Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

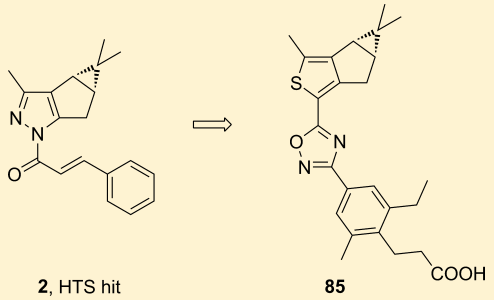
# Novel $S1P_1$ Receptor Agonists – Part 1: From Pyrazoles to Thiophenes

Martin H. Bolli,* Claus Müller, Boris Mathys, Stefan Abele, Magdalena Birker, Roberto Bravo, Daniel Bur, Patrick Hess, Christopher Kohl, David Lehmann, Oliver Nayler, Markus Rey, Solange Meyer, Michael Scherz, Gunther Schmidt, Beat Steiner, Alexander Treiber, Jörg Velker, and Thomas Weller

Drug Discovery Chemistry, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, Allschwil CH-4123, Switzerland

Supporting Information

**ABSTRACT:** From a high-throughput screening campaign aiming at the identification of novel $S1P_1$ receptor agonists, the pyrazole derivative **2** emerged as a hit structure. Medicinal chemistry efforts focused not only on improving the potency of the compound but in particular also on resolving its inherent instability issue. This led to the discovery of novel bicyclo[3.1.0]-hexane fused thiophene derivatives. Compounds with high affinity and selectivity for $S1P_1$ efficiently reducing the blood lymphocyte count in the rat were identified. For instance, compound **85** showed $EC_{50}$ values of 7 and 2880 nM on $S1P_1$ and $S1P_3$, respectively, had favorable pharmacokinetic properties in rat and dog, distributed well into brain tissue, and efficiently and dose dependently reduced the blood lymphocyte count in the rat. After oral administration to spontaneously hypertensive rats, the $S1P_1$ selective compound **85** showed no effect on mean arterial blood pressure and affected the heart rate during the wake phase of the animals only.




## INTRODUCTION

With the recent approval of FTY720 (**1**, fingolimod, Figure 1) for the treatment of relapsing remitting multiple sclerosis (MS),

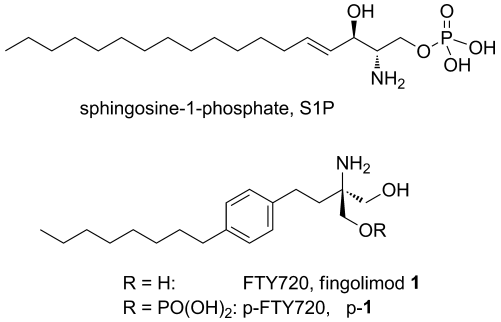



**Figure 1.** Structures of sphingosine-1-phosphate, FTY720 (fingolimod, **1**), and p-FTY720 (p-**1**).

a first sphingosine-1-phosphate (S1P, Figure 1) receptor agonist is now available to patients suffering from this debilitating disease.[1−3] After oral administration, the prodrug **1** is phosphorylated to p-FTY720 (p-**1**) and is thus rendered a nonselective agonist of the five known S1P receptors.[4−7] It has been shown that sphingosine-1-phosphate receptor 1 ($S1P_1$) agonism leads to sequestration of lymphocytes in lymph nodes and hereby prevents these immune cells from causing inflammation and tissue damage.[8−11] Two hypotheses have been proposed to explain the observed effects. In a first hypothesis, the tightening of the lymphatic endothelial cell junctions was postulated to be the main reason for the inability of the lymphocytes to leave the lymphoid tissue.[5,12−16] In a second hypothesis, the S1P concentration gradient[17] that exists between blood plasma and lymph was proposed as a driving force for lymphocytes to exit the lymph nodes and re-enter systemic circulation.[18−21] The activation of the $S1P_1$ receptor by synthetic agonists is thought to lead to receptor internalization and thus desensitization of the cell toward the external S1P gradient. According to this hypothesis, the $S1P_1$ receptor agonists therefore behave as functional antagonists. Indeed, $S1P_1$ receptor antagonists have recently been shown to also induce lymphocyte sequestration in rodents.[22−25]

In addition to the benefit of lymphocyte sequestration, several reports also proposed direct effects of S1P receptor agonism in brain[26−33] and other tissues.[34] While agonism of the $S1P_1$, and possibly the $S1P_5$ receptor,[30,35−37] are beneficial in the context of treating MS, activation of the $S1P_3$ receptor is deemed an undesired property. Several reports showed that activation of the $S1P_3$ receptor leads to heart rate reduction,[38−43] vaso- and bronchoconstriction,[42,44] blood pressure increase,[45] pulmonary epithelial leakage,[46] and fibrosis[7] in rodents. More recently, however, Hamada et al. have shown that, in the rat, selectivity against $S1P_3$ is necessary but not sufficient for a compound to be devoid of effects on heart rate.[47] Furthermore, Fryer et al.[45] carefully compared heart rate and arterial blood pressure recordings of Sprague−Dawley rats after iv infusion of the nonselective compound **1**

**Received:** September 18, 2013

   dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

with those obtained after treatment with the $S1P_3$ receptor sparing compound BAF312 (siponimod).[48] These analyses suggest that, in the rat, the short-lasting heart rate decrease seen upon iv infusion of the two agonists is caused by $S1P_1$ receptor agonism while the arterial blood pressure increase observed with **1** is associated with $S1P_3$ activation. However, it is noteworthy that Fryer et al. observed no heart rate reduction after oral administration of **1** and siponimod to Sprague–Dawley rats. There is also a growing body of evidence suggesting that $S1P_1$ receptor activation triggers a transient heart rate reduction in humans.[49−54]

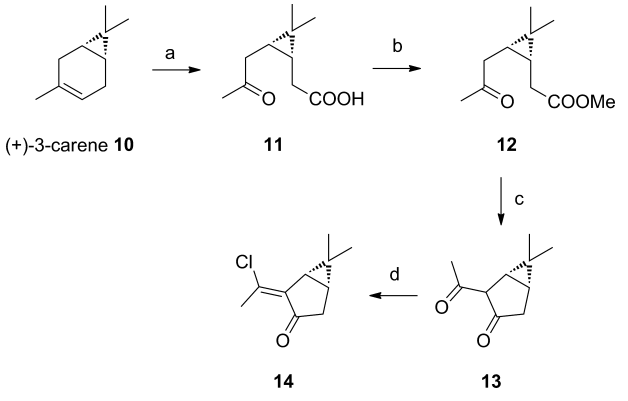
The studies summarized above defined the key properties we deemed desirable for $S1P_1$ agonists suitable for clinical development. Hence, a potential drug candidate needs to be selective against the $S1P_3$ receptor. In the rat, the pharmacokinetic (PK) properties of the compound should allow oral dosing in the low milligram range to obtain sustained 24 h maximal lymphocyte count (LC) reduction. The ability of an $S1P_1$ agonist to penetrate brain tissue was seen as a potential benefit when treating central nervous system related autoimmune diseases. Furthermore, we selected the spontaneously hypertensive rat to assess the cardiovascular safety profile of the compound. In the following paragraphs, we describe our efforts aiming at identifying compounds fulfilling the above-mentioned requirements. Compound **85**, which emerged from these efforts, showed $EC_{50}$ values of 7 and 2880 nM on the $S1P_1$ and $S1P_3$ receptors, respectively, had favorable PK properties in rat and dog, distributed well into brain tissue, and efficiently and dose dependently reduced the blood LC in the rat. After oral administration to spontaneously hypertensive rats, the $S1P_1$ selective compound **85** showed no effect on mean arterial blood pressure and affected the heart rate during the wake phase of the animals only.

## RESULTS AND DISCUSSION

**Synthesis.** The chlorovinyl ketone **14** represents a key intermediate in the syntheses of the compounds discussed in this account, and its preparation[55−58] started from (+)-3-carene **10** (Scheme 1). Ozonolysis followed by oxidative workup furnished the heptanoic acid derivative **11**. Its corresponding ester **12** was cyclized in a Dieckmann reaction to form the cyclopentanone derivative **13**, which was then chlorinated under Appel conditions[59] to yield the desired chlorovinyl ketone **14**.

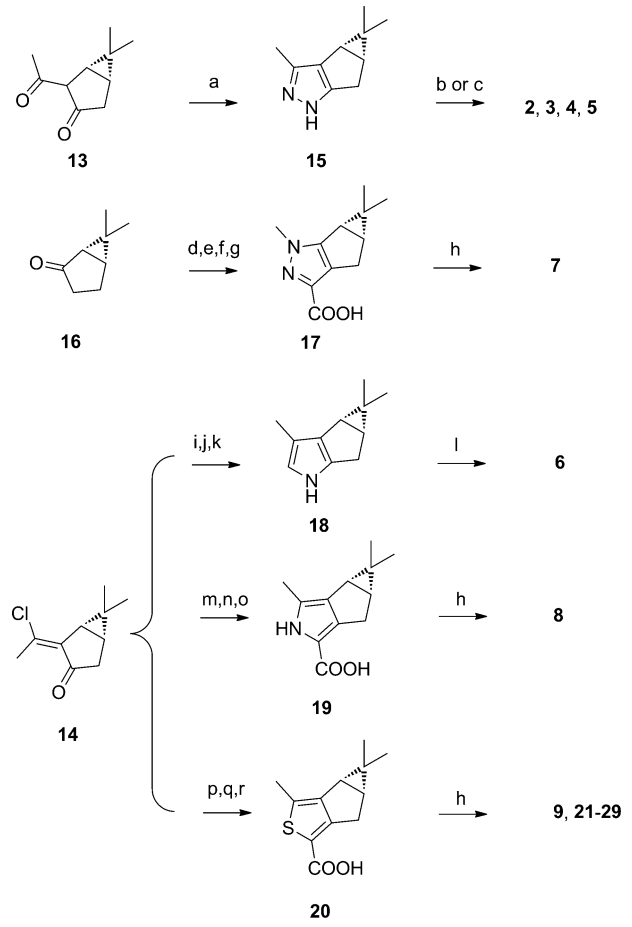
Treating diketone **13** with hydrazine hydrate in a mixture of methanol and acetic acid furnished the desired pyrazole scaffold **15**[60] that was then reacted with either a cinnamic acid derivative, a dihydrocinnamic acid, or 2-methoxybenzylamine in the presence of carbonyl di-imidazole (CDI) to give the corresponding amides **2**, **3**, and **4**, or urea **5**, respectively (Scheme 2).

**Scheme 1. Preparation of the Key Intermediate Chlorovinyl Ketone 14**[a]




[a]Reagents and conditions: (a) (i) $O_3$, MeOH, −65 °C, (ii) $H_2O_2$, rt to 35 °C, 2 h, 45−65%; (b) $SOCl_2$, MeOH, 0 °C, 30 min, 70%; (c) NaOMe, MeOH, reflux 1 h, 90% crude; (d) $PPh_3$, $CCl_4$, $CHCl_3$, 65 °C 1−3 h, 46−68%.

**Scheme 2. Preparation of Pyrazole, Pyrrole, and Thiophene Derivatives**[a]




[a]Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, AcOH, MeOH, rt, 1 h, 67%; (b) (dihydro-) cinnamic acid, EDC or TBTU, THF or DMF, 45−57% (compounds **2**−**4**); (c) 2-methoxy-benzyl amine, CDI, THF, rt, 1 h, then **15**, 50 °C, 1 h, 32% (compound **5**); (d) diethyl oxalate, KOtBu, THF, rt, 1 h; (e) MeOH, HOAc, $NH_2NH_2$ $H_2O$, rt, 30 min, 14% over two steps; (f) MeI, $Cs_2CO_3$, DMF, 0 °C to rt, 2 h; (g) 2 N aq LiOH, $H_2O$, $CH_3CN$, 45 °C, 1 h, 25% (over two steps, + 27% of N2-methyl isomer); (h) (1) appropriate benzylamine, Hünig's base, TBTU, DMF, rt, 4 h, 12−75%; for **27**, **28**: (2) 2-bromo-ethanol, 2 N NaOH, MeOH 85 °C, 24 h, 21−54%; (i) diethyl 2-aminomalonate HCl, NaOEt, EtOH, rt, 1 h; (j) 2 N aq LiOH, EtOH, 75 °C, 16 h; (k) TFA, DCM, rt, 15 min, 31−62% (over 3 steps); (l) NaHMDS, pentafluorophenyl 3-(2-methoxyphenyl)propanoate, THF, rt, 15 min, 34%; (m) glycine ethyl ester HCl, $Et_3N$, $NaHCO_3$, EtOH, 70 °C, 15 h; (n) NaOEt, EtOH, microwave 130 °C, 10 min, 25%; (o) 2 N aq LiOH, EtOH, 65 °C, 18 h, 96%; (p) NaOEt, $HSCH_2COOEt$, EtOH, rt, 1 h; (q) NaOEt, EtOH, 75 °C, 1 h; (r) 2 N aq LiOH, EtOH, 75 °C, 2 h, rt, 16 h, 67% over 3 steps.


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

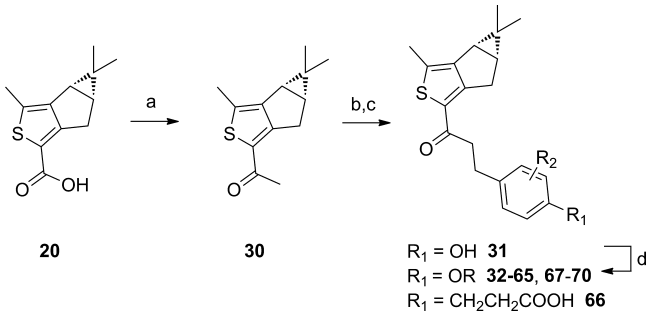
The preparation of the isomeric pyrazole scaffold **17** started from ketone **16**.[61,62] Hence, Claisen condensation of **16** with diethyl oxalate under basic conditions gave the corresponding pyruvate intermediate that, upon treatment with hydrazine hydrate in methanol and acetic acid, cyclized to the desired pyrazole scaffold.[63,64] Reacting the pyrazole intermediate with MeI in the presence of $Cs_2CO_3$ furnished the N-alkylated pyrazole scaffold as an approximately 1:1 mixture of the two regioisomers. The mixture was separated by preparative high-performance liquid chromatography (HPLC; X-terra column), and structural assignment was confirmed by X-ray crystal structure analysis of one of the two isomers (see the Supporting Information). Ester hydrolysis under basic conditions gave the pyrazole carboxylic acid **17** that was coupled with 2-methoxybenzylamine to produce amide **7**.

Reacting chlorovinyl ketone **14** with diethyl 2-amino-malonate[65] in the presence of sodium ethoxide followed by LiOH-mediated ester hydrolysis produced the 2-carboxylic acid derivative of pyrrole **18**, which upon exposure to TFA in DCM, rapidly decarboxylated to furnish pyrrole **18**.[66] N-acylation to give **6** was achieved with moderate yield by reacting deprotonated pyrrole **18** with the appropriate carboxylic acid as its pentafluorophenyl ester. Pyrrole carboxylic acid **19** was prepared by reacting chlorovinyl ketone **14** with ethyl glycinate[67,68] in the presence of $Et_3N$ and $NaHCO_3$ at 70 °C. Cyclization was achieved under microwave irradiation at 130 °C in the presence of sodium ethoxide. Lithium hydroxide mediated ester hydrolysis furnished the desired pyrrole carboxylic acid **19**. As this compound has a tendency to slowly decarboxylate, it is best purified at the ester stage. Amide coupling was achieved under standard conditions using TBTU as the coupling reagent and gave the target compound **8** in 12% yield. Finally, by treating chlorovinyl ketone **14** with methyl mercaptoacetate in the presence of sodium methoxide first at room temperature then at 75 °C followed by hydrolyzing the ester intermediate using aqueous LiOH produced the thiophene carboxylic acid **20** in good yield.[69] As before, amide formation to obtain compounds **9** and **21**−**29** was achieved under standard coupling conditions using TBTU as the activating agent.[70,71]

Starting from carboxylic acid **20**, the preparation of thiophene derivatives incorporating a propanone linker involved three steps (Scheme 3). First, the carboxylic acid was transformed into the corresponding methylketone by treating **20** with MeLi. Progress of the reaction had to be carefully monitored to avoid overreaction to the tertiary alcohol. Then, condensation of methylketone **30** with an appropriately substituted benzaldehyde and subsequent reduction of the thus formed propenone using Pd/C and hydrogen gas furnished the propanone derivative of general structure **31** in usually good yield. As indicated in step (d) in Scheme 3, further transformation of the phenol furnished the target compounds **32**−**65** and **67**−**70**. The carboxylic acid derivative **66** was obtained in a similar fashion by condensing 3-(4-formyl-2,6-dimethylphenyl)propenoic acid[71] to ketone **30** followed by reducing the corresponding propenone intermediate.

