71	80%	29	26	59%	12	<5
17	66%	72	62	82%	31	<5	82%	17	<5
18	79%	200	120	87%	140	58	82%	27	18
19	82%	9.1	15	48%	1.4	2.3	16%	<1	<1
20	72%	87	43	76%	16	10	44%	<5	<5
21	37%	33	570	0%	0	190	—	—	—
22	30%	110	ND	27%	18	ND	4.4%	4.1	ND
23	9.3%	48	12	2.1%	37	19	—	—	—
24	31%	97	39	63%	68	<5	30%	<5	<5

^a Estimated $T_{1/2}$ from oral dosing. All results are a mean of 2 rats. BL; blood lymphocytes, lymph. red.; lymphocyte reduction, phosphate lymphocyte reduction relative to vehicle.

Table 2 *In vitro* potency of selected active metabolites

	S1P ₁ GTPγS (EC ₅₀ , nM)	S1P ₁ GTPγS (Y _{max})	S1P ₁ GTPγS F or P	S1P ₃ GTPγS (EC ₅₀ , nM)
10-P	19	79%	P	>3000
12-P	21	79%	P	>3000
14-P	10	98%	F	>3000
15-P	30	58%	P	>3000
17-P	12	100%	F	>3000
18-P	1.0	110%	F	190
19-P	20	58%	P	>3000
21-P	130	49%	P	>3000

All results are an average of 2 experiments.

F – full agonist, P = partial agonist.

Table 3 Rat PK/PD relationship of various aromatic tricyclic S1P₁ modulators

R =

Me-CH₂-CH₂-CH₂-O- 25

Me-CH₂-CH₂-CH₂-O- 26

Me-CH₂-CH₂-CH₂-CH₂-O- 27

Me-CH₂-CH₂-CH₂-S- 28

	4 h			24 h			48 h			Parent <i>in vivo</i> T _{1/2} ^a (h)
	Lymph. red.	Parent (nM)	Phos. (nM)	Lymph. red.	Parent (nM)	Phos. (nM)	Lymph. red.	Parent (nM)	Phos. (nM)	
25	65%	28	5.1	77%	21	<5	59%	12	<5	14
26	80%	31	ND	80%	17	ND	68%	8.3	ND	35
27	73%	81	260	83%	78	250	86%	41	160	41
28	69%	15	10	75%	8.0	8.1	59%	<5	<5	6

^a Estimated T_{1/2} from oral dosing. All result are a mean of 2 rats. BL; blood lymphocytes, lymph. red.; lymphocyte reduction, phos.; phosphate lymphocyte reduction relative vehicle.

an estimated T_{1/2} from oral dosing over a period of 72 h.¹⁵ The circulating levels of parent and corresponding active phosphate at three time points are presented in Table 1. Aliphatic sidechains of varying length (C3–C7) were prepared as the initial comparators to bicyclic analogue 2 (see compounds 10–14, Table 1). Relative to 2, this series demonstrated a shorter *in vivo* T_{1/2} (e.g. 12 vs. 2; molecules of same side chain length, see Fig. 2 for numbering). In general, longer aliphatic chains led to longer oral T_{1/2}.¹⁶ Consistent with this observation, longer aliphatic side chains had a more robust effect on lymphocyte reduction at 48 h. Increased carbon length also resulted in full agonism at S1P₁ of the phosphate metabolites *in vitro*, an undesirable feature (see 14-P, Table 2).¹⁷ Thiazole 12 provided a balance between efficacy at 24 h, S1P₁ partial agonism, and favorable oral T_{1/2}. In order to reduce the *in vivo* T_{1/2} further, heteroatoms were introduced on the side chain of 12 (see, 15, 19, and 21, Table 1). In all cases, the estimated T_{1/2} was reduced compared to 12. Oxythiazole 15 and 16 showed desirable properties although 16 has similar T_{1/2}

