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Discovery of Novel 1,2,4-Thiadiazole Derivatives as Potent, Orally Active Agonists of Sphingosine 1-Phosphate Receptor Subtype 1 ($S1P_1$)

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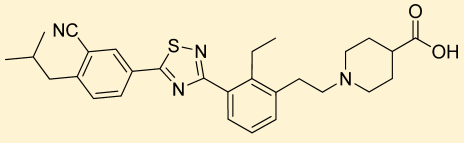
Supporting Information

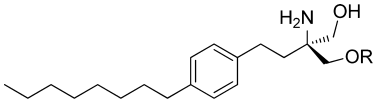
ABSTRACT: A novel series of 1,2,4-thiadiazole compounds was discovered as selective $S1P_1$ agonists. The extensive structure−activity relationship studies for these analogues were reported. Among them, **17g** was identified to show high in vitro potency with reasonable free unbound fraction in plasma (F_u > 0.5%), good brain penetration (BBR > 0.5), and desirable pharmacokinetic properties in mouse and rat. Oral administration of 1 mg/kg **17g** resulted in significant peripheral lymphocytes reduction at 4 h after dose and rapid lymphocytes recovery at 24 h. **17g** showed a transient lymphopenia profile in the repeated dose study in mouse. In addition, **17g** also demonstrated efficacy comparable to that of FTY720 (**1**) in the mouse EAE model of MS.



17g

INTRODUCTION

Multiple sclerosis (MS) is a debilitating, progressive autoimmune disorder in the CNS as a result of inflammation mediated neural cell damage.[1] As the leading cause of neurological disability and impairment in young adults (aged 20−40 years), MS is believed to affect around 2.5 million individuals worldwide.[2,3] Most MS patients (~85%) are diagnosed with the relapsing−remitting form of the disease.[4] Currently, all available drugs on the market are administered by injection except for fingolimod (FTY720, **1**, Figure 1), which was recently approved as the first oral drug for RRMS.[3]



R = H: FTY720 **1**
R = $PO(OH)_2$: FTY720-p **2**

Figure 1. Structures of FTY720 (**1**) and its phosphate (**2**).

The therapeutic activity of **1** could be mainly due to its ability to reduce lymphocyte infiltration into CNS by sequestration of peripheral T-cells and B-cells in secondary lymphoid organs.[3,5] In addition, **1** may act directly on neural cells to enhance remyelination in cerebellar slices with lysolecithin-induced demyelination[6] and to attenuate astrogliosis in animal models of MS.[7] The pharmacological efficacy of **1** is believed to result from the agonism activity of its monophosphate metabolite (FTY720-p, **2**, Figure 1) to $S1P_1$,[8] a G-protein-coupled receptor that regulates peripheral lymphocyte trafficking[9] and affects neural cell survival.[10] $S1P_1$ receptor has then emerged as an attractive molecular target for the discovery of new therapeutic agents for autoimmune diseases.

2 is a nonselective $S1P_1$ agonist over the other S1P receptors ($S1P_3$, $S1P_4$, and $S1P_5$),[8b] and agonism of $S1P_3$ is thought to be related to hemodynamic and pulmonary side effects seen in clinical trials.[11] Thus, clinical compounds were optimized toward $S1P_1$ agonists with increased selectivity over $S1P_3$, including prodrug 2-amino-2-[2-[2-chloro-4-[[3-(phenylmethoxy)phenyl]thio]phenyl]ethyl]-1,3-propanediol hydrochloride (KRP-203),[12] as well as direct $S1P_1$ agonists (*E*)-1-(4-(1-(((4-cyclohexyl-3-(trifluoromethyl)benzyl)oxy)imino)-ethyl)-2-ethylbenzyl)azetidine-3-carboxylic acid (BAF312, proposed structure)[13] and (*Z*,*Z*)-5-[3-chloro-4-((2R)-2,3-dihydroxypropoxy)benzylidene]-2-propylimino-3-*o*-tolylthiazolidin-4-one (ACT128800).[14]

There have been extensive SAR studies on structurally diverse $S1P_1$ receptor agonists reported to date,[15] among which oxadiazole derivatives were demonstrated to be potent and selective $S1P_1$ receptor agonists with good pharmacokinetic properties.[16] Most recently, a novel class of oxadiazole analogues were discovered from this laboratory as selective $S1P_1$ receptor agonists that showed efficacy comparable to that of **1** in the mouse EAE model, an animal model for multiple sclerosis, by oral dosing (**3** as a representative, Figure 2).[17] However, there were several issues associated with this compound that potentially can delay its clinical development. Even though compound **3** showed reasonable CNS penetration (BBR > 0.5), its high protein binding (F_u = 0.04%)[18] indicates a

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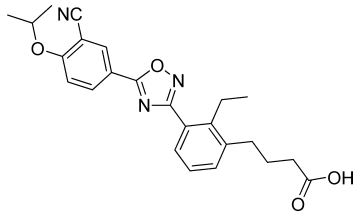


3 pEC_{50} (S1P1): 9.7

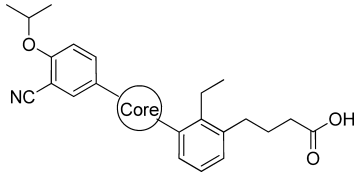
Figure 2. Lead compound **3**.

low free drug concentration in the brain. In addition, the literature-reported opening of the oxadiazole ring under certain circumstances was also a concern to us with regard to further development of compound **3**.[19] Thus, a more stable compound with high CNS penetration balanced with reasonable plasma protein binding should be developed. Herein, we report the discovery of a novel 1,2,4-thiadiazole series as potent, orally active $S1P_1$ agonists with improved plasma protein binding (F_u > 0.5%) and high CNS penetration (BBR > 0.5) for the treatment of MS.

RESULTS AND DISCUSSION

We first turned our attention to the replacement of the oxadiazole core by other aromatics/heteroaromatics to improve its stability. The opening potential of the oxadiazole ring could be represented by its low aromatic index (AI, 39 for oxadiazole and 100 for benzene), an indication of aromaticity of five- or six-membered ring heterocycles.[20] Thus, analogues containing different heterocyclic aromatic cores with higher aromaticity (Table 1, AI > 45) were synthesized and evaluated in the in vitro $S1P_1$ assay. Even though all 21 cores synthesized (**4a**–**u**) showed reduced $S1P_1$ receptor agonism activities in the cell

Table 1. $S1P_1$ Potencies with Different Aromatic/Heteroaromatic Cores



cmpd	core	pEC_{50} ($S1P_1$)[a]	AI[b]
3		9.7	39
4a		8.9	63
4b		8.6	72
4c		8.9	72
4d		8.5	50
4e		8.5	64
4f		8.4	66
4g		8.3	47
4h		6.2	73
4i		5.6	73
4j		<5.0	73

cmpd	core	pEC_{50} ($S1P_1$)[a]	AI[b]
4k		<5.0	64
4l		8.8	100
4m		9.0	84
4n		7.6	84
4o		7.5	86
4p		7.5	86
4q		6.2	79
4r		<5.5	84
4s		<5.5	84
4t		<5.5	84
4u		<5.5	86

[a]Data are the average of at least two determinations. [b]AI data are from ref 20.

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Table 2. Physicochemical and Biological Properties of Compounds 3, 4a, 4b, 4d, 4e, 4f, 4l, and 4m[a]

		pEC$_{50}$								
compd	AI[b]	S1P$_1$	S1P$_3$	cLogP	permeability (nm/s)	solubility (μg/mL)	pIC$_{50}$(P450)	F_u (%) (human)[d]	BBR (1 h)[d]	BBR (4 h)[d]
3	39	9.7	<5	5.1	345	46	all <5[c]	0.04*	0.53	0.68
4a	63	8.9	<5	5	230	256	all <5[c]	0.51	0.21	0.19
4b	72	8.6	<5	5.3	320	160	5.3 (2C9)	0.21	0.36	0.52
4d	50	8.5	<5	4.2	240	187	5.1 (2C9)	0.21*	0.11	0.09
4e	64	8.5	<5	6.1	220	125	5.4 (2C9)	0.10	0.38	0.35
4f	66	8.4	<5	7.3	300	115	5.8 (2C9)	0.02*	1.0	1.5
4l	100	8.8	<5	7.3	310	154	5.4 (2C9)	0.01*	0.93	0.95
4m	84	9	<5	5.1	190	161	5.2 (2D6)	0.19	0.47	0.48

[a]S1P$_1$, S1P$_3$ assay data are the average of at least two determinations. [b]AI data are from ref 20. [c]All >5 except for the five CYP enzymes that were included in the assay panel: 1A2, 2D6, 2C9, 2C19, and 3A4. [d]F_u in human plasma unless otherwise indicated by an asterisk; (*) F_u in rat plasma.

based FRET assay,[21] quite a few of them demonstrated reasonable potency (**4a**−**g**, **4l**, and **4m**) with pEC$_{50}$ > 8.0. We then further evaluated these compounds for their physicochemical and biological properties in order to prioritize the chemical series for further SAR studies (**4g** was excluded because of its low AI).

