ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

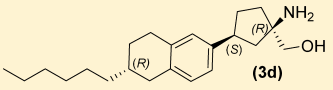
Identification and Preclinical Pharmacology of BMS-986104: A Differentiated $S1P_1$ Receptor Modulator in Clinical Trials

T. G. Murali Dhar,* Hai-Yun Xiao, Jenny Xie, Lois D. Lehman-McKeeman, Dauh-Rurng Wu, Marta Dabros, Xiaoxia Yang, Tracy L. Taylor, Xia D. Zhou, Elizabeth M. Heimrich, Rochelle Thomas, Kim W. McIntyre, Bethanne Warrack, Hong Shi, Paul C. Levesque, Jia L. Zhu, James Hennan, Praveen Balimane, Zheng Yang, Anthony M. Marino, Georgia Cornelius, Celia J. D'Arienzo, Arvind Mathur, Ding Ren Shen, Mary Ellen Cvijic, Luisa Salter-Cid, Joel C. Barrish, Percy H. Carter, and Alaric J. Dyckman

Research and Development, Bristol-Myers Squibb Company, Princeton, New Jersey 08543-4000, United States

Supporting Information

ABSTRACT: Clinical validation of S1P receptor modulation therapy was achieved with the approval of fingolimod (Gilenya, **1**) as the first oral therapy for relapsing remitting multiple sclerosis. However, **1** causes a dose-dependent reduction in the heart rate (bradycardia), which occurs within hours after first dose. We disclose the identification of clinical compound BMS-986104 (**3d**), a novel $S1P_1$ receptor modulator, which demonstrates ligand-biased signaling and differentiates from **1** in terms of cardiovascular and pulmonary safety based on preclinical pharmacology while showing equivalent efficacy in a T-cell transfer colitis model.



Differentiated $S1P_1$ receptor modulator

KEYWORDS: *GPCR, S1P1, S1P3, biased signaling*

Lymphocyte infiltration from blood into sites of inflammation is critical to the pathogenesis of autoimmune diseases and allograft rejection. Gilenya (FTY720, **1**) blocks lymphocyte migration through sequestration of lymphocytes in the thymus and secondary lymphoid organs, leading to a marked lymphopenia.[1] Compound **1** is a pro-drug; its phosphorylated form, FTY-P (**1-P**), binds four out of the five S1P receptors (S1P-1, 3, 4, 5) and elicits a full agonist response in functional assays such as GTP-S binding, ERK phosphorylation, cAMP, and calcium mobilization. Among these four receptors, $S1P_1$ has been shown to be critically involved in lymphocyte trafficking and agonism of this receptor is responsible for the peripheral blood lymphopenia believed to be key to the efficacy seen with **1**.[2,3]

Clinical studies have demonstrated a side effect profile of **1** that includes cardiovascular effects (transient bradycardia, sustained blood pressure elevation) as well as a decline in pulmonary function.[4] In rodent studies, $S1P_3$ activity was shown to play a role in some of the observed acute toxicity of nonselective S1P receptor agonists, including bradycardia, hypertension, and bronchoconstriction.[5,6] As agonism of $S1P_3$ does not appear to contribute to efficacy, the identification of $S1P_1$ agonists sparing of $S1P_3$ has been a primary emphasis of many research programs in this area.[7] However, clinical studies with S1P agonists with selectivity for $S1P_1$ over $S1P_3$ have suggested that in humans the heart rate reduction effects are controlled at least in part through agonism of $S1P_1$.[8] Additionally, through the course of our own studies it was discovered that simply abolishing $S1P_3$ agonism was not sufficient to eliminate the acute and chronic pulmonary toxicity elicited in rodents by **1** or by selective $S1P_1$ full agonists, findings that led us to discontinue our efforts related to $S1P_1$ full agonists and seek alternative profiles that could overcome these liabilities.[9] In this letter we describe the identification of a differentiated $S1P_1$ receptor modulator, BMS-986104 (**3d**), which distinguishes itself from **1** in terms of cardiovascular and pulmonary safety based on preclinical pharmacology while showing equivalent efficacy in a T-cell transfer colitis model.