as amino alcohol 12. While analogue 15 had a short T_{1/2} (5 h), it had a rapid onset of lymphopenia with robust lymphocyte reduction at 4 h. Long chain oxythiazoles such as 17 and 18 showed sustained lymphopenia beyond 48 h and their corresponding phosphates exhibited undesired full agonistic properties (see 17-P and 18-P, Table 2). On the other hand, sulfur incorporation (19–20, Table 1) further reduced T_{1/2}. The lymphocyte reduction observed with 19 is noteworthy since robust (4 h) to moderate (24 h) affects were seen with very low levels of parent or phosphate in circulation. The presence of unidentified active metabolites, one possible explanation for the sustained pharmacodynamic (PD; lymphopenia) effects, was not investigated for these compounds. Alternatively, the lipophilic pocket of S1P₁ did not seem to accommodate amines 21 and 22 both *in vivo* and *in vitro* (Tables 1 and 2). Exploration beyond alkyl groups to aromatic (23–24, Table 1) side chains was not promising.

Fully aromatic tricyclic S1P₁ modulators were also prepared (Table 3). In general, the *in vivo* T_{1/2} are slightly longer

than the corresponding unsaturated system allowing for pronounced and sustained (≥ 24 h) lymphopenia. For example, tricyclic compounds 25 and 28 showed longer $T_{1/2}$ than their unsaturated equivalent 15 and 19.

Based upon the combined profile of short estimated $T_{1/2}$, partial agonism at $S1P_1$, and differentiated lymphopenia induction potential, thiazoles 12, 15, and 19 were advanced into rat pulmonary safety studies (Fig. 3). In these studies, the level of protein in the lungs following a bronchoalveolar lavage (BAL) was recorded as a marker of compound-induced vascular leakage and pulmonary edema.¹⁸ Relative to its estimated *in vivo* ED_{50} (estimated effective dose to give 50% lymphopenia at 24 h), thiazole 12 did not increase BAL protein levels up to $10\times ED_{50}$. Minimal BAL increases were noted at $30\times ED_{50}$ with more pronounced effects observed at $60\times ED_{50}$. On the other hand, comparable level of protein was observed with only $1\times ED_{50}$ for oxythiazole 15 while amino alcohol 19 showed marked increase of protein level at $5\times$ and $15\times ED_{50}$. In order to place these results in perspective, amino alcohol 18, whose phosphate is an $S1P_1$ full agonist with $S1P_3$ activity (Table 2), showed a robust $\sim 5\times$ increase in BAL protein level at $20\times ED_{50}$.

Concurrent with lung safety studies, the corresponding phosphates (12P, 15P, and 19P) were evaluated in cultured human cardiomyocytes derived from inducible pluripotent stem cells at 1 nM and 10 nM; an *in vitro* cardiac safety assay that predicted the effect of fingolimod on human heart rate at clinically relevant concentrations.¹⁹ Fig. 4 reports the beating rate reduction after 20 minutes of drug treatment, when steady state was reached. Considering that all three phosphates are nearly equipotent partial agonists of $S1P_1$, we con-

Fig. 4 Cardiomyocyte beating rate reduction 20 minutes after drug treatment. Average of 3–6 experiments. SE represented with bars.

sidered the maximally desirable beating rate change at these concentrations to be -15% and -30% , respectively. Oxythiazole 15-P reduced spontaneous beat rate to a lesser extent at those concentration (-16% @ 1 nM and -36% at 10 nM). Thiazoles 12-P and 19-P elicited a more robust beating rate reduction but still improved over fingolimod (1-P) (-38% @ 1 nM and -62% @ 10 nM).⁸

Conclusions

In conclusion, the successful hybridization of two distinct scaffolds led to the identification of $S1P_1$ modulators which exhibited appreciable lymphocyte reduction *in vivo*. *In vivo* $T_{1/2}$ was reduced to provide more attractive profiles with respect to the potential for long term infection risk reduction. Structure activity studies revealed that shorter chains provided weak and short-lived lymphocyte reductions whereas longer chains provided robust and sustained lymphocyte effects, albeit now with associated $S1P_1$ full agonism. Three analogues, 12, 15 and 19 were evaluated in additional safety studies *in vivo* and *in vitro*. Thiazole 12 showed superior pulmonary safety over thiazoles 15 and 19. Although the corresponding phosphate of 12 (12-P) led to a decrease in beating rate in an *in vitro* cardiomyocyte assay, this reduction was improved over the one observed with fingolimod (1-P). Overall, the preclinical safety profile of thiazole 12/12-P compare favorably to the marketed fingolimod (1).

