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2,5-Disubstituted pyrrolidine carboxylates as potent, orally active sphingosine-1-phosphate (S1P) receptor agonists

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Abstract—A series of 2,5-*cis*-disubstituted pyrrolidines were synthesized and evaluated as S1P receptor agonists. Compounds **15**–**21** were identified with good selectivity over $S1P_3$ which lowered circulating lymphocytes after oral administration in mice.

The sphingosine-1-phosphate-1 ($S1P_1$) receptor has recently emerged as a novel molecular target for immunosuppression.[1] Systemic administration of S1P agonists results in the sequestration of peripheral blood lymphocytes (PBLs) in secondary lymphoid organs, which prevents their access to transplanted or non-lymphoid tissues.[2] This pharmacodynamic phenomenon is putatively responsible for the immunosuppressive efficacy of this class of compounds.[3]

Work from these laboratories has shown that the 2,5-disubstituted pyrrolidine (±)-**1** and diaryl-1,2,4-oxadiazole **2** are potent agonists of S1P receptors.[4,5] In addition, compound **2** and its analogs were found to have exceptional selectivity against $S1P_3$, a receptor subtype that mediates acute cardiovascular toxicity in rodents.[6] Based on these results, we sought to combine the salient features of the oxadiazole-based lipophilic domain of compounds like **2** and the pyrrolidine scaffold in (±)-**1** with the aim of affording potent, selective, and orally active $S1P_1$ agonists (Fig. 1).

In order to modify both the 2- and 5-positions of the pyrrolidine scaffold, a flexible synthesis of these disubstituted pyrrolidines was designed, starting with (±)-pyrrolglutamic acid **3**. Schemes 1–3 illustrate our synthetic approach. Sequential protection of (±)-**3** under standard conditions gave the *N*-*tert*-butoxycarbamoyl methyl ester (±)-**4**.[7] Regioselective addition to the amide carbonyl with 4-cyanophenylmagnesium chloride gave ketone (±)-**5**.[8] Treatment of (±)-**5** with trifluoroacetic acid effected ring closure to the corresponding pyrroline, which was subsequently reduced with sodium cyanoborohydride to provide the diastereomeric (±)-*cis*- and (±)-*trans*-pyrrolidines (±)-**6a**,**b**. These diastereomers were separated by flash chromatography and assignment of their relative stereochemistries was secured by ID nOe experiments.[9] Protection of the pyrrolidine nitrogen afforded nitriles (±)-**7a**,**b**.

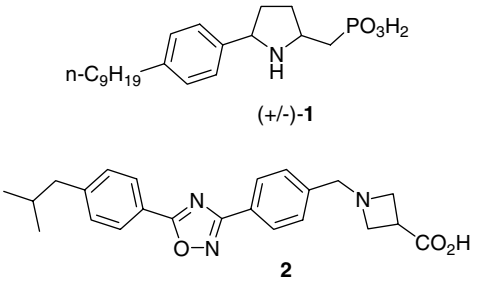

Figure 1.

Keywords: Pyrrolidines; Oxadiazole; S1P receptor; Immunosuppressants.
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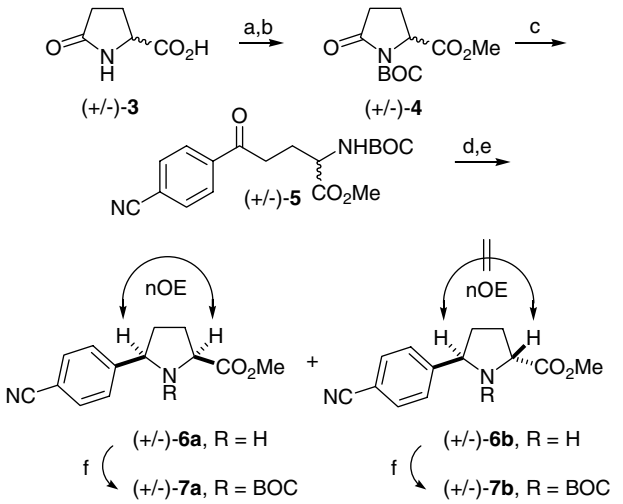
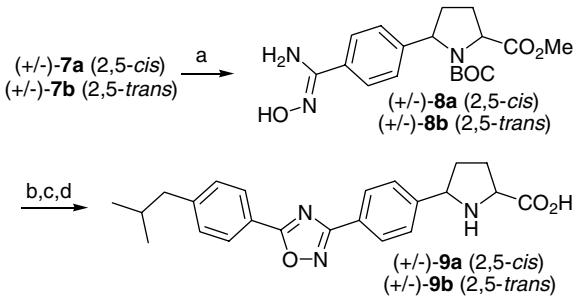
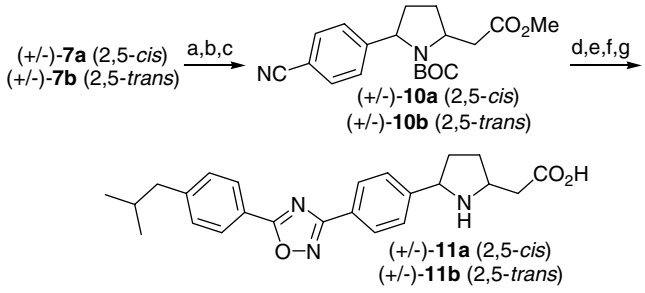


