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Pharmacophore-Based Design of Novel Oxadiazoles as Selective Sphingosine-1-phosphate (S1P) Receptor Agonists with in vivo Efficacy

Anna Quattropani,*[a] Wolfgang H. B. Sauer,[b] Stefano Crosignani,[a] Jerome Dorbais,[a] Patrick Gerber,[a] Jerome Gonzalez,[a] Delphine Marin,[a] Mathilde Muzerelle,[a] Fanny Beltran,[c] Anthony Nichols,[c] Katrin Georgi,[d] Manfred Schneider,[d] Pierre-Alain Vitte,[e] Valerie Eligert,[e] Laurence Novo-Perez,[e] Jennifer Hantson,[e] Sebastien Nock,[e] Susanna Carboni,[e] Adriano Luis Soares de Souza,[e] Jean-François Arrighi,[e] Ursula Boschert,[e] and Agnes Bombrun[a]

Sphingosine-1-phosphate (S1P) receptor agonists have shown promise as therapeutic agents for multiple sclerosis (MS) due to their regulatory roles within the immune, central nervous system, and cardiovascular system. Here, the design and optimization of novel [1,2,4]oxadiazole derivatives as selective S1P receptor agonists are described. The structure–activity relationship exploration was carried out on the three dominant segments of the series: modification of the polar head group (P), replacement of the oxadiazole linker (L) with different five-membered heterocycles, and the use of diverse 2,2′-disubstituted biphenyl moieties as the hydrophobic tail (H). All three segments have a significant impact on potency, S1P receptor subtype selectivity, physicochemical properties, and in vitro absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of the compounds. From these optimization studies, a selective $S1P_1$ agonist, *N*-methyl-*N*-(4-{5-[2-methyl-2′-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)glycine (**45**), and a dual $S1P_{1,5}$ agonist, *N*-methyl-*N*-(3-{5-[2′-methyl-2-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}-benzyl)glycine (**49**), emerged as frontrunners. These compounds distribute predominantly in lymph nodes and brain over plasma and induce long lasting decreases in lymphocyte count after oral administration. When evaluated head-to-head in an experimental autoimmune encephalomyelitis mouse model, together with the marketed drug fingolimod, a pan-S1P receptor agonist, $S1P_{1,5}$ agonist **49** demonstrated comparable efficacy while $S1P_1$-selective agonist **45** was less potent. Compound **49** is not a prodrug, and its improved property profile should translate into a safer treatment of relapsing forms of MS.

Introduction

Sphingosine-1-phosphate (S1P) receptors form a class of G-protein-coupled receptors (GPCRs) with five subtypes, denoted as $S1P_1$ to $S1P_5$. These receptors are regulated by S1P and have important regulatory functions in normal physiology and disease processes, particularly involving the immune, the central nervous system (CNS), and the cardiovascular system.[1] Within the immune system, down regulation of $S1P_1$ prevents the egress of B and T cells from lymph nodes (LN) into the lymphatic circulation. This is especially relevant in certain autoimmune diseases, including multiple sclerosis (MS), in which demyelination and brain atrophy occur due to the presence of autoreactive lymphocytes within the CNS. Accordingly, $S1P_1$-directed pharmacologic interventions that aim to retain these autoreactive lymphocytes in the LN and thus prevent their recirculation and subsequent infiltration into the CNS have been investigated as a means of preventing disease progression in patients with MS.[2] S1P receptor agonists have also proven useful, as shown by sphingolipid-like immunomodulator fingolimod (FTY-720, **Ia**; Figure 1), approved by the US Food and Drug Administration (FDA) for use in relapsing remitting multiple sclerosis (RRMS) patients since 2010.[3]

Fingolimod is converted in vivo into its phosphate (**Ib**), which binds to $S1P_{1,3-5}$. It has been shown to modulate the activity of $S1P_1$ in patients with MS and is believed to decrease immune cell infiltration into the CNS, consistent with its previously established effects in animal models of the disease.[4a]

[a] *Dr. A. Quattropani,[+] Dr. S. Crosignani, J. Dorbais, Dr. P. Gerber, J. Gonzalez, D. Marin, Dr. M. Muzerelle, Dr. A. Bombrun*
Chemistry Department, Merck Serono S.A.
9 Chemin des Mines, 1202 Geneva (Switzerland)
E-mail: anna.quattropani@asceneuron.com

[b] *Dr. W. H. B. Sauer*
Computational Chemistry, Merck Serono S.A.
9 Chemin des Mines, 1202 Geneva (Switzerland)

[c] *F. Beltran, Dr. A. Nichols*
Lead Discovery Technologies, Merck Serono S.A.
9 Chemin des Mines, 1202 Geneva (Switzerland)

[d] *Dr. K. Georgi, Dr. M. Schneider*
Early ADME, Merck Serono S.A.
9 Chemin des Mines, 1202 Geneva (Switzerland)

[e] *Dr. P.-A. Vitte, V. Eligert, L. Novo-Perez, J. Hantson, S. Nock, Dr. S. Carboni, Dr. A. L. S. de Souza, Dr. J.-F. Arrighi, Dr. U. Boschert*
Pharmacology/Neuroimmunology, Merck Serono S.A.
9 Chemin des Mines, 1202 Geneva (Switzerland)

[+] *Present Address: Asceneuron S.A., EPFL Innovation Park, 1015 Lausanne (Switzerland)*

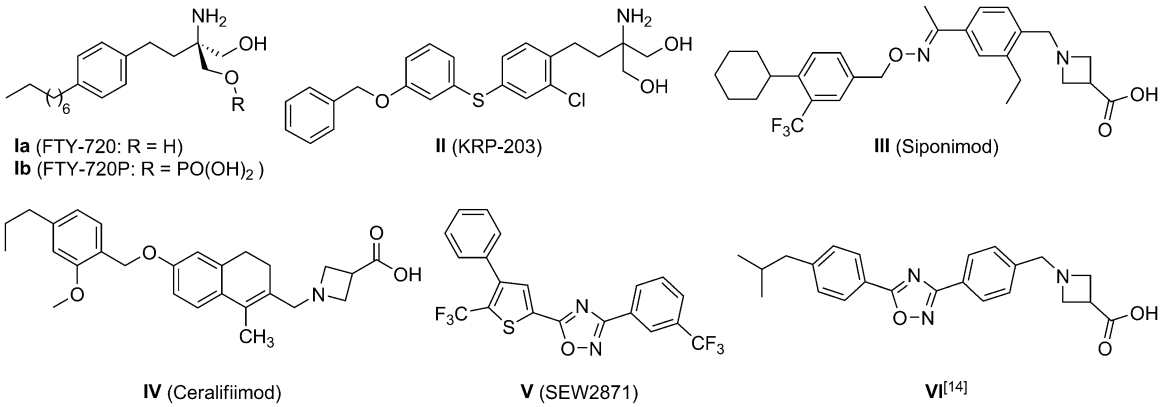


Figure 1. Structures of known $S1P_1$ agonists; **I** and **II** require phosphorylation to achieve activity and are administered as prodrugs.

The potential mode of action underlying the therapeutic activity of fingolimod in RRMS patients is the $S1P_1$-receptor-dependent inhibition of T and B cell egress from lymph nodes,[2] although fingolimod, as well as more selective $S1P_{1,5}$ agonists such as siponimod (BAF312, **III**)[5] and ceralifimod (ONO-4641, **IV**),[6] cross the blood–brain barrier and therefore might also have additional direct CNS effects.[4] The $S1P_5$ receptor has a more restricted expression profile in immune cells and is predominantly expressed on oligodendrocytes in the white matter tracts of the CNS, suggesting a potential role in NK cell trafficking and in myelin repair.[7] Phase 2 clinical trial results of siponimod[8] and ceralifimod[9] suggest that $S1P_3$ and $S1P_4$ targeting are not needed for therapeutic efficacy as measured using magnetic resonance imaging (MRI). Agonism of $S1P_3$ was thought to be related to heart and pulmonary side effects in fingolimod clinical trials,[3] but $S1P_{1,5}$-selective agonist siponimod has shown a similar safety profile in clinical trials.[8b] Nevertheless, extensive research is still focused on $S1P_3$-sparing agonists to avoid potential side effects.[10]

In the competitive search of $S1P_1$ receptor agonists, analogues of FTY-720 and FTY-720-phosphate monoester have been extensively explored in academia and industry as illustrated in Figure 1 by examples of biological tools compounds, prodrugs and non-prodrugs.[11] In our efforts to find novel chemical starting points for S1P receptor drug discovery, we report herein the design and evaluation of carboxylic acid derivatives of oxadiazoles as selective non-prodrug S1P receptor agonists.

Our investigation was built on our internal knowledge of phosphate ester isosteres. Fluorobenzoic acid was first selected as a phosphate ester replacement, due to its druglike properties demonstrated in previous programs.[12] Its combination with hydrophobic chains via an oxadiazole ring, key linker of SEW2871 (**V**), a known reference compound used for in vitro assays, was evaluated.[13] Privileged lipophilic chains for S1P mimics included substituted phenyl with aliphatic, such as isobutyl[14,15] and cyclohexyl,[15] and aromatic substituents.[15,16] This article focuses on generic structures **1** and **2**, covering compounds **3**–**50**, with **45** and **49** emerging as front runners, and the ultimate selection of **49** as a clinical candidate (Figure 2). Structure–activity relationship (SAR) and optimization studies were focused towards S1P receptor selectivity, CNS penetration, and lymphopenia in mouse.

Herein, the rational design and synthesis of a novel oxadiazole series as $S1P_1$ agonists are described together with the

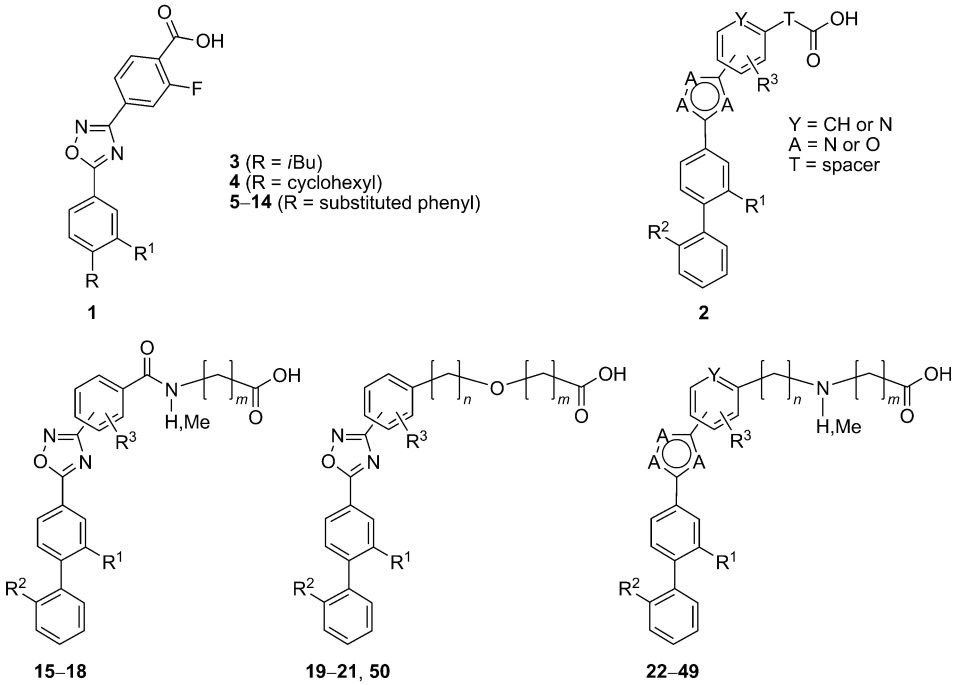


Figure 2. $S1P_1$ agonists disclosed in this article with **1** and **2** as generic structure.

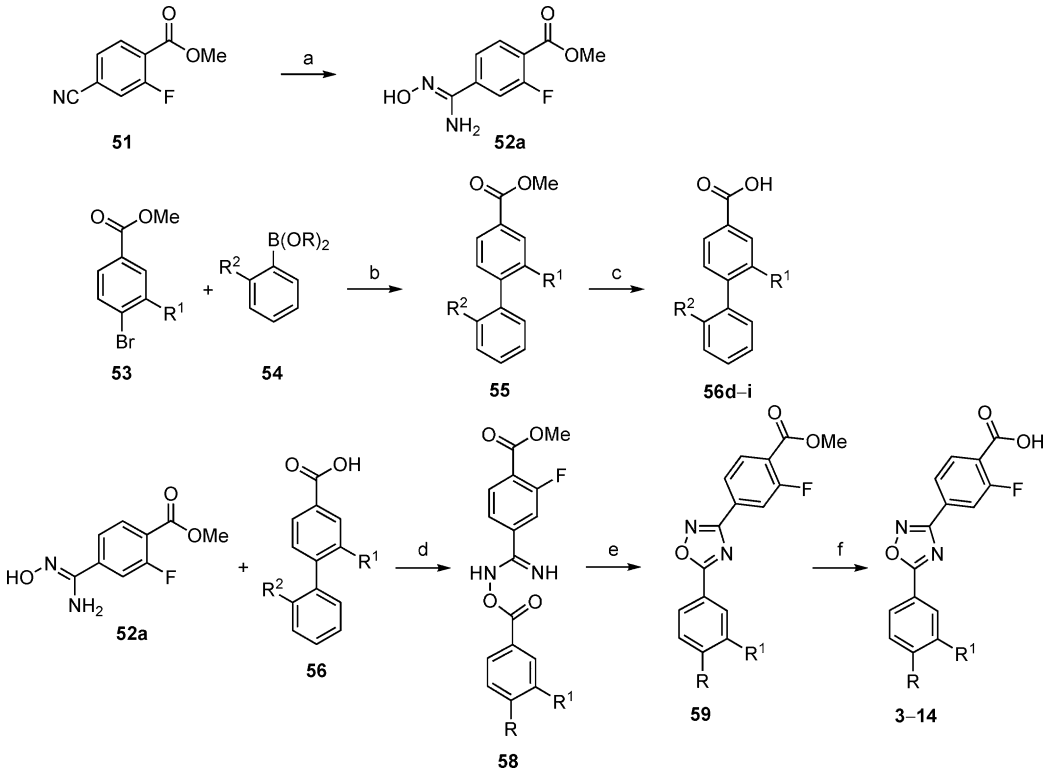
identified SAR, the physicochemical properties, the in vitro absorption, distribution, metabolism, and excretion (ADME) properties, and the results of pharmacological evaluation in a lymphopenia pharmacokinetic /pharmacodynamic (PK/PD) model. Selected compounds were further assessed, based on selectivity and safety, in vivo in a myelin oligodendrocyte glycoprotein peptide (MOG_{35-55})-induced experimental autoimmune encephalomyelitis (EAE) murine disease model relevant for the treatment of MS.

Rational Design and Synthesis

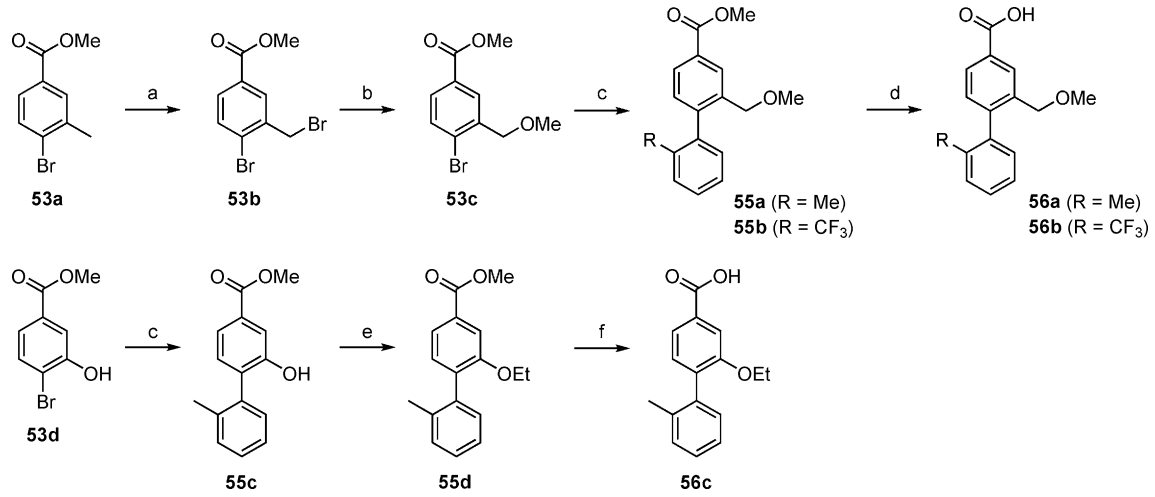
Starting from compounds **1**, mimics of the lipohilic chain of sphingosine were explored, maintaining the fluorobenzoic acid head group and oxadiazole linker. Our aim was to optimize the potency on the $S1P_1$ receptor, the selectivity versus the $S1P_3$ receptor, as well as overall druglike properties for oral absorption and CNS exposure. Computed property ranges in line with good oral bioavailability[17] and CNS exposure[18] were used to guide our optimization, in particular overall lipophilicity calculated at pH 7.4 ($cLog\,D_{7.4} < 3$), topological polar surface area (TPSA) (< 90 Å^2), number of hydrogen-bond donors (HBD = 1–2) and basicity for the amino-containing compounds ($pK_a < 8$).

A simple synthetic strategy in hand (Scheme 1) allowed us to explore the SAR of compounds **1**. The general synthetic method used for the preparation of the oxadiazole fluorobenzoic acid derivatives (**1**) was the coupling and cyclization of an amidoxime and a carboxylic acid. Amidoxime **52a** was prepared from the addition of aqueous hydroxylamine to aromatic nitrile **51**. The hydrophobic tail (H) consisted of a phenyl substituted with aliphatic (e.g., cyclohexyl, isobutyl) or aromatic groups; starting materials that were not commercially available were synthesized independently. In particular, the 2,2′-disubstituted bis-aryl carboxylic acid derivatives (**56**) were prepared by palladium-catalyzed Suzuki cross-coupling reactions, followed by ester saponification. Subsequent 1,2,4-oxadiazole formation provided a series of derivatives **59**, leading to compounds **3**–**14** after saponification. Diverse conditions for oxadiazole formation were used successfully in the progression of the project.[19] The optimal conditions that were applicable from milligram to multigram scale involved 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU)-mediated coupling of carboxylic acid **56** with amidoxime **52a** at room temperature in *N*,*N*-dimethylformamide (DMF). The cyclization of intermediate **58** was then performed either by conventional heating overnight or under microwave irradiation for 30 min.

In the synthesis of 2,2′-disubstituted bis-aryl carboxylic acid **56**, all aryl bromide and boronic acids were commercially available, except for acids **56a**,**b**,**c**, where the aryl bromides needed to be prepared (Scheme 2). For the preparation of compounds **56a** and **56b**, methyl 4-bromo-3-(methoxymethyl)benzoate **53c** was obtained in two steps, benzylic bromination of methyl 4-bromo-3-methylbenzoate **53a** followed by its substitution with methanol. Compounds **56a** and **56b** were obtained after two additional steps. Compound **56c** was pre-



Scheme 1. General synthetic scheme for the preparation of 1,2,4-oxadiazole flurorobenzoic acid analogues **1**. *Reagents and conditions*: a) H_2NOH in 50% H_2O, EtOH, RT, 15 h, 90%; b) $Pd(PPh_3)_4$, K_2CO_3, toluene/H_2O (1:1), 110 °C, 1–15 h, 59–95%; c) NaOH or LiOH, THF/H_2O (1:1), between RT and 100 °C, 1–15 h, 59–95%; d) Optimal conditions: HATU, DIEA, DMF, 0 °C→RT, 2–3 h, quantitative; e) toluene/DMF (1:1), 95 °C, 15 h or CH_3CN, 150 °C, microwave irradiation (full power, 400 W), 30 min, 34–85%; f) aq NaOH, THF/MeOH (1:1), RT, 1–15 h, 68–98%.



Scheme 2. Synthesis of 2,2′-disubstituted bis-aryl carboxylic acid **56a–c**. *Reagents and conditions*: a) NBS, AIBN, CH_3CN, reflux, 2 d, 62%; b) MeOH, reflux, 4 d, 94%; c) when R = Me: *o*-tolylboronic acid; when R = CF_3: 2-(trifluoromethyl)phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene/H_2O (1:1), 110 °C, 1–15 h, 63–95%; d) NaOH, EtOH/H_2O (1:1), 60 °C, 1 h, 74–92%; e) EtBr, K_2CO_3, CH_3CN, 50 °C, 2 d, 98%; f) LiOH, THF/H_2O (1:1), RT, 15 h, 93%.

pared from methyl 4-bromo-3-hydroxybenzoate **53d**. After Suzuki cross-coupling with *ortho*-tolylboronic acid, phenol alkylation with ethyl bromide was performed in acetonitrile in the presence of potassium carbonate. Saponification of the methyl ester gave **56c** that was used in the synthesis of the oxadiazole.