As shown in Table 2, these compounds should have cores more stable than the 1,2,4-oxadiazole core, as indicated by their AI (all >50, compared to 39 of 1,2,4-oxadiazole). They are all selective S1P$_1$ receptor agonists over S1P$_3$ (pEC$_{50}$ < 5)[22] with good permeability and solubility properties. None of them raised drug−drug interaction concerns, as evidenced by their lack of to low inhibitory activities against CYP enzymes (2C9, 2C19, 2D6, and 3A4). However, the compound with the 1,3,4-oxadiazole core (**4d**) showed low CNS penetration property (mouse, ip dosing) with BBR of 0.11 and 0.09 at 1 and 4 h, respectively. Since the brain exposure is critical for a CNS drug, **4d** is thus deprioritized from further progress. On the other hand, analogues with the thiophene and phenyl cores (**4f** and **4l**) are highly brain penetrable (BBR > 0.9 at both 1 and 4 h), whereas their developability was predicted to be poor (cLogP = 7.3) according to Lipinski's rule.[23] For this reason, **4f** and **4l** were also deprioritized. It is noteworthy that most of the compounds (except for **4a**) showed high protein binding property. We decided to resolve this issue at a later stage, knowing several strategies are available in literature to improve protein binding.[24] For further comparison, the four compounds with the 1,3,4-thiadiazole core (**4a**), the 1,2,4-thiadiazole core (**4b**), the thiazole core (**4e**), and the pyrimidine core (**4m**) were progressed to in vivo studies.

Mouse pharmacokinetic studies for the four compounds revealed a desirable PK profile (Table 3). They all had good oral bioavailability (>75%), moderate to long half-lives (1.5−8.0 h), and moderate to high blood exposure (2.60−11.5 μg·h/mL). Thus, all compounds were suitable for further evaluation in in vivo pharmacological studies. It is well established that S1P$_1$ agonism causes lymphocyte retention in the secondary lymphoid organs, leading to rapid onset of lymphopenia. In the acute mouse lymphopenia model where the number of peripheral blood lymphocytes was measured at 4 and 24 h after a single oral administration (Experimental Section), these compounds (1 mg/kg) caused a pharmacologically relevant lymphocyte reduction to 15−38% of baseline levels at 4 h, along with rapid recovery in lymphocytes counts (88%, 63%, and 103% of baseline lymphocyte levels by 24 h for **4b**, **4e**, and **4m**, respectively; **4a** showed sustained lymphopenia effect with <44% lymphocyte recovery). These compounds were further evaluated in the mouse EAE model.[25] At a daily oral dose of 1 mg/kg starting from disease onset (indicated by a nonzero mean clinical score), compounds **4b** and **4m** demonstrated efficacy comparable to that of **1** (1 mg/kg) (Figure 3b). Compounds **4a** and **4e**, however, showed no/marginal beneficial effect in the EAE model (Figure 3a and Figure 3b). Thus, the 1,2,4-thiadiazole series (**4b**) and the pyrimidine series (**4m**), which demonstrated efficacy in both lymphopenia and EAE models, were selected for further structure−activity relationship studies. In this paper, we will focus on the 1,2,4-thiadiazole series because of space limitation.

Table 3. Mouse Pharmacokinetic and Lymphopenia Studies for Compounds 4a, 4b, 4e, and 4m

compd	mouse PK	lymphocyte counts (relative to baseline)
4a	$T_{1/2}$ = 3.8 h; $F \approx$ 100%	37.9% (4 h),
	AUC$_{0-24h}$ = 5.94 μg·h/mL	43.6% (24 h)
4b	$T_{1/2}$ = 1.5 h; F = 87%	22.7% (4 h),
	AUC$_{0-24h}$ = 2.60 μg·h/mL	87.9% (24 h)
4e	$T_{1/2}$ = 3.6 h; $F \approx$ 100%	15.3% (4 h),
	AUC$_{0-24h}$ = 8.34 μg·h/mL	62.6% (24 h)
4m	$T_{1/2}$ = 8.0 h; F = 78%	34.9% (4 h),
	AUC$_{0-24h}$ = 11.5 μg·h/mL	103.2% (24 h)

We first examined the effects of substitutions on the left-hand side aromatic group of **4b** on the S1P$_1$ receptor potency (**4b**−**5h**, Table 4). Replacements of the cyano group of **4b** with either chlorine (**5a**) or trifluoromethyl (**5b**) substitutions retained potency. The isopropoxyl group could also be replaced by the isobutyl (**5c**) functionality with similar or increased potency. A slight decrease of S1P$_1$ agonist activity was observed once an N atom was introduced to the phenyl ring (**5d**). The replacement of the isopropoxyl functionality was tolerated with the phenyl ring (**5h**) or an electron-donating piperidine group (**5g**), compared to **5b**. However, when more heteroatoms (O or N) were introduced to the piperidine ring, a significant decrease of S1P$_1$ potency was observed (**5e** and **5f**). SAR for substitutions on the left-hand aromatic ring suggested that isopropoxyl and isobutyl groups are preferred at the para position and cyano, chloro, and trifluoromethyl functionalities are preferred at the meta position. As mentioned previously, one problem of the 1,2,4-thiadiazole series is their high plasma protein binding (F_u of 0−0.32% for **4b**−**5h**), which may contribute to the disconnection between the in vitro potency and the in vivo efficacy. A well-established solution to improve the protein binding is to introduce polarity to the molecule. We first tried to introduce an N atom to the right-hand aromatic ring (**5i**−**l**, Table 4). However, this led to a significant decrease

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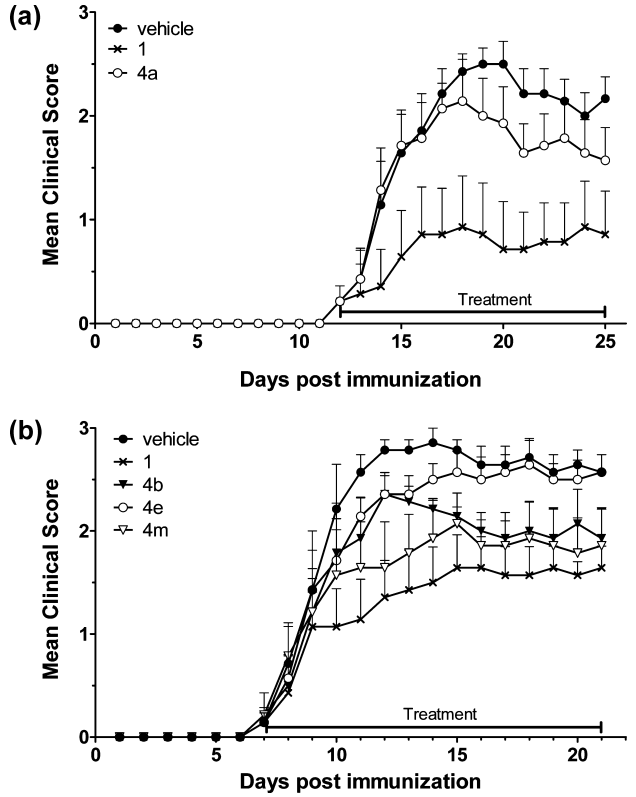


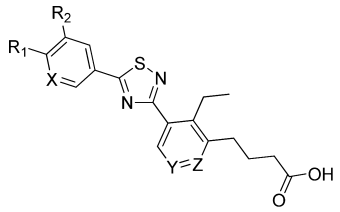
Figure 3. (a) Treatment efficacy of compound **4a** in mouse EAE. (b) Treatment efficacy of compounds **4b**, **4e**, and **4m** in mouse EAE.

in the $S1P_1$ receptor potency ($S1P_1$ pEC_{50} of 6.4−7.6) without any significant improvement in plasma protein binding (F_u of 0.12−0.19% for **5i**−**l**). We then turned our attention to introduce polarity (such as N) to the carboxylic side chain for better potency and plasma protein binding.

In order to efficiently explore the SAR of the carboxylic acid side chain, convergent syntheses of the general structures **12** and **17** were developed, where the diversity (amino acid side chains) was introduced at the last step (Scheme 1). Suzuki coupling of the commercially available starting material 5-chloro-3-bromo-1,2,4-thiadiazole (**6**) with boronic ester **7** provided diaryl bromide **8**. The second Suzuki coupling of **8** with boronic ester **9** afforded aldehyde **10**. The final step of the reductive amination with different amino acids **11** gave benzylic amino acid analogues **12** with generally acceptable yield. For the synthesis of homobenzylic amino acid derivatives, the boronic ester for the second Suzuki coupling is methyl enoether **13**. After acid catalyzed hydrolysis of the methyl enoether **14**, aldehyde **15** was obtained with a moderate to good yield. Reductive amination then afforded homobenzylic amino acid derivatives **17**.

