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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3564–3568

2-Aryl(pyrrolidin-4-yl)acetic acids are potent agonists of sphingosine-1-phosphate (S1P) receptors

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Received 18 January 2006; revised 23 March 2006; accepted 24 March 2006
Available online 18 April 2006

Abstract—A series of 2-aryl(pyrrolidin-4-yl)acetic acids were synthesized and their biological activities were evaluated as agonists of S1P receptors. These analogs were able to induce lowering of lymphocyte counts in the peripheral blood of mice and were found to have good overall pharmacokinetic properties in rat.

Immunosuppressants that dampen the immune response are used to prevent the rejection of allografts after transplantation and to treat autoimmune disorders. Current immunotherapies (e.g., calcineurin inhibitors, antimetabolites, antiproliferatives, and monoclonal antibodies to T lymphocytes and cytokines) have narrow therapeutic windows; their chronic usage can have limitations due to the risk of side effects (e.g., infections, nephrotoxicity, and lymphoproliferative disorders).[1] Development of immunosuppressants with improved safety profiles based on known modes of drug action and/or new mechanisms is highly desired.

FTY720 (**1**, Fig. 1) is a novel immunosuppressant that has a unique mode of action. FTY720 is a prodrug for monophosphate **2**, which binds to and is an agonist of four out of the five known sphingosine-1-phosphate (S1P) receptors (which are members of the superfamily of seven transmembrane G protein-coupled receptors).[2] Agonism of $S1P_1$ alters lymphocyte trafficking by sequestering lymphocytes into secondary lymphoid organs,[3] while agonism of $S1P_3$ has been linked to bradycardia in rodents.[4] FTY720 has shown immunosuppressive efficacy in animal models[5] and is currently in

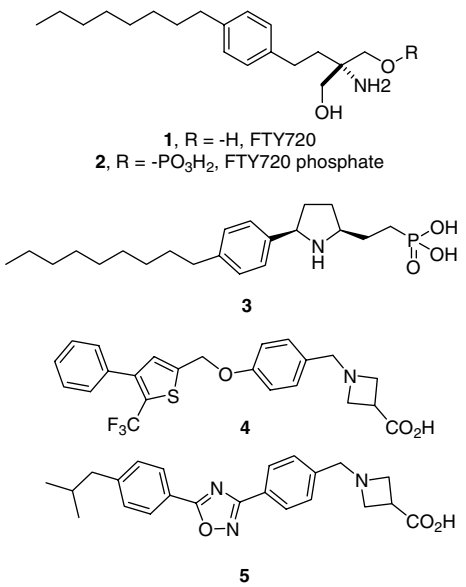

Figure 1. Structures of FTY720 (**1**), FTY720-phosphate (**2**), amino phosphonic acid analog (**3**), and two lead azetidine-3-carboxylic acids **4** and **5**.

Keywords: Immunosuppressants; S1P agonists; (Pyrrolidin-4-yl)acetic acids; Peripheral lymphocyte lowering.
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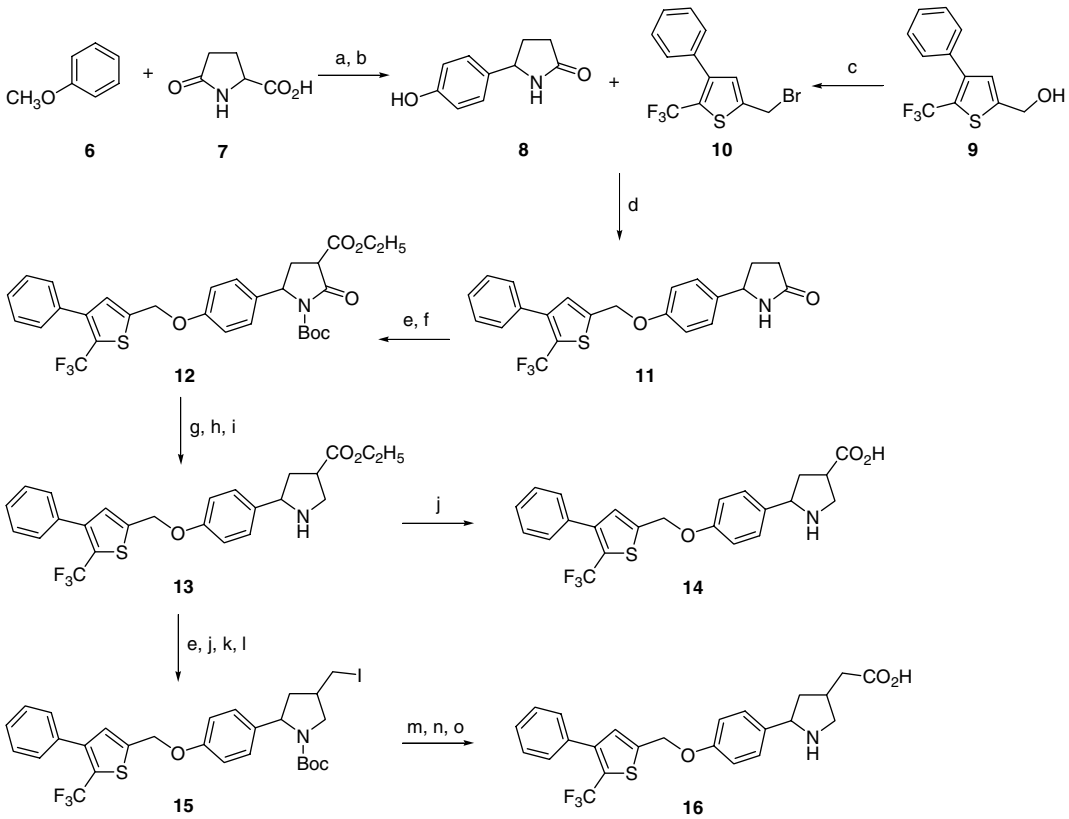
doi:10.1016/j.bmcl.2006.03.090