To support our medicinal chemistry progression, a pharmacophore model was constructed using Catalyst HipHop[20] from nine diverse $S1P_1$ ligands from the literature and internal screening. Five common pharmacophoric features were identified (Figure 3): three hydrophobic motifs and two hydrogen-bond acceptors (shown with the putative donor locations on the receptor). Their geometric arrangement is described in the Experimental Section.

This model provided two important hints for modifications around compounds **1**: 1) The orientation of the hydrophobic centers of the hydrophobic tail (H), which could be filled optimally with a 2,2′-disubstitued biphenyl, one by the interior aromatic ring and the other (terminal) hydrophobic center by the

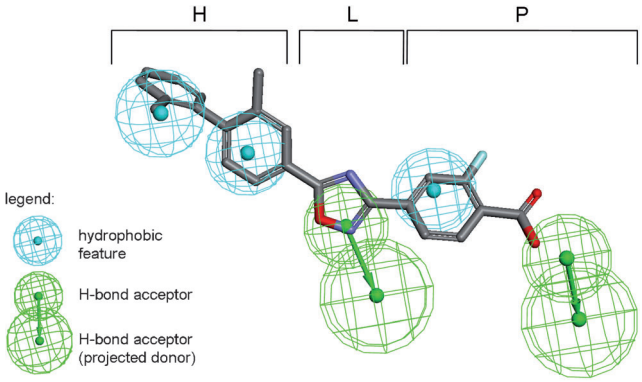
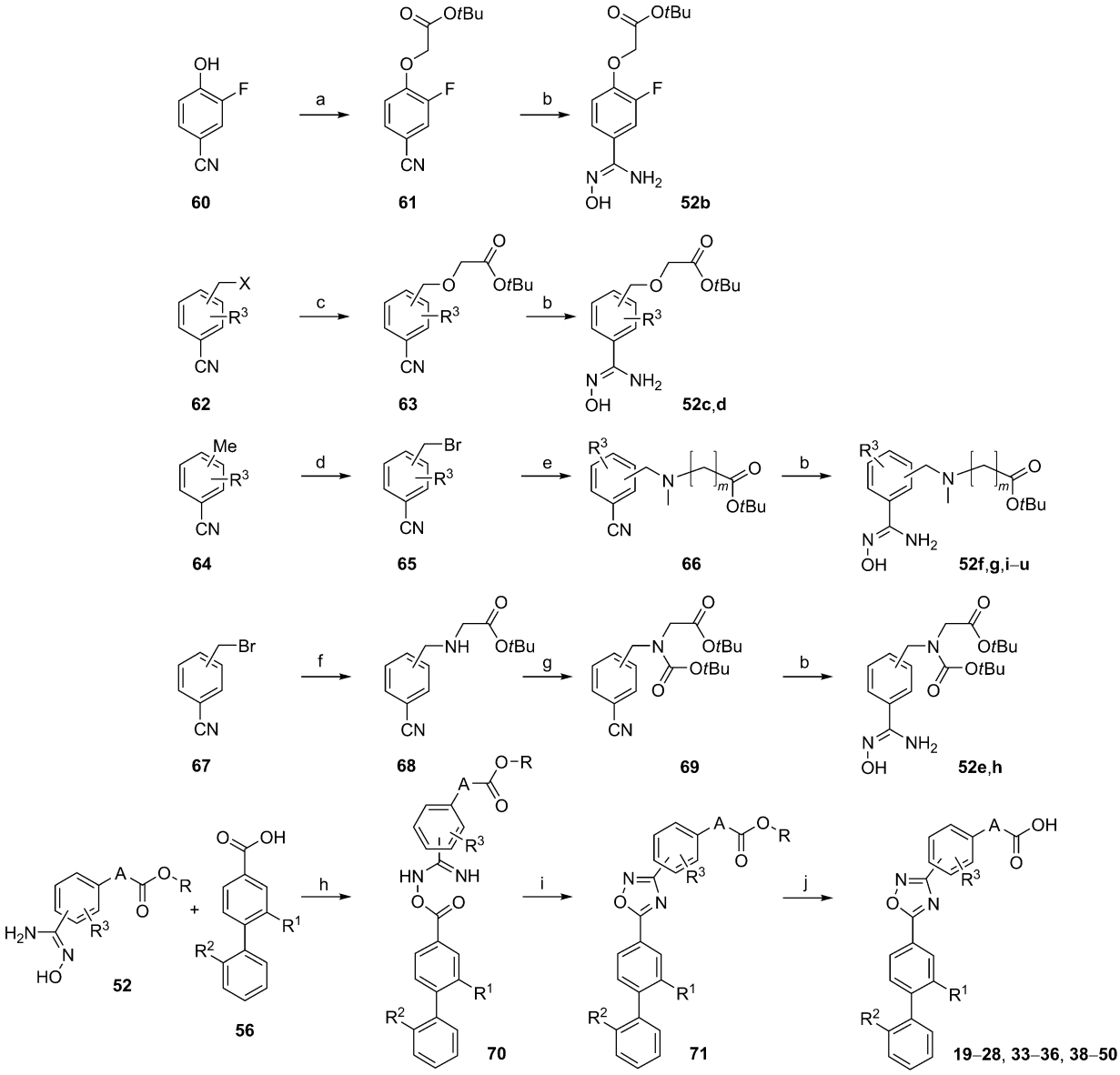


Figure 3. Compound **1** mapped onto a pharmacophore model derived from literature and in-house data on $S1P_1$ modulator characteristics; correspondence to the hydrophobic tail (H), oxadiazole linker (L) and polar head group (P) as indicated.

2′-substituent of the second phenyl, kept in a favorable conformation by the 2-substituent of the first; 2) The distance between the hydrophobic and the acceptor feature of the head group (P); for the fluorobenzoic acid, originally selected as phosphate monoester mimetic, this distance was identified as being too great to be covered by the aromatic ring and a carboxyl oxygen. This led to the exploration of diverse spacers between the two parts of P (compounds **15**–**28**).[21] To this end, three spacers (namely ether, amine and amide) between the carboxyl groups and the aromatic nitrile were explored (Scheme 3). Some oxygen-containing spacers were evaluated first. Phenoxyacetic acid *tert*-butyl ester analogue **61** was prepared by O-alkylation of 3-fluoro-4-hydroxy-benzonitrile **60** with *tert*-butyl bromoacetate in the presence of cesium carbonate. Benzyl ether analogues **63** were prepared by alkylation of corresponding benzyl alcohol **62** (X = OH) or by addition of 2-*tert*-butyl glycolate to benzyl bromide **62** (X = Br) under phase-transfer conditions (Scheme 3).[22]

Nitrogen-containing spacers were evaluated next. To access polysubstituted head groups ($R^3 \neq H$), several benzyl bromide reagents were prepared by radical bromination of the corresponding methyl-substituted aromatic nitrile precursor using *N*-bromosuccinimide (NBS) in the presence of α,α′-azoisobutyronitrile (AIBN). The resulting benzyl bromide derivatives (**65**) were reacted with sarcosine *tert*-butyl ester hydrochloride or *tert*-butyl *N*-methyl-β-alaninate[23] in the presence of potassium carbonate. The same synthetic pathway could be applied successfully for the synthesis of pyridine equivalent **52p**. For the synthesis of secondary amines analogues, *tert*-butyl glycinate was added to benzyl bromide **67**. The resulting secondary amine was protected with a *tert*-butyloxycarbonyl (boc) group that was maintained until the end of the synthesis. These different aromatic nitriles were transformed into the corresponding amidoximes (**52b**–**u**), which were used in the synthesis of the 1,2,4-oxadiazole derivatives. For some analogues (**70**), cyclization at 150 °C was in competition with the cleavage of the oxime bond, yielding the liberation of carboxylic acid **56** and

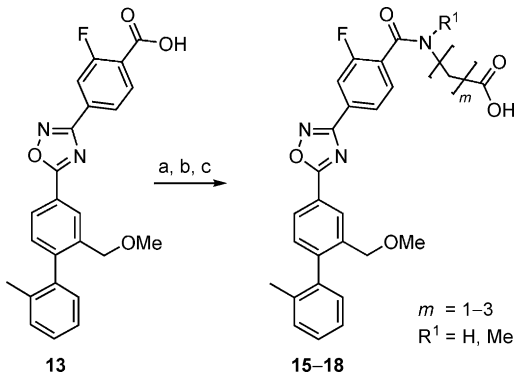


Scheme 3. Reactions of substituted aromatic nitrile as head group precursors. *Reagents and conditions*: a) *tert*-butyl bromoacetate, Cs_2CO_3, CH_3CN, RT, 15 h, 97%; b) H_2NOH in 50% H_2O, EtOH, RT, 15 h, 77–97%; c) when X = OH: *tert*-butyl bromoacetate; when X = Br: 2-*tert*-butyl glycolate, $Bu_4N\ SO_4H$, aq NaOH, toluene, RT, 15 h, 60–70%; d) NBS, AIBN, CH_3CN, reflux, 15 h, 65–75%; e) sarcosine *tert*-butyl ester hydrochloride or *N*-methyl-β-alaninate, K_2CO_3, CH_3CN, RT, 15 h, 85–98%; f) *tert*-butyl glycinate, K_2CO_3, CH_3CN, RT, 15 h, 60–86%; g) Boc_2O, DIEA, CH_2Cl_2, 3.5 h, 75–77%; h) Optimal conditions: HATU, DIEA, DMF, 0 °C→RT, 2–3 h, quantitative; i) toluene/DMF (1:1), 95 °C, 15 h or CH_3CN, 150 °C, microwave irradiation (full power, 400 W), 30 min, 34–85%; j) when R = Me or Et: aq NaOH, THF/MeOH (1:1), RT, 1–15 h, 68–98%; when R = *t*Bu: HCl, dioxane, RT, 15 h, 54–95%.

the amidine equivalent of **52**, as reported under hydrogenation conditions.[24] This could be prevented by performing this step at lower temperature (95 °C) and longer reaction time of 15 hours.

Furthermore, the amide bond was assessed as a spacer. Amide derivatives **15**–**18** were prepared by coupling the acid chloride of compound **13** with diverse amino esters (Scheme 4). The last step consisted of ester hydrolysis performed either under basic conditions for methyl and ethyl esters or under acidic conditions for *tert*-butyl esters.

Although the 1,2,4-oxadiazole heterocycle was first used for direct comparison with known $S1P_1$ agonists, the replacement of the oxadiazole linker (L) was obviously challenged for potency, selectivity and importantly physical and pharmaceutical profile.[25] Regioisomers of oxadiazoles, triazoles and thiadiazoles were synthesized. The alternative 1,2,4-oxadiazole isomers such as compound **29** were prepared from benzoic acid **73** and amidoxime **75**. Addition of sarcosine *tert*-butyl ester to 3-(bromomethyl)benzoic acid **72** gave compound **73**. Independently, **56a** was transformed into the corresponding aromatic nitrile **74** in three steps: acyl chloride formation, ammonia addition, and dehydration of the resulting primary amide. Addition of aqueous hydoxyamine yielded amidoxime **75**. Previously described conditions for the formation of the 1,2,4-oxadiazole and *tert*-butyl ester deprotection gave compound **29**. Acyl hydrazone **77** was obtained by coupling of **56a** with boc-



Scheme 4. Synthesis of amides **15**–**18**. *Reagents and conditions*: a) oxalyl chloride, DMF (cat), CH_2Cl_2, 0 °C→RT, 2 h, quantitative; b) $HNR^1(CH_2)_nCOOMe$, DIEA, THF, RT, 15 h, 36–63%; c) aq NaOH, THF/MeOH (1:1), RT, 1–3 h, 52–97%.

protected hydrazine, followed by boc deprotection. It was then coupled with acid **73** by using HATU as a coupling agent, yielding **78**, which was used as a common intermediate for the synthesis of 1,3,4-oxadiazole **30** and 1,3,4-thiadiazole **31**. Compound **30** was obtained by treatment of **78** with anhydride trifluoroacetate and 1-methylimidazole, followed by ester hydrolysis. Reaction of **78** with Lawesson's reagent at 120 °C under microwave irradiation gave compound **31** after ester hydrolysis. Triazole **81** was prepared from acyl hydrazone **77** and aromatic nitrile **80** at 200 °C under microwave irradiation in the presence of potassium carbonate. Compound **32** was isolated after hydrolysis of the *tert*-butyl ester.

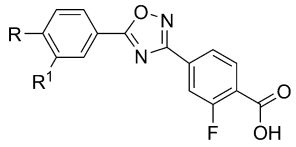
With the different synthetic pathways described above, all combinations incorporating hydrophobic biphenyls as H, heterocycles as L, and another hydrophobic site substituted by a polar head group for P, are accessible, enabling us to optimize this novel chemical series regarding $S1P_1$ potency, S1P receptor selectivity, ADME and safety profile.

Structure–Activity Relationship Study

All compounds synthesized in this program were first tested in radioligand binding assays on $S1P_1$ and $S1P_3$, to optimize the potency on $S1P_1$ and the selectivity versus $S1P_3$. In the first steps of optimization, the lipophilic part of the phenyl-[1,2,4]oxadiazole fluorobenzoic acid derivatives was optimized (Table 1). As reported, the *n*-octyl chain at the phenyl ring of fingolimod can be replaced by a wealth of lipophilic residues.[26] In our series, alkyl substituents show only moderate activity (data not shown), and the isobutyl substituent (compound **3**) was one of the best. Better potency was obtained with a cyclohexyl group (compound **4**). Its replacement by a phenyl group significantly decreased the potency (cf., compound **5**). The introduction of substituents in the 2 or 2′ position of the biphenyl moiety allowed recovery of the initial potency of **3** and **4** (cf., compounds **6** and **7**). The breakthrough in potency was obtained with a 2,2′-disubstitution as in compounds **8**–**14**, leading to K_i values below 50 nM.

Mapping of early compounds onto the pharmacophore matched the distal hydrophobic feature against the *ortho* substituent of the terminal phenyl ring. This required the angle between the two rings to be at least 70°, which led us to concentrate on 2,2′-substituted systems.[27] The SAR showed that electron-donating (e.g., OMe) and electron-withdrawing (e.g., CF_3) groups were tolerated in both positions (see compounds **9**–**12**). Further elaboration around compound **9**, with the introduction of a distal ether group, yielded compound **13** with potency below 10 nM and greater than 700-fold selectivity for $S1P_1$ over $S1P_3$. For this compound **13**, *c*Log $D_{7.4}$ and TPSA values were 1.9 and 85.5 Å^2, respectively, which is in the desirable range for a CNS drug.[18] The position of the ether functionality was probed by moving it to the 2′-position of the biphen-

Table 1. Structure–activity relationships of fluoro benzoic acid analogues **3**–**14**.[a]



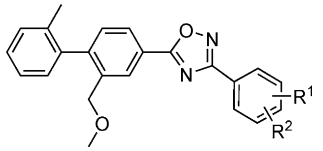
Compd	R^1	R	K_i [nM] $S1P_1$	K_i [nM] $S1P_3$	*c*Log $D_{7.4}$	TPSA [Å^2]
3	H	*iso*-Bu	460 ± 57	> 20000	1.5	76.2
4	H	Cy	145 ± 21	4050 ± 1600	2.1	76.2
5	H	Ph	4900 ± 4800	> 20000	1.5	76.2
6	Me	Ph	90.5 ± 4.9	> 20000	2.2	76.2
7	H	2-Me-Ph	530 ± 99	> 20000	2.0	76.2
8	Me	2-Me-Ph	28.5 ± 3.5	4950 ± 1800	2.8	76.2
9	OMe	2-Me-Ph	28.5 ± 0.7	> 20000	1.7	85.5
10	CF_3	2-Me-Ph	43 ± 1.4	2300 ± 420	2.9	76.2
11	Me	2-OMe-Ph	31.5 ± 3.5	> 20000	1.9	85.5
12	Me	2-CF_3-Ph	22 ± 5.7	> 20000	2.9	76.2
13	CH_2OMe	2-Me-Ph	2.9 ± 0.2	2200 ± 280	1.9	85.5
14	Me	2-CH_2OMe-Ph	145 ± 7.1	> 20000	1.9	85.5

[a] K_i values are the mean ± SD of three replicates and a minimum of two independent experiments. Calculated overall lipophilicity at pH 7.4 (*c*Log $D_{7.4}$) and topological polar surface area (TPSA).

yl moiety, yielding **14**; this modification resulted in a substantial drop in $S1P_1$ potency. Other modifications in the substitution of the biphenyl moiety of the [1,2,4]oxadiazole fluorobenzoic acid series did not yield an improvement in potency on $S1P_1$ or selectivity over $S1P_3$, confirming compound **13** as the most potent, with more than 700-fold selectivity versus $S1P_3$.

The choice of diverse spacers (ether, amines and amides) was guided and filtered by the pharmacophore hypothesis presented in Figure 3; the results are summarized in Table 2. In the exploration of an alternative to the fluorobenzoic acid initially used as a head group, fluorobenzoic amide analogues were explored first as straightforward modifications. The distance defined by the number of methylene groups between the carboxylic acid group and the fluorobenzoic amide functionality was varied incrementally to optimize $S1P_1$ binding potency and $S1P_3$ selectivity (cf., **15**–**17**). The length of the chain

Table 2. Structure–activity relationships of the spacer between the hydrophobic site and the polar head group (P) in derivatives **15**–**28**.[a]



Compd	R^1	R^2	K_i [nM] $S1P_1$	K_i [nM] $S1P_3$	*c*Log $D_{7.4}$	basic pK_a	TPSA [Å^2]
15	4-CONHCH$_2$COOH	3-F	2.08 ± 0.22	440 ± 270	0.8	–	114.6
16	4-CONH(CH$_2$)$_2$COOH	3-F	1.71 ± 0.09	186 ± 30	1.5	–	114.6
17	4-CONH(CH$_2$)$_3$COOH	3-F	2.7 ± 0.9	445 ± 64	1.6	–	114.6
18	4-CON(CH$_3$)(CH$_2$)$_2$COOH	3-F	10.5 ± 5.0	177 ± 120	1.4	–	105.8
19	4-OCH$_2$COOH	3-F	1.93 ± 0.31	270[b]	1.0	–	94.7
20	4-CH$_2$OCH$_2$COOH	3-F	1.2[b]	440[b]	0.7	–	94.7
21	3-CH$_2$OCH$_2$COOH	H	0.81 ± 0.19	1040 ± 85	1.2	–	94.7
22	4-CH$_2$NHCH$_2$COOH	H	0.35 ± 0.23	200 ± 73	3.1	8.0	97.5
23	4-CH$_2$N(CH$_3$)CH$_2$COOH	H	0.40 ± 0.24	865 ± 520	2.0	7.7	88.7
24	4-CH$_2$N(CH$_3$)(CH$_2$)$_2$COOH	H	0.23 ± 0.14	108 ± 7	2.7	7.8	88.7
25	3-CH$_2$NHCH$_2$COOH	H	0.76 ± 0.05	25.5 ± 0.7	3.8	8.3	97.5
26	3-CH$_2$N(CH$_3$)CH$_2$COOH	H	0.27 ± 0.12	75.3 ± 6.2	2.8	8.3	88.7
27	3-CH$_2$N(CH$_3$)(CH$_2$)$_2$COOH	H	0.24 ± 0.01	25.5 ± 0.7	3.4	8.0	88.7
28	2-CH$_2$N(CH$_3$)CH$_2$COOH	H	107 ± 74	> 20000	2.7	8.4	88.7

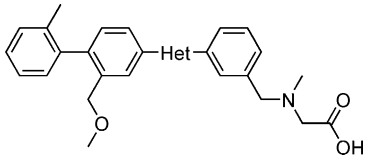
[a] K_i values are the mean ± SD of three replicates and a minimum of two independent experiments. Calculated overall lipophilicity at pH 7.4 (*c*Log $D_{7.4}$), bacicity of amino-containing compounds (pK_a) and topological polar surface area (TPSA). [b] Value corresponds to the mean of three replicates of a single experiment.