Having developed a robust synthetic procedure for various amino acid analogues, we started to investigate the right side of SAR. Trifluoromethyl substitution on the left-hand phenyl ring was our first focus (**12a**−**d** and **17a**−**d**) because of the higher potency. As shown in Table 5, straight chain benzylic amino acid derivatives (**12a** and **12b**) showed moderate $S1P_1$ agonist activities. Extending the side chain by one carbon to homobenzylic amino acid analogues provided much improved potency (**17a** and **17b**). Cyclic amino acids resulted in either similar or higher potency (**12c** and **12d**, respectively). Further extending the side chain to their homobenzylic analogues led to

Table 4. SAR of the Left-Hand and Right-Hand Aromatic Rings and the Human Plasma Protein Binding Data Measured as F_u



cmpd	R_1	R_2	X	Y	Z	pEC_{50} ($S1P_1$)[a]	F_u (%) (human)[b]
4b	i-PrO	CN	CH	CH	CH	8.6	0.21
5a	i-PrO	Cl	CH	CH	CH	8.4	0.15
5b	i-PrO	CF_3	CH	CH	CH	8.7	0.014
5c	i-Bu	CN	CH	CH	CH	8.9	0.03
5d	i-PrO	CF_3	N	CH	CH	8.3	0.031
5e		CF_3	CH	CH	CH	<5.5	0.32*
5f		CF_3	CH	CH	CH	6.9	0.06*
5g		CF_3	CH	CH	CH	8.7	0.001
5h		CF_3	CH	CH	CH	8.3	0*
5i	i-PrO	CF_3	CH	N	CH	7.6	0.12
5j	i-PrO	Cl	CH	N	CH	6.6	0.19
5k	i-Bu	CN	CH	N	CH	6.4	0.14
5l	i-Bu	CN	CH	CH	N	6.8	0.19

[a]Data are the average of at least two determinations. [b]F_u in human plasma unless otherwise indicated by an asterisk; (*) F_u in rat plasma.

a substantial increase of activity (piperidine analogue **17c**) or a similar activity (azetidine analogue **17d**). However, these compounds (**12a**−**d** and **17a**−**d**) still showed high protein binding except for **12a**. Apparently, a further lipophilicity reduction of these compounds is needed. We then turned our attention to replace the trifluoromethyl group with less lipophilic Cl and CN. As the straight chain amino acid derivatives provided limited exposure probably because of high clearance, the cyclic amino acid analogues were chosen to be explored. As expected, replacement of the trifluoromethyl group with Cl (**12e**, **17e**, and **17f**) or CN (**12f**, **17g**, and **17h**) resulted in much improved protein binding property with F_u at 0.45−1.28%. In addition, all of them showed excellent $S1P_1$ potency (pEC_{50} > 9.0) except for the two benzylic azetidine derivatives (**12e** and **12f**). The carboxylic acid functionality proved to be critical for $S1P_1$ activity. Replacement or removal of the carboxylic acid resulted in dramatic decrease in potency (**17i** and **17j**). All compounds (**12a**−**f** and **17a**−**h**) proved to be selective $S1P_1$ receptor agonists versus $S1P_3$ (>1000 fold).

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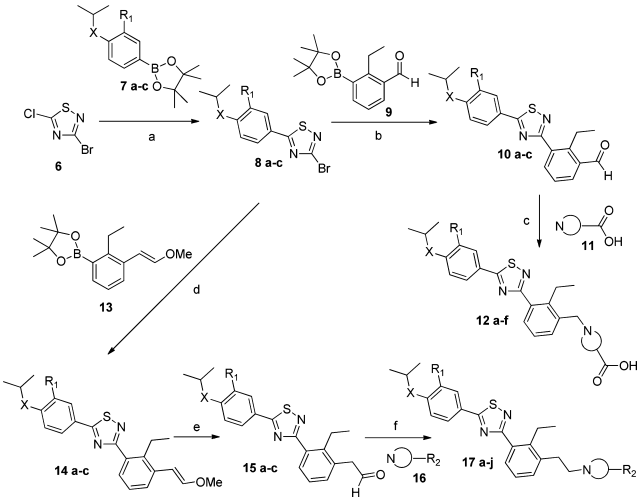
Scheme 1. General Synthetic Procedure for the 1,2,4-Thiadiazole Amino Acid Derivatives 12 and 17[a]



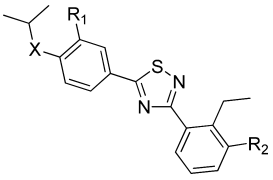
[a](a) **7**, $PdCl_2(dppf)$, K_3PO_4, 53−97%. (b) **9**, $Pd(PPh_3)_4$, K_3PO_4, 77−94%. (c) **11**, $NaBH(OAc)_3$, HOAc, 10−60%. (d) **13**, $Pd(PPh_3)_4$, K_3PO_4, 65−98%. (e) HCl, 80−100%. (f) **16**, $NaBH(OAc)_3$, HOAc, 10−60%.

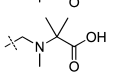
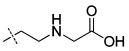
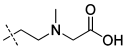
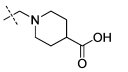
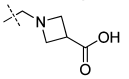
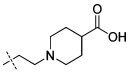
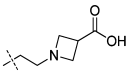
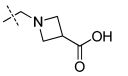
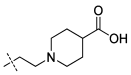
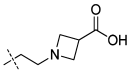
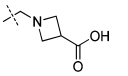
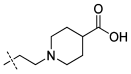
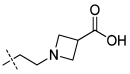
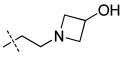
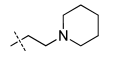
Among these compounds, **17e** and **17g** demonstrated good overall balanced profile ($S1P_1$ pEC_{50} > 9.0; F_u > 0.4%; BBR > 0.5, Table 6) and were then selected for further in vitro and in vivo evaluation.

In a mouse CNS penetration study, both **17e** and **17g** showed good BBR (1.49 and 0.68, respectively, at 4 h after dosing; Table 6). Mouse PK studies revealed that both compounds had decent oral bioavailability (~40%) and moderate to long half-lives (15.6 and 5.3 h for **17e** and **17g**, respectively). In rat, **17e** also showed a much longer half-life (27.7 h) than **17g** (6.2 h). Again, both compounds demonstrated good bioavailability (>50%) in rat. Encouraged by their mouse and rat PK profile, we evaluated both compounds in in vivo efficacy studies. In the acute mouse lymphopenia model, both compounds turned out to be efficacious at 1 mg/kg (62−83% lymphocyte reduction at 4 h). On the basis of the lymphocyte counts measured by 24 h, compound **17g** (72% of baseline at 24 h) had a transient effect whereas compound **17e** resulted in a sustained lymphopenia profile (only 40% of baseline at 24 h). Thus, duration of the PD effect produced by these compounds seemed to correlate with their half-lives. As expected, both compounds demonstrated good stability and no glutathione (GSH) adducts were observed in the GSH conjugation study. Neither compound showed inhibitory activity in the human CYP enzymes (1A2, 2D6, 2C9, 2C19, and 3A4), indicating that there is no drug−drug interaction concern. They were both progressed to the chronic mouse EAE evaluation. To our delight, both compounds were efficacious at a daily oral dose of 1 mg/kg (Figure 4a and 4b).

Compound **17g** was further progressed in both single and repeat dosing lymphopenia studies to assess its PK−PD relationship to facilitate human dose selection. The single dose rat PK−PD study demonstrated a dose-dependent lymphocyte reduction and later on reversal over a 60 h period (Figure 5a), with a calculated IC_{50} of 12 ng/mL. In addition, the repeat-dose study showed that at the EAE-efficacious dose of 1 mg/kg, compound **17g** rapidly produced a daily lymphocyte reduction at the range 20−60% of baseline over the treatment period (Figure 5b). Perhaps more important,

Table 5. SAR for Amino Acid Side Chains[a]



cmpd	X	R_1	R_2	pEC_{50} ($S1P_1$)	pEC50 ($S1P_3$)	F_u (%) (human)[b]
12a	O	CF_3		8.0	<5	0.49
12b	O	CF_3		7.0	<5	0.004
17a	O	CF_3		10.8	<5.5	0.13
17b	O	CF_3		10.7	<5.5	0.21
12c	O	CF_3		7.8	<5	0.38*
12d	O	CF_3		9.6	5.6	0.26
17c	O	CF_3		10.0	5.6	0.17
17d	O	CF_3		9.6	5.5	0.25
12e	O	Cl		8.4	<5.5	0.96
17e	O	Cl		9.8	<5.5	0.45
17f	O	Cl		9.6	<5.5	0.67
12f	CH_2	CN		8.1	<5.5	1.28
17g	CH_2	CN		9.1	<5.5	0.64
17h	CH_2	CN		9.1	5.6	0.71
17i	CH_2	CN		6.7	<5.5	0.091
17j	CH_2	CN		5.8	<5	0.017

[a]$S1P_1$, $S1P_3$ assay data are the average of at least two determinations. [b]F_u in human plasma unless otherwise indicated by an asterisk; (*) F_u in rat plasma.

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Table 6. In Vitro and in Vivo Data for 17e and 17g

	17e	17g
BBR[a]	0.52 (1 h)	0.23 (1 h)
	1.48 (4 h)	0.65 (4 h)
mouse PK	F_{po} = 40%	F_{po} = 45%
	$T_{1/2}$ = 15.6 h	$T_{1/2}$ = 5.3 h
rat PK[b]	F_{po} = 70%	F_{po} = 50%
	$T_{1/2}$ = 27.7 h	$T_{1/2}$ = 6.2 h
lymphopenia[a]	16% (4 h),	23% (4 h),
	40% (24 h)	72% (24 h)
CYP (1A2, 2D6, 2C9, 2C19, 3A4)	all pIC_{50} < 5	all pIC_{50} < 5
GSH conjugates	not observed	not observed

[a]See Experimental Section. [b]Male Sprague–Dawley (for iv, 1 mg/kg, 1%DMSO in 10% HP-β-CD (w/v), N = 3; for po, 2 mg/kg, 1% DMSO in 1% MC (w/v), N = 3).