Phase III clinical trials for the prevention of rejection of kidney after transplantation and in Phase II for the treatment of multiple sclerosis.[6]

Studies from these laboratories have previously identified a series of 3-(*N*-alkylamino)propyl-phosphonic acids[7] as well as conformationally constrained analogs of those compounds (e.g., **3**, $S1P_1$ $IC_{50} = 0.1$ nM),[8] as potent agonists of $S1P_1$ that induce peripheral lymphocyte lowering in mice after iv administration. Replacement of the phosphonic acid in these earlier compounds with other acid groups, such as carboxylate, was investigated with the hope of increasing oral bioavailability, but these structural changes were found to decrease binding to $S1P_1$ in instances where their lipophilic tails were kept constant.[7] The rational combination of leads identified from high-throughput screening of the Merck sample collection and scaffolds selected from the conformationally constrained 3-aminopropyl-phosphonic acids led to the discovery of a series of azetidine-3-carboxylic acids, such as **4**[9] and **5**,[10] as potent, selective, and orally bioavailable $S1P_1$ receptor agonists. This paper describes the design and synthesis of a novel series of $S1P_1$ receptor agonists that utilize (pyrrolidin-4-yl)acetic acid as a zwitterionic pharmacophore and that have properties comparable to azetidine-3-carboxylic acid analogs **4** and **5**.

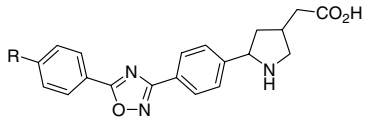
The synthesis of pyrrolidine-4-carboxylic acid and (pyrrolidin-4-yl)acetic acid analogs that have a pendant substituted thiophene group similar to that found in **4** is shown in Scheme 1. Decarboxylative arylation of anisole with pyroglutamic acid using Eaton's reagent[11] followed by demethylation furnished phenol **8**. Alkylation with bromide **10** (prepared from alcohol **9**[9]) gave lactam **11**. *N*-Boc protection of **11** followed by acylation with ethyl chloroformate provided **12** as a 1:1 *cis*:*trans* mixture.[12] Carbonyl reduction was effected by removal of the *N*-Boc group, treatment with Meerwein's salt, and reduction with $NaCNBH_3$ to give pyrrolidine **13**. Saponification of **13** gave pyrrolidine-4-carboxylic acid **14** as a 1:1 *cis*:*trans* mixture of diastereomers. Hydrolysis of *N*-Boc protected **13** led to a carboxylic acid, which was reduced to an alcohol and subsequently converted into iodide **15**. Cyanide displacement, acid hydrolysis, and removal of the *N*-Boc furnished 5-aryl(pyrrolidin-4-yl)acetic acid **16** as a 1:1 *cis*:*trans* mixture of diastereomers. S1P receptor binding studies[2a] showed that (pyrrolidin-3-yl)acetic acid **16** was a more potent $S1P_1$ receptor agonist than was pyrrolidine-4-carboxylic acid **14** (Table 1). Based on these data, (pyrrolidin-4-yl)acetic acid analogs were targeted for further investigation.