Notes and references

- 1 J. G. Cyster and S. R. Schwab, *Annu. Rev. Immunol.*, 2012, 30, 69–94.
- 2 For a review, see; H. Rosen and E. J. Goetzl, *Nat. Rev. Immunol.*, 2005, 5, 560–570.
- 3 A. Dyckman, *Annu. Rep. Med. Chem.*, 2012, 47, 195–207.
- 4 S. Mandala, R. Hajdu, J. Bergstorm, E. Quackenbush, J. Xie, J. Milligan, R. Thornton, G.-J. Shei, D. Card, C. A. Keohane,

Fig. 3 Protein level in rat lungs following BAL. Average of 3 rats. SD represented with bars.

- M. Rosenbach, J. Hale, C. L. Lynch, K. Rupprecht, W. Parsons and H. Rosen, *Science*, 2002, **296**, 346–349.
- 5 J. Chun and H. Hartung, *Clin. Neuropharmacol.*, 2010, **33**, 91–101.
 - 6 V. Brinkmann, M. D. Davis, C. E. Heise, R. Albert, S. Cottens, R. Hof, C. Bruns, E. Prieschl, T. Baumruker, P. Hiestand, C. A. Foster, M. Zollinger and K. R. Lynch, *J. Biol. Chem.*, 2002, **277**, 21453–21457.
 - 7 L. Kappos, J. Antel, G. Comi, X. Montalban, P. O'Connor, C. H. Polman, T. Haas, A. A. Korn, G. Karlsson and E. W. Radue, *N. Engl. J. Med.*, 2006, **355**, 1124–1140.
 - 8 T. G. M. Dhar, H.-Y. Xiao, J. Xie, L. D. Lehman-McKeeman, D.-R. Wu, M. Dabros, X. Yang, T. L. Taylor, X. D. Zhou, E. M. Heimrich, R. Thomas, K. W. McIntyre, B. Warrack, H. Shi, P. C. Levesque, J. L. Zhu, J. Hennen, P. Balimane, Z. Yang, A. M. Marino, G. Cornelius, C. J. D'Arienzo, A. Mathur, D. R. Shen, M. E. Cvijic, L. Salter-Cid, J. C. Barrish, P. H. Carter and A. J. Dyckman, *ACS Med. Chem. Lett.*, 2016, **7**, 283–288.
 - 9 (a) M. Forrest, S.-Y. Sun, J. Bergstorm, D. Card, G. Doherty, J. Hale, C. Keohane, C. Meyers, J. Milligan, S. Mills, N. Nomura, H. Rosen, M. Rosenbach, G.-J. Shei, I. I. Singer, M. Tian, V. White, J. Xie, R. L. Proia and S. Mandala, *J. Pharmacol. Exp. Ther.*, 2004, **309**, 758–768; (b) Z. Li, Z. Chen, J. J. Hale, C. L. Lynch, S. G. Mills, T. Hajdu, C. A. Keohane, M. J. Rosenbach, J. A. Milligan, G. J. Shei, G. Chrebet, S. A. Parent, J. Bergstrom, D. Card, M. Forrest, E. J. Quackenbush, L. A. Wickham, H. Vargas, R. M. Evans, H. Rosen and S. Mandala, *J. Med. Chem.*, 2005, **48**, 6169–6173; (c) P. Gergely, B. Nuesslein-Hildesheim, D. Guerini, V. Brinkmann, M. Traebert, C. Bruns, S. Pan, N. S. Gray, K. Hinterding, N. G. Cooke, A. Groenewegen, A. Vitaliti, T. Sing, O. Luttringer, J. Yang, A. Gardin, N. Wang, W. J. Crumb, M. Saltzman, M. Rosenberg and E. Wallstrom, *Br. J. Pharmacol.*, 2012, **167**, 1035–1047.
 - 10 L. Lehman-McKeeman, A. J. Dyckman, T. L. Taylor, X. Yang, D.-R. Shen, R. L. Thomas, V. Borowski, H. Tang, E. M. Heimrich, H.-Y. Xiao, J. L. Gilmore, J. Sheppeck, T. G. M. Dhar, G. Cornelius, A. M. Marino, L. Lecureux, C. D'Arienzo, H. Sun, Z. Yang, D. Kukral, D. M. Banas, S. J. Suchard, M. E. Cvijic, K. W. McIntyre, L. Salter-Cid and J. Xie, *Manuscript in preparation*.