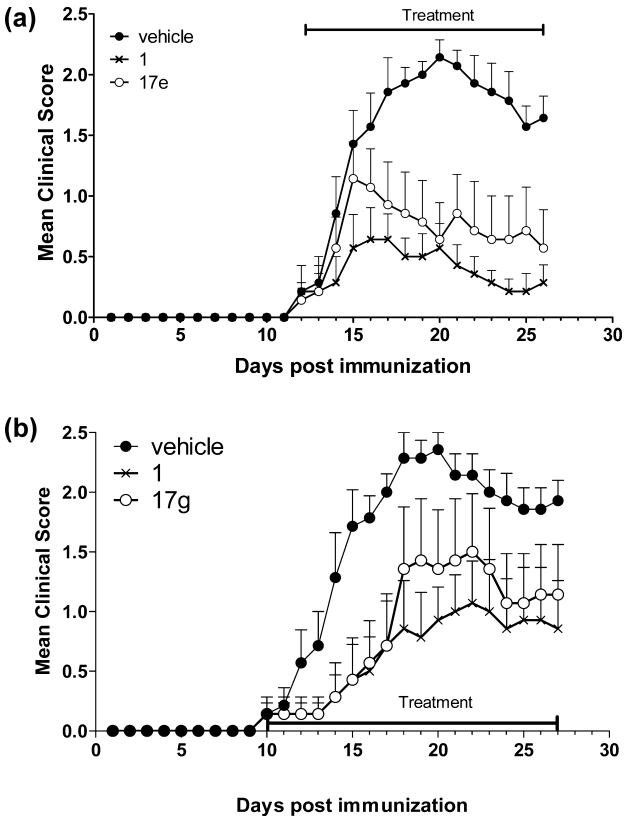

Figure 4. (a) Treatment efficacy of compound **17e** in mouse EAE. (b) Treatment efficacy of compound **17g** in mouse EAE.

lymphocyte counts recovered to normal range (~70% of baseline) 3 days after treatment cessation.

CONCLUSION

We have discovered a novel 1,2,4-thiadiazole series of compounds as selective $S1P_1$ agonists. Extensive SAR studies led to the discovery of compound **17g**, a potent $S1P_1$ agonist (pEC_{50} > 9) with reasonable F_u in plasma (0.64%), good brain penetration (BBR > 0.5), and desirable PK properties in rodent species. Oral administration of 1 mg/kg of **17g** resulted in significant peripheral lymphocyte reduction at 4 h after dose and rapid lymphocyte recovery at 24 h. A transient lymphopenia profile for **17g** was observed in the repeat dosing lymphopenia study. In addition, **17g** demonstrated comparable efficacy to **1** in the mouse EAE model of MS.

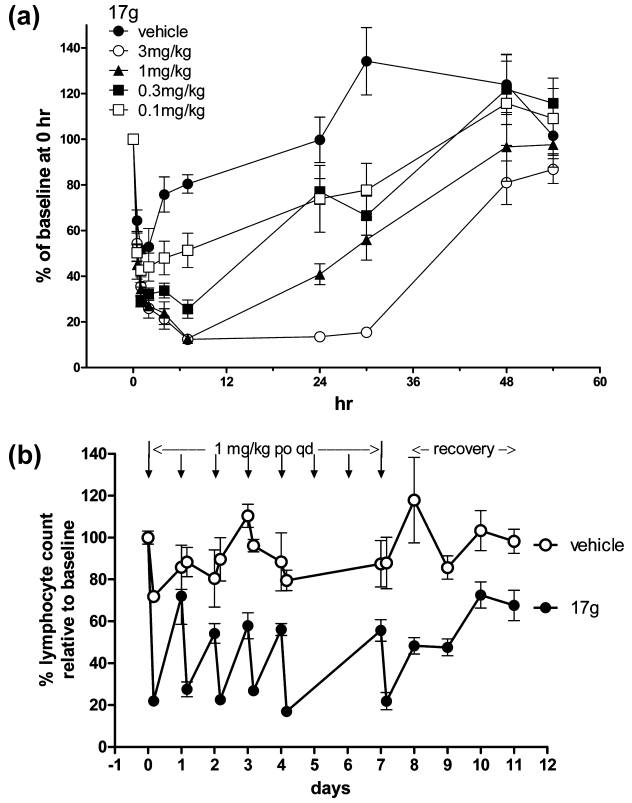

Figure 5. (a) Dose-dependent lymphopenia produced by single ascending dose of compound **17g**. (b) Chronic lymphopenia elicited by repeat dosing of compound **17g**.

EXPERIMENTAL SECTION

Chemistry. Compounds not described below were purchased from commercial vendors or as previously reported. Compound purity was determined using LC/MS analysis. Purification of the compounds was carried out by conventional methods such as chromatography and/or recrystallization using suitable solvents. Chromatographic methods include column chromatography, flash chromatography, and MDAP (mass directed autopurification system).

^{1}H NMR spectral data were recorded on a Bruker 400 NMR spectrometer operating at 400 MHz. ^{13}C NMR spectra were recorded at 100 MHz. $CDCl_3$ is deuteriochloroform, and DMSO-d_6 is hexadeuteriodimethylsulfoxide. Chemical shifts are given in parts per million (δ) downfield from the internal standard tetramethylsilane (TMS) or the NMR solvent. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in hertz. High resolution mass spectrometry (HRMS) was operated in positive mode of electrospray ionization (ESI) at an orthogonal acceleration time-of-flight (oa-TOF) SYNAPT G2 HDMSTM (Waters, Manchester, U.K.). Solutions (500 ng/mL in ACN/H_2O (1:1, v/v)) were introduced via infusion at a flow rate of 5 μL/min to acquire accurate mass. LC/MS (Agilent 1200SL-6110) analysis was conducted for all assayed compounds in either acidic or basic conditions. (1) Acidic conditions refer to water containing 0.05% TFA/acetonitrile as mobile phase on an Agilent SB-C18 column (1.8 μm, 4.6 mm × 30 mm) with MS instrument and photodiode array detector. The following conditions were used: a gradient from 5% to 95% in 4 min (or 6 min) and held at 95% for 1 min; UV detection at 214 and 254 nm; a flow rate of 1.5 mL/min; full scan; mass range from 100 to 1000 amu. (2) Basic conditions refer to water containing 10 mM aqueous NH_4HCO_3/acetonitrile as mobile phase on a Waters XBridge C18 column (3.5 μm, 4.6 mm × 50 mm) with MS instrument and photodiode array detector. The following conditions were used: a gradient from 5% to

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95% in 5 min and held at 95% for 1 min; UV detection at 214 and 254 nm; a flow rate of 2 mL/min; full scan; mass range from 100 to 1000 amu. All the assayed compounds possess ≥95% purity determined using LC/MS analysis. Column chromatography was performed on Isco or Biotage instrument using a prepacked silica gel column, a detector with UV wavelength at 254 nm, and mixed solvents. MDAP equipped with 2489 UV detector, 2767 sample manager, 2545 pump, and 3100 single quadrupole mass spectrometer was performed on Sunfire Prep C18 column (5 μm, 19 mm × 50 mm) using water containing 0.05% TFA/acetonitrile as mobile phase. The following conditions were used: a gradient from 5% to 95% in 8 min and held in 95% for 2 min; a flow rate of 30 mL/min.

3-Bromo-5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (8a). To a solution of 2-(3-chloro-4-isopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **7a**[26] (500 mg, 1.51 mmol), 3-bromo-5-chloro-1,2,4-thiadiazole **6** (604 mg, 3.03 mmol), and tripotassium phosphate (964 mg, 4.54 mmol) in DMF (3 mL) and water (0.6 mL) was added $PdCl_2(dppf)-CH_2Cl_2$ adduct (618 mg, 0.76 mmol). The vessel was sealed and heated in a microwave reactor at 80 °C for 1 h. After cooling, the mixture was concentrated in vacuo, and the residue was purified by chromatography on silica gel with petroleum/ethyl acetate (4:1) to give 294 mg (53%) of **8a** as a white solid. ESMS *m/z*: 366.9 $(M + H)^+$.

3-Bromo-5-(3-chloro-4-isopropoxyphenyl)-1,2,4-thiadiazole (8b). Compound **8b** (6.2 g, 66%) was prepared from 2-(3-chloro-4-isopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **7b**[26] (6 g, 28 mmol) and **6** (11.2 g, 56 mmol) in the same manner as described for **8a**. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.44 (d, *J* = 6.40 Hz, 6 H), 4.69 (dt, *J* = 12.05, 6.40 Hz, 1 H), 7.01 (d, *J* = 8.66 Hz, 1 H), 7.78 (dd, *J* = 8.28, 2.26 Hz, 1 H), 7.99 (d, *J* = 2.26 Hz, 1 H). ESMS *m/z*: 333.0 $(M + H)^+$.

5-(3-Bromo-1,2,4-thiadiazol-5-yl)-2-isobutylbenzonitrile (8c). Compound **8c** (1.14 g, 88%) was prepared from 2-(2-methylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-benzonitrile **7c**[26] (1 g, 3.51 mmol) and **6** (1.399 g, 7.01 mmol) in the same manner as described for **8a**. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.00 (d, *J* = 6.78 Hz, 6 H), 2.01–2.10 (m, 1 H), 2.81 (d, *J* = 7.53 Hz, 2 H), 7.47 (d, *J* = 8.28 Hz, 1 H), 8.06 (dd, *J* = 8.28, 1.88 Hz, 1 H), 8.23 (d, *J* = 1.88 Hz, 1 H). ESMS *m/z*: 321.9 $(M + H)^+$.