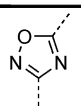
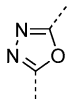
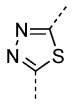
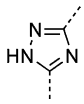
(1–3 carbon atoms) had no real impact on $S1P_1$ potency, with all compounds exhibiting $S1P_1$ activity close to that of compound **13**, and yielded a drop in selectivity over $S1P_3$ of a factor ranging from 100- to 300-fold. To decrease the number of hydrogen-bond-donor groups, N-alkylation of **16** was undertaken, yielding **18**, which exhibited decreased $S1P_1$ potency compared with **16**. All fluorobenzoic amide analogues possess a calculated TPSA greater than 90 Å^2, encouraging us to explore alternative linkers.

Ether derivatives such as 2-fluoro-phenoxyacetic acid combined with the lipophilic part of **13** yielded **19**, with similar $S1P_1$ potency. The shift of the heteroatom to the benzylic position improved $S1P_3$ selectivity (cf. **20** and **21** vs **19**); more than 1000-fold selectivity could be obtained with the head group in the *meta* position (compound **21**). Replacement of the ether by an amine yielded an improvement in $S1P_1$ potency with K_i values below 0.5 nM (cf., **22**–**27**). Secondary or tertiary benzylic amines substituted with acetic or propionic acids exhibited similar binding potency on $S1P_1$. On the other hand, such modifications also had a significant impact on $S1P_3$ selectivity. The best $S1P_3$ selectivity was obtained with tertiary benzylic amines, substituted with an acetic acid, as illustrated with **23** versus **22** and **24**, and **26** versus **25** and **27**. In contrast to the benzylic ethers, amino acids gave better selectivity in the *para* rather than the *meta* position (cf. **23** vs **26**). Compounds **23** and **26** were characterized as having $S1P_3$ selectivity of greater than 2000- and 280-fold, respectively, with comparable or slightly higher *c*Log $D_{7.4}$ values versus fluorobenzoic acid analogues, TPSA values of 89 Å^2, and calculated pK_a for the tertiary amines of 7.7 and 8.3, respectively. As expected from the pharmacophore hypothesis, *ortho*-substituted amino acid **28** was much less active on $S1P_1$.

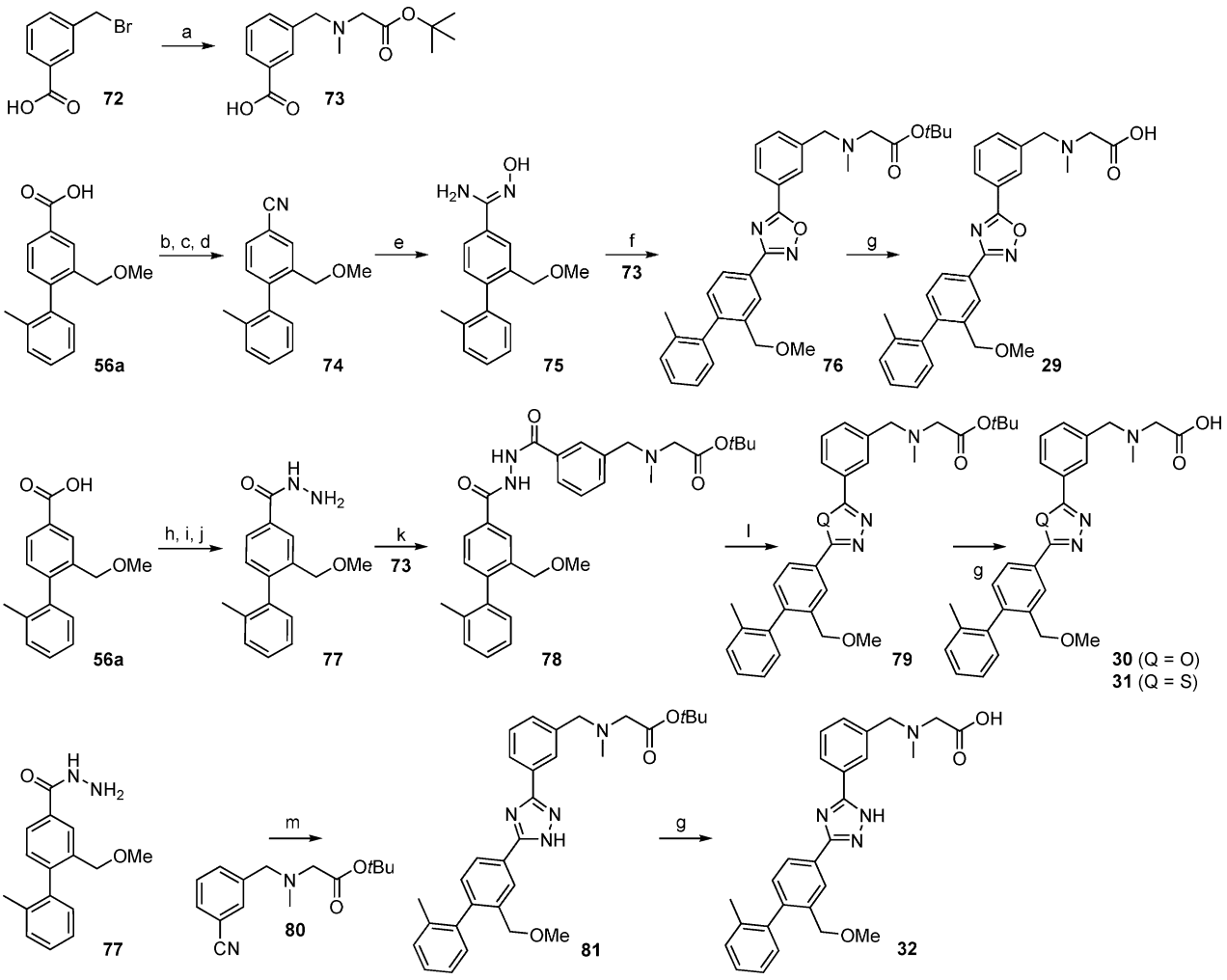
The replacement of the central [1,2,4]oxadiazole by alternative five-membered ring heterocycles was evaluated with the synthesis of a small set of compounds **29**–**32** (Scheme 5). These analogues showed an order of magnitude lower potency compared with compound **26** (Table 3). Surprisingly, none of these analogues exhibited a decrease in lipophilicity. In particular, the shift in lipophilicity of the [1,3,4]-oxadiazole compound **30** versus compound **26** was opposite to the reported impact of such an isomer on the lipophilicity.[25] These results encouraged us to keep the [1,2,4]oxadiazole as the central core.

Table 3. Structure–activity relationships of the linker heterocycle (Het) in derivatives **29**–**32**.[a]



Compd	Het	K_i [nM] $S1P_1$	K_i [nM] $S1P_3$	*c*Log $D_{7.4}$	basic pK_a	TPSA [Å^2]
29		1.49 ± 0.11	470 ± 42	2.8	8.4	88.7
30		2.19 ± 0.61	> 20000	3.5	8.4	88.7
31		0.78 ± 0.40	395 ± 7	4.0	8.4	75.5
32		3.87 ± 1.30	5900 ± 283	3.3	8.6	91.3

[a] K_i values are the mean ± SD of three replicates and a minimum of two independent experiments. Calculated overall lipophilicity at pH 7.4 (*c*Log $D_{7.4}$), bacicity of amino-containing compounds (pK_a) and topological polar surface area (TPSA).



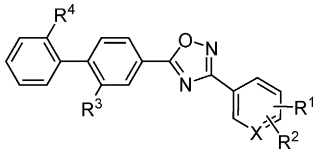
Scheme 5. Synthesis of alternative 5-membered ring heterocycle L. *Reagents and conditions*: a) sarcosine *tert*-butyl ester hydrochloride, K_2CO_3, CH_3CN, 60 °C, 6 h, 97%; b) oxalyl chloride, DMF (cat), CH_2Cl_2, 0 °C→RT, 3 h, quantitative; c) 0.5 M NH_3 in dioxane, toluene, RT, 15 min, 91%; d) $(CF_3CO)_2O$, pyridine, dioxane, RT, 3 h, 67%; e) 50% NH_2OH in H_2O, EtOH, RT, 15 h, 90%; f) 1. HATU, DIEA, DMF, RT, 2 h; 2. toluene/DMF (1:1), 95 °C, o/n, or CH_3CN, 150 °C, microwave irradiation (full power, 400 W), 30 min, 36%; g) HCl, dioxane, RT, 2–15 h, 68–88%; h) oxalyl chloride, DMF (cat), toluene, 0 °C→RT, 3 h, quantitative; i) $H_2NNHBoc$, DIEA, CH_2Cl_2, RT, 15 h, quantitative; j) TFA, CH_2Cl_2, RT, 5 h, 98%; k) HATU, DIEA, DMF, RT, 3 h, 35%; l) when Q = O: $(CF_3CO)_2O$, 1-methylimidazole, CH_2Cl_2, RT, 2 h, 69%; when Q = S: Lawesson's reagent, THF, 120 °C, microwave irradiation (full power, 400 W), 30 min, 52%; m) K_2CO_3, *n*BuOH, 200 °C, microwave irradiation (full power, 400 W), 2 h, 15%.

A last set of compounds (Table 4) explored modifications of the lipophilic part and substitution of the head group, keeping the tertiary benzylic amine substituted with an acetic acid as phosphate mimetic as in compounds **23** and **26**. Starting from compound **23**, small lipophilic substituents were tolerated on the head group, such as a fluoro or a trifluoromethyl group (see compounds **33**–**36**). These groups yielded a slight increase in $S1P_1$ potency without considerable impact on the basicity of the tertiary amine, with calculated pK_a values ranging from 7.7 to 8.1. Pyridine analogue **37**, selected as a bioisosteric replacement of 3-fluoro analogue **33**, showed a significant decrease in lipophilicity and tertiary amine basicity, but a high TPSA, coupled with a drop in $S1P_1$ potency.

For the *meta*-substituted series, starting with compound **26**, the impact of a fluorine substituent on $S1P_1$ potency and $S1P_3$ selectivity was probed by moving it around the aromatic head group (see compounds **38**– **41**). Substitution on position 5 was the best tolerated regarding $S1P_1$ potency, with compound **40** characterized by a K_i value of 0.2 nM but only 112-fold selectivity versus $S1P_3$. Its replacement with a trifluoromethyl group yielded compound **42**, which exhibited a one-log drop in $S1P_1$ potency and an important increase in lipophilicity with a *c*Log $D_{7.4}$ value of 3.8 versus 2.8 for compound **26**.

Further elaboration of the hydrophobic tail was explored with compounds **43**–**50**. Displacement of the benzylic ether to the phenolic position led to compound **43** with potency comparable with compound **23** but poorer $S1P_3$ selectivity. The introduction of the trifluoromethyl group on the biphenyl moiety, as exemplified in compound **44** with a *c*Log $D_{7.4}$ value of 2.2, had less impact on the lipophilicity than on the head group (cf. compound **42**). Its $S1P_1$ potency and $S1P_3$ selectivity were comparable with those of compound **23**. Further improvement in $S1P_3$ selectivity was obtained by removing the ether moiety, resulting in compounds **45** and **46** with $S1P_3$ K_i

Table 4. Structure–activity relationships around compounds **23** and **26**, with the modifications of the lipophilic part and substitution of the head group, and the impact of these structural modification on the physicochemical properties of the resultant derivatives **33**–**50**.[a]



Compd	R^1	R^2	X	R^3	R^4	K_i [nM] $S1P_1$	K_i [nM] $S1P_3$	*c*Log $D_{7.4}$	basic pK_a	TPSA [Å^2]
33	4-$CH_2N(CH_3)CH_2COOH$	3-F	CH	CH_2OMe	Me	0.22 ± 0.12	128 ± 27	2.1	7.8	88.7
34	4-$CH_2N(CH_3)CH_2COOH$	2-F	CH	CH_2OMe	Me	0.33 ± 0.06	320 ± 42	1.5	7.9	88.7
35	4-$CH_2N(CH_3)CH_2COOH$	3-CF_3	CH	CH_2OMe	Me	0.89 ± 0.16	1500 ± 490	2.9	8.0	88.7
36	4-$CH_2N(CH_3)CH_2COOH$	2-CF_3	CH	CH_2OMe	Me	0.49 ± 0.01	1800 ± 420	2.9	8.1	88.7
37	4-$CH_2N(CH_3)CH_2COOH$	H	N	CH_2OMe	Me	1.93 ± 0.65	1650 ± 210	0.6	7.2	101.6
38	3-$CH_2N(CH_3)CH_2COOH$	2-F	CH	CH_2OMe	Me	0.27 ± 0.04	140 ± 13	2.4	8.2	88.7
39	3-$CH_2N(CH_3)CH_2COOH$	4-F	CH	CH_2OMe	Me	0.75 ± 0.28	220[b]	3.0	8.2	88.7
40	3-$CH_2N(CH_3)CH_2COOH$	5-F	CH	CH_2OMe	Me	0.20 ± 0.07	22.2 ± 0.4	2.6	8.1	88.7
41	3-$CH_2N(CH_3)CH_2COOH$	6-F	CH	CH_2OMe	Me	1.86 ± 0.12	1550 ± 71	2.4	8.4	88.7
42	3-$CH_2N(CH_3)CH_2COOH$	5-CF_3	CH	CH_2OMe	Me	2.96 ± 0.26	7550 ± 2190	3.8	8.2	88.7
43	4-$CH_2N(CH_3)CH_2COOH$	H	CH	OCH_2Me	Me	0.31 ± 0.15	244 ± 17	2.4	7.7	88.7
44	4-$CH_2N(CH_3)CH_2COOH$	H	CH	CH_2OMe	CF_3	0.66 ± 0.06	1400 ± 170	2.2	7.6	88.7
45	4-$CH_2N(CH_3)CH_2COOH$	H	CH	Me	CF_3	1.09 ± 0.38	> 20000	3.0	7.6	79.5
46	4-$CH_2N(CH_3)CH_2COOH$	H	CH	Me	Me	0.84 ± 0.08	> 20000	2.8	7.7	79.5
47	4-$CH_2N(CH_3)CH_2COOH$	H	CH	CF_3	Me	0.42 ± 0.12	847 ± 230	3.0	7.7	79.5
48	4-$CH_2N(CH_3)CH_2COOH$	3-F	CH	CF_3	Me	0.71 ± 0.21	470 ± 160	3.1	7.8	79.5
49	3-$CH_2N(CH_3)CH_2COOH$	H	CH	CF_3	Me	0.48 ± 0.21	252 ± 93	3.8	8.3	79.5
50	3-CH_2OCH_2COOH	H	CH	CF_3	Me	0.98 ± 0.14	603 ± 24	2.2	–	85.5

[a] K_i values are the mean ± SD of three replicates and a minimum of two independent experiments. Calculated overall lipophilicity at pH 7.4 (*c*Log $D_{7.4}$), bacicity of amino-containing compounds (pK_a) and topological polar surface area (TPSA). [b] Value corresponds to the mean of three replicates of a single experiment.

values greater than 20000 nM while keeping $S1P_1$ potency similar to that of compound **44**.

Exchange of the biphenyl substituents of **45** led to compound **47** with improved affinities for both $S1P_1$ as well as $S1P_3$. However, the selectivity factor of greater than 2000 was retained. Combining this same lipophilic part with previously investigated head groups yielded compounds **48**–**50.** These compounds all demonstrated good $S1P_1$ potency with K_i values ranging from 0.48 to 0.98 nM and $S1P_3$ selectivity factors between 520 and 662 fold. Solid-state structures of both the parent and the chloride salt of compound **49** have been solved by single-crystal X-ray analyses, and the structures revealed conformations consistent with expectations and the pharmacophore hypothesis.[28] The SAR study of these compounds indicated clearly that each moiety has an impact on potency, $S1P_3$ selectivity, and overall physicochemical properties.

In vitro Selectivity and Early ADMET Profile

From among the most potent H–P fragment combinations, compounds **21**, **26**, **40**, **45**, **48**, **49** and **50** were selected for further characterization. We evaluated their selectivity versus $S1P_{3-5}$ isoforms in radioligand binding assays, early ADME focused on metabolism (intrinsic clearance), cytochrome P450 (CYP) interactions, unbound fraction, and early safety (Table 5).

For all compounds tested, only weak activity on $S1P_4$ was found (selectivity factor versus $S1P_1$ ≥1000 fold).[29] Most analogues showed similar potency on $S1P_1$ and $S1P_5$, except for compounds **45** and **50**, which showed selectivity factors of 1000 and 500, respectively. The high selectivity of compound **45** was unexpected taking into account the similarity of $S1P_1$ and $S1P_5$ binding pocket residues.[21b]

In vitro metabolism studies with liver microsomes indicated that the metabolic clearance of the evaluated compounds is low in human, rat and mouse (Table 5). CYP interaction by inhibition and induction was also evaluated, aiming to predict the possibility of drug–drug interactions with other co-medicated drugs. CYP inhibition by the seven selected compounds was found to be weak, with IC_{50} values greater than 10 μM, except for compounds **21**, **26** and **48**, which were consequently deprioritized (Table 5). CYP induction was measured for compounds **45** and **49**, and minor inhibitory activity was found for compound **49** against CYP 2B at 100 μM. All compounds are extensively bound to plasma proteins (> 99.4% for human, rat and mouse plasma), and low unbound fractions in brain (< 0.1%) were measured for compounds **45**, **48** and **49**.

Early safety and toxicity evaluation of this selection of benzylic amine and ether derivatives was carried out. The compounds were tested on hERG channel in an electrophysiology assay (patch clamp assay), yielding K_i values greater than 10 μM, except for compounds **45** and **49** with K_i values of 4 μM and 9 μM, respectively. Considering the unbound fraction

Table 5. Selectivity and in vitro ADME profiling of compounds **21**, **26**, **40**, **45**, **48**, **49** and **50**.

	Compd **21**	Compd **26**	Compd **40**	Compd **45**	Compd **48**	Compd **49**	Compd **50**
K_i [nM][a]							
$S1P_1$	0.81 ± 0.19	0.27 ± 0.12	0.20 ± 0.07	1.09 ± 0.38	0.71 ± 0.21	0.48 ± 0.21	0.98 ± 0.14
$S1P_3$	1040 ± 85	75.3 ± 6.2	22.2 ± 0.4	> 20000	470 ± 160	251.6 ± 93	603 ± 24
$S1P_4$	N.D.	N.D.	381 ± 270	5550 ± 490	895 ± 290	605 ± 210	> 20000
$S1P_5$	3.5 ± 0.7	2.50 ± 0.55	12.9 ± 7.3	1020 ± 250	36.0 ± 9.9	8.8 ± 4.6	530 ± 230
Cl_{int}[b] [µL/min mg^{-1}]							
human	< 10	29	< 10	< 10	18	< 10	< 10
rat	< 10	18	12	< 10	< 10	< 10	< 10
mouse	N.D.	N.D.	N.D.	< 10	12	< 10	N.D.
CYP Inh.[c]	2C19 = 6.7	3A4 = 7.3	> 10	> 10	2C8 = 3.9	> 10	> 10
IC_{50} [µM]	2C8 = 9.3						
Fraction unbound							
plasma (human)	N.D.	0.63	0.21	0.08	< 0.05	0.11	< 0.05
plasma (rat)	N.D.	0.6	0.14	0.06	0.08	0.19	< 0.05
plasma (mouse)	N.D.	0.48	0.21	0.07	0.06	0.14	< 0.05
brain (mouse)	N.D.	N.D.	N.D.	0.04	0.02	0.02	N.D.
hERG[d]	13 ± 5%	N.D.	24 ± 4%	4000 nM	11300 nM	9000 nM	6 ± 4%
Genotoxicity[e]	N.D.	AT−	MNT−	AT−	AT−	AT−	MNT−

[a] K_i are the mean ± SD of three replicates and a minimum of two independent experiments. N.D. = not determined. [b] Intrinsic clearance (Cl_{int}) determined against human, rat and mouse microsomes. [c] Inhibition of cytochrome P450 (CYP) isoforms; values are > 10 µM against all isoforms evaluated unless specified otherwise. [d] hERG inhibition was determined using a patch clamp assay; data represent the K_i value [nM] or % inhibition at 10 µM. [e] Genotoxicity was evaluated in vitro using a micronucleus (MNT) or Ames (AT) test; all compounds tested gave negative results, presented as MNT− or AT−.

available in vivo, the cardiovascular risk associated with hERG inhibition is estimated to be acceptable for all compounds. Furthermore, when evaluated in vitro for genotoxicity using a micronucleus or Ames test, all compounds were found to be negative (Table 5).