In our search for $S1P_1$ agonists that could further dissociate efficacy from toxicity, we evaluated a range of compounds with unique S1P receptor profiles, including cyclopentyl constrained analogues described in the literature as partial agonists of $S1P_1$ with activity on $S1P_3$ dependent upon the stereochemistry of the benzylic center (Figure 1, **2a** and **2b**).[10] Authors of this letter show that the alcohol prodrug of these compounds evoked lymphopenia after oral administration in mice and a stereoisomeric mixture of **2a**/**2b** had minimal effects on heart rate changes in rodents relative to **1**. As discussed earlier, effects in rodent on bronchoconstriction and heart rate is mediated by the $S1P_3$ receptor and may not be relevant in a clinical setting where $S1P_1$ is believed to play a role. We decided to explore the unique attributes of the cyclopentyl head piece in terms of properties that are more significant from a clinical perspective (markers of pulmonary toxicity as well as evaluation of predicted cardiovascular function with a human-relevant in vitro system). In addition, we decided to explore the functional

Received: November 22, 2015
Accepted: January 19, 2016

DOI: 10.1021/acsmedchemlett.5b00448
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

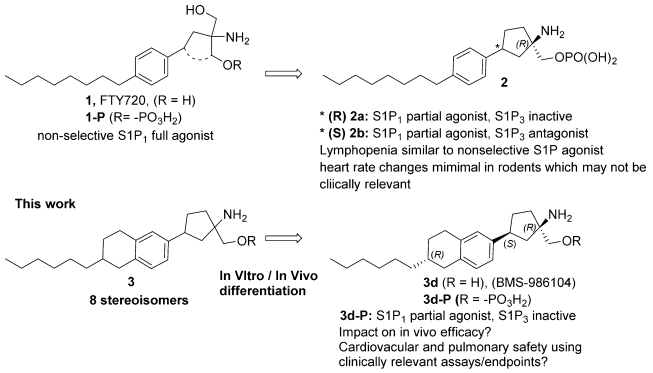
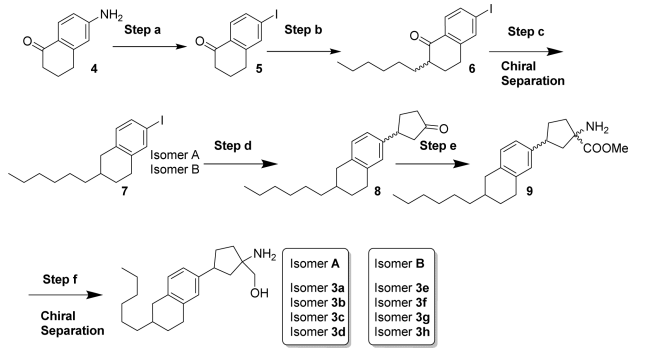


Figure 1. Rationale for the synthesis of **3d**.

consequences (in terms of $S1P_1/S1P_3$ activity) of conformationally restricting the side chain of **2** in the form of a tetralin ring system (Figure 1, **3**).

The synthesis of compounds **3a–h** is shown in Scheme 1 wherein the hexyl side chain was installed in a nonselective

Scheme 1[a]



[a]Reagents and conditions: (a) $NaNO_2$, H_2SO_4, HOAc, H_2O, 0 °C, 10 min; then KI, H_2O, 0 °C, 30 min, RT, 1 h, 69%. (b) KO^{t}Bu, Et_3B, $CH_3(CH_2)_5I$, THF, RT, 4 h, 23%. (c) Step 1, Et_3SiH, TFA, 50 °C, 15 h, 95%; step 2, chiral SFC using AD-H column and 10% MeOH in CO_2 eluent. (d) Cyclopent-2-enol, $Pd(OAc)_2$, Bu_4NCl, KOAc, DMF, 80 °C, 2.5 h, 75%. (e) Step 1, NH_3, NaCN, NH_4Cl, MeOH, RT, 2 days; step 2, HCl, H_2O, HOAc, dioxane, 100 °C, 9 h; step 3, MeOH, $SOCl_2$, 70 °C, 7 h; 43% for 3 steps. (f) Step 1, $NaBH_4$, EtOH, DCM, RT, overnight, 100%; step 2, chiral SFC using AD-H column and 12% MeOH in CO_2 with 0.1% DEA eluent; then, chiral SFC using AS-H column and 12% of MeOH–CH_3CN (1:1) in CO_2 with 0.5% DEA eluent.

manner to afford iodophenyl enantiomers **A** and **B**, which were separated by chiral SFC. Each isomer (**A**/**B**) was then independently carried through the same sequence to arrive at two separate mixtures of four diastereomeric compounds. Chiral chromatographic separation of each mixture afforded the individual isomers **3a–d** and **3e–h**.

Single crystals of the *R*-(−)-mandelic acid salt of **3d** were obtained and analyzed by X-ray diffraction. Two conformers were present, differing mainly in the orientation of the hydroxy methyl group and alignment of the *n*-hexyl chain. The absolute configuration of **3d** was determined to be **RSR** (see Supporting Information).