The preparation of thiophene derivatives incorporating a five-membered heteroaromatic ring as linker between the thiophene and the phenyl ring is outlined in Schemes 4 and 5. The 5-thiophen-2-yl-1,2,4-oxadiazole derivative **78** was prepared by coupling thiophene-2-carboxylic acid **20** with *N*,4-dihydroxy-3,5-dimethylbenzamidine using TBTU as the activating agent. Cyclization of the hydroxyamidine ester intermediate

**Scheme 3. Preparation of 1-Thien-2-yl-propan-1-one Derivatives (for $R_1$ and $R_2$, see Tables 2 and 3)[a]**

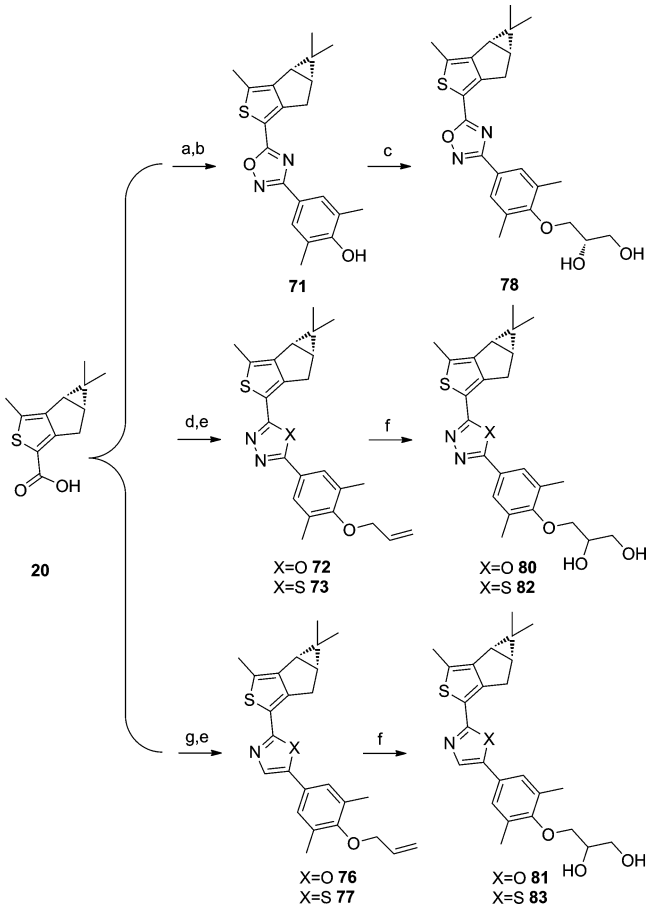



[a]Reagents and conditions: (a) MeLi, diethyl ether, 35 °C, 1−2 h, 44−77%; (b) substituted benzaldehyde, KOH, EtOH, 60−80 °C, 4−6 h; 31%; or substituted benzaldehyde, ∼6 N HCl in 2-propanol, EtOH, rt, 20 h, 42−90%; or 3-(4-formyl-2,6-dimethyl-phenyl)-acrylic acid, 10% w/v NaOH in MeOH, rt, 3 d, 40% (for **66**); (c) $H_2$, Pd/C, EtOH, THF, DIPEA (for **66**), rt, 1−18 h, 31−89%; (d) 2-bromoethanol (**33**−**45**), 3-bromopropanol (**46**) or (*R*)- or (*S*)-3-chloro-propane-1,2-diol (**47** and **48**), 2 N aq NaOH, NaI, 2-propanol, 70−90 °C, 4−24 h, 34−87%; or (2,2-dimethyl-1,3-dioxan-5-yl)methanol, $Ph_3P$, DEAD, THF, rt, 24 h, 67%, then 6 N HCl in 2-propanol, $H_2O$, THF, 1 h, 40% (**49**), or **41** or **46**, methanesulfonyl chloride, Hünig's base, DCM, rt, 1 h, 84−95%, then appropriate amine or amino acid, Hünig's base, DMF, 70−85 °C, 7−22 h, 46−73% (**50**−**56** and **58**−**60**), or epichlorohydrin, 3 N aq NaOH, 2-propanol, rt, 18 h, 63−77%, then ethanolamine, glycine or azetidine-3-carboxylic acid, EtOH, $H_2O$, Hünig's base, 65 °C, 3−5 h, 34−67% (**57**, **61**, and **62**); **54**, glycolic acid, Hünig's base, TBTU, DCM, rt, 2 h, 35% (**63**); or **31** and epichlorohydrin as for **61**, then 7 N $NH_3$ in MeOH, 60−65 °C, 2−4 h, 64−85%, then glycolic acid, Hünig's base, TBTU, DCM, rt, 1 h, 57% (**64**); or bromoacetic acid, 3 N NaOH, 2-propanol, 70 °C, 24 h; 15−24% (**65**); or **65**, appropriate amine, TBTU, Hünig's base, DMF, rt, 30 min, 53−75% (**67**−**69**); or **50**, methanesulfonyl chloride, Hünig's base, DCM, rt, 0.5−2 h, 34% (**70**).

to the 1,2,4-oxadiazole **71** was achieved by heating the crude coupling product in dioxane at 100 °C.[72,73] The glycerol side chain in **78** was introduced by alkylating the phenol **71** with (*R*)-glycerol acetonide under Mitsunobu conditions followed by acid-mediated cleavage of the acetal protective group or by reacting **71** with (*S*)-3-chloropropane-1,2-diol. Coupling thiophene-2-carboxylic acid **20** with 4-(allyloxy)-3,5-dimethyl-benzoic acid hydrazide followed by cyclization using Burgess' reagent furnished the 1,3,4-oxadiazole **72**. On the other hand, using Lawesson's reagent in the cyclization step gave access to the corresponding 1,3,4-thiadiazole **73**.[73−75] Osmium tetroxide mediated dihydroxylation of the allylic side chain delivered compounds **80** and **82** as 1:1 mixtures of the two epimers at the glycerol side chain. Coupling of thiophene-2-carboxylic acid **20** with 2-amino-4′-allyloxy-3′,5′-dimethyl-acetophenone followed by cyclization effected by either Burgess' or Lawesson's reagent gave access to the oxazole **76** or the thiazole **77**, respectively.[75−77] As before, dihydroxylation of the allylic ether furnished the desired diols **81** and **83** as a 1:1 mixture of epimers. The 3-thiophen-2-yl-1,2,4-oxadiazole derivative **79** (Scheme 5) was prepared in analogy to **78**. Thus, reaction of chlorovinyl ketone **14** with thioacetic acid cyanomethyl ester under basic conditions furnished the 2-cyano-thiophene derivative that, after reaction with hydroxylamine hydrochloride, gave *N*-hydroxyamidine **74**.[73,78] Coupling of **74** with 3,5-dimethyl-4-hydroxy-benzoic acid followed by cyclization under Dean−Stark conditions produced the 1,2,4-oxadiazole **75**


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

**Scheme 4. Preparation of Thiophene Derivatives with Five-Membered Heteroaromatic Linkers[a]**

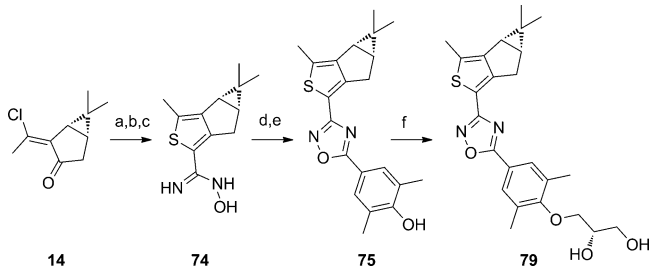



[a]Reagents and conditions: (a) *N*,4-Dihydroxy-3,5-dimethylbenzamidine, TBTU, Hünig's base, DMF, rt, 1−6 h; (b) dioxane or toluene, 100 °C, 18−24 h, 39−71% (over 2 steps); (c) (1) (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol, $Ph_3P$, DEAD, THF, rt, 24 h, 66% (2) 6 N HCl in 2-propanol, $H_2O$, THF, rt, 30 min, 59%; or 3-chloropropane-1,2-diol, 2 N NaOH, isopropanol, 65 °C, 10 h, 50%; (d) 4-(allyloxy)-3,5-dimethylbenzoic acid hydrazide, HOBt, EDC, $Et_3N$, DCM, 0 °C, 16 h, 82%; (e) Burgess reagent, THF, microwave irradiation 110 °C, 5 min, 58-70% (for **72** and **76**) or Lawesson reagent, THF, microwave irradiation 110 °C, 5 min, 30−41% (for **73** and **77**); (f) $OsO_4$, NMO, acetone, $H_2O$, rt, 16−18 h, 15−48%; (g) 2-amino-4′-allyloxy-3′,5′-dimethyl-acetophenone,[77] HOBt, EDC HCl, $Et_3N$, DCM, rt, 5 h, 51%.

in good yield. Alkylation of the phenol moiety with (*S*)-3-chloro-propane-1,2-diol concluded the synthesis of **79**.

**Early in Vitro Structure−Activity Relationship Studies Addressing the Instability of Pyrazole 2.** From a high-throughput screening (HTS) campaign based on FLIPR technology using CHO cells stably expressing a chimeric $S1P_1$-$G_{\alpha qs}$ construct, compound **2** emerged as a hit structure. In a GTP$\gamma$S assay using membranes of CHO cells overexpressing the human $S1P_1$ receptor, this compound showed an $EC_{50}$ value of 16 nM, and small changes in its structure led to analogues, such as compounds **3** or **4**, with single digit nanomolar activities on $S1P_1$ (Figure 2). Compounds **3** and **4** were more than 100-fold selective against $S1P_3$. However, it soon became evident that the stability of the acyl pyrazole moiety, in particular in aqueous media, is insufficient for these molecules to serve as drugs.[79] Urea analogues such as compound **5** retained at least some of the affinity for $S1P_1$

**Scheme 5. Preparation of Thiophene Derivatives with Five-Membered Heteroaromatic Linkers[a]**




[a]Reagents and conditions: (a) thioacetic acid *S*-cyanomethylester, 2 N aq NaOH, THF, rt, 3 h; (b) 2 N aq NaOH, THF, 90 °C, 4 h, 59% (over 2 steps); (c) KOtBu; $HONH_2$−HCl, MeOH, rt, 5 h, 85%; (d) 3,5-dimethyl-4-hydroxy benzoic acid, TBTU, Hünig's base, DMF, rt, 2−3 h, 46%; (e) toluene, 110 °C, 35 h, 86%; or dioxane, 60 °C, 24 h, 41%; (f) (*S*)-3-chloro-propane-1,2-diol, 2 N aq NaOH, 2-propanol, 65 °C, 10 h, 18−38%.

but did not significantly ameliorate the instability problem. Similarly, replacing the pyrazole moiety by a pyrrole ring (e.g., pyrrole **6**, Figure 3) delivered potent $S1P_1$ agonists for which in vivo efficacy on blood lymphocyte count reduction could be measured after oral dosing, but the compounds were still prone to hydrolyze in aqueous media. Analogues wherein the acyl group was attached to a carbon rather than a nitrogen atom of the five-membered heterocycle, as in compounds **7** and **8**, for instance, lost affinity for the $S1P_1$ receptor (Figure 3).

However, replacing the pyrrole in compound **8** by a thiophene furnished stable compounds with improved affinity for the $S1P_1$ receptor (e.g., compound **9**, Figure 3). We therefore set out to explore the structure−activity relationship (SAR) of these novel thiophene derivatives in more detail.

**In Vitro SAR of Bicyclo[3.1.0]hexane-fused Thiophene Derivatives.** Easy access to the thiophene-2-carboxamides, such as **9**, facilitated exploration of the SAR around the phenyl ring. First, several examples incorporating either a chlorine or a fluorine atom, a methyl or a methoxy group were prepared. Among these monosubstituted derivatives, the 2-methoxy compound **9** was clearly the most potent representative (data not shown). In a second step, we introduced another methoxy group to the phenyl ring (Table 1). As it turned out, this additional group was only tolerated in the 4-position (compound **22**). In order to reduce the lipophilicity of the compound, we attached polar chains to the phenyl moiety. As illustrated by compound **25**, a glycolic acid moiety was not tolerated in position 4. Similarly, an ethylene glycol attached to position 2 or 3 led to inactive compounds (**26**, **27**). However, attaching the ethylene glycol to position 4 of the phenyl ring was tolerated (compound **28**) and in combination with a 2-methoxy substituent gave rise to compound **28** that showed a similar $S1P_1$ receptor affinity but a clearly reduced lipophilicity (clogP = 4.4) when compared to analogues **9** and **22** (both clogP = 5.2).

Meanwhile, we established the chemistry to get access to thiophene derivatives incorporating a ketone function in the linker (Scheme 3), and we prepared a set of compounds making use of the SAR knowledge acquired with the thiophene-2-carboxamides (Table 2). We therefore prepared compound **32** incorporating an ethylene glycol side chain in position 4 of the phenyl ring. As already observed with the two pyrazoles **4** and **5** (Figure 2), compound **32** lacking a nitrogen in the linker


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

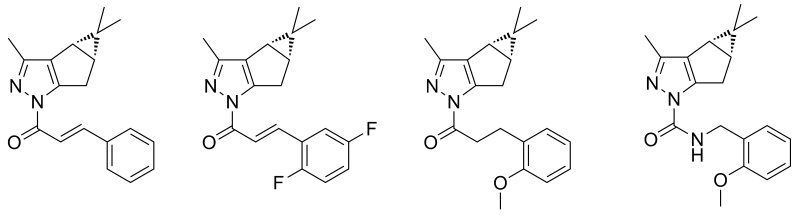



**Figure 2.** Structure and in vitro activity of the HTS hit **2** and close analogues thereof.

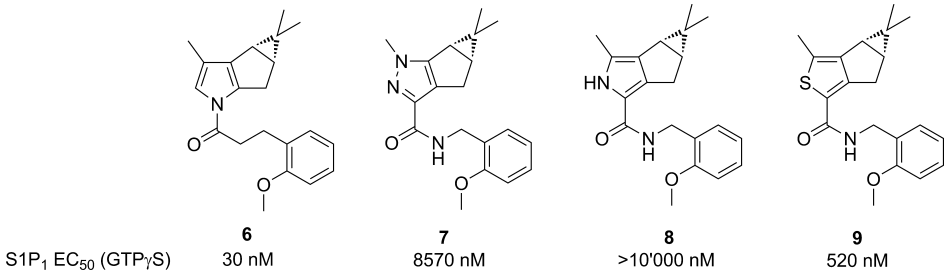
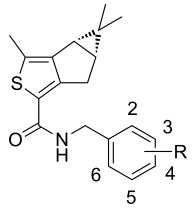



**Figure 3.** Some examples of close analogues of the HTS hit **1** prepared to overcome the instability of **2** in aqueous media.

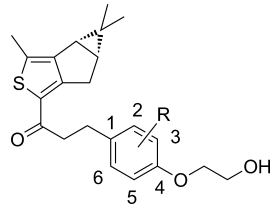
**Table 1. SAR of the Substituents around the Phenyl Ring in the Thiophene-2-carboxamide Series**



| compound | R | $EC_{50}$ $S1P_1$[a] [nM] | $EC_{50}$ $S1P_3$[a] [nM] |
|---|---|---|---|
| **9** | 2-methoxy | 520 | >10 000 |
| **21** | 2,3-dimethoxy | 7490 | >10 000 |
| **22** | 2,4-dimethoxy | 450 | >10 000 |
| **23** | 2,5-dimethoxy | 7280 | >10 000 |
| **24** | 2,6-dimethoxy | >10 000 | >10 000 |
| **25** | 2-methoxy-4-(carboxymethoxy) | >10 000 | >10 000 |
| **26** | 2-(2-hydroxyethoxy) | >10 000 | >10 000 |
| **27** | 3-(2-hydroxyethoxy) | >10 000 | >10 000 |
| **28** | 4-(2-hydroxyethoxy) | 1800 | >10 000 |
| **29** | 2-methoxy-4-(2-hydroxyethoxy) | 282 | >10 000 |

[a]$EC_{50}$ values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$;[80] $EC_{50}$ values represent geometric mean values of at least three independent measurements in duplicate. For details see the Experimental Section.

