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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**.

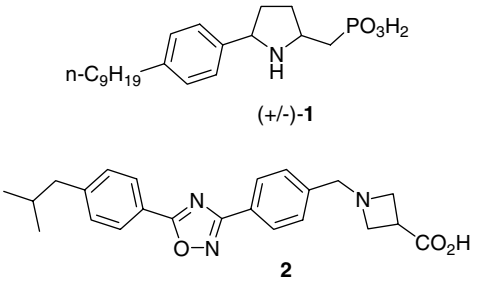

Figure 1.

Keywords: Pyrrolidines; Oxadiazole; S1P receptor; Immunosuppressants.
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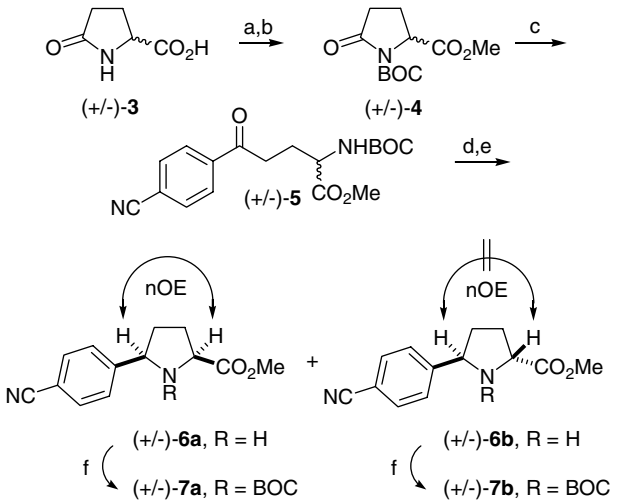
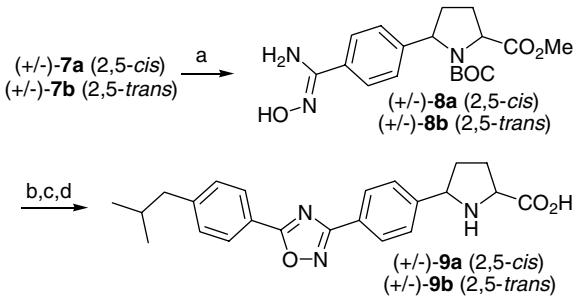
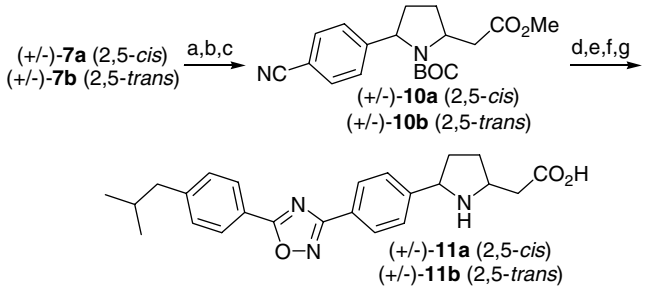


Scheme 1. Reagents and conditions: (a) Amberlyst-15, CH_3OH, 50 °C; (b) Boc_2O, Et_3N, DMAP (78%, two steps); (c) 4-cyanophenylmagnesium chloride, −40 °C; (d) TFA, CH_2Cl_2; (e) $NaBH_3CN$, HCl, CH_3OH (**6a**: 38%, **6b**: 24%, three steps); (f) Boc_2O, CH_2CI_2 (**7a**: 96%, **7b**: 80%).

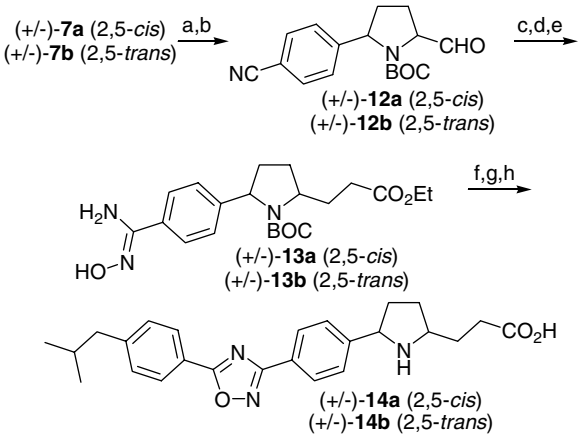


Scheme 2. Reagents and conditions: (a) NH_2OH, Et_3N, CH_3OH ((±)-**7a**: 93%, (±)-**7b** 73%); (b) EDC, 4-(2-methylpropyl)phenylbenzoic acid, CH_3CN, rt then 120 °C, 15 h; (c) TFA, CH_2Cl_2; (d) NaOH, CH_3OH ((±)-**9a**: 51%, (±)-**9b**: 34%, three steps).



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