 - 11 T. G. M. Dhar, H.-Y. Xiao, S. H. Watterson, S. S. Ko, A. J. Dyckman, C. M. Langevine, J. Das and R. J. Cherney, PCT Int. Appl., WO 2011059784, 2011; For SAR discussion of related compounds, see: H.-Y. Xiao, S. H. Watterson, C. M. Langevine, A. S. Srivastava, S. S. Ko, Y. Zhang, R. J. Cherney, W.-W. Guo, J. L. Gilmore, J. E. Sheppeck, D.-R. Wu, P. Li, D. Ramasamy, P. Arunachalam, A. Mathur, T. L. Taylor, D. J. Shuster, K. W. McIntyre, D.-R. Shen, M. Yarde, M. E. Cvijic, A. M. Marino, P. V. Balimane, Z. Yang, D. M. Banas, G. Cornelius, C. J. Darienzo, L. Lehman-McKeeman, L. M. Salter-Cid, J. Xie, J. C. Barrish, P. H. Carter, A. J. Dyckman T. G. M. Dhar, *J. Med. Chem.*, 2016, **59**, 9837–9854.
 - 12 H.-Y. Xiao, A. J. Dyckman, Z. Xiao, M. G. Yang, T. G. M. Dhar, J. L. Gilmore and D. Marcoux, PCT Int. Appl., WO 2016028959A1, 2016, 20160225.
 - 13 For selected references, see; (a) S. Bhat, J. S. Shim and J.-O. Liu, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2733–2737; (b) M. D. Chordia, L. J. Murphree, T. L. Macdonald, J. Linden and R. A. Olsson, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1563–1566.
 - 14 For seminal papers, see; (a) A. Furstner, A. Leitner, M. Mendez and H. Krause, *J. Am. Chem. Soc.*, 2002, **124**, 13856–13863; (b) G. Seidel, D. Laurich and A. Furstner, *J. Org. Chem.*, 2004, **69**, 3950–3952.
 - 15 All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH Publication No. 85-23, revised 2011).
 - 16 Although the reason behind the longer estimated apparent *in vivo* $T_{1/2}$ observed with longer chains has not been studied in details, it is possible that the additional lipophilicity rendered by the long sider aliphatic chain could affect compound turnover, volume of distribution, phosphorylation and protein binding, leading to the longer estimated apparent $T_{1/2}$.
 - 17 The agonistic nature (full vs. partial) of the compounds have been assigned based on their efficacy ($Y_{\max}$) in a GTP γ S binding assay.
 - 18 (a) B. S. Shea, S. F. Brooks, B. A. Fontaine, J. Chun, A. D. Luster and A. Tager, *Am. J. Respir. Cell Mol. Biol.*, 2010, **43**, 662–673; (b) S. Sammani, L. Moreno-Vinassco, T. Mirzapioazova, P. A. Singleton, E. T. Chiang, C. L. Evenoski, T. Wang, B. Mathew, A. Husain, J. Moitra, X. Sun, L. Nunez, J. R. Jacobson, S. M. Dudek, V. Natarajan and J. G. N. Garcia, *Am. J. Respir. Cell Mol. Biol.*, 2010, **43**, 394–402.
 - 19 J. M. Khan, A. R. Lyon and S. E. Harding, *Br. J. Pharmacol.*, 2013, **169**, 304–317.