**Table 2. SAR of the Ethylene Glycol Substituted Phenyl Ring in the 1-Thien-2-yl propan-1-one Series**



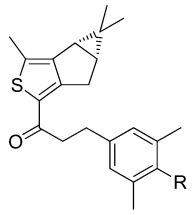
| compound | R | $EC_{50}$ $S1P_1$[a] [nM] | $EC_{50}$ $S1P_3$[a] [nM] |
|---|---|---|---|
| **32** | none | 15.0 | 1610 |
| **33** | 2-methoxy | 4.6 | 2780 |
| **34** | 2-chloro | 6.5 | 5200 |
| **35** | 2,6-dimethoxy | 3110 | >10 000 |
| **36** | 2,6-dimethyl | 902 | >10 000 |
| **37** | 3-methoxy | 28.6 | >10 000 |
| **38** | 3-methyl | 5.8 | 2590 |
| **39** | 3-chloro | 9.8 | 4630 |
| **40** | 3,5-dimethoxy | 232 | >10 000 |
| **41** | 3,5-dimethyl | 2.6 | 879 |
| **42** | 3,5-dichloro | 1.1 | 4250 |
| **43** | 3-chloro-5-methyl | 1.8 | 1350 |
| **44** | 3-ethyl-5-methyl | 4.4 | >10 000 |
| **45** | 2,3,5-trimethyl | 23.7 | >10 000 |

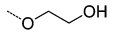
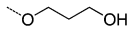
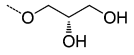
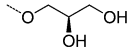
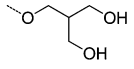
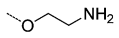
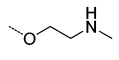
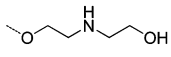
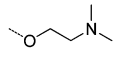
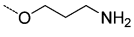
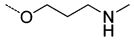
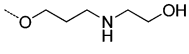
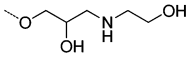
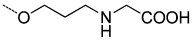
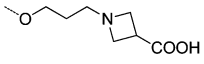
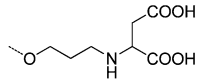
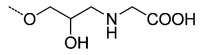
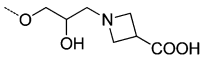
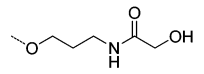
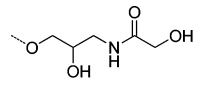
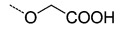
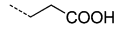
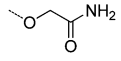
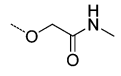
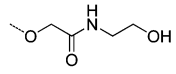
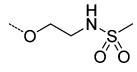
[a]$EC_{50}$ values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$;[80] $EC_{50}$ values represent geometric mean values of at least three independent measurements in duplicate. For details see the Experimental Section.

turned out to be considerably more potent on $S1P_1$ when compared to its amide linked analogue **28**. While keeping the ethylene glycol moiety in position 4 constant, we therefore evaluated the effect of an additional substituent at the phenyl ring in more detail. As illustrated with compounds **33** and **34**, a substituent in position 2 further improved the potency of the compound on $S1P_1$ and slightly decreased the activity on $S1P_3$. In contrast, as shown with compounds **35** and **36**, a 2,6-disubstitution seriously hampered the affinity of the compound for $S1P_1$. A substituent in the 3-position had a moderate effect on the $S1P_1$ potency (compounds **37**–**39**) only. Interestingly, while a 3,5-dimethoxy (**40**) substitution pattern resulted in a marked loss in affinity for $S1P_1$, 3,5-dichloro (**42**) and in particular 3,5-dimethyl (**41**) and 3-chloro-5-methyl (**43**) substitutions furnished highly potent $S1P_1$ receptor agonists with good selectivity against $S1P_3$. Among the various 3,5-disubstitutions evaluated, the 3,5-dichloro (**42**) and the 3-ethyl-5-methyl (**44**) patterns gave the best selectivity against $S1P_3$. Compound **45** illustrates that combining the 3,5-disubstitution


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

**Table 3. SAR of Polar Side Chains in the 4-Position of the Phenyl Ring**



| compound | R | $EC_{50}$ $S1P_1$[a] [nM] | $EC_{50}$ $S1P_3$[a] [nM] |
|---|---|---|---|
| **41** |  | 2.6 | 879 |
| **46** |  | 4.7 | 1800 |
| **47** |  | 0.8 | 623 |
| **48** |  | 1.4 | 489 |
| **49** |  | 6.3 | 2200 |
| **50** |  | 1.4 | 407 |
| **51** |  | 2.6 | 780 |
| **52** |  | 5.1 | 1040 |
| **53** |  | 10.7 | 2840 |
| **54** |  | 6.6 | 4200 |
| **55** |  | 6.8 | 5030 |
| **56** |  | 13.4 | 8420 |
| **57**[b] |  | 4.8 | 7470 |
| **58** |  | 1.1 | 201 |
| **59** |  | 2.8 | 1070 |
| **60**[b] |  | 3.5 | 348 |
| **61**[b] |  | 1.5 | 597 |
| **62**[b] |  | 2.0 | 1000 |
| **63** |  | 0.9 | 555 |
| **64**[b] |  | 1.3 | 437 |
| **65** |  | 300 | >10000 |
| **66** |  | 17.8 | 5210 |
| **67** |  | 0.6 | 875 |
| **68** |  | 3.4 | 870 |
| **69** |  | 4.4 | 3410 |
| **70** |  | 3.3 | 566 |

[a]$EC_{50}$ values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$;[80] $EC_{50}$ values represent geometric mean values of at least three independent measurements in duplicate. For details see the Experimental Section. [b]Compound represents a 1:1 mixture of epimers with respect to the polar side chain.

with a 2-substituent reduces the activity of the compound on $S1P_1$, indicating that, at the phenyl ring, either a 2- or a 3,5-substitution is optimal for $S1P_1$ receptor affinity.

Keeping the 3,5-dimethyl substitution pattern at the phenyl ring constant, we then evaluated the effect of a large variety of polar side chains in position 4 (Table 3). As compared to the ethylene glycol **41**, the propylene glycol analogue **46** was slightly less potent on both $S1P_1$ and $S1P_3$. Introducing an additional hydroxy group to this side chain gave the 2,3-dihydroxy-propoxy derivatives **47** and **48**. As illustrated by these two compounds, the chirality in the side chain had a minimal effect on the potency of the compound. As observed in other series (data not shown), the (*S*)-configured side chain was slightly more potent on $S1P_1$ while both epimers showed comparable affinity for $S1P_3$. The achiral diol **49** was less potent than diols **47** and **48**. Replacing the alcohol function in **46** by a primary (compound **50**) or secondary amine (compounds **51** and **52**) again furnished highly potent $S1P_1$ agonists with good selectivity against $S1P_3$. The tertiary amine **53** represented a slightly less potent $S1P_1$ receptor agonist. Compounds **54**, **55**,

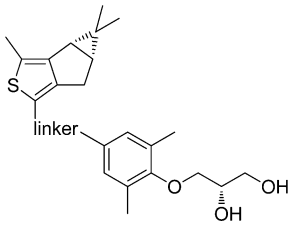

dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

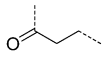
and **56** incorporating an amino-propoxy chain were less potent on $S1P_1$ but also more selective against $S1P_3$ when compared to their corresponding amino-ethoxy analogues **50**, **51**, and **52**, respectively. As seen with compounds **46** and **47**, introducing an additional hydroxy group to the side chain to give compound **57** improved the affinity for $S1P_1$. The three examples **58**–**60** illustrate that incorporating an amino acid into the side chain was tolerated and furnished highly potent $S1P_1$ receptor agonists. Even the two carboxylic acid groups in glutamate derivative **60** were well tolerated by the receptor. In this series, however, an additional hydroxy group attached to the amino-propoxy unit had only minimal effect on the potency of the compound and selectivity (compare **61** and **62** with **58** and **59**, respectively). In view of the above data, it is not surprising that also amides **63** and **64** were potent and selective $S1P_1$ receptor agonists. Interestingly, there is a marked difference in potency between the two carboxylic acids **65** and **66**. While the glycolic acid derivative **65** showed only moderate potency on the $S1P_1$ receptor (in line with observations made with compound **25**), the propanoic acid derivative **66** showed significant affinity for $S1P_1$. The glycolic acid amide derivatives **68**, **69**, and in particular, **67** represent highly potent and selective $S1P_1$ receptor agonists. Finally, as illustrated by compound **70**, a sulfonamide moiety was also tolerated by $S1P_1$. In brief, the compounds compiled in Table 3 illustrate that a large variety of polar side chains attached to the 4-position of the phenyl ring are tolerated by the $S1P_1$ receptor. The wide range of functional groups tolerated in this part of the molecule clearly allows for fine-tuning of the physicochemical properties of the compound.

With a last set of compounds compiled in Table 4, we explored the SAR of the linker between the thiophene and the phenyl ring. Inspired by the structure of $S1P_1$ selective agonist SEW2871[11], the propanone moiety was replaced by an oxadiazole and other five-membered heteroaromates. Interestingly, as illustrated by compounds **78**–**80**, replacing the propanone linker by an oxadiazole had little impact on the affinity of the compound for $S1P_1$. However, all three oxadiazole derivatives were markedly more potent on $S1P_3$ and were therefore clearly less selective when compared to **47**. The oxazole **81** had a similar receptor affinity profile as its structurally closest oxadiazole analogues **78** and **80**. In contrast, the thiadiazole **82** and the thiazole **83** were both significantly less potent $S1P_1$ agonists.

**In Vivo Efficacy Assessment.** Several compounds incorporating a carene-derived thiophene scaffold were assessed for their ability to reduce the peripheral blood LC in the rat, and a few examples are listed in Table 5. The compounds were administered orally at a dose of 10 mg/kg to male Wistar rats, and the blood LC was measured shortly before and 3, 6, and 24 h after compound administration. A LC reduction of greater than or equal to 60% was considered to be the maximal effect that is observed under the described experimental conditions.[80] As illustrated by the compounds in Table 5, a large variety of side chains attached to position 4 of the phenyl ring led to in vivo active compounds. Clear differences in the pharmacodynamic profiles could be observed. For instance, while the propanone-linked compounds incorporating a mono- (**46**) or dialcohol (**47**), an amino acid (**61**), or a glycolamide (**64**) in the side chain reached maximal efficacy at 3 h and already showed partial recovery of LC at 6 h, compounds incorporating an amine or an amino alcohol side chain (**52** and **55**–**57**) reached maximal efficacy at 6 h. At 24 h, only amino alcohols

**Table 4. SAR of the Linker between the Thiophene and the Phenyl Ring**



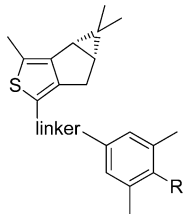

| compound | linker | $EC_{50}$ $S1P_1$[a] [nM] | $EC_{50}$ $S1P_3$[a] [nM] |
|---|---|---|---|
| **47** |  | 0.8 | 623 |
| **78** |  | 1.1 | 40.6 |
| **79** |  | 0.7 | 19.9 |
| **80**[b] |  | 0.7 | 65.3 |
| **81**[b] |  | 1.0 | 110 |
| **82**[b] |  | 34.2 | >10000 |
| **83**[b] |  | 37.8 | >10000 |

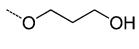
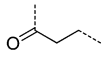
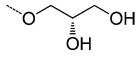
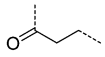
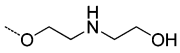
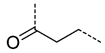
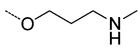
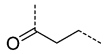
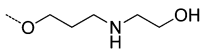
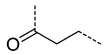
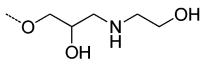
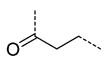
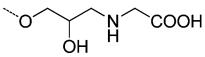
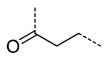
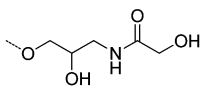
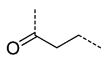
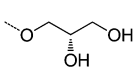
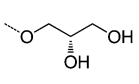
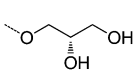
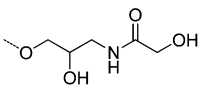
[a] $EC_{50}$ values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$;[80] $EC_{50}$ values represent geometric mean values of at least three independent measurements in duplicate. For details see the Experimental Section. [b] 1:1 mixture of epimers at 2,3-dihydroxy-propoxy side chain.

**56** and **57** showed significant LC reductions. On the other hand, the four oxadiazole-linked compounds **78**–**80** and **84** already reached maximal efficacy at 3 h post-administration, and the LC remained maximally reduced at 6 h. Similar to the propanone-linked analogue **47**, the LC recovered 24 h after administration of the three compounds incorporating a glycerol side chain (**78**–**80**). On the other hand, the glycolamide **84** showed maximal LC reductions for 24 h and therefore displayed a much longer duration of action when compared to the propanone-linked analogue **64**. Studies with additional analogues revealed that compounds **47** and **64** and **78** and **84** are prototypical with respect to the in vivo behavior of propanone- and oxadiazole-linked thiophene derivatives, respectively. In part two of this communication series, we further illustrate that oxadiazole-linked compounds consistently show a higher LC reduction efficacy due to a more sustained PK when compared to their propanone analogues.[81]


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX–XXX

Table 5. Effect on Blood LC after Oral Administration of 10 mg/kg Compound to Male Wistar Rats




| compound | R | linker | %LC 3 h | %LC 6 h | %LC 24 h |
|---|---|---|---|---|---|
| 46 |  |  | -58 | -47 | -4 |
| 47 |  |  | -63 | -48 | 12 |
| 52 |  |  | -32 | -58 | -26 |
| 55 |  |  | -39 | -59 | 10 |
| 56 |  |  | -37 | -62 | -42 |
| 57 |  |  | -23 | -66 | -54 |
| 61 |  |  | -42 | -26 | 34 |
| 64 |  |  | -57 | -50 | 18 |
| 78 |  |  | -72 | -76 | -27 |
| 79 |  |  | -69 | -74 | -14 |
| 80 |  |  | -76[a] | -78[a] | 10[a] |
| 84[b] |  |  | -66 | -69 | -73 |

[a]Data of pure (*S*)-enantiomer. [b]This compound showed $EC_{50}$ values of 1.3 and 7.0 nM for $S1P_1$ and $S1P_3$, respectively.

The potency on $S1P_1$ and selectivity against $S1P_3$ was then further optimized in the oxadiazole series by combining a 3-ethyl-5-methyl substitution pattern with a propanoic acid chain in position 4 at the phenyl ring. This led to compound **85** showing $EC_{50}$ values of 7 and 2880 nM for $S1P_1$ and $S1P_3$, respectively (Figure 4 and Table 6). A dose response of LC change in rats was established with oxadiazole **85** (Figure 4). Administration of 0.3 mg/kg **85** to Wistar rats displayed minimal if any blood lymphocyte sequestration. Administration of 1, 3, and 10 mg/kg had a clear, dose-dependent effect on the number of circulating lymphocytes. Rapid reversibility of the LC was observed at 1 and 3 mg/kg. At 10 mg/kg, the LC was maximally reduced for 24 h. For the three active doses of **85**, the reduction of the LC measured at 3 h demonstrates rapid onset of action. A PK experiment with oxadiazole **85** in the rat revealed that the compound was absorbed almost completely and reached a $C_{max}$ of 4000 ng/mL at 2 h after administration (Table 7). A rather low clearance and a volume of distribution


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

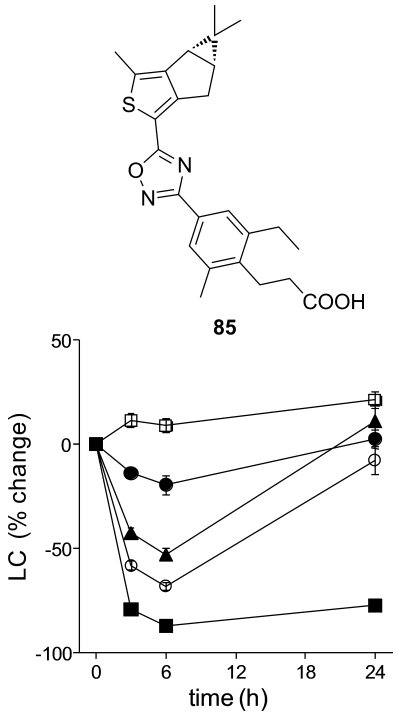



**Figure 4.** Change of blood lymphocyte count after oral administration of **85** to male Wistar rats. □ vehicle ($n$ = 5); ● 0.3 mg/kg ($n$ = 5); ▲ 1 mg/kg ($n$ = 5); ○ 3 mg/kg ($n$ = 5); ■ 10 mg/kg ($n$ = 5). For experimental details see the Supporting Information.

**Table 6. In Vitro Potency Profile (GTPγS $EC_{50}$ Values) of Compound 85 on Human (h) and Rat (r) S1P Receptors**

| | $hS1P_1$ | $hS1P_2$ | $hS1P_3$ | $hS1P_4$ | $hS1P_5$ | $rS1P_1$ | $rS1P_3$ |
|---|---|---|---|---|---|---|---|
| $EC_{50}$[a] [nM] | 7.0 | >10 000 | 2880 | >10 000 | 275 | 4.4 | 6110 |
| $\sigma_g$[b] | 1.8 | – | 1.8 | – | 2.6 | 2.3 | 1.7 |
| $n$[c] | 11 | 7 | 12 | 7 | 10 | 3 | 3 |

[a]$EC_{50}$ values as determined in a GTPγS assay using membranes of CHO cells expressing either $S1P_1$ or $S1P_3$;[80] $EC_{50}$ values represent geometric mean values of at least three independent measurements in duplicate. For details see the Experimental Section. [b]Geometric standard deviation. [c]Number of independent measurements.

clearly exceeding total body water resulted in a half-life of 4.9 h. The PK profile therefore showed a good correlation with the LC reductions in the rat.

The PK profile in the Beagle dog was comparable (Table 7). The compound was rapidly absorbed reaching high plasma concentrations. Clearance in the dog was lower than in the rat leading to an even longer half-life despite a smaller volume of distribution. Compound **85** distributed well into brain tissue reaching concentrations of 220, 2880, and 410 ng/g at 2, 6, and 24 h, respectively, after oral administration of 10 mg/kg to Wistar rats.

**Cardiovascular Effects in Spontaneously Hypertensive Rats.** In a next step, compound **85** was administered to conscious spontaneously hypertensive rats (SHR) equipped with a telemetry system allowing noninvasive monitoring of mean arterial blood pressure (MAP) and heart rate (HR).[82,83] The compound was administered at doses of 3, 10, 30, and 100 mg/kg in the morning, that is, at the end of the wake phase of the animals. None of the doses induced a MAP increase that was observed with **1** using the same experimental conditions (Figures B and C, Supporting Information). However, the HR recordings 24 h before (control) and after administration of compound **85** shown in Figure 5 clearly document that oral administration of compound **85** to SHR affects HR. As illustrated by the data summarized in Figure 6, the amplitude of the HR reduction as well as the overall extent of the effect as expressed by the area between the curves (ABC) followed a clear dose response. A dose of 3 mg/kg had no significant effect on HR. With the doses of 10, 30, and 100 mg/kg, the HR remained unchanged for the first 8 h after oral administration (sleep phase of the rats). However, about 8 h after dosing, a dose-dependent difference (ABC) between the HR of control and treated rats started to manifest, and the effect persisted for 8−10 h (i.e., for the entire wake phase of the animals). In fact, the HR increase normally observed during the wake phase of nontreated rats was completely abolished in animals treated with 30 and 100 mg/kg of compound **85**. At a dose of 10 mg/kg, the HR was maximally 23 bpm lower than that of the control at about 9 h after compound administration. On the basis of the PK experiment and the plasma exposure data obtained from the LC experiment, plasma concentrations of **85** were 4−6 μM at this time point and therefore at best reached the $EC_{50}$ value for the rat $S1P_3$ receptor. At 12 h, plasma concentrations were clearly below the $EC_{50}$ value on $S1P_3$. At 30 and 100 mg/kg, the HRs were 32 and 37 bpm lower than those in the control rats, respectively. The ABC reached 250 bpm × h at 10 mg/kg and appeared to reach a plateau with 350 and 380 bpm × h at 30 and 100 mg/kg, respectively.