The identification of **3d** from the mixture of stereoisomers relied on initial in vitro evaluation of phosphorylation potential followed by an in vivo "PK/PD/Tox" screening approach, with lymphopenia as the pharmacodynamic (PD) marker and an increase in bronchoalveolar lavage (BAL) protein as the marker of vascular leakage and pulmonary edema.[11,12] It was anticipated based on previously published work[10] that the stereoisomeric configuration would influence the efficiency of the metabolic conversion of the amino-alcohol prodrugs to the corresponding phosphates, which are the active S1P agonists. To facilitate the identification of the most active isomers, the extent of phosphorylation of the compounds was evaluated after incubation in mouse whole blood. The appearance of the phosphorylated compound was measured after 4 h by LC/MS/MS to determine the relative extent of phosphate ester formation. As shown in Table 1, two compounds within each

Table 1. In Vitro and in Vivo Differentiation of Stereoisomers 3a–3h

compound number	mouse whole blood phosphate area ratio at 4 h[a]	mouse PK[b] phosphate blood concentration from 10 mg/kg dose of parent: 4 h (nM)	24 h (nM)
3a	0.07	<9.8 (bllq)	14
3b	1.60	67	475
3c	0.14	12	46
3d	0.60	41	202
3e	0.03		
3f	0.60		
3g	0.02		
3h	0.17		

[a]LC/MS/MS area ratio of phosphorylated material vs nonphosphorylated material after 4 h incubation. [b]Compounds were administered by oral gavage as solutions in polyethylene glycol 300 (PEG300).

of Isomer **A** and Isomer **B** series demonstrated superior phosphorylation (**3b**, **3d** and **3f**, **3h**). Within Isomer **A** series, the predictive utility of the mouse whole blood assay was confirmed by a PK study in mouse to measure phosphate formation after oral administration of the alcohol compounds **3a–3d** at 10 mg/kg. As shown in Table 1, **3b** and **3d** demonstrated substantially greater levels of phosphate metabolites at both the 4 h and the 24 h time points than did the corresponding diastereomers **3a** or **3c**.

The mouse blood lymphocyte reduction model provided PD data (Table 2) in line with the in vitro and PK results shown in

Table 2. Pharmacodynamic Differentiation of Stereoisomers

	blood lymphocyte reduction in mouse: 24 h post 1 mg/kg dose[b]					
	3a	**3b**	**3c**	**3d**	**3f**	**3h**
vehicle[a] ($\times 10^3$/mm^3)		5.39			5.86	
compound ($\times 10^3$/mm^3)	4.76	0.54	4.4	0.7	1.2	2.37
% reduction	12%	90%	18%	87%	80%	60%

[a]Vehicle: PEG300. [b]Compounds were administered by oral gavage as solutions in PEG 300. Circulating lymphocyte levels were monitored 24 h postdose.

Table 1. Single isomers **3a** and **3c** showed very minor reductions in circulating lymphocytes at 24 h after a 1 mg/kg dose (12% and 18%), whereas single isomers **3b** and **3d** afforded a maximal response for this assay (90% and 88%). Within the Isomer **B** series, only compounds **3f** and **3h** (those predicted to be better phosphorylated in the mouse blood

DOI: 10.1021/acsmedchemlett.5b00448
ACS Med. Chem. Lett. XXXX, XXX, XXX–XXX

Table 3. Effects of Compounds 1 and 3d on Blood Lymphocyte Counts and BAL Protein in mice (5 Mice/Dose Group)[a]

		1 (mg/kg)			3d (mg/kg)		
	vehicle[b]	0.5	10	30	0.5	10	30
BL	8.02 ± 0.42 (1) 5.13 ± 0.57 (3d)	1.28 ± 0.11	0.92 ± 0.31	0.29 ± 0.08	1.65 ± 0.15	0.51 ± 0.07	0.52 ± 0.05
BAL	8.20 ± 0.37 (1) 8.30 ± 0.30 (3d)	7.50 ± 0.29	34.2 ± 4.18	71.0 ± 30.4	7.67 ± 0.67	8.25 ± 0.63	10.0 ± 1.22

[a]BL = blood lymphocytes ($\times 10^3$/mm^3); BAL = BAL protein (mg/dL). [b]Water (1); 18.4% (w/v) hydroxypropyl-β-cyclodextrin (HP-β-CD) in 13.8 mM citric acid pH 4 (3d).

assay) were evaluated in the mouse PD model. Lymphocyte reductions from Isomer **B** analogues **3f** and **3h** (80% and 60%) were notably lower in comparison to the Isomer **A** analogues **3b** and **3d**, highlighting the impact of the stereochemistry at the carbon on the tetralin ring anchoring the *n*-hexyl side chain.