Scheme 1. Reagents and conditions: (a) Amberlyst-15, CH_3OH, 50 °C; (b) Boc_2O, Et_3N, DMAP (78%, two steps); (c) 4-cyanophenylmagnesium chloride, −40 °C; (d) TFA, CH_2Cl_2; (e) $NaBH_3CN$, HCl, CH_3OH (**6a**: 38%, **6b**: 24%, three steps); (f) Boc_2O, CH_2CI_2 (**7a**: 96%, **7b**: 80%).

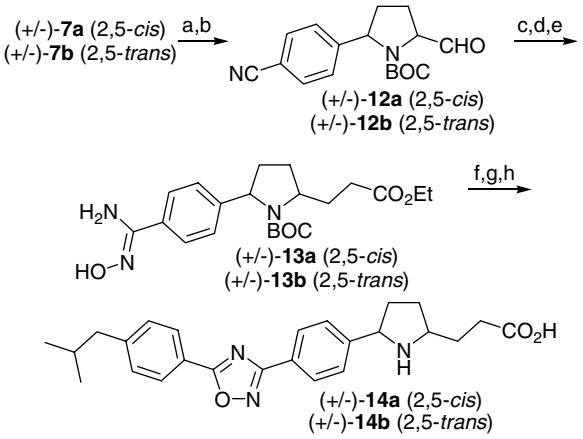


Scheme 2. Reagents and conditions: (a) NH_2OH, Et_3N, CH_3OH ((±)-**7a**: 93%, (±)-**7b** 73%); (b) EDC, 4-(2-methylpropyl)phenylbenzoic acid, CH_3CN, rt then 120 °C, 15 h; (c) TFA, CH_2Cl_2; (d) NaOH, CH_3OH ((±)-**9a**: 51%, (±)-**9b**: 34%, three steps).



Scheme 3. Reagents and conditions: (a) LiOH, $THF/CH_3OH/H_2O$; (b) *i*-BuOCOCl, Et_3N, then CH_2N_2; (c) AgOBz, Et_3N, CH_3OH ((±)-**10a**: 52%, (±)-**10b**: 14%, three steps); (d) NH_2OH, Et_3N, CH_3OH; (e) EDC, 4-(2-methylpropyl)-phenylbenzoic acid, CH_3CN, rt then 120 °C, 15 h; (f) TFA, CH_2Cl_2; (g) NaOH, CH_3OH ((±)-**11a**: 45%, (±)-**11b**: 36%, four steps).

Nitriles (±)-**7a**,**b** were valuable intermediates for the synthesis of the pyrrolidine carboxylate homologs (±)-**9a**, **9b**, **11a**, **11b**, **14a**, and **14b** (Schemes 2–4). Preparation of the α-amino acids (±)-**9a** and (±)-**9b** is outlined in Scheme 2. Independent treatment of nitriles (±)-**7a** and



Scheme 4. Reagents and conditions: (a) $LiBH_4$, THF; (b) $(COC1)_2$, DMSO, then Et_3N, −78 °C to rt; (c) ethyl(triphenylphosphoranylidine) acetate, $PhCH_3$; (d) H_2, 10% Pd–C, CH_3OH; (e) NH_2OH, Et_3N, CH_3OH ((±)-**7a**: 11%, (±)-**7b** 16%, four steps); (f) EDC, 4-(2-methylpropyl)-phenylbenzoic acid, CH_3CN, rt then 120 °C, 15 h; (g) TFA, CH_2Cl_2; (h) NaOH, CH_3OH ((±)-**14a**: 68%, (±)-**14**: 22%, three steps).