We then speculated that the late onset of the effect on HR could be due to the slow formation of an active metabolite. However, the observation that compounds **56** and **57** (both tested at 100 mg/kg, for **57** see Figure D Supporting Information), but also **1** (Figure 6, Figure E Supporting Information) displayed a very similar behavior in SH rats precluded this interpretation. It is important to note that maximal HR changes were clearly more pronounced with **1** than with compound **85** (Figure 6) and that with **1** first signs of HR changes manifested earlier, that is, 3 h after dosing already. As p-**1** is a potent agonist of the $S1P_3$ receptor[4,5,45] while compound **85** is highly selective for $S1P_1$ (Table 6), part of the HR effect observed with **1** can be attributed to $S1P_3$ receptor

**Table 7. Pharmacokinetic Data of Compound 85**

| | pharmacokinetic parameters[a] | | | | | |
|---|---|---|---|---|---|---|
| species | $F$ [%] | $C_{max}$ ($t_{max}$) [ng/mL]([h]) | $AUC_{0-24h}$ [(ng·h)/mL] | $t_{1/2}$ [h] | clearance [mL/(min·kg)] | $V_{ss}$ [L/kg] |
| rat | 97 | 4000 (2) | 40 800 | 4.9 | 4.0 | 1.6 |
| dog | 62 | 3160 (2) | 39 300 | 6.9 | 0.8 | 0.45 |

[a]$F$ = bioavailability; $C_{max}$ = maximal plasma concentration; $t_{max}$ = time at which $C_{max}$ was reached; $t_{1/2}$ = half-life determined with iv experiment; $V_{ss}$ = volume of distribution; oral dosing: 10 mg/kg in 3 Wistar rats, 3 mg/kg in 2 Beagle dogs; iv dosing: 1 mg/kg in 2 rats, 2 dogs; for experimental details see the Supporting Information.


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

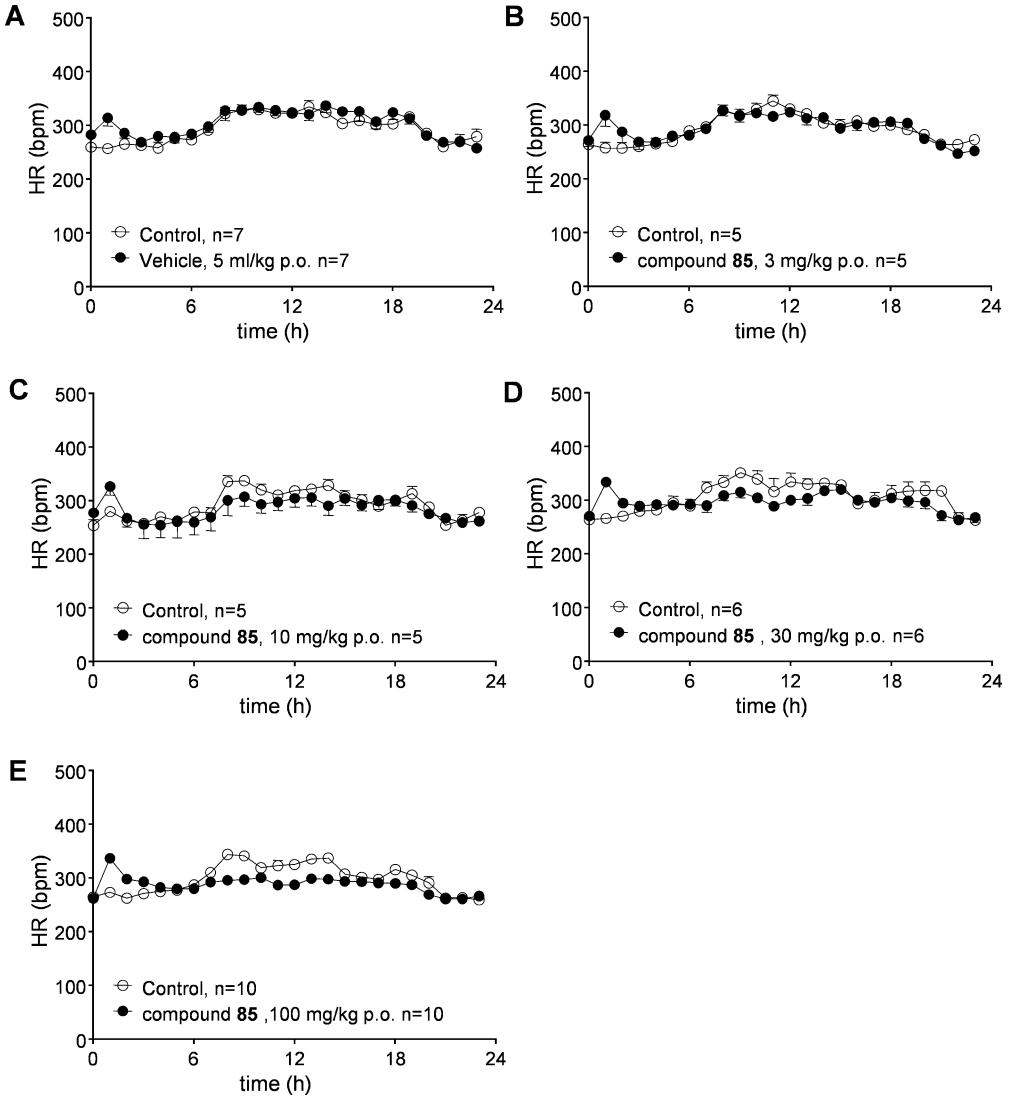



**Figure 5.** HR in beats per minute (bpm) after oral administration of (A) vehicle, (B) 3, (C) 10, (D) 30, and (E) 100 mg/kg **85** to male SHR. The HR increase observed during the first 2 h is an artifact of the oral gavage. For experimental details see the Supporting Information and Hess et al.[84]

agonism. Fryer et al.[45] observed a clear but only short-lasting HR reduction in anesthetized normotensive Sprague–Dawley rats after infusion of nonselective **1** and $S1P_{1/5}$ selective BAF312. However, these authors report that **1** and BAF312 had no significant effect on HR after oral dosing to conscious Sprague–Dawley rats. In contrast, we observed a significant, dose-dependent effect on HR during the wake phase of male SH rats. Our experiments therefore corroborate the observation by Hamada et al.[47] that, in the rat, selectivity against $S1P_3$ alone is not sufficient for a compound to be devoid of effects on HR.

At this point, it remains unclear whether the HR effects observed in the SH rat with compound **85** are indeed associated with $S1P_1$ receptor agonism. Further experiments will be needed to address this question and may shed light on why effects on HR manifest during the wake phase of the rats only and hence significantly differ from the rapid but only transient HR reduction observed in humans.[49−54]

## ■ CONCLUSIONS

Starting from the hydrolytically susceptible HTS hit **2**, we developed stable, potent, and selective $S1P_1$ receptor agonists wherein the pyrazole in **2** was replaced by a thiophene. SAR studies of these novel $S1P_1$ agonists revealed that a ketone linker yields significantly more potent compounds as compared to analogues incorporating an amide between the thiophene and the phenyl ring. Replacing the linear propanone linker by an oxadiazole maintained the affinity for $S1P_1$ but also increased the potency on $S1P_3$. A large variety of side chains in position 4 of the phenyl ring are tolerated, and either an additional 2- or a 3,5-substitution is optimal for $S1P_1$ agonistic activity. Within a given series of analogues, the 3-ethyl-5-methyl substitution provided compounds with the most pronounced selectivity against $S1P_3$. The tolerance of a diverse set of polar side chains in position 4 as well as various linkers between the thiophene and the phenyl ring offered a handle to fine-tuning the physicochemical and pharmacodynamic properties of the compound. From the above SAR studies, the acid **85** emerged as a potent $S1P_1$ receptor agonist with high selectivity against $S1P_3$. In Wistar rats, this compound showed an $ED_{50}$ of about 5 mg/kg for the sequestration of circulating lymphocytes at 24 h after oral compound administration. The compound was well absorbed in the rat and the dog, rapidly reached high plasma concentrations, and was cleared slowly. Despite its high selectivity against $S1P_3$, compound **85** showed a marked


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

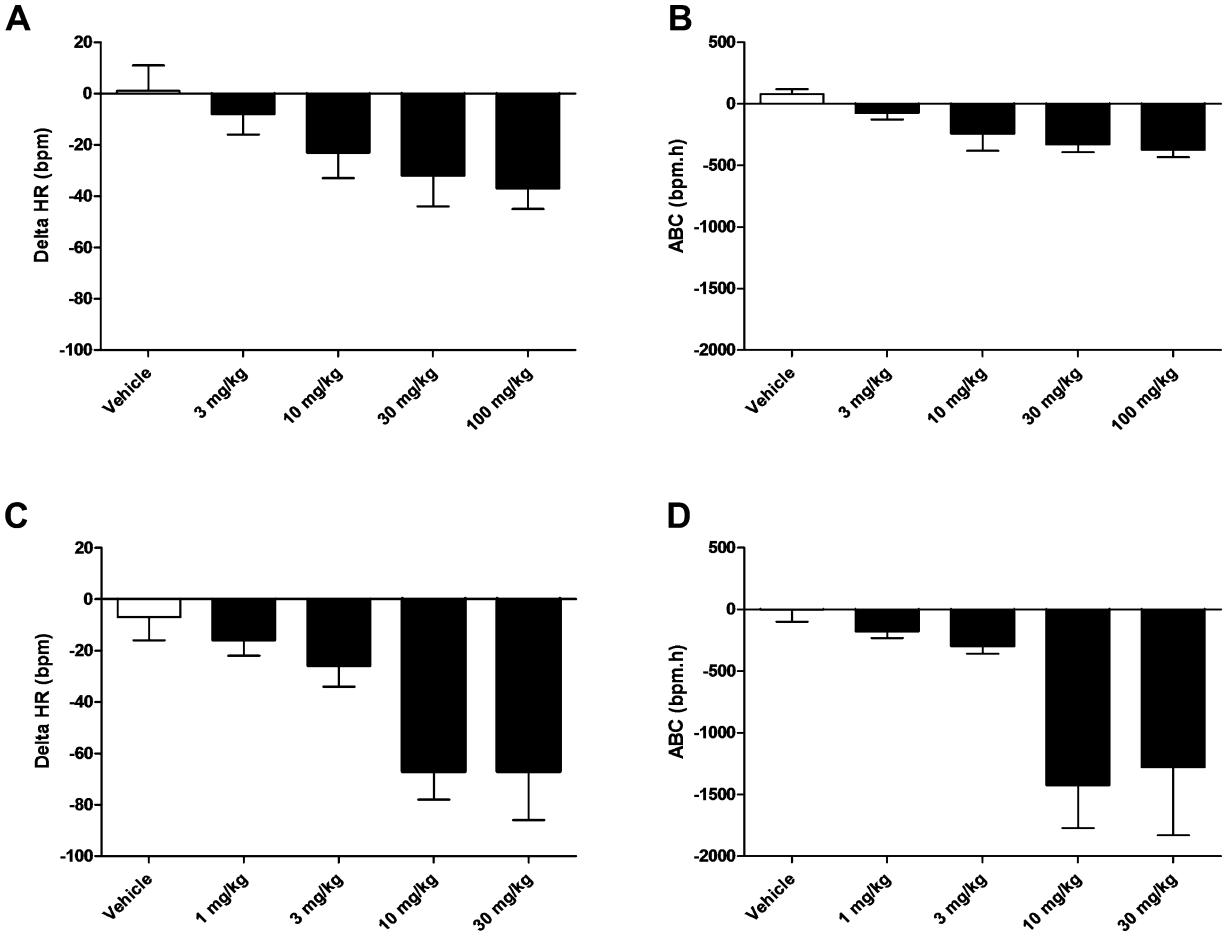



**Figure 6.** Results of dose response experiments of compounds (A, B) **85** and (C, D) **1** in male SHR. Maximal HR changes (ΔHR in bpm, A, C) and area between curves (ABC, in bpm h; B, D).

inhibition of the HR increase that is usually observed during the wake phase of nontreated male SH rats. Further studies to better understand the factors influencing the HR and efforts to identify compounds devoid of this activity are certainly warranted.

## 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 liquid chromatography–mass spectrometry (LC-MS). LC-MS: 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 (TFA), within 1 min; flow: 4.5 mL/min; 40 °C; $t_R$ is given in min. 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: 1.2 mL/min; 60 °C. According to these LC-MS analyses, final compounds showed a purity of greater than 95% (UV at 230 and at 214 nm). Chiral integrity was proven by HPLC (chiral stationary phase): Hardware from UltiMate instrument series (Dionex): HPG-3200SD binary pump, WPS-3000 autosampler, TCC-3200 thermostatted column compartment, DAD-3000 detector, SRD-3400 degasser. ValveMate 2 (Gilson) solvent valves; column, solvent and retention time ($t_R$) as indicated, DEA = diethyl amine, TFA = trifluoroacetic acid, at 25 °C, flow 1 mL/min. No racemisation/epimerization was observed during the synthesis of the target compounds. LC-HRMS: Analytical pump: Waters Acquity Binary, Solvent Manager, MS: SYNAPT G2MS, source temperature: 150 °C, desolvation temperature: 400 °C; desolvatation gas flow: 400 L/h; cone gas flow: 10 L/h; extraction cone: 4 RF; lens: 0.1 V; sampling cone: 30; capillary: 1.5 kV; high-resolution mode; gain: 1.0; MS function: 0.2 s per scan, 120–1000 amu in full scan, centroid mode. Lock spray: Leucine enkephalin 2 ng/mL (556.2771 Da) scan time 0.2 s with interval of 10 s and average of 5 scans; DAD: Acquity UPLC PDA Detector. Column: Acquity UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm from Waters, thermostatted in the Acquity UPLC Column Manager at 60 °C. Eluents: water and 0.05% formic acid; B: acetonitrile and 0.05% formic acid. Gradient: 2–98% B over 3.0 min. Flow: 0.6 mL/min. Detection: UV 214 nm and MS, $t_R$ is given in minutes. Compound purity and identity was further confirmed by NMR spectroscopy (Varian Oxford; $^1$H (300 MHz) or $^{13}$C (75 MHz) or 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, coupling constants are given in Hz). Several compounds have been prepared in a combinatorial library format on a 15–50 μmol scale. For those compounds, $^1$H NMR spectra were acquired using nondeuterated 10 mM DMSO stock solutions submitted for biological testing.[85] 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 two frequencies are not always accurate. The numbers of


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX–XXX

protons given in the description represent observed values. In some cases, signals close to either of the two solvent signals were not visible as they were suppressed by irradiation. Compound purification: compounds were purified by either flash column chromatography (CC) on silica gel 60 (Fluka Sigma−Aldrich, Switzerland), or 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 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% MeOH in water to 100% MeOH). X-ray diffraction: To determine the molecular structure of the 2-methyl isomer of compound **17**, a crystal of the compound was mounted on a Bruker Nonius diffractometer equipped with a CCD detector, and reflections were measured using monochromatic Mo Kα radiation. The structure was solved by direct methods using SIR92, and refinement was performed with CRYSTALS. Full matrix least-squares refinement was performed with anisotropic temperature factors for all atoms except hydrogen that were included at calculated positions with isotropic temperature factors. Coordinates, anisotropic temperature factors, and bond lengths and angles were deposited with the Cambridge Crystallographic Data Centre; CCDC code: 969024. 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: 4.5 mL/min; and (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 1.2 mL/min. In addition, important compounds were analyzed by LC-HRMS as described above. Purity and identity of the target compounds was further corroborated by NMR spectroscopy, and chiral integrity was proven by HPLC using chiral stationary phases. No racemisation/epimerization was observed during the synthesis of the target compounds. According to these LC-MS analyses, final compounds showed a purity of greater than or equal to 95% (UV at 230 and at 214 nm).