2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)benzaldehyde (10a). To a solution of **8a** (0.93 g, 2.53 mmol) in DMF (6 mL) and water (1.5 mL) were added 2-ethyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde **9**[17] (0.659 g, 2.53 mmol), tripotassium phosphate (1.344 g, 6.33 mmol), and $Pd(Ph_3P)_4$ (0.585 g, 0.507 mmol). The reaction vessel was sealed and heated with a microwave reactor at 120 °C for 15 min. After cooling, the reaction mixture was filtered and the solid was washed with ethyl acetate (2 × 15 mL). The filtrate and the washing solution were combined. The combined solution was washed with water (2 × 20 mL), then dried over anhydrous sodium sulfate. The dried solution was concentrated in vacuo, and the residue was purified by chromatography on silica gel with petroleum/ethyl acetate (9:1) to give 1 g (94%) of **10a** as a brown oil. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (t, *J* = 7.40 Hz, 3 H), 1.44 (d, *J* = 6.02 Hz, 6 H), 3.39 (q, *J* = 7.40 Hz, 2 H), 4.78 (dt, *J* = 12.11, 6.02 Hz, 1 H), 7.14 (d, *J* = 8.78 Hz, 1 H), 7.50 (t, *J* = 7.78 Hz, 1 H), 8.02 (dd, *J* = 7.78, 1.51 Hz, 1 H), 8.12–8.17 (m, 2 H), 8.24 (d, *J* = 2.01 Hz, 1 H), 10.48 (s, 1 H). ESMS *m/z*: 421.1 $(M + H)^+$.

3-(5-(3-Chloro-4-isopropoxyphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylbenzaldehyde (10b). Compound **10b** (450 mg, 78%) was prepared from **8b** (500 mg, 1.51 mmol) and **9** (390 mg, 1.51 mmol) in the same manner as described for **10a**. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (t, *J* = 7.40 Hz, 3 H), 1.45 (d, *J* = 6.02 Hz, 6 H), 3.38 (q, *J* = 7.40 Hz, 2 H), 4.71 (dt, *J* = 12.11, 6.02 Hz, 1 H), 7.05 (d, *J* = 8.78 Hz, 1 H), 7.49 (t, *J* = 7.65 Hz, 1 H), 7.88 (dd, *J* = 8.53, 2.26 Hz, 1 H), 8.01 (dd, *J* = 7.65, 1.38 Hz, 1 H), 8.08 (d, *J* = 2.01 Hz, 1 H), 8.14 (dd, *J* = 7.78, 1.51 Hz, 1 H), 10.48 (s, 1 H). ESMS *m/z*: 387.1 $(M + H)^+$.

5-(3-(2-Ethyl-3-formylphenyl)-1,2,4-thiadiazol-5-yl)-2-isobutylbenzonitrile (10c). Compound **10c** (520 mg, 89%) was prepared from **8c** (500 mg, 1.55 mmol) and **9** (424 mg, 1.63 mmol) in the same manner as described for **10a**. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 1.01 (d, *J* = 6.78 Hz, 6 H), 1.38 (t, *J* = 7.34 Hz, 3 H), 2.03–2.10 (m, 1 H), 2.82 (d, *J* = 7.53 Hz, 2 H), 3.40 (q, *J* = 7.34 Hz, 2 H), 7.47–7.54 (m, 2 H), 8.04 (d, *J* = 7.53 Hz, 1 H), 8.12–8.19 (m, 2 H), 8.31 (d, *J* = 1.88 Hz, 1 H), 10.48 (s, 1 H). ESMS *m/z*: 376.1 $(M + H)^+$.

2-((2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)benzyl)(methyl)amino)acetic Acid (12a). To a solution of **10a** (80 mg, 0.19 mmol) in DCM (6 mL) were added *N*-methylglycine (51 mg, 0.57 mmol) and acetic acid (0.022 mL, 0.38 mmol). The reaction solution was stirred at room temperature overnight. Sodium triacetoxyborohydride (81 mg, 0.38 mmol) was added, and stirring continued for another 2 h. The reaction mixture was concentrated in vacuo, and the residue was purified by MDAP to give 35 mg (30%) of **12a** as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 (t, *J* = 7.28 Hz, 3 H), 1.34 (d, *J* = 6.02 Hz, 6 H), 2.74 (s, 3 H), 3.08 (q, *J* = 7.28 Hz, 2 H), 4.11 (s, 2 H), 4.42 (s, 2 H), 4.96 (dt, *J* = 11.98, 6.02 Hz, 1 H), 7.47 (t, *J* = 7.65 Hz, 1 H), 7.54 (d, *J* = 8.78 Hz, 1 H), 7.68 (d, *J* = 7.28 Hz, 1 H), 7.90 (d, *J* = 7.28 Hz, 1 H), 8.26 (d, *J* = 2.26 Hz, 1 H), 8.33 (dd, *J* = 8.66, 2.13 Hz, 1 H). HRMS $C_{24}H_{27}N_3O_3F_3S$ $(M + H)^+$ calcd 494.1725, found 494.1728. LC/MS: t_R = 4.23 min, >95%, *m/z* 494.2 $(M + H)^+$.

2-((2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)benzyl)(methyl)amino)-2-methylpropanoic Acid (12b). To a solution of **10a** (650 mg, 1.55 mmol) in MeOH (10 mL) at 0 °C was added sodium borohydride (117 mg, 3.09 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in ethyl acetate (50 mL). The solution was washed with water and dried over anhydrous sodium sulfate. The dried solution was concentrated in vacuo to give the crude alcohol. The alcohol was dissolved in DCM (10 mL), and perbromomethane (615 mg, 1.86 mmol) was added at 0 °C, followed by a solution of triphenylphosphine (608 mg, 2.32 mmol) in DCM (10 mL). The reaction solution was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residue was purified by chromatography on silica gel with petroleum/ethyl acetate (4:1) to give 400 mg (53%) of 3-(3-(bromomethyl)-2-ethylphenyl)-5-(4-isopropoxy-3-(trifluoromethyl)-phenyl)-1,2,4-thiadiazole. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (t, *J* = 7.40 Hz, 3 H), 1.44 (d, *J* = 6.02 Hz, 6 H), 3.14 (q, *J* = 7.40 Hz, 2 H), 4.67 (s, 2 H), 4.77 (dt, *J* = 12.11, 6.02 Hz, 1 H), 7.13 (d, *J* = 8.78 Hz, 1 H), 7.30–7.36 (m, 1 H), 7.50 (dd, *J* = 7.53, 1.25 Hz, 1 H), 7.90 (dd, *J* = 7.78, 1.25 Hz, 1 H), 8.14 (dd, *J* = 8.66, 2.13 Hz, 1 H), 8.24 (d, *J* = 2.01 Hz, 1 H). ESMS *m/z*: 485.0 $(M + H)^+$.

To a solution of the above bromide (100 mg, 0.21 mmol) in DMF (5 mL) were added potassium carbonate (85 mg, 0.62 mmol) and 2-methyl-2-(methylamino)propanoic acid (29.0 mg, 0.25 mmol). The reaction mixture was heated at 60 °C overnight. After cooling, the reaction mixture was diluted with ethyl acetate (20 mL). The organic fraction was separated, washed with water, concentrated, and purified by MDAP to give 27 mg (21%) of **12b** as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 1.16 (t, *J* = 7.28 Hz, 3 H), 1.34 (d, *J* = 6.02 Hz, 6 H), 1.51 (s, 6 H), 2.58 (s, 3 H), 2.99 (q, *J* = 7.28 Hz, 2 H), 4.96 (dt, *J* = 11.98, 6.02 Hz, 1 H), 5.42 (s, 2 H), 7.42 (t, *J* = 7.65 Hz, 1 H), 7.53 (d, *J* = 9.03 Hz, 1 H), 7.60 (d, *J* = 7.53 Hz, 1 H), 7.89 (d, *J* = 7.53 Hz, 1 H), 8.26 (s, 1 H), 8.29–8.37 (m, 1 H), 9.31 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.4, 173.5, 170.7, 158.5, 158.1, 157.8, 142.2, 133.8, 133.7, 132.8, 131.6, 125.9, 121.7, 118.8, 118.5, 115.8, 71.7, 65.8, 60.9, 27.6, 22.2, 21.5, 21.1, 15.5. HRMS $C_{26}H_{31}N_3O_3F_3S$ $(M + H)^+$ calcd 522.2038, found 522.2048. LC/MS: t_R = 4.67 min, >95%, *m/z* 522.2 $(M + H)^+$.