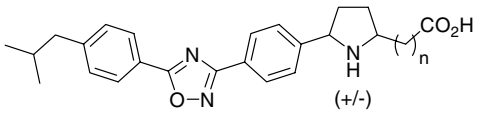
(±)-**7b** with hydroxylamine gave the amidoximes (±)-**8a** and (±)-**8b**. These intermediates were *O*-acylated and thermally cyclized and dehydrated to afford the corresponding 1,2,4-oxadiazoles. Deprotection of the *N*-*tert*-butoxycarbamate and methyl ester moieties afforded compounds (±)-**9a** and (±)-**9b**.

The homologation of nitriles (±)-**7a** and (±)-**7b** was accomplished through an Arndt-Eistert sequence[10] to give esters (±)-**10a** and (±)-**10b** (Scheme 3). Installation of the oxadiazole and deprotection vide infra furnished the desired β-amino acids (±)-**11a** and (±)-**11b**.

Preparation of the γ-amino acids (±)-**14a** and (±)-**14b** is outlined in Scheme 4. Selective reduction of the esters of (±)-**7a** and (±)-**7b** with lithium borohydride[11] gave the corresponding alcohols, which were oxidized using the Swern protocol[12] to furnish aldehydes (±)-**12a** and (±)-**12b**. Reaction with ethyl(triphenylphosphoranylidine) acetate, followed by hydrogenation and reaction with hydroxylamine, gave amidoximes (±)-**13a** and (±)-**13b**. Once again, oxadiazole formation followed by sequential deprotection afforded compounds (±)-**14a** and (±)-**14b**.

Binding affinities for new compounds were evaluated for each of the five known sphingosine-1-phosphate receptors ($S1P_{1-5}$) in radioligand competitive binding assays using [^{33}P]S1P expressed in Chinese hamster ovary (CHO) cell membranes.[1] S1P receptor agonism was determined by measurement of ligand-induced [^{35}S]-5′-*O*-3-thiotriphosphate (GTPγS) binding. All new compounds were found to be agonists of the $SlP_{1,3,5}$ receptors and to have minimal affinity for the $S1P_2$ receptor subtype. Values for binding (IC_{50}) and functional (EC_{50}) assays were in agreement to a factor of 4, thus only IC_{50} values for $S1P_{1,3-5}$ receptors will be displayed for new compounds (see Tables 1 and 2). The ability of selected compounds to lower circulating PBLs

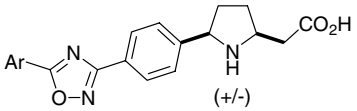
Table 1. S1P receptor affinities of carboxylate homologs

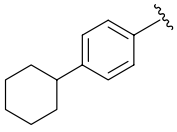
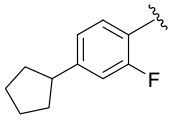
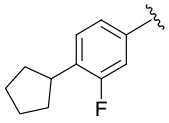
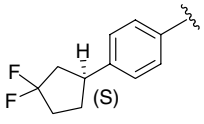
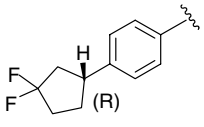


Compound	*n*	Stereo	IC_{50} (nM)			
			$S1P_1$	$S1P_3$	$S1P_4$	$S1P_5$
9a	0	*cis*	75	>10,000	1000	1280
9b	0	*trans*	17	10,000	300	180
11a	1	*cis*	3.3	>10,000	1000	200
11b	1	*trans*	8.0	>10,000	700	100
14a	2	*cis*	4.5	>10,000	540	30
14b	2	*trans*	5.7	6700	630	80

Compounds (±)-**9**–**14** were >10 μM for the $S1P_2$ receptor.

Table 2. Binding affinities of compounds **15**–**21**



Compound	Ar	IC_{50} (nM)			
		$S1P_1$	$S1P_3$	$S1P_4$	$S1P_5$
15		0.7	1700	350	20
16		2.7	>10,000	1400	60
17		2.0	7600	2100	30
18		0.8	2200	720	16
19		1.2	2500	570	13
20	**19** diastereomer 1	2.9	7800	1500	19
21	**19** diastereomer 2	0.64	3800	720	15

Compounds (±)-**15**–**19**, **20** and **21** were >10 μM for the $S1P_2$ receptor.