**In Vitro Potency Assessment.** Data ($EC_{50}$) are given as geometric means ($X_{geo}$) with geometric standard deviation ($\sigma_g$). The upper and lower 95% confidence limits are calculated as $X_{geo}*\sigma_g^2$ and $X_{geo}/\sigma_g^2$, 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 $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, or $S1P_5$ or rat $S1P_1$ 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, 0.5 μM (for $S1P_2$, $S1P_5$), 1 μM (for $S1P_1$, $S1P_4$) or 3 μM GDP (for $S1P_3$), 2.5% DMSO, and 50 pM $^{35}$S-GTPγS. Test compounds were dissolved, 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 filterplates 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 the 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. PK in the rat. Wistar rats (RCC Ltd., Biotechnology and Animal Breeding Division, Füllinsdorf, Switzerland) were used for PK experiments after an acclimatization period of at least 7 days after delivery. Animals foreseen for iv dosing underwent surgery to receive a catheter implanted into the jugular vein. Animals foreseen for oral dosing did not undergo surgery, and 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. 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. Serial blood samples of 0.25 mL each were taken predose and at 30 min, 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 separated by centrifugation and stored at −20 °C. All animals had free access to food during the entire duration of the experiments. Plasma samples from the rat were analyzed using liquid chromatography coupled to mass spectrometry (LC-MS-MS) after protein precipitation with MeOH and centrifugation at 3220*g* for 20 min at 4 °C. Using 1.25 μL of plasma on the column, lower and upper limits of quantification were 41 and 10 000 ng/mL for compound **85**, respectively. PK parameters were estimated with the WinNonlin software (Pharsight Corporation, Mountain View, CA, USA) using noncompartmental analysis. Brain penetration in the rat. At 2, 6, and 24 h after dosing, male Wistar rats ($n$ = 2) were anaesthetised 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 trough the carotid. The whole brain was then removed and homogenized in an equal volume of ice-cold 0.1 M sodium phosphate buffer, pH 7.4, using a IKA-WERKE ultraturrax 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. PK in the dog. The PK profile of compound **85** was determined in the male Beagle dog. The compound was administered iv at a dose of 1 mg/kg as a solution in miced micelles and orally at a dose of 3 mg/kg as a dispersion in succinylated gelatin (7.5% w/v) in water. In vivo efficacy of the target compounds was assessed by measuring the circulating lymphocytes after oral administration of 3−100 mg/kg 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 the LC at a given time point and the predose value (= 100%). All data are presented as 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 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% DMSO was administred to the animals by gavage. A mixture of 95% succinylated gelatin (7.5% w/v) in water and 5% DMSO served as the vehicle. Telemetric in vivo studies.[82−84] The effect of compound **85** on MAP and HR was assessed by oral administration of **85** as a suspension in succinylated gelatin (7.5% w/v) in water to male SHR equipped with a telemetric system recording arterial blood pressure and HR. Compound administation took place in the morning, that is, during the sleep phase of the animals. For both of these parameters, each animal served as its own control by using the data of the last 24 h before treatment. An area between curve (ABC) was calculated between the control and the treatment period to assess the efficacy of the compound. Maximal mean arterial blood pressure change was extracted from the moving average over 6 h of the blood pressure recordings.


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

**(3b*S*,4a*R*)-3,4,4-Trimethyl-3b,4,4a,5-tetrahydrocyclopropa-[3,4]cyclopenta[1,2-*c*]thiophene-1-carboxylic Acid (20).** To a solution of sodium (2.80 g, 122 mmol) in ethanol (400 mL), a solution of mercapto-acetic acid ethyl ester (14.64 g, 122 mmol) in ethanol (40 mL) was added. The solution was stirred for 5 min before (1*S*,5*R*)-2-(1-chloro-(*E*)-ethylidene)-6,6-dimethyl-bicyclo[3.1.0]-hexan-3-one (15.0 g, 81.2 mmol) in ethanol (40 mL) was added dropwise. The solution became slightly warm (approximately 30 °C) and turned orange to brown. A fine precipitate formed. Stirring was continued at rt for 1 h. Then, a solution of sodium (2.24 g, 97.5 mmol) in ethanol (75 mL) was added rapidly, and the mixture was heated to 75 °C for 1 h. A 2 N aq solution of LiOH (75 mL) was added and stirring was continued at 75 °C for 2 h and then at rt for 16 h. About two-thirds of the solvent was removed in vacuo, and the remaining mixture was diluted with water (250 mL) and extracted with DCM (200 mL). The organic extract was washed twice with 1 N aq (100 mL). The combined aqueous layers are acidified by adding 2N aq HCl and extracted three times with diethyl ether (3 × 300 mL). The organic extracts are dried over $MgSO_4$ and evaporated. The remaining residue was suspended in acetonitrile, filtered, washed with additional acetonitrile, and dried under high vacuum to give thiophene carboxylic acid **20** (12.02 g, 67%) as a pale yellow to beige crystalline powder. LC-MS: $t_R$ = 0.95 min, $[M + 1]^+$ = 223.00 (calcd 223.08). $^1H$ NMR ($CDCl_3$): $\delta$ 3.04−2.92 (m, 1 H), 2.83 (d, $J$ = 19.3 Hz, 1 H), 2.39 (s, 3 H), 1.91−1.87 (m, 2 H), 1.13 (s, 3 H), 0.73 (s, 3 H). $^{13}C$ NMR ($CDCl_3$): $\delta$ 168.4, 160.1, 146.8, 137.2, 117.5, 36.2, 30.0, 29.1, 26.6, 22.8, 14.4, 14.1.

**(3b*S*,4a*R*)-*N*-(2-Methoxybenzyl)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]thiophene-1-carboxamide (9).** A solution of (3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclo-propa[3,4]cyclopenta[1,2-*c*]thiophene-1-carboxylic acid **20** (30.0 mg, 135 μmol), TBTU (47.7 mg, 148 μmol), and Hünig's base (57.6 mg, 445 μmol) in DMF (1.2 mL) was stirred at rt for 30 min before a solution of 2-methoxy-benzylamine (3.3 mg, 0.022 mmol) in DMF (0.2 mL) was added. The mixture was allowed to stand at rt for 1 h before it was subjected to purification by preparative HPLC (Grom-Sil 120 ODS-4-HE, 30 mm × 75 mm, 10 μm particle size, gradient of 20−95% acetonitrile in water containing 0.5% formic acid) to give **9** (45 mg, 98%) as a colorless lyophilizate. LC-MS: $t_R$ = 1.08 min, $[M + 1]^+$ = 342.18 (calcd 342.15). $^1H$ NMR ($H_6$-DMSO, solvent suppression): $\delta$ 7.64 (t br, $J$ = 4.8 Hz, 1 H), 7.17 (t, $J$ = 7.7 Hz, 1 H), 7.06 (d, $J$ = 7.3 Hz, 1 H), 6.83 (t, $J$ = 7.3 Hz, 1 H), 4.32 (d, $J$ = 5.2 Hz, 2 H), 3.79 (s, 3 H), 2.93 (dd, $J$ = 6.3, 18.3 Hz, 1 H), 2.74 (d, $J$ = 18.5 Hz, 1 H), 2.31 (s, 3 H), 1.86−1.99 (m, 2 H), 1.08 (s, 3 H), 0.68 (s, 3 H). LC-HRMS: $t_R$ = 2.14 min, $[M + H]/z$ = 342.1528, found 342.1530.

**(3b*S*,4a*R*)-*N*-(3-(2-Hydroxyethoxy)benzyl)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]-thiophene-1-carboxamide (27).** (a) To a solution of thiophene carboxylic acid **20** (222 mg, 1.00 mmol) in DMF (15 mL), TBTU (353 mg, 1.10 mmol) and Hünig's base (427 mg, 3.30 mmol) was added. The mixture was allowed to stand at rt for 20 min before 3-hydroxybenzylamine (160 mg, 1.00 mmol) and Hünig's base (142 mg, 1.10 mmol) were added as a solution in DMF (1.5 mL). The mixture was stirred at rt for 3 h, treated with formic acid (2 mL), and directly separated by preparative HPLC (Phenomenex AQUA, 30 mm × 75 mm, gradient of acetonitrile in water containing 0.5% of formic acid) to give (3b*S*,4a*R*)-*N*-(3-hydroxybenzyl)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]thiophene-1-carboxamide (126 mg, 38%) as a pale yellow resin. LC-MS: $t_R$ = 0.97 min, $[M + 1]^+$ = 328.25 (calcd 328.14). $^1H$ NMR ($H_6$-DMSO, solvent suppression): $\delta$ 9.35 (s, 1 H), 7.94 (t br, $J$ = 5.6 Hz, 1 H), 7.09 (t, $J$ = 7.8 Hz, 1 H), 6.64−6.71 (m, 2 H), 6.60 (d, $J$ = 8.3 Hz, 1 H), 4.28 (d, $J$ = 6.1 Hz, 2 H), 2.92 (dd, $J_1$ = 18.6 Hz, $J_2$ = 6.6 Hz, 1 H), 2.77 (d, $J$ = 18.3 Hz, 1 H), 2.32 (s, 3 H), 1.84−2.02 (m, 2 H), 1.08 (s, 3 H), 0.68 (s, 3 H).

(b) To a solution of the above phenol (7.5 mg, 23 μmol) in MeOH (1 mL) and 2 M aq NaOH (0.1 mL), a catalytic amount of NaI and 2-bromoethanol (11.5 mg, 92 μmol) were added. The mixture was stirred at 85 °C for 24 h before another portion of 2 M aq NaOH was added, and the mixture was separated by preparative HPLC (Waters XTerra Prep MS C18 19 mm × 50 mm 5 μm, gradient of acetonitrile in water containing 0.85% of $Et_2NH$) to give **27** (1.83 mg, 21%) as a colorless resin. LC-MS: $t_R$ = 0.97 min, $[M + 1]^+$ = 372.15 (calcd 372.16); $^1H$ NMR ($H_6$-DMSO, solvent suppression): $\delta$ 7.96 (t, $J$ = 5.9 Hz, 1 H), 7.21 (t, $J$ = 8.1 Hz, 1 H), 6.78−6.87 (m, 2 H), 6.78 (d, $J$ = 7.8 Hz, 1 H), 4.90 (t, $J$ = 4.9 Hz, 1 H), 4.32 (d, $J$ = 5.1 Hz, 2 H), 3.91−3.97 (m, 2 H), 3.65−3.73 (m, 2 H), 2.92 (dd, $J_1$ = 18.6 Hz, $J_2$ = 6.1 Hz, 1 H), 2.75 (d, $J$ = 18.6 Hz, 1 H), 2.31 (s, 3 H), 1.86−1.96 (m, 2 H), 1.07 (s, 3 H), 0.66 (s, 3 H).

**1-((3b*S*,4a*R*)-3,4,4-Trimethyl-3b,4,4a,5-tetrahydrocyclo-propa[3,4]cyclo-penta[1,2-*c*]thiophen-1-yl)ethanone (30).** To a solution of **20** (12.0 g, 54 mmol) in diethyl ether (600 mL), MeLi (1.6 M, 72 mL, 115 mmol, 1.6 M in diethyl ether) was slowly added. Progress of the reaction was carefully monitored by LC-MS. Upon completion of the addition, stirring was continued at rt for 10 min. The reaction was quenched by carefully adding the mixture to ice/water (400 mL). The organic layer was separated, dried over $MgSO_4$, and filtered, and the solvent was evaporated. The crude product was purified by CC on silica gel eluting with heptane/EA 4:1 to give the title compound (8.81 g, 74%) as a pale yellow oil. LC-MS: $t_R$ = 1.03 min, $[M + 1]^+$ = 221.20 (calcd 221.10). $^1H$ NMR ($CDCl_3$): $\delta$ 3.00 (ddd, $J$ = 1.8, 4.7, 18.8 Hz, 1 H), 2.80 (d, $J$ = 18.8 Hz, 1 H), 2.38 (s, 6 H), 1.93−1.90 (m, 2 H), 1.14 (s, 3 H), 0.74 (s, 3 H).

**3-(4-(2-Hydroxyethoxy)phenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]-thiophen-1-yl)propan-1-one (32).** (a) A solution of 4-hydroxybenzaldehyde (346 mg, 2.84 mmol) and **30** (500 mg, 2.27 mmol) in ethanol (20 mL) and approximately 6 N HCl in isopropanol (4 mL) was stirred at rt for 20 h. The dark brown solution was diluted with diethyl ether and washed with saturated aq $NaHCO_3$ solution and water. The aqueous phases are extracted with diethyl ether. The combined organic extracts are dried over $MgSO_4$ and evaporated. The crude product was purified by crystallization from MeOH to give (*E*)-3-(4-hydroxyphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydro-cyclopropa[3,4]cyclopenta[1,2-*c*]thio-phen-1-yl)prop-2-en-1-one (694 mg, 75%) as an olive powder. LC-MS: $t_R$ = 1.05 min, $[M + 1]^+$ = 325.22 (calcd 325.13). $^1H$ NMR ($CDCl_3$): $\delta$ 7.70 (d, $J$ = 15.8 Hz, 1 H), 7.51 (d, $J$ = 8.8 Hz, 2 H), 7.10 (d, $J$ = 15.2 Hz, 1 H), 6.89 (d, $J$ = 8.2 Hz, 2 H), 5.72 (s, 1 H), 3.13 (dd, $J$ = 5.9, 18.8 Hz, 1 H), 2.94 (d, $J$ = 18.8 Hz, 1 H), 2.42 (s, 3 H), 1.98−1.89 (m, 2 H), 1.13 (s, 3 H), 0.74 (s, 3 H).

(b) A mixture of (*E*)-3-(4-hydroxyphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydro-cyclopropa[3,4]cyclopenta[1,2-c]-thiophen-1-yl)prop-2-en-1-one (690 mg, 2.13 mmol) and Pd/C (200 mg, 10% Pd) in ethanol (25 mL) and THF (25 mL) was stirred at rt for 3 h under $H_2$ (1.5 bar). The mixture was filtered, the filtrate was evaporated, and the crude product was purified by CC on silica gel eluting with heptane/EA 7:3 to give 3-(4-hydroxyphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclo-propa[3,4]cyclopenta[1,2-*c*]thiophen-1-yl)propan-1-one **31** (616 mg, 88%) as a colorless foam. LC-MS: $t_R$ = 1.05 min, $[M + 1]^+$ = 327.24 (calcd 327.14). $^1H$ NMR ($CDCl_3$): $\delta$ 7.11−7.05 (m, 2 H), 6.78−6.70 (m, 2 H), 4.75 (s, 1 H), 3.04−2.90 (m, 5 H), 2.78 (d, $J$ = 18.8 Hz, 1 H), 2.37 (s, 3 H), 1.91−1.85 (m, 2 H), 1.11 (s, 3 H), 0.70 (s, 3 H).

(c) Alkylation was carried out as described for **33**. LC-MS: $t_R$ = 1.05 min, $[M + 1]^+$ = 371.19 (calcd 371.17). $^1H$ NMR ($H_6$-DMSO, solvent suppression): $\delta$ 7.08 (d, $J$ = 8.0 Hz, 2 H), 6.77 (d, $J$ = 7.9 Hz, 2 H), 4.88 (t, $J$ = 5.4 Hz, 1 H), 3.91 (t, $J$ = 4.4 Hz, 2 H), 3.64−3.72 (m, 2 H), 2.92−2.96 (m, 1 H), 2.76 (t, $J$ = 7.5 Hz, 2 H), 2.71 (d, $J$ = 18.8 Hz, 1 H), 2.32 (s, 3 H), 1.88−1.92 (m, 2 H), 1.08 (s, 3 H), 0.65 (s, 3 H). LC-HRMS: $t_R$ = 1.44 min, $[M + H]/z$ = 371.1680, found 371.1684.

**3-(4-(2-Hydroxyethoxy)-2-methoxyphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclo-penta[1,2-*c*]thiophen-1-yl)propan-1-one (33).** (a) A stirred solution of (1a*S*,5a*R*)-1-(1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclopropa[a]pen-talen-4-yl)-ethanone (535 mg, 1.70 mmol), 4-hydroxy-2-methoxybenzaldehyde (335 mg, 2.20 mmol), and NaOH (2.60 g, 65.0 mmol) in MeOH (25 mL) was heated to 70 °C for 4 h. The mixture was diluted with water (150 mL) and extracted with diethyl ether (75 mL). The pH of the aqueous phase was adjusted to


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

pH = 10 by adding saturated aq $NH_4Cl$ solution, and the solution was extracted twice with DCM (2 × 70 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was separated by preparative HPLC to afford 3-(4-hydroxy-2-methoxy-phenyl)-1-((1a*S*,5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclo-propa[a]pentalen-4-yl)-propenone (450 mg, 75%) as a yellow solid. LC-MS: $t_R$ = 1.06 min, $[M + 1]^+$ = 355.20 (calcd 355.14). $^1H$ NMR ($D_6$-DMSO): $\delta$ 10.15 (s, 1 H), 7.73 (d, $J$ = 15.2 Hz, 1 H), 7.55 (d, $J$ = 8.8 Hz, 1 H), 7.23 (d, $J$ = 15.8 Hz, 1 H), 6.47−6.40 (m, 2 H), 3.84 (s, 3 H), 3.12 (dd, $J$ = 6.4, 18.8 Hz, 1 H), 2.88 (d, $J$ = 18.8 Hz, 1 H), 2.07 (s, 3 H), 2.02−1.93 (m, 2 H), 1.11 (s, 3 H), 0.70 (s, 3 H).

(b) To a solution of 3-(4-hydroxy-2-methoxy-phenyl)-1-((1a*S*,5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclo-propa[a]-pentalen-4-yl)-propenone (443 mg, 1.25 mmol) in ethanol (30 mL) was added Pd/C (450 mg, 10% Pd). The resulting suspension was stirred at rt for 2 h under 1 atm $H_2$. The mixture was filtered over Celite, and the filtrate was evaporated. The residue was purified by preparative HPLC to give 3-(4-hydroxy-2-methoxy-phenyl)-1-((1a*S*,5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclopropa[a]-pent-alen-4-yl)-propan-1-one (162 mg, 36%) as a slightly yellow resin. LC-MS: $t_R$ = 1.06 min, $[M + 1]^+$ = 357.10 (calcd 357.15). $^1H$ NMR ($CD_3OD$): $\delta$ 6.88 (d, $J$ = 8.2 Hz, 1 H), 6.37 (d, $J$ = 2.3 Hz, 1 H), 6.26 (dd, $J$ = 2.3, 8.2 Hz, 1 H), 3.76 (s, 3 H), 3.00−2.74 (m, 6 H), 2.04 (s, 3 H), 1.98−1.89 (m, 2 H), 1.12 (s, 3 H), 0.70 (s, 3 H).