Compounds **3b** and **3d** were each able to drive maximal reduction in the mouse PD model at 24 h after a single 1 mg/kg dose. The phosphate level detected at 24 h postdose was 27 nM for **3d** and 47 nM for **3b**. As **3d** was able to drive the desired pharmacodynamic effect with lower active drug exposure, it was selected for further advanced evaluation, including assessment of pulmonary toxicity and predictive cardiovascular safety.

As shown in Table 3, when **1** and **3d** (0.5, 10, and 30 mg/kg) were dosed orally to mice, a significant decrease in the number of lymphocytes was noted for both compounds at all doses after 24 h. However, the extent of BAL protein elevation was different for both compounds; while **1** increased BAL protein significantly at 10 and 30 mg/kg doses, BAL changes were insignificant with all doses of **3d**.

We have also shown that the BAL changes observed with **1** as well as $S1P_3$ sparing $S1P_1$ full agonists eventually progresses to early fibrotic changes in rats.[9] Although, the severity of the lung changes observed in preclinical species has not yet directly translated to humans, data from the clinical trials indicated that **1** caused a mild dose-dependent reduction in FEV1 (forced expiratory volume in 1 s)[4] and DLCO (carbon monoxide diffusing capacity) values. Clearly, an $S1P_1$ modulator devoid of any pulmonary effects may be a preferred entity in the clinic.

Since **3d** is a prodrug, we extensively characterized the phosphate metabolite **3d-P** in various in vitro assays to understand the factors contributing to its differentiated profile (Table 4). As is clear from Table 4, **1-P** and **3d-P** are equipotent in the $S1P_1$ binding assay and act as full agonists in the cAMP functional assay. However, in the following three assays, compound **3d-P** shows a differentiated profile compared to **1-P**: (i) in the $S1P_1$ internalization assay, **3d-P** is clearly a partial agonist compared to **1-P**; (ii) in the ERK-P assay, although both **1-P** and **3d-P** are full agonists, from a potency perspective, **3d-P** is ∼1000-fold weaker than **1-P**; and (iii) **3d-P** is a partial $S1P_1$ agonist in the $S1P_1$ GTPγS assay with maximum efficacy that reaches 81% in relation to the maximum efficacy of the endogenous ligand. In addition, **3d-P** did not show activity in the $S1P_3$ GTPγS assay when run in the antagonist mode. The ligand bias by **3d-P** in the internalization and the ERK-P assays as well as in the $S1P_1$ and $S1P_3$ GTPγS assays may impact signaling through the $S1P_1$ and $S1P_3$ receptors and contribute to the differentiated in vivo profile seen with the compound. In addition, the partial agonist profile in the internalization assay may reflect the improved pulmonary safety in preclinical studies (BAL protein elevation) since maintaining some level of S1P/$S1P_1$ signaling may be crucial for controlling vascular tone.[13]

Table 4. 1-P vs 3d-P in Vitro and in Vivo Pharmacological Data

assay	1-P	3d-P
$hS1P_1$ binding (IC_{50}, nM)	0.014 ± 0.006 (n = 6)	0.010 ± 0.004 (n = 7)
$hS1P_1$ cAMP (EC_{50}, nM), Y_{max}	0.005 ± 0.001 (n = 3), 100%	0.006 ± 0.002 (n = 3), 98%
$hS1P_1$ internalization (EC_{50},nM), Y_{max}	0.070 ± 0.011 (n = 7), 100%	0.114 ± 0.020 (n = 5), 68%
$hS1P_1$ ERK-P (EC_{50}, nM), Y_{max}	0.0056 ± 0.0005 (n = 4),100%	8.16 ± 3.62 (n = 4) , 100%
$hS1P_1$ GTPγS (EC_{50}, nM), Y_{max} (agonist)	0.377 ± 0.096 (n = 6), 95%	0.901 ± 0.358 (n = 7), 81%
$hS1P_3$ GTPγS (EC_{50}, nM)	3.57 ± 2.80 (n = 7),	>1000 (n = 7)
$hS1P_3$ GTPγS (IC_{50}, nM), (antagonist)	NA	>1000 (n = 3)
$hS1P_4$ GTPγS (EC_{50}, nM)	0.09 (n = 2)	10.5 (n = 2)
$hS1P_5$ GTPγS (EC_{50}, nM)	0.4 (n = 2)	10.7 (n = 2)
mouse lymphopenia (EC_{50}, nM)	5.6 ± 2.5	7.9 ± 0.9
mouse lymphopenia (24 h; ED_{50}, mg/kg)	0.017	0.12 ± 0.04
mouse T cell transfer colitis: maximal efficacious dose	1 mg/kg daily of 1	5 mg/kg every other day of 3d