Selectivity of compounds **45** and **49** was evaluated by screening against a panel of receptors, ion channels, transporters and kinases. No appreciable activities at concentrations below 1 µM were identified, except for compound **45**, which exhibited binding activity on H_1 and H_2 receptors with a K_i value of 500 nM.[30] Overall, these pharma profiles suggest a low potential for off target effects for these derivatives, in spite of the frequent use of oxadiazole in drug discovery programs across diverse target classes and therapeutic areas.[25]

PK/PD Lymphopenia and EAE Models

Decrease of peripheral lymphocyte count has frequently been used as a PD biomarker for $S1P_1$ agonist efficacy in autoimmune diseases such as MS. This model was thus employed to further characterize the optimized compounds in vivo. Decrease in circulating blood lymphocytes was measured at 48 hours after a single oral gavage, along with PK parameters. This PK/PD experiment also provided data for the total drug exposure over time as estimated by the area under the curve (AUC_∞), the maximum plasma concentration (C_{max}), the elimination half-life ($t_{1/2}$), the clearance (Cl) to bioavailability (F) ratio, and brain and lymph node (LN) exposure (Table 6).

Table 6. Lymphopenia and pharmacokinetic (PK) parameters after oral (po) administration to mice.[a]

	Compd **40**	Compd **45**	Compd **49**	Compd **50**
Lymphopenia at 48 h[b]				
at 30 mg kg^{-1}	68 ± 3	75 ± 1	71 ± 2	29 ± 7
at 10 mg kg^{-1}	−1 ± 8	70 ± 3	57 ± 3	16 ± 3
at 3 mg kg^{-1}	3 ± 7	12 ± 9	36 ± 5	12 ± 4
PK parameters (30 mg kg^{-1})[c]				
AUC_∞ [h* ng mL^{-1}]	202751	265151	106171	227668
C_{max} [ng mL^{-1}]	10293	13267	8530	22433
t_{max} [h]	4	2	2	2
$t_{1/2}$ [h]	5.3	14.9	7.8	6.3
Cl/F [L kg^{-1} h^{-1}]	0.15	0.1	0.3	0.1
Exposure [ng mL^{-1}]				
Plasma at 24 h	3823	5701	1429	849
Plasma at 48 h	123	1576	145	124
LN at 24 h	3143	6103	3414	3765
LN at 48 h	85	1309	386	501
Brain at 24 h	1504	16172	4275	4536
Brain at 48 h	37	6944	332	648

[a] Test compound was administered orally to female C57BL/6 mice as a suspension in 0.5% carboxymethylcellulose (CMC)/0.25% Tween20 in water. [b] Values represent the percentage of the basal value; each treatement group was comprised of $n = 8$ animals; values are expressed as the mean ± SEM. [c] PK parameters: total drug exposure in plasma over time (AUC_∞); maximum plasma concentration (C_{max}); time taken to reach C_{max} (t_{max}); elimination half-life ($t_{1/2}$); clearance (Cl)/bioavailability (F) ratio; compound exposure in plasma, lymph nodes (LN) and brain.

A dose of 30 mg kg^{-1} was initially selected for PK parameter determination and lymphocyte counts at 48 hours (Table 6). A long lasting lymphopenic effect was targeted in view of better efficacy.[31] At this dose, significant lymphopenia was observed for all selected compounds in correlation with plasma exposure. Higher distribution in LN and brain compared with plasma was measured for all compounds with the exception of **40**.

We recognized that some of the efficacy of fingolimod in MS could be due to actions on S1P receptors in the CNS, therefore the brain penetration of these compounds can be considered an asset.[4] Accumulation in lymphoid tissues was also reported for fingolimod (**Ia**) and has been linked to its prolonged efficacy on the $S1P_1$ receptor.[31] This benchmark compound demonstrated profound lymphopenia (Figure 4), due to its very specific, albeit well-described, PK profile.

Candidates for the subsequent EAE disease model were selected after lymphopenia evaluation at lower concentrations. Only compounds **45** and **49** demonstrated efficacious lymphopenia at 10 mg kg^{-1} and to a lower extent at 3 mg kg^{-1} (Table 6 and Figure 4); these two compounds also exhibited the longest half-life. These results make the compound pair **45** and **49**, two very similar $S1P_1$ agonists from the same chemical series but with significantly different $S1P_5$ activity, a very promising probe set for investigations aiming to dissect the contributions of $S1P_5$ to $S1P_1$-mediated biological effects.

Compound **49**, an $S1P_{1,5}$ agonist, and compound **45**, an $S1P_1$-selective agonist, were benchmarked head to head with fingolimod (FTY-720, **Ia**) in an experimental autoimmune encephalomyelitis (EAE) murine disease model.[32] This model mimics MS by inducing a demyelinating autoimmune response by utilizing MOG_{35-55} as an autoantigen.[33] Leukopenia and plasma exposure were determined 24 hours after the last dosing. $S1P_{1,5}$ agonist **49** demonstrated efficacy comparable with fingolimod at doses of 1 and 3 mg kg^{-1} for 3611 days (Figure 5 D,E). The selective $S1P_1$ agonist, compound **45**, was less efficacious than fingolimod even at 3 mg kg^{-1} and a treatment period of 44 days (Figure 5 A,B). Interestingly, leukopenia measured 24 hours after the last dosing was similar for both compounds **45** and **49** (Figure 5 C,F). As plasma exposures are consistently higher for compound **45** versus compound **49**, the superiority of compound **49** cannot be attributed to favourable PK.[34] This difference in efficacy in the MOG_{35-55}-induced EAE model is revealing. The main distinction of compound **45** is its lack of activity against $S1P_5$, whose involvement in rodent MS models has been disputed.[7] The head-to-head comparison of analogues of the same series, with similar $S1P_1$ potencies, physicochemical and ADME properties but different S1P receptor affinity profiles, opens the opportunity to address this question in detail. In the case at hand, however, the presented results led to the selection of compound **49** as a clinical candidate.

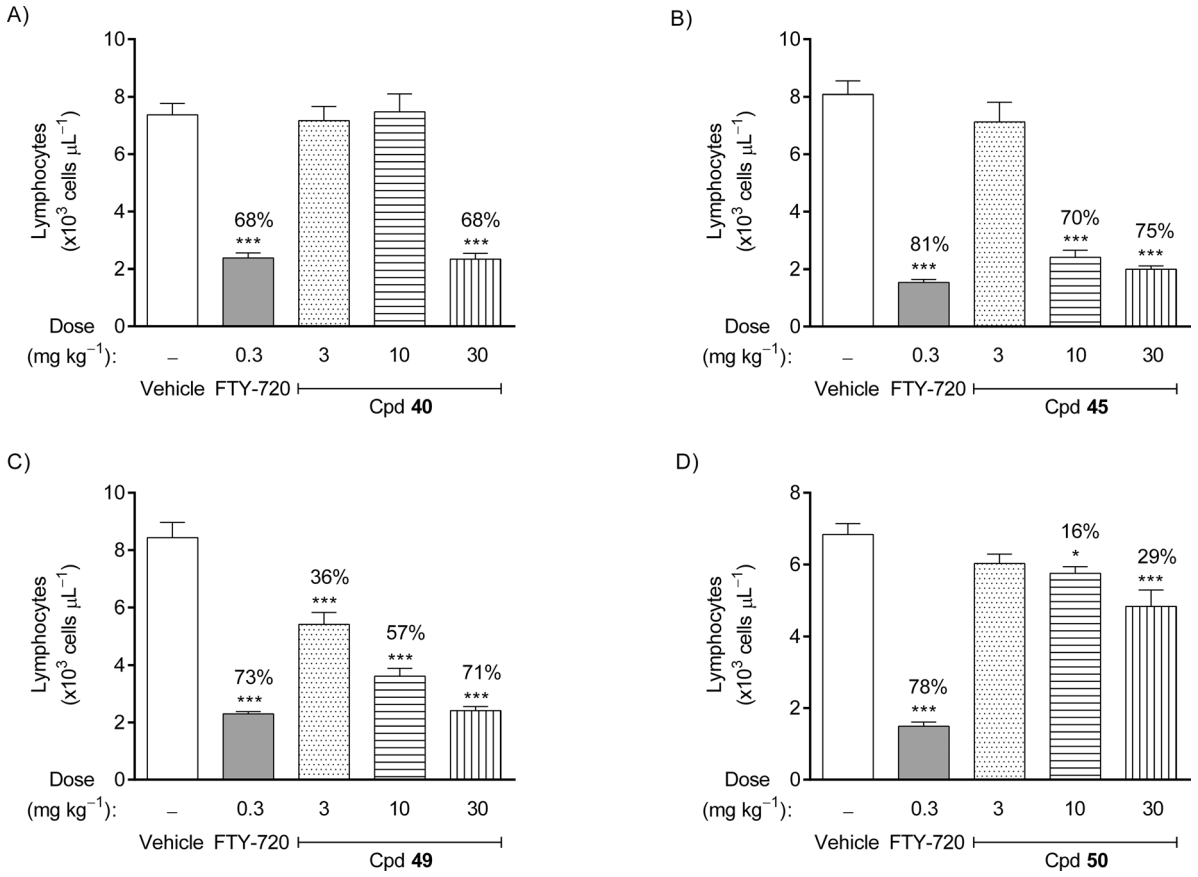


Figure 4. Treatment with S1P receptor agonists induced lymphopenia in C57BL/6 mice. Mice received oral treatment with compound **40** (A), **45** (B), **49** (C) or **50** (D), and blood lymphocytes were counted 48 h later. Each treatment group was comprised of $n = 8$ animals. Statistical analysis was performed by ANOVA followed by Newman–Keuls multiple comparison test (* $P < 0.05$; *** $P < 0.001$). Values are expressed as the mean ± SEM.

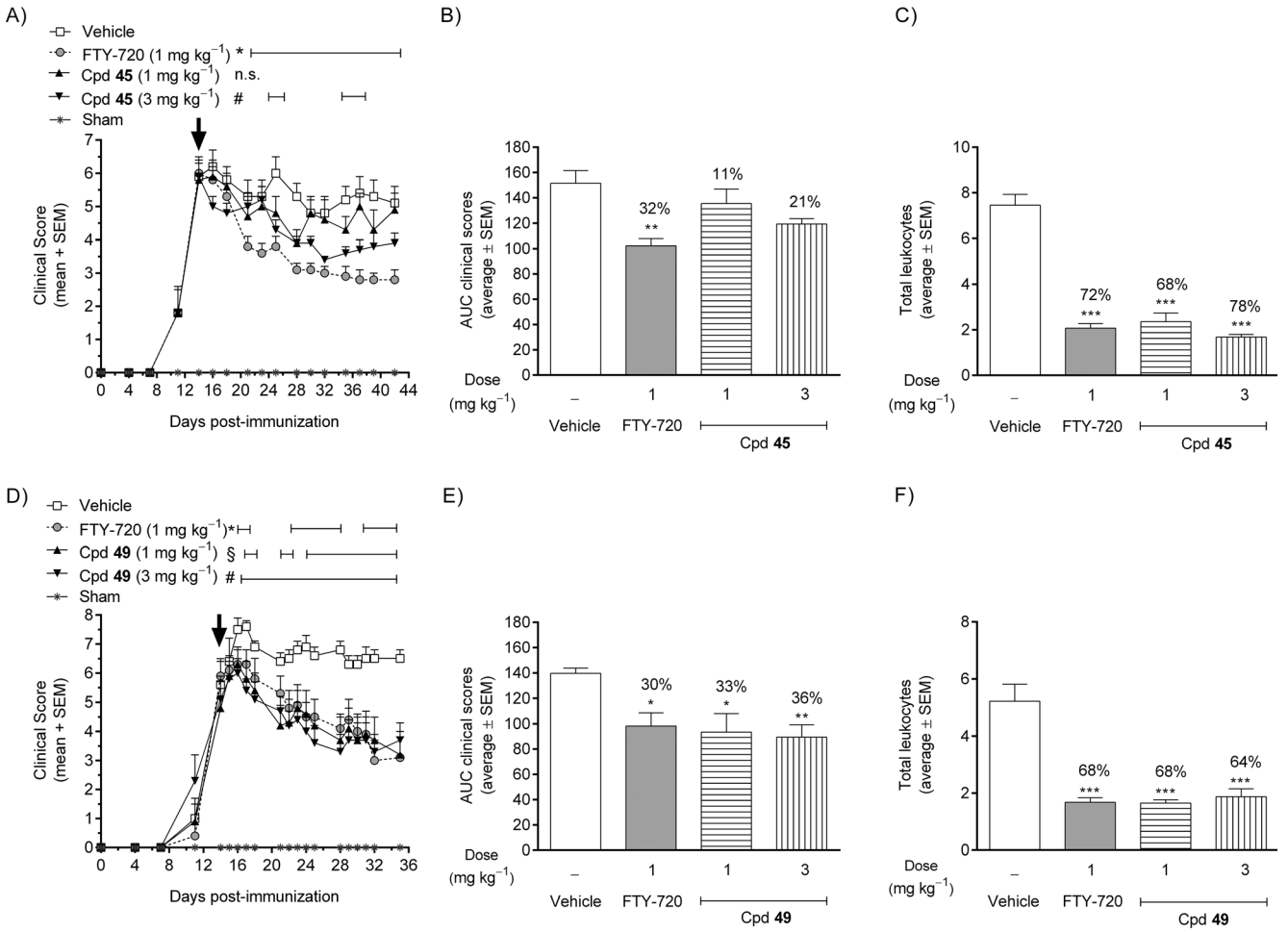


Figure 5. Treatment with compound **45** or **49** induced a therapeutic effect in the MOG_{35-55}-induced EAE model in C57BL/6 mice. Mice were immunized with MOG_{35-55} and treated with **45** (A, B and C), **49** (D, E and F) or FTY-720 (A–F) at the indicated dose from day 14 post-immunization until study termination, as described in the Experimental Section. The arrow indicates the day of the beginning of treatment. Each treatment group was comprised of *n* animals: vehicle ($n=12$ in A, $n=8$ in D), FTY-720 at 1 mg kg^{-1} ($n=12$ in A, $n=8$ in D), **45** or **49** at 1 and 3 mg kg^{-1} ($n=12$ and 9, respectively), Sham ($n=5$). A and D) Data were analyzed by the Kruskal–Wallis method followed by Dunn's multiple comparisons test. The bars placed above the graph are in line with each treatment group to indicate the period during which a statistically significant difference has been found in comparison with the vehicle-treated group as follows: * $P<0.05$ for vehicle vs FTY-720 at 1 mg kg^{-1}; § $P<0.05$ for vehicle vs **45** or **49** at 1 mg kg^{-1}; # $P<0.05$ for vehicle vs **45** or **49** at 3 mg kg^{-1}. B and E) The clinical efficacy was analyzed by measuring the area under the curve (AUC) of the clinical score from beginning of treatment until study termination. AUC data were analyzed by the Kruskal–Wallis method followed by Dunn's multiple comparisons test (* $P<0.05$; ** $P<0.01$ vs vehicle group). C and F) Upon termination of the experiment, 24 h after the last administration of compound, blood was harvested, and total leukocytes were counted for assessment of the leukopenia. Leukopenia data were analyzed by ANOVA followed by a Newman–Keuls multiple comparison test (** $P<0.01$; *** $P<0.001$). For B, C, E and F, data for the inhibition (%) versus the vehicle group are shown above each bar. Values are expressed as mean ± SEM.

Conclusions

This report summarizes the genesis of a series of novel oxadiazoles as selective S1P receptor agonists, its design and optimization supported by a pharmacophore model and calculated physicochemical properties. An extensive SAR study of 48 derivatives allowed the association of fragments and their combinations with their impact on selectivity, early toxicology and PK/PD. After close examination of the data, compounds **45** and **49** were identified as $S1P_1$- and $S1P_{1,5}$-selective frontrunners, respectively. These compounds induced long-lasting decreases in lymphocyte count after oral administration and showed preferential distribution in lymph nodes and brain. In a head-to-head comparison in an MOG_{35-55}-induced EAE murine model, only compound **49** demonstrated efficacy comparable with the marketed agent, fingolimod, which led to its selection as a clinical candidate. Compound **49** is not a prodrug, and its improved property profile, most notably its selectivity, should translate into a safer treatment of relapsing forms of multiple sclerosis.

Experimental Section

Computational methods

$S1P_1$ pharmacophore: A Catalyst pharmacophore model (Figure 6) was constructed using HipHop[20] from nine diverse $S1P_1$ ligands from the literature and internal screening efforts. The model contains three hydrophobic centers and two hydrogen-bond acceptors with their projected donor sites. In an

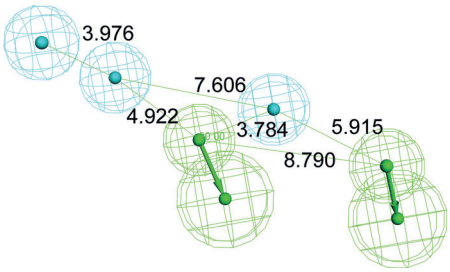


Figure 6. Geometrical arrangement of the pharmacophore; distances between features in Å; colour coding as in Figure 3.

a posteriori validation against 178 S1P receptor modulators from a recent comprehensive Review article,[26] the model was able to retrieve up to 148 (83%) of the known actives.

Computed physical properties: Topological polar surface area (TPSA), pK_a and *c*Log *D* values were calculated using ACD/Labs PhysChem, version 12.01 (2009), from Advanced Chemistry Development Inc., Toronto, Canada, www.acdlabs.com.

Synthetic procedures

General: The commercially available starting materials used in the following experimental description were purchased from Aldrich, Fluka, Acros or ABCR unless otherwise noted. ^{1}H NMR spectra were recorded on BRUKER spectrometers, models DPX-300, AV-II or AV-III 400 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent peak; multiplicity is denoted using standard abbreviations, and coupling constants (*J*) are reported in Hertz (Hz). Microwave reactions were performed in a single-mode microwave reactor Emrys Optimiser from Personal Chemistry or a single-mode microwave reactor Initiator 60 from Biotage in sealed reaction vessels. Elemental analyses were performed on an Erba Science 11108 CHN analyzer.

HPLC: Analyses were performed on a Waters 2695 instrument equipped with a Waters 996 photodiode array detector. Trifluoroacetic acid (TFA), formic acid (HCOOH), CH_3CN and H_2O were used as eluents.

Method A: column: X-Bridge C_8 (50×4.6 mm 3.5 μm); flow rate: 2 mL min^{-1}; mobile phase: H_2O + 0.1% TFA (solvent A) and CH_3CN + 0.1% TFA (solvent B); conditions (A/B): 95:5→5:95 over 8 mins.

Method B: column: ATLANTIS C_{18} (50×4.6 mm, 5 μm); flow rate: 1 mL min^{-1}; mobile phase: H_2O + 0.1% HCOOH (solvent A) and CH_3CN (solvent B); conditions (A/B): 90:10→0:100 over 4 min, then held at 100% solvent B for 2 mins.

Method C: column: Phenomenex Luna C_{18} (2) (100×4.6 mm, 5 μm) plus guard cartridge; flow rate: 2 mL min^{-1}; mobile phase: H_2O + 0.1% HCOOH (solvent A) and CH_3CN + 0.1% HCOOH (solvent B); conditions (A/B): 95:5→5:95 over 3.5 min, then held at 5:95 for 2 min.

Method D: column: Waters Xbridge C_{18} (2) (250×4.6 mm, 5 μm) plus guard cartridge; flow rate: 1 mL min^{-1}; mobile phase: 10 mM NH_4HCO_3 in H_2O (solvent A) and CH_3CN (solvent B); conditions (A/B): 95:5→0:100 over 19.5 min, then held at 100% solvent B for 4 min.

Method E: column: Waters Xterra MS C_{18} (100×4.6 mm, 5 μm) plus guard cartridge; flow rate: 2 mL min^{-1}; mobile phase: 10 mM NH_4HCO_3 in H_2O (solvent A) and CH_3CN (solvent B); conditions (A/B): 95:5→5:95 over 3.5 min, then held at 5:95 for 1.5 min.

Preparative HPLC: Purifications were performed with a mass-directed autopurification (MDAP) FractionLynx from Waters equipped with a sunfire prep C_{18} OBD column (19×100 mm 5 μm), unless otherwise noted. All HPLC purifications were performed with a gradient of H_2O (0.1% HCOOH)/CH_3CN (0.1% HCOOH) 95:5→0:100 over 15 to 20 min with a flow rate of 20 mL min^{-1}.