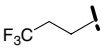
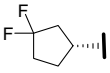
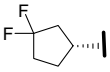
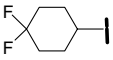
The synthesis of (pyrrolidin-4-yl)acetic acid analogs based on **5** is shown in Scheme 2. Conversion of phenol **8** to the corresponding triflate followed by palladium



Scheme 1. Reagents and conditions: (a) P_2O_5, CH_3SO_3H, 100 °C (54%); (b) BBr_3, CH_2Cl_2, −78 °C to 0 °C (80%); (c) CBr_4, PPh_3, CH_2Cl_2, rt (100%); (d) K_2CO_3, CH_3CN, 60 °C (96%); (e) $(Boc)_2O$, DMAP, CH_2Cl_2, rt (90%); (f) 1—$LiN(TMS)_2$, THF, −78 °C; 2—$ClCO_2Et$, THF, −78 °C (83%); (g) HCl, EtOH, rt (81%); (h) $(CH_3)_3O{\cdot}BF_4$, CH_2Cl_2, rt (94%); (i) $NaCNBH_3$, CH_3OH, pH 3–4, rt (97%); (j) NaOH, EtOH (100%); (k) 1—$ClCO_2Et$, Et_3N, THF, 0 °C to rt; 2—$NaBH_4$, THF, H_2O, 0 °C to rt (93%); (l) I_2, PPh_3, imidazole, CH_2Cl_2, rt (56%); (m) NaCN, DMSO, 60 °C (68%); (n) 40% KOH, CH_3OH, H_2O, 100 °C (100%); (o) 20% CF_3CO_2H, CH_2Cl_2, rt (16%).

Table 1. Inhibition (IC_{50}, nM) of ^{33}P-S1P binding to S1P receptors[a,b]



Compound	R	Stereo	$S1P_1$	$S1P_3$	$S1P_4$	$S1P_5$
4			1.2	530	1600	23
5			0.6	12,000	70	1.0
14			25	4800	2700	130
16			2.2	2100	390	390
22a		*trans*	5.7	>1000	150	300
22b		*cis*	7.9	1500	160	140
22c		*trans*	1.0	>1000	120	68
22d		*trans*	1.4	3100	110	57
22e		*trans*	5.5	>1000	230	50
22f		*trans* chiral 1	1.2	690	32	5.1
22g		*trans* chiral 2	0.8	1100	800	11
22h		*trans*	2.0	460	110	21

[a] Displacement of ^{33}P-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as means for $n = 3$ measurements. SD were generally within ±20% of the average.

[b] $S1P_2$ IC_{50} values for new compounds were all greater than 10 μM.

catalyzed cyanation[13] and *N*-Boc protection gave lactam **17**. Allylation of **17** furnished a 76:3:1 mixture of readily separable 3,5-*trans*, 3,5-*cis* and *bis*-allyl adducts. The higher *trans* stereoselectivity presumably resulted from the favorable approaching of allyl iodide to the less-hindered face of the enolate intermediate of **17**. The double bond of **18** was oxidized under Sharpless' conditions[14] and subsequent methylation provided pyrrolidinone **19**. Conversion of **19** to pyrrolidine **20** was achieved using the procedures analogous to those described in Scheme 1. Treatment of nitrile **20** with hydroxylamine furnished *N*-hydroxyamidine **21**, which was coupled to various benzoic acids using EDC followed by dehydration at elevated temperature and removal of the *N*-Boc to provide the amino acids exemplified in Table 2. Most of the derivatized benzoic acids used to prepare the new analogs were either commercially available or synthesized as described previously.[8]