In order to confirm that the differentiated profile of **3d** does not compromise its in vivo efficacy, we tested the compound in the $CD45RB^{hi}CD4^+$ T-cell transfer colitis model of inflammatory bowel disease (IBD). We chose to test **3d** in this model since leukocyte infiltration into the inflamed intestine is fundamental to disease development and perpetuation in IBD. In addition, a significant overexpression of the $S1P_1$ receptor is observed in the colon biopsy samples of patients. Taking advantage of the compound's long pharmacokinetic half-life ($t_{1/2}$) in mouse (Table 5), we decided to dose the compound in an alternate day dosing regimen. While offering flexibility in dosing schedules, the impact of a long half-life on sustained PD effects will be further evaluated in the clinic.

Figure 2 shows a dose-dependent inhibition of the body weight loss and the shortening of the colon length by 5 weeks of treatment with **3d** in this colitis model. Statistical significance was reached in 5 mg/kg treatment group of **3d** and 1 mg/kg/day treatment group of **1** vs vehicle control with regard to both body weight and colon length measurements. Histological evaluation of the colon showed that treatment with **3d** reduced overall colon inflammation and tissue damage in a dose-dependent fashion. The colon gene expression levels for pro-inflammatory cytokines (IFN-γ and IL-2), leukocyte markers (CD4, B220, and CD11b), and IFN signature genes (Fcgr4, Ifit1, and OASL2) by RT-PCR were significantly reduced in 5 mg/kg dose group compared to vehicle controls. Overall, the in vivo and gene expression data clearly shows efficacy comparable

DOI: 10.1021/acsmedchemlett.5b00448
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 5. Pharmacokinetic Parameters for Compounds 3d and 3d-P in Mouse

	mouse[a]	
parameter	3d	3d-P
po dose (mg/kg)	1[b]	
iv dose (mg/kg)	0.5[c]	
C_{max} (μM), po	0.021	0.049
T_{max} (h), po	6.0	24
$AUC_{0\text{-last}}$ (μM·h), po	1.0[d]	4.6[d]
$AUC_{0\text{-inf}}$ (μM·h), po	1.6	5.2
$t_{1/2}$ (h), iv	37	56
MRT (h), iv	50	88
Cl (mL/min/kg), iv	20	
V_{ss} (L/kg), iv	60	
F_{po} (%)	65	

[a]Composite blood concentration−time profiles were constructed for PK analysis. [b]Vehicle: 90% PEG300, 5% ethanol, 5% TPGS (D-alpha-tocopheryl polyethylene glycol succinate). [c]Vehicle: 18.4% hydroxypropyl beta cyclodextrin, 81.6% citrate buffer. [d]The last time point was 72 h for **3d** and 168 h for **3d-P**.

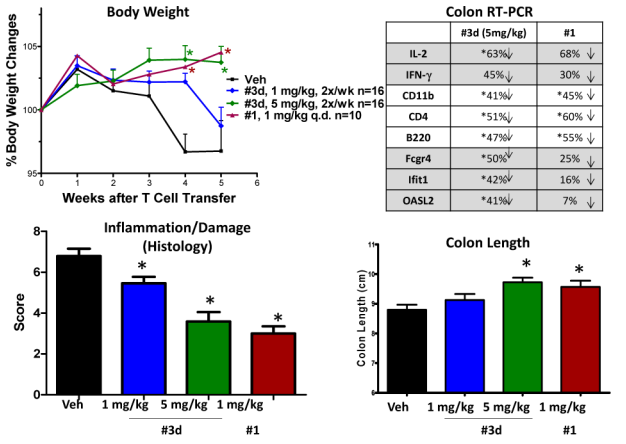


Figure 2. Inhibition of T cell transfer colitis by **3d** in mice.

to FTY in all measures and demonstrates that the differentiated profile of **3d** does not impact its performance in vivo.

Having established that compound **3d** is differentiated from **1** in terms of pulmonary safety without compromising in vivo efficacy, we proceeded to evaluate the cardiovascular safety profile in relation to **1**. As mentioned earlier, in most preclinical species, the $S1P_3$ receptor plays a predominant role in the regulation of heart rate, and as such, compounds that are selective for $S1P_1$ over $S1P_3$ show reduced potential for bradycardia in these models. However, selective $S1P_1$ agonists cause bradycardia in humans, and research suggests that the guinea pig heart is also sensitive to $S1P_1$-selective compounds, making it more similar to humans than mice, rats, or dogs.[14] In isolated guinea pig hearts, perfusion of clinically relevant concentrations of **1-P** (1 nM) decreased ventricular rate (32 ± 2 bpm) and induced 3° atrial-ventricular (AV) block. Siponimod, a selective, full agonist of $S1P_1$, also decreased ventricular rate and caused 3° AV block in the guinea pig heart at clinically relevant concentrations of 100 and 300 nM, respectively (data not shown).[15,16] In direct contrast, 100 nM **3d-P** elicited no effects on heart rate or AV conduction, and suprapharmacologic concentrations of 1000 nM were required to elicit significant bradycardia and 3° AV block, thereby establishing a clear differentiation from **1-P** and $S1P_1$-selective agonists.