Mass spectrometry: Liquid chromatography (LC)/MS analyses were performed on a Waters Alliance 2795 coupled with a ZSpray™ mass detector (ZMD) [electrospray (ES)] equipped with a Waters X-Bridge column (C_8 30×2.1 mm 3.5 μm). Ultra-high-performance liquid chromatography (UHPLC)/MS analyses were performed on a Waters Acquity coupled with Waters Acquity S70QD (ES) equipped with a Waters Acquity BEH column (C_{18} 50×2.1 mm 1.7 μm), using the following conditions: CH_3CN/H_2O (NH_4OAc 10 mM), 5 to 100% (2–3 min), max plot 230–400 nm.

Methyl 4-[amino(hydroxyimino)methyl]-2-fluorobenzoate (52a): Methyl 4-cyano-2-fluorobenzoate (17.76 g, 99.1 mmol) was suspended in EtOH (200 mL). NH_2OH (50% in H_2O, 30 mL, 495 mmol) was added, and the resulting yellow suspension was stirred at RT overnight. The suspension was filtered, and the remaining residue was rinsed with EtOH (2×50 mL) and dried in vacuo, affording **52a** as a white solid (18.91 g, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.09 (s, 1H), 7.95–7.89 (m, 1H), 7.70–7.62 (m, 2H), 6.05 (s, 2H), 3.89 ppm (s, 3H); UHPLC/MS: *m/z*: 210.9 [*M*−H]$^-$, 212.9 [*M*+H]$^+$; HPLC (method A): t_R = 0.97 min (purity: 100%).

***tert*-Butyl {4-[amino(hydroxyimino)methyl]-2-fluorophenoxy}acetate (52b)**: *Step 1*: 3-Fluoro-4-hydroxybenzonitrile (2 g, 14.59 mmol) was dissolved in CH_3CN (120 mL). $CsCO_3$ (5.70 g, 17.50 mmol) and *tert*-butyl bromoacetate (2.26 mL, 15.32 mmol) were added, and the mixture was stirred at RT for 12 h. The reaction mixture was concentrated, and the crude mixture was diluted with EtOAc (50 mL) and washed with water (3×30 mL) then with brine (30 mL), dried over $MgSO_4$, filtered and concentrated, affording *tert*-butyl (4-cyano-2-fluorophenoxy)acetate as a yellow solid (3.58 g, 97%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.91–7.84 (m, 1H), 7.68–7.65 (m, 1H), 7.37–7.24 (m, 1H), 4.92 (s, 2H), 1.42 ppm (s, 9H); UHPLC/MS: *m/z*: 250.2 [*M*−H]$^-$; HPLC (method A): t_R = 4.72 min (purity: 97.6%).

Step 2: Compound **52b** was prepared following the procedure described for **52a**, starting from *tert*-butyl (4-cyano-2-fluorophenoxy)acetate. It was isolated as a white solid (3.57 g, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.61 (br s, 1H), 7.51–7.41 (m, 2H), 7.09–7.03 (t, *J* = 8.84 Hz, 1H), 5.81 (br s, 2H), 4.78 (s, 2H), 1.42 ppm (s, 9H); UHPLC/MS: *m/z*: 285.1 [*M*+H]$^+$; HPLC (method A): t_R = 2.91 min (purity: 94.3%).

***tert*-Butyl ({4-[(*Z*)-amino(hydroxyimino)methyl]-2-fluorobenzyl}oxy)acetate (52c)**: *Step 1*: A solution of NaOH (20 g) in H_2O (40 mL) was added to a mixture of 2-*tert*-butyl glycolate (1.38 g, 10.47 mmol) and tetrabutylammonium hydrogen sulfate (317 mg, 0.93 mmol) in toluene (40 mL). 4-Cyano-2-fluorobenzylbromide (2.00 g, 9.34 mmol) was added, and the resulting mixture was stirred at RT overnight. The layers were separated. The aqueous layer was extracted with EtOAc (2×30 mL). The organic layers were combined, washed with saturated aq NH_4Cl (30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. After purification by flash chromatography (EtOAc/heptane,

10:90→60:40), *tert*-butyl [(4-cyano-2-fluorobenzyl)oxy]acetate was obtained as a colorless oil (1.58 g, 64%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=7.87 (dd, J=9.9, 1.4 Hz, 1H), 7.74 (dd, J=7.9, 1.5 Hz, 1H), 7.71–7.64 (m, 1H), 4.68 (s, 2H), 4.12 (s, 2H), 1.43 ppm (s, 9H); UHPLC/MS: m/z: 283.3 $[M+NH_4]^+$; HPLC (method A): t_R= 4.38 min (purity: 99.9%).

Step 2: Compound **52c** was prepared following the procedure described for **52a**, starting from *tert*-butyl [(4-cyano-2-fluorobenzyl)-oxy]acetate. It was isolated as a colorless oil (758 mg, 96%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.79 (s, 1H), 7.57–7.39 (m, 3H), 5.90 (s, 2H), 4.59 (s, 2H), 4.05 (s, 2H), 1.43 ppm (s, 9H); UHPLC/MS: m/z: 299.1 $[M+H]^+$; HPLC (method A): t_R=2.92 min (purity: 97.7%).

***tert*-Butyl ({3-[amino(hydroxyimino)methyl]benzyl}oxy)acetate (52d)**: Compound **52d** was prepared following the procedure described for **52c**, starting from 3-cyanobenzyl alcohol (3 g, 22.53 mmol) and *tert*-butyl bromoacetate (3.73 mL, 25.23 mmol). It was isolated as a white powder (4.48 g, 91%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.62 (s, 1H), 7.65 (br s, 1H), 7.63–7.54 (m, 1H), 7.41–7.31 (m, 2H), 5.80 (s, 2H), 4.54 (s, 2H), 4.04 (s, 2H), 1.43 ppm (s, 9H); UHPLC/MS: m/z: 279.2 $[M-H]^-$, 281.2 $[M+H]^+$; HPLC (method A): t_R=2.75 min (purity: 100%).

***tert*-Butyl *N*-{4-[amino(hydroxyimino)methyl]benzyl}-*N*-(*tert*-butoxycarbonyl)glycinate (52e)**: *Step 1*: To a solution of 4-cyanobenzyl bromide (1.50 g, 7.65 mmol) in CH_3CN (25 mL) was added K_2CO_3 (2.11 g, 15.3 mmol) and *tert*-butyl glycinate (1.20 g, 9.18 mmol). The reaction mixture was stirred at RT overnight. The solvent was removed in vacuo, and the resulting mixture was diluted with water (20 mL), extracted with EtOAc (2×25 mL), washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (cyclohexane/EtOAc, 90:10→40:60), affording *tert*-butyl *N*-(4-cyanobenzyl)glycinate as colorless oil (1.14 g, 60%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=7.78 (d, J=8.3 Hz, 2H), 7.52 (d, J=8.3 Hz, 2H), 3.79 (s, 2H), 3.19 (s, 2H), 2.60 (br s, 1H), 1.41 ppm (s, 9H); UHPLC/MS: m/z: 247.0 $[M+H]^+$; HPLC (method A): t_R=2.17 min (purity: 97.2%).

Step 2: A mixture of *tert*-butyl *N*-(4-cyanobenzyl)glycinate (1.1 g, 4.5 mmol), di-*tert*-butyl dicarbonate (1.1 g, 4.9 mmol) and DIEA (1.1 mL, 6.7 mmol) was prepared in CH_2Cl_2 (22 mL) and stirred at RT for 3.5 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with saturated aq $NaHCO_3$ (2×20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to afford *tert*-butyl *N*-(*tert*-butoxycarbonyl)-*N*-(4-cyanobenzyl)glycinate as a colorless oil (1.2 g, 77%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=7.84–7.78 (m, 2H), 7.48 (d, J=8.0 Hz, 2H), 4.50–4.45 (m, 2H), 3.93–3.83 (m, 2H), 1.41–1.29 ppm (m, 18H); UHPLC/MS: m/z: 347.1 $[M+H]^+$; HPLC (method A): t_R=5.10 min (purity: 100.0%).

Step 3: ***Compound* 52e** was prepared following the procedure described for **52a**, starting from *tert*-butyl *N*-(*tert*-butoxycarbonyl)-*N*-(4-cyanobenzyl)glycinate. It was isolated as a colorless oil (1.26 g, 97%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.59 (s, 1H), 7.65–7.60 (m, 2H), 7.26 (d, J=8.3 Hz, 2H), 5.77 (s, 2H), 4.40 (s, 2H), 3.84–3.75 (m, 2H), 1.41–1.34 ppm (m, 18H); UHPLC/MS: m/z: 380.1 $[M+H]^+$; HPLC (method A): t_R=3.31 min (purity: 98.1%).

***tert*-Butyl *N*-{4-[amino(hydroxyimino)methyl]benzyl}-*N*-methylglycinate (52f)**: *Step 1*: A mixture of 4-(bromomethyl)benzonitrile (1.50 g, 7.65 mmol), sarcosine *tert*-butyl ester hydrochloride (1.67 g, 9.18 mmol) and K_2CO_3 (3.17 g, 22.95 mmol) in CH_3CN (25 mL) was stirred at RT overnight. The reaction mixture was concentrated in vacuo. The residue was diluted with water (25 mL) and extracted with EtOAc (2×25 mL). The combined organic layers were washed with water (25 mL), dried over $MgSO_4$, filtered and the solvent was removed in vacuo to give *tert*-butyl *N*-(4-cyanobenzyl)-*N*-methylglycinate as a yellow oil (1942 g, 98%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=7.79 (d, J=8.4 Hz, 2H), 7.51 (d, J=8.4 Hz, 2H), 3.71 (s, 2H), 3.20 (s, 2H), 2.23 (s, 3H), 1.42 ppm (s, 9H); UHPLC/MS: m/z: 260.9 $[M+H]^+$; HPLC (method A): t_R=2.27 min (purity: 88%).

Step 2: Compound **52f** was prepared following the procedure described for **52a**, starting from *tert*-butyl *N*-(4-cyanobenzyl)-*N*-methylglycinate, which was used as prepared in step 1 without further purification. Compound **52f** was isolated as a white powder (12.54 g, 95%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.58 (s, 1H), 7.62 (d, J=8.3 Hz, 2H), 7.28 (d, J=8.3 Hz, 2H), 5.78 (s, 2H), 3.62 (s, 2H), 3.15 (s, 2H), 2.24 (s, 3H), 1.42 ppm (s, 9H); UHPLC/MS: m/z: 294.0 $[M+H]^+$; HPLC (method A): t_R=1.33 min (purity: 90%).

***tert*-Butyl *N*-{4-[amino(hydroxyimino)methyl]benzyl}-*N*-methyl-β-alaninate (52g)**: Compound **52g** was prepared following the procedure described for **52f**, starting from 4-cyanobenzyl bromide and *tert*-butyl *N*-methyl-β-alaninate.[30] It was isolated as a yellow oil (1.6 g, 77%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.57 (br s, 1H), 7.61 (d, J=8.2 Hz, 2H), 7.27 (d, J=8.2 Hz, 2H), 5.77 (s, 2H), 3.51–3.45 (m, 2H), 2.59 (t, J=7.0 Hz, 2H), 2.38 (t, J=7.0 Hz, 2H), 2.10 (s, 3H), 1.40 ppm (s, 9H); UHPLC/MS: m/z: 308.0 $[M+H]^+$; HPLC (method A): t_R=1.52 min (purity: 82.3%).

{*tert*-Butoxycarbonyl-[3-(*N*-hydroxycarbamimidoyl)benzyl]amino}acetic acid *tert*-butyl ester (52h): *Step 1*: A solution of glycine-*tert*-butyl ester (5 g, 38 mmol), 3-cyanobenzaldehyde (5 g, 0.0381 mol) in toluene (100 mL) was heated at reflux under N_2 for 2 h. After evaporation of the solvent, the reaction mass was redissolved in dry MeOH (100 mL). $NaBH_4$ (2.17 g, 57.1 mmol) was added in portions at 0 °C, and the resulting mixture was stirred at RT for 12 h. Water was added (100 mL), and the aqueous layer was extracted with CH_2Cl_2 (2×100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to afford *tert*-butyl [(3-cyanobenzyl)amino]acetate as a yellow oil (8.1 g, 86%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.71–7.72 (m, 2H), 7.63–7.65 (m, 1H), 7.53–7.55 (m, 1H), 5.40–5.41 (m, 1H), 4.53–4.54 (m, 2H), 3.17 (s, 2H), 1.39 ppm (s, 9H).

Step 2: Compound **52h** was prepared following the procedure described for **52e**, starting from *tert*-butyl [(3-cyanobenzyl)amino]acetate. It was isolated as a pale yellow oil (3.8 g, 99%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=9.59 (s, 1H), 7.53–7.56 (m, 2H), 7.29–7.33 (m, 1H), 7.24–7.26 (m, 1H), 5.76 (s, 2H), 4.37–4.39 (s, 2H), 3.74 (s, 2H), 1.35–1.37 ppm (s, 18H); UHPLC/MS: m/z: 380.0 $[M+H]^+$; HPLC (method B): t_R=5.91 min (purity: 90.3%).

***tert*-Butyl [{3-[amino(hydroxyimino)methyl]benzyl}(methyl)amino]acetate (52i)**: Compound 52**i** was prepared following the procedure described for **52f**, starting from 3-(bromomethyl)benzonitrile. It was isolated as a white powder (8.5 g, 84%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=9.57 (s, 1H), 7.59 (s, 1H), 7.54–7.51 (m, 1H), 7.31–7.28 (m, 2H), 5.75 (s, 2H), 3.61 (s, 2H), 3.15 (s, 2H), 2.23 (s, 3H), 1.41 ppm (s, 9H); UHPLC/MS: m/z: 294.0 $[M+H]^+$; HPLC (method A): t_R=3.31 min (purity: 97.5%).

***tert*-Butyl 3-[{3-[amino(hydroxyimino)methyl]benzyl}(methyl)amino]propanoate (52j)**: Compound **52j** was prepared following the procedure described for **52f**, starting from 3-(bromomethyl)-benzonitrile and *tert*-butyl *N*-methyl-β-alaninate.[30] It was isolated as a white gummy solid (4.5 g, 84%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=9.56 (s, 1H), 7.51–7.53 (m, 2H), 7.27–7.31 (m, 2H), 5.74 (s, 2H), 3.44 (s, 2H), 2.55–2.59 (m, 2H), 2.34–2.38 (m, 2H), 2.09

(s, 3H), 1.37 ppm (s, 9H); UHPLC/MS: *m/z*: 308.2 $[M+H]^+$; HPLC (method B): $t_R = 5.18$ min (purity: 96.5%).

***tert*-Butyl *N*-{2-[(*Z*)-amino(hydroxyimino)methyl]benzyl}-*N*-methylglycinate (52k)**: Compound **52k** was prepared following the procedure described for **52f**, starting from 2-(bromomethyl)benzonitrile. It was isolated as a white solid (3.47 g, 81%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.39$ (s, 1H), 7.45–7.24 (m, 4H), 6.07 (s, 2H), 3.67 (s, 2H), 3.16 (s, 2H), 2.25 (s, 3H), 1.40 ppm (s, 9H); UHPLC/MS: *m/z*: 294.0 $[M+H]^+$; HPLC (method A): $t_R = 1.99$ min (purity: 90.0%).

***tert*-Butyl *N*-{4-[amino(hydroxyimino)methyl]-2-fluorobenzyl}-*N*-methylglycinate (52l)**: Compound **52l** was prepared following the procedure described for **52f**, starting from 4-cyano-2-fluorobenzyl bromide. It was isolated as white powder (323 g, 89%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.74$ (s, 1H), 7.51 (dd, $J = 8.0$, 1.6 Hz, 1H), 7.45–7.36 (m, 2H), 5.87 (s, 2H), 3.70 (s, 2H), 3.17 (s, 2H), 2.26 (s, 3H), 1.42 ppm (s, 9H); UHPLC/MS: *m/z*: 312.3 $[M+H]^+$; HPLC (method A): $t_R = 1.36$ min (purity: 97.3%).

2-Fluoro-*N*-hydroxy-4-hydroxymethyl-benzamidine (52m): Compound **52m** was prepared following the procedure described for **52a**, starting from 2-fluoro-4-(hydroxymethyl)benzonitrile. It was isolated as a white powder (12.54 g, 95%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.62$ (s, 1H), 7.48 (t, $J = 7.7$ Hz, 1H), 7.18 (d, $J = 3.7$ Hz, 1H), 7.16 (s, 1H), 5.79 (s, 2H), 5.42–5.35 (m, 1H), 4.55 ppm (d, $J = 5.8$ Hz, 2H); LC/MS: *m/z*: 185 $[M+H]^+$; HPLC (method D): $t_R = 8.16$ min (purity: 97.0%).

***tert*-Butyl 2-((4-(*N*′-hydroxycarbamimidoyl)-2-(trifluoromethyl)benzyl)(methyl)amino)acetate (52n)**: *Step 1*: A solution of 4-methyl-3-(trifluoromethyl)benzonitrile (1.85 g, 10 mmol), *N*-bromosuccinimide (2.14 g, 12.0 mmol) and α,α′-azoisobutyronitrile (AIBN) (0.033 g, 0.2 mmol) in CH_3CN (40 mL) was heated at reflux for 18 h. The reaction mixture was diluted with EtOAc (40 mL) and water (25 mL). The resulting suspension was filtered, and the filtrate was separated. The organic phase was washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (*iso*-hexane/EtOAc, 90:10→40:60). The resulting product was mixed with sarcosine *tert*-butyl ester hydrochloride (0.516 g, 2.84 mmol) and K_2CO_3 (0.980 g, 7.1 mmol) in CH_3CN (10 mL). The mixture was heated at 70 °C for 18 h. The solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL), washed with brine (15 mL), dried over $MgSO_4$ and filtered. The solvent was evaporated in vacuo, and the resulting product was purified by flash chromatography (*iso*-hexane/EtOAc, 90:10→40:60) to afford *tert*-butyl 2-((4-cyano-2-(trifluoromethyl)benzyl)(methyl)amino)acetate (0.543 g, 70%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.15$ (d, $J = 8.2$ Hz, 1H), 7.90 (s, 1H), 7.83 (d, $J = 8.2$ Hz, 1H), 3.90 (s, 2H), 3.26 (s, 2H), 2.37 (s, 3H), 1.48 ppm (s, 9H).

Step 2: Compound **52n** was prepared following the procedure described for **52a**, starting from *tert*-butyl 2-((4-cyano-2-(trifluoromethyl)benzyl)(methyl)amino)acetate. It was isolated as white solid (0.460 g, 77%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.97$ (d, $J = 8.1$ Hz, 1H), 7.89 (s, 1H), 7.78 (d, $J = 8.1$ Hz, 1H), 4.86 (br s, 2H), 3.86 (s, 2H), 3.23 (s, 2H), 2.37 (s, 3H), 1.48 ppm (s, 9H); LC/MS: *m/z*: 362 $[M+H]^+$, 360 $[M-H]^-$; HPLC (method E): $t_R = 3.38$ min (purity: 84.8%).

***tert*-Butyl 2-((4-(*N*′-hydroxycarbamimidoyl)-3-(trifluoromethyl)benzyl)(methyl)amino)acetate (52o)**: Compound **52o** was prepared following the procedure described for **52n**, starting from 4-methyl-2-(trifluoromethyl)benzonitrile. It was isolated as an off-white solid (0.233 g, 78%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.72$ (s, 1H), 7.63–7.52 (m, 2H), 4.84 (s, 2H), 3.76 (s, 2H), 3.19 (s, 2H), 2.37 (s, 3H), 1.48 ppm (s, 9H); LC/MS: *m/z*: 362 $[M+H]^+$; HPLC (method E): $t_R = 2.36$ min (purity: 93.24%)

***tert*-Butyl 2-(((5-(*N*′-hydroxycarbamimidoyl)pyridin-2-yl)methyl)(methyl)amino)acetate (52p)**: Compound **52p** was prepared following the procedure described for **52f**, starting from 6-bromomethylnicotinonitrile. It was isolated as an off-white solid (1.87 g, 77%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.81$ (s, 1H), 8.79 (d, $J = 2$ Hz, 1H), 8.03 (dd, $J = 8.2$, 2.2 Hz, 1H), 7.48 (d, $J = 8.2$ Hz, 1H), 5.97 (br s, 2H), 3.80 (s, 2H), 2.32 (s, 3H), 1.46 ppm (s, 9H).

