Identification and Preclinical Pharmacology of BMS-986104: A Differentiated S1P₁ Receptor Modulator in Clinical Trials

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S 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 S1P₁ 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.

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, S1P₁ 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, S1P₃ 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 S1P₃ does not appear to contribute to efficacy, the identification of S1P₁ agonists sparing of S1P₃ has been a primary emphasis of many research programs in this area.⁷ However, clinical studies with S1P agonists with selectivity for S1P₁ over S1P₃ have suggested that in humans the heart rate reduction effects are controlled at least in part through agonism of S1P₁.⁸ Additionally, through the course of our own studies it was discovered that simply abolishing S1P₃ agonism was not sufficient to eliminate the acute and chronic pulmonary toxicity

elicited in rodents by **1** or by selective S1P₁ full agonists, findings that led us to discontinue our efforts related to S1P₁ full agonists and seek alternative profiles that could overcome these liabilities.⁹ In this letter we describe the identification of a differentiated S1P₁ 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 S1P₁ 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 S1P₁ with activity on S1P₃ 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 S1P₃ receptor and may not be relevant in a clinical setting where S1P₁ 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

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Figure 1. Rationale for the synthesis of 3d.

consequences (in terms of S1P₁/S1P₃ 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

^aReagents and conditions: (a) NaNO₂, H₂SO₄, HOAc, H₂O, 0 °C, 10 min; then KI, H₂O, 0 °C, 30 min, RT, 1 h, 69%. (b) KO^tBu, Et₃B, CH₃(CH₂)₅I, THF, RT, 4 h, 23%. (c) Step 1, Et₃SiH, TFA, 50 °C, 15 h, 95%; step 2, chiral SFC using AD-H column and 10% MeOH in CO₂ eluent. (d) Cyclopent-2-enol, Pd(OAc)₂, Bu₄NCl, KOAc, DMF, 80 °C, 2.5 h, 75%. (e) Step 1, NH₃, NaCN, NH₄Cl, MeOH, RT, 2 days; step 2, HCl, H₂O, HOAc, dioxane, 100 °C, 9 h; step 3, MeOH, SOCl₂, 70 °C, 7 h; 43% for 3 steps. (f) Step 1, NaBH₄, EtOH, DCM, RT, overnight, 100%; step 2, chiral SFC using AD-H column and 12% MeOH in CO₂ with 0.1% DEA eluent; then, chiral SFC using AS-H column and 12% of MeOH–CH₃CN (1:1) in CO₂ 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 work¹⁰ 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 (blq)	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		

^aLC/MS/MS area ratio of phosphorylated material vs nonphosphorylated material after 4 h incubation. ^bCompounds 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 (×10 ³ /mm ³)			5.39			5.86
compound (×10 ³ /mm ³)	4.76	0.54	4.4	0.7	1.2	2.37
% reduction	12%	90%	18%	87%	80%	60%

^aVehicle: PEG300. ^bCompounds 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

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

	vehicle ^b	1 (mg/kg)			3d (mg/kg)		
		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

^aBL = blood lymphocytes ($\times 10^3/\text{mm}^3$); BAL = BAL protein (mg/dL). ^bWater (**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 S1P₃ sparing S1P₁ 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 S1P₁ 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 S1P₁ 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 S1P₁ 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 S1P₁ agonist in the S1P₁ GTP γ S 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 S1P₃ GTP γ S 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 S1P₁ and S1P₃ GTP γ S assays may impact signaling through the S1P₁ and S1P₃ 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

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 GTP γ S (EC ₅₀ , nM), Y _{max} (agonist)	0.377 ± 0.096 (n = 6), 95%	0.901 ± 0.358 (n = 7), 81%
hS1P ₃ GTP γ S (EC ₅₀ , nM)	3.57 ± 2.80 (n = 7),	>1000 (n = 7)
hS1P ₃ GTP γ S (IC ₅₀ , nM), (antagonist)	NA	>1000 (n = 3)
hS1P ₄ GTP γ S (EC ₅₀ , nM)	0.09 (n = 2)	10.5 (n = 2)
hS1P ₅ GTP γ S (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

maintaining some level of S1P/S1P₁ signaling may be crucial for controlling vascular tone.¹³

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 S1P₁ 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

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

parameter	mouse ^a	
	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-last} (μM·h), po	1.0 ^d	4.6 ^d
AUC _{0-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	

^aComposite blood concentration–time profiles were constructed for PK analysis. ^bVehicle: 90% PEG300, 5% ethanol, 5% TPGS (D-α-tocopheryl polyethylene glycol succinate). ^cVehicle: 18.4% hydroxypropyl beta cyclodextrin, 81.6% citrate buffer. ^dThe 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 S1P₃ receptor plays a predominant role in the regulation of heart rate, and as such, compounds that are selective for S1P₁ over S1P₃ show reduced potential for bradycardia in these models. However, selective S1P₁ agonists cause bradycardia in humans, and research suggests that the guinea pig heart is also sensitive to S1P₁-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 S1P₁, 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 S1P₁-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 S1P₁ and S1P₃ 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 S1P₁ 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 S1P₁ 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/acsmmedchemlett.5b00448.

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

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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; GTP γ S, guanosine 5'-O-[gamma-thio]triphosphate; IFN, interferon; RT-PCR, real-time polymerase chain reaction

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