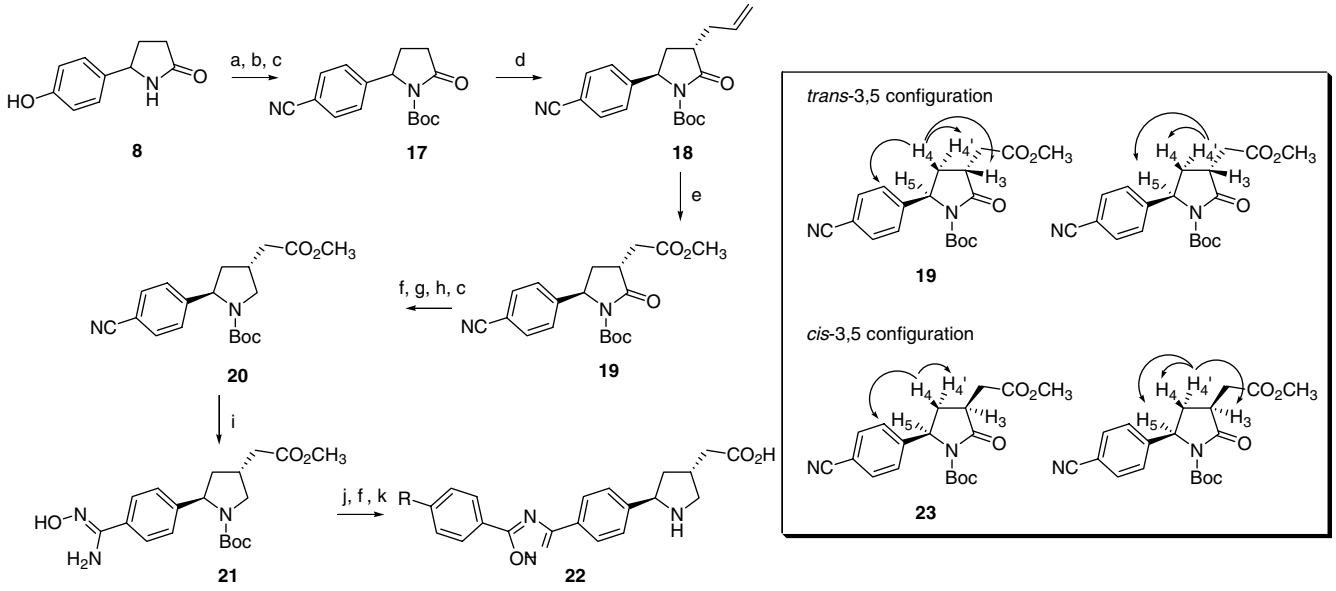
The *cis* ester **23** was prepared by racemizing the *trans* allyl adduct **18** using LiHMDS at −78 °C and then converting the resulting allyl group to methyl ester **23**. Transformation of **23** to the final amino acid was accomplished using the procedure analogous to that of **19**. The configuration of methyl acetates on C-3 of pyrrolidone relative to the aryl group on C-5 was determined using 1D NOE experiments (Scheme 2, inset). Direct NOE effects between H_3 and H_5 on the *cis* analog were not observed. The *cis* and the *trans* configurations were, therefore, assigned based on their distinct NOE patterns generated by irradiating H_4 and $H_{4'}$, respectively.

The S1P receptor binding affinities (IC_{50}) for new compounds were determined in competitive binding assays in transfected Chinese hamster ovary (CHO) cell membranes expressing S1P receptors with ^{33}P-labeled S1P as the ligand.[2a] The functional activities (EC_{50}) were determined by measuring the binding of ^{35}S-GTPγS to S1P receptors expressed in CHO cell membranes.[2a] All of the new pyrrolidine acids were found to be full agonists (in a range of 70–120% of max) of $S1P_{1,3,5}$ receptors and inverse agonists of $S1P_4$ receptor; none of the new compounds were found binding to $S1P_2$ receptor ($IC_{50} > 10$ μM).[15] Compound-induced peripheral blood lymphocyte (PBL) lowering in mice was measured by the reduction percentage of the absolute PBL counts determined at a 3-h time point after the oral administration of the test compound in comparison to those from vehicle controls.[2a] The murine PBL lowering has been previously shown to correlate with immunosuppressive efficacy in rodents.[16]

The S1P receptor binding data for pyrrolidine-4-carboxylic acid **14** and (pyrrolidin-4-yl)acetic acid **16**, both of which have substituted thiophene ether side chain, are shown in Table 1. The binding affinity of pyrrolidine-4-carboxylic acid **14** for $S1P_1$ is about one order of magnitude less than that of (pyrrolidin-4-yl)acetic acid **16**, indicating that (pyrrolidin-4-yl)acetic acid provides the more optimal spacial orientation between the amino group and the carboxylic acid. Compound **16** is about 1000-fold selective for $S1P_1$ over $S1P_3$ and has modest affinity for both $S1P_4$ and $S1P_5$. Compound **16** was also found to lower peripheral lymphocyte count in mice after a relative high oral dose (39% vs control after a 10 mpg/kg po dose).