(a marker for immunosuppressive efficacy) was determined in mice, at 3 h post oral administration. Quantification of circulating PBLs in this manner also provides a gauge on the overall pharmacokinetic properties of new compounds.[13]

The α-amino acids (±)-**9a** and (±)-**9b** were found to be moderately potent $S1P_1$ agonists. Homologation to the β-amino acids (±)-**11a** and (±)-**11b** led to an improvement in SlP_1 potency, with the *cis*-isomer (±)-**11a** being roughly 2-fold more potent than the *trans*-isomer

Table 3. Rat pharmacokinetics[a] for selected compounds

Compound	Rat pharmacokinetic parameters
11a	Cl_p = 6.6 mL/min/kg, V_d = 2.9 L/kg, $t_{1/2}$ = 4.1 h; % F = 48
11b	Cl_p = 8.4 mL/min/kg, V_d = 3.0 L/kg, $t_{1/2}$ = 3.5 h; % F = 40
21	Cl_p = 9.7 mL/min/kg, V_d = 2.3 L/kg, $t_{1/2}$ = 2.2 h; % F = 48

[a] Sprague–Dawley rats (n = 2); 1.0 mg/kg iv; 2.0 mpk po.

(±)-**11b**. Additionally, the *cis*-isomer (±)-**11a** was more selective for the remaining S1P subtypes (>3000-fold over $S1P_3$, >300-fold over $S1P_4$, and >60-fold over $S1P_5$); such trends had been observed for analogs of **2**[5] and with 2,4-(disubstituted)pyrrolidine S1P receptor agonists,[14] but were somewhat less pronounced. The γ-amino acids (±)-**14a** and (±)-**14b** were approximately 2-fold less potent than β-amino acids (±)-**11a** and (±)-**11b** with excellent selectivity against $S1P_3$ and $S1P_4$, although both compounds had an increased affinity for the $S1P_5$ receptor.

Administration of either pyrrolidine (±)-**11a** or (±)-**11b** to mice at 10 mpk resulted in a near maximal lowering of circulating PBLs and both of these compounds exhibited favorable pharmacokinetics in the rat (Table 3). Based on the in vitro potency and selectivity of the *cis*-isomer (±)-**11a**, additional analogs were prepared in this series. Chemistry was carried out as outlined in Scheme 3, starting with the amidoxime derived from nitrile (±)-**10a** and some select substituted benzoic acids.[15] S1P receptor data for these compounds are listed in Table 2.

4-(Cycloalkyl)phenyl substituents appended to the 5-position of the 1,2,4-oxadiazole ring had been previously demonstrated to either maintain or enhance S1P receptor affinity as compared to the corresponding 4-(2-methylpropyl)phenyl analogs in other series of S1P receptor agonists. The same trend was observed for the compounds in this study (Table 2). Cyclohexyl analog (±)-**15** and the diastereomeric mixtures **18** and **19** were all found to have $S1P_1$ IC_{50} values of 1 nM or less. Since the (*R*)-(2,2-difluoro)cyclopentyl analog **19** appeared to be more potent in the mouse peripheral lymphocyte lowering (PLL) screening assay; separation of this compound by chiral HPLC[16] afforded the single diastereomers **20** and **21**. Analog **21** was found to be a potent $S1P_1$ agonist ($S1P_1$ IC_{50} = 0.6 nM) with greater than 5900-fold selectivity over $S1P_3$ and 1100-fold selectivity over the $S1P_4$ receptor. Compound **21** displayed good rat pharmacokinetic properties (Table 3) and its PLL pharmacodynamic ED_{50} value in the mouse was approximately 0.3 mg/kg after oral administration.

In short, we have demonstrated that a rational combination of compounds (±)-**1** and **2** led to the identification of a series of *cis*-2,5-disubstituted pyrrolidine carboxylates as agonists of the $S1P_1$ receptor, with good to exceptional selectivity over $S1P_{2,3,4}$ subtypes. Select compounds have also been shown to lower circulating PBLs after oral administration in mice and display encouraging pharmacokinetic profiles in rats.

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15. (4-Cyclohexyl)phenylbenzoic acid was purchased from Lancaster synthesis. For the preparation of benzoic acids used in the synthesis of compounds **16**–**19**, see Ref. 4.
16. Chiral HPLC was performed on the *N*-*tert*-butoxycarbonylmethyl ester of compound **19** (Chiralpak OD column, 2 × 25 cm, 10% EtOH/heptane, 8.0 mL/min, λ = 254 nm, peak 1 = 21.9 min; peak 2 = 26.1 min). Sequential deprotection afforded compounds **20** and **21**.