***tert*-Butyl *N*-{2-fluoro-3-[(hydroxyamino)(imino)methyl]benzyl}-*N*-methylglycinate (52q)**: Compound **52q** was prepared following the procedure described for **52n**, starting from 2-fluoro-3-methylbenzonitrile. It was isolated as a yellow oil (1 g, 85%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.57$ (s, 1H), 7.49–7.30 (m, 2H), 7.22–7.09 (m, 1H), 5.77 (s, 2H), 3.70 (d, $J = 1.5$ Hz, 2H), 3.18 (s, 2H), 2.26 (s, 3H), 1.42 ppm (s, 9H); UHPLC/MS: *m/z*: 312.0 $[M+H]^+$; HPLC (method A): $t_R = 1.09$ min (purity: 92%).

***tert*-Butyl *N*-{5-[amino(hydroxyimino)methyl]-2-fluorobenzyl}-*N*-methylglycinate (52r)**: Compound **52r** was prepared following the procedure described for **52f**, starting from 5-cyano-2-fluorobenzyl bromide. It was isolated as a colorless oil (602 mg, quantitative): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.61$ (br s, 1H), 7.73 (dd, $J = 7.3$, 2.3 Hz, 1H), 7.62–7.56 (m, 1H), 7.20–7.12 (m, 1H), 5.81 (s, 2H), 3.71 (s, 2H), 3.20 (s, 2H), 2.28 (s, 3H), 1.43 ppm (s, 9H); UHPLC/MS: *m/z*: 310.1 $[M-H]^-$, 312.2 $[M+H]^+$; HPLC (method A): $t_R = 1.52$ min (purity: 94%).

***tert*-Butyl *N*-{3-[amino(hydroxyimino)methyl]-5-fluorobenzyl}-*N*-methylglycinate (52s)**: Compound **52s** was prepared following the procedure described for **52n**, starting from 3-fluoro-5-methylbenzonitrile. It was isolated as a yellow solid (850 mg, 75%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 9.75$ (br s, 1H), 7.49 (s, 1H), 7.34 (m, 1H), 7.13 (m, 1H), 5.87 (br s, 2H), 3.65 (s, 2H), 3.19 (s, 2H), 2.25 (s, 3H), 1.43 ppm (s, 9H); UHPLC/MS: *m/z*: 311.9 $[M+H]^+$, 310.0 $[M-H]^-$; HPLC (method A): $t_R = 1.89$ min (purity: 53%).

***tert*-Butyl *N*-{3-[amino(hydroxyimino)methyl]-6-fluorobenzyl}-*N*-methylglycinate (52t)**: Compound **52t** was prepared following the procedure described for **52n**, starting from 2-fluoro-5-methylbenzonitrile. It was isolated as a colorless oil (870 mg, 78%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.59$ (s, 1H), 7.42 (dd, $J = 7.1$, 2.3 Hz, 1H), 7.37–7.25 (m, 1H), 7.17 (dd, $J = 10.6$, 8.4 Hz, 1H), 5.78 (s, 2H), 3.59 (s, 2H), 3.15 (s, 2H), 2.22 (s, 3H), 1.41 ppm (s, 9H); UHPLC/MS: *m/z*: 312.1 $[M+H]^+$; HPLC (method A): $t_R = 1.23$ min (purity: 70%).

***tert*-Butyl *N*-[3-[amino(hydroxyimino)methyl]-5-(trifluoromethyl)benzyl]-*N*-methylglycinate (52u)**: Compound **52u** was prepared following the procedure described for **52n**, starting from 3-methyl-5-(trifluoromethyl)benzonitrile. It was isolated as a colorless oil (790.0 mg, 83%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.82$ (s, 1H), 7.95–7.83 (m, 2H), 7.70–7.59 (m, 1H), 5.98 (s, 2H), 3.72 (s, 2H), 3.20 (s, 2H), 2.25 (s, 3H), 1.41 ppm (s, 9H); UHPLC/MS: *m/z*: 362.2 $[M+H]^+$, 360.2 $[M-H]^-$; HPLC (method A): $t_R = 1.90$ min (purity: 95.5%).

2,2′-Dimethyl-1,1′-biphenyl-4-carboxylic acid (56d): *Step 1*: To a solution of methyl 4-bromo-3-methylbenzoate (ABCR) (15 g, 65 mmol) in toluene (200 mL) and H_2O (200 mL), was added *o*-tolylboronic acid (10.68 g, 78 mmol) followed by K_2CO_3 (45.25 g, 32.7 mmol) and $Pd(PPh_3)_4$ (3.78 g, 3.3 mmol). The mixture was degassed with N_2 and then heated at reflux (120 °C) for 6 h. After

completion, the reaction mixture was cooled to RT. The organic phase was separated and evaporated in vacuo. The crude compound was passed through a silica gel (60–120 mesh) column using hexane as an eluent to obtain methyl 2,2′-dimethyl-1,1′-biphenyl-4-carboxylate as a white solid (15 g, 95%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.91 (s, 1H), 7.83–7.81 (m, 1H), 7.33–7.30 (m, 2H), 7.28–7.26 (m, 1H), 7.25–7.22 (m, 1H), 7.07–7.05 (m, 1H), 3.84 (s, 3H), 2.04 (s, 3H), 1.97 ppm (s, 3H); LC/MS: *m/z*: 240.9 $[M+H]^+$; HPLC (method A): t_R = 3.01 min (purity: 98.71%).

Step 2: To a solution of methyl 2,2′-dimethyl-1,1′-biphenyl-4-carboxylate (15 g, 62.2 mmol) in THF (100 mL) was added 10% aq NaOH (100 mL), and the mixture was heated at 100 °C overnight. THF was removed in vacuo, and the aqueous residue was washed with EtOAc (100 mL). The aqueous layer was then acidified with 3 N HCl to pH 2–3 and extracted with CH_2Cl_2 (2×100 mL). The combined organic phase was washed with water (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to obtain **56d** as a white solid (13.5 g, 95%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.89 (br s, 1H), 7.89 (s, 1H), 7.82–7.80 (m, 1H), 7.32–7.23 (m, 3H), 7.19–7.11 (m, 1H), 7.07–7.05 (m, 1H), 2.04 (s, 3H), 1.98 ppm (s, 3H); LC/MS: *m/z*: 227.0 $[M+H]^+$; HPLC (method B): t_R = 4.1 min (purity: 99.6%).

2-Methoxy-2′-methylbiphenyl-4-carboxylic acid (56e): Compound **56e** was prepared following the procedure described for **56d**, starting from methyl 4-bromo-3-methoxybenzoate. It was isolated as a beige solid (1.95 g, 83%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.06 (s, 1H), 7.66–7.61 (m, 2H), 7.31–7.23 (m, 4H), 7.14 (d, *J* = 7.28 Hz, 1H), 3.80 (s, 3H), 2.07 ppm (s, 3H); UHPLC/MS: *m/z*: 240.9 $[M-H]^-$; HPLC (method A): t_R = 4.05 min (purity: 97.3%).

2′-Methyl-2-(trifluoromethyl) biphenyl-4-carboxylic acid (56f): Compound **56f** was prepared following the procedure described for **56d**, starting from methyl 4-bromo-3-(trifluoromethyl)benzoate. It was isolated as off-white solid (4.07 g, 94%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.55 (br s, 1H), 8.31 (s, 1H), 8.25 (d, *J* = 7.90 Hz, 1H), 7.50 (d, *J* = 7.90 Hz 1H), 7.37–7.12 (m, 4H), 1.99 ppm (s, 3H); UHPLC/MS: *m/z*: 278.9 $[M-H]^-$; HPLC (method A): t_R = 4.57 min (purity: 98.7%).

2′-Methoxy-2-methylbiphenyl-4-carboxylic acid (56g): Compound **56g** was prepared following the procedure described for **56d**, starting from 2-methoxyphenylboronic acid. It was isolated as a brown solid (1.12 g, 59%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.89 (s, 1H), 7.86 (s, 1H), 7.81 (dd, *J* = 7.99, 1.23 Hz, 1H), 7.46–7.40 (m, 1H), 7.26–7.23 (d, *J* = 7.68 Hz, 1H), 7.16–7.04 (m, 3H), 3.75 (s, 3H), 2.13 ppm (s, 3H); UHPLC/MS: *m/z*: 240.9 $[M-H]^-$; HPLC (method A): t_R = 4.05 min (purity: 98.5%).

2-Methyl-2′-(trifluoromethyl)biphenyl-4-carboxylic acid (56h): Compound **56h** was prepared following the procedure described for **56d**, starting from 2-(trifluoromethyl)benzeneboronic acid. It was isolated as a white powder (22.5 g, 92%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.00 (s, 1H), 7.87 (m, 2H), 7.80 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.75–7.71 (m, 1H), 7.64–7.61 (m, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 1H), 2.02 ppm (s, 3H); UHPLC/MS: *m/z*: 279.0 $[M-H]^-$; HPLC (method A): t_R = 4.4 min (purity: 100%).

2-(Methoxymethyl)-2′-methyl biphenyl-4-carboxylic acid (56a): *Step 1*: To a solution of methyl 4-bromo-3-methylbenzoate (50 g, 218.27 mmol) in $CHCl_3$ (1 L) under inert atmosphere were added *N*-bromosuccinimide (NBS) (46.62 g, 261.93 mmol) in one portion and AIBN (0.72 g, 4.37 mmol). The mixture was stirred at 70 °C for 2 days. The reaction mixture was cooled to RT and water (500 mL) was added. The organic layer was separated and washed with saturated aq $NaHCO_3$ (50 mL), water (340 mL), brine (500 mL), dried over $MgSO_4$, and filtered. After evaporation of the solvent, a yellow solid was isolated. It was washed with pentane (2×500 mL), affording methyl 4-bromo-3-(bromomethyl)benzoate as a yellow solid (42 g, 62%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.24 (d, *J* = 1.91 Hz, 1H), 7.88–7.82 (m, 2H), 4.87 (s, 2H), 3.91 ppm (s, 3H); HPLC (method A): t_R = 4.44 min (purity: 97.9%).

Step 2: A solution of methyl 4-bromo-3-(bromomethyl)benzoate (37.50 g, 121.77 mmol) in MeOH (1.125 L) was heated at reflux for 4 days. The reaction was concentrated and then was partitioned between EtOAc (500 mL) and water (200 mL). The organic layer was washed with 5% aq $NaHCO_3$ (200 mL), brine (200 mL), dried over $MgSO_4$, and filtered. After evaporation of the solvent, methyl 4-bromo-3-(methoxymethyl)benzoate was isolated as a beige solid (29.8 g, 94%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.06–8.05 (m, 1H), 7.83 (d, *J* = 1.23 Hz, 2H), 4.54–4.51 (m, 2H), 3.90 (s, 3H), 3.45 ppm (s, 3H); UHPLC/MS: *m/z*: 227.2 $[M-H]^-$; HPLC (method A): t_R = 4.42 min (purity: 93.0%).

Step 3: A mixture of methyl 4-bromo-3-(methoxymethyl)benzoate (40 g, 154.38 mmol), *o*-tolylboronic acid (23.09 g, 169.82 mmol), K_2CO_3 (106.68 g, 771.90 mmol) and $Pd(PPh_3)_4$ (1.78 g, 1.54 mmol) was dissolved in toluene (200 mL) and water (200 mL) under inert atmosphere. The reaction mixture was purged with vacuum, degassed with N_2, and then heated at reflux for 1 h. The reaction mixture was cooled to RT, filtered over a pad of celite and washed with EtOAc (1 L). The filtrate was concentrated to afford a yellow oil, which was taken up in EtOAc (800 mL). The organic layer was washed with saturated aq $NaHCO_3$ (250 mL), water (250 mL) and brine (250 mL), dried over $MgSO_4$, filtered and concentrated affording methyl 2-(methoxymethyl)-2′-methylbiphenyl-4-carboxylate as a yellow oil (41.9 g, quantitative): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.11 (dq, *J* = 1.9, 0.6 Hz, 1H), 7.93 (ddd, *J* = 7.8, 2.2, 0.6 Hz, 1H), 7.38–7.21 (m, 4H), 7.09 (dt, *J* = 7.0, 1.1 Hz, 1H), 4.21–4.04 (m, 2H), 3.89 (s, 3H), 3.19 (s, 3H), 2.04–1.92 ppm (m, 3H); UHPLC/MS: *m/z*: 271.2 $[M+H]^+$; HPLC (method A): t_R = 5.34 min (purity: 89.4%).

*Step 4:*A solution of methyl 2-(methoxymethyl)-2′-methylbiphenyl-4-carboxylate (40 g, 147.97 mmol), which was used as isolated in step 3 without further purification, in EtOH (1.2 L) at RT was treated with 5 M aq NaOH (88.78 mL, 443.90 mmol). The reaction mixture was stirred at 60 °C for 1 h. It was cooled to RT and concentrated to give a yellow solid, which was dissolved in water (800 mL), and the aqueous phase was washed with EtOAc (2×400 mL). The aqueous phase was acidified with concentrated HCl (40 mL) to pH 2 and then extracted with EtOAc (2×400 mL). The combined organic extracts were washed with brine (2×400 mL), dried over $MgSO_4$, filtered and concentrated affording compound **56a** as a yellow solid (35.1 g, 92%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.99 (br s, 1H), 8.09 (s, 1H), 7.92–7.89 (m, 1H), 7.33–7.22 (m, 4H), 7.10–7.08 (m, 1H), 4.11 (m, 2H), 3.18 (s, 3H), 1.99 ppm (s, 3H); UHPLC/MS: *m/z*: 255.2 $[M-H]^-$; HPLC (method A): t_R = 4.52 min (purity: 96.4%).

2′-(Methoxymethyl)-2-methylbiphenyl-4-carboxylic acid (56i): Compound **56i** was prepared following the procedure described for **56d**, starting from (2-methoxymethylphenyl)boronic acid. It was isolated as a beige solid (1.74 g, 78%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.95 (br s, 1H), 7.92–7.91 (m, 1H), 7.84–7.81 (dd, 1H), 7.55–7.52 (m, 1H), 7.47–7.38 (m, 2H), 7.25–7.22 (m, 1H), 7.15–7.14 (m, 1H), 4.09–4.06 (m, 2H), 3.17 (s, 3H), 2.07 ppm (s, 3H); UHPLC/MS: *m/z*: 255.2 $[M-H]^-$; HPLC (method A): t_R = 4.35 min (purity: 96.8%).

2-Ethoxy-2′-methyl-1,1′-biphenyl-4-carboxylic acid (56c): *Step 1*: A mixture of methyl 4-bromo-3-hydroxybenzoate (CombiBlocks, 25 g, 108 mmol), *o*-tolylboronic acid (22 g, 162 mmol), anhydrous

K_2CO_3 (44 g, 324 mmol) and $Pd(PPh_3)_4$ (6.25 g, 5.4 mmol) was dissolved in toluene (500 mL) and water (100 mL) under an inert atmosphere. The reaction mixture was heated at 110 °C for 12 h. The reaction mixture was cooled at RT, filtered through a Celite pad. The filtrate was washed with 10% aq $NaHCO_3$ (1×300 mL), water (1×300 mL) and brine (2×300 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by chromatography (pet ether/EtOAc, 90:10→20:80), affording methyl 2-hydroxy-2′-methyl-1,1′-biphenyl-4-carboxylate as pale yellow solid (20 g, 77%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.85 (s, 1H), 7.52 (s, 1H), 7.45–7.43 (m, 1H), 7.25–7.20 (m, 3H), 7.15–7.13 (m, 1H), 7.11–7.09 (m, 1H), 3.84 (s, 3H), 2.09 ppm (s, 3H).

Step 2: To a stirred solution of methyl 2-hydroxy-2′-methyl-1,1′-biphenyl-4-carboxylate (10 g, 41.2 mmol) in anhydrous CH_3CN (100 mL) was added anhydrous K_2CO_3 (17.1 g, 123.6 mmol) followed by EtBr (15.4 mL, 206 mmol). The reaction mixture was heated at 50 °C for 48 h, and was then cooled to RT and filtered. The filtrate was concentrated in vacuo affording methyl 2-ethoxy-2′-methyl-1,1′-biphenyl-4-carboxylate as a brown oil (11 g, 98%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.70–7.68 (m, 1H), 7.62 (s, 1H), 7.29–7.16 (m, 5H), 4.09–4.03 (m, 2H), 3.95 (s, 3H), 2.15 (s, 3H), 1.35–1.25 ppm (m, 3H).

Step 3: To a stirred solution of methyl 2-ethoxy-2′-methyl-1,1′-biphenyl-4-carboxylate (11 g, 40.6 mmol) in a mixture of THF (100 mL) and water (10 mL) was added LiOH (6.82 g, 162.7 mmol) in portions. After 24 h at RT, the reaction mixture was evaporated, and the residue was taken up in water (15 mL). This solution was acidified to pH 1 with concentrated HCl and extracted with EtOAc (2×20 mL). The organic layer was washed with brine (1×20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo affording **56c** as a pale yellow solid (9.7 g, 93%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 13.02 (br s, 1H), 7.57 (m, 2H), 7.25–7.18 (m, 4H), 7.11–7.08 (m, 1H), 4.06–4.01 (m, 2H), 2.05 (s, 3H), 1.18 ppm (m, 3H); UHPLC/MS: *m/z*: 255.0 $[M-H]^-$; HPLC (method B): t_R = 4.99 min (purity: 98.7%).

2-(Methoxymethyl)-2′-(trifluoromethyl)biphenyl-4-carboxylic acid (56b): Compound **56b** was prepared following the procedure described for **56a**, replacing *o*-tolylboronic acid with 2-(trifluoromethyl)phenylboronic acid in step 3. It was isolated as a pale yellow solid (6.2 g, 74%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 13.08 (s, 1H), 8.06 (s, 1H), 7.87–7.81 (m, 2H), 7.73–7.68 (m, 1H), 7.65–7.61 (m, 1H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.27 (d, *J* = 7.9 Hz, 1H), 4.11–4.03 (m, 2H), 3.15 ppm (s, 3H); UHPLC/MS: *m/z*: 309.0 $[M-H]^-$; HPLC (method A): t_R = 4.65 min (purity: 99.6%).

3-[(*tert*-Butoxycarbonylmethyl-methylamino)methyl]benzoic acid lithium (73): *Step 1*: DIEA (11.14 mL, 65.48 mmol) was added to a mixture of methyl 3-(bromomethyl)benzoate (5.00 g, 21.83 mmol) and sarcosine *tert*-butyl ester hydrochloride (3965 mg, 21.83 mmol) in CH_3CN (250 mL). The resulting solution was heated at 60 °C for 6 h. The solvents were evaporated, and water (75 mL) was added to the residue. The mixture was extracted with EtOAc (2×75 mL). The combined organic phases were washed with brine (40 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to afford methyl 3-{[(2-*tert*-butoxy-2-oxoethyl)(methyl)amino]methyl}benzoate as yellow oil (6.21 g, 97%): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03–7.97 (m, 1H), 7.97–7.90 (m, 1H), 7.63–7.54 (m, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 3.91 (s, 3H), 3.72 (s, 2H), 3.18 (s, 2H), 2.36 (s, 3H), 1.56–1.37 ppm (m, 9H); UHPLC/MS: *m/z*: 294.4 $[M+H]^+$; HPLC (method A): t_R = 2.53 min (purity: 93.1%).

Step 2: A solution of methyl 3-{[(2-*tert*-butoxy-2-oxoethyl)(methyl)-amino]methyl}benzoate (6.2 g, 21.2 mmol, 1.00 equiv), used as prepared without further purification, in THF (60.00 mL), MeOH (40.00 mL) and water (20.00 mL) was treated with LiOH (0.98 g, 23.29 mmol, 1.10 equiv). The reaction mixture was stirred at RT for 16 h. The solvents were removed under vacuo, affording compound **73** as hygroscopic white foam that was used as a reagent without further purification (19.9 mmol, 94%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.82 (s, 1H), 7.80–7.70 (m, 1H), 7.28–7.19 (m, 2H), 3.60 (s, 2H), 3.14 (s, 2H), 2.23 (s, 3H), 1.42 ppm (s, 9H); UHPLC/MS: *m/z*: 280.3 $[M+H]^+$. 278.2 $[M-H]^-$; HPLC (method A): t_R = 2.05 min (purity: 84.3%).