1-(2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)benzyl)piperidine-4-carboxylic Acid (12c). To a solution of **10a** (80 mg, 0.19 mmol) in DCM (5 mL) and MeOH (5 mL) were added ethyl 4-piperidinecarboxylate (90 mg, 0.57 mmol) and acetic acid (0.022 mL, 0.38 mmol). The reaction solution was stirred at room temperature overnight. Sodium triacetoxyborohydride (81 mg, 0.381 mmol) was added, and stirring continued for another 2 h. After removal of the solvent, the residue was dissolved in ethyl acetate (50 mL). The organic fraction was separated, washed with water (2 × 10 mL), and concentrated to afford the ester. The ester was

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dissolved in isopropanol (5 mL) and water (5 mL), and NaOH (0.5 M in water) (1.90 mL, 0.95 mmol) was added. The reaction solution was heated at 90 °C for 2 h. After removal of the solvent, the residue was acidified to pH 3−4, extracted with ethyl acetate (2 × 20 mL). The combined organic phases were dried over sodium sulfate and concentrated, and the residue was purified by MDAP to give 44 mg (36%) **12c** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.08 (t, J = 7.40 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 1.77 (q, J = 12.30 Hz, 2 H), 2.08 (d, J = 13.30 Hz, 2 H), 3.04 (q, J = 6.78 Hz, 2 H), 3.12−3.26 (m, 2 H), 3.41−3.50 (m, 3 H), 4.38−4.54 (m, 2 H), 4.96 (dt, J = 12.05, 6.02 Hz, 1 H), 7.44−7.57 (m, 2 H), 7.71 (d, J = 7.53 Hz, 1 H), 7.91 (d, J = 7.53 Hz, 1 H), 8.26 (d, J = 2.01 Hz, 1 H), 8.33 (dd, J = 8.78, 2.01 Hz, 1 H), 9.24 (br s, 1 H), 12.58 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.5, 174.6, 173.4, 158.5, 158.1, 143.7, 133.7, 133.3, 132.4, 126.4, 125.8, 124.5, 121.7, 118.9, 118.5, 115.8, 71.8, 56.1, 51.2, 38.0, 25.3, 22.7, 21.5, 16.0. HRMS $C_{27}H_{31}N_3O_3F_3S$ (M + H)$^+$ calcd 534.2038, found 534.2041. LC/MS: t_R = 4.26 min, >95%, m/z 534.3 (M + H)$^+$.

1-(2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)benzyl)azetidine-3-carboxylic Acid (12d). Compound **12d** (44 mg, 37%) was prepared from **10a** (100 mg, 0.24 mmol) and methyl azetidine-3-carboxylate, HCl salt (72 mg, 0.48 mmol) in the same manner as described for **12c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.12 (t, J = 7.40 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 2.93 (q, J = 7.40 Hz, 2 H), 3.18−3.28 (m, 3 H), 3.42 (t, J = 6.65 Hz, 2 H), 3.66 (s, 2 H), 4.95 (dt, J = 12.05, 6.02 Hz, 1 H), 7.30 (t, J = 7.65 Hz, 1 H), 7.45 (d, J = 6.53 Hz, 1 H), 7.52 (d, J = 8.78 Hz, 1 H), 7.67−7.73 (m, 1 H), 8.24 (d, J = 2.01 Hz, 1 H), 8.31 (dd, J = 8.78, 2.01 Hz, 1 H). LC/MS: t_R = 4.27 min, >95%, m/z 506.1 (M + H)$^+$.

1-(3-(5-(3-Chloro-4-isopropoxyphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylbenzyl)azetidine-3-carboxylic Acid (12e). Compound **12e** (40 mg, 33%) was prepared from **10b** (100 mg, 0.26 mmol) and methyl azetidine-3-carboxylate, HCl salt (59 mg, 0.39 mmol) in the same manner as described for **12c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.11 (t, J = 7.28 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 2.93 (q, J = 7.28 Hz, 2 H), 3.19−3.28 (m, 3 H), 3.42 (t, J = 6.53 Hz, 2 H), 3.67 (s, 2 H), 4.85 (dt, J = 12.05, 6.02 Hz, 1 H), 7.26−7.32 (m, 1 H), 7.37 (d, J = 8.78 Hz, 1 H), 7.44 (d, J = 7.28 Hz, 1 H), 7.71 (d, J = 7.03 Hz, 1 H), 8.00 (dd, J = 8.66, 2.13 Hz, 1 H), 8.13 (d, J = 2.01 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.2, 174.3, 174.1, 156.0, 141.7, 136.8, 132.6, 130.7, 129.7, 128.8, 127.9, 125.6, 123.1, 123.0, 115.5, 71.6, 60.4, 56.6, 33.6, 22.0, 21.7, 15.3. HRMS $C_{24}H_{27}N_3O_3SCl$ (M + H)$^+$ calcd 472.1462, found 472.1462. LC/MS: t_R = 4.04 min, >95%, m/z 472.2 (M + H)$^+$.

1-(3-(5-(3-Cyano-4-isobutylphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylbenzyl)azetidine-3-carboxylic Acid (12f). Compound **12f** (51 mg, 33%) was prepared from **10c** (100 mg, 0.27 mmol) and methyl azetidine-3-carboxylate, HCl salt (42 mg, 0.28 mmol) in the same manner as described for **12c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (d, J = 6.78 Hz, 6 H), 1.10 (t, J = 7.40 Hz, 3 H), 1.99 (dt, J = 13.49, 6.78 Hz, 1 H), 2.78 (d, J = 7.28 Hz, 2 H), 3.00 (q, J = 7.03 Hz, 2 H), 3.66 (quin, J = 8.28 Hz, 1 H), 4.19−4.38 (m, 4 H), 4.58 (s, 2 H), 7.46 (t, J = 7.65 Hz, 1 H), 7.58 (d, J = 7.53 Hz, 1 H), 7.70 (d, J = 8.03 Hz, 1 H), 7.90 (d, J = 7.28 Hz, 1 H), 8.33 (dd, J = 8.16, 1.88 Hz, 1 H), 8.54 (d, J = 1.76 Hz, 1 H), 10.31 (br s, 1 H), 13.19 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.1, 173.6, 158.1, 149.0, 142.7, 133.1, 132.0, 131.8, 131.5, 128.5, 126.4, 117.2, 113.2, 55.6, 54.4, 42.7, 32.3, 29.7, 22.4, 22.0, 15.7. HRMS $C_{26}H_{29}N_4O_2S$ (M + H)$^+$ calcd 461.2011, found 461.2011. LC/MS: t_R = 4.11 min, >95%, m/z 461.1 (M + H)$^+$.

3-(2-Ethyl-3-(2-methoxyvinyl)phenyl)-5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (14a). Compound **14a** (580 mg, 98%) was prepared from **8a** (487 mg, 1.33 mmol) and 2-(2-ethyl-3-(2-methoxyvinyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **13**[26] (573 mg, 1.99 mmol) in the same manner as described for **10a** as a colorless oil except that the reaction time was 10 min. ESMS m/z: 449.1 (M + H)$^+$.

5-(3-Chloro-4-isopropoxyphenyl)-3-(2-ethyl-3-(2-methoxyvinyl)phenyl)-1,2,4-thiadiazole (14b). Compound **14b** (313 mg, 65%) was prepared from **8b** (388 mg, 1.16 mmol) and **13** (335 mg, 1.16 mmol) in the same manner as described for **10a** as a colorless oil except that the reaction time was 10 min. ESMS m/z: 415.1 (M + H)$^+$.

5-(3-(2-Ethyl-3-(2-methoxyvinyl)phenyl)-1,2,4-thiadiazol-5-yl)-2-isobutylbenzonitrile (14c). Compound **14c** (928 mg, 66%) was prepared from **8c** (1.13 g, 3.51 mmol) and **13** (1.11 g, 3.86 mmol) in the same manner as described for **10a** as an oil except that the reaction temperature was 110 °C. ESMS m/z: 404.2 (M + H)$^+$.

2-(2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)phenyl)acetaldehyde (15a). To a solution of **14a** (140 mg, 0.31 mmol) in THF (10 mL) under nitrogen was added HCl (0.2 mL, 0.40 mmol). The reaction mixture was heated at 70 °C for 5 h. After the mixture was cooled, the solvent was concentrated under reduced pressure. The remaining trace hydrochloric acid was removed under high vacuum for 2 h to give 108 mg (80%) of **15a**, which was used for the following step without further purification. ESMS m/z: 435.2 (M + H)$^+$.

2-(3-(5-(3-Chloro-4-isopropoxyphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylphenyl)acetaldehyde (15b). Compound **15b** (300 mg, 99%) was prepared from **14b** (313 mg, 0.76 mmol) in the same manner as described for **15a**. ESMS m/z: 401.1 (M + H)$^+$.

5-(3-(2-Ethyl-3-(2-oxoethyl)phenyl)-1,2,4-thiadiazol-5-yl)-2-isobutylbenzonitrile (15c). Compound **15c** (990 mg, 100%) was prepared from **14c** (1.03 g, 2.55 mmol) in the same manner as described for **15a**. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 1.00 (d, J = 6.40 Hz, 6 H), 1.19 (t, J = 7.34 Hz, 3 H), 2.03−2.09 (m, 1 H), 2.81 (d, J = 7.15 Hz, 2 H), 2.97 (q, J = 7.34 Hz, 2 H), 3.86 (s, 2 H), 7.32−7.38 (m, 2 H), 7.45−7.49 (m, 1 H), 7.89 (d, J = 7.15 Hz, 1 H), 8.14 (d, J = 7.91 Hz, 1 H), 8.30 (s, 1 H), 9.79 (s, 1 H). ESMS m/z: 390.2 (M + H)$^+$.