Additionally, the potential for **3d** to produce bradycardia in humans was evaluated in cultured human cardiomyocytes derived from inducible pluripotent stem cells (iPSCs). Cardiomyocytes differentiated from iPSCs show concentration-dependent responses to cardioactive drugs, including beating frequency and contractility, which are consistent with clinical effects.[17] Additionally, these cells express $S1P_1$ and $S1P_3$ receptors (results not shown). Compound **1-P** showed a concentration-dependent decrease in cardiomyocyte beating rate, with a no-effect concentration of 0.1 nM. At 1 nM drug, **1-P** decreased cardiomyocyte beating rate by 35%, whereas **3d-P** was without effect at 1 nM (Figure 3). Compound **3d-P** was

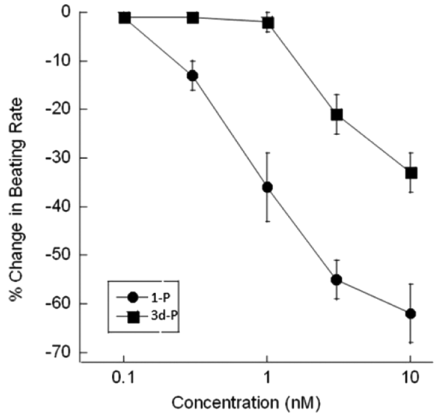


Figure 3. Differential effects of **1-P** and **3d-P** in cultured human cardiomyocytes.

further differentiated from **1-P** in human cells as beating rates were decreased at suprapharmacologic concentrations, but to a lesser magnitude than the changes observed with **1-P**. Collectively, data obtained in the guinea pig heart and human cardiomyocyte models, which were used to mitigate species differences in S1P receptor pharmacology, demonstrate a reduced risk for cardiovascular liability by **3d** in human.

In conclusion, we have demonstrated that compound **3d** is a $S1P_1$ receptor modulator, which is differentiated from **1** in terms of pulmonary and cardiovascular safety, based on preclinical pharmacology. From an efficacy point of view, **3d** induces maximal lymphopenia comparable to **1** in vivo. Furthermore, **3d** demonstrates comparable level of efficacy to **1** in a $CD45RB^{hi}CD4^{+}$T-cell transfer colitis model. These results confirm that lymphopenia and efficacy can be fully achieved without complete desensitization of the $S1P_1$ receptors. On the basis of its preclinical cardiovascular and pulmonary safety profiles and comparable efficacy to **1** in a preclinical model of IBD, compound **3d** was advanced into Phase I clinical trials.[18−26]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00448.

Synthetic procedures and complete characterization data for compound **3d** and assay protocols; single crystal X-ray crystal structure of **3** (PDF)

DOI: 10.1021/acsmedchemlett.5b00448
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

AUTHOR INFORMATION

Corresponding Author

*Phone: 609-252-4158. E-mail: murali.dhar@bms.com.

Author Contributions

The manuscript contains contributions from all authors.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

S1P, sphingosine 1-phosphate; GTP, guanosine-5′-triphosphate (GTP); cAMP, cyclic adenosine monophosphate; SAR, structure−activity relationship; PK, pharmacokinetic; Tox, toxicity; GTPγS, guanosine 5′-*O*-[gamma-thio]triphosphate; IFN, interferon; RT-PCR, real-time polymerase chain reaction

REFERENCES

(1) Chun, J.; Hartung, H. Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. *Clin. Neuropharmacol.* **2010**, *33*, 91−101.

(2) Mandala, S.; Hajdu, R.; Bergstorm, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C. A.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* **2002**, *296*, 346−349.

(3) 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. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J. Biol. Chem.* **2002**, *277*, 21453−21457.

(4) Kappos, L.; Antel, J.; Comi, G.; Montalban, X.; O'Connor, P.; Polman, C. H.; Haas, T.; Korn, A. A.; Karlsson, G.; Radue, E. W. Oral fingolimod (FTY720) for relapsing multiple sclerosis. *N. Engl. J. Med.* **2006**, *355*, 1124−1140.

(5) Forrest, M.; Sun, S.-Y.; Bergstorm, 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.; 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.