***tert*-Butyl *N*-{3-[(2-{[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-carbonyl}hydrazino) carbonyl]benzyl}-*N*-methylglycinate (77)**: *Step 1*: Oxalyl chloride (2.5 mL, 29.3 mmol, 1.5 equiv) and DMF (30 μL, 0.4 mmol, 0.02 equiv) were added at RT to a solution of **56a** (5.0 g, 19.5 mmol, 1.0 equiv) in dry toluene (100 mL). The reaction mixture was stirred for 3 h and then concentrated in vacuo affording the corresponding acyl chloride as a yellow oil (5.4 g, quantitative). The acyl chloride was immediately dissolved in CH_2Cl_2 (100 mL), and Et_3N (8.2 mL, 58.5 mmol, 3.0 equiv) was added followed by hydrazinecarboxylic acid *tert*-butyl ester (2.8 g, 21.5 mmol, 1.1 equiv). The reaction was stirred at RT for 18 h. Water (100 mL) was added, and the aqueous layer was separated and extracted with CH_2Cl_2 (2×100 mL). The combined organic extracts were washed with saturated aq $NaHCO_3$ (75 mL), dried over $MgSO_4$, filtered and concentrated to afford *N*′-(2-methoxymethyl-2′-methyl-biphenyl-4-carbonyl)hydrazinecarboxylic acid *tert*-butyl ester as a yellow oil (7.3 g, quantitative): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.28 (s, 1H), 8.96 (s, 1H), 8.01 (s, 1H), 7.93–7.67 (m, 2H), 7.40–7.00 (m, 4H), 4.11 (s, 2H), 3.18 (s, 3H), 1.99 (s, 3H), 1.44 ppm (s, 9H); UHPLC/MS: *m/z*: 371.4 $[M+H]^+$, 369.3 $[M-H]^-$; HPLC (method A): t_R = 4.34 min (purity: 98.0%).

Step 2: A solution of *N*′-(2-methoxymethyl-2′-methyl-biphenyl-4-carbonyl)hydrazinecarboxylic acid *tert*-butyl ester (7.3 g, 19.5 mmol, 1.0 equiv) in CH_2Cl_2 (100 mL) was cooled to 0 °C, and TFA (100 mL) was then added dropwise. The reaction was stirred for 5 h at RT, and then the solvents were removed in vacuo. The resulting yellow oil was resuspended in CH_2Cl_2 (50 mL) and water (50 mL). $NaHCO_3$ was added as solid until gas evolution ceased and the aqueous layer reached pH 8. The organic layer was separated and washed with water (1×50 mL) and brine (2×50 mL), dried over $MgSO_4$, filtered and concentrated to afford compound **77** as a yellow oil (5.2 g, 98%): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98–7.90 (m, 1H), 7.77 (dd, *J* = 7.9, 1.9 Hz, 1H), 7.60 (s, 1H), 7.41–7.20 (m, 4H), 7.16–7.03 (m, 1H), 4.51–4.09 (m, 4H), 3.30 (s, 3H), 2.05 ppm (s, 3H); UHPLC/MS: *m/z*: 271.3 $[M+H]^+$, 269.3 $[M-H]^-$.

***N*′-Hydroxy-2-(methoxymethyl)-2′-methylbiphenyl-4-carboximidamide (75)**: *Step 1*: Oxalyl chloride (1.25 ml, 14.7 mmol) and DMF (15 μL, 0.2 mmol) were added at RT to a solution of **56a** (2.5 g, 9.75 mmol) in dry toluene (50 mL). The reaction mixture was stirred for 3 h and then concentrated in vacuo. Toluene (50 mL) and 0.5 M NH_3 in dioxane (106 mL, 53 mmol) were added, and the reaction mixture was stirred at RT for 15 min. The solvents were removed in vacuo, and the resulting white solid was washed with pentane (200 mL), then water (200 mL) and pentane (2×100 mL) to afford 2-(methoxymethyl)-2′-methylbiphenyl-4-carboxamide as a white solid (2.5 g, 91%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.05 (br s, 1H), 8.02 (d, *J* = 1.5 Hz, 1H), 7.86–7.83 (m, 1H), 7.38 (br s, 1H), 7.32–7.22 (m, 3H), 7.18 (d, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 7.3 Hz, 1H), 4.10 (s, 2H), 3.16 (s, 3H), 1.99 ppm (s, 3H); UHPLC/MS: *m/z*: 256.2 $[M+H]^+$, 254.2 $[M-H]^-$; HPLC (method A): t_R = 3.47 min (purity: 98.5%).

Step 2: To a mixture of 2-(methoxymethyl)-2′-methylbiphenyl-4-carboxamide (306 mg, 1.2 mmol, 1.0 equiv) in dioxane (6 mL) was added pyridine (190 μL, 2.0 equiv). The solution was cooled to 0 °C and trifluoroacetic anhydride (200 μL, 1.4 mmol, 1.2 equiv) was added. The resulting solution was stirred at RT for 3 h. The reaction mixture was diluted with Et_2O (50 mL) and washed with water (50 mL). Aqueous layer was extracted with Et_2O (2×50 mL). The combined organic layers were washed with saturated aq $NaHCO_3$ (2×100 mL) and brine (100 mL). The organic phase was then dried over $MgSO_4$, filtered and concentrated to afford 2-(methoxymethyl)-2′-methylbiphenyl-4-carbonitrile (190 mg, 67%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.89 (m, 1H), 7.84–7.80 (m, 1H), 7.35–7.23 (m, 4H), 7.08 (d, J = 7.3 Hz, 1H), 4.13 (d, J = 12.8 Hz, 1H), 4.07 (d, J = 12.8 Hz, 1H), 3.17 (s, 3H), 1.98 ppm (s, 3H); HPLC (method A): t_R = 4.79 min (purity: 94.9%).

Step 3: Amidoxime **75** was prepared following the procedure described for **52a**, starting from 2-(methoxymethyl)-2′-methylbiphenyl-4-carbonitrile. It was isolated as white solid (1.9 g, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.64 (s, 1H), 7.82 (d, J = 1.6 Hz, 1H), 7.65–7.62 (m, 1H), 7.32–7.21 (m, 3H), 7.12–7.06 (m, 2H), 5.84 (br s, 2H), 4.08 (s, 2H), 3.15 (s, 3H), 2.00 ppm (s, 3H); UHPLC/MS: *m/z*: 271.2 [M+H]$^+$; HPLC (method A): t_R = 2.72 min (purity: 98.7%).

2-Fluoro-4-[5-(4-isobutyl-phenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid (3): *Step 1*: 4-Isobutyl-benzoic acid (138.6 mg, 0.78 mmol), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) (295.7 mg, 0.78 mmol) and DIEA (0.27 mL, 1.56 mmol) were mixed in DMF (5 mL), and the reaction mixture stirred at RT for 15 min. Compound **52a** (150 mg, 0.71 mmol) was added, and the reaction mixture was stirred at RT for 3 h. Toluene (5 mL) was added, and the reaction mixture was stirred at 95 °C overnight. It was diluted with EtOAc (20 mL) and washed with 1 N aq HCl (20 mL). The resulting crude product was purified by flash chromatography (EtOAc/cyclohexane, 10:90→60:40), affording 2-fluoro-4-[5-(4-isobutyl-phenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester as colorless oil (100 mg, 40%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.18–8.08 (m, 3H), 8.06–8.01 (m, 1H), 7.99–7.92 (m, 1H), 7.52–7.42 (d, J = 7.9 Hz, 2H), 3.91 (s, 3H), 2.64–2.54 (d, J = 7.1 Hz, 2H), 2.01–1.82 (m, 1H), 0.98–0.84 ppm (d, J = 6.7 Hz, 6H); UHPLC/MS: *m/z*: 355.4 [M+H]$^+$; HPLC (method A): t_R = 6.14 min (purity: 92.3%).

Step 2: 2-Fluoro-4-[5-(4-isobutyl-phenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid methyl ester (100 mg, 0.28 mmol) was disolved in THF/MeOH (5 mL, 1:1), and 5 N aq NaOH (0.58 mL, 4.23 mmol) was added. The reaction mixture stirred for 2 h at RT and was then concentrated. CH_2Cl_2 (20 mL) was added, and the resulting solution was washed with 5 M aq HCl (20 mL), dried over $MgSO_4$, filtered and concentrated, affording compound **3** as a white powder with acceptable purity (65 mg, 68%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.17–8.05 (m, 3H), 8.04–7.99 (m, 1H), 7.96–7.88 (dd, J = 11.1, 1.5 Hz, 1H), 7.53–7.39 (d, J = 8.1 Hz, 2H), 2.63–2.54 (d, J = 7.2 Hz, 2H), 2.02–1.83 (m, 1H), 0.96–0.81 ppm (d, J = 6.6 Hz, 6H); UHPLC/MS: *m/z*: 339.4 [M−H]$^-$, 341.4 [M+H]$^+$; HPLC (method A): t_R = 5.34 min (purity: 99.8%).

4-[5-(2,2′-Dimethylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]-2-fluoro-benzoic acid (8): *Step 1*: Compound **56d** (814 mg, 3.60 mmol), **52a** (637 mg, 3 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (690 mg, 3.60 mmol) were dissolved in THF (10 mL) and CH_3CN (10 mL) in a 20 mL microwave vial under an inert atmosphere. The reaction mixture was stirred overnight at RT. DIEA (1.22 mL, 7.20 mmol) was added, and the mixture was heated at 150 °C for 30 min under microwave irradiation (full power, 400 W). The reaction mixture was concentrated, EtOAc (50 mL) was added to the residue, and the mixture was washed with 0.1 N aq HCl (2×25 mL), saturated aq $NaHCO_3$ (25 mL), dried over $MgSO_4$, and filtered. After evaporation of the solvents, the resulting crude product was purified by flash chromatography (cyclohexane/EtOAc, 9.5/0.5), affording methyl 4-[5-(2,2′-dimethylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]-2-fluorobenzoate as a white powder (495 mg, 34%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.17–8.16 (m, 1H), 8.12 (d, J = 7.2 Hz, 1H), 8.29–8.04 (m, 2H), 7.98 (dd, J = 11.1, 2.5 Hz, 1H), 7.39–7.27 (m, 4H), 7.14–7.11 (m, 1H), 3.91 (s, 3H), 2.13 (s, 3H), 2.03 ppm (s, 3H); LC/MS: *m/z*: 403.0 [M+H]$^+$; HPLC (method A): t_R = 6.23 min (purity: 93.5%).

Step 2: Methyl 4-[5-(2,2′-dimethylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]-2-fluorobenzoate (495 mg, 1.23 mmol) was dissolved in THF (10 mL) and MeOH (10 mL). 5 M aq NaOH (1.23 mL, 6.15 mmol) was added, and the mixture was stirred at RT overnight. 5 N aq HCl (1.23 mL, 6.15 mmol) was added, and the mixture was concentrated. The aqueous residue was diluted with CH_2Cl_2 (50 mL) and was washed with water (2×25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated, affording compound **8** as a light yellow solid (457 mg, 95%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.16–8.01 (m, 4H), 7.94 (dd, J = 11.1, 1.4 Hz, 1H), 7.38–7.26 (m, 4H), 7.13–7.11 (m, 1H), 2.13 (s, 3H), 2.03 ppm (s, 3H); LC/MS: *m/z*: 386.9 [M−H]$^-$, 388.7 [M+H]$^+$; HPLC (method A): t_R = 5.38 min (purity: 98.0%); Anal. calcd for $C_{23}H_{17}N_2O_3F$: C 71.13, H 4.41, N 7.21, found: C 71.26, H 4.75, N 6.83.

4-[5-(4-Cyclohexyl-phenyl)-[1,2,4]oxadiazol-3-yl]-2-fluoro-benzoic acid (4): Compound **4** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with 4-cyclohexylbenzoic acid. It was isolated as a white solid (34.8 mg, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.55 (s, 1H), 8.17–7.97 (m, 3H), 7.95–7.87 (m, 2H), 7.6–7.45 (m, 2H), 2.6–2.8 (m, 1H), 1.92–1.65 (m, 5H), 1.55–1.15 ppm (m, 5H); LC/MS: *m/z*: 367.0 [M+H]$^+$; HPLC (method A): t_R = 5.55 min (purity: 99.6%).

4-(5-Biphenyl-4-yl-[1,2,4]oxadiazol-3-yl)-2-fluoro-benzoic acid (5): Compound **5** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with 4-phenylbenzoic acid. It was isolated as an off-white solid (64 mg, 88%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.68 (s, 1H), 8.45–8.20 (m, 2H), 8.15–7.95 (m, 5H), 7.9–7.75 (m, 2H), 7.70–7.35 ppm (m, 3H); LC/MS: *m/z*: 361.0 [M+H]$^+$; HPLC (method A): t_R = 4.91 min (purity: 95.7%).

2-Fluoro-4-[5-(2-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]benzoic acid (6): Compound **6** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with 3-methyl-4-phenylbenzoic acid. It was isolated as an off-white solid (64 mg, 88%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.61 (s, 1H), 8.10–7.83 (m, 5H), 7.62–7.30 (m, 6H), 2.37 ppm (s, 3H); LC/MS: *m/z*: 373.0 [M−H]$^-$; 375.0 [M+H]$^+$; HPLC (method A): t_R = 5.19 min (purity: 99.3%).

2-Fluoro-4-[5-(2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]benzoic acid (7): Compound **7** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with 4-(2-methylphenyl)benzoic acid. It was isolated as an off-white solid (36.8 mg, 98%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.61 (s, 1H), 8.23–8.31 (m, 2H), 8.01–8.14 (m, 2H), 7.98–7.91 (m, 1H), 7.70–7.62 (m, 2H), 7.38–7.25 (m, 4H), 2.49 ppm (s, 3H); LC/MS: *m/z*: 373.0 [M−H]$^-$; 375.0 [M+H]$^+$; HPLC (method A): t_R = 5.17 min (purity: 97.6%).

2-Fluoro-4-[5-(2-methoxy-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]benzoic acid (9): Compound **9** was prepared following the

procedure described for compound **8**, replacing carboxylic acid **56d** with **56e**. It was isolated as an off-white solid (56 mg, 79%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.62 (s, 1H), 8.15–8.02 (m, 2H), 7.97 (dd, J=11.1, 1.5 Hz, 1H), 7.88 (dd, J=7.7, 1.6 Hz, 1H), 7.82 (d, J=1.6 Hz, 1H), 7.41 (d, J=7.8 Hz, 1H), 7.35–7.21 (m, 3H), 7.19–7.12 (m, 1H), 3.89 (s, 3H), 2.09 ppm (s, 3H); LC/MS: *m/z*: 403.0 [M−H]$^-$; 405.0 [M+H]$^+$; HPLC (method A): t_R=5.14 min (purity: 96.5%).

2-Fluoro-4-{5-[2′-methyl-2-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoic acid (10): Compound **10** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with **56f**. It was isolated as a white solid (109 mg, 85%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.62 (br s, 1H), 8.56–8.49 (m, 2H), 8.14–7.97 (m, 3H), 7.67 (d, J=8.0 Hz, 1H), 7.41–7.26 (m, 3H), 7.17 (d, J=7.4 Hz, 1H), 2.02 ppm (s, 3H); LC/MS: *m/z*: 440.9 [M−H]$^-$; HPLC (method A): t_R=5.51 min (purity: 99.0%).

2-Fluoro-4-[5-(2′-methoxy-2-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]benzoic acid (11): Compound **11** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with **56g**. It was isolated as an off-white solid (45 mg, 98%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.60 (br s, 1H), 8.14–7.99 (m, 4H), 7.98–7.90 (m, 1H), 7.47–7.36 (m, 2H), 7.21–7.11 (m, 2H), 7.10–7.02 (m, 1H), 3.74 (s, 3H), 2.18 ppm (s, 3H); LC/MS: *m/z*: 403 [M−H]$^-$; 405.0 [M+H]$^+$; HPLC (method A): t_R=5.11 min (purity: 97.1%).

2-Fluoro-4-{5-[2-methyl-2′-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoic acid (12): Compound **12** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with **56h**. It was isolated an off-white solid (75.9 mg, 89%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.60 (br s, 1H), 7.97 (s, 1H), 7.95–7.80 (m, 3H), 7.79–7.66 (m, 2H), 7.63–7.53 (m, 1H), 7.53–7.43 (m, 2H), 7.27–7.16 (m, 2H), 2.25 ppm (s, 3H); LC/MS: *m/z*: 441.0 [M−H]$^-$; 443.4 [M+H]$^+$; HPLC (method A): t_R=5.40 min (purity: 98.2%).

2-Fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoic acid (13): *Step 1*: Carboxylic acid **56a** (4 g, 15.61 mmol) was dissolved in dry toluene (60 mL) under N_2 at RT, then oxalyl chloride (1.98 mL, 23.41 mmol) was added in one portion followed by DMF (0.024 mL). The reaction mixture was stirred at RT for 3 h. The reaction mixture was concentrated to afford a yellow oil, which was then redissolved in toluene (40 mL) and added dropwise at RT over 15 min to a mixture of amidoxime **52a** (3.31 g, 15.61 mmol) in pyridine (20 mL) and toluene (20 mL) under an inert atmosphere. The reaction mixture was stirred at RT for 12 h, then heated at reflux for 24 h. The reaction mixture was cooled to RT and concentrated, affording a beige solid. This solid was suspended in MeOH (40 mL) and filtered, affording methyl 2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoate as a beige solid (5.58 g, 82%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.33 (m, 1H), 8.19–7.98 (m, 4H), 7.44–7.28 (m, 4H), 7.16–7.13 (m, 1H), 4.24–4.14 (m, 2H), 3.91 (s, 3H), 3.25 (s, 3H), 2.03 ppm (s, 3H); UHPLC/MS: *m/z*: 433.1 [M+H]$^+$; HPLC (method A): t_R=5.94 min (purity: 94.9%).

Step 2: A solution of methyl 2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoate (16 g, 37 mmol) in THF (400 mL) and MeOH (400 mL) at RT was treated with 5 M aq NaOH (37 mL, 184.99 mmol). The reaction mixture was stirred at RT for 12 h. The reaction mixture was concentrated to give a yellow solid. Water (600 mL) was added, and the aqueous phase was washed with EtOAc (250 mL). The aqueous phase was then acidified to pH 2 with concentrated HCl and extracted with EtOAc (2× 300 mL). The combined organic phase was washed with brine (2× 300 mL), dried over $MgSO_4$, filtered and concentrated, affording compound **13** as a beige solid (11.66 g, 75%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.64 (br s, 1H), 8.33 (d, J=1.41 Hz, 1H), 8.17 (dd, J=7.92, 1.4 Hz, 1H), 8.13–8.03 (m, 2H), 7.96 (dd, J=11.10, 1.17 Hz, 1H), 7.43 (d, J=7.91 Hz, 1H), 7.37–7.27 (m, 3H), 7.15 (d, J=7.03 Hz, 1H), 4.25–4.14 (m, 2H), 3.25 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 419.1 [M+H]$^+$, 417.2 [M−H]$^-$; HPLC (method A): t_R=5.19 min (purity: 99.2%).

2-Fluoro-4-{5-[2′-(methoxymethyl)-2-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoic acid (14): Compound **14** was prepared following the procedure described for compound **8**, replacing carboxylic acid **56d** with **56i**. It was isolated as a white powder (88 mg, 84%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.61 (br s, 1H), 8.17 (d, J=1.0 Hz, 1H), 8.13–8.02 (m, 3H), 7.96 (dd, J=11.1, 1.3 Hz, 1H), 7.56–7.53 (m, 1H), 7.48–7.39 (m, 3H), 7.20–7.17 (m, 1H), 4.14 (d, J=12.0 Hz, 1H), 4.09 (d, J=11.9 Hz, 1H), 3.13 (s, 3H), 2.14 ppm (s, 3H); UHPLC/MS: *m/z*: 417.2 [M−H]$^-$, 419.2 [M+H]$^+$; HPLC (method A): t_R=5.38 min (purity: 98.2%).

***N*-(2-Fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoyl)glycine (15)**: *Step 1*: To a solution of compound **13** (2170 mg, 5.2 mmol) in dry CH_2Cl_2 (40 mL), oxalyl chloride (1.32 mL, 15.6 mmol) was added followed by dry DMF (0.040 mL). The reaction mixture was stirred at RT for 2 h. It was then concentrated to afford 2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoyl chloride as a white powder (2300 mg, 100%). Part of this product (125 mg, 0.29 mmol) was redissolved in dry THF (5 mL). DIEA (0.17 mL, 1 mmol) and glycine methyl ester hydrochloride (71.85 mg, 0.57 mmol) were added. The mixture was stirred overnight at RT. The solvents were evaporated, and the crude residue was purified by flash chromatography (cyclohexane/EtOAc, 90:10→40:60), affording methyl *N*-(2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoyl)glycinate as an orange solid (50 mg, 36%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.42 (m, 1H), 8.25 (t, J=8 Hz, 1H), 8.16 (dd, J=1.9, 7.9 Hz, 1H), 8.11 (dd, J=1.5, 7.9 Hz, 1H), 8.01 (dd, J=1.4, 12.4 Hz, 1H), 7.39–7.22 (m, 5H), 7.12 (m, 1H), 4.30 (d, J=4.8 Hz, 2H), 4.22 (d, J=2 Hz, 2H), 3.81 (s, 3H), 3.33 (s, 3H), 2.07 (s, 3H), 1.37 (d, J=6.8 Hz, 3H), 1.28 ppm (t, J=7.1 Hz, 3H); UHPLC/MS: *m/z*: 490.2 [M+H]$^+$ 488.3 [M−H]$^-$; HPLC (method A): t_R=5.22 min (purity: 100%).