2-((2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)phenethyl)amino)acetic Acid (17a). Compound **17a** (5.6 mg, 11%) was prepared from **15a** (45 mg, 0.10 mmol) and glycinate (21 mg, 0.21 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.13 (t, J = 7.40 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 2.91 (q, J = 7.40 Hz, 2 H), 3.02 (s, 4 H), 3.22 (s, 2 H), 4.95 (dt, J = 12.05, 6.02 Hz, 1 H), 7.27−7.40 (m, 2 H), 7.52 (d, J = 9.03 Hz, 1 H), 7.68−7.72 (m, 1 H), 8.24 (d, J = 2.01 Hz, 1 H), 8.31 (dd, J = 8.78, 2.01 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.2, 174.0, 158.4, 141.4, 136.8, 133.6, 132.8, 131.4, 129.5, 126.1, 125.9, 121.8, 118.5, 115.7, 71.7, 50.0, 47.8, 29.1, 22.2, 21.5, 15.7. HRMS $C_{24}H_{27}N_3O_3F_3S$ (M + H)$^+$ calcd 494.1725, found 494.1726. LC/MS: t_R = 3.37 min, >95%, m/z 494.2 (M + H)$^+$.

2-((2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)phenethyl)(methyl)amino)acetic Acid (17b). Compound **17b** (9.2 mg, 17%) was prepared from **15a** (45 mg, 0.10 mmol) and ethyl 2-(methylamino)acetate (24.27 mg, 0.21 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.13 (t, J = 7.28 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 2.58 (s, 3 H), 2.88−2.97 (m, 4 H), 3.32 (s, 4 H), 4.95 (dt, J = 12.05, 6.02 Hz, 1 H), 7.30 (t, J = 7.65 Hz, 1 H), 7.38 (d, J = 6.78 Hz, 1 H), 7.53 (d, J = 8.78 Hz, 1 H), 7.67 (d, J = 7.78 Hz, 1 H), 8.25 (d, J = 1.76 Hz, 1 H), 8.32 (dd, J = 8.78, 2.01 Hz, 1 H). HRMS $C_{25}H_{29}N_3O_3F_3S$ (M + H)$^+$ calcd 508.1882, found 508.1888. LC/MS: t_R = 3.36 min, >95%, m/z 508.2 (M + H)$^+$.

1-(2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)phenethyl)piperidine-4-carboxylic Acid (17c). Compound **17c** (5 mg, 9%) was prepared from **15a** (45 mg, 0.10 mmol) and ethyl 4-piperidinecarboxylate (323 mg, 0.21 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.13 (t, J = 7.28 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 1.49−1.61 (m, 2 H), 1.80 (d, J = 13.80 Hz, 2 H), 2.01−2.10 (m, 2 H), 2.12−2.18 (m, 1 H), 2.81−2.94 (m, 8 H), 4.96 (dt, J = 12.11, 6.02 Hz, 1 H), 7.27 (t, J = 7.53 Hz, 1 H), 7.36 (d, J = 7.78 Hz, 1 H), 7.53 (d, J = 9.03 Hz, 1 H), 7.64 (dd, J = 7.65, 1.38 Hz, 1 H), 8.25 (d, J = 2.01 Hz, 1 H), 8.32 (dd, J = 8.66, 2.13 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.6, 1774.7, 158.8, 141.5, 139.7, 134.1, 134.1, 133.0, 132.2, 129.3, 126.3, 122.3, 122.3, 116.2, 72.2, 60.5, 52.9, 30.1, 28.6, 22.8, 21.9, 16.1. HRMS $C_{28}H_{33}N_3O_3F_3S$ (M + H)$^+$ calcd

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548.2195, found 548.2205. LC/MS: t_R = 3.48 min, >95%, m/z 548.3 $(M + H)^+$.

1-(2-Ethyl-3-(5-(4-isopropoxy-3-(trifluoromethyl)phenyl)-1,2,4-thiadiazol-3-yl)phenethyl)azetidine-3-carboxylic Acid (17d). Compound **17d** (7 mg, 13%) was prepared from **15a** (45 mg, 0.10 mmol) and azetidinecarboxylate (24 mg, 0.21 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.12 (t, J = 7.28 Hz, 3 H), 1.34 (d, J = 6.02 Hz, 6 H), 2.58−2.64 (m, 2 H), 2.64−2.71 (m, 2 H), 2.89 (q, J = 7.28 Hz, 2 H), 3.12−3.21 (m, 5 H), 4.95 (dt, J = 12.05, 6.02 Hz, 1 H), 7.24−7.30 (m, 1 H), 7.33−7.38 (m, 1 H), 7.53 (d, J = 8.78 Hz, 1 H), 7.65 (dd, J = 7.53, 1.25 Hz, 1 H), 8.24 (d, J = 2.26 Hz, 1 H), 8.31 (dd, J = 8.66, 2.13 Hz, 1 H). HRMS $C_{26}H_{29}N_3O_3F_3S$ $(M + H)^+$ calcd 520.1882, found 520.1884. LC/MS: t_R = 3.43 min, >95%, m/z 520.2 $(M + H)^+$.

1-(3-(5-(3-Chloro-4-isopropoxyphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylphenethyl)piperidine-4-carboxylic Acid (17e). Compound **17e** (40 mg, 24%) was prepared from **15b** (100 mg, 0.25 mmol) and ethyl 4-piperidinecarboxylate (78 mg, 0.50 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.14 (t, J = 7.28 Hz, 3 H), 1.35 (d, J = 6.02 Hz, 6 H), 1.65−2.08 (m, 2 H), 2.10−2.18 (m, 2 H), 2.87−2.98 (m, 2 H), 2.98−3.17 (m, 4 H), 3.22−3.35 (m, 3 H), 3.70 (d, J = 11.80 Hz, 2 H), 4.86 (dt, J = 12.05, 6.02 Hz, 1 H), 7.29−7.46 (m, 3 H), 7.69−7.78 (m, 1 H), 8.02 (dd, J = 8.53, 2.26 Hz, 1 H), 8.14 (d, J = 2.26 Hz, 1 H), 9.56 (br s, 1 H), 12.66 (br s, 1 H). HRMS $C_{27}H_{33}N_3O_3SCl$ $(M + H)^+$ calcd 514.1931, found 514.1935. LC/MS: t_R = 3.39 min, >95%, m/z 514.2 $(M + H)^+$.

1-(3-(5-(3-Chloro-4-isopropoxyphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylphenethyl)azetidine-3-carboxylic Acid (17f). Compound **17f** (32 mg, 21%) was prepared from **15b** (100 mg, 0.25 mmol) and methyl 3-azetidinecarboxylate (57 mg, 0.50 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.12 (t, J = 7.40 Hz, 3 H), 1.35 (d, J = 6.02 Hz, 6 H), 2.86−2.96 (m, 4 H), 3.38−3.44 (m, 2 H), 3.60−3.66 (m, 1 H), 4.18−4.39 (m, 4 H), 4.86 (dt, J = 12.05, 6.02 Hz, 1 H), 7.31−7.46 (m, 3 H), 7.71−7.76 (m, 1 H), 8.02 (dd, J = 8.66, 2.13 Hz, 1 H), 8.14 (d, J = 2.26 Hz, 1 H), 10.17 (br s, 1 H), 13.21 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.3, 173.9, 156.0, 141.5, 135.6, 132.9, 131.5, 129.8, 128.7, 128.0, 126.1, 123.1, 122.9, 115.5, 71.6, 55.4, 55.1, 32.4, 27.0, 22.3, 21.6, 15.6. HRMS $C_{25}H_{29}N_3O_3SCl$ $(M + H)^+$ calcd 486.1618, found 486.1620. LC/MS: t_R = 3.32 min, >95%, m/z 486.2 $(M + H)^+$.

1-(3-(5-(3-Cyano-4-isobutylphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylphenethyl)piperidine-4-carboxylic Acid (17g). Compound **17g** (86 mg, 49%) was prepared from **15c** (110 mg, 0.28 mmol) and ethyl 4-piperidinecarboxylate (58 mg, 0.37 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (d, J = 6.53 Hz, 6 H), 1.15 (t, J = 7.28 Hz, 3 H), 1.69−2.03 (m, 3 H), 2.13 (d, J = 13.05 Hz, 2 H), 2.78 (d, J = 7.28 Hz, 2 H), 2.94 (q, J = 7.28 Hz, 2 H), 3.00−3.16 (m, 4 H), 3.25−3.32 (m, 2 H), 3.53 (d, J = 12.55 Hz, 1 H), 3.70 (d, J = 11.80 Hz, 2 H), 7.33−7.40 (m, 1 H), 7.40−7.46 (m, 1 H), 7.70 (d, J = 8.28 Hz, 1 H), 7.73−7.79 (m, 1 H), 8.33 (dd, J = 8.16, 1.88 Hz, 1 H), 8.53 (d, J = 1.76 Hz, 1 H), 9.44 (br s, 1 H), 12.62 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.0, 174.6, 174.1, 149.0, 141.5, 135.7, 132.8, 131.8, 131.5, 130.2, 128.5, 126.2, 117.2, 113.2, 51.1, 42.7, 38.0, 29.8, 26.6, 25.5, 22.4, 22.0, 15.8. HRMS $C_{29}H_{35}N_4O_2S$ $(M + H)^+$ calcd 503.2481, found 503.2491. LC/MS: t_R = 4.15 min, >95%, m/z 503.2 $(M + H)^+$.