The receptor binding data of new (pyrrolidin-4-yl)acetic acids having derivatized oxadiazole ring are also shown in Table 1. The racemic 2,4-*trans* analog **22a** was determined to be slightly more $S1P_1$ potent than the *cis* analog **22b**; this was found to generally hold for this series of compounds. For other *trans* analogs, sterically more-hindered groups, such as cyclopentyl and cyclohexyl groups, enhanced $S1P_1$ binding affinities by about half a log unit. Fluorination of the alkyl groups slightly enhanced receptor binding affinities but decreased the selectivity for $S1P_1$ over $S1P_3$. The former, but not the latter, was observed of analogs of **5**.[10]

Several of the new (pyrrolidin-4-yl)acetic acid analogs were selected for further evaluation in the mouse PBL lowering assay. Analogs **22b**, **22c**, **22d**, **22g**, and **22h** were all found to induce a PBL response after oral administration, with **22f**, **22g**, and **22h** being able to



Scheme 2. Reagents and conditions: (a) $PhN(Tf)_2$, EtN-*i*-Pr_2, DMF, rt (88–100%); (b) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 80 °C; (c) $(Boc)_2O$, DMAP, CH_2Cl_2, rt (over two steps, 61–88%); (d) 1—LDA, THF, −78 °C; 2—allyl iodide, THF, −78 °C (76–86%); (e) 1—$RuCl_3{\cdot}xH_2O$, $NaIO_4$, CCl_4:CH_3CN:H_2O, rt; 2—$TMSCHN_2$, benzene, CH_3OH, rt (over two steps, 94%); (f) 20% CF_3CO_2H, CH_2Cl_2, rt (89%); (g) $(CH_3)_3O{\cdot}BF_4$, CH_2Cl_2, rt; (h) $NaCNBH_3$, CH_3OH, pH 3–4, rt (85–100%); (i) $NH_2OH{\cdot}HCl$, $NaHCO_3$, CH_3OH, reflux (74–93%); (j) derivatized benzoic acid, EDC, CH_3CN, 1 h at rt and 16 h at 120 °C (40–60%); (k) NaOH, EtOH, rt (100%). The relative configurations of substitutes on the pyrrolidinone ring were assigned based on 1D NOE ^1H NMR experiments (inset). Curved arrows point away from the irradiated proton to the proton that exhibits NOE effect.

Table 2. Rat pharmacokinetic data (2.0 mpk po, 1.0 mpk iv) for selected S1P receptor agonists[a]

Compound	PK parameters
4	Cl_p = 12.3 mL/min/kg, V_{dss} = 10.0 L/kg, $t_{1/2}$ = 6.7 h, %F = 79
5	Cl_p = 4.1 mL/min/kg, V_{dss} = 2.8 L/kg, $t_{1/2}$ = 8.5 h, %F = 67
22a	Cl_p = 7.4 mL/min/kg, V_{dss} = 6.6 L/kg, $t_{1/2}$ = 10.7 h, %F = 79
22b	Cl_p = 3.4 mL/min/kg, V_{dss} = 2.9 L/kg, $t_{1/2}$ = 10.9 h, %F = 27
22g	Cl_p = 2.1 mL/min/kg, V_{dss} = 1.3 L/kg, $t_{1/2}$ = 5.6 h, %F = 36
22h	Cl_p = 3.5 mL/min/kg, V_{dss} = 2.2 L/kg, $t_{1/2}$ = 4.4 h, %F = 23

[a] Plasma compound concentrations used to calculate pharmacokinetic parameters were obtained after iv administration (1.0 mpk) and po administration (2.0 mpk) of test compounds to male Sprague–Dawley rats (n = 2), respectively.

do so after doses of less than 1.0 mpk po.[17] The pharmacodynamic ED_{50} value for **22g** in this assay was determined to be approximately 0.3 mpk po, making it comparable to **4** (ED_{50} = 0.4 mpk po). Rat pharmacokinetics for selected compounds (Table 2) appeared to parallel those for analogs of **5**, with isobutyl analogs **22a** and **22b** as well as fluoroalkyl analogs **22g** and **22h** all being low clearance compounds with good oral bioavailability.

In summary, a series of 2-aryl(pyrrolidin-4-yl)acetic acids have been identified as potent and selective $S1P_1$ receptor agonists. Unlike the previously described amino phosphonic acids,[7,8] these amino acids exhibit good overall pharmacokinetic properties in rat and effectively lower peripheral lymphocytes in mice after oral administration. This work demonstrates that a (pyrrolidin-4-yl)acetic acid scaffold can be utilized to afford potent and selective $S1P_1$ receptor agonists. Further, this also supports the continued investigation of this structurally novel scaffold as a potential replacement for the zwitterionic azetidine-3-carboxylic acid pharmacophore of S1P receptor agonists such as **4** and **5**.

Acknowledgments

We thank Ms. Susan Li and Dr. Larry Colwell for coordinating the rat pharmacokinetic studies.

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17. Compounds **22f** and **22g** were prepared by chiral separation of their corresponding racemic methyl ester on Chiralcel OD 2×25 cm column (eluted with EtOH/heptane (v:v = 15:85) at rate of 8.0 mL/min), followed by saponification.