(6) Li, Z.; Chen, Z.; Hale, J. J.; Lynch, C. L.; Mills, S. G.; Hajdu, T.; 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. Discovery of Potent 3,5-Diphenyl-1,2,4-oxadiazole Sphingosine-1-phosphate-1 ($S1P_1$) Receptor Agonists with Exceptional Selectivity against $S1P_2$ and $S1P_3$. *J. Med. Chem.* **2005**, *48*, 6169−6173.

(7) Dyckman, A. Recent Advances in the Discovery and Development of Sphingosine-1-Phosphate-1 Receptor Agonists. *Annu. Rep. Med. Chem.* **2012**, *47*, 195−207.

(8) Gergely, P.; Nuesslein-Hildesheim, B.; Guerini, D.; Brinkmann, V.; Traebert, M.; Bruns, C.; Pan, S.; Gray, N. S.; Hinterding, K.; Cooke, N. G.; Groenewegen, A.; Vitaliti, A.; Sing, T.; Luttringer, O.; Yang, J.; Gardin, A.; Wang, N.; Crumb, W. J., Jr.; Saltzman, M.; Rosenberg, M.; Wallstrom, E. The selective sphingosine 1-phosphate receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate. *Br. J. Pharmacol.* **2012**, *167*, 1035−1047.

(9) Lehman-McKeeman, L.; Dyckman, A. J.; Taylor, T. L.; Yang, X.; Shen, D.-R.; Thomas, R. L.; Borowski, V.; Tang, H.; Heimrich, E. M.; Xiao, H.-Y.; Gilmore, J. L.; Sheppeck, J.; Dhar, T. G. M.; Cornelius, G.; Marino, A. M.; Lecureux, L.; D'Arienzo, C.; Sun, H.; Yang, Z.; Kukral, D.; Banas, D. M.; Suchard, S. J.; Cvijic, M. E.; McIntyre, K. W.; Salter-Cid, L.; Xie, J. Manuscript in preparation.

(10) 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.

(11) Shea, B. S.; Brooks, S. F.; Fontaine, B. A.; Chun, J.; Luster, A. D.; Tager, A. Prolonged Exposure to Sphingosine 1−Phosphate Receptor-1 Agonists Exacerbates Vascular Leak, Fibrosis, and Mortality after Lung Injury. *Am. J. Respir. Cell Mol. Biol.* **2010**, *43*, 662−673.

(12) Sammani, S.; Moreno-Vinassco, L.; Mirzapoiazova, T.; Singleton, P. A.; Chiang, E. T.; Evenoski, C. L.; Wang, T.; Mathew, B.; Husain, A.; Moitra, J.; Sun, X.; Nunez, L.; Jacobson, J. R.; Dudek, S. M.; Natarajan, V.; Garcia, J. G. N. Differential Effects of Sphingosine 1−Phosphate Receptors on Airway and Vascular Barrier Function in the Murine Lung. *Am. J. Respir. Cell Mol. Biol.* **2010**, *43*, 394−402.

(13) Oo, M. L.; Chang, S.-H.; Thangada, S.; Wu, M.-T.; Rezaul, K.; Blaho, V.; Hwang, S.-I.; Han, D. K.; Hla, T. Engagement of S1P(1)-degradative mechanisms leads to vascular leak in mice. *J. Clin. Invest.* **2011**, *121*, 2290−2300.

(14) Rey, M.; Hess, P.; Clozel, M.; Delahaye, S.; Gatfield, J.; Nayler, O.; Steiner, B. Desensitization by progressive up-titration prevents first-dose effects on the heart: Guinea pig study with ponesimod, a selective S1P1 receptor modulator. *PLoS One* **2013**, *8* (9), e74285.

(15) Legangneux, E.; Gardin, A.; Johns, D. Dose titration of BAF312 attenuates the initial heart rate reducing effect in healthy subjects. *Br. J. Clin. Pharmacol.* **2012**, *75*, 831−841.

(16) Gergely, P.; Nuesslein-Hildesheim, B.; Guerini, D.; Brinkmann, V.; Traebert, M.; Bruns, C.; Pan, S.; Gray, N. S.; Hinterding, K.; Cooke, N. G.; Groenewegen, A.; Vitaliti, A.; Sing, T.; Luttringer, O.; Yang, J.; Gardin, A.; Wang, N.; Crumb, W. J.; Saltzman, M.; Rosenberg, M.; Wallstrom, E. The selective sphingosine 1-phosphate receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate. *Br. J. Pharmacol.* **2012**, *167*, 1035−1047.

(17) Khan, J. M.; Lyon, A. R.; Harding, S. E. The case for induced pluripotent stem cell-derived cardiomyocytes in pharmacological screening. *Br. J. Pharamacol.* **2013**, *169*, 304−317.