(c) To a solution of the above phenol (80 mg, 0.225 mmol) in isopropanol (7 mL) and 2 N aq NaOH (2.8 mL), NaI (10 mg) and 2-bromoethanol (156 mg, 1.25 mmol) were added. The mixture was stirred at 65 °C for 24 h before it was treated with acetic acid (0.3 mL) and concentrated. The crude product was dissolved in MeOH (8 mL) and purified by HPLC (Waters Xterra MS 18, 75 mm × 30 mm i.d., 10 $\mu$m, gradient of acetonitrile in water containing 0.74% diethylamine) to give the title compound (81 mg, 90%) as a pale yellow resin. LC-MS: $t_R$ = 1.06 min, $[M + 1]^+$ = 401.18 (calcd 401.18). $^1H$ NMR ($CDCl_3$): $\delta$ 7.06 (d, $J$ = 8.2 Hz, 1 H), 6.47 (d, $J$ = 2.3 Hz, 1 H), 6.41 (dd, $J_1$ = 8.2 Hz, $J_2$ = 2.3 Hz, 1 H), 4.05−4.09 (m, 2 H), 3.92−3.98 (m, 2 H), 3.81 (s, 3 H), 2.90−3.01 (m, 5 H), 2.79 (d, $J$ = 18.8 Hz, 1 H), 2.38 (s, 3 H), 1.86−1.90 (m, 2 H), 1.12 (s, 3 H), 0.72 (s, 3 H). LC-HRMS: $t_R$ = 2.11 min, $[M + H]/z$ = 401.1786, found 401.1790.

**(2*R*/*S*)-3-((2-Hydroxy-3-((2-hydroxyethyl)amino)propoxy)-3,5-dimethylphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]thiophen-1-yl)-propan-1-one (57).** (a) A solution of 3,5-dimethyl-4-hydroxybenzaldehyde (2.21 g, 14.7 mmol) and **30** (2.70 g, 12.3 mmol) in ethanol (50 mL) and approximately 6 N HCl in isopropanol (25 mL) was stirred at rt for 90 min. The dark brown solution was diluted with diethyl ether and washed with a 1:1 mixtue of 1 N aq NaOH and saturated aq $NaHCO_3$ solution and water. The aqueous phases were extracted with diethyl ether. The combined organic extracts were dried over $MgSO_4$ and evaporated. The crude product was purified by CC on silica gel eluting with heptane/EA 7:3 to give (*E*)-3-(4-hydroxy-3,5-dimethylphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclo-propa[3,4]cyclopenta[1,2-*c*]thio-phen-1-yl)prop-2-en-1-one (3.28 g, 76%) as a yellow powder. LC-MS: $t_R$ = 1.12 min, $[M + 1]^+$ = 353.31 (calcd 353.16). $^1H$ NMR ($CDCl_3$): $\delta$ 7.65 (d, $J$ = 15.8 Hz, 1 H), 7.24 (s, 2 H), 7.06 (d, $J$ = 15.8 Hz, 1 H), 5.08 (s, 1 H), 3.12 (dd, $J$ = 5.9, 18.8, 1 H), 2.95 (d, $J$ = 18.8 Hz, 1 H), 2.41 (s, 3 H), 2.28 (s, 6 H), 1.96−1.89 (m, 2 H), 1.13 (s, 3 H), 0.75 (s, 3 H).

(b) A mixture of (*E*)-3-(4-hydroxy-3,5-dimethylphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclo-penta[1,2-*c*]thiophen-1-yl)prop-2-en-1-one (3.0 g, 8.51 mmol) and Pd/C (500 mg, 10% Pd) in ethanol (50 mL) and THF (50 mL) was stirred at rt for 4 h under $H_2$ (1.5 bar). The mixture was filtered, the filtrate was evaporated, and the crude product was purified by CC on silica gel eluting with heptane/EA 1:1 to give 3-(4-hydroxy-3,5-dimethylphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydro-cyclopropa[3,4]cyclopenta[1,2-*c*]thiophen-1-yl)propan-1-one **31** (3.0 g, 99%) as a yellow foam. LC-MS: $t_R$ = 1.11 min, $[M + 1]^+$ = 355.33 (calcd 355.17). $^1H$ NMR ($CDCl_3$): $\delta$ 6.84 (s, 2 H), 4.62 (s, 1 H), 3.05−2.76 (m, 5 H), 2.39 (s, 3 H), 2.23 (s, 6 H), 1.94−1.87 (m, 2 H), 1.13 (s, 3 H), 0.72 (s, 3 H).

(c) A solution of **31** (10.6 g, 29.9 mmol) in isopropanol (100 mL) and 3 N aq. NaOH (50 mL) was treated with epichlorohydrin (8.31 g, 89.8 mmol). The dark red reaction mixture was stirred at rt for 24 h. The mixture was diluted with diethyl ether (300 mL) and washed with saturated aq. $NaHCO_3$ followed by water. The organic layer was dried over $MgSO_4$ and evaporated. The crude product was purified by CC on silica gel eluting with heptane/EA 4:1 to give 3-(3,5-dimethyl-4-(oxiran-2-ylmethoxy)phenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]thiophen-1-yl)propan-1-one (9.57 g, 78%, mixture of diastereoisomers) as an almost colorless oil. LC-MS: $t_R$ = 1.16 min, $[M + 1]^+$ = 411.17 (calcd 411.20). $^1H$ NMR ($CDCl_3$): $\delta$ 6.85 (s, 2 H), 4.04−3.97 (m, 1 H), 3.77−3.69 (s, 1 H), 3.52−3.44 and 3.38−3.32 (2m, 1 H), 3.05−2.84 (m, 6 H), 2.79 (d, $J$ = 18.8 Hz, 1 H), 2.72−2.68 (m, 1 H), 2.38 (s, 3 H), 2.26 (s, 6 H), 1.92−1.86 (m, 2 H), 1.11 (s, 3 H), 0.71 (s, 3 H).

(d) A solution of 3-(3,5-dimethyl-4-(oxiran-2-ylmethoxy)phenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclo-penta[1,2-*c*]thiophen-1-yl)propan-1-one (4.55 g, 11.1 mmol) in ethanol (50 mL) was treated with ethanolamine (2.71 g, 44.3 mmol) The reaction mixture was stirred at 65 °C for 3 h before it was diluted with diethyl ether (400 mL) and washed twice with water (2 × 200 mL). The aqueous washings were extracted back with diethyl ether (400 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by MPLC eluting with 90−50% water in MeOH. Product containing fractions were extracted with diethyl ether. The organic extract was dried over $MgSO_4$, filtered, and evaporated to give the title compound **57** (3.51 g, 67%) as a pale yellow foam. LC-MS: $t_R$ = 0.88 min, $[M + 1]^+$ = 472.27 (calcd 472.25). HPLC with chiral stationary phase (Chiralpak AD-H 250 mm × 4.6 mm i.d., 5 $\mu$m; 50% ethanol, 50% MeOH containing 0.05% DEA): $t_R$ = 6.7 min, 52%, $t_R$ = 8.0 min, 48%. $^1H$ NMR ($CDCl_3$): $\delta$ 6.85 (s, 2 H), 4.21−4.30 (m, 1 H), 3.81 (t, $J$ = 5.0 Hz, 2 H), 3.77 (d, $J$ = 5.2 Hz, 2 H), 3.55 (s br, 4 H), 2.85−3.07 (m, 9 H), 2.81 (d, $J$ = 18.8 Hz, 1 H), 2.39 (s, 3 H), 2.24 (s, 6 H), 1.88−1.93 (m, 2 H), 1.13 (s, 3 H), 0.72 (s, 3 H). $^{13}C$ NMR ($CDCl_3$): $\delta$ 191.9, 156.0, 153.4, 147.2, 136.9, 136.5, 130.6, 129.2, 128.9, 74.1, 68.6, 60.1, 51.7, 51.2, 42.2, 36.1, 29.9, 29.8, 29.7, 25.4, 22.7, 16.3, 14.4, 14.1. LC-HRMS: $t_R$ = 1.52 min, $[M + H]/z$ = 472.2521, found 472.2530.

***N*-((2*R*/*S*)-3-(2,6-Dimethyl-4-(3-oxo-3-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydro-cyclopropa[3,4]cyclopenta[1,2-*c*]thiophen-1-yl)propyl)phenoxy)-2-hydroxy-propyl)-2-hydroxyacetamide (64).** (a) A solution of 3-(3,5-dimethyl-4-(oxiran-2-ylmethoxy)phenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]thiophen-1-yl)propan-1-one (800 mg, 1.95 mmol) in 6 N $NH_3$ in MeOH (10 mL) was stirred at 60 °C for 4 h in a sealed vessel. The crude product was purified on preparative TLC plates using DCM containing 15% of MeOH to give 3-(4-(3-amino-2-hydroxypropoxy)-3,5-dimethylphenyl)-1-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydro-cyclopropa[3,4] cyclopenta[1,2-*c*]thiophen-1-yl)propan-1-one (554 mg, 66%) as a colorless foam. LC-MS: $t_R$ = 0.87 min, $[M + 1]^+$ = 428.05 (calcd 428.23).

(b) To a solution of the above amine (70 mg, 164 $\mu$mol) in DCM (3 mL) was added Hünig's base (85 mg, 655 $\mu$mol), TBTU (74 mg, 229 $\mu$mol), and glycolic acid (19 mg, 246 $\mu$mol), and the reaction mixture was stirred at rt for 1 h before it was separated on preparative TLC plates with DCM containing 10% of MeOH to give **64** (45 mg, 57%) as a pale yellow resin. LC-MS: $t_R$ = 0.98 min, $[M + 1]^+$ = 486.04 (calcd 486.23). HPLC with chiral stationary phase (Chiralpak AD-H 250 mm × 4.6 mm i.d., 5 $\mu$m; 80% heptane containing 0.05% DEA, 20% ethanol containing 0.05% DEA): $t_R$ = 12.1 min, 47%, $t_R$ = 13.9 min, 53%. $^1H$ NMR ($CDCl_3$): $\delta$ 6.91 (t br, $J$ = 5.0 Hz, 1 H), 6.86 (s, 2 H), 4.09−4.20 (m, 3 H), 3.68−3.85 (m, 3 H), 3.41−3.52 (m, 1 H), 3.19 (s br, 1 H), 2.84−3.01 (m, 5 H), 2.79 (d, $J$ = 18.8 Hz, 1 H), 2.38 (s, 3 H), 2.24 (s, 6 H), 1.84−1.94 (m, 2 H), 1.11 (s, 3 H), 0.71 (s, 3 H). LC-HRMS: $t_R$ = 1.36 min, $[M + H]/z$ = 486.2314, found 486.2318.

**(*S*)-3-(2,6-Dimethyl-4-(5-((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydro-cyclopropa[3,4]cyclopenta[1,2-*c*]-**


dx.doi.org/10.1021/jm4014373 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

**thiophen-1-yl)-1,2,4-oxadiazol-3-yl)phenoxy)-propane-1,2-diol (78).** (a) To dry MeOH (190 mL) was carefully added K-*tert*-butylate (18.68 g, 166 mmol) followed by hydroxylamine hydrochloride (9.92 g, 143 mmol). The suspension was stirred for 30 min before 3,5-dimethyl-4-hydroxybenzonitrile (7.00 g, 47.6 mmol) was added. The mixture was refluxed for 32 h; then, the suspension was diluted by adding 2 N aq HCl. The solution was extracted twice with DCM (100 mL). The aqueous layer was basified (pH 9) by adding solid $NaHCO_3$ and extracted five times with DCM followed by four times with EA. The combined organic layers were dried over $Na_2SO_4$ and evaporated to dryness to give 4,*N*-dihydroxy-3,5-dimethyl-benzamidine (7.9 g, 92%) as a colorless solid. LC-MS: $t_R$ = 0.62 min, $[M + 1]^+$ = 181.14 (calcd 181.10). $^1H$ NMR ($D_6$-DMSO): $\delta$ 9.25 (s, 1 H), 8.36 (s, 1 H), 7.21 (s, 2 H), 5.54 (s, 2 H), 2.14 (s, 6 H).

(b) A solution of **20** (3.00 g, 13.5 mmol), TBTU (4.77 g, 14.9 mmol), and DIPEA (7.62 mL, 44.5 mmol) in DMF (30 mL) was stirred for 10 min at rt before 4,*N*-dihydroxy-3,5-dimethyl-benzamidine (2.68 g, 14.9 mmol) was added. The solution was stirred for further 20 min, formic acid (6 mL) was added, and the solution was chromatographed by preparative HPLC (Grom-Sil 120 ODS-4-HE, 30 mm × 75 mm, gradient of acetonitrile in water containing 0.5% formic acid) to give 4-hydroxy-3,5-dimethyl-*N*-(((3b*S*,4a*R*)-3,4,4-trimethyl-3b,4,4a,5-tetrahydrocyclopropa[3,4]cyclopenta[1,2-*c*]-thiophene-1-carbonyl) oxy)benzimidamide (4.1 g, 79%) as a colorless solid. LC-MS: $t_R$ = 1.03 min, $[M + 1]^+$ = 385.18 (calcd 385.16). $^1H$ NMR ($D_6$-DMSO): $\delta$ 8.65 (s, 1 H), 7.30 (s, 2 H), 6.38 (s br, 2 H), 3.04(dd, *J* = 5.9, 18.8 Hz, 1 H), 2.75 (d, *J* = 18.8 Hz, 1 H), 2.36 (s, 3 H), 2.18 (s, 6 H), 2.01−1.88 (m, 2 H), 1.10 (s, 3 H), 0.70 (s, 3 H).

(c) A suspension of the above hydroxyamidine ester (4.0 g, 10.4 mmol) in toluene (400 mL) was stirred at 100 °C for 24 h before the solvent was removed under reduced pressure. The residue was dissolved in DCM and purified by CC on silica gel eluting with hexane/EA 5:1 to give **71** (1.5 g, 39%) as a colorless solid. LC-MS: $t_R$ = 1.37 min, $[M + 1]^+$ = 367.13 (calcd 367.15). $^1H$ NMR ($CDCl_3$): $\delta$ 7.75 (s, 2 H), 4.90 (s, 1 H), 3.12 (dd, *J* = 5.9, 18.8 Hz, 1 H), 2.94 (d, *J* = 18.8 Hz, 1 H), 2.44 (s, 3 H), 2.32 (s, 6 H), 2.02−1.94 (m, 2 H), 1.16 (s, 3 H), 0.77 (s, 3 H).

(d) To a solution of **71** (10 mg, 27 $\mu$mol) in isopropanol (1 mL), 3-chloropropane-1,2-diol (15 mg, 135 $\mu$mol) and 2 N aq NaOH (0.2 mL) were added. The reaction mixture was shaken for 10 h at 65 °C and then directly separated by preparative HPLC (Waters XTerra Prep MS C18 19 mm × 50 mm, 5 $\mu$m, gradient of acetonitrile in water containing 0.85% of diethylamine) to give **78** (6 mg, 50%) as a colorless lyophilizate. LC-MS: $t_R$ = 1.02 min, $[M + 1]^+$ = 440.88 (calcd 441.18). HPLC with chiral stationary phase (Chiralpak AD-H 250 mm × 4.6 mm i.d., 5 $\mu$m; 50% ethanol, 50% MeOH containing 0.1% TFA): $t_R$ = 10.3 min, 100%, ((*R*)-epimer: $t_R$ = 12.4 min). $^1H$ NMR ($CDCl_3$): $\delta$ 7.78 (s, 2 H), 4.08−4.18 (m, 1 H), 3.78−3.92 (m, 4 H), 3.11 (dd, $J_1$ = 6.0 Hz, $J_2$ = 18.8 Hz, 1 H), 2.94 (d, *J* = 18.7 Hz, 1 H), 2.71 (d, *J* = 4.4 Hz, 1 H), 2.43 (s, 3 H), 2.35 (s, 6 H), 2.06 (s br, 1 H), 1.92−2.01 (m, 2 H), 1.15 (s, 3 H), 0.76 (s, 3 H). $^{13}C$ NMR ($CDCl_3$): $\delta$ 171.4, 168.2, 157.5, 156.7, 147.0, 135.2, 131.4, 128.4, 122.8, 111.0, 73.3, 71.0, 63.8, 36.4, 30.1, 28.8, 26.6, 22.9, 16.3, 14.5, 13.9. LC-HRMS: $t_R$ = 2.39 min, $[M + H]/z$ = 441.1848, found 441.1848.

**3-{2-Ethyl-6-methyl-4-[5-((1a*S*,5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclopropa[a]pentalen-4-yl)-[1,2,4]oxadiazol-3-yl]-phenyl}-propionic Acid (85).** (a) 2-Ethyl-6-methylaniline (15.0 g, 111 mmol) was added to an ice-cold solution of $H_2SO_4$ (150 mL) in water (250 mL). 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. 50% aq. $H_2SO_4$ (200 mL) was added, and stirring was continued at rt for 18 h. The mixture was extracted with DCM, and 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-methyl-phenol (8.6 g, 57%) as a crimson oil. LC-MS: $t_R$ = 0.89 min. $^1H$ NMR ($CDCl_3$): $\delta$ 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-methyl-phenol (8.40 g, 61.7 mmol) and hexamethylene tetraamine (12.97 g, 92.5 mmol) in acetic acid (60 mL) and water (14 mL) was heated to 115 °C. The water was distilled off at 117 °C and collected with a Dean−Stark apparatus. Then, the water separator was replaced by a reflux condensor, and the mixture was refluxed for 3 h. The mixture was cooled to rt, diluted with water (100 mL), and extracted with EA. The organic extract was washed with saturated aq $NaHCO_3$, dried over $MgSO_4$, and evaporated. The remaining solid was dissolved in EA and treated with heptane to initialize crystallization. The solid material was collected and dried to give 3-ethyl-4-hydroxy-5-methyl-benzaldehyde (3.13 g, 31%) as a colorless crystalline powder. $^1H$ NMR ($CDCl_3$): $\delta$ 9.83 (s, 1 H), 7.58−7.53 (m, 2 H), 5.30 (s br, 1 H), 2.69 (q, *J* = 7.6 Hz, 2 H), 2.32 (s, 3 H), 1.28 (t, *J* = 7.6 Hz, 3 H).