1-(3-(5-(3-Cyano-4-isobutylphenyl)-1,2,4-thiadiazol-3-yl)-2-ethylphenethyl)azetidine-3-carboxylic Acid (17h). Compound **17h** (24 mg, 14%) was prepared from **15c** (110 mg, 0.28 mmol) and methyl azetidine-3-carboxylate, HCl salt (45 mg, 0.30 mmol) in the same manner as described for **12c**, giving a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (d, J = 6.78 Hz, 6 H), 1.13 (t, J = 7.28 Hz, 3 H), 1.99 (dt, J = 13.49, 6.78 Hz, 1 H), 2.78 (d, J = 7.28 Hz, 2 H), 2.87−2.97 (m, 4 H), 3.38−3.44 (m, 2 H), 3.57−3.68 (m, 1 H), 4.18−4.34 (m, 4 H), 7.33−7.39 (m, 1 H), 7.42−7.47 (m, 1 H), 7.70 (d, J = 8.03 Hz, 1 H), 7.74−7.79 (m, 1 H), 8.33 (dd, J = 8.16, 1.88 Hz, 1 H), 8.53 (d, J = 1.76 Hz, 1 H), 10.06 (br s, 1 H), 13.14 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.9, 174.0, 158.3, 157.9, 148.9, 141.5, 135.6, 132.7, 131.7, 131.6, 131.5, 129.9, 128.5, 126.1, 117.2, 113.1, 55.3, 54.9, 42.7, 32.3, 29.7, 26.9, 22.3, 21.9, 15.6. HRMS $C_{27}H_{31}N_4O_2S$ $(M + H)^+$ calcd 475.2168, found 475.2176. LC/MS: t_R = 4.10 min, >95%, m/z 475.1 $(M + H)^+$.

5-(3-(2-Ethyl-3-(2-(3-hydroxyazetidin-1-yl)ethyl)phenyl)-1,2,4-thiadiazol-5-yl)-2-isobutylbenzonitrile (17i). To a solution of 3-azetidinol (38 mg, 0.51 mmol) and **15c** (100 mg, 0.26 mmol) in DCM (10 mL), which was prestirred at room temperature for 20 min, was added sodium triacetoxyborohydride (108 mg, 0.51 mmol). The reaction mixture was stirred at room temperature for 2 h. Afterward, the reaction was quenched with water. The reaction mixture was concentrated in vacuo, and the residue was purified by MDAP to give 20 mg (17%) of **17i** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.93 (d, J = 6.53 Hz, 6 H), 1.11 (t, J = 7.40 Hz, 3 H), 1.98 (dt, J = 13.36, 6.53 Hz, 1 H), 2.57−2.64 (m, 2 H), 2.64−2.79 (m, 6 H), 2.90 (q, J = 7.40 Hz, 2 H), 3.52−3.58 (m, 2 H), 4.11−4.20 (m, 1 H), 5.30 (d, J = 5.52 Hz, 1 H), 7.26 (t, J = 7.65 Hz, 1 H), 7.35 (d, J = 7.03 Hz, 1 H), 7.67 (dd, J = 7.40, 3.89 Hz, 2 H), 8.31 (dd, J = 8.28, 1.51 Hz, 1 H), 8.50 (s, 1 H). HRMS $C_{26}H_{31}N_4OS$ $(M + H)^+$ calcd 447.2219, found 447.2217. LC/MS: t_R = 3.29 min, >95%, m/z 447.2 $(M + H)^+$.

5-(3-(2-Ethyl-3-(2-(piperidin-1-yl)ethyl)phenyl)-1,2,4-thiadiazol-5-yl)-2-isobutylbenzonitrile (17j). Compound **17j** (48 mg, 32%) was prepared from **15c** (100 mg, 0.26 mmol) and piperidine (109 mg, 1.28 mmol) in the same manner as described for **17i** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.94 (d, J = 6.53 Hz, 6 H), 1.15 (t, J = 7.40 Hz, 3 H), 1.34−1.48 (m, 1 H), 1.59−1.79 (m, 3 H), 1.88 (d, J = 14.31 Hz, 2 H), 1.99 (dt, J = 13.36, 6.53 Hz, 1 H), 2.78 (d, J = 7.28 Hz, 2 H), 2.90−3.00 (m, 4 H), 3.07−3.16 (m, 2 H), 3.27 (dt, J = 12.05, 4.77 Hz, 2 H), 3.61 (d, J = 11.54 Hz, 2 H), 7.33−7.40 (m, 1 H), 7.40−7.45 (m, 1 H), 7.70 (d, J = 8.03 Hz, 1 H), 7.76 (dd, J = 7.53, 1.25 Hz, 1 H), 8.33 (dd, J = 8.16, 1.88 Hz, 1 H), 8.53 (d, J = 1.76 Hz, 1 H), 9.39 (br s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.9, 174.1, 158.2, 157.9, 149.0, 141.5, 135.9, 132.8, 129.9, 128.5, 126.2, 118.6, 117.2, 115.6, 113.1, 56.4, 52.1, 42.7, 29.7, 26.5, 22.7, 22.3, 21.9, 21.4, 15.7. HRMS $C_{28}H_{35}N_4S$ $(M + H)^+$ calcd 459.2582, found 459.2592. LC/MS: t_R = 3.55 min, >95%, m/z 459.3 $(M + H)^+$.

CNS Penetration Study (BBR) in Male Mice after ip Administration. Six male C57BL/6 mice received an ip injection of the test compound at 2 mg/kg. The dose was prepared on the day of administration in a suspension (DMSO/1% methylcellulose = 1:99 as the vehicle) at a concentration of 0.2 mg/mL (dose volume of 10 mL/kg). At 1, 2, and 4 h after ip administration, blood samples (50 μL per animal) from two of the animals were collected. An amount of 30 μL of blood was then diluted with 90 μL of water. Immediately after blood collection, whole brains of the two animals were quickly removed and rinsed with cold H_2O and surface vasculature was ruptured and blotted with dry tissue. Brain tissue was homogenized with 3× PBS (w/v). Blood and brain samples were analyzed by LC/MS/MS. The measured concentrations were corrected for dilution factor, and then the brain-to-blood ratio (BBR) was determined based on the ratio of brain concentration and blood concentration at each time point (1, 2, and 4 h):

$$\mathrm{BBR} = \frac{C_{\mathrm{brain}}}{C_{\mathrm{blood}}}$$

Lymphopenia Study in Male Mice after po Administration. Animals (n = 3/group) were treated once with various doses of the compounds (and vehicle) via oral gavage. At predetermined time points (including immediately prior to compound administration), 50 μL of peripheral blood was obtained and the number of lymphocytes therein was determined by flow cytometry and expressed as relative percentages of the baseline value at time 0. For repeat dose studies, animals were dosed daily for a week and lymphocyte counts were determined either just prior to or 4 h after dosing every day except on days 5 and 6, which were the weekend.

EAE Induction and Treatment. For induction and treatment of EAE, male C57BL/6 mice (6−8 weeks old) were purchased from the

Shanghai Laboratory Animal Center and were maintained under pathogen-free conditions. Mice were immunized subcutaneously with a synthetic peptide (300 μg) of myelin oligodendrocyte glycoprotein (MOG) corresponding to residues 35−55 (GL Biochem, Shanghai, China). Immunization was performed by mixing MOG peptide in complete Freund adjuvant containing 5 mg/mL heat-killed H37Ra, strain of *Mycobacterium tuberculosis* (Difco Laboratories, Detroit, MI). Pertussis toxin (200 ng, List Biological Laboratories, Campbell, CA) in PBS was administered iv on the day of immunization and 48 h later. For treatment of EAE, compounds or vehicle (DMSO in PBS) was administered at 1 mg/kg po daily from day 10 after immunization onward. Mice were examined daily and scored for disease severity using the following standard scale: 0, no clinical signs; 1, limp tail; 2, paraparesis (weakness, incomplete paralysis of one or two hind limbs); 3, paraplegia (complete paralysis of two hind limbs); 4, paraplegia with forelimb weakness or paralysis; 5, moribund or death. The animal use protocol was approved by Institutional Animal Care and Use Committee of GSK R&D China.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis procedures and spectral data for compounds **3**, **4a**−**u**, and **5a**−**l**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AI, aromatic index; AUC, area under curve; BBR, brain to blood ratio; CNS, central nervous system; CYP, cytochrome P450; DMSO, dimethylsulfoxide; EAE, experimental autoimmune encephalomyelitis; *F*, oral bioavailability; FRET, fluorescence resonance energy transfer; F_u, free unbound fraction; GSH, glutathione; HP-β-CD, 2-hydroxypropyl-β-cyclodextrin; ip, intraperitoneal; MC, methylcellulose; MS, multiple sclerosis; PD, pharmacodynamic; $PdCl_2$(dppf), 1,1′-bis(diphenylphosphino)ferrocenepalladium(II) dichloride; Pd(PPh_3)$_4$, tetrakis(triphenylphosphine)palladium(0); PK, pharmacokinetic; po, oral; RRMS, relapsing-remitting multiple sclerosis; $S1P_1$, sphingosine 1-phosphate receptor 1; $S1P_3$, sphingosine 1-phosphate receptor 3; $S1P_4$, sphingosine 1-phosphate receptor 4; $S1P_5$, sphingosine 1-phosphate receptor 5; SAR, structure−activity relationship; $T_{1/2}$, half-life

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