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# SAR studies of 3-arylpropionic acids as potent and selective agonists of sphingosine-1-phosphate receptor-1 ($S1P_1$) with enhanced pharmacokinetic properties

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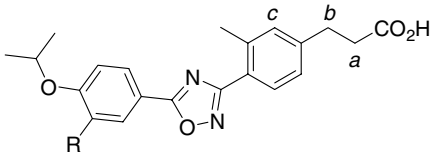
**Abstract**—Structure–activity relationship (SAR) studies of 3-arylpropionic acids—a class of novel $S1P_1$ selective agonists—by introducing substitution to the propionic acid chain and replacing the adjacent phenyl ring with pyridine led to a series of modified 3-arylpropionic acids with enhanced half-life in rat. These analogs (e.g., cyclopropanecarboxylic acids) exhibited longer half-life in rat than did unmodified 3-arylpropionic acids. This result suggests that metabolic oxidation at the propionic acid chain, particularly at the C3 benzylic position of 3-arylpropionic acids, is probably responsible for their short half-life in rodent.


Recent studies have demonstrated that agonism of sphingosine-1-phosphate receptor-1 ($S1P_1$)—one of the five known S1P G-protein coupled receptors (GPCRs)—leads to the sequestration of peripheral lymphocytes into secondary lymphoid organs and results in observed immunosuppressive efficacy in preclinical animal models.[1] FTY720 (i.e., 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride) is phosphorylated in vivo to a monophosphate, which binds tightly to $S1P_{1,3,4,5}$ receptors as a non-selective $S1P_1$ agonist.[2] FTY720 has progressed in clinical trials for the prevention of transplanted kidney rejection and for the treatment of multiple sclerosis.[3]

We have recently discovered that 3-arylpropionic acids (exemplified by **1a** and **1b**, Fig. 1) are potent $S1P_1$ agonists with high selectivity against $S1P_{2\text{-}5}$ receptors.[4,5] SAR studies of the left-hand side pendant phenyl ring and the internal five-membered heterocycle ring revealed several structural features that afford high $S1P_1$ selectivity against all other known S1P subtypes. These analogs can lower peripheral lymphocytes in mice; one of them has been shown to prolong skin allograft in rat, demonstrating immunosuppressive efficacy in that model similar to that of FTY720. In spite of various substituents on the pendant phenyl ring and different five-membered heterocyclic replacements of the internal 1,2,4-oxadizole ring, these 3-arylpropionic acids shared similar pharmacokinetic characteristics; among them, short half-life in rat is undesirable.[6] This paper describes SAR studies around the propionic acid group and the adjacent phenyl ring. These modified 3-arylpropionic acids generally have longer half-life in rat, suggesting that the short half-life of this class of novel $S1P_1$ agonists is largely




**1a R = $CF_3$**
**1b R = CN**

**Figure 1.** Structures of lead 3-phenylpropionic acids (**1a** and **1b**) as $S1P_1$-selective agonists, where alphabetic letters indicate positions for modification.

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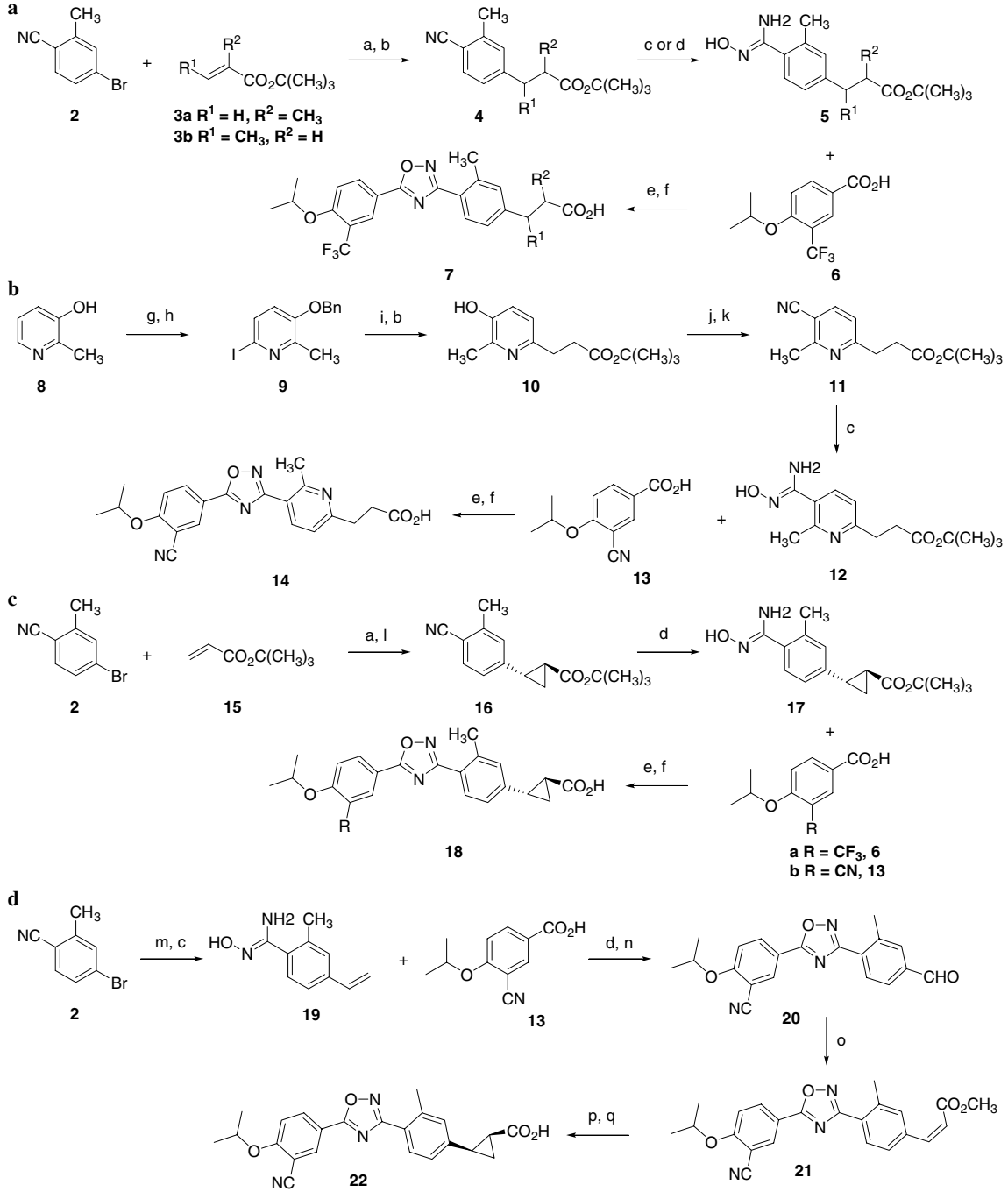
due to the metabolic oxidation on the propionic acid chain, especially at the C3 benzylic position (position *b*, Fig. 1).

Scheme 1 describes the syntheses of modified 3-arylpropionic acids. Heck coupling of bromide **2** with *tert*-butyl methacrylate, **3a**, and subsequent hydrogenation gave **4a** (Scheme 1a). Nitrile **4a** was treated with hydroxylamine, condensed with benzoic acid **6**, and hydrolyzed to provide C2 methyl analog **7a** (position *a*, Fig. 1). The C3 methyl analog of **1a** (position *b*, Fig. 1) was prepared in a similar fashion from *tert*-butyl crotonate **3b**. In this synthetic sequence, amidoxime **5b** could only be obtained in good yield under microwave conditions, with




**Scheme 1.** Reagents and conditions: (a) $Pd_2(dba)_3$, 2-(di-*tert*-butylphosphino)diphenyl, $Cy_2NCH_3$, 1,4-dioxane, 80 °C (100%); (b) 10% Pd/C, $H_2$ (1 atm), $CH_3OH$, rt (80%); (c) $NH_2OH{\cdot}HCl$, $NaHCO_3$, $C_2H_5OH$, 80 °C (70–100%); (d) $NH_2OH$ (aq), 1,4-dioxane, $C_2H_5OH$, microwave, 180 °C (30–50%); (e) *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, $CH_3CN$, rt (4 h) to 100 °C (14 h) (14–80%); (f) 20% TFA, $CH_2Cl_2$, rt (50–100%); (g) $I_2$, $Na_2CO_3$, $H_2O$, rt (58%); (h) BnBr, $K_2CO_3$, acetone, reflux (93%); (i) *tert*-butyl acrylate, $Pd(OAc)_2$, $Bu_4NCl$, $NaHCO_3$, 4 Å MS, DMF, 60 °C (85%); (j) $PhN(Tf)_2$, Hünig's base, $CH_2Cl_2$, rt (95%); (k) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 85 °C (100%); (l) $(CH_3)_3S(O)I$, NaH, DMSO, rt to 50 °C (44–50%); (m) tributyl(vinyl)tin, $Pd[P(Bu)_3]_2$, CsF, dioxane, rt to 100 °C (78–89%); (n) cat. $OsO_4$, NMO, $NaIO_4$, THF:$H_2O$ (v:v=3:1), rt (89–98%); (o) $(CH_3O)C(O)CH_2P(O)(OCH_2CF_3)_2$, $KN(TMS)_2$, 18-Crown-6, THF, −78 °C (65%); (p) $CH_2N_2$, $Pd(OAc)_2$, $Et_2O$, $CH_2Cl_2$, 0 °C (41%); (q) NaOH, $C_2H_5OH$, rt (50%).

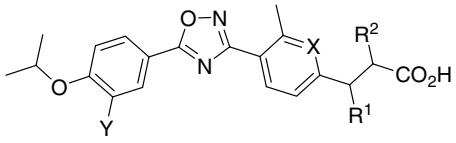
**5b** formed only in trace amounts using conventional thermal heating. The mechanism for this discrepancy is not fully understood. Jeffery-Heck coupling[7] of 2-iodopyridine **9** (from 3-hydroxy-2-methyl-pyridine **8** in two steps)[8] and *tert*-butyl acrylate followed by hydrogenation delivered phenol **10** (Scheme 1b). Triflation of the resulting phenol and cyanation gave nitrile **11**, which was subsequently converted to amidoxime **12**, condensed with benzoic acid **13**, and deprotected to afford pyridine analog **14** (position *c*, Fig. 1). Heck coupling of bromide **2** and *tert*-butyl acrylate afforded *trans*-cinnamate **16**, which was treated with trimethylsulfoxonium iodide[9] to yield (±)-*trans*-cyclopropane **16**, tethering positions *a* and *b* (Fig. 1) through a methylene group (Scheme 1c). Again only under microwave conditions amidoxime **17** was formed in good yield. Compound **17** was readily converted to (±)-*trans*-cyclopropanepropionic acids **18** in three steps. Vinylation[10] of bromide **2** and subsequent treatment with hydroxylamine gave amidoxime **19**, which was condensed with benzoic acid **13** and oxidized to provide aldehyde **20** (Scheme 1d). Formation of *cis*-cinnamate **21** was achieved using the Still modified Horner-Emmons olefination in good yield.[11] Cyclopropanation of **21** followed by saponification gave (±)-*cis*-cyclopropanepriopionic acid **22**.

Table 1 compares the functional binding affinities ($EC_{50}$) of $S1P_{1,3,5}$ receptors,[12] the pharmacodynamic $ED_{50}$ values of peripheral lymphocyte lowering (PLL) in mice,[13] and the rat pharmacokinetic properties of **1a** and **1b** to those of modified 3-arylpropionic acids. Like **1a** and **1b**, all modified analogs appeared to be potent, subnanomolar $S1P_1$ full agonists. The selectivity against $S1P_3$ receptor was more pronounced when the pendant phenyl ring was substituted by a C3-cyano group instead of a C3-$CF_3$ group (i.e., **18a** vs **18b**). This trend was observed in the 3-arylpropionic acid series as well (i.e., **1a** vs **1b**). These structural modifications, however, did not appear to significantly alter the binding selectivity of $S1P_1$ receptor against either $S1P_3$ receptor or $S1P_5$ receptor. All modified 3-arylpropionic acids in this study induced PLL in mice at low dosage (<0.7 mpk).

Among pharmacokinetic properties, these modified 3-arylpropionic acids generally showed low clearances, low volumes of distribution, and good bioavailability in rat, but their half-lives were profoundly different. Compound **7a** having a methyl group at C2 showed slight half-life prolongation in comparison to **1a**. In contrast, **7b** with a methyl group at C3 benzylic position had half-life extended three-fold compared to **1a**, suggesting that the metabolism of 3-arylpropionic acids is more sensitive to the substitution at C3 than at C2, probably due to a steric effect. Replacement of the adjacent phenyl ring with a pyridine group extended half-life more than two-fold, implicating that such replacement slows the metabolic oxidation at the C3 benzylic position of 3-arylpropionic acids. The replacement of the propionic acid by a cyclopropane carboxylic acid led to substantial half-life enhancement (about five-fold or more); the cyclopropane ring is known to be more metabolically stable. Together, these data indicate that metabolic oxidation of the propionic acid group, especially at the benzylic C3 position, is responsible for the short half-life of this series of compounds observed in rodent.

In conclusion, a series of modified 3-arylpropionic acids has been designed and synthesized as potent and selective $S1P_1$ agonists. All these analogs induced PLL at

**Table 1.** S1P functional binding affinities ($EC_{50}$, nM),[a] murine peripheral lymphocyte lowering (PLL),[b] and rat pharmacokinetic data[c] for selected S1P receptor agonists




| Compound | $R^1$, $R^2$ | X | Y | $S1P_1$ | $S1P_3$ | $S1P_5$ | PLL $ED_{50}$ (mg/kg, po) | Rat PK |
|---|---|---|---|---|---|---|---|---|
| **1a** | H, H | –CH– | $CF_3$ | 0.08 | 110 | 40 | 0.3 | $Cl_p$ = 8.7, $Vd_{ss}$ = 1.1, $t_{1/2}$ = **1.1**, %$F$ = 88 |
| **1b** | H, H | –CH– | CN | 0.08 | 1100 | 6.5 | 0.1 | $Cl_p$ = 2.3, $Vd_{ss}$ = 0.2, $t_{1/2}$ = **0.7**, %$F$ = 53 |
| **7a** | H, $CH_3$ | –CH– | $CF_3$ | 0.3 | 400 | 9.9 | 0.1 | $Cl_p$ = 7.0, $Vd_{ss}$ = 1.5, $t_{1/2}$ = **1.7**, %$F$ = 82 |
| **7b** | $CH_3$, H | –CH– | $CF_3$ | 0.12 | 184 | 3.9 | 0.1 | $Cl_p$ = 2.9, $Vd_{ss}$ = 0.6, $t_{1/2}$ = **3.1**, %$F$ = 100 |
| **14** | H, H | –N– | CN | 0.44 | 2760 | 28.6 | 0.2 | $Cl_p$ = 1.2, $Vd_{ss}$ = 0.3, $t_{1/2}$ = **2.4**, %$F$ = 93 |
| **18a** | –$CH_2$– | –CH– | $CF_3$ | 0.21 | 123 | 5.1 | 0.2 | $Cl_p$ = 2.4, $Vd_{ss}$ = 1.1, $t_{1/2}$ = **6.3**, %$F$ = 88 |
| **18b** | –$CH_2$– | –CH– | CN | 0.45 | >10,000 | 13.6 | 0.2 | $Cl_p$ = 1.0, $Vd_{ss}$ = 0.4, $t_{1/2}$ = **5.9**, %$F$ = 90 |
| **18b′**[d] | –$CH_2$– enant. 1 | –CH– | CN | 0.31 | 2600 | 5.2 | 0.2 | $Cl_p$ = 3.4, $Vd_{ss}$ = 1.0, $t_{1/2}$ = **4.9**, %$F$ = 84 |
| **18b″**[d] | –$CH_2$– enant. 2 | –CH– | CN | 0.93 | 2660 | 88.4 | 0.3 | n.d. |
| **22** | –$CH_2$– | –CH– | CN | 0.8 | >1000 | 37.7 | 0.7 | $Cl_p$ = 1.2, $Vd_{ss}$ = 0.7, $t_{1/2}$ = **7.4**, %$F$ = 89 |

[a] Ligand-induced uptake of [$^{35}$S]-GTPγS on CHO cell membranes expressing human S1P receptors. Data are reported as means for $n$ = 3 measurements. SD were generally within ±20% of the average.

[b] The pharmacodynamic $ED_{50}$ value is the effective dose of test compound that induces 50% of maximal peripheral lymphocyte lowering (PLL) in mice.

[c] 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. The units for $Cl_p$, $Vd_{ss}$, and $t_{1/2}$ are mL/min/kg, L/kg, and hour, respectively.

[d] Enantiomers **18b′** and **18b″** were prepared by chiral separation of (±)-*tert*-butyl ester of **18b** on Chiralcel OD 4.6 × 25 cm column (eluted with EtOH/heptane (v:v = 1:9) at a rate of 0.5 mL/min), followed by hydrolysis.

low dosage in mice and exhibited similar pharmacokinetic properties in rat with the exception of half-life. In rodent, half-life is highly sensitive to the structural modification on the propionic acid side chain as well as the replacement of the adjacent phenyl ring with a pyridine group. This result suggests that metabolic oxidation of the propionic acid group, especially at the C3 benzylic position, is largely responsible for the short half-life of 3-arylpropionic acids in rodent.

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6. Compound **1a** also exhibited short half-life in beagle dog (0.5 mpk iv, 1.0 mk po): $Cl_p$ = 12.6 mL/min/kg, $Vd_{ss}$ = 0.5 L/kg, $t_{1/2}$ = 1.4 h, %$F$ = 70.
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12. The S1P functional binding affinities ($EC_{50}$) for the test compounds were determined by measuring the binding of [$^{35}$S]-GTP$\gamma$S to S1P receptors expressed in transfected Chinese hamster ovary (CHO) cell membranes. The S1P receptor binding affinities ($IC_{50}$) were determined in competitive binding assays in CHO membranes expressing S1P receptors with $^{33}$P-labelled S1P as the ligand. (See Ref. 2a for detailed experiment protocols.) We found that, like the 3-arylpropionic acids, these modified 3-arylpropionic acids appeared to be more potent when evaluated in the functional assay than in the competitive binding assay. The reason for such affinity shift is not fully understood. We found that their $EC_{50}$ values served as a better guide for interpreting in vivo activity. All 3-arylpropionic acids and their modified analogs are found to be inactive against $S1P_{2,4}$ receptors ($IC_{50}$ > 10 μM).
13. The pharmacodynamic $ED_{50}$ value is the effective dose of test compound that induces 50% of maximal peripheral lymphocyte lowering (PLL) in mice. Compound-induced PLL was determined by measuring the reduction percentage of the absolute peripheral lymphocyte counts at a three-hour time point after the oral administration of the test compound in comparison to those from vehicle controls (see Ref 2a for detailed experimental protocols).