(c) To an ice-cooled solution of 5-ethyl-4-hydroxy-3-methylbenzaldehyde (10.0 g, 60.9 mmol) in DCM (50 mL) and pyridine (15 mL), trifluoromethanesulfonic acid anhydride (18.9 g, 67 mmol) was added over a period of 20 min. Upon complete addition, the ice bath was removed, and the reaction was stirred for a further 2 h at rt. The mixture was diluted with DCM (150 mL), washed three times with water, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash chromatography on silica gel eluting with heptane/EA 9:1 to give trifluoro-methanesulfonic acid 2-ethyl-4-formyl-6-methyl-phenyl ester (10.75 g, 60%) as a pale yellow oil. LC-MS: $t_R$ = 1.07 min. $^1H$ NMR ($CDCl_3$): $\delta$ 9.98 (s, 1 H), 7.70 (s, 1 H), 7.66 (s, 1 H), 2.85 (q, *J* = 10.1 Hz, 2 H), 2.48 (s, 3 H), 1.30 (t, *J* = 10.2 Hz, 3 H).

(d) To a stirred solution of the above triflate (10.7 g, 36.1 mmol) in dry DMF (75 mL) was sequentially added triethylamine (7.3 g, 72.2 mmol), methyl acrylate (31.1 g, 361 mmol), DPPP (819 mg, 1.99 mmol), and $Pd(OAc)_2$ (405 mg, 1.81 mmol) under nitrogen. The mixture was stirred at 115 °C for 5 h, cooled to rt, diluted with diethyl ether (350 mL), and washed twice with 1 N aq HCl and once with saturated aq $NaHCO_3$ solution. The organic extract was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash chromatography on silica gel eluting with heptane/EA 19:1 to give 3-(2-ethyl-4-formyl-6-methyl-phenyl)-acrylic acid methyl ester (5.93 g, 71%) as a colorless liquid. LC-MS: $t_R$ = 0.99 min. $^1H$ NMR ($CDCl_3$): $\delta$ 9.97 (s, 1 H), 7.83 (d, *J* = 16.4 Hz, 1 H), 7.60 (s, 1 H), 7.57 (s, 1 H), 6.09 (d, *J* = 16.4 Hz, 1 H), 3.84 (s, 3 H), 2.72 (q, *J* = 7.5 Hz, 2 H), 2.39 (s, 3 H), 1.22 (t, *J* = 7.5 Hz, 3 H).

(e) A suspension of 3-(2-ethyl-4-formyl-6-methyl-phenyl)-acrylic acid methyl ester (5.93 g, 25.53 mmol) in MeOH (140 mL) and 2 N aq NaOH (45 mL) was stirred at rt for 1 h. The MeOH was evaporated, and the aqueous solution was extracted twice with DCM. The aqueous layer was acidified with 37% aq HCl. The precipitate that formed was collected, washed with water, and dried. The product was further purified by recrystallization from EA (100 mL) to give 3-(2-ethyl-4-formyl-6-methyl-phenyl)-acrylic acid (4.2 g, 75%) as yellow crystals. LC-MS: $t_R$ = 0.87 min. $^1H$ NMR ($CDCl_3$): $\delta$ 9.98 (s, 1 H), 7.97 (d, *J* = 16.4 Hz, 1 H), 7.62 (s, 1 H), 7.59 (s, 1 H), 6.14 (d, *J* = 16.4 Hz, 1 H), 2.75 (q, *J* = 7.5 Hz, 2 H), 2.42 (s, 3 H), 1.24 (t, *J* = 7.5 Hz, 3 H).

(f) To a solution of 3-(2-ethyl-4-formyl-6-methyl-phenyl)-acrylic acid (2.75 g, 12.6 mmol) and DIPEA (1.8 g, 13.8 mmol) in ethanol (80 mL), Pd/C (275 mg, 10% Pd, moistened with 50% water) was added. The mixture was stirred for 16 h at rt under 1 atm of $H_2$. The catalyst was filtered off, and the filtrate was concentrated. The residue was dissolved in EA, washed with 2 N aq HCl followed by 1 N aq HCl and brine. The organic extract was dried over $Na_2SO_4$, filtered, and evaporated to give 3-(2-ethyl-4-hydroxymethyl-6-methyl-phenyl)-propionic acid (2.8 g, 99%) as a white solid. LC-MS: $t_R$ = 0.76 min. $^1H$ NMR ($CDCl_3$): $\delta$ 7.06 (s, 1 H), 7.04 (s, 1 H), 4.62 (s, 2 H), 2.97−3.05 (m, 2 H), 2.67 (q, *J* = 7.5 Hz, 2 H), 2.47−2.55 (m, 2 H), 2.35 (s, 3 H), 1.24 (t, *J* = 7.5 Hz, 3 H).

(g) A solution of 3-(2-ethyl-4-hydroxymethyl-6-methyl-phenyl)-propionic acid (2.8 g, 12.6 mmol) in acetic acid (50 mL) was treated with $MnO_2$ (3.9 g, 45.4 mmol), and the resulting mixture was stirred at 80 °C for 4 h. The mixture was filtered, and the filtrate was concentrated. The crude product was purified by CC on silica gel eluting with DCM to give 3-(2-ethyl-4-formyl-6-methyl-phenyl)-


dx.doi.org/10.1021/jm4014373 | J. Med. Chem. XXXX, XXX, XXX−XXX

propionic acid (1.76 g, 63%) as a beige solid. LC-MS: $t_R$ = 0.86 min. $^1$H NMR ($CDCl_3$): $\delta$ 9.93 (s, 1 H), 7.57 (s, 1 H), 7.53 (s, 1 H), 3.03−3.12 (m, 2 H), 2.74 (q, $J$ = 7.5 Hz, 2 H), 2.49−2.57 (m, 3 H), 2.43 (s, 3 H), 1.28 (t, $J$ = 7.6 Hz, 3 H).

(h) A solution of 3-(2-ethyl-4-formyl-6-methyl-phenyl)-propionic acid (1.67 g, 7.58 mmol) and hydroxylamine hydrochloride (780 mg, 11.36 mmol) in 1-methyl-2-pyrrolidone was heated to 80 °C for 30 min in the microwave (300 W, active cooling during irradiation). The reaction mixture was diluted with diethyl ether and washed with water and brine. The organic extract was dried over $Na_2SO_4$, filtered, and evaporated to give 3-(4-cyano-2-ethyl-6-methyl-phenyl)-propionic acid (1.55 g, 94%) as a beige solid. LC-MS: $t_R$ = 0.89 min. $^1$H NMR ($D_6$-DMSO): $\delta$ 12.25 (s, 1 H), 7.45 (s, 2 H), 2.91−2.84 (m, 2 H), 2.67−2.59 (m, 2 H), 2.35−2.30 (m, 5 H), 1.14 (t, $J$ = 7.6 Hz, 3 H).

(i) Potassium *tert*-butoxide (2.71 g, 24.1 mmol) was carefully dissolved in MeOH (25 mL). To this solution, hydroxylamine hydrochloride (1.44 g, 20.7 mmol) followed by 3-(4-cyano-2-ethyl-6-methyl-phenyl)-propionic acid (1.50 g, 6.90 mmol) dissolved in MeOH (7.5 mL) was added. The mixture was refluxed for 8 h, and the solvent was evaporated. The residue was dissolved in 2 N aq HCl and extracted with EA. The pH of the aqueous phase was adjusted to pH 5 by adding saturated aq $NaHCO_3$, and the mixture was extracted three times with EA. The combined organic extracts were dried over $Na_2SO_4$, filtered, evaporated, and dried to give 3-[2-ethyl-4-(*N*-hydroxycarbamimidoyl)-6-methyl-phenyl]-propionic acid (1.4 g, 81%) as a white solid. LC-MS: $t_R$ = 0.60 min, $[M + 1]^+$ = 251.17 (calcd 251.14). $^1$H NMR ($D_6$-DMSO): $\delta$ 9.48 (s br, 1 H), 7.33 (s, 1 H), 7.31 (s, 1 H), 5.72 (s br, 1 H), 2.83−2.89 (m, 2 H), 2.63 (q, $J$ = 7.4 Hz, 2 H), 2.31−2.37 (m, 2 H), 2.30 (s, 3 H), 1.18 (t, $J$ = 7.4 Hz, 3 H).

(j) To a solution of (1a*S*, 5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclopropa[a]pentalene-4-carboxylic acid **20** (270 mg 1.22 mmol) in DMF (3 mL), TBTU (390 mg, 1.22 mmol) and DIPEA (518 mg, 4.0 mmol) were added. The reaction mixture was stirred at rt for 5 min before a solution of 3-[2-ethyl-4-(*N*-hydroxycarbamimidoyl)-6-methyl-phenyl]-propionic acid (305 mg, 1.22 mmol) in DMF (2 mL) was added. Stirring was continued at rt for 1 h. The mixture was diluted with formic acid (0.5 mL) and acetonitrile (5 mL) and separated by preparative HPLC (Grom-Sil 120 ODS-4-HE, 30 mm × 75 mm, 10 $\mu$m, 10−95% acetonitrile in water containing 0.5% formic acid) to afford (1a*S*,5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclopropa[a]pentalene-4-carboxylic acid *N*-(3-ethyl-5-methyl-4-(2-carboxy-ethyl)-*N*-hydroxybenzamidine) ester (260 mg, 47%) as a white solid. LC-MS: $t_R$ = 1.05 min, $[M + 1]^+$ = 455.32.

(k) A suspension of (1a*S*,5a*R*)-1,1,2-trimethyl-1,1a,5,5a-tetrahydro-3-thia-cyclopropa[a]pentalene-4-carboxylic acid *N*-(3-ethyl-5-methyl-4-(2-(hydroxy-carboxy)-ethyl)-*N*-hydroxybenzamidine) ester (255 mg, 0.561 mmol) in toluene (10 mL) was heated to 85 °C for 24 h and then at 105 °C for 3 days. The mixture was cooled to rt, and the solvent was evaporated. The residue was dissolved in DMF and separated by preparative HPLC (Grom-Sil 120 ODS-4-HE, 30 mm × 75 mm, 10 $\mu$m, 70−100% acetonitrile in water containing 0.5% formic acid) to afford title compound **85** (150 mg, 61%) as a white crystalline solid. LC-MS: $t_R$ = 1.19 min, $[M + 1]^+$ = 437.28 (calcd 437.19). $^1$H NMR ($D_6$-DMSO): $\delta$ 12.26 (s, 1 H), 7.64 (s, 1 H), 7.63 (s, 1 H), 3.08 (dd, $J$ = 6.4, 19.3 Hz, 1 H), 2.94−2.89 (m, 2 H), 2.84 (d, $J$ = 18.2 Hz, 1 H), 2.69 (q, $J$ = 7.6 Hz, 2 H), 2.41 (s, 3 H), 2.39−2.33 (m, 5 H), 2.05 (d, $J$ = 5.8 Hz, 1 H), 1.99 (t, $J$ = 5.8 Hz, 1 H), 1.19 (t, $J$ = 7.6 Hz, 3 H), 1.11 (s, 3 H), 0.70 (s, 3 H). $^{13}$C NMR ($D_6$-DMSO): $\delta$ 174.1, 171.1, 168.3, 156.9, 147.2, 143.2, 141.2, 137.6, 135.8, 126.9, 125.3, 124.3, 110.5, 36.2, 34.0, 29.9, 28.7, 26.6, 25.7, 24.6, 22.8, 19.9, 16.0, 14.8, 14.0. LC-HRMS: $t_R$ = 2.56 min, $[M + H]/z$ = 437.1899, found 437.1896.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental details on synthesis and characterization of all target compounds and corresponding building blocks not described in the main text are given; the structure of compound **17** as determined by single crystal X-ray analysis; figures showing mean arterial blood pressure (MAP) recordings in telemetrized SHR after administration of compound **85**, and heart rate recordings in SHR after administration of compounds **1** and **57**. This material is available free of charge via the Internet at http://pubs.acs.org.

### Accession Codes

The corresponding data set has been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, United Kingdom, http://www.ccdc.cam.ac.uk/, under the following deposition number: 969024 (isomer of compound **17**, see the Supporting Information).

## ■ AUTHOR INFORMATION


### Corresponding Author

*Phone: +41 61 565 65 65; fax: +41 61 565 65 00; e-mail: martin.bolli@actelion.com.


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS


The authors gratefully acknowledge their colleagues Céline Bortolamiol, Maxime Boucher, Stéphane Delahaye, Julien Grimont, Hakim Hadana, Julie Hoerner, Benedikt Hofstetter, François LeGoff, Heidemarie Kletzl, Daniel Leuenberger, Claire Maciejasz, Céline Mangold, Katalin Menyhart, Matthias Merrettig, Christine Metzger, Christine Seeger, Jürgen Seifert, Virginie Sippel, Mireille Tena Stern, Marco Tschanz, Gaby von Aesch, Daniel Wanner, and Aude Weigel for the excellent work done and Martine Clozel and Walter Fischli for support.


## ■ ABBREVIATIONS USED

AcOH, acetic acid; AUC, area under the curve; BuLi, butyl lithium; CC, column chromatography; CDI, carbonyldiimidazole; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DMF, dimethylformamide; DPPP, 1,3-bis-(diphenylphosphino)propane; EA, ethyl acetate; EDC, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide; HOBt, *N*-hydroxybenzotriazole; LC, lymphocyte count; LDA, lithium diisopropyl amide; MeOH, methanol; NMO, *N*-methylmorpholine-*N*-oxide; PK, pharmacokinetics; PD, pharmacodynamics; $PPh_3$, triphenylphosphine; SAR, structure−activity relationship; SHR, spontaneously hypertensive rats; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; THF, tetrahydrofuran; S1P, sphingosine 1-phosphate

## ■ REFERENCES


(1) Kappos, L.; Antel, J.; Comi, G.; Montalban, X.; O'Connor, P.; Polman, C. H.; Haas, T.; Korn, A. A.; Karlsson, G.; Radue, E. W. Oral Fingolimod (FTY720) for Relapsing Multiple Sclerosis. *N. Engl. J. Med.* **2006**, *355*, 1124−1140.

(2) 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.

(3) Cohen, J. A.; Chun, J. Mechanisms of Fingolimod's Efficacy and Adverse Effects in Multiple Sclerosis. *Ann. Neurol.* **2011**, *69*, 759−777.

(4) 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.