(18) Refs 18−26. Other $S1P_1$ modulators in clinical trials. Ponesimod (ACT-128800). 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.; Müller, C.; Nayler, O.; Scherz, M.; Schmidt, G.; Sippel, V.; Steiner, B.; Strasser, D.; Treiber, A.; Weller, T. 2-Imino-thiazolidin- 4-one derivatives as potent, orally active S1P1 receptor agonists. *J. Med. Chem.* **2010**, *53*, 4198−4211.

(19) Siponimod (BAF312). Pan, S.; Gray, N.; Gao, W.; Mi, Y.; Fan, Y.; Wang, X.; Tuntland, T.; Che, J.; Lefebvre, S.; Chen, Y.; Chu, A.; Hinterding, K.; Gardin, A.; End, P.; Heining, P.; Bruns, C.; Cooke, N. G.; Nuesslein-Hildesheim, B. Discovery of BAF312 (siponimod), a potent and selective S1P receptor modulator. *ACS Med. Chem. Lett.* **2013**, *4*, 333−337.

(20) Ceralifimod (ONO-4641). Bar-Or, A.; Zipp, F.; Scaramozza, M.; Vollmer, T.; Due, B.; Thangavelu, K.; Fischer, T.; Selmaj, K. Effect of Ceralifimod (ONO-4641), a Sphingosine-1-Phosphate Receptor-1 and −5 Agonist, on Magnetic Resonance Imaging Outcomes in Patients with Multiple Sclerosis: Interim Results from the Extension of the DreaMS Study. *Neurology* **2014**, *82*, 161.

(21) CS-0777 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. Discovery of CS-0777: a potent, selective, and orally active S1P1 agonist. *ACS Med. Chem. Lett.* **2011**, *2*, 368−372.

(22) KRP-203. 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. KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and hear allografts. *Circulation* **2005**, *111*, 222−229.

(23) Ozanimod (RPC1063). Olson, A.; Hartung, J.; Timony, G.; Peach, R.; Boehm, M.; Rosen, H.; Smith, H.; Pan, C.; Brooks, J.; Gujrathu, S. Safety and Tolerability of Orally Administered RPC1063,

DOI: 10.1021/acsmedchemlett.5b00448
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

a Novel S1P1 Receptor Modulator, in Healthy Adult Volunteers, Results of a Phase 1 Study. *Neurology* **2013**, *80*, 178

(24) Amiselimod (MT-1303). Kappos, L.; Arnold, D.; BarOr, A.; Camm, J.; Derfuss, T.; Kieseier, B.; Sprenger, T.; Greenough, K.; Ni, P.; Harada, T. Results of MOMENTUM, a randomised, double blind, placebo controlled phase 2 trial with MT1303, a novel selective sphingosine 1phosphate receptor 1 (S1P1) modulator, in relapsing remitting MS. Neurology 31st Congr Eur Comm Treat Res Multiple Scler (ECTRIMS) (Oct 7−10, Barcelona) 2015, Abst 227a.

(25) APD334. Buzard, D. J.; Kim, S. H.; Lopez, L.; Kawasaki, A.; Zhu, X.; Moody, J.; Thoresen, T.; Calderon, I.; Ullman, B.; Han, S.; Lehmann, J.; Gharbaoui, T.; Sengupta, D.; Calvano, L.; Montalban, A. G.; Ma, Y.-A.; Sage, C.; Gao, Y.; Semple, G.; Edwards, J.; Barden, J.; Morgan, M.; Chen, W.; Usmani, K.; Chen, C.; Sadeque, A.; Christopher, R. J.; Thatte, J.; Fu, L.; Solomon, M.; Mills, D.; Whelan, K.; Al-Shamma, H.; Gatlin, J.; Le, M.; Gaidarov, I.; Anthony, T.; Unett, D. J.; Blackburn, A.; Rueter, J.; Stirn, S.; Behan, D. P.; Jones, R. P. Discovery of APD334: Design of a clinical stage functional antagonist of the sphingosine-1-phosphate-1 receptor. *ACS Med. Chem. Lett.* **2014**, *5*, 1313−1317.

(26) GSK2018682. Xu, J.; Gray, F.; Henderson, A.; Hicks, K.; Yang, J.; Thompson, P.; Oliver, J. Safety, pharmacokinetics, pharmacodynamics, and bioavailability of GSK2018682, a sphingosine-1-phosphate receptor modulator, in healthy volunteers. *Clinical Pharmacology in Drug Development* **2014**, *3*, 170−178.

DOI: 10.1021/acsmedchemlett.5b00448
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX