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Novel $S1P_1$ Receptor Agonists – Part 3: From Thiophenes to Pyridines

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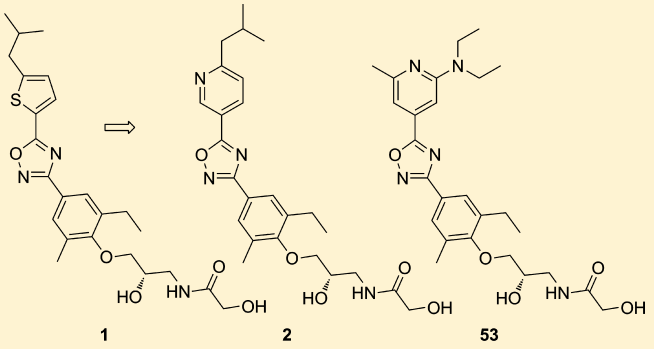
Supporting Information

ABSTRACT: In preceding communications we summarized our medicinal chemistry efforts leading to the identification of potent, selective, and orally active active $S1P_1$ agonists such as the thiophene derivative **1**. As a continuation of these efforts, we replaced the thiophene in **1** by a 2-, 3-, or 4-pyridine and obtained less lipophilic, potent, and selective $S1P_1$ agonists (e.g., **2**) efficiently reducing blood lymphocyte count in the rat. Structural features influencing the compounds' receptor affinity profile and pharmacokinetics are discussed. In addition, the ability to penetrate brain tissue has been studied for several compounds. As a typical example for these pyridine based $S1P_1$ agonists, compound **53** showed EC_{50} values of 0.6 and 352 nM for the $S1P_1$ and $S1P_3$ receptor, respectively, displayed favorable PK properties, and penetrated well into brain tissue. In the rat, compound **53** maximally reduced the blood lymphocyte count for at least 24 h after oral dosing of 3 mg/kg.



INTRODUCTION

Synthetic sphingosine 1-phosphate 1 ($S1P_1$) receptor agonists hold great promise for treating inflammatory disorders and autoimmune diseases,[1−3] and the number of clinical trials investigating their safety and efficacy is growing rapidly. While fingolimod[4−7] has approval to treat relapsing multiple sclerosis, siponimod,[8−10] ponesimod,[11,12] and ONO-4641 (structure undisclosed)[13,14] successfully completed phase II and RPC-1063 (structure undisclosed)[15] and MT-1303 (structure undisclosed)[16] recently entered phase II clinical trials for this debilitating disease. The last two compounds are also evaluated in patients suffering from ulcerative colitis[17] and inflammatory bowel disease,[18] respectively. Results of an open-label phase I study in MS patients treated with CS-0777 have been published recently.[19,20] In addition, siponimod is currently studied in patients suffering from secondary progressive multiple sclerosis[21] as well as in patients with polymyositis and dermatomyositis.[22] Ponesimod, on the other hand, successfully completed a phase II trial for chronic plaque psoriasis.[23,24] Furthermore, fingolimod's ability to reduce graft rejection has been studied in renal transplant patients,[25] and KRP-203[26] was investigated in patients with subacute cutaneous lupus erythematosus[27] and refractory ulcerative colitis.[28] APD334 (structure undisclosed)[29,30] recently entered phase I clinial trials. Additional molecules emerged from discovery programs at Almirall,[31,32] Glaxo,[33−35] Bristol-Myers Squibb,[36] e.g.[37] and other companies,[38−41] but it is not known whether they entered clinical development yet.

The lysophospholipid sphingosine 1-phosphate (S1P) is the natural ligand of five G-protein-coupled receptors named $S1P_1$−$S1P_5$.[42] S1P−S1P receptor signaling is involved in many cellular responses including survival, proliferation, differentiation, adhesion, migration, and chemotaxis[43−45] and leads to angiogenesis, endothelial barrier enhancement, airway and blood vessel constriction, alveolar epithelial barrier disruption, heart rate modulation, neurite extension, and bone homeostasis.[46−49] The physiological role of S1P receptor signaling has been studied in several organs and tissues such as the nervous system,[50−52] the lung,[46,53] the cardiovascular system,[54−58] the cells of the immune system,[59−61] and bone tissue,[62] and S1P receptor activation has been implicated in many pathological situations such as autoimmunity, inflammation, cardiovascular disorders, and cancer.[3,60,61,63−66]

S1P plays an important role in the motility of various cell types,[62,67,68] in particular of the immune system.[69−72] Lymphocytes continuously circulate through the body, thereby monitoring the body for microbial intruders or aberrant cells and home to lymph nodes and Peyer's patches.[73] In order to leave these secondary lymphoid organs and return to

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circulation, lymphocytes follow the gradient of S1P that exists between lymph and blood. This chemotactic event is driven by the S1P/$S1P_1$ receptor signaling axis.[69,74−76] Synthetic $S1P_1$ agonists have been shown to trigger internalization of the $S1P_1$ receptor, thereby abolishing the lymphocyte's ability to sense the S1P gradient.[76,77] As a consequence, lymphocytes are sequestered to lymph nodes and no longer migrate to other organs. Interfering with lymphocyte trafficking by $S1P_1$ receptor modulation represents an attractive approach to treat a variety of lymphocyte dependent autoimmune diseases, as demonstrated in several clinical trials. In contrast, activation of the $S1P_3$ receptor is deemed undesirable, as it has been associated with effects on heart rate,[78−84] vaso- and bronchoconstriction,[82,85] hypertension,[84] and enhancement of fibrosis[86−88] in animal studies.

In addition to the peripheral immunomodulatory action of $S1P_1$ receptor agonists, more recent studies identified modulation of the $S1P_1$, $S1P_3$, or $S1P_5$ receptors on neural cells like astrocytes and oligodendrocytes as a nonimmunologic central nervous system (CNS) effect possibly contributing to the efficacy of synthetic S1P receptor agonists in multiple sclerosis.[6,89−95] Following these arguments, brain penetration of a synthetic $S1P_1$ receptor agonist may be beneficial for a compound's efficacy in CNS related diseases such as multiple sclerosis. On the other hand, for the treatment of peripheral diseases such as psoriasis or Crohn's disease, CNS penetration of a S1P receptor agonist is not needed or may even be undesired. Hence, the utility of a compound for different diseases may depend on its ability to penetrate brain tissue, and we therefore characterized some of our most advanced compounds with respect to their potential to penetrate the brain.

Previously we described the discovery of a novel series of selective $S1P_1$ receptor agonists incorporating a 5-isobutyl substituted thiophene.[96] A prototypical example, compound **1** (Figure 1), had EC_{50} values of 0.7 and 320 nM in a GTPγS

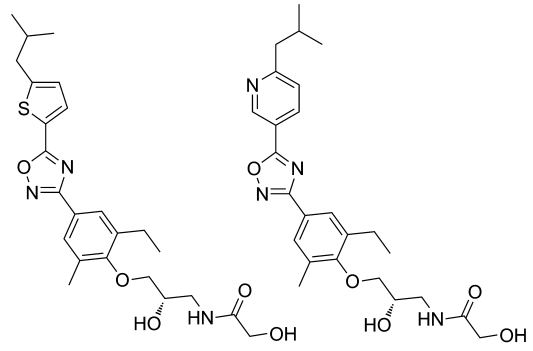


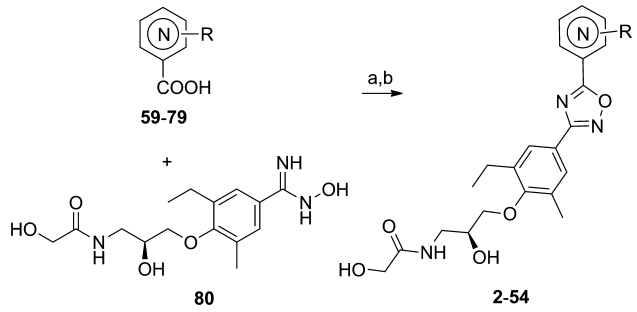
Figure 1. Structures of thiophene based $S1P_1$ receptor agonist **1** and a first 3-pyridine analogue **2**.

assay for $S1P_1$ and $S1P_3$, respectively. With a clogP value of 3.97 this thiophene derivative is rather lipophilic. As high lipophilicity has been shown to be associated with compound liabilities such as target promiscuity, hERG inhibition, poor aqueous solubility, metabolic instability, or high protein binding,[97−101] we embarked on a program in which the thiophene head of our $S1P_1$ receptor agonists was replaced by a pyridine. A first such analogue, pyridine derivative **2**, has a clogP of 2.74 and showed an EC_{50} value of 5.7 nM for $S1P_1$, making the pyridine an attractive alternative to the thiophene in **1**. In the following sections we describe our detailed studies aiming at the discovery of pyridine containing $S1P_1$ receptor agonists meeting the following requirements: the compound has a lower clogP than thiophene **1** (ideally <3), is highly potent on $S1P_1$ (EC_{50} < 5 nM) with an affinity of >250 nM for $S1P_3$ and a more than 100-fold selectivity against this receptor, and shows maximal lymphocyte count reduction for at least 24 h when administered at a dose of 10 mg/kg to Wistar rats. For compounds fulfilling these criteria the ability to penetrate the brain would then be measured to assess their utility in different diseases.

RESULTS AND DISCUSSION

Synthesis. The target compounds **2**−**54** for this study were usually prepared by coupling each of the pyridine carboxylic acids **59**−**79** with *N*-hydroxybenzamidine **80** followed by cyclizing the hydroxyamidine ester intermediate to the desired oxadiazole (Scheme 1). *N*-Hydroxybenzamidine **80** was

Scheme 1. Assembling the Central Oxadiazole Ring[a]



[a]Reagents and conditions: (a) HOBt, EDC HCl, THF, rt, 18 h; or TBTU, Hünig's base, DMF, rt, 1 h; (b) dioxane, 80 °C, 18 h, 6−76% (two steps).

prepared by formylating phenol **81** in a Duff reaction to give benzaldehyde **82** (Scheme 2). Reacting **82** with hydroxylamine hydrochloride in NMP under microwave irradiation furnished benzonitrile **83** which was then alkylated with (*R*)-glycidol under Mitsunobu conditions. Epoxide **84** was reacted with ammonia in methanol to give amino alcohol **85**. Coupling of **85** with glycolic acid to give **86** established the side chain attached to the benzene ring. Finally, nitrile **86** was reacted with hydroxylamine hydrochloride to afford the desired building block **80**. In some cases the polar side chain attached to the para position of the phenyl moiety was introduced after the oxadiazole ring had been established. In this case, the appropriate pyridine carboxylic acid was reacted with 3-ethyl-*N*,4-dihydroxy-5-methylbenzamidine and the side chain was established in analogy to the steps outlined in Scheme 2 (for details see Supporting Information). For details on the synthesis of compounds **55**−**58** (Table 7) see Supporting Information.

Prototypical syntheses of the various pyridinecarboxylic acids are exemplified in Schemes 3, 4, 5, and 6. Alkyl groups could be introduced to the pyridines by making use of transition metal catalyzed cross-coupling reactions developed by Suzuki,[102−104] Negishi,[104] and Fürstner.[105,106] The choice of the reaction type was generally driven by the availability of the corresponding alkyl electrophile. Alkylamines were introduced either by simple thermal displacement or by a Pd-catalyzed Buchwald−Hartwig

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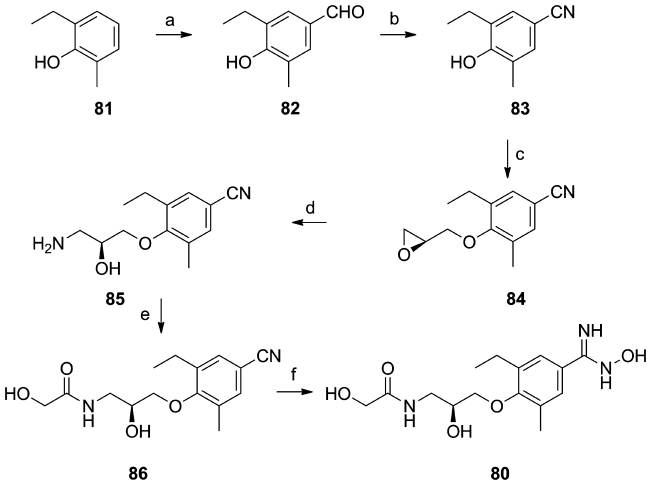
Scheme 2. Preparation of the *N*-Hydroxybenzamidine Building Block 80[a]

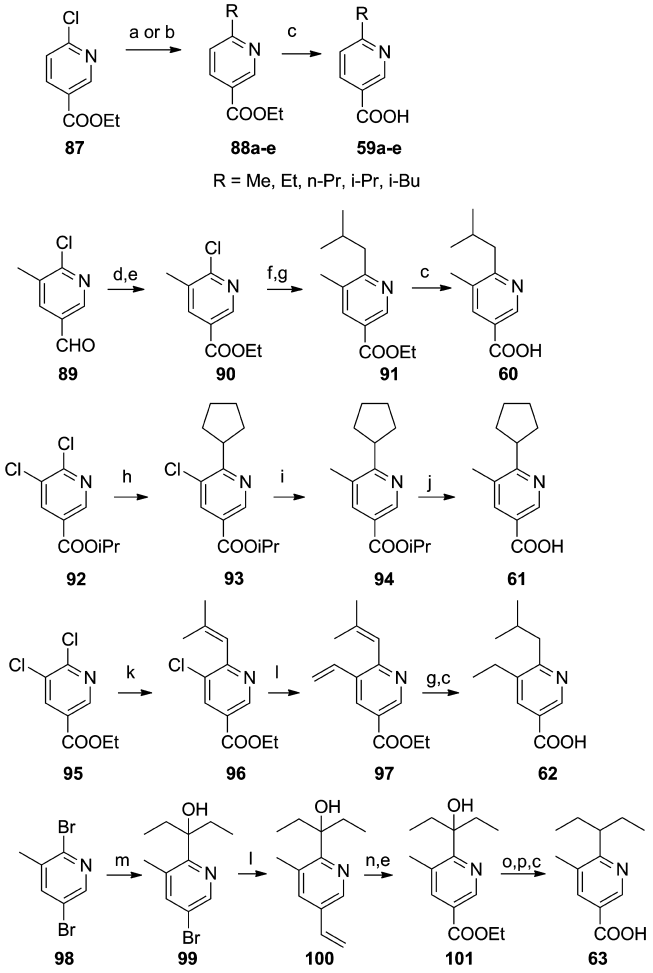


[a]Reagents and conditions: (a) hexamethylenetetraamine, HOAc, H_2O, 120 °C, Dean–Stark 2–3 h, 53–97%; (b) $HONH_2$ HCl, NMP, microwave 80–100 °C, 0.5–3 h; 86–95%; (c) (*R*)-glycidol, PPh_3, DEAD, THF, 0–20 °C, 18 h, 72–86%; (d) 7 N NH_3 in MeOH, 65 °C, 18 h (sealed vessel), quantitative (crude); (e) glycolic acid, EDC HCl, HOBt, rt, 18 h, 51–90%; (f) $HONH_2$ HCl, $NaHCO_3$ or Et_3N, MeOH or EtOH 60–80 °C, 18 h, 60–87%.

reaction.[107] For more details see Supporting Information. Scheme 3 illustrates the synthesis of some nicotinic acids. 6-Alkylnicotinic acid esters **88a–e** were obtained by reacting ethyl 6-chloronicotinate **87** either with the appropriate alkenyl boronic ester under Suzuki conditions followed by catalytic hydrogenation or with the appropriate Grignard reagent under Fürstner conditions. Ester cleavage under acidic conditions afforded the 6-alkylnicotinic acid building blocks **59a–e**. 6-Alkyl-5-methylnicotinic acids (e.g., **60**) were prepared in a similar fashion starting from ethyl 5-methyl-6-chloronicotinate **90** employing 2,4,6-tri-(2-alkenyl)cyclotriboroxanepyridine complexes or alkenylboronic acid pinacol esters under Suzuki conditions.[108] Catalytic hydrogenation of the ethyl 6-alkenylnicotinate intermediate furnished the corresponding ethyl 6-alkyl-5-methylnicotinate (e.g., **91**) which upon acidic ester cleavage afforded the desired nicotinic acid building block (e.g., **60**). Ethyl nicotinate **90** was prepared by oxidizing aldehyde **89**[109] with hydrogen peroxide and subsequent esterification of the resulting nicotinic acid carboxylic acid. In an alternative approach, isopropyl 5,6-dichloronicotinate **92** was first reacted with cyclopentylmagnesium bromide at room temperature in the presence of $Fe(acac)_3$ to give isopropyl 5-chloro-6-cyclopentylnicotinate **93** which was then converted to nicotinate **94** using dimethylzinc at 75–85 °C. Saponification yielded nicotinic acid **61**. Reacting ethyl 5,6-dichloronicotinate **95** with 2,4,6-tri(2-methylprop-1-en-1-yl)cyclotriboroxane-pyridine complex under aqueous Suzuki conditions furnished 5-chloronicotinate **96** which was then subjected to anhydrous Suzuki conditions in the presence of 2,4,6-trivinylcyclo-triboroxanepyridine complex to afford 5,6-dialkenylnicotinate **97**. Hydrogenation and ester cleavage furnished 5-ethyl-6-isobutylnicotinic acid **62**.

In the case of the 3-pentyl derivative **63** the above approaches were unsuccessful. We therefore followed an alternative strategy. Thus, 2,5-dibromo-3-methylpyridine **98** was lithiated using 1.1 equiv of *n*-butyllithium and then reacted

Scheme 3. Preparation of 6-Alkylnicotinic Acids[a]



[a]Reagents and conditions: (a) 2-alkenyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $Pd(PPh_3)_4$, PPh_3, 2 M aq K_2CO_3, 80 °C, 18 h, 66%, then 5 bar of H_2, Pd/C, methanol, 18 h, 46%; (b) alkylmagnesium bromide, $Fe(acac)_3$, THF, NMP, −75 °C, 1–4 h, 47–93% (c) 4–7 N aq HCl, 65–80 °C, 18–24 h, 91–97%; (d) 50% H_2O_2, HCOOH, 0 °C, 15 h, 47–87%; (e) EtOH, H_2SO_4, or TMSCl, reflux 18–42 h, 65–92%; (f) 2,4,6-tri(2-methylpropenyl)cyclotriboroxanepyridine complex or alkenylboronic acid pinacol ester, Ph_3P, $Pd(PPh_3)_4$, DME or dioxane, 2 M aq K_2CO_3, 90 °C, 20 h, 71–73%; (g) Pd/C, H_2, MeOH, THF, rt, 15 h, 61–99%; (h) cyclopentylmagnesium bromide, $Fe(acac)_3$, THF, NMP, 0 °C, 1 h, rt, 18 h, 78%; (i) Me_2Zn, $Pd(dppf)Cl_2$, dioxane, 75–85 °C, 3–18 h, 36–58%; (j) 2 M aq LiOH, MeOH or EtOH, rt, 1–2 h, 83–88%; (k) 2,4,6-tri(2-methylpropenyl)-cyclotriboroxanepyridine complex, Ph_3P, $Pd(PPh_3)_4$, DME, 2 M aq K_2CO_3, 100 °C, 4.5 h, 92%; (l) 2,4,6-trivinylcyclotriboroxanepyridine complex, Cs_2CO_3, tri-*tert*-butylphosphine, $Pd_2(dba)_3$, or PPh_3, Pd-$(PPh_3)_4$, dioxane, 100 °C, 16–40 h, 59–67%; (m) *n*-BuLi, 3-pentanone, THF, −70 °C, 2 h, 37%; (n) $KMnO_4$, acetone, rt, 24 h, quantitative crude; (o) Burgess reagent, dioxane, 80 °C, 1 h microwave, 76%; (p) 1 bar of H_2, Pd/C, THF/methanol 1:1, rt, 4 h, 80%.

with 3-pentanone to form 5-bromopyridine **99**, which was converted to vinylpyridine **100** using trivinylcyclotriboroxane-pyridine complex under Suzuki conditions. The olefin in **100** was cleaved by $KMnO_4$, and the resulting acid was esterified to give nicotinate **101**. Treating **101** with Burgess reagent under microwave irradiation effected dehydration, and the corresponding unsaturated product was hydrogenated using H_2 on

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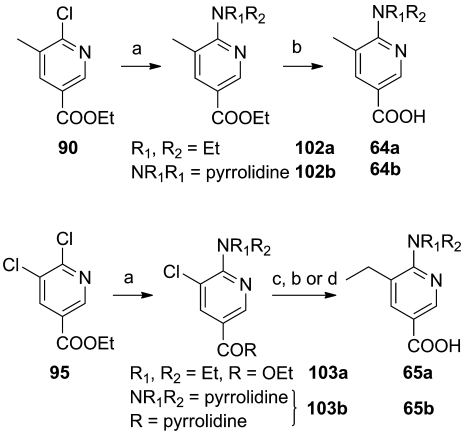
Scheme 4. Preparation of 6-Aminonicotinic Acids[a]



[a]Reagents and conditions: (a) R_1R_2NH, 80−100 °C, 1 day to 3 weeks, 85−99%; (b) 4−7 N aq HCl, 65−80 °C, 18−24 h, quantitative; (c) Et_2Zn, Pd(dppf)Cl_2, dioxane, 75 °C, 18−24 h, 84% to quantitative; (d) 3 N aq NaOH, MeOH, 75 °C, 18 h, 79%.

Pd/C. Saponification of this material finally furnished the desired 6-(3-pentyl)nicotinic acid derivative **63**.

6-Aminonicotinic acids **64a**,**b** were obtained from their esters **102a**,**b** which were prepared by heating 6-chloronicotinate **90** in a sealed vessel with a large excess of the corresponding amine (Scheme 4). Alternatively, 5,6-dichloronicotinate **95** was reacted with diethylamine to form ethyl 5-chloro-6-aminonicotinate **103a**. When pyrrolidine was used as the amine component, the ester in **95** was transformed to the corresponding pyrrolidine amide **103b**. Pd-catalyzed Negishi coupling of **103a**,**b** with diethylzinc and subsequent ester hydrolysis afforded the 6-amino-5-ethylnicotinic acids **65a**,**b**.

The synthesis of prototypical 4,5-, 5,6-, and 4,6-disubstituted picolinic acids is outlined in Scheme 5. Reacting 2,5-dibromopyridine **104** or **108** with trivinylcyclotriboroxane-pyridine complex selectively furnished the corresponding 2-vinylpyridines **105** and **109** in good yields. Potassium permanganate mediated oxidation established the carboxylic acids which were esterified to deliver **106** and **110**. A second Suzuki reaction using 2,4,6-tri(2-methylpropenyl)-cyclotriboroxanepyridine complex produced the 5-alkenylpicolinic acid derivatives **107** and **111**. Catalytic hydrogenation and subsequent ester cleavage completed the synthesis of the two picolinic acids **66** and **67**. Negishi coupling of 5-bromo-4-methylpicolinate **106** with cyclopentylzinc bromide furnished ester **112** which was cleaved under basic conditions to give cyclopentyl-4-methylpicolinic acid **68**. 5-Diethylaminopicolinic acids **69** and **70** were prepared by reacting 5-bromopicolinates **113** and **114**, respectively, with diethylamine under Buchwald−Hartwig conditions and subsequent ester hydrolysis. Although the yields of these cross-coupling reactions were rather low, sufficient material could be isolated for the purpose of our studies. The preparation of 4,6-disubstituted picolinic acids followed a strategy similar to the one pursued for the 3,4- and 2,3-disubstituted picolinic acids **66** and **67**. Hence, 2,6-dichloro-4-methylpyridine **115** was first converted to 2-chloro-4-methyl-6-vinylpyridine **116** and then oxidized and esterified to give 2-chloropicolinate **117**. Suzuki cross-coupling of this material with 2,4,6-tri(2-methylpropenyl)cyclotriboroxanepyridine complex furnished the 6-isobutenylpicolinate **118** which, after hydrogenation and ester hydrolysis, produced picolinic acid **71**.

The isomeric picolinic acid **72** was prepared following the same strategy starting from 2,4-dibromopicoline **119**. Thus, a vinyl group was introduced to position 6 of **119** via Suzuki reaction to give **120** which was then oxidized and esterified to form picolinate **121**. Suzuki reaction using 2,4,6-tri(2-methylpropenyl)cyclotriboroxanepyridine complex, catalytic hydrogenation, and saponification furnished the desired compound **72**. Ethyl 4-bromo-6-methylpicolinate **121** could also be prepared via an alternative route making use of the Minisci reaction.[110−112] To this end, 4-bromopicoline **123** was treated with ammonium peroxydisulfate in methanol containing a small amount of sulfuric acid to give hydroxymethylpyridine **124** in moderate yield. Oxidation of the primary alcohol with $KMnO_4$ and esterification of the resulting acid gave picolinate **121**. The 6-diethylaminopicolinic acid **73** was obtained by Buchwald reaction of 6-chloropicolinate **125** and subsequent saponification. The isomeric acid **74** was obtained by thermal displacement of the 4-bromo substituent of acid **126**. To enhance purification, the acid was temporarily transformed to its ethyl ester.

Finally, Scheme 6 illustrates the synthesis of 2-substituted and 2,6-disubstituted isonicotinic acids. The preparation of the 2-substituted representatives **75a**−**h** started from 2-haloisonicotinic ester **127** and involved a Negishi or a Fürstner type cross-coupling reaction with the appropriate alkylzinc or alkylmagnesium halide, respectively, and subsequent ester cleavage. The 6-methylisonicotinic acids **76a**−**g** were prepared in a similar fashion via Negishi or Suzuki reaction of the 2-halo-6-methylisonicotinate **129**. 2-Alkyl-6-ethylisonicotinic acids **77a**−**d** were prepared starting from ethyl 2,6-dichloroisonicotinate **131** by two consecutive Negishi couplings. Although the bulkier alkyl group was introduced first, the monochloroisonicotinates **132a**−**d** were usually contaminated with 10−25% of the corresponding double alkylation product. In the case of Negishi reactions employing 3-pentylzinc bromide, formation of a significant amount of the isomeric 2-(pentan-2-yl)isonicotinates clearly hampered the purification of the reaction products. Hence, Suzuki reaction and subsequent catalytic hydrogenation as employed to prepare 6-methyl isonicotinic esters **130a**−**e** often gave better results when compared to a Negishi or Fürstner cross-coupling approach.

2-Amino-6-methylisonicotinic acids **78a**,**b** and **79** were prepared starting from 2-chloro-6-methyl- or 2,6-dichloroisonicotinic acid ester **134** or **136**, respectively. A large excess of the amine was usually employed when 2-chloro-6-methyl isonicotinate **134** was reacted under thermal, noncatalyzed conditions. However, the use of a large excess of pyrrolidine led to the formation of the corresponding amide (e.g., **135b**) which then had to be cleaved under basic conditions to give the desired acid (**78b**). A much smaller excess of the amine could be used if the reaction was carried out under Pd-catalyzed Buchwald conditions (e.g., **135a**). In addition, 1 equiv of diethylamine was sufficient to efficiently convert *tert*-butyl 2,6-dichloroisonicotinate **136** to the corresponding monochloride **137**. 6-Chloropicolinate **137** was then treated with dimethylzinc to afford the 6-methyl substituted isonicotinate **138** which upon ester cleavage delivered the isonicotinic acid **79**.

In Vitro SAR Discussion. As mentioned in the introduction, we wanted to reduce the overall lipophilicity and amphiphilic nature of our $S1P_1$ agonists such as **1** (Figure 1). In our previous studies,[113,96] we learned that a large variety of polar side chains are tolerated in the 4-position of the phenyl ring of **1**. This offered a starting point to improve the

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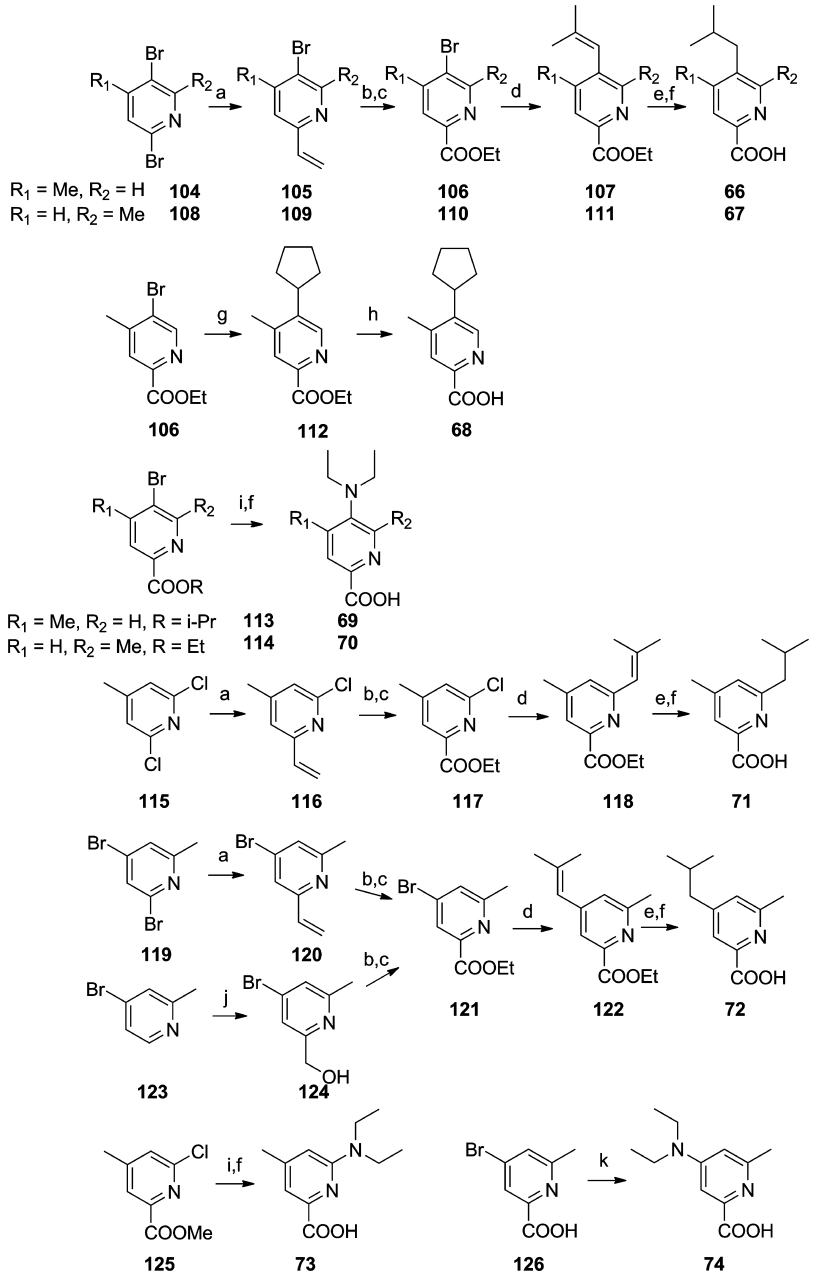
Scheme 5. Preparation of Picolinic Acids[a]



[a]Reagents and conditions: (a) 2,4,6-trivinylcyclotriboroxanepyridine complex, 2 M aq K_2CO_3, $Pd(PPh_3)_4$, DME, 80 °C, 15 h, 49−99%; (b) $KMnO_4$, acetone, water, rt, 3 days, quantitative crude; (c) EtOH, H_2SO_4 or TMSCl, 70 °C, 18 h, 40−95%; (d) 2,4,6-tri(2-methylpropenyl)cycloboroxane-pyridine complex, $Pd(PPh_3)_4$, 2 M aq K_2CO_3, DME, 80 °C, 6 h, 37−68%; (e) H_2, Pd/C, THF, EtOH, rt, 15 h, 95%; (f) 6−7 N aq HCl, 65 °C, 48 h, 64−94%; (g) cyclopentylzinc bromide, $Pd(dppf)Cl_2$, dioxane, 65 °C, 18 h, 28−70% (h) 2 M aq LiOH, dioxane, 75 °C, 5 h, 15−88%; (i) Et_2NH, $Pd(OAc)_2$, xanthphos, Cs_2CO_3, dioxane, 90−110 °C, 18−24 h, 1−9%; (j) MeOH, H_2SO_4, ammonium peroxydisulfate, 75 °C, 2 h, 19−28%; (k) Et_2NH, BuOH, reflux 16 h; EtOH, H_2SO_4, 70 °C, 18 h; 6 N aq HCl, 65 °C, 18 h, 40% (three steps).

compounds' physicochemical properties. In a next step, we explored whether polar groups were also tolerated in the headgroup. In this account, we focus our discussion on the replacement of the thiophene by a pyridine. For the following SAR discussion the (*S*)-3-(3-ethyl-4-(2-hydroxy-3-(2-hydroxyacetamido)propoxy)-5-methylphenyl)-1,2,4-oxadiazole part was kept constant, as this moiety reliably brought about high affinity and selectivity for $S1P_1$ and high in vivo efficacy.

A set of 3-pyridines representing close analogues of compound **1** is compiled in Table 1. As evident from the clogP values listed in Table 1, even pyridines bearing large and lipophilic substituents such as the 3-pentyl derivative **11** or the cyclopentylpyridines **12** and **14** are less liphophilic than thiophene **1**. The closest analogue of thiophene **1**, the isobutylpyridine **2**, had a lower clogP (2.75 vs 3.97) and showed an EC_{50} of 5.7 nM for $S1P_1$, demonstrating that replacing the thiophene in **1** by a pyridine reduces the compound's lipophilicity while retaining its affinity for the $S1P_1$ receptor. The $S1P_{1/3}$ selectivity ratio is comparable for the two compounds **1** and **2**. The potency on $S1P_1$ and $S1P_3$ remained unaffected when the isobutyl chain in **2** was replaced by an *n*-propyl (**4**) or isopropyl (**5**) group. However, when the alkyl

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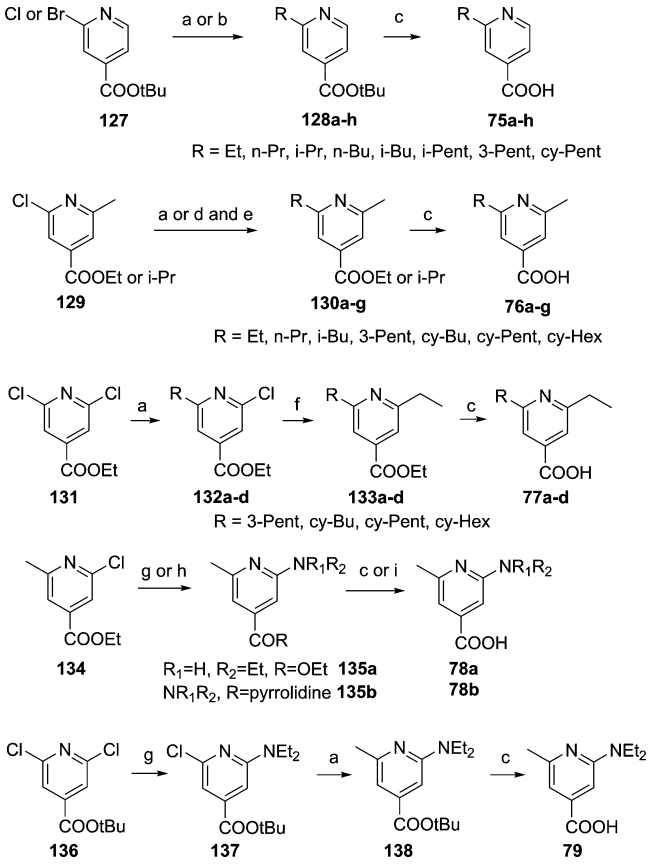
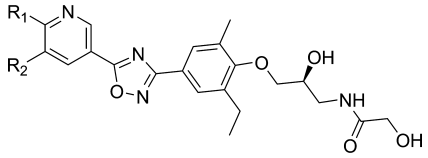
Scheme 6. Preparation of Pyridine-4-carboxylic Acids[a]



[a]Reagents and conditions: (a) alkylzinc bromide or chloride, or dialkylzinc, $Pd(dppf)Cl_2$, dioxane, 75−80 °C, 2−24 h, 21−84%; (b) alkylmagnesium bromide or chloride, $Fe(acac)_3$, THF, NMP, −75 °C, 1 h, rt, 1 h, 26−40%; (c) 6−7 M aq HCl, 60−95 °C, 3−20 h, 37% to quantitative; (d) 2,4,6-trialkenylcycloboroxanepyridine complex, PPh_3, $Pd(PPh_3)_4$, 2 M aq K_2CO_3, DME, 75−90 °C, 6−20 h, 50−95%; (e) H_2, Pd/C, THF, MeOH, rt, 5−16 h, 98%; (f) Et_2Zn, $Pd(dppf)Cl_2$, dioxane, 75−80 °C, 1−2 h, 79−82%; (g) diethylamine, 70−100 °C, 2−72 h, 95% to quantitative; (h) ethylamine, Cs_2CO_3, $Pd(OAc)_2$, Xanthphos, dioxane, 90 °C, 15 h, 44−47%; (i) 3 N aq NaOH, EtOH, 60 °C, 72 h; 87%.

group was further shortened to an ethyl (**6**) or a methyl (**7**) group, a significant potency loss was observed. As shown with compounds **8** and **10**, introducing a methyl group to position 5 of the pyridine clearly enhanced the compound's affinity for $S1P_1$ and $S1P_3$. An ethyl group in position 5 brought a further potency gain on both receptors, and several compounds (e.g., **13**, **14**, **16**) reached EC_{50} values close to 0.1 nM for $S1P_1$ and about 25 nM for $S1P_3$. While the (*R*)-enantiomer of **2**, compound **3**, was significantly less potent in particular on $S1P_1$, the two enantiomers **8** and **9** showed an almost identical affinity profile. As shown with compound **11**, replacing the isobutyl chain in **8** by a 3-pentyl group had no significant effect on $S1P_1$ but significantly increased the compound's selectivity against $S1P_3$. Interestingly, the cyclopentyl derivative **12** was clearly less selective against $S1P_3$ when compared to its open chain analogue **11**. Replacing the 3-pentyl group in **11** by diethylamine to give aminopyridine **15** was well tolerated by $S1P_1$ and slightly improved the compound's affinity for $S1P_3$. Conversely, exchanging the cyclopentyl group in **12** by a pyrrolidine to give **17** resulted in a marked affinity loss on both

Table 1. SAR of 3-Pyridines (Nicotinic Acid Derivatives)



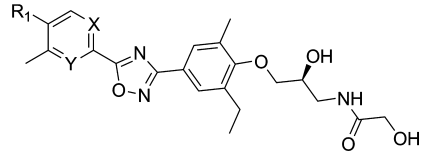
compd	R_1	R_2	clogP	EC_{50} [nM][a] $S1P_1$	EC_{50} [nM][a] $S1P_3$
1	*b*		3.97	0.7	320
2	isobutyl	H	2.75	5.7	1710
3[c]	isobutyl	H	2.75	89	5740
4	*n*-propyl	H	2.41	4.6	1770
5	isopropyl	H	2.37	3.9	2900
6	ethyl	H	1.94	23	>10000
7	methyl	H	1.59	248	>10000
8	isobutyl	Me	3.06	1.0	318
9[c]	isobutyl	Me	3.06	1.9	519
10	isopropyl	Me	2.68	0.1	429
11	pent-3-yl	Me	3.61	0.9	2630
12	cyclopentyl	Me	3.20	0.1	25
13	isobutyl	Et	3.42	0.1	29
14	cyclopentyl	Et	3.56	0.2	24
15	diethylamino	Me	2.83	2.1	616
16	diethylamino	Et	3.18	0.3	30
17	*N*-pyrrolidine	Me	2.24	23	1230
18	*N*-pyrrolidine	Et	2.59	2.8	176

[a]EC_{50} values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$.[11] EC_{50} values represent the geometric mean of at least three independent measurements. [b]For structure, see Figure 1. [c](*R*)-Enantiomer.

receptors. As observed with the alkyl substituted pyridines, the 5-ethyl analogues **16** and **18** were clearly more potent on $S1P_1$ and $S1P_3$ when compared to their 5-methyl analogues **15** and **17**, respectively.

In a next step we turned our attention to replacing the thiophene in **1** by a number of substituted 2-pyridines bearing two substituents in either an ortho or a meta relationship. A few examples illustrating their SAR are listed in Tables 2 and 3. As judged by their clogP values, the most lipophilic 2-pyridines in

Table 2. SAR of 4,5- and 5,6-Disubstituted Pyridin-2-yl Derivatives (Picolinic Acid Derivatives)



compd	R_1	X	Y	clogP	EC_{50} [nM][a] $S1P_1$	EC_{50} [nM][a] $S1P_3$
19	isobutyl	N	CH	3.02	0.2	154
20	isobutyl	CH	N	3.12	4.4	3080
21	cyclopentyl	N	CH	3.15	0.2	106
22	cyclopentyl	CH	N	3.26	0.8	860
23	diethylamino	N	CH	2.41	0.2	617
24	diethylamino	CH	N	2.52	9.5	5960

[a]EC_{50} values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$.[11] EC_{50} values represent the geometric mean of at least three independent measurements.

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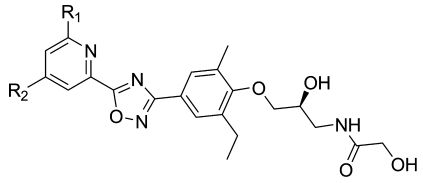
Table 3. SAR of 4,6-Disubstituted Pyridin-2-yl Derivatives (Picolinic Acid Derivatives)



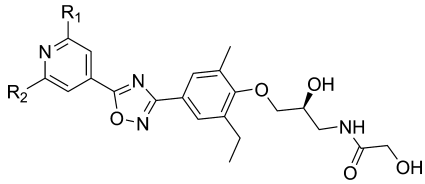
				EC_{50} [nM][a]	
compd	R_1	R_2	clogP	$S1P_1$	$S1P_3$
25	isobutyl	methyl	3.12	0.5	125
26	methyl	isobutyl	3.12	1.3	139
27	cyclopentyl	methyl	3.26	0.2	866
28	methyl	cyclopentyl	3.26	0.9	258
29	diethylamino	methyl	2.89	1.3	429
30	methyl	diethylamino	2.52	54	5240

[a]EC_{50} values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$.[11] EC_{50} values represent the geometric mean of at least three independent measurements.

Tables 2 and 3 (e.g., **22** and **28**) are less lipophilic than thiophene **1**. The 4,5-disubstituted 2-pyridines **19**, **21**, and **23** are all highly potent $S1P_1$ agonists with affinities and selectivity profiles very close to those of their 3-pyridine analogues **8**, **12**, and **15**, respectively (Table 1). Compound **21** appears to be somewhat more selective against $S1P_3$ when compared to its analogue **12**. The 5,6-disubstituted 2-pyridines **20**, **22**, and in particular **24** were less potent on both S1P receptors when compared to the corresponding 3-pyridine analogues **8**, **12**, and **15**, respectively. In the series of the 4,6-disubstituted 2-pyridines, compounds **25**−**28** were highly potent $S1P_1$ agonists, and no relevant difference in the affinity profile was seen between isomers (i.e., **25** vs **26**, **27** vs **28**). This is in contrast to diethylaminopyridines **29** and **30** where the 6-methyl substituted isomer **30** was significantly less potent than 4-methyl isomer **29**. A similar trend was observed between compounds **23** and **24**.

The observation that incorporation of 4,6-disubstituted 2-pyridines led to potent and selective $S1P_1$ agonists prompted us to extend our study to 4-pyridine derivatives. Table 4 compiles some prototypical examples illustrating their SAR. As observed with the 2- and the 3-pyridine derivatives, the 4-pyridines were generally less lipophilic when compared to thiophene **1** and only examples incorporating rather large lipophilic substituents (e.g., as in **48**, **51**) showed a clogP as high as the one of thiophene **1**. The least lipophilic compound of this series, the unsubstituted 4-pyridine **31**, gave only weak activity on the $S1P_1$ receptor. However, the compound's affinity for $S1P_1$ very rapidly improved with increasing length of a 2-alkyl substituent, and the 2-ethylpyridine **33** already represented a highly potent and selective $S1P_1$ agonist. A further increase in the length of the alkyl group brought a less profound gain in $S1P_1$ affinity. The *n*-butyl (**36**) and in particular the isopentyl (**38**) derivative were in fact less potent when compared to their isomers **37** and **39**, indicating that the ideal alkyl substituent may be branched but should not exceed more than three consecutive carbons in the chain. In general, there is a trend for compounds **32**−**40** to become more potent on $S1P_3$ with increasing size of the alkyl substituent. As for $S1P_1$, the isopentylpyridine **38** appears to mark the turn of this trend.

Table 4. SAR of 2- and 2,6-(Di)Substituted Pyridin-4-yl Derivatives (Isonicotinic Acid Derivatives)



				EC_{50} [nM][a]	
compd	R_1	R_2	clogP	$S1P_1$	$S1P_3$
31	H	H	1.17	1110	>10000
32	methyl	H	1.59	86	>10000
33	ethyl	H	1.94	2.1	9950
34	*n*-propyl	H	2.41	0.7	1120
35	isopropyl	H	2.37	0.3	3940
36	*n*-butyl	H	2.87	1.8	700
37	isobutyl	H	2.75	0.2	757
38[b]	isopentyl	H	3.21	8.4	3730
39	pent-3-yl	H	3.30	0.1	1340
40	cyclopentyl	H	2.88	0.1	736
41	ethyl	Me	2.36	0.3	1532
42	*n*-propyl	Me	2.83	0.4	310
43	isobutyl	Me	3.17	0.1	33
44	pent-3-yl	Me	3.71	0.2	46
45	cyclobutyl	Me	2.98	0.1	24
46	cyclopentyl	Me	3.30	0.2	47
47	cyclohexyl	Me	3.62	1.0	149
48	pent-3-yl	Et	4.07	0.2	3.6
49	cyclobutyl	Et	3.34	0.1	5.0
50	cyclopentyl	Et	3.65	0.3	2.4
51	cyclohexyl	Et	3.98	2.0	25
52	ethylamino	Me	2.07	0.4	868
53	diethylamino	Me	2.93	0.6	352
54	*N*-pyrrolidinyl	Me	2.34	1.9	307

[a]EC_{50} values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$.[11] EC_{50} values represent the geometric mean of at least three independent measurements. [b]Racemate.

Compounds **41**−**47** bearing an additional 6-methyl substituent were all highly potent $S1P_1$ agonists, indicating that the additional methyl group was well tolerated by this receptor. The 2-ethyl-6-methyl derivative **41** appears to be slightly more potent when compared to the corresponding des-methyl analogue **33**. In the case of the *n*-propylpyridine **42**, the affinity gain on $S1P_1$ was not significant. The additional 6-methyl group markedly increased the activity of compounds **41**−**47** on $S1P_3$ (compare **44** with **39**, **46** with **40**). A further gain in potency on $S1P_3$ was observed when the 6-methyl group was replaced by an ethyl group. As a consequence, compounds **48**−**51** were almost equipotent on both S1P receptors.

The activity of the alkylaminopyridines **52** and **53** on $S1P_1$ was nearly identical to the one of the corresponding alkylpyridine analogues **42** and **44**, while the pyrrolidine derivative **54** was less potent than its cyclopentane analogue **46**. On the other hand, the aminopyridines had lower clogP values and showed a clearly reduced affinity for $S1P_3$ when compared to the corresponding alkylpyridines (compare **53** with **44** or **54** with **46**), making them a particularly attractive subclass of compounds.

In brief, all substituted pyridine isomers constituted potent $S1P_1$ agonists. More specifically, we have seen that in the 3-

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pyridine series potent $S1P_1$ agonists were obtained if the substituent at position 6 of the pyridine consisted of at least three carbon atoms. An additional substituent in position 5 improved the affinity for both $S1P_1$ and $S1P_3$ at least 5-fold. In the series of the 2-pyridine derivatives, compounds incorporating a 4,5-, a 5,6-, or a 4,6-substitution pattern have been studied. While the 4,5- and the 4,6-disubstituted 2-pyridine compounds led to highly potent $S1P_1$ agonists, compounds with a 5,6-disubstitution pattern were slightly less active. In the 4-pyridines, an ethyl substituent in position 2 was sufficient to obtain highly potent $S1P_1$ agonists. A larger substituent in postion 2 and/or an additional substituent in position 6 mainly improved the compound's affinity for $S1P_3$. The amino-4-pyridine derivatives had lower clogP values and were usually more selective against $S1P_3$ when compared to the corresponding alkylpyridine analogues.

Pharmacokinetics and Pharmacodynamics. In the body, $S1P_1$ agonists lead to sequestration of circulating lymphocytes to lymph nodes and lymphoid tissue. Blood lymphocyte numbers can be measured easily by means of hemocytometry and therefore represent an attractive biomarker to assess a compound's in vivo activity. Hence, the ability to reduce the blood lymphocyte count (LC) was studied with several of the highly potent $S1P_1$ agonists discussed above. While a high potency on $S1P_1$ was required for a compound to be included in the pharmacodynamic experiment, we did not restrict these studies to highly selective compounds only in order to obtain a representative data set for each pyridine isomer subseries. The compounds were administered orally at a dose of 10 mg/kg to Wistar rats and the blood LC was measured shortly before and 3, 6, and 24 h after compound administration. A LC reduction of ≥60% was considered to be the maximal effect observable under the experimental conditions. A sustained 24 h plasma exposure resulting in sustained maximal LC reduction appeared desirable from a compound efficacy and safety perspective. In humans, a transient heart rate reduction after oral dosing of (selective) $S1P_1$ receptor agonists has been observed.[8,114−116] Model studies in guinea pigs suggested that the $S1P_1$ receptor is rapidly desensitized upon activation by a $S1P_1$ receptor agonist and sustained plasma concentrations of the agonist protect the animal from second dose effects on heart rate.[117] Table 5 summarizes our results.

At 3 h all compounds tested displayed maximal LC reduction, indicating a rapid onset of action irrespective of the pyridine isomer series the compound belongs to. In general, maximal LC reduction was maintained for 24 h. Notable exceptions were the two closely related 2-pyridines **26** and **28** and the 4-pyridine **39**. Although these three compounds were highly potent on $S1P_1$, they did not significantly lower LC at 24 h.

In the LC experiments with the cyclopentyl substituted 2-pyridines **21** and **28**, compound concentrations were determined in plasma (Table 6). The data show a good PK−PD correlation. In line with its long duration of action, compound **21** still showed nearly micromolar plasma concentrations at 24 h. On the other hand, compound **28** reached micromolar concentrations 3 and 6 h after administration but was more rapidly cleared and almost completely removed from circulation at 24 h. Metabolic stability testing using rat liver microsomes (RLM) revealed that compound **28** is less stable when compared to the corresponding isomer **21** (Tables 5 and 6), explaining its shorter duration of action in vivo. Similar differences observed between the microsomal stability of the two isobutyl-2-pyridines **19** and **26** may also explain the corresponding differences in the compounds' PD profiles. Rat microsomal stability data revealed that the pent-3-ylpyridine **39** is less stable than its cyclopentyl analogue **40** (Table 5), offering an explanation of why compound **39** showed a shorter duration of action relative to analogue **40**. A similar difference in microsomal stability between pent-3-ylpyridines **11** and **44** and their cyclopentyl analogues **12** and **46**, respectively, indicated that the pent-3-yl residue in general leads to metabolically less stable compounds than the cyclopentyl analogues (Table 5).

Table 5. Effect on Blood LC after Oral Administration of 10 mg/kg to Wistar Rats and in Vitro Intrinsic Clearance Measured in Rat Liver Microsomes

		% LC after dose			
compd	type	3 h	6 h	24 h	CL_{int} in RLM[a] [(μL/min)/mg protein]
8[b]	3-pyridine	−68	−61	−69	nd
11	3-pyridine	nd	nd	nd	253
12	3-pyridine	−75	−81	−85	8
13[b]	3-pyridine	−55	−61	−66	nd
15	3-pyridine	−59	−61	−67	56
19	2-pyridine	−70	−74	−65	29
21	2-pyridine	−68	−68	−71	29
25	2-pyridine	−69	−74	−61	17
26	2-pyridine	−64	−67	−20	48
27	2-pyridine	−67	−69	−50	21
28	2-pyridine	−67	−63	9	85
39	4-pyridine	−71	−71	7	112
40	4-pyridine	−70	−73	−71	40
41	4-pyridine	−67	−74	−81	2.0
42	4-pyridine	−61	−67	−73	5.7
43	4-pyridine	−62	−65	−71	27
44	4-pyridine	−59	−63	−53	65[c]
46	4-pyridine	−67	−73	−76	17[d]
47	4-pyridine	−57	−59	−67	nd
50	4-pyridine	−62	−67	−68	nd
51	4-pyridine	−66	−65	−70	nd
53	4-pyridine	−64	−64	−70	1.0[e]
54	4-pyridine	−73	−76	−82	nd

[a]Intrinsic clearance in the presence of rat liver microsomes (RLM) at 1 μM compound concentration. [b]Compound tested as racemate. [c]Intrinsic clearance at 0.5 and 0.1 μM **44** was 107 and 233 μL/(min mg), respectively. [d]Intrinsic clearance at 0.5 and 0.1 μM **46** was 20 and 12 μL/(min mg), respectively. [e]Intrinsic clearance at 0.5 and 0.1 μM **53** was 5 and 13 μL/(min mg), respectively.

Table 6. Effect on Lymphocyte Count (LC) and Plasma Concentration after Oral Administration of 10 mg/kg Compounds 21 and 28 to Wistar Rats (n = 6)

	3 h		6 h		24 h		
compd	plasma concn [nM]	LC [%]	plasma concn [nM]	LC [%]	plasma concn [nM]	LC [%]	CL_{int} in RLM[a] [(μL/min)/mg protein]
21	4910	−68	3690	−68	800	−71	29
28	1890	−67	1070	−63	9	9	85

[a]Intrinsic clearance determined in rat liver microsomes at 1 μM compound concentration. Experiments at 0.5 and 0.1 μM gave comparable results indicating that metabolic processes are not saturated under the assay conditions.

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Table 7. Pharmacokinetic Data of Compounds 44, 46 and 53

		pharmacokinetic parameters[a]								
		oral dosing				intravenous dosing				
compd	species[b]	dose [mg/kg]	C_{max} [ng/mL] (t_{max}, [h])	AUC_{0-24h} [ng h/mL]	F [%]	dose [mg/kg]	AUC_{0-inf} [ng h/mL]	Cl [(mL/min)/kg]	V_{ss} [L/kg]	$t_{1/2}$ [h]
44	rat	10	804 (0.5)	1760	126[c]	0.5	69.9	120	3.4	0.6
		1	BLQ							
46	rat	1	49 (3.0)	436	39	0.5	566	16	7.3	5.8
53	rat	10	1090 (2.0)	16500[d]	52	1	3200	5.4	3.5	8.3
	dog	3	546 (1.0)	3250	32	1	3350	5.0	1.2	3.4
	cynomolgus	1	97 (2.0)	866	16	1	5450	3.1	1.1	4.8

[a] F = bioavailability; C_{max} = maximal concentration reached in plasma; t_{max} = time at which C_{max} was reached, $t_{1/2}$ = half-life determined with iv experiment, Cl = clearance, V_{ss} = volume of distribution. [b] Oral dosing in three Wistar rats, two Beagle dogs, or three cynomolgus monkeys; iv dosing in two Wistar rats, two Beagle dogs, or three cynomolgus monkeys; for experimental details, see Supporting Information. [c] Bioavailability exceeds 100% because of saturation of the metabolism of compound **44** in the 10 mg/kg oral dose experiment. [d] AUC_{0-32h}.

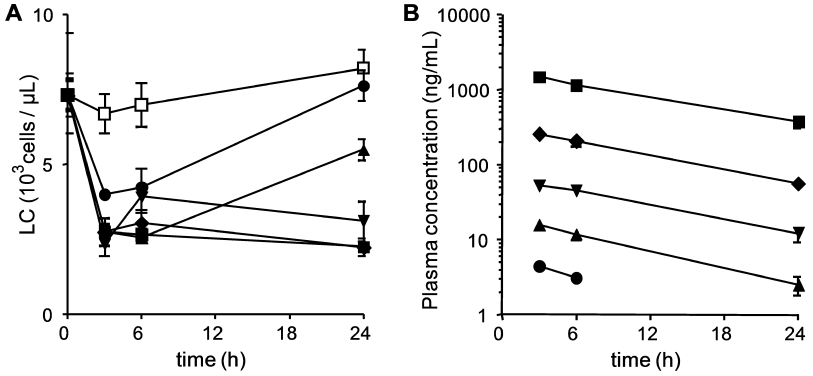

Figure 2. LC (A) and plasma concentrations (B) of a dose response experiment with amino pyridine **53** in Wistar rats: □, vehicle (n = 5); ●, 0.1 mg/kg (n = 5); ▲, 0.3 mg/kg (n = 5); ▼, 1 mg/kg (n = 5); ◆, 3 mg/kg (n = 5); ■, 10 mg/kg (n = 5). (A) LC fully recovered 96 h after administration of all doses tested (data not shown). (B) 24 h after administration of 0.1 mg/kg and 96 h after administration of 0.3, 1, 3, and 10 mg/kg plasma concentrations were below the limit of quantification (BLQ) of 1.5 ng/mL.

Interestingly, the enhanced in vitro clearance of 65 (µL/min)/mg of the pent-3-yl derivative **44** was not clearly reflected in a significantly shorter duration of action in vivo. Pharmacokinetic assessment of **44** revealed that this compound was indeed cleared very rapidly from the body with a clearance value in the range of liver blood flow (Table 7). When the compound was administered orally at a dose of 1 mg/kg, no significant exposure in the plasma was detected. At a dose of 10 mg/kg, however, plasma concentrations reached 804 ng/mL and therefore clearly exceeded dose proportionality, suggesting possible saturation of compound metabolism. A closer inspection of the in vitro clearance revealed that the intrinsic clearance of compound **44** was already saturated under the assay conditions chosen (1 µM compound concentration). Repeating the microsomal stability assay at lower concentrations of **44** showed that the unsaturated in vitro clearance is >250 (µL/min)/mg (see Table 5). From substrate depletion studies using RLM the Michaelis constant (K_M) was estimated to be <0.5 µM (see Supporting Information). Pent-3-ylpyridine **44** therefore is best characterized as a high clearance compound for which the metabolic process is saturated at relatively low concentrations. The pharmacokinetic behavior of the pent-3-yl derivative **44** is in clear contrast to the one of its close analogues **46** and **53** (Table 7). Cyclopentyl analogue **46** not only had a lower in vitro and in vivo clearance than **44** but also showed no sign of blocking its own metabolism in vitro at concentrations up to 1 µM (Tables 5 and 7). Interestingly, replacing the pent-3-yl group in **44** by a diethylamino moiety to give **53** completely abolished the metabolic instability of **44**. RLM data of **53** indicated very low intrinsic clearance (K_M = 0.06 µM), although saturation was occurring at 1 µM (Table 5). A PK experiment in the rat revealed that compound **53** was rapidly and well absorbed, slowly cleared, and easily distributed into tissue (Table 7).

In brief, several compounds listed in Table 4 meet the requirements we set forth for a compound to qualify for more detailed studies. For instance, compounds **15**, **40**, **41**, **42**, **53** were all highly potent on $S1P_1$, displayed an EC_{50} of >250 nM on $S1P_3$, and were >100-fold selective against this receptor. In particular, the last three compounds were further characterized by a very low in vitro clearance and produced a maximal LC reduction in the rat lasting for at least 24 h. In the following paragraphs we chose compound **53** to illustrate the additional experiments that were carried out to assess the compounds' viability as candidates for preclinical development.

First, a clear dose response of compound **53** on LC could be established in the rat (Figure 2A). At a dose of 0.1 mg/kg, aminopyridine **53** showed a substantial reduction of LC at 3 and 6 h. Maximal LC count reduction was achieved for 6 h with a dose of 0.3 mg, and a dose of 3 mg/kg maximally reduced LC for at least 24 h. At all doses studied, LC completely recovered within 96 h after compound administration. As shown in Figure 2B, plasma concentrations were dose proportional over the dose range of 0.1−10 mg/kg at each time point measured. The exposure data further suggest that a plasma concentration of

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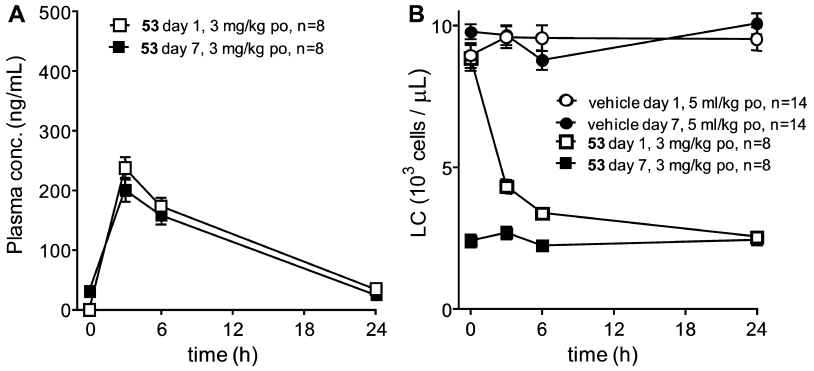
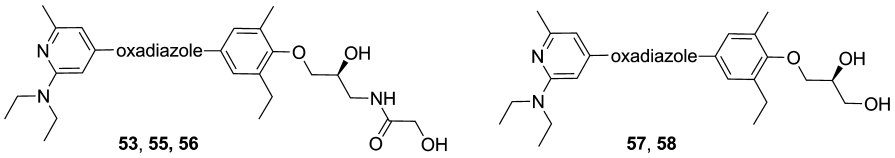


Figure 3. Plasma concentrations (A) and blood LC (B) at days 1 and 7 after repeated daily dosing of 3 (mg/kg)/day compound **53** to Wistar rats.

Table 8. Plasma and Brain Concentrations of Compounds 53, 55, 56, 57, and 58 after Oral Administration to Wistar Rats[a]



compound	oxadiazole	clogP	2 h plasma [ng/mL]	2 h brain [ng/mL]	6 h plasma [ng/mL]	6 h brain [ng/mL]	24 h plasma [ng/mL]	24 h brain [ng/mL]
53		2.93	2170	269	1775	653	412	487
55		2.93	875	140	918	369	230	301
56		3.00	815	46	510	52	8	3
57		3.86	608	435	254	271	22	34
58		3.93	820	600	315	215	4	2

[a]10 mg/kg administered orally as a suspension in 7.5% aqueous gelatin.

about 12 ng/mL (25 nM) pyridine **53** is sufficient to induce maximal reduction of the number of circulating lymphocytes.

A dose of 3 mg/kg was then selected for a multiple dose experiment with aminopyridine **53**. The compound was administered once daily (t = 0 h) on 7 consecutive days to Wistar rats, and plasma concentrations of **53** and LC were measured (Figure 3). At corresponding time points, plasma concentrations of **53** were nearly identical on days 1 and 7 and were in good agreement with the concentrations found in the single dose experiment, indicating that the compound did not accumulate upon repeated daily dosing of 3 mg/kg. Sustained maximal LC reduction was observed between 6 h after the first dose (day 1) and 24 h after the last dose (day 7) with no sign of tachyphylaxis.

In a next step, pharmacokinetic studies with compound **53** were extended to the Beagle dog and the cynomolgus monkey (Table 7). Peak plasma concentrations (C_{max}) and total plasma exposure (AUC) were comparable for all three species when corrected for the dose. As in the rat, low clearance was observed in the dog and the monkey. However, the half-life of **53** in the dog and the monkey was shorter than in the rat because of a smaller volume of distribution, and bioavailability indicated less efficient absorption in these two species.

Brain Penetration. Several research groups reported beneficial effects of $S1P_1$ receptor agonism in brain tissue, in particular in animal models of multiple sclerosis.[6,89−95] For the treatment of CNS related autoimmune diseases, it thus appears advantageous for a synthetic S1P receptor agonists to be able to penetrate the brain. On the other hand, CNS penetration of a S1P receptor agonist is not needed or may even be undesired when treating peripheral diseases such as psoriasis or Crohn's disease. To support the decision on the utility of our most interesting compounds in different diseases, their ability to penetrate the brain was studied. For the following discussion, we selected the aminopyridine **53** and a few close analogues to illustrate how compound permeability and plasma exposure influence brain exposure. Similar observations were made with compound **41** and the corresponding analogues (data not shown). A number of physicochemical parameters, biochemical assays, and computational models have been proposed to predict a compound's ability to pass the blood−brain barrier (BBB).[118−124] For example, Wager et al. recently proposed the central nervous system multiparameter optimization (CNS MPO) tool.[125] The clogP, clogD, PSA, M_w, the number of hydrogen bond donors, and the pK_a values of known CNS drugs were analyzed to design a scoring algorithm to classify the compounds according to their potential to cross the BBB. Wager et al. reported that 90% of CNS drugs show a PSA of <86 Å^2 and a M_w of <427 and incorporate one hydrogen bond donor only.[126] Other groups proposed similar values to be

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optimal for brain penetration.[120,122] In view of these observations, aminopyridine **53** clearly classified as a compound with a low potential to cross the BBB, as it has a PSA of 134 Å^2, a M_w of 497, and three hydrogen bond donors and scored only 2.9 out of 6 points using the CNS MPO tool. On the other hand, the smallest cross section of the aminopyridine **53** as calculated according to Seelig et al.[127] is 56 Å^2 and thus is clearly below the proposed maximal projection area of 80 Å^2 still allowing good permeability.[118] Plasma and brain concentrations of **53** were then measured after oral administration of 10 mg/kg to Wistar rats (Table 8). In line with the previous PK experiment, plasma concentration of **53** reached a C_{max} of 2170 ng/mL very rapidly and was still about 400 ng/mL at 24 h. Interestingly, high concentrations of **53** were also found in brain tissue. At first sight, this result appeared to contradict the above predictions. In the brain, however, C_{max} was reached much later ($t_{max} \approx 6$ h) than in plasma ($t_{max} = 2$ h). At 24 h brain concentrations were even slightly higher than in plasma, indicating that for compound **53** crossing the BBB is a rather slow process in both directions. Hence, the predictions were indeed correct in the sense that **53** crosses the BBB only slowly. The high and sustained plasma exposure of **53** compensated for the low permeability, i.e., the slow BBB passage of the compound. A similar result was obtained with the [1,2,4]oxadiazole isomer **55**. As with **53**, sustained high plasma concentrations allowed reaching significant brain concentrations despite slow permeation across the BBB. In contrast, the [1,3,4]oxadiazole **56** showed a more rapid decline of the plasma concentrations which clearly hampered building up of significant brain concentrations. (For a detailed discussion of property differencies between oxadiazole isomers see Boström et al.[128]) Compared to **53**, the two glycerol derivatives **57** and **58** are characterized by a reduced molecular weight (440) and PSA (105 Å^2) and a smaller number of hydrogen bond donors. With a CNS MPO score of 3.2 and 3.6 the glycerol derivatives **57** and **58** respectively show a more desirable property profile for brain penetration. Indeed, brain concentrations of the two glycerol derivatives **57** and **58** followed plasma concentrations very closely confirming a more rapid crossing of the BBB of these two compounds. As a consequence of the enhanced permeability of **57** and **58**, significant brain concentrations were observed at 2 and 6 h after compound administration but no sustained 24 h brain exposure was achieved. The different behavior of compounds **53** and **56** with respect to brain penetration makes them interesting tools to assess the contribution of $S1P_1$ agonism to the treatment efficiency in diseases affecting the brain. In particular, the above studies suggest that compound **53** might be well suited for the treatment of diseases for which brain penetration is important, while compound **56** may be useful for diseases for which brain penetration is not necessary or even undesirable.

CONCLUSIONS

Aiming at reducing the lipophilicity of thiophene based $S1P_1$ agonists such as compound **1**, we replaced the thiophene in **1** by a 2-, 3-, or 4-pyridine. This led to the discovery of a new series of less lipophilic $S1P_1$ receptor agonists with all three pyridine isomers furnishing potent compounds. Increasing the size of a first alkyl or alkylamino substituent in the para- or meta-position to the oxadiazole improved the compound's affinity rapidly for the $S1P_1$ and to a lesser extent for the $S1P_3$ receptor. When a second alkyl group was attached to the pyridine, the compound's potency on $S1P_1$ and $S1P_3$ was further enhanced. This time, the affinity gain was more pronounced on $S1P_3$. Representatives from all three pyridine isomer series maximally reduced blood LC for at least 24 h when administered at a dose of 10 mg/kg to Wistar rats. Enhanced in vitro clearance in rat liver microsomes may explain the shorter duration of action observed for compounds **26**, **28**, and **39**. Compound **44** showed a high clearance in vitro and in vivo. However, the metabolism was saturated at relatively low compound concentrations, explaining why at a dose of 10 mg/kg this compound was still able to maximally reduce LC for almost 24 h. From the above studies several compounds appeared interesting for further characterization, as they were less lipophilic than thiophene **1**, displayed high affinity and selectivity for $S1P_1$, and sustained maximal reduction of blood LC in the rat. As an example, aminopyridine **53** has a clogP which is 1 log unit lower than the one of thiophene **1**, showed an EC_{50} of 0.6 nM on $S1P_1$, and was more than 500-fold selective against $S1P_3$. In addition, this compound was highly efficacious in reducing blood LC in Wistar rats. In the following, the aminopyridine **53** was chosen to illustrate the more detailed charaterization process. A dose response experiment revealed that for **53** the ED_{50} for maximal LC reduction at 24 h after administration is about 0.5 mg/kg in the rat. Furthermore, compound **53** showed favorable PK properties in the Wistar rat and Beagle dog, showed acceptable PK behavior in the cynomolgus monkey, and is considered suitable for once daily dosing in humans. High concentrations of **53** were found in rat brain tissue after oral administration, making this compound an interesting candidate for further studies in particular in CNS related diseases or disorders.

EXPERIMENTAL SECTION

Chemistry. All reagents and solvents were used as purchased from commercial sources (Sigma-Aldrich, Switzerland; Lancaster Synthesis GmbH, Germany; Acros Organics, USA). Moisture sensitive reactions were carried out under an argon atmosphere. Progress of the reactions was followed either by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F_{254} on glass plates) or by LC−MS.

LC−MS parameters were the following: Finnigan MSQ plus or MSQ surveyor (Dionex, Switzerland), with HP 1100 binary pump and DAD (Agilent, Switzerland), column Zorbax SB-AQ, 3.5 μm, 120 Å, 4.6 mm × 50 mm (Agilent), gradient 5−95% acetonitrile in water containing 0.04% of trifluoroacetic acid within 1 min, flow of 4.5 mL/min. t_R is given in min. The asterisk (∗) denotes basic conditions: gradient 5−95% acetonitrile in water containing 0.04% of ammonium hydroxide within 1 min, flow of 4.5 mL/min; 40 °C. t_R is given in min. UV detection was at at 230, 254, and 280 nm.

Purity of all final compounds was checked by an additional LC−MS analysis on a Waters Acquity UPLC system equipped with an ACQ-PDA detector, an ACQ-ESL detector, and an ACQ-SQ detector: column ACQUITY UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm; gradient 2−98% acetonitrile containing 0.045% formic acid in water containing 0.05% formic acid over 1.8 min; flow of 1.2 mL/min; 60 °C. According to these LC−MS analyses, final compounds showed a purity of >95% (UV at 230 and at 214 nm).

Chiral integrity was proven by HPLC (chiral stationary phase). Hardware was from UltiMate instrument series (Dionex): HPG-3200SD binary pump, WPS-3000 autosampler, TCC-3200 thermostated column compartment, DAD-3000 detector, SRD-3400 degasser, ValveMate 2 (Gilson) solvent valves. Column, solvent, and retention time (t_R) are as indicated, DEA = diethylamine, TFA = trifluoroacetic acid, at 25 °C, flow 1 mL/min. No racemization was observed during the synthesis of the target compounds.

LC−HRMS parameters were the following: analytical pump Waters Acquity binary, Solvent Manager, MS, SYNAPT G2 MS, source

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temperature of 150 °C, desolvatation temperature of 400 °C, desolvatation gas flow of 400 L/h; cone gas flow of 10 L/h, extraction cone of 4 RF; lens 0.1 V; sampling cone 30; capillary 1.5 kV; high resolution mode; gain of 1.0, MS function of 0.2 s per scan, 120−1000 amu in full scan, centroid mode. Lock spray: keucine enkephalin, 2 ng/mL (556.2771 Da), scan time of 0.2 s with interval of 10 s and average of 5 scans; DAD: Acquity UPLC PDA detector. Column was an Acquity UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm from Waters, thermostated in the Acquity UPLC column manager at 60 °C. Eluents were the following: water + 0.05% formic acid; B, acetonitrile + 0.05% formic acid. Gradient was 2−98% B over 3.0 min. Flow was 0.6 mL/min. Detection was at UV 214 nm. For MS, t_R is given in min.

Purity of all target compounds was assessed using the two independent LC−MS methods described above: (1) a Zorbax SB-AQ, 5 μm, 120 Å, 4.6 mm × 50 mm (Agilent) column, eluting with a gradient of 5−95% acetonitrile in water containing 0.04% of trifluoroacetic acid, within 1 min, flow of 4.5 mL/min; (2) an ACQUITY UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm column, eluting with a gradient of 2−98% acetonitrile containing 0.045% formic acid in water containing 0.05% formic acid over 1.8 min; flow of 1.2 mL/min. In addition, important compounds were analyzed by LC−HRMS as described above. Purity and identity of the target compounds were further corroborated by NMR spectroscopy, and chiral integrity was proven by HPLC using chiral stationary phases. No racemization/epimerization was observed during the synthesis of the target compounds. According to these LC−MS analyses, final compounds showed a purity of ≥95% (UV at 230 and at 214 nm).

For NMR spectroscopy, instruments used were the following: Varian Oxford, ^{1}H (300 MHz) or ^{13}C (75 MHz); Bruker Avance II, 400 MHz UltraShield, ^{1}H (400 MHz), ^{13}C (100 MHz). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintuplet), h (hextet), hept (heptuplet), or m (multiplet). br = broad, and coupling constants are given in Hz. Several compounds have been prepared in a combinatorial library format on a 15−50 mmol scale. For those compounds ^{1}H NMR spectra were acquired using nondeuterated 10 mM DMSO stock solutions submitted for biological testing.[129] The solvent and water signals were suppressed by irradiation at 2.54 and 3.54 ppm, respectively. As a consequence, signal integrals close to those frequencies are not always accurate. The numbers of protons given in the description represent observed values.

Compounds were purified by flash column chromatography (CC) on silica gel 60 (Fluka Sigma-Aldrich, Switzerland), by preparative TLC glass plates coated with silica gel 60 F_{254} (0.5 mm), by preparative HPLC (Waters XBridge Prep C18, 5 μm, OBD, 19 mm × 50 mm, or Waters X-terra RP18, 19 mm × 50 mm, 5 μm, gradient of acetonitrile in water containing 0.4% of formic acid, flow of 75 mL/min), or by MPLC (Labomatic MD-80-100 pump, linear UVIS-201 detector, column 350 mm × 18 mm, Labogel-RP-18-5s-100, gradient 10% methanol in water to 100% methanol).

clogP values were calculated using an algorithm developed in-house and published online at openmolecules.org.[130]

In Vitro Potency Assessment. Data (EC_{50}) are given as geometric mean values (X_{geo}) with geometric standard deviation (σ_g). The upper and lower 95% confidence limits are calculated as $X_{geo}\sigma_g^2$ and X_{geo}/σ_g^2, respectively (results not shown).

GTPγS binding assays were performed in 96-well polypropylene microtiter plates in a final volume of 200 μL. Membrane preparations of CHO cells expressing recombinant human $S1P_1$ or $S1P_3$ receptors were used. Assay conditions were 20 mM Hepes, pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 0.1% fatty acid free BSA, 1 or 3 μM GDP (for $S1P_1$ or $S1P_3$, respectively), 2.5% DMSO, and 50 pM ^{35}S-GTPγS. Test compounds were dissolved and diluted and preincubated with the membranes, in the absence of ^{35}S-GTPγS, in 150 μL of assay buffer at room temperature for 30 min. After addition of 50 μL of ^{35}S-GTPγS in assay buffer, the reaction mixture was incubated for 1 h at room temperature. The assay was terminated by filtration of the reaction mixture through a Multiscreen GF/C plate, prewetted with ice-cold 50 mM Hepes, pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 0.4% fatty acid free BSA, using a cell harvester. The filter plates were then washed with ice-cold 10 mM Na_2HPO_4/NaH_2PO_4 (70%/30%, w/w) containing 0.1% fatty acid free BSA. Then the plates were dried at 50 °C and sealed, 25 μL of MicroScint20 was added, and membrane-bound ^{35}S-GTPγS was determined on the TopCount. Specific ^{35}S-GTPγS binding was determined by subtracting nonspecific binding (the signal obtained in the absence of agonist) from maximal binding (the signal obtained with 10 μM S1P). The EC_{50} of a test compound is the concentration of a compound inducing 50% of specific binding.

Intrinsic metabolic clearance (CL_{int}) was determined by substrate depletion experiments, with a default starting concentration of 1 μM in the presence of 0.5 mg/mL rat liver microsomes (RLM) in 100 mM sodium phosphate buffer at pH 7.4. Incubations were initated by the addition of an NADPH regenerating system containing D-glucose 6-phosphate, NADPH, and glucose 6-phosphate dehydrogenase. All incubations were conducted by shaking reaction mixtures under air at 37 °C. Aliquots (0.1 mL) were removed at 0, 2.5, 5, 10, and 15 min and terminated by addition to 0.1 mL of methanol on ice. After protein precipitation by centrifugation, the remaining concentrations were analyzed by liquid chromatography coupled to mass spectrometry (LC−MS/MS). CL_{int} was calculated from the concentration remaining versus time, fitted to a first order decay constant versus time. K_M values from substrate consumption experiments were determined by plotting the first order decay constants versus the substrate concentration on a linear−log plot and fitting the following equation:[131]

$$\text{decay constant} = \mathrm{CL}_{\mathrm{int}[S]\to 0}\left\{\frac{[S]}{[S] + K_M}\right\}$$

Male Wistar rats (RccHan:WIST) were obtained from Harlan (Venray, The Netherlands) and used for pharmacokinetic experiments after an acclimatization period of at least 7 days. The body weight of the rats was about 250 g at the day of the experiment. Two days prior to dosing, rats were anesthetized via inhalation of the gas anesthetic isoflurane (4−5% for induction and 1.5−3% for maintenance) in 100% O_2. Buprenorphine was dosed as analgesic at 0.03 mg/kg sc half an hour before the operation. Catheters were implanted into jugular vein and carotid artery under aseptic conditions to allow for multiple serial blood sampling. Animals foreseen for oral dosing did not undergo surgery, but blood samples were taken sublingually under light anesthesia with isoflurane. Compounds were administered intravenously via the tail vein at doses of 1 mg/kg body weight formulated as solutions in an aqueous mixed micellar vehicle based on phospholipids and bile acids (mixed micelles). Oral administration at doses of 10 mg/kg was performed by gavage. Oral formulations were dispersions prepared by addition of a DMSO stock solution of the compounds to succinylated gelatin (7.5% w/v) in water.

For pharmacokinetics in the dog, the pharmacokinetic profile of compound **53** was determined in the fasted male Beagle dog (n = 2 dogs). The compound was administered iv at a dose of 1 mg/kg as a solution in mixed micelles and orally at a dose of 3 mg/kg as a dispersion in succinylated gelatin (7.5% w/v) in water.

For pharmacokinetics in the monkey, the pharmacokinetic profile of compound **53** was determined in the male cynomolgus monkey (n = 3). The compound was administered iv as a short infusion at a dose of 1 mg/kg as a solution in mixed micelles and orally at a dose of 1 mg/kg as a dispersion in 0.25% methyl cellulose in water with 0.05% Tween 80.

For bioanalysis and pharmacokinetics, serial blood samples of 0.25 mL each were taken from each individual animal to obtain a complete concentration vs time profile per animal. Blood samples were taken predose and at 30 min and 1, 2, 3, 4, 6, 8, and 24 h postdose into vials containing EDTA as anticoagulant. For the iv applications, additional samples were obtained 2, 10, and 20 min after dosing. Plasma was prepared from blood samples by centrifugation and stored at −20 °C and analyzed for drug concentrations using LC−MS/MS after protein precipitation. Pharmacokinetic parameters were estimated with the WinNonlin software (Pharsight Corporation, Mountain View, CA, USA) using noncompartmental analysis.

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For brain penetration in the rat, at 2, 6, and 24 h after dosing, male Wistar rats (n = 2) were anaesthetized with 5% isoflurane and sacrificed by opening the diaphragm. A blood sample was taken. Plasma was prepared, and the brain was slowly perfused with 10 mL of 0.9% NaCl through the carotid. The whole brain was then removed and homogenized in an equal volume of ice-cold 0.1 M Na phosphate buffer, pH 7.4, using a IKA-WERKE Ultra-Turrax T25 tissue homogenizer for 10 s, and the brain homogenate was snap frozen in liquid nitrogen. Drug concentrations were then determined as described for plasma, using a calibration curve from blanco brain homogenate.

The in vivo efficacy of the target compounds was assessed by measuring the circulating lymphocytes after oral administration of 3−100 mg/kg of a target compound to normotensive male Wistar rats. The animals were housed in climate-controlled conditions with a 12 h light/dark cycle and had free access to normal rat chow and drinking water. Blood was collected before and 3, 6, and 24 h after drug administration. Full blood was subjected to hematology using Beckman Coulter Ac·T 5diff CP (Beckman Coulter International SA, Nyon, Switzerland). The effect on lymphocyte count (% LC) was calculated for each animal as the difference between LC at a given time point and the predose value (=100%). All data are presented as the mean ± SEM. Statistical analyses were performed by analysis of variance (ANOVA) using Statistica (StatSoft) and the Student−Newman−Keuls procedure for multiple comparisons. The null hypothesis was rejected when $p < 0.05$. Because of interindividual variability and the circadian rhythm of the number of circulating lymphocytes, a compound showing relative changes in the range of −20% to +40% is considered inactive. A lymphocyte count (LC) reduction in the range of −60% to −75% represents the maximal effect to be observed under the conditions of the experiment. For formulation, the compounds were dissolved in DMSO. This solution was added to a stirred solution of succinylated gelatin (7.5% w/v) in water. The resulting milky suspension containing a final concentration of 5% of DMSO was administred to the animals by gavage. A mixture of 95% of succinylated gelatin (7.5% w/v) in water and 5% of DMSO served as vehicle.

6-Isobutyl-5-methylnicotinic Acid (60). (a) Phosphoroxychloride (183 mL, 2 mol) was heated at 90 °C, and a mixture of commercially available 2-methyl-2-butenenitrile (73 g, 0.9 mol) and DMF (154 mL, 2 mol) was added slowly while keeping the temperature at 100−110 °C. The mixture was stirred at 110 °C for 15 h, cooled to room temperature, and diluted with DCM (500 mL). The mixture was cooled at 0 °C and carefully quenched with water (500 mL). The phases were separated, and the aqueous phase was extracted with DCM (total of 800 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was crystallized from cyclohexane to provide 6-chloro-3-formyl-5-methylpyridine **89**[109] (28.3 g, 20%) as slightly yellow crystals. LC−MS: t_R = 0.76 min, $[M + 1]^+$ = 156.14. ^{1}H NMR (DMSO-d_6): δ 10.09 (s, 1 H), 8.78 (s, 1 H), 8.24 (s, 1 H), 2.43 (s, 3 H).

(b) A solution of **89** (10 g, 64 mmol) in formic acid (200 mL) was cooled at 0 °C, and an aqueous 50 wt % solution of H_2O_2 in water (9.6 mL, 360 mmol) was added at this temperature. The mixture was stirred at 0 °C for 15 h, carefully diluted with water (200 mL), and extracted with DCM (8 × 100 mL). The combined organic extracts were washed with 1 M aqueous HCl (100 mL) (checked for remaining peroxide), dried ($MgSO_4$), filtered, and evaporated. The residue was dried to give 6-chloro-5-methylnicotinic acid (9.56 g, 87%). LC−MS: t_R = 0.72 min, $[M + 1]^+$ = 172.0. ^{1}H NMR ($CDCl_3$): δ 8.94 (d, J = 2.0 Hz, 1 H), 8.23 (d, J = 2.3 Hz, 1 H), 2.49 (s, 3 H).

(c) A solution of 6-chloro-5-methylnicotinic acid (13.85 g, 80.75 mmol) in dry EtOH (200 mL) containing some drops of concentrated H_2SO_4 was stirred at reflux for 2 days. The solution was cooled to room temperature, the solvent evaporated, the residue dissolved in EA (200 mL) and washed with a solution of saturated aqueous Na_2CO_3 (2 × 80 mL), 1 M aqueous $KHSO_4$ (2 × 80 mL), and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to give 6-chloro-5-methylnicotinic acid ethyl ester **90** (12.65 g, 79%) as a solid. LC−MS: t_R = 0.92 min, $[M + 1]^+$ = 200.10. ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7.0 Hz, 3 H), 2.46 (s, 3 H), 4.43 (q, J = 7.3 Hz, 2 H), 8.16 (m, 1 H), 8.84 (d, J = 2.0 Hz, 1 H).

(d) To a solution of **90** (4.98 g, 24.9 mmol), 2,4,6-tri(2-methylpropenyl)cyclotriboroxanepyridine complex (5.74 g, 17.7 mmol, prepared in analogy to a procedure given by F. Kerins and D. F. O'Shea[108]), and PPh_3 (1.15 g, 4.4 mmol) in DME (60 mL), a solution of 2 M aqueous K_2CO_3 (20 mL) was added. The mixture was degassed and flushed with N_2 before $Pd(PPh_3)_4$ (460 mg, 0.4 mmol) was added. The mixture was stirred at 90 °C for 20 h before it was cooled to room temperature, diluted with EA (150 mL), and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL). The organic extract was dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by FC (SiO_2, heptane−EA) to give 5-methyl-6-(2-methylpropenyl)nicotinic acid ethyl ester (3.98 g, 73%) as an orange oil. LC−MS: t_R = 0.72 min, $[M + 1]^+$ = 220.15. ^{1}H NMR ($CDCl_3$): δ 9.04 (d, J = 1.9 Hz, 1 H), 8.04 (d, J = 1.6 Hz, 1 H), 6.37 (s, 1 H), 4.41 (q, J = 7.1 Hz, 2 H), 2.34 (s, 3 H), 2.008 (s, 3 H), 2.006 (s, 3 H), 1.42 (t, J = 7.1 Hz, 3 H).

(e) 5-Methyl-6-(2-methylpropenyl)nicotinic acid ethyl ester (3.98 g, 18.2 mmol) was dissolved in THF (100 mL) and MeOH (100 mL). Pd/C (500 mg, 10% Pd) was added as a slurry in THF (5 mL), and the mixture was stirred under 1 atm of H_2 at room temperature for 15 h. The catalyst was filtered off and the filtrate was evaporated to give 6-isobutyl-5-methylnicotinic acid ethyl ester **91** (3.76 g, 93%) as a colorless oil. LC−MS: t_R = 0.75 min, $[M + 1]^+$ = 222.15. ^{1}H NMR ($CDCl_3$) δ 0.97 (d, J = 6.8 Hz, 6 H), 1.42 (t, J = 7.3 Hz, 3 H), 2.20 (hept, J = 6.8 Hz, 1H), 2.38 (s, 3 H), 2.75 (d, J = 7.0 Hz, 2 H), 4.41 (q, J = 7.3 Hz, 2 H), 8.03 (d, J = 1.8 Hz, 1 H), 9.00 (d, J = 2.0 Hz, 1 H).

(f) A solution of **91** (3.75 g, 16.95 mmol) in 12.5% aqueous HCl (50 mL) was stirred at 65 °C for 24 h before the solvent was evaporated. The residue was dried under high vacuum to give 6-isobutyl-5-methylnicotinic acid hydrochloride **60** (3.55 g, 91%) as a white powder. LC−MS: t_R = 0.57 min, $[M + 1]^+$ = 194.25. ^{1}H NMR ($CDCl_3$): δ 9.23 (s, 1 H), 8.77 (s, 1 H), 3.17 (d, J = 7.5 Hz, 2 H), 2.62 (s, 3 H), 2.35 (hept, J = 6.3 Hz, 1 H), 1.09 (d, J = 6.6 Hz, 6 H).

6-Cyclopentyl-5-methylnicotinic Acid (61). (a) A solution of 5,6-dichloronicotinic acid (50 g, 260 mmol) and TMSCl (8.49 g, 781 mmol) in isopropanol (500 mL) was stirred at 60 °C for 18 h. The mixture was concentrated, and the residue was partitioned between EA and saturated aqueous $NaHCO_3$ solution. The organic extract was separated, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified on silica gel, eluting with heptane/EA 9:1 to give 5,6-dichloronicotinic acid isopropyl ester **92** (56.2 g, 92%) as a white solid. LC−MS: t_R = 1.01 min, $[M + 1]^+$ = 233.89. ^{1}H NMR (DMSO-d_6): δ 8.85 (d, J = 2.0 Hz, 1 H), 8.49 (d, J = 2.0 Hz, 1 H), 5.18 (hept, J = 6.3 Hz, 1 H), 1.35 (d, J = 6.3 Hz, 6 H).

(b) To a solution of **92** (2.15 g, 9.19 mmol) in THF (30 mL) were added NMP (3 mL) and $Fe(acac)_3$ (162 mg, 0.459 mmol). The mixture was cooled to 0 °C before cyclopentylmagnesium bromide (9.2 mL of 2 M solution in diethyl ether) was added. The dark red to black reaction mixture was stirred at 0 °C for 1 h, then at room temperature for 18 h. The reaction was quenched carefully by adding water (30 mL), then extracted twice with EA (2 × 60 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA 9:1 to give isopropyl 5-chloro-6-cyclopentylnicotinate **93** (1.93 g, 78%) which was still contaminated with 5,6-dichloronicotinic acid isopropyl ester as a pale yellow oil. LC−MS: t_R = 0.87 min, $[M + 1]^+$ = 268.2. ^{1}H NMR (DMSO-d_6): δ 8.95 (d, J = 1.8 Hz, 1 H), 8.21 (d, J = 1.9 Hz, 1 H), 5.16 (hept, J = 6.3 Hz, 1 H), 3.66 (quint, J = 7.8 Hz, 1 H), 1.95−2.05 (m, 2 H), 1.74−1.88 (m, 4 H), 1.59−1.73 (m, 2 H), 1.34 (d, J = 6.2 Hz, 6 H).

(c) To a solution of the above **93** (1.93 g, 7.21 mmol) and $Pd(dppf)Cl_2$ (60 mg, 74 μmol) in dioxane (30 mL) was added dimethylzinc (13.3 mL of a 1.2 M solution in toluene). The mixture was stirred at 75 °C for 18 h before the reaction was quenched at room temperature by carefully adding water (50 mL). The mixture was extracted twice with EA (2 × 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA 9:1 to give

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isopropyl 6-cyclopentyl-5-methylnicotinate **94** (600 mg, 36%, containing the corresponding methyl ester as impurity) as a pale yellow oil. LC−MS: t_R = 0.61 min, $[M + 1]^+$ = 248.31. ^{1}H NMR ($CDCl_3$): δ 9.01 (s, 1 H), 7.99 (s, 1 H), 5.27 (hept, J = 6.5 Hz, 1 H), 3.40 (quint, J = 7.5 Hz, 1 H), 2.41 (s, 3 H), 1.95−2.07 (m, 2 H), 1.83−1.95 (m, 4 H), 1.65−1.78 (m, 2 H), 1.38 (d, J = 6.2 Hz, 6 H).

(d) A solution of the above **94** (600 mg, 2.43 mmol) in MeOH (8 mL) and 2 M aqueous LiOH (2 mL) was stirred at room temperature for 2 h. The mixture was concentrated, diluted with water (15 mL) and EA (40 mL), and then neutralized by adding 2 N aqueous HCl (approximately 2 mL). The organic phase was separated, and the aqueous phase was extracted three more times with EA (3 × 40 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, concentrated, and dried to give the title compound (412 mg, 83%) as a pale yellow oil. LC−MS: t_R = 0.35 min, $[M + 1]^+$ = 206.39. ^{1}H NMR ($CDCl_3$): δ 9.14 (s, 1 H), 8.13 (s, 1 H), 3.45 (quint, J = 7.3 Hz, 1 H), 2.46 (s, 3 H), 2.01−2.11 (m, 2 H), 1.87−2.01 (m, 4 H), 1.64−1.84 (m, 2 H).

5-Cyclopentyl-4-methylpicolinic Acid (68). (a) Under argon, Pd(dppf)Cl_2 (161 mg, 0.198 mmol) and cyclopentylzinc bromide (39 mL of a 0.5 M solution in THF) were added to a solution of ethyl 5-bromo-4-methylpicolinate **107** (4.73 g, 19.4 mmol) in dioxane (250 mL). The mixture was stirred at 65 °C for 2 h before another portion of Pd(dppf)Cl_2 (161 mg, 0.198 mmol) and cyclopentylzinc bromide (19.5 mL) was added. Stirring was continued at 65 °C for 18 h. The mixture was cooled to room temperature before the reaction was quenched by carefully adding water (200 mL) and saturated aqueous $NaHCO_3$ solution (150 mL). The mixture was extracted six times with EA (6 × 100 mL) and DCM (100 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give crude ethyl 5-cyclopentyl-4-methylpicolinate **113** (3.96 g) as an orange oil containing about 10% of the corresponding cyclopentyl ester. LC−MS: t_R = 0.85 min, $[M + 1]^+$ = 234.05. Cyclopentyl ester, LC−MS: t_R = 0.95 min, $[M + 1]^+$ = 274.06.

(b) A solution of **113** (3.96 g, 17.0 mmol) in dioxane (2 mL) and 2 M aqueous LiOH (50 mL) was stirred at 75 °C for 5 h. The mixture was cooled to room temperature before it was extracted with EA. The aqueous phase was acidified by adding 1 N HCl and then extracted with EA. The second organic extract was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by MPLC (ODS-AQ), eluting with a gradient of methanol in water to give **68** (532 mg, 15%) as a beige solid. LC−MS: t_R = 0.37 min, $[M + 1]^+$ = 206.31. ^{1}H NMR (DMSO-d_6): δ 8.52 (s, 1 H), 7.92 (s, 1 H), 3.21−3.31 (m, 1 H), 2.44 (s, 3 H), 1.99−2.11 (m, 2 H), 1.75−1.88 (m, 2 H), 1.56−1.75 (m, 4 H).

2-Methyl-6-(pentan-3-yl)isonicotinic Acid (76d). (a) To a suspension of 2-chloro-6-methylisonicotinic acid (20.0 g, 117 mmol) in isopropanol (80 mL) was added H_2SO_4 (5 mL) dropwise. The mixture became warm (40 °C). The mixture was stirred for 24 h at room temperature, then at 90 °C for 28 h before the solvent was removed in vacuo. The residue was dissolved in diethyl ether (200 mL), washed with saturated aqueous $NaHCO_3$ solution (3 × 50 mL) followed by brine (3 × 50 mL), dried over Na_2SO_4, filtered, and concentrated to give 2-chloro-6-methylisonicotinic acid isopropyl ester (21.0 g) as a colorless oil which slowly crystallizes. LC−MS: t_R = 0.97 min, $[M + 1]^+$ = 214.05. ^{1}H NMR (CD_3OD). δ 7.64 (s, 1 H), 7.61 (s, 1 H), 5.21 (hept, J = 6.2 Hz, 1 H), 2.54 (s, 3 H), 1.37 (d, J = 6.3 Hz, 6 H).

(b) A solution of 2-chloro-6-methylisonicotinic acid isopropyl ester (2.0 g, 9.36 mmol) in dioxane (75 mL) was degassed and put under argon before Pd(dppf)Cl_2 (229 mg, 0.281 mmol) was added. At room temperature, a 0.5 M solution of 1-ethylpropylzinc bromide in THF (46.8 mL, 23.4 mmol) was added dropwise to the mixture. The mixture was stirred at 80 °C for 16 h before the reaction was quenched by adding ice-cold water (200 mL). A precipitate forms, and the mixture was diluted with EA (200 mL) and filtered through Celite. The filtrate was transferred into a separatory funnel. The organic phase was collected, and the aqueous phase was extracted with EA (120 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA 9:1 to 4:1 to give isopropyl 2-(1-ethylpropyl)-6-methylisonicotinate **130d** (1.6 g, 69%) as a yellow oil containing a few percent of isopropyl 2-methyl-6-(pentan-2-yl)-isonicotinate. LC−MS: t_R = 0.79 min, $[M + 1]^+$ = 250.14. ^{1}H NMR (DMSO-d_6): δ 0.70 (t, J = 7.3 Hz, 6 H), 1.33 (d, J = 6.3 Hz, 6 H), 1.58−1.70 (m, 4 H), 2.51 (s, 3 H), 2.55−2.63 (m, 1 H), 5.15 (hept, J = 5.8 Hz), 7.39 (s, 1 H), 7.49 (s, 1 H).

(c) A solution of **130d** (1.54 g, 6.18 mmol) in 25% aqueous HCl (60 mL) was stirred at 65 °C for 16 h. The solvent was removed in vacuo and the residue was dissolved in dioxane and concentrated again to give 2-(1-ethyl-propyl)-6-methylisonicotinic acid **76d** as hydrochloride salt (1.70 g) in the form of a brownish solid. LC−MS: t_R = 0.62 min, $[M + 1]^+$ = 208.52. ^{1}H NMR (CD_3OD): δ 8.23 (s, 1 H), 8.19 (s, 1 H), 3.00 (d, J = 7.4 Hz, 2 H), 2.88 (s, 3 H), 2.10−2.25 (m, 1 H), 1.05 (d, J = 6.6 Hz, 6 H).

2-Cyclopentyl-6-methylisonicotinic Acid (76f). (a) Under argon, Pd(dppf)Cl_2 (200 mg, 0.245 mmol) was added to a solution of 2-chloro-6-methylisonicotinic acid ethyl ester **129** (4.80 g, 24.0 mmol) in dioxane (60 mL). A solution of cyclopentylzinc chloride (50 mL, 24.0 mmol, ∼2 M solution in THF) was added dropwise. The mixture was stirred at 75 °C for 2 h before it was cooled to room temperature, carefully diluted with water, and extracted twice with EA. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA 9:1 to give 2-cyclopentyl-6-methylisonicotinic acid ethyl ester **130f** (3.96 g, 71%) as an oil. LC−MS: t_R = 0.72 min, $[M + 1]^+$ = 234.11. ^{1}H NMR ($CDCl_3$): δ 7.56 (s, 1 H), 7.52 (s, 1 H), 4.42 (q, J = 7.1 Hz, 2 H), 3.18−3.29 (m, 1 H), 2.61 (s, 3 H), 2.06−2.18 (m, 2 H), 1.66−1.93 (m, 6 H), 1.43 (t, J = 7.1 Hz, 3 H).

(b) A solution of **130f** (3.96 g, 17.0 mmol) in 25% aqueous HCl (50 mL) was stirred at 75 °C for 16 h. The solvent was removed in vacuo and the remaining residue was dried under high vacuum to give **76f** as a hydrochloride salt (4.12 g, quantitative) in the form of a white solid. LC−MS: t_R = 0.54 min, $[M + 1]^+$ = 206.08. ^{1}H NMR (DMSO-d_6): δ 7.90 (s, 1 H), 7.85 (s, 1 H), 3.79 (s br, 2 H), 3.41−3.53 (m, 1 H), 2.73 (s, 3 H), 2.07−2.17 (m, 2 H), 1.63−1.89 (m, 6 H).

2-Cyclopentyl-6-ethylisonicotinic Acid (77c). The title compound (7.19 g) was prepared in analogy to **77a** (see Supporting Information). LC−MS: t_R = 0.59 min, $[M + 1]^+$ = 220.00. ^{1}H NMR (DMSO-d_6): δ 7.85 (s, 1 H), 7.83 (s, 1 H), 3.47−3.57 (m, 1 H), 3.02 (q, J = 7.5 Hz, 2 H), 2.06−2.16 (m, 2 H), 1.71−1.88 (m, 4 H), 1.64−1.71 (m, 2 H), 1.29 (t, J = 7.5 Hz, 3 H).

2-(Diethylamino)-6-methylisonicotinic Acid (79). (a) A mixture of *tert*-butyl 2,6-dichloroisonicotinate **136** (11.3 g, 45.4 mmol) and diethylamine (3.32 g, 45.4 mmol) was stirred at 100 °C for 72 h in a sealed vessel. The mixture was cooled to room temperature, diluted with water (50 mL), and extracted three times with EA (3 × 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA 9:1 to give *tert*-butyl 2-chloro-6-(diethylamino)isonicotinate **137** (13.3 g, quantitative) as a pale yellow oil. LC−MS: t_R = 1.15 min, $[M + 1]^+$ = 285.05. ^{1}H NMR (DMSO-d_6): δ 6.88 (d, J = 0.9 Hz, 1 H), 6.80 (d, J = 0.8 Hz, 1 H), 3.50 (q, J = 7.0 Hz, 4 H), 1.54 (s, 9 H), 1.11 (t, J = 7.0 Hz, 6 H).

(b) To solution of **137** (6.70 g, 23.5 mmol) and Pd(dppf)Cl_2 (196 mg, 240 μmol) in dioxane (50 mL) under argon was added dimethylzinc (70.6 mL, 1 M solution in THF). The mixture was stirred at 75 °C for 16 h before it was cooled to room temperature. The reaction was quenched by carefully adding water. The mixture was extracted twice with EA. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA to give *tert*-butyl 2-(diethylamino)-6-methylisonicotinate **138** (2.54 g, 41%) as a yellow oil. LC−MS: t_R = 0.81 min, $[M + 1]^+$ = 265.09.

(c) A solution of **138** (2.53 g, 9.57 mmol) in 25% aqueous HCl (50 mL) was stirred at 80 °C for 24 h. The mixture was concentrated and dried under high vacuum to give the hydrochloride salt of **79** (2.45 g, quantitative) as a yellow oil. LC−MS: t_R = 0.48 min, $[M + 1]^+$ = 209.42. ^{1}H NMR (DMSO-d_6): δ 7.17 (s, 1 H), 6.97 (s, 1 H), 3.70 (q br, J = 7.0 Hz, 2 H), 2.56 (s, 3 H), 1.17 (t, J = 6.8 Hz, 6 H).

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***N*-((*S*)-3-[2-Ethyl-4-(*N*-hydroxycarbamimidoyl)-6-methylphenoxy]-2-hydroxypropyl)-2-hydroxyacetamide (80).** (a) To an ice-cold solution of H_2SO_4 (150 mL) in water (250 mL) was added 2-ethyl-6-methylaniline (15.0 g, 111 mmol). The solution was treated with ice (150 g) before a solution of $NaNO_2$ (10.7 g, 155 mmol) in water (150 mL) and ice (50 g) was added dropwise. The mixture was stirred at 0 °C for 1 h. Then 50% aqueous H_2SO_4 (200 mL) was added and stirring was continued at room temperature for 18 h. The mixture was extracted with DCM. The organic extracts were dried over $MgSO_4$ and evaporated. The crude product was purified by CC on silica gel, eluting with heptane/EA 9:1 to give 2-ethyl-6-methylphenol **81** (8.6 g, 57%) as a crimson oil. LC−MS: t_R = 0.89 min. ^{1}H NMR ($CDCl_3$): δ 7.03−6.95 (m, 2 H), 6.80 (t, J = 7.6 Hz, 1 H), 4.60 (s, 1 H), 2.64 (q, J = 7.6 Hz, 2 H), 2.25 (s, 3 H), 1.24 (t, J = 7.6 Hz, 3 H).

(b) A solution of 2-ethyl-6-methylphenol **81** (200 g, 1.47 mol) and hexamethylenetetraamine (206 g, 1.47 mol) in acetic acid (1600 mL) and water (264 mL) was heated to reflux. The condensate was removed using a Dean−Stark apparatus until about 1200 mL of condensate was collected. The reaction mixture was cooled to room temperature, and water (1000 mL) was added. The thick suspension was filtered and the collected solid was dried at 60 °C under vacuum (10 mbar) to give 3-ethyl-4-hydroxy-5-methylbenzaldehyde **82** (191 g, 79%) as an orange solid. LC−MS: t_R = 0.86 min, $[M + 1 + CH_3CN]^+$ = 206.27. ^{1}H NMR ($CDCl_3$): δ 9.84 (s, 1 H), 7.59 (s, 1 H), 7.57 (s, 1 H), 4.64 (s br, 1 H), 2.71 (q, J = 7.5 Hz, 2 H), 2.34 (s, 3 H), 1.30 (t, J = 7.5 Hz, 3 H).

(c) A solution of **82** (5.32 g, 32.4 mmol) and hydroxylamine hydrochloride (3.38 g, 48.6 mmol) in NMP (35 mL) was stirred for 3 h at 80 °C under microwave irradiation (300 W, continuous cooling).[132] The mixture was diluted with water and extracted twice with diethyl ether. The organic extracts were washed with 2 N aqueous HCl, saturated aqueous $NaHCO_3$ solution, and brine. The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with heptane/EA 3:2 to give 3-ethyl-4-hydroxy-5-methylbenzonitrile **83** as a pale yellow solid (4.80 g, 92%). LC−MS: t_R = 0.90 min. ^{1}H NMR ($CDCl_3$): δ 1.24 (t, J = 7.6 Hz, 3 H), 2.26 (s, 3 H), 2.63 (q, J = 7.6 Hz, 2 H), 5.19 (s, 1 H), 7.30 (s, 2 H).

(d) To a solution of **83** (5.06 g, 31.4 mmol) in THF (80 mL) were added PPh_3 (9.06 g, 34.5 mmol) and (*R*)-glycidol (2.29 mL, 34.5 mmol). The mixture was cooled to 0 °C before DEAD in toluene (15.8 mL, 34.5 mmol) was added. The mixture was stirred for 18 h while warming up to room temperature. The solvent was evaporated and the crude product was purified by CC on silica gel, eluting with heptane/EA 7:3 to give (*S*)-3-ethyl-5-methyl-4-(oxiran-2-ylmethoxy)-benzonitrile **84** (5.85 g, 86%) as a yellow oil. LC−MS: t_R = 0.96 min, $[M + 42]^+$ = 259.08. ^{1}H NMR ($CDCl_3$): δ 7.38 (s, 1 H), 7.35 (s, 1 H), 4.12−4.19 (m, 1 H), 3.73−3.80 (m, 1 H), 3.36−3.42 (m, 1 H), 2.90−2.96 (m, 1 H), 2.68−2.77 (m, 3 H), 2.34 (s, 3 H), 1.26 (t, J = 7.6 Hz, 3 H).

(e) The above epoxide **84** (5.85 g, 26.9 mmol) was dissolved in 7 N NH_3 in methanol (250 mL), and the solution was stirred at 65 °C for 18 h. The solvent was evaporated to give crude (*S*)-4-(3-amino-2-hydroxypropoxy)-3-ethyl-5-methylbenzonitrile **85** (6.23 g, quantitative) as a yellow oil. LC−MS: t_R = 0.66 min, $[M + 1]^+$ = 235.11. ^{1}H NMR (DMSO-d_6): δ 7.54 (s, 2 H), 4.96 (s br, 1 H), 3.77−3.83 (m, 1 H), 3.68−3.77 (m, 2 H), 2.59−2.75 (m, 4 H), 2.28 (s, 3 H), 1.58 (s br, 2 H), 1.17 (t, J = 7.5 Hz, 3 H).

(f) To a solution **85** (6.23 g, 26.6 mmol) were added glycolic acid (2.43 g, 31.9 mmol), HOBt (4.31 g, 31.9 mmol), and EDC hydrochloride (6.12 g, 31.9 mmol). The mixture was stirred at room temperature for 18 h before it was diluted with saturated aqueous $NaHCO_3$ and extracted twice with EA. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC with DCM containing 8% of methanol to give (*S*)-*N*-[3-(4-cyano-2-ethyl-6-methylphenoxy)-2-hydroxypropyl]-2-hydroxyacetamide **86** (7.03 g, 90%) as a yellow oil. LC−MS: t_R = 0.74 min, $[M + 1]^+$ = 293.10. ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.5 Hz, 3 H), 2.32 (s, 3 H), 2.69 (q, J = 7.5 Hz, 2 H), 3.48−3.56 (m, 3 H), 3.70−3.90 (m, 3 H), 4.19 (s, br, 3 H), 7.06 (m, 1 H), 7.36 (s, 1 H), 7.38 (s, 1 H).

(g) To a solution of **86** (19.6 g, 67 mmol) in methanol (500 mL) were added hydroxylamine hydrochloride (9.32 g, 134 mmol) and $NaHCO_3$ (11.3 g, 134 mmol).[133] The resulting suspension was stirred at 65 °C for 18 h. The mixture was filtered, and the filtrate was concentrated. The residue was dissolved in water (20 mL) and EA (300 mL). The aqueous phase was separated and extracted three times with EA. The combined organic extracts were dried over $MgSO_4$, filtered, concentrated, and dried to give the title compound **80** as a white solid (18.9 g, 87%). LC−MS: t_R = 0.51 min, $[M + 1]^+$ = 326.13. ^{1}H NMR (DMSO-d_6): δ 1.17 (t, J = 7.4 Hz, 3 H), 2.24 (s, 3H), 2.62 (q, J = 7.4 Hz, 2 H), 3.23 (m, 1 H), 3.43 (m, 1 H), 3.67 (m, 2 H), 3.83 (s, 2 H), 3.93 (m, 1 H), 5.27 (s br, 1 H), 5.58 (s br, 1 H), 5.70 (s, 2 H), 7.34 (s, 1 H), 7.36 (s, 1 H), 7.67 (m, 1 H), 9.46 (s br, 1H).

Method A: (*S*)-*N*-(3-(4-(5-(2-Cyclopentyl-6-ethylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-2-hydroxypropyl)-2-hydroxyacetamide (50). To a solution of 2-cyclopentyl-6-ethylisonicotinic acid hydrochloride **77c** (187 mg, 0.73 mmol) in DMF (20 mL) was added Hünig's base (284 mg, 2.19 mmol) followed by TBTU (210 mg, 0.65 mmol). The mixture was stirred at room temperature for 10 min before *N*-((*S*)-3-[2-ethyl-4-(*N*-hydroxycarbamimidoyl)-6-methylphenoxy]-2-hydroxypropyl)-2-hydroxyacetamide **80** (251 mg, 0.77 mmol) was added. Stirring was continued at room temperature for 2 h. The mixture was diluted with EA (100 mL) and washed three times with saturated $NaHCO_3$ solution (2 × 30 mL). The organic extract was concentrated, and the residue was dissolved in dioxane (20 mL). The mixture was stirred at 100 °C for 16 h before it was concentrated. The crude product was purified on preparative HPLC (Waters XBridge, gradient of MeCN in water containing 0.5% NH_3) to give **53** (281 mg, 76%) as beige wax. LC−MS: t_R = 0.66 min, $[M + 1]^+$ = 509.28. ^{1}H NMR ($CDCl_3$): δ 7.87 (s, 1 H), 7.86 (s, 1 H), 7.76 (s, 1 H), 7.73 (s, 1 H), 7.21 (t, J = 5.9 Hz, 1 H), 4.18−4.25 (m, 3 H), 3.82−3.93 (m, 2 H), 3.74−3.82 (m, 2 H), 3.60 (s br, 1 H), 3.49−3.58 (m, 1 H), 3.27−3.39 (m, 1 H), 2.95 (q, J = 7.5 Hz, 2 H), 2.75 (q, J = 7.5 Hz, 2 H), 2.39 (s, 3 H), 2.13−2.21 (m, 2 H), 1.73−1.95 (m, 6 H), 1.39 (t, J = 7.6 Hz, 3 H), 1.32 (t, J = 7.5 Hz, 3 H). ^{13}C NMR ($CDCl_3$): δ 174.5, 174.0, 168.8, 166.9, 164.3, 157.3, 137.6, 131.60, 131.55, 128.3, 126.6, 122.5, 116.8, 116.2, 74.2, 69.9, 62.1, 48.1, 42.3, 33.6, 31.4, 25.8, 22.8, 16.4, 14.8, 13.8. LC−HRMS: t_R = 2.21 min, $[M + H]/z$ = 509.2764, found = 509.2762.

Method B: *N*-((*S*)-3-{4-[5-(2-Diethylamino-6-methylpyridin-4-yl)[1,2,4]oxadiazol-3-yl]-2-ethyl-6-methylphenoxy}-2-hydroxypropyl)-2-hydroxyacetamide (53). For an alternative, large scale synthesis, see Schmidt et al.[134] (a) A solution of 2-(diethylamino)-6-methylisonicotinic acid hydrochloride **79** (6.96 g, 28.4 mmol), Hünig's base (11.0 g, 85.3 mmol), and TBTU (9.13 g, 28.4 mmol) in DCM (100 mL) was stirred at room temperature for 10 min before 3-ethyl-*N*,4-dihydroxy-5-methylbenzimidamide (5.52 g, 28.4 mmol) was added. Stirring was continued at room temperature for 1 h. The mixture was diluted with DCM, washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in dioxane (100 mL), and the mixture was stirred at 100 °C for 18 h. The solvent was evaporated and the crude product was purified by CC on silica gel, eluting with heptane/EA 5:1 to give 4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenol (6.40 g, 61%) as a yellow solid. LC−MS: t_R = 0.90 min, $[M + 1]^+$ = 367.16. ^{1}H NMR ($CDCl_3$): δ: 7.85 (s, 2 H), 7.10 (s, 1 H), 7.01 (s, 1 H), 4.98 (s, 1 H), 3.63 (q, J = 7.0 Hz, 4 H), 2.73 (q, J = 7.4 Hz, 2 H), 2.49 (s, 3 H), 2.36 (s, 3 H), 1.33 (t, J = 7.5 Hz, 3 H), 1.25 (t, J = 6.9 Hz, 6 H).

(b) To a solution of 4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenol (580 mg, 1.58 mmol) in isopropanol (15 mL) and 3 M aqueous NaOH (3 mL) was added (*R*)-epichlorohydrine (439 mg, 4.75 mmol), and the mixture was stirred at room temperature for 24 h before another portion of (*R*)-epichlorohydrine (439 mg, 4.75 mmol) was added. Stirring was continued for 24 h. The mixture was diluted with EA (100 mL) and washed with saturated aqueous $NaHCO_3$ solution. The organic extract was dried over $MgSO_4$, filtered, and concentrated. The crude product

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was purified by CC on silica gel using heptane/EA 4:1 to give (*S*)-*N*,*N*-diethyl-4-(3-(3-ethyl-5-methyl-4-(oxiran-2-ylmethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-6-methylpyridin-2-amine (450 mg, 67%) as a yellow oil. LC−MS: t_R = 0.94 min, $[M + 1]^+$ = 423.19. ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1 H), 7.87 (s, 1 H), 7.10 (s, 1 H), 7.01 (s, 1 H), 4.18−4.27 (m, 1 H), 3.92 (d, J = 5.2 Hz, 2 H), 3.58−3.70 (m, 6 H), 2.77 (q, J = 7.6 Hz, 2 H), 2.50 (s, 3 H), 2.41 (s, 3 H), 1.33 (t, J = 7.5 Hz, 3 H), 1.25 (t, J = 7.0 Hz, 6 H).

(c) A solution of (*S*)-*N*,*N*-diethyl-4-(3-(3-ethyl-5-methyl-4-(oxiran-2-ylmethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-6-methylpyridin-2-amine (5.70 g, 13.5 mmol) in 7 M NH_3 in MeOH (100 mL) was stirred in a sealed vessel at 60 °C for 24 h. The mixture was concentrated and the crude product was purified by CC on silica gel, eluting with DCM/MeOH 10:1 containing a small amount of 7 M NH_3 in MeOH to give (*S*)-1-amino-3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)propan-2-ol (2.33 g, 39%) as a yellow oil. LC−MS: t_R = 0.70 min, $[M + 1]^+$ = 440.25. ^{1}H NMR δ: 7.88 (s, 1 H), 7.87 (s, 1 H), 7.10 (s, 1 H), 7.00 (s, 1 H), 3.97−4.05 (m, 1 H), 3.85−3.93 (m, 2 H), 3.62 (q, J = 7.0 Hz, 4 H), 3.23 (s, 1 H), 3.03 (dd, J_1 = 12.8 Hz, J_2 = 3.8 Hz, 1 H), 2.93 (dd, J_1 = 12.8 Hz, J_2 = 7.0 Hz, 1 H), 2.77 (q, J = 7.5 Hz, 2 H), 2.49 (s, 3 H), 2.40 (s, 3 H), 1.32 (t, J = 7.5 Hz, 3 H), 1.24 (t, J = 7.0 Hz, 6 H).

(d) To a solution of (*S*)-1-amino-3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-propan-2-ol (2.33 g, 5.30 mmol) in DCM (25 mL) were added glycolic acid (443 mg, 5.83 mmol) and HOBt (788 mg, 5.83 mmol). The mixture was stirred for 10 min before EDC·HCl (1.12 g, 5.83 mmol) was added. The mixture was stirred at room temperature for 1 h before it was diluted with water and brine. The mixture was extracted twice with EA. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by CC on silica gel, eluting with DCM/MeOH 10:1 to give **56** (2.20 g, 83%) as a yellow foam. LC−MS: t_R = 0.78 min, $[M + 1]^+$ = 498.22. HPLC with chiral stationary phase (Chiralpak AD-H 250 mm × 4.6 mm i.d., 5 μm; 85% hexane, 15% ethanol containing 0.1% DEA): t_R = 12.3 min, 100% ((*R*)-enantiomer, t_R = 10.2 min). ^{1}H NMR (DMSO-d_6): δ 7.79 (s, 2 H), 7.70 (t, J = 5.5 Hz, 1 H), 7.07 (s, 1 H), 6.99 (s, 1 H), 5.56 (t, J = 5.5 Hz, 1 H), 5.31 (d, J = 5.1 Hz, 1 H), 3.93−4.00 (m, 1 H), 3.84 (d, J = 5.2 Hz, 2 H), 3.70−3.81 (m, 2 H), 3.58 (q, J = 6.5 Hz, 4 H), 3.39−3.48 (m, 1 H), 3.20−3.29 (m, 1 H), 2.73 (q, J = 7.3 Hz, 2 H), 2.43 (s, 3 H), 2.35 (s, 3 H), 1.22 (t, J = 7.5 Hz, 3 H), 1.16 (t, J = 6.9 Hz, 6 H). LC-HRMS: t_R = 1.86 min, $[M + H]/z$ = 498.2716, found = 498.2717.

***N*-((*S*)-3-{2-Ethyl-4-[5-(6-isobutyl-5-methylpyridin-3-yl)-[1,2,4]oxadiazol-3-yl]-6-methylphenoxy}-2-hydroxypropyl)-2-hydroxyacetamide (8).** **8** was obtained with method A, using nicotinic acid **60**. Colorless resin (17 mg, 20%). LC−MS: t_R = 0.83 min, $[M + 1]^+$ = 483.23. HPLC with chiral stationary phase (Chiralpak AD-H 250 × 4.6 mm i.d., 5 μm; 80% heptane containing 0.05% DEA, 20% ethanol containing 0.05% DEA): t_R = 12.9 min, 98% ((*R*)-enantiomer, t_R = 11.3 min, 2%). ^{1}H NMR (DMSO-d_6): δ 9.08 (d, J = 2.0 Hz, 1 H), 8.30 (d, J = 1.7 Hz, 1 H), 7.79 (s, 2 H), 7.72 (t, J = 5.8 Hz, 1 H), 5.58 (t, J = 5.7 Hz, 1 H), 5.33 (d, J = 5.2 Hz, 1 H), 3.93−4.01 (m, 1 H), 3.84 (d, J = 5.7 Hz, 2 H), 3.70−3.79 (m, 2 H), 3.40−3.48 (m, 1 H), 3.20−3.29 (m, 1 H), 2.69−2.78 (m, 4 H), 2.42 (s, 3 H), 2.34 (s, 3 H), 2.19 (hept, J = 6.8 Hz, 1 H), 1.22 (t, J = 7.5 Hz, 3 H), 0.94 (d, J = 6.6 Hz, 6 H). LC−HRMS: t_R = 1.93 min, $[M + H]/z$ = 483.2607, found = 483.2612.

***N*-((*S*)-3-{4-[5-(6-Cyclopentyl-5-methylpyridin-3-yl)[1,2,4]-oxadiazol-3-yl]-2-ethyl-6-methylphenoxy}-2-hydroxypropyl)-2-hydroxyacetamide (12).** **12** was obtained with method A, using nicotinic acid **61**. Pale yellow oil (105 mg, 73%). LC−MS: t_R = 0.75 min, $[M + 1]^+$ = 495.31. ^{1}H NMR ($CDCl_3$): δ 9.20 (d, J = 2.0 Hz, 1 H), 8.20 (d, J = 1.5 Hz, 1 H), 7.87 (s, 1 H), 7.85 (s, 1 H), 7.17 (t, J = 5.8 Hz, 1 H), 4.18−4.25 (m, 3 H), 3.76−3.93 (m, 3 H), 3.40−3.57 (m, 2 H), 2.74 (q, J = 7.5 Hz, 2 H), 2.49 (s, 3 H), 2.38 (s, 3 H), 2.00−2.11 (m, 2 H), 1.88−2.00 (m, 4 H), 1.70−1.81 (m, 2 H), 1.31 (t, J = 7.5 Hz, 3 H). LC−HRMS: t_R = 2.11 min, $[M + H]/z$ = 495.2607, found = 495.2609.

***N*-((*S*)-3-{4-[5-(5-Cyclopentyl-4-methylpyridin-2-yl)[1,2,4]-oxadiazol-3-yl]-2-ethyl-6-methylphenoxy}-2-hydroxypropyl)-2-hydroxyacetamide (21).** **21** was obtained with method A, using picolinic acid **68**. Beige resin (79 mg, 22%). LC−MS: t_R = 0.75 min, $[M + 1]^+$ = 495.34. ^{1}H NMR (CD_3OD): δ 8.62 (s, 1 H), 8.18 (s, 1 H), 8.00 (t, J = 6.0 Hz, 1 H), 7.90 (s, 1 H), 7.87 (s, 1 H), 4.11−4.19 (m, 1 H), 4.04 (s, 2 H), 3.84−3.92 (m, 2 H), 3.63−3.71 (m, 1 H), 3.37−3.52 (m, 2 H), 2.81 (q, J = 7.5 Hz, 2 H), 2.55 (s, 3 H), 2.41 (s, 3 H), 2.13−2.24 (m, 2 H), 1.68−1.99 (m, 6 H), 1.32 (t, J = 7.5 Hz, 3 H). LC−HRMS: t_R = 1.95 min, $[M + H]/z$ = 495.2607, found = 495.2609.

***N*-[(*S*)-3-(2-Ethyl-4-{5-[2-(1-ethylpropyl)-6-methylpyridin-4-yl][1,2,4]oxadiazol-3-yl}-6-methylphenoxy)-2-hydroxypropyl]-2-hydroxyacetamide (44).** **44** was obtained with method A, using isonicotinic acid **76d**. Pale yellow solid (836 mg, 34%). LC−MS: t_R = 0.94 min, $[M + 1]^+$ = 497.19. HPLC with chiral stationary phase (Chiralpak AD-H 250 mm × 4.6 mm i.d., 5 μm; 80% heptane containing 0.05% DEA, 20% ethanol containing 0.05% DEA): t_R = 9.5 min, 100% ((*R*)-enantiomer, t_R = 7.5 min). ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1 H), 7.89 (s, 1 H), 7.75 (s, 1 H), 7.67 (s, 1 H), 6.99 (t, J = 5.5 Hz, 1 H), 4.19−4.27 (m, 3 H), 3.92 (dd, J_1 = 9.6 Hz, J_2 = 4.7 Hz, 1 H), 3.85 (dd, J_1 = 9.5 Hz, J_2 = 6.3 Hz, 1 H), 3.80 (ddd, J_1 = 13.8 Hz, J_2 = 6.0 Hz, J_3 = 2.5 Hz, 1 H), 3.54 (ddd, J_1 = 14.1 Hz, J_2 = 7.3 Hz, J_3 = 5.5 Hz, 1 H), 3.32 (d, J = 4.5 Hz, 1 H), 2.76 (q, J = 7.5 Hz, 2 H), 2.70 (s, 3 H), 2.55 (s br, 1 H), 2.41 (s, 3 H), 1.80 (quint, J = 7.4 Hz, 4 H), 1.33 (t, J = 7.6 Hz, 3 H), 0.85 (t, J = 7.4 Hz, 6 H). ^{13}C NMR ($CDCl_3$): δ 174.4, 173.8, 168.9, 166.7, 159.3, 157.3, 137.7, 131.6, 131.5, 128.4, 126.6, 122.5, 118.3, 116.9, 74.2, 70.0, 62.1, 51.5, 42.3, 28.1, 24.6, 22.9, 16.5, 14.8, 12.1. LC−HRMS: t_R = 2.01 min, $[M + H]/z$ = 497.2764, found = 497.2767.

***N*-((*S*)-3-{4-[5-(2-Cyclopentyl-6-methylpyridin-4-yl)[1,2,4]-oxadiazol-3-yl]-2-ethyl-6-methylphenoxy}-2-hydroxypropyl)-2-hydroxyacetamide (46).** **46** was obtained with method A, using isonicotinic acid **76f**. Colorless oil (40 mg, 47%). LC−MS: t_R = 0.83 min, $[M + 1]^+$ = 495.30. HPLC with chiral stationary phase (Chiralpak AD-H 250 mm × 4.6 mm i.d., 5 μm; 80% heptane containing 0.05% DEA, 20% ethanol containing 0.05% DEA): t_R = 13.6 min, 100% ((*R*)-enantiomer, t_R = 10.5 min). ^{1}H NMR ($CDCl_3$): δ: 7.89 (s, 1 H), 7.88 (s, 1 H), 7.76 (s, 1 H), 7.73 (s, 1 H), 7.03 (t, J = 5.5 Hz, 1 H), 4.18−4.27 (m, 3 H), 3.91 (dd, J_1 = 9.6 Hz, J_2 = 4.7 Hz, 1 H), 3.85 (dd, J_1 = 9.5 Hz, J_2 = 6.3 Hz, 1 H), 3.80 (ddd, J_1 = 14.2 Hz, J_2 = 6.7 Hz, J_3 = 3.3 Hz, 1 H), 3.49−3.58 (m, 1 H), 3.38 (s br, 1 H), 3.25−3.35 (m, 1 H), 2.76 (q, J = 7.6 Hz, 2 H), 2.68 (s, 3 H), 2.40 (s, 3 H), 2.12−2.22 (m, 2 H), 1.73−1.95 (m, 6 H), 1.33 (t, J = 7.5 Hz, 3 H). ^{13}C NMR ($CDCl_3$): δ 174.4, 173.3, 168.9, 167.0, 159.2, 157.3, 137.7, 131.7, 131.6, 128.4, 126.7, 122.5, 118.2, 116.0, 74.1, 70.2, 62.2, 48.2, 42.3, 33.7, 25.8, 24.6, 22.9, 16.5, 14.8. LC−HRMS: t_R = 1.98 min, $[M + H]/z$ = 495.2607, found = 495.2600.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details on the synthesis and characterization of all target compounds and all intermediates not given above as well as data and a brief comment on mean arterial blood pressure and heart rate effects of compound **53** in spontaneously hypertensive rats are given. 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

AUC, area under the curve; BuLi, butyllithium; CC, column chromatography; DCM, dichloromethane; DEAD, diethyl azodicarbocylate; DMF, dimethylformamide; EA, ethyl acetate; EDC, 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide; HOBt, *N*-hydroxybenzotriazole; LC, lymphocyte count; LC−MS, (high pressure) liquid chromatography combined with mass spectrometry; PK, pharmacokinetics; PD, pharmacodynamics; RLM, rat liver microsome; SAR, structure−activity relationship; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; THF, tetrahydrofuran; S1P, sphingosine 1-phosphate

■ REFERENCES

(1) Chun, J.; Rosen, H. Lysophospholipid receptor as potential drug targets in tissue transplantation and autoimmune diseases. *Curr. Pharm. Des.* **2006**, *12*, 161−171.

(2) Nixon, G. F. Sphingolipids in inflammation: pathological implications and potential therapeutic targets. *Br. J. Pharmacol.* **2009**, *158*, 982−993.

(3) Obinata, H.; Hla, T. Sphingosine 1-phosphate in coagulation and inflammation. *Semin. Immunopathol.* **2012**, *34*, 73−91.

(4) Brinkmann, V.; Billich, A.; Baumruker, T.; Heining, P.; Schmouder, R.; Francis, G.; Aradhye, S.; Burtin, P. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat. Rev. Drug Discovery* **2010**, *9*, 883−897.

(5) Kappos, L.; Radue, E.-W.; O'Connor, P.; Polman, C.; Hohlfeld, R.; Calabresi, P.; Selmaj, K.; Agoropoulou, C.; Leyk, M.; Zhang-Auberson, L.; Burtin, P. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N. Engl. J. Med.* **2010**, *362*, 387−401.

(6) Cohen, J. A.; Chun, J. Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis. *Ann. Neurol.* **2011**, *69*, 759−777.

(7) Gasperini, C.; Ruggieri, S. Development of oral agent in the treatment of multiple sclerosis: how the first available oral therapy, fingolimod will change theratpeutic paradigm approach. *Drug Des., Dev. Ther.* **2012**, *6*, 175−186.

(8) Gergely, P.; Nuesslein-Hildesheim, B.; Guerini, D.; Brinkmann, V.; Traebert, M.; Bruns, C.; Pan, S.; Gray, N. S.; Hinterding, K.; Cooke, N. G.; Groenewegen, A.; Vitaliti, A.; Sing, T.; Luttringer, O.; Yang, J.; Gardin, A.; Wang, N.; Crumb, W. J. J.; Saltzmann, M.; Rosenberg, M.; Wallström, E. The selective S1P receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate: translation from preclinical to clinical studies. *Br. J. Pharmacol.* **2012**, *167*, 1035−1047.

(9) Pan, S.; Gray, N.; Gao, W.; Mi, Y.; Fan, Y.; Wang, X.; Tuntland, T.; Che, J.; Lefebvre, S.; Chen, Y.; Chu, A.; Hinterding, K.; Gardin, A.; End, P.; Heining, P.; Bruns, C.; Cooke, N. G.; Nuesslein-Hildesheim, B. Discovery of BAF312 (siponimod), a potent and selective S1P receptor modulator. *ACS Med. Chem. Lett.* **2013**, *4*, 333−337.

(10) Selmaj, K.; Li, D. K.; Hartung, H. P.; Hemmer, B.; Kappos, L.; Freedman, M. S.; Stuve, O.; Rieckmann, P.; Montalban, X.; Ziemssen, T.; Auberson, L. Z.; Pohlmann, H.; Mercier, F.; Dahlke, F.; Wallstrom, E. Siponimod for patients with relapsing-remitting multiple sclerosis (BOLD): an adaptive, dose-ranging, randomised, phase 2 study. *Lancet Neurol.* **2013**, *12*, 756−767.

(11) Bolli, M. H.; Abele, S.; Binkert, C.; Bravo, R.; Buchmann, S.; Bur, D.; Gatfield, J.; Hess, P.; Kohl, C.; Mangold, C.; Mathys, B.; Menyhart, K.; Müller, C.; Nayler, O.; Scherz, M.; Schmidt, G.; Sippel, V.; Steiner, B.; Strasser, D.; Treiber, A.; Weller, T. 2-imino-thiazolidin-4-one derivatives as potent, orally active S1P1 receptor agonists. *J. Med. Chem.* **2010**, *53*, 4198−4211.

(12) Freedman, M. S.; Olsson, T.; Melanson, M.; Fernández, O.; Boster, A.; Bach, D.; Berkani, O.; Mueller, M.; Sidorenko, T.; Pozzilli, C. Dose-Dependent Effect of Ponesimod, an Oral, Selective Sphingosine 1-Phosphate Receptor-1 Modulator, on Magnetic Resonance Imaging Outcomes in Patients with Relapsing−Remitting Multiple Sclerosis. Presented at the 28th Congress of the European Commitee for Treatment and Research in Multiple Sclerosis (ECTRIMS 2012), Lyon, France, Oct 10−13, 2012; P923.

(13) Kurata, H.; Kusumi, K.; Otsuki, K.; Suzuki, R.; Kurono, M.; Tokuda, N.; Takada, Y.; Shioya, H.; Mizuno, H.; Komiya, T.; Ono, T.; Hagiya, H.; Minami, M.; Nakade, S.; Habashita, H. Structure−activity relationsphip studies of S1P agonists with a dihydronaphthalene scaffold. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 144−148.

(14) Komiya, T.; Sato, K.; Shioya, H.; Inagaki, Y.; Hagiya, H.; Kozaki, R.; Imai, M.; Takada, Y.; Maeda, T.; Kurata, H.; Kurono, M.; Suzuki, R.; Otsuki, K.; Habashita, H.; Nakade, S. Efficacy and immunomodulatory actions of ONO-4641, a novel selective agonist for sphingosine 1-phosphate receptors 1 and 5, in preclinical models of multiple sclerosis. *Clin. Exp. Immunol.* **2013**, *171*, 54−62.

(15) ClinicalTrials.gov. Efficacy and Safety Study of RPC1063 in Relapsing Multiple Sclerosis Patients. http://clinicaltrials.gov/show/NCT01628393 (accessed July 2013).

(16) ClinicalTrials.gov. Dose Finding Study of MT-1303. http://clinicaltrials.gov/show/NCT01742052 (accessed Jan 2013).

(17) ClinicalTrials.gov. Efficacy and Safety Study of RPC1063 in Ulcerative Colitis. http://clinicaltrials.gov/show/NCT01647516 (accessed July 2013).

(18) ClinicalTrials.gov. Efficacy, Tolerability, Pharmacokinetcs and Pharmacodynamics Study of MT-1303 in Subjects with Inflammatory Bowel Disease. http://clinicaltrials.gov/show/NCT01666327 (accessed Jan 2013).

(19) Nishi, T.; Miyazaki, S.; Takemoto, T.; Suzuki, K.; Iio, Y.; Nakajima, K.; Ohnuki, T.; Kawase, Y.; Nara, F.; Inaba, S.; Izumi, T.; Yuita, H.; Oshima, K.; Doi, H.; Inoue, R.; Tomisato, W.; Kagari, T.; Shimozato, T. Discovery of CS-0777: a potent, selective, and orally active $S1P_1$ agonist. *ACS Med. Chem. Lett.* **2011**, *2*, 368−372.

(20) Moberly, J. B.; Ford, D. M.; Zahir, H.; Chen, S.; Mochizuki, T.; Truitt, K. E.; Vollmer, T. L. Pharmacological effects of CS-0777, a selective sphingosine 1-phosphate receptor-1 modulator: results from a 12-week, open-label pilot study in multiple sclerosis patients. *J. Neuroimmunol.* **2012**, *246*, 100−107.

(21) ClinicalTrials.gov. Exploring the Efficacy and Safety of Siponimod in Patients with Secondary Progressive Multiple Sclerosis (EXPAND). http://clinicaltrials.gov/show/NCT01665144 (accessed Dec 2012).

(22) ClinicalTrials.gov. Efficacy and Tolerability of BAF312 in Patients with Polymyositis and Dermatomyositis. http://clinicaltrials.gov/show/NCT01148810 (accessed Dec 2012).

(23) ClinicalTrials.gov. ACT-128800 in Patients with Moderate to Severe Chronic Plaque Psoriasis. http://clinicaltrials.gov/show/NCT01208090 accessed Aug 2012.

(24) Media Releases. Actelion's Ponesimod Successful in Mid-Stage Trial in Patients with Moderate to Severe Chronic Plaque Psoriasis. http://www.actelion.com/en/investors/media-releases/index.page?pageId=2&language=en (accessed December 18 2012).

(25) Budde, K.; Schütz, M.; Glander, P.; Peters, H.; Waiser, J.; Liefeldt, L.; Neumayer, H. H.; Böhler, T. FTY720 (fingolimod) in renal transplantation. *Clin. Transplant.* **2006**, *20* (Suppl. 17), 17−24.

(26) Shimizu, H.; Takahashi, M.; Kaneko, T.; Murakami, T.; Hakamata, Y.; Kudou, S.; Kishi, T.; Fukuchi, K.; Iwanami, S.; Kuriyama, K.; Yasue, T.; Enosawa, S.; Matsumoto, K.; Takeyoshi, I.; Morishita, Y.; Kobayashi, E. KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and hear allografts. *Circulation* **2005**, *111*, 222−229.

(27) ClinicalTrials.gov. Safety and Efficacy of KRP203 in Subacute Cutaneous Lupus Erythematosus. http://clinicaltrials.gov/show/NCT01294774 (accessed July 2013).

(28) ClinicalTrials.gov. Efficacy & Safety in Moderately Active Refractory Ulcerative Colitis Patients. http://clinicaltrials.gov/show/NCT01375179 (accessed July 2013).
(29) Buzard, D.; Han, S.; Thoresen, L.; Moody, J.; Lopez, L.; Kawasaki, A.; Schrader, T.; Sage, C.; Gao, Y.; Edwards, J.; Barden, J.; Thatte, J.; Fu, L.; Solomon, M.; Liu, L.; Al-Shamma, H.; Gatlin, J.; Le, M.; Xing, C.; Espinola, S.; Jones, R. M. Discovery and characterization of potent and selective 4-oxo-4-(5-(5-phenyl-1,2,4-oxadiazol-3-yl)-indolin-1-yl)butanoic acids as $S1P_1$ agonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6013−6018.
(30) Buzard, D. J.; Han, S.; Lopez, L.; Kawasaki, A.; Moody, J.; Thoresen, L.; Ullman, B.; Lehmann, J.; Calderon, I.; Zhu, X.; Gharbaoui, T.; Sengupta, D.; Krishnan, A.; Gao, Y.; Edwards, J.; Barden, J.; Morgan, M.; Usmani, K.; Chen, C.; Sadeque, A.; Thatte, J.; Solomon, M.; Fu, L.; Whelan, K.; Liu, L.; Al-Shamma, H.; Gatlin, J.; Le, M.; Xing, C.; Espinola, S.; Jones, R. M. Fused tricyclic indoles as S1P(1) agonists with robust efficacy in animal models of autoimmune disease. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4404−4409.
(31) Aguilar, N.; Mir, M.; Grima, P. M.; Lopez, M.; Segarra, V.; Esteban, L.; Moreno, I.; Godessart, N.; Tarrason, G.; Domenech, T.; Vilella, D.; Armengol, C.; Cordoba, M.; Sabate, M.; Casals, D.; Dominguez, M. Discovery of a novel class of zwitterionic, potent, selective and orally active S1P(1) direct agonists. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7672−7676.
(32) Almirall. Product Development Pipeline. http://www.almirall.com/webcorp2/cda/ImD_03_02.jsp (accessed Jan 2013).
(33) Demont, E. H.; Arpino, S.; Bit, R. A.; Campbell, C. A.; Deeks, N.; Desai, S.; Dowell, S. J.; Gaskin, P.; Gray, J. R. J.; Harrison, L. A.; Haynes, A.; Heightman, T. D.; Holmes, D. S.; Humphreys, P. G.; Kumar, U.; Morse, M. A.; Orborne, G. J.; Panchal, T.; Philpott, K. L.; Taylor, S.; Watson, R.; Willis, R.; Witherington, J. Discovery of a brain-penetrant $S1P_3$-sparing direct agonist of the $S1P_1$ and $S1P_5$ receptors efficacious at low oral dose. *J. Med. Chem.* **2011**, *54*, 6724−6733.
(34) Ren, F.; Deng, G.; Wang, H.; Luan, L.; Meng, Q.; Xu, Q.; Xu, H.; Xu, X.; Zhang, H.; Zhao, B.; Li, C.; Guo, T. B.; Yang, J.; Zhang, W.; Zhao, Y.; Jia, Q.; Lu, H.; Xiang, J.-N.; Elliott, J. D.; Lin, X. Discovery of novel 1,2,4-thiadiazole derivatives as potent, orally active agonists of sphingosine 1-phosphate subtype 1 ($S1P_1$). *J. Med. Chem.* **2012**, *55*, 4286−4295.
(35) Taylor, S.; Gray, J. R. J.; Willis, R.; Deeks, N.; Haynes, A.; Campbell, C.; Gaskin, P.; Leavens, K.; Demont, E.; Dowell, S.; Cryan, J.; Morse, M.; Patel, A.; Garden, H.; Witherington, J. The utility of pharmacokinetic-pharmacodynamic modeling in the discovery and optimization of selective $S1P_1$ agonists. *Xenobiotica* **2012**, *42*, 671−686.
(36) Bristol-Myers Squibb. In the Pipeline. http://www.bms.com/research/pipeline/Pages/default.aspx (accessed Jan 2013).
(37) Cherney, R. J.; Wang, Z. Heterocyclic Compounds as S1P1 Agonists for the Treatment of Autoimmune and Vascular Diseases. WO2012061459, May 10, 2012.
(38) Buzard, D. J.; Thatte, J.; Lerner, M.; Edwards, J.; Jones, R. M. Recent progress in the development of selective $S1P_1$ receptor agonists for the treatment of inflammatory and autoimmune disorders. *Expert Opin. Ther. Pat.* **2008**, *18*, 1141−1159.
(39) Bolli, M. H.; Lescop, C.; Nayler, O. Synthetic sphingosine 1-phosphate receptor modulators: opportunities and potential pitfalls. *Curr. Top. Med. Chem.* **2011**, *11*, 726−757.
(40) Dyckman, A. J. Recent advances in the discovery and development of sphingosine-1-phosphate-1 receptor agonists. *Annu. Rep. Med. Chem.* **2012**, *47*, 195−207.
(41) Roberts, E.; Guerrero, M.; Urbano, M.; Rosen, H. Sphingosine 1-phosphate receptor agonists: a patent review (2010−2012). *Expert Opin. Ther. Pat.* **2013**, *23*, 817−841.
(42) Chun, J.; Goetzl, E. J.; Hla, T.; Igarashi, Y.; Lynch, K. R.; Moolenaar, W.; Pyne, S.; Tigyi, G. International Union of Pharmacology. XXXIV. Lysophospholipid receptor nomenclature. *Pharmacol. Rev.* **2002**, *54*, 265−269.
(43) Meyer-zu-Heringdorf, D.; Jakobs, K. H. Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. *Biochim. Biophys. Acta* **2006**, *1768*, 923−940.
(44) Brinkmann, V. Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology. *Pharmacol. Ther.* **2007**, *115*, 84−105.
(45) Takabe, K.; Paugh, S. W.; Milstien, S.; Spiegel, S. "Inside-out" signaling of sphingosine-1-phosphate: therapeutic targets. *Pharmacol. Rev.* **2008**, *60*, 181−195.
(46) Brinkmann, V.; Baumruker, T. Pulmonary and vascular pharmacology of sphingosine 1-phosphate. *Curr. Opin. Pharmacol.* **2006**, *6*, 244−250.
(47) Hait, N. C.; Oskeritzian, C. A.; Paugh, S. W.; Milstien, S.; Spiegel, S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. *Biochim. Biophys. Acta* **2006**, *1758*, 2016−2026.
(48) Alvarez, S. E.; Milstien, S.; Spiegel, S. Autocrine and paracrine roles of sphingosine-1-phosphate. *Trends Endocrinol. Metab.* **2007**, *18*, 300−307.
(49) Marsolais, D.; Rosen, H. Chemical modulators of sphingosine-1-phosphate receptors as barrier-oriented therapeutic molecules. *Nat. Rev. Drug Discovery* **2009**, *8*, 297−307.
(50) Okada, T.; Kajimoto, T.; Jahangeer, S.; Nakamura, S. Sphingosine kinase/sphingosine 1-phosphate signalling in central nervous system. *Cell. Signalling* **2009**, *21*, 7−13.
(51) Milstien, S.; Gude, D.; Spiegel, S. Sphingosine 1-phosphate in neural signalling and function. *Acta Paediatr.* **2007**, *96*, 40−43.
(52) Dev, K. K.; Mullershausen, F.; Mattes, H.; Kuhn, R. R.; Bilbe, G.; Hoyer, D.; Mir, A. Brain sphingosine-1-phosphate receptors: implication for FTY720 in the treatment of multiple sclerosis. *Pharmacol. Ther.* **2008**, *117*, 77−93.
(53) Uhlig, S.; Gulbins, E. Sphingolipids in the lungs. *Am. J. Respir. Crit. Care Med.* **2008**, *178*, 1100−1114.
(54) Augé, N.; Nègre-Salvayre, A.; Salvayre, R.; Levade, T. Sphingomyelin metabolites in vascular cell signaling and atherogenesis. *Prog. Lipid Res.* **2000**, *39*, 207−229.
(55) Alewijnse, A. E.; Peters, S. L. M.; Michel, M. C. Cardiovascular effects of sphingosine-1-phosphate and other sphingomyelin metabolites. *Br. J. Pharmacol.* **2004**, *143*, 666−684.
(56) Yatomi, Y. Sphingosine 1-phosphate in vascular biology: possible therapeutic strategies to control vascular disease. *Curr. Pharm. Des.* **2006**, *12*, 575−587.
(57) Rosen, H.; Sanna, M. G.; Cahalan, S. M.; Gonzalez-Cabrera, P. J. Tipping the gatekeeper: S1P regulation of endothelial barrier function. *Trends Immunol.* **2007**, *28*, 102−107.
(58) Michel, M. C.; Mulders, A. C. M.; Jongsma, M.; Alewijnse, A. E.; Peters, S. L. M. Vascular effects of sphingolipids. *Acta Paediatr.* **2007**, *96*, 44−48.
(59) Rosen, H.; Goetzl, E. J. Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network. *Nat. Rev. Immunol.* **2005**, *5*, 560−570.
(60) Oskeritzian, C. A.; Milstien, S.; Spiegel, S. Sphingosine-1-phosphate in allergic responses, asthma and anaphylaxis. *Pharmacol. Ther.* **2007**, *115*, 390−399.
(61) Rivera, J.; Proia, R. L.; Olivera, A. The alliance of sphingosine-1-phosphate and its receptors in immunity. *Nat. Rev. Immunol.* **2008**, *8*, 753−763.
(62) Ishii, M.; Egen, J. G.; Klauschen, F.; Meier-Schellersheim, M.; Saeki, Y.; Vacher, J.; Proia, R. L.; Germain, R. N. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature* **2009**, *458*, 524−528.
(63) Alewijnse, A. E.; Peters, S. L. M. Sphingolipid signalling in the cardiovascular system: good, bad or both? *Eur. J. Pharmacol.* **2008**, *585*, 292−302.
(64) Chi, H. Sphingosine-1-phosphate and immune regulation: trafficking and beyond. *Trends Pharmacol. Sci.* **2010**, *32*, 16−24.
(65) Pyne, N. J.; Pyne, S. Sphingosine 1-phosphate and cancer. *Nat. Rev. Cancer* **2010**, *10*, 489−503.

(66) Maceyka, M.; Harikumar, K. B.; Milstien, S.; Spiegel, S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol.* **2012**, *22*, 50−60.
(67) Yu, H.; Okada, T.; Kobayashi, M.; Abo-Elmatty, D. M.; Jahangeer, S.; Nakamura, S.-i. Roles of extracellular and intracellular sphingosine 1-phosphate in cell migration. *Genes Cells* **2009**, *14*, 597−605.
(68) Li, C.; Zheng, S.; You, H.; Liu, X.; Lin, M.; Yang, L.; Li, L. Sphingosine 1-phosphate (S1P)/S1P receptors are involved in human liver fibrosis by action on hepatic myofibroblasts motility. *J. Hepatol.* **2011**, *54*, 1205−1213.
(69) Allende, M. L.; Dreier, J. L.; Mandala, S.; Proia, R. L. Expression of the sphingosine 1-phosphate receptor, $S1P_1$, on T-cells controls thymic emigration. *J. Biol. Chem.* **2004**, *279*, 15396−15401.
(70) Weigert, A.; Weis, N.; Bruene, B. Regulation of macrophage function by sphinosine-1-phosphate. *Immunobiology* **2009**, *214*, 748−760.
(71) Allende, M. L.; Tuymetova, G.; Lee, B. G.; Bonifacino, E.; Wu, Y.-P.; Proia, R. L. S1P1 receptor directs the release of immature B cells from bone marrow into blood. *J. Exp. Med.* **2010**, *207*, 1113−1124.
(72) Rathinasamy, A.; Czeloth, N.; Pabst, O.; Förster, R.; Bernhardt, G. The origin and maturity of dendritic cells determine the pattern of sphingosine 1-phosphate receptors expressed and required for efficient migration. *J. Immunol.* **2010**, *185*, 4072−4081.
(73) Cyster, J. G.; Schwab, S. R. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu. Rev. Immunol.* **2012**, *30*, 69−94.
(74) Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G.-J.; Card, D.; Keohane, C. A.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* **2002**, *296*, 346−349.
(75) Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J. Biol. Chem.* **2002**, *277*, 21453−21457.
(76) Matloubian, M.; Lo, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Proia, R. L.; Cyster, J. G. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* **2004**, *427*, 355−360.
(77) Cyster, J. G. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu. Rev. Immunol.* **2005**, *23*, 127−159.
(78) Hale, J. J.; Doherty, G.; Toth, L.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.-J.; Chrebet, G.; Bergstrom, J.; Card, D.; Forrest, M.; Sun, S.-Y.; West, S.; Xie, H.; Nomura, N.; Rosen, H.; Mandala, S. Selecting against $S1P_3$ enhances the acute cardiovascular tolerability of 3-(*N*-benzyl)-aminopropylphosphonic acid S1P receptor agonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3501−3505.
(79) Forrest, M.; Sun, S.-Y.; Hajdu, R.; Bergstrom, J.; Card, D.; Doherty, G.; Hale, J.; Keohane, C.; Meyers, C.; Milligan, J.; Mills, S.; Nomura, N.; Rosen, H.; Rosenbach, M.; Shei, G.-J.; Singer, I. I.; Tian, M.; West, S.; White, V.; Xie, J.; Proia, R. L.; Mandala, S. Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate receptor agonists in rodents are mediated via distinct receptor subtypes. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 758−768.
(80) Sanna, M. G.; Liao, J.; Jo, E.; Alfonso, C.; Ahn, M.-Y.; Peterson, M. S.; Webb, B.; Lefebvre, S.; Chun, J.; Gray, N.; Rosen, H. Sphingosine 1-phosphate (S1P) receptor subtypes $S1P_1$ and $S1P_3$, respectively, regulate lymphocyte recirculation and heart rate. *J. Biol. Chem.* **2004**, *279*, 13839−13848.
(81) Salomone, S.; Potts, E. M.; Tyndall, S.; Ip, P. C.; Chun, J.; Brinkmann, V.; Waeber, C. Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools. *Br. J. Pharmacol.* **2008**, *153*, 140−147.
(82) Murakami, A.; Taksugi, H.; Ohnuma, S.; Koide, Y.; Sakurai, A.; Takeda, S.; Hasegawa, T.; Sasamori, J.; Konno, T.; Hayashi, K.; Watanabe, Y.; Mori, K.; Sato, Y.; Takahashi, A.; Mochizuki, N.; Takakura, N. Sphingosine 1-phosphate (S1P) regulates vascular contraction via $S1P_3$ receptor: investigation based on a new $S1P_3$ receptor antagonist. *Mol. Pharmacol.* **2010**, *77*, 704−713.
(83) Hamada, M.; Nakamura, M.; Kiuchi, M.; Marukawa, K.; Tomatsu, A.; Shimano, K.; Sato, N.; Sugahara, K.; Asayama, M.; Takagi, K.; Adachi, K. Removal of sphingosine 1-phosphate receptor-3 ($S1P_3$) agonism is essential, but inadequate to obtain immunomodulating 2-aminopropane-1,3-diol $S1P_1$ agonists with reduced effect on heart rate. *J. Med. Chem.* **2010**, *53*, 3154−3168.
(84) Fryer, R. M.; Muthukumarana, A.; Harrison, P. C.; Nodop Mazurek, S.; Chen, R. R.; Harrington, K. E.; Dinallo, R. M.; Horan, J. C.; Patnaude, L.; Modis, L. K.; Reinhart, G. A. The clinically-tested S1P receptor agonists, FTY720 and BAF312, demonstrate subtype-specific bradycardia (S1P(1)) and hypertension (S1P(3)) in rat. *PLoS One* **2012**, *7*, e52985.
(85) Lucke, S.; Levkau, B. Endothelial functions of sphingosine-1-phosphate. *Cell. Physiol. Biochem.* **2010**, *26*, 87−96.
(86) Takuwa, N.; Ohkura, S.; Takashima, S.; Ohtani, K.; Okamoto, Y.; Tanaka, T.; Hirano, K.; Usui, S.; Wang, F.; Du, W.; Yoshioka, K.; Banno, Y.; Sasaki, M.; Ichi, I.; Okamura, M.; Sugimoto, N.; Mizugishi, K.; Nakanuma, Y.; Ishii, I.; Takamura, M.; Kaneko, S.; Kojo, S.; Satouchi, K.; Mitumori, K.; Chun, J.; Takuwa, Y. S1P3-mediated cardiac fibrosis in sphingosine kinase 1 transgenic mice involves reactive oxygen species. *Cardiovasc. Res.* **2009**, *85*, 484−493.
(87) Sobel, K.; Menyhart, K.; Killer, N.; Renault, B.; Bauer, Y.; Studer, R.; Steiner, B.; Bolli, M. H.; Nayler, O.; Gatfield, J. Sphingosine-1-phosphate (S1P) receptor agonists mediate pro-fibrotic responses in normal human lung fibroblasts via $S1P_2$ and $S1P_3$ receptors and Smad-independent signaling. *J. Biol. Chem.* **2013**, *288*, 14839−14851.
(88) Pyne, N. J.; Dubois, G.; Pyne, S. Role of sphingosine 1-phosphate and lysophosphatidic acid in fibrosis. *Biochim. Biophys. Acta* **2013**, *1831*, 228−38.
(89) Foster, C. A.; Howard, L. M.; Schweitzer, A.; Persohn, E.; Hiestand, P. C.; Balatoni, B.; Reuschel, R.; Beerli, C.; Schwartz, M.; Billich, A. Brain penetration of the oral immunomodulatory drug FTY720 and its phosphorylation in the central nervous system during experimental autoimmune encephalomyelitis: consequences for mode of action in multiple sclerosis. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 469−476.
(90) Balatoni, B.; Storch, M. K.; Swoboda, E.-M.; Schönborn, V.; Koziel, A.; Lambrou, G. N.; Hiestand, P. C.; Weissert, R.; Foster, C. A. FTY720 sustains and restores neuronal function in the DA rat model of MOG-induced experimental autoimmune encephalomyelitis. *Brain Res. Bull.* **2007**, *74*, 307−316.
(91) Brinkmann, V. FTY720 (fingolimod) in multiple sclerosis: therapeutic effects in the immune and central nervous system. *Br. J. Pharmacol.* **2009**, *158*, 1173−1182.
(92) Coelho, R. P.; Saini, H. S.; Sato-Bigbee, C. Sphingosine-1-phosphate and oligodendrocytes: from cell development to the treatment of multiple sclerosis. *Prostaglandins Other Lipid Mediators* **2010**, *91*, 139−144.
(93) Miron, V. E.; Ludwin, S. K.; Darlington, P. J.; Jarjour, A. A.; Soliven, B.; Kennedy, T. E.; Antel, J. P. Fingolimod (FTY720) enhances remyelination following demyelination of organotypic cerebellar slices. *Am. J. Pathol.* **2010**, *176*, 2682−2694.
(94) Choi, J. W.; Gardell, S. E.; Herr, D. R.; Rivera, R.; Lee, C.-W.; Noguchi, K.; Teo, S. T.; Yung, Y. C.; Lu, M.; Kennedy, G.; Chun, J. FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 ($S1P_1$) modulation. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 751−756.
(95) Groves, A.; Kihara, Y.; Chun, J. Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. *J Neurol. Sci.* **2013**, *328*, 9−18.

(96) Bolli, M. H.; Velker, J.; Müller, C.; Mathys, B.; Birker, M.; Bravo, R.; Bur, D.; de Kanter, R.; Hess, P.; Kohl, C.; Lehmann, D.; Meyer, S.; Nayler, O.; Rey, M.; Scherz, M.; Steiner, B. Novel $S1P_1$ receptor agonists - Part 2: From bicyclo[3.1.0]hexane fused thiophenes to isobutyl substituted thiophenes. *J. Med. Chem.* **2013**, DOI: 10.1021/jm401456d.
(97) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and developement settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−25.
(98) Hann, M. M. Molecular obesity, potency and other addictions in drug discovery. *MedChemComm* **2011**, *2*, 349−355.
(99) Waring, M. J.; Johnstone, C. A quantitative assessment of hERG liability as a function of lipophilicity. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1759−1764.
(100) Edwards, M. P.; Price, D. A. Role of physicochemical properties and ligand lipophilicity efficiency in addressing drug safety risks. *Annu. Rep. Med. Chem.* **2010**, *45*, 380−391.
(101) Walters, W. P. Going further than Lipinski's rule in drug design. *Expert Opin. Drug. Discovery* **2012**, *7*, 99−107.
(102) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457−2483.
(103) Doucet, H. Suzuki−Miyaura cross-coupling reactions of alkylboronic acid derivatives or alkyltrifluoroborates with aryl, alkenyl or alkyl halides and triflates. *Eur. J. Org. Chem.* **2008**, *2008*, 2013−2030.
(104) Jana, R.; Pathak, T. P.; Sigman, M. S. Advances in transition metal (Pd,Ni,Fe)-catalyzed cross-coupling reactions using alkyl-organometallics as reaction partners. *Chem. Rev.* **2011**, *111*, 1417−1492.
(105) Fürstner, A.; Leitner, A. Iron-catalyzed cross-coupling reactions of alkyl-Grignard reagents with aryl chloride, tosylates and triflates. *Angew. Chem., Int. Ed.* **2002**, *41*, 609−612.
(106) Fürstner, A.; Leitner, A.; Méndez, M.; Krause, H. Iron-catalyzed cross coupling reactions. *J. Am. Chem. Soc.* **2002**, *124*, 13856−13863.
(107) Hartwig, J. F. Transition metal catalyzed synthesis of arylamines and aryl ethers from aryl halides and triflates: scope and mechanism. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046−2067.
(108) Kerins, F.; O'Shea, D. F. Generation of substituted styrenes via Suzuki cross-coupling of aryl halides with 2,4,6-trivinylcyclotriboroxane. *J. Org. Chem.* **2002**, *67*, 4968−4971.
(109) Beck, G.; Heitzer, H. Verfahren zur Herstellung von 2-Halogenpyridinaldehyden und Neue 2-Halogenpyridinaldehyde. EP0702003, Mar 20, 1996.
(110) Buratti, W.; Gardini, G. P.; Minisci, F.; Bertini, F.; Galli, R.; Perchinunno, M. Nucleophilic character of alkyl radicals. V: Selective homolytic α-oxyalkylation of heteroaromatic bases. *Tetrahedron* **1971**, *27*, 3655−3668.
(111) Minisci, F. Novel applications of free-radical reactions in preparative organic chemistry. *Synthesis* **1973**, *1973*, 1−24.
(112) Duncton, M. A. J. Minisci reactions: versatile CH-functionalizations for medicinal chemists. *MedChemComm* **2011**, *2*, 1135−1161.
(113) Bolli, M. H.; Müller, C.; Mathys, B.; Abele, S.; Birker, M.; Bravo, R.; Bur, D.; Hess, P.; Kohl, C.; Lehmann, D.; Nayler, O.; Rey, M.; Meyer, S.; Scherz, M.; Schmidt, G.; Steiner, B.; Treiber, A.; Velker, J.; Weller, T. Novel $S1P_1$ receptor agonists—Part 1: From pyrazoles to thiophenes. *J. Med. Chem.* **2013**, *56*, 9737−9755.
(114) Brossard, P.; D'Ambrosio, D.; Ipek, M.; Dingemanse, J. Effects on Heart Rate of Three Different Up-Titration Regimens of Ponesimod, a Selective $S1P_1$ Receptor Modulator, and of Re-Initiation of Treatment in Healthy Male and Female Subjects. Presented at the 28th Congress of the European Commitee for Treatment and Research in Multiple Sclerosis (ECTRIMS 2012), Lyon, France, Oct 10−13, 2012; P468.
(115) Gergely, P.; Wallström, E.; Nuesslein-Hildesheim, B.; Bruns, C.; Zécri, F. J.; Cooke, N. G.; Traebert, M.; Tuntland, T.; Rosenberg, M.; Saltzman, M. Phase I Study with the Selective S1P1/S1P5 Receptor Modulator BAF312 Indicates That S1P1 Rather Than S1P3 Mediates Transient Heart Rate Reduction in Humans. Presented at the 25th Congress of the European Committe for Treatment and Research in Multiple Sclerosis (ECTRIMS), Düsseldorf, Germany, Sep 9−12, 2009; P437.
(116) Wallström, E.; Gergely, P.; Nuesslein-Hildesheim, B.; Zécri, F. J.; Cooke, N. G.; Bruns, C.; Luttringer, O.; Sing, T.; Groenewegen, A.; Rosenberg, M.; Saltzman, M. BAF312, a Selective S1P1/S1P5 Receptor Modulator, Effectively Reduces Absolute Lymphocyte Counts in Human Volunteers and Demonstrates the Relevance of S1P1 in Mediating a Transient Heart Rate Reduction. Presented at the 62nd Annual Meeting of the American Academy of Neurology, Toronto, Ontario, Canada, Apr 10−17, 2010; PO5.052.
(117) Rey, M.; Hess, P.; Clozel, M.; Delahaye, S.; Gatfield, J.; Nayler, O.; Steiner, B. Desensitization by progressive up-titration prevents first-dose effects on the heart: guinea pig study with ponesimod, a selective S1P1 receptor modulator. *PLoS One* **2013**, *8*, e74285.
(118) Fischer, H.; Gottschlich, R.; Seelig, A. Blood−brain barrier permeation: molecular parameters governing passive diffusion. *J. Membr. Biol.* **1998**, *165*, 201−211.
(119) Van De Waterbeemd, H.; Camenisch, G.; Folkers, G.; Chretien, J. R.; Raevsky, O. A. Estimation of blood−brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. *J. Drug Targeting* **1998**, *6*, 151−165.
(120) Clark, D. E. Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood−brain barrier penetration. *J. Pharm. Sci.* **1999**, *88*, 815−821.
(121) Norinder, U.; Haeberlein, M. Computational approaches to the prediction of the blood−brain distribution. *Adv. Drug Delivery Rev.* **2002**, *54*, 291−313.
(122) Hitchcock, S. A.; Pennington, L. D. Structure−brain exposure relationships. *J. Med. Chem.* **2006**, *49*, 7559−7583.
(123) Abbott, N. J.; Dolman, D. E. M.; Patabendige, A. K. Assays to predict drug permeation across the blood−brain barrier, and distribution to brain. *Curr. Drug Metab.* **2008**, *9*, 901−910.
(124) Wager, T. T.; Villalobos, A.; Verhoest, P. R.; Hou, X.; Shaffer, C. L. Strategies to optimize the brain availability of central nervous system drug candidates. *Expert Opin. Drug Discovery* **2011**, *6*, 371−381.
(125) Wager, T. T.; Hou, X.; Verhoest, P. R.; Villalobos, A. Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. *ACS Chem. Neurosci.* **2010**, *1*, 435−449.
(126) Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. Defining desirable central nervous system drug space through the alignment of molecular properties, in vitro ADME, and safety attributes. *ACS Chem. Neurosci.* **2010**, *1*, 420−434.
(127) Gerebtzoff, G.; Seelig, A. In silico prediction of blood−brain barrier permeation using the calculated molecular cross-sectional area as main parameter. *J. Chem. Inf. Model.* **2006**, *46*, 2638−50.
(128) Boström, J.; Hogner, A.; Llinàs, A.; Wellner, E.; Plowright, A. T. Oxadiazoles in medicinal chemsitry. *J. Med. Chem.* **2012**, *55*, 1817−1830.
(129) Grimont, J.; Le Goff, F.; Bourquin, G.; Silva, J. Quality Assessment and Concentration Determination of Actelion's Compound Solutions by Automated LCMS and Quantitative NMR. Presented at MipTec 2010—The Leading European Event for Drug Discovery, Basel, Switzerland, Sep 20−24, 2010.
(130) openmolecules.org. Property Explorer Applet. http://www.openmolecules.org/propertyexplorer/applet (accessed October 2013).
(131) Obach, R. S.; Reed-Hagen, A. E. Measurement of Michaelis constants for cytochrome P450-mediated biotransformation reactions using a substrate depletion approach. *Drug Metab. Dispos.* **2002**, *30*, 831−837.

(132) Chakraborti, A. K.; Kaur, G. One-pot synthesis of nitriles from aldehydes under microwave irradiation: influence of the medium and mode of microwave irradiation on product formation. *Tetrahedron* **1999**, *55*, 13265−13268.
(133) Meyer, E.; Joussef, A. C.; Gallardo, H. Synthesis of new 1,2,4- and 1,3,4-oxadiazole derivatives. *Synthesis* **2003**, *2003*, 899−905.
(134) Schmidt, G.; Reber, S.; Bolli, M. H.; Abele, S. Practical and scalable synthesis of $S1P_1$ receptor agonist ACT-209905. *Org. Process Res. Dev.* **2012**, *16*, 595−604.