(5) 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.
(6) Billich, A.; Bornancin, F.; Dévay, P.; Mechtcheriakova, D.; Urtz, N.; Baumruker, T. Phosphorylation of the Immunomodulatory Drug FTY720 by Sphingosine Kinases. *J. Biol. Chem.* **2003**, *278*, 47408−47415.
(7) 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.
(8) 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.
(9) 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.
(10) Hale, J. J.; Neway, W.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach, M.; Milligan, J.; Shei, G.-J.; Chrebet, G.; Bergstrom, J.; Card, D.; Koo, G. C.; Koprak, S. L.; Jackson, J. J.; Rosen, H.; Mandala, S. Potent S1P Receptor Agonists Replicate the Pharmacologic Actions of the Novel Immune Modulator FTY720. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3351−3355.
(11) Jo, E.; Sanna, M. G.; Gonzalez-Cabrera, P. J.; Thangada, S.; Tigyi, G.; Osborne, D. A.; Hla, T.; Parrill, A. L.; Rosen, H. S1P1-Selective In Vivo-Active Agonists from High-Throughput Screening: Off-the-Shelf Chemical Probes of Receptor Interactions, Signaling, and Fate. *Chem. Biol.* **2005**, *12*, 703−715.
(12) McVerry, B. J.; Garcia, J. G. N. In vitro and in Vivo Modulation of Vascular Barrier Integrity by Sphingosine 1-Phosphate: Mechanistic Insights. *Cell. Signalling* **2005**, *17*, 131−139.
(13) Wei, S. H.; Rosen, H.; Matheu, M. P.; Sanna, M. G.; Wang, S.-K.; Jo, E.; Wong, C.-H.; Parker, I.; Cahalan, M. D. Sphingosine 1-Phosphate Type 1 Receptor Agonism Inhibits Transendothelial Migration of Medullary T Cells to Lymphatic Sinuses. *Nat. Immunol.* **2005**, *6*, 1228−1235.
(14) Rosen, H.; Alfonso, C.; Surh, C. D.; McHeyzer-Williams, M. G. Rapid Induction of Medullary Thymocyte Phenotypic Maturation and Egress Inhibition by Nanomolar Sphingosine 1-Phosphate Receptor Agonist. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10907−10912.
(15) Sanna, M. G.; Wang, S.-K.; Gonzalez-Cabrera, P. J.; Don, A.; Marsolais, D.; Matheu, M. P.; Wei, S. H.; Parker, I.; Jo, E.; Cheng, W.-C.; Cahalan, M. D.; Wong, C.-H.; Rosen, H. Enhancement of Capillary Leakage and Restoration of Lymphocyte Egress by a Chiral S1P1 Antagonist in Vivo. *Nat. Chem. Biol.* **2006**, *8*, 434−441.
(16) Marsolais, D.; Rosen, H. Chemical Modulators of Sphingosine-1-phosphate Receptors as Barrier-Oriented Therapeutic Molecules. *Nat. Rev. Drug Discovery* **2009**, *8*, 297−307.
(17) Hla, T.; Venkataraman, K.; Michaud, J. The Vascular S1P Gradient—Cellular Sources and Biological Significance. *Biochim. Biophys. Acta* **2008**, *1781*, 477−482.
(18) Dorsam, G.; Graeler, M. H.; Seroogy, C.; Kong, Y.; Voice, J. K.; Goetzl, E. J. Transduction of Multiple Effects of Sphingosine 1-Phosphate (S1P) on T Cell Functions by the $S1P_1$ G Protein-Coupled Receptor. *J. Immunol.* **2003**, *171*, 3500−3507.
(19) Schwab, S. R.; Cyster, J. G. Finding a Way Out: Lymphocyte Egress from Lymphoid Organs. *Nat. Immunol.* **2007**, *8*, 1295−1301.
(20) Pappu, R.; Schwab, S. R.; Cornelissen, I.; Pereira, J. P.; Regard, J. B.; Xu, Y.; Camerer, E.; Zheng, Y.-W.; Huang, Y.; Cyster, J. G.; Coughlin, S. R. Promotion of Lymphocyte Egress into Blood and Lymph by Distinct Sources of Sphingosine-1-Phosphate. *Science* **2007**, *316*, 295−298.
(21) Sensken, S.-C.; Nagarajan, M.; Bode, C.; Graeler, M. H. Local Inactivation of Spingosine 1-Phosphate in Lymph Nodes Induces Lymphopenia. *J. Immunol.* **2011**, *186*, 3432−3440.
(22) Berst, F.; Grosche, P.; Janser, P.; Zecri, F.; Bollbuck, B. N-Biaryl (Hetero) Arylsulphonamide Derivatives Useful in the Treatment of Diseases Mediated by Lymphocyte Interactions. WO2008028937 A1, March 13, 2008.
(23) Tarrasón, G.; Aulí, M.; Mustafa, S.; Dolgachev, V.; Domènech, M. T.; Prats, N.; Domínguez, M.; López, R.; Auilar, N.; Calbet, M.; Pont, M.; Milligan, G.; Kunkel, S. L.; Godessart, N. The Sphingosine-1-phosphate Receptor-1 Antagonist, W146, Causes Early and Short-Lasting Peripheral Blood Lymphophenia in Mice. *Int. Immunopharmacol.* **2011**, *11*, 1773−1779.
(24) Fujii, Y.; Ohtake, H.; Ono, N.; Hara, T.; Sakurai, T.; Takahashi, S.; Takayama, T.; Fukasawa, Y.; Shiozawa, F.; Tsukahara, N.; Hirayama, T.; Igarashi, Y.; Goitsuka, R. Lymphopenia Induced by a Novel Selective $S1P_1$ Antagonist Structurally Unrelated to S1P. *Biochim. Biophys. Acta* **2012**, *1821*, 600−606.
(25) Fujii, Y.; Hirayama, T.; Ohtake, H.; Ono, N.; Inoue, T.; Sakurai, T.; Takayama, T.; Matsumoto, K.; Tsukahara, N.; Hidano, S.; Harima, N.; Nakazawa, K.; Igarashi, Y.; Goitsuka, R. Amelioration of Collagen-Induced Arthritis by a Novel $S1P_1$ Antagonist with Immunomodulatory Activities. *J. Immunol.* **2012**, *188*, 206−215.
(26) 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. Pharmcol. Exp. Ther.* **2007**, *323*, 469−476.
(27) 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.
(28) 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.
(29) Miron, V. E.; Schubart, A.; Antel, J. P. Central Nervous System-Directed Effects of FTY720 (Fingolimod). *J. Neurol. Sci.* **2008**, *274*, 13−17.
(30) Miron, V. E.; Hall, J. A.; Kennedy, T. E.; Soliven, B.; Antel, J. P. Cyclical and Dose-Dependent Responses of Adult Human Mature Oligodendrocytes to Fingolimod. *Am. J. Pathol.* **2008**, *173*, 1143−1152.
(31) Chun, J.; Hartung, H.-P. Mechanism of Action of Oral Fingolimod (FTY720) in Multiple Sclerosis. *Clin. Neuropharmacol.* **2010**, *33*, 91−101.
(32) Lee, C. W.; Choi, J. W.; Chun, J. Neurological S1P Signalling as an Emerging Mechanism of Action of Oral FTY720 (Fingolimod) in Multiple Sclerosis. *Arch. Pharm. Res.* **2010**, *33*, 1567−1574.
(33) Kipp, M.; Amor, S. FTY720 on the Way from the Base Camp to the Summit of the Mountain: Relevance for Remyelination. *Mult. Scler. J.* **2012**, *18*, 258−263.
(34) Awad, A. S.; Rouse, M. D.; Khutsishvili, K.; Huang, L.; Bolton, W. K.; Lynch, K. R.; Okusa, M. D. Chronic Sphingosine 1 - Phosphate 1 Receptor Activation Attenuates Early-Stage Diabetic Nephropathy Independent of Lymphocytes. *Kidney Int.* **2011**, *79*, 1090−1098.
(35) 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.
(36) Mattes, H.; Dev, K. K.; Bouhelal, R.; Barske, C.; Gasparini, F.; Guerini, D.; Mir, A. K.; Orain, D.; Osinde, M.; Picard, A.; Dubois, C.; Tasdelen, E.; Haessig, S. Design and Synthesis of Selective and Potent Orally Active S1P5 Agonists. *ChemMedChem* **2010**, *5*, 1693−1696.
(37) 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.

(38) 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.
(39) 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.
(40) 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.
(41) 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.
(42) 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.
(43) Saha, A. K.; Yu, X.; Lin, J.; Lobera, M.; Shardendu, A.; Chereku, S.; Schutz, N.; Segal, D.; Marantz, Y.; McCauley, D.; Middleton, S.; Siu, J.; Bürli, R. W.; Buys, J.; Horner, M.; Salyers, K.; Schrag, M.; Vargas, H. M.; Xu, Y.; McElvain, M.; Xu, H. Benzofuran Derivatives as Potent, Orally Active S1P1 Receptor Agonists: A Preclinical Lead Molecule for MS. *ACS Med. Chem. Lett.* **2011**, *2*, 97−101.
(44) Lucke, S.; Levkau, B. Endothelial Functions of Sphingosine-1-phosphate. *Cell. Physiol. Biochem.* **2010**, *26*, 87−96.
(45) 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.
(46) Gon, Y.; Wood, M. R.; Kiosses, W. B.; Jo, E.; Sanna, M. G.; Chun, J.; Rosen, H. $S1P_3$ Receptor-Induced Reorganization of Epithelial Tight Junctions Compromises Lung Barrier Integrity and Is Potentiated by TNF. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9270−9275.
(47) 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.
(48) 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.
(49) Brinkmann, V. FTY720 (Fingolimod) in Multiple Sclerosis: Therapeutic Effects in the Immune and Central Nervous System. *Br. J. Pharmacol.* **2009**, *158*, 1173−1182.
(50) Brossard, P.; Hofmann, S.; Cavallaro, M.; Halabi, A.; Dingemanse, J. Entry-into-Humans Study with ACT-128800, a Selective S1P1 Receptor Agonist: Tolerability, Safety, Pharmacokinetics, and Pharmacodynamics. *Clin. Pharmcol. Ther.* 2009, S63−S64, PII-87; Annual Meeting of the American Society for Clinical Pharmacology & Therapeutics, March 18−21, 2009, Washington DC, USA; ASCPT:Alexandria, VA.
(51) 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. 25th Congress of the European Committe for Treatment and Research in Multiple Sclerosis (ECTRIMS), Sept 9−12, 2009, Düsseldorf, Germany; p P437.
(52) 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. In 62nd Annual Meeting of the American Academy of Neurology, Toronto, Ontario, Canada, 2010; p PO5.052.
(53) 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.
(54) Brossard, P.; Derendorf, H.; Xu, J.; Maatouk, H.; Halabi, A.; Dingemanse, J. Pharmacokinetics and Pharmacodynamics of Ponesimod, a Selective S1P Receptor Modulator, in the First-in-Human Study. *Br. J. Clin. Pharmacol.* [Online early access]. DOI 10.1111/bcp.12129. Published Online: April 18, 2013. http://onlinelibrary.wiley.com/doi/10.1111/bcp.12129/abstract;jsessionid=BB7009BA3B1862F516180A38B14839B0.f03t03
(55) Popov, S. A.; Denisov, A. Y.; Gatilov, Y. V.; Bagryanskaya, I. Y.; Tkachev, A. V. Synthesis of New Chiral Heterocycles of the Pyrazole and 2-Isoxazoline Types Form (+)-3-Carene. *Tetrahedron: Asymmetry* **1994**, *5*, 479−489.
(56) Popov, S. A.; Tkachev, A. V. Synthesis of 2-Alkyl and 2-Aryl Pyrimidines from β-Chlorovinyl Ketones of Cyclopentanone Type. *Synth. Commun.* **2001**, *31*, 233−243.
(57) Gajcy, K.; Librowski, T.; Lochynski, S. Concise Synthesis fo Non-protein Amino Acid from Natural Product *via* Schmidt Reaction. *Lett. Org. Chem.* **2009**, *6*, 186−188.
(58) Gajcy, K.; Pekala, J.; Frackowiak-Wojtasek, B.; Librowski, T.; Lochynski, S. Stereochemistry of Terpene Derivatives. Part 7: Novel Rigidified Amino Acids from (+)-3-Carene Designed as Chiral GABA Analogues. *Tetrahedron: Asymmetry* **2010**, *21*, 2015−2020.
(59) Appel, R. Tertiary Phosphane/Tetrachloromethane, a Versatile Reagent for Chlorination, Dehydration, and P-N Linkage. *Angew. Chem., Int. Ed.* **1975**, *14*, 801−811.
(60) Popov, S. A.; Tkachev, A. V. New Chiral Agents for Resolution of Racemic *cis*-Permethric and *cis*-Z-Cyhalothric Acids. *Tetrahedron: Asymmetry* **1995**, *6*, 1013−1018.
(61) Walkowicz, M.; Lochynski, S.; Walkowicz, C. Stereochemistry of Bicyclo[3.1.0]hexane Derivatives. Part XII. Optically Active 6,6-Dimethylbicyclo[3.1.0]hexan-2-ols. *Pol. J. Chem.* **1981**, *55*, 135−140.
(62) Malkov, A. V.; Pernazza, D.; Bell, M.; Bella, M.; Massa, A.; Teply, F.; Meghani, P.; Kocovsky, P. Synthesis of New Chiral 2,2′-Bipyridine Ligands and Their Application in Copper-Catalyzed Asymmetric Allylic Oxidation and Cyclopropanation. *J. Org. Chem.* **2003**, *68*, 4727−4742.
(63) Boatman, D. P.; Schrader, T. O.; Semple, G.; Skinner, P. J.; Jung, J.-K. Fused Pyrazole Derivatives and Methods of Treatment of Metabolic-Related Disorders Thereof. WO2006069242, Jun 29, 2006.
(64) Boatman, P. D.; Schrader, T. O.; Kasem, M.; Johnson, B. R.; Skinner, P. J.; Jung, J.-K.; Xu, J.; Cherrier, M. C.; Webb, P. J.; Semple, G.; Sage, C. R.; Knudsen, J.; Chen, R.; Taggart, A. K.; Carballo-Jane, E.; Richman, J. G. Potent Tricyclic Pyrazole Tetrazole Agonists of the Nicotinic Acid Receptor (GPR109a). *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2797−2800.

(65) Paine, J. B.; Brough, J. R.; Buller, K. K.; Erikson, E. E. Regioselectivity of Pyrrole Synthesis from Diethyl Aminomalonate and 1,3-Diketones: Further Observations. *J. Org. Chem.* **1987**, *52*, 3986−3993.
(66) Bolli, M.; Bur, D.; Clozel, M.; Fischli, W.; Lehmann, D.; Mathys, B.; Mueller, C.; Nayler, O.; Scherz, M.; Weller, T. 4-Carbonyl Substituted 1,1,2-Trimethyl-1A,4,5,5A-Tetrahydro-1H-4-Aza-Cyclopropa[a]pentalene Derivatives as Agonists for the G-Protein Coupled Receptor S1P1/EDG1 and Immunsuppressant Agents. WO2005123677, Dec 29, 2005.
(67) Alberola, A.; Andres, J. M.; Gonzalez, A.; Pedrosa, R.; Vicente, M. The Reaction of $\beta$-Aminoenones with $\alpha$-Amino Derivatives. Synthesis of 2-Functionalized Pyrroles. *Heterocycles* **1990**, *31*, 1049−1058.
(68) Hombrecher, H. K.; Horter, G. Synthesis of Pyrroles via Ethyl N-(3-Oxo-1-alkenyl)glycinates. *Synthesis* **1990**, *1990*, 389−391.
(69) Alberola, A.; Andres, J. M.; Gonzalez, A.; Pedrosa, R.; Pradanos, P. Regioselective Synthesis of 2-Functionalized Thiophenes by Condensation of $\alpha$-Mercapto Compounds with $\beta$-Amino Enone Derivatives. *Synth. Commun.* **1990**, *20*, 2537−2547.
(70) Bolli, M.; Bur, D.; Clozel, M.; Fischli, W.; Lehmann, D.; Mathys, B.; Mueller, C.; Nayler, O.; Scherz, M.; Weller, T. 1, 1A, 5, 5A-Tetrahydro-3-thia-cycloprop[a] pentalenes: Tricyclic Thiophene Derivatives as S1P1/EDG1 Receptor Agonists. WO2006010544, Feb 2, 2006.
(71) Bolli, M.; Lehmann, D.; Mathys, B.; Mueller, C.; Nayler, O.; Velker, J.; Weller, T. Novel Thiophene Derivatives. WO2006100635, Sep 28, 2006.
(72) Diana, G. D.; Rudewicz, P.; Pevear, D. C.; Nitz, T. J.; Aldous, S. C.; Aldous, D. J.; Robinson, D. T.; Draper, R.; Dutko, F. J.; Aldi, C.; Gendron, G.; Oglesby, R. C.; Volkots, D. L.; Reuman, M.; Bailey, R. R.; Czerniak, R.; Block, T.; Roland, R.; Oppermann, J. Picornavirus Inhibitors: Trifluoromethyl Substitution Provides a Global Protective Effect agains Hepatic Metabolism. *J. Med. Chem.* **1995**, *38*, 1355−1371.
(73) Bolli, M.; Lehmann, D.; Mathys, B.; Mueller, C.; Nayler, O.; Velker, J.; Weller, T. Novel Thiophene Derivatives as Sphingosine-1-phosphate Receptor Agonists. WO2006100633, Sep 28, 2006.
(74) Rasmussen, P. B.; Pedersen, U.; Thomsen, I.; Yde, B.; Lawesson, S.-O. Studies on Organophosphorus Compounds XLIX. An Improved Method for the Preparation of 2,5-Disubstituted 1,3,4-Thiadiazoles and 1,3,4-Thiadiazole-2(3H)-Thiones. *Bull. Soc. Chim. Fr.* **1985**, *1985*, II/62−II/65.
(75) Kiryanov, A. A.; Sampson, P.; Seed, A. J. Synthesis of 2-Alkoxy-Substituted Thiophenes, 1,3-Thiazoles, and Related S-Heterocycles via Lawesson's Reagent-Mediated Cyclization under Microwave Irradiation: Applications for Liquid Crystal Synthesis. *J. Org. Chem.* **2001**, *66*, 7925−7929.
(76) Brain, C. T.; Paul, J. M. Rapid Synthesis of Oxazoles under Microwave Conditions. *Synlett* **1999**, *1999*, 1642−1644.
(77) Holub, J. M.; O'Toole-Colin, K.; Getzel, A.; Argenti, A.; Evans, M. A.; Smith, D. C.; Dalglish, G. A.; Rifat, S.; Wilson, D. L.; Taylor, B. M.; Miott, U.; Glersaye, J.; Lam, K. S.; McCranor, B. J.; Berkowitz, J. D.; Miller, R. B.; Lukens, J. R.; Krumpe, K.; Gupton, J. T.; Burnham, B. S. Lipid-Lower Effects of Ethyl 2-Phenacyl-3-aryl-1H-pyrrole-4-carboxylates in Rodents. *Molecules* **2004**, *2004*, 135−157.
(78) Hedegaard, B.; Mortensen, J. Z.; Lawesson, S.-O. *Tetrahedron* **1971**, *27*, 3853−3859.
(79) Zécri, F. J.; Albert, R.; Landrum, G.; Hinterding, K.; Cooke, N. G.; Guerini, D.; Streiff, M.; Bruns, C.; Nuesslein-Hildesheim, B. Pyrazole Derived from (+)-3-Carene; a Novel Potent, Selective Scaffold for Sphingosine-1-phosphate ($S1P_1$) Receptor Agonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 35−37.
(80) 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.
(81) 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., unpublished work.
(82) Brockway, B. P.; Mills, P. A.; Azar, S. H. A New Method for Continuous Chronic Measurement and Recording of Blood Pressure, Heart Rate and Activity in the Rat via Radio-Telemetry. *Clin. Exp. Hypertens., Part A* **1991**, *A13*, 885−895.
(83) Guiol, C.; Ledoussal, C.; Surgé, J.-M. A Radiotelemetry System for Chronic Measurement of Blood Pressure and Heart Rate in the Unrestrained Rat Validation of the Method. *J. Pharmcol. Toxicol. Methods* **1992**, *28*, 99−105.
(84) Hess, P.; Clozel, M.; Clozel, J.-P. Telemetry Monitoring of Pulmonary Arterial Pressure in Freely Moving Rats. *J. Appl. Physiol.* **1996**, *81*, 1027−1032.
(85) 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. MipTec - The Leading European Event for Drug Discovery, Basel, Switzerland, Sept 20−24, 2010.