Step 2: To a solution of methyl *N*-(2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoyl)glycinate (0.1806 mg, 0.369 mmol) in MeOH (4 mL), 5 M aq NaOH (0.37 mL, 1.85 mmol) was added. The reaction was stirred at RT for 1 h. EtOAc (20 mL) was added, and the organic phase was washed with 1 N aq HCl (15 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated to afford compound **15** as a white solid (143.9 mg, 82%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=(m, 1H), 8.34 (m, 1H), 8.19 (dd, J=1.9, 8 Hz, 1H), 8.07 (dd, J=1.5, 8 Hz, 1H), 7.98 (dd, J=1.5, 11 Hz, 1H), 7.91 (t, J=7.7 Hz, 1H), 7.44 (d, J=8 Hz, 1H), 7.39–7.24 (m, 3H), 7.15–7.11 (m, 1H), 4.23 (d, J=12.9 Hz, 1H), 4.17 (d, J=12.9 Hz, 1H), 3.96 (d, J=6 Hz, 2H), 3.26 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 476.1 [M+H]$^+$ 474.3 [M−H]$^-$; HPLC (method A): t_R=4.63 min (purity: 99.9%).

***N*-(2-Fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoyl)-β-alanine (16)**: Compound **16** was prepared following the procedure described for compound **15**, replacing glycine methyl ester hydrochloride with β-alanine methyl ester hydrochloride. It was isolated as a white powder (1490.0 mg, 82%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.58 (m, 1H), 8.33 (br s, 1H), 8.18 (dd, J=1.7, 8 Hz, 1H), 8.03 (dd, J=1.7, 8 Hz, 1H), 7.95 (d,

J=10.6 Hz, 1H), 7.84 (t, J=7.6 Hz, 1H), 7.44 (d, J=7.9 Hz, 1H), 7.40–7.22 (m, 3H), 7.15 (m, 1H), 4.23 (d, J=12.9 Hz, 1H), 4.17 (d, J=12.9 Hz, 1H), 3.49 (m, 2H), 3.25 (s, 3H), 2.53–2.45 (m, 2H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 490.3 $[M+H]^+$ 488.4 $[M-H]^-$; HPLC (method A): t_R=4.83 min (purity: 99.7%).

4-[(2-Fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzoyl)amino]butanoic acid (17): Compound **17** was prepared following the procedure described for compound **15**, replacing glycine methyl ester hydrochloride with methyl 4-aminobutyrate hydrochloride. It was isolated as a white solid (135 mg, 52%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=12.10 (br s, 1H), 8.57–8.53 (m, 1H), 8.33 (br s, 1H), 8.19 (dd, J=1.8, 7.9 Hz, 1H), 8.03 (dd, J=1.5, 7.9 Hz, 1H), 7.95 (dd, J=1.3, 10.5 Hz, 1H), 7.82 (t, J=7.5 Hz, 1H), 7.43 (d, J=7.9 Hz, 1H), 7.39–7.24 (m, 3H), 7.15–7.11 (m, 1H), 4.23 (d, J=12.9 Hz, 1H), 4.17 (d, J=12.9 Hz, 1 J), 3.30–3.22 (m, 2H), 3.25 (s, 3H), 3.21–3.13 (m, 2H), 2.31 (t, J=7.3 Hz, 2H), 2.04 (s, 3H), 1.77 ppm (q, J=7.3 Hz, 2H); UHPLC/MS: *m/z*: 504.2 $[M+H]^+$ 502.3 $[M-H]^-$; HPLC (method A): t_R=4.8 min (purity: 99%).

3-({2-Fluoro-4-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-benzoyl}-methylamino)propionic acid (18): Compound **18** was prepared following the procedure described for compound **15**, replacing glycine methyl ester hydrochloride with methyl *N*-methyl-β-alaninate hydrochloride. It was isolated as a white solid (42 mg, 97%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ= 12.37 (s, 1H), 8.34 (d, J=2.3 Hz, 1H), 8.18 (dd, J=8.0, 2.0 Hz, 1H), 8.10–7.92 (m, 2H), 7.69–7.59 (m, 1H), 7.44 (d, J=8.0 Hz, 1H), 7.40–7.23 (m, 3H), 7.15 (d, J=7.3 Hz, 1H), 4.32–4.07 (m, 2H), 3.70 (t, J= 7.2 Hz, 1H), 3.43 (t, J=7.2 Hz, 1H), 3.25 (s, 3H), 3.02 (s, 1H), 2.90 (s, 2H), 2.61 (t, J=7.2 Hz, 1H), 2.54–2.50 (m, 1H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 504.2 $[M+H]^+$. 502.2 $[M-H]^-$; HPLC (method A): t_R=4.84 min (purity: 98.3%).

(2-Fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}phenoxy)acetic acid (19): *Step 1*: Using the procedure described for compound **8** (step 1), replacing the carboxylic acid **56d** with **56a** and amidoxime **52a** with **52b**, *tert*-butyl (2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}phenoxy)acetate was isolated as a white powder (167 mg, 66%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.41 (d, J=1.3 Hz, 1H), 8.15 (dd, J=7.8, 1.8 Hz, 1H), 7.98–7.91 (m, 2H), 7.36–7.23 (m, 4H), 7.13 (d, J=7.1 Hz, 1H), 7.03–6.97 (m, 1H), 4.68 (s, 2H), 4.22 (s, 2H), 3.33 (s, 3H), 2.07 (s, 3H), 1.49 ppm (s, 9H); UHPLC/MS: *m/z*: 505.2 $[M+H]^+$; HPLC (method A): t_R=6.36 min (purity: 98.6%).

Step 2: To *tert*-butyl (2-fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}phenoxy)acetate (116 mg, 0.23 mmol), obtained in step 1, was added a 4 M HCl in dioxane (2 mL, 8 mmol). The mixture was stirred at RT for 16 h. The solvents were concentrated to dryness to afford compound **19** as a white powder (98 mg, 95%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=13.24 (br s, 1H), 8.31 (s, 1H), 8.16 (d, J=8.2 Hz, 1H), 7.92 (s, 1H), 7.89 (s, 1H), 7.42 (d, J=8.0 Hz, 1H), 7.35–7.30 (m, 4H), 7.15 (d, J=7.1 Hz, 1H), 4.93 (s, 2H), 4.25–4.14 (m, 2H), 3.25 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 447.2 $[M-H]^-$, 449.1 $[M+H]^+$; HPLC (method A): t_R=5.18 min (purity: 100.0%); Anal. calcd for $C_{25}H_{21}N_2O_5F\cdot0.1H_2O$: C 66.69, H 4.75, N 6.22, found: C 66.53, H 4.71, N 6.18.

{2-Fluoro-4-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-benzyloxy}-acetic acid (20): Compound **20** was prepared following the procedure described for compound **19**, replacing amidoxime **52b** with **52c**. It was isolated as a dark yellow oil (61.3 mg, 20%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.42 (s, 1H), 8.16 (d, J=7.4 Hz, 1H), 8.01 (d, J=7.9 Hz, 1H), 7.92 (d, J= 10.5 Hz, 1H), 7.59 (t, J=7.6 Hz, 1H), 7.30–7.34 (m, 3H), 7.25 (s, 1H), 7.13 (d, J=7.5 Hz, 1H), 4.80 (s, 2H), 4.23 (s, 2H), 4.22 (s, 2H), 3.33 (s, 3H), 2.08 ppm (s, 3H); UHPLC/MS: *m/z*: 463.3 $[M+H]^+$; HPLC (method A): t_R=5.77 min (purity: 97.9%).

[(3-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)oxy]acetic acid (21): Compound **21** was prepared following the procedure described for compound **19**, replacing amidoxime **52b** with **52d**. It was isolated as a colorless oil (298 mg, 82%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=12.75 (br s, 1H), 8.33 (d, J=1.5 Hz, 1H), 8.18 (dd, J=7.9, 1.8 Hz, 1H), 8.13 (br s, 1H), 8.06–8.02 (m, 1H), 7.61–7.55 (m, 2H), 7.43 (d, J=8.0 H, 1H), 7.37–7.26 (m, 3H), 7.15 (d, J=7.0 Hz, 1H), 4.68 (s, 2H), 4.23 (s, 2H), 4.17 (s, 2H), 3.25 (s, 3H), 2.04 ppm (s, 3H); LC/MS: *m/z*: 445.3 $[M+H]^+$. 443.4 $[M-H]^-$; HPLC (method A): t_R=5.18 min (purity: 97.5%).

***N*-(4-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)glycine, hydrochloride salt (22)**: Compound **22** was prepared following the procedure described for compound **3** (step 1), replacing carboxylic acid **56d** with **56a** and amidoxime **52a** with **52e**. The resulting *N*-Boc-protected *tert*-butyl ester derivative was treated with 4 M HCl solution in dioxane similarly to the procedure described for compound **19** (step 2), affording compound **22** as a white powder (172 mg, 84%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.33 (d, J=1.5 Hz, 1H), 8.21–8.15 (m, 3H), 7.77 (d, J=8.5 Hz, 2H), 7.44 (d, J=8.1 Hz, 1H), 7.39–7.27 (m, 3H), 7.15 (d, J=7.0 Hz, 1H), 4.28 (s, 2H), 4.26–4.14 (m, 2H), 3.90 (s, 2H), 3.26 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 442.1 $[M-H]^-$, 444.0 $[M+H]^+$; HPLC (method A): t_R=3.96 min (purity: 99.7%).

***N*-(4-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (23)**: Compound **23** was prepared following the procedure described for compound **22**, replacing amidoxime **52e** with **52f**. It was isolated as a white powder (168 mg, 86%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.33 (d, J=1.4 Hz, 1H), 8.24–8.15 (m, 3H), 7.79 (s, 1H), 7.77 (s, 1H), 7.44 (d, J=7.9 Hz, 1H), 7.38–7.27 (m, 3H), 7.15 (d, J=7.0 Hz, 1H), 4.43 (s, 2H), 4.26–4.14 (m, 2H), 4.06 (s, 2H), 3.26 (s, 3H), 2.78 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 456.1 $[M-H]^-$, 458.0 $[M+H]^+$; HPLC (method A): t_R=4.01 min (purity: 99.5%).

***N*-(4-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methyl-β-alanine, hydrochloride salt (24)**: Compound **24** was prepared following the procedure described for compound **22**, replacing amidoxime **52e** with **52g**. It was isolated as a white powder (37 mg, 54%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.33 (s, 1H), 8.25–8.15 (m, 3H), 7.84 (d, J=8.0 Hz, 2H), 7.47–7.27 (m, 4H), 7.15 (d, J=7.1 Hz, 1H), 4.45 (s, 2H), 4.26–4.14 (m, 2H), 3.41–3.23 (m, 5H), 2.88 (t, J=7.2 Hz, 2H), 2.69 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 470.2 $[M-H]^-$, 472.1 $[M+H]^+$; HPLC (method A): t_R=4.03 min (purity: 99.1%).

***N*-(3-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)glycine, hydrochloride salt (25)**: Compound **25** was prepared following the procedure described for compound **22**, replacing amidoxime **52e** with **52h**. It was isolated as a white powder (250 mg, 78%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.34 (dd, J=6.3, 1.8 Hz, 2H), 8.18 (dq, J=7.8, 1.8 Hz, 2H), 7.81–7.63 (m, 2H), 7.45 (d, J=8.0 Hz, 1H), 7.37 (dd, J=5.1, 1.4 Hz, 2H), 7.35–7.25 (m, 1H), 7.20–7.09 (m, 1H), 4.31 (s, 2H), 4.28–4.11 (m, 2H), 3.89 (s, 2H), 3.26 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 442.3 $[M-H]^-$, 444.3 $[M+H]^+$; HPLC (method A): t_R=3.85 min (purity: 96.8%).

***N*-(3-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (26)**: Compound **26** was prepared following the procedure described for

compound **13** (step 1), replacing amidoxime **52a** with **52i**. The resulting *tert*-butyl ester was hydrolysed with 4 M HCl in dioxane similarly to the procedure described for compound **19** (step 2), affording compound **26** as a white powder (1.4 g, 85%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.35–8.34 (m, 2H), 8.22 (d, J=1.8 Hz, 1H), 8.17 (dd, J=7.9, 1.8 Hz, 1H), 7.83 (d, J=7.8 Hz, 1H), 7.72 (t, J=7.7 Hz, 1H), 7.44 (d, J=8.0 Hz, 1H), 7.37–7.27 (m, 3H), 7.15 (d, J=7.2 Hz, 1H), 4.52 (s, 2H), 4.25–4.13 (m, 4H), 3.26 (s, 3H), 2.82 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 456.4 [*M*−H]$^-$, 458.4 [*M*+H]$^+$; HPLC (method A): t_R=4.45 min (purity: 98.6%); Anal. calcd for $C_{27}H_{28}O_4N_3Cl$: C 65.65, H 5.71, N 8.51, Cl 7.18, found: C 65.36, H 5.65, N 8.49, Cl 7.08.

***N*-(3-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methyl-β-alanine, hydrochloride salt (27)**: Compound **27** was prepared following the procedure described for compound **26**, replacing amidoxime **52i** with **52j**. It was isolated as an off-white powder (60 mg, 26%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=12.74 (br s, 1H), 10.38 (br s, 1H), 8.36–8.33 (m, 1H), 8.23–8.20 (m, 1H), 8.18–8.15 (m, 1H), 7.87–7.84 (m, 1H), 7.72 (t, J=7.8 Hz, 1H), 7.44 (d, J=7.9 Hz, 1H), 7.37–7.28 (m, 3H), 7.15–7.13 (m, 1H), 4.48 (br s, 2H), 4.23 (d, J=12.7 Hz, 1H), 4.16 (d, J=12.7 Hz, 1H), 3.25 (s, 3H), 2.89–2.84 (m, 2H), 2.70 (s, 3H), 2.03 ppm (s, 3H); UHPLC/MS: *m/z*: 472.4 [*M*+H]$^+$; HPLC (method A): t_R=4.53 min (purity: 97.4%).

***N*-(2-{5-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (28)**: *Step 1*: HATU (217 mg, 0.57 mmol) was added to a solution of carboxylic acid **56a** (154 mg, 0.60 mmol) and DIEA (0.215 mL, 1.26 mmol) in anhydrous DMF (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 15 min. Amidoxime **52k** (176 mg, 0.60 mmol) was added in one portion, and the mixture was stirred 2 h at 0 °C. The reaction was diluted with EtOAc (10 mL) and washed with water (2×5 mL) and brine (3×5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was taken up in CH_3CN (5 mL) and heated at 150 °C for 30 min under microwave irradiation (full power, 400 W). The reaction mixture was concentrated in vacuo. After purification by flash chromatography (cyclohexane/EtOAc, 90:10→40:60), *tert*-butyl *N*-(2-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycinate was obtained as a brown oil (127 mg, 41%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.31 (d, J=1.5 Hz, 1H), 8.16 (dd, J=7.9, 1.9 Hz, 1H), 7.91 (dd, J=7.6, 1.3 Hz, 1H), 7.67–7.56 (m, 2H), 7.49 (dt, J=7.4, 1.6 Hz, 1H), 7.42 (d, J=7.9 Hz, 1H), 7.38–7.26 (m, 3H), 7.15 (d, J=7.0 Hz, 1H), 4.25–4.13 (m, 2H), 4.06 (s, 2H), 3.24 (s, 3H), 3.17 (s, 2H), 2.22 (s, 3H), 2.04 (s, 3H), 1.41 ppm (s, 9H); UHPLC/MS: *m/z*: 514.1 [*M*+H]$^+$; HPLC (method A): t_R=4.83 min (purity: 90.3%).

Step 2: A solution of *tert*-butyl *N*-(2–5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-ylbenzyl)-*N*-methylglycinate (103 mg, 0.20 mmol) in 4 N HCl in dioxane (2.5 mL, 10 mmol) was stirred at RT for 7 h. The reaction mixture was concentrated in vacuo. The residue was taken up in CH_3CN (2 mL), and a white solid precipitated out. The precipitate was isolated by filtration and dried in vacuo to give compound **28** as a white powder (69 mg, 70%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.36 (d, J=1.4 Hz, 1H), 8.22 (dd, J=7.9, 1.7 Hz, 1H), 8.18–8.13 (m, 1H), 7.91–8.87 (m, 1H), 7.78–7.71 (m, 2H), 7.45 (d, J=7.8 Hz, 1H), 7.39–7.27 (m, 3H), 7.15 (d, J=7.1 Hz, 1H), 4.87 (s, 2H), 4.26–4.14 (m, 4H), 3.25 (s, 3H), 2.89 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 456.1 [*M*−H]$^-$, 458.0 [*M*+H]$^+$; HPLC (method A): t_R=3.90 min (purity: 99.4%).

({3-[3-(2-Methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-5-yl]-benzyl}-methylamino)acetic acid, hydrochloride salt (29): Compound **29** was prepared following the procedure described for compound **28**, replacing amidoxime **52k** with **75** and carboxylic acid **56a** with **73**. It was isolated as a white powder (163 mg, 88%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.47 (t, J=1.7 Hz, 1H), 8.32 (dt, J=7.9, 1.3 Hz, 1H), 8.28–8.22 (m, 1H), 8.08 (dd, J=7.9, 1.8 Hz, 1H), 7.92 (dt, J=7.8, 1.4 Hz, 1H), 7.78 (t, J=7.8 Hz, 1H), 7.45–7.23 (m, 4H), 7.21–7.06 (m, 1H), 4.54 (br s, 2H), 4.32–4.02 (m, 4H), 3.24 (s, 3H), 2.83 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 458.4 [*M*+H]$^+$, 456.5 [*M*−H]$^-$; HPLC (method A): t_R=4.01 min (purity: 99.4%).

***tert*-Butyl *N*-{3-[(2-{[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-carbonyl}hydrazino)carbonyl]benzyl}-*N*-methylglycinate (78)**: To a solution of **73** (2.9 g, 10.0 mmol) in DMF (30 mL) was added DIEA (5.2 mL, 30.0 mmol) and HATU (5.7 g, 15.0 mmol). After 10 min at RT, intermediate **77** (2.7 g, 10.0 mmol) was added and the mixture was stirred at RT for 3 h. Water was added (30 mL), and the mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine (3×20 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The orange oily residue was purified by flash chromatography (heptane/EtOAc, 60:40) affording **78** as a yellow oil (1.8 g, 35%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.57 (d, J=13.1 Hz, 2H), 8.19–8.02 (m, 1H), 7.98–7.77 (m, 3H), 7.60–7.42 (m, 2H), 7.40–7.19 (m, 4H), 7.12 (dd, J=8.1, 1.3 Hz, 1H), 4.14 (s, 2H), 3.72 (s, 2H), 3.28–3.13 (m, 5H), 2.28 (s, 3H), 2.02 (s, 3H), 1.44 ppm (s, 9H); UHPLC/MS: *m/z*: 530.6 [*M*−H]$^-$, 532.6 [*M*+H]$^+$; HPLC (method A): t_R=3.62 min (purity: 89.8%).

({3-[5-(2-Methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,3,4]oxadiazol-2-yl]-benzyl}-methylamino)acetic acid, hydrochloride salt (30): *Step 1*: Compound **78** (200 mg, 0.38 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5.00 mL) and 1-methylimidazole (90 μL, 1.1 mmol) was added. The reaction mixture was cooled to 0 °C, and then trifluoromethanesulfonic anhydride (93 μL, 0.56 mmol) was added. The solution was stirred at RT for 2 h. Water (5 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2×5 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (heptane/EtOAc, 90:10→40:60) affording ({3-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,3,4]oxadiazol-2-yl]-benzyl}methylamino)acetic acid *tert*-butyl ester as a yellow oil (134 mg, 69%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.37–8.30 (m, 1H), 8.20–8.03 (m, 3H), 7.67–7.58 (m, 1H), 7.58–7.47 (m, 1H), 7.38–7.21 (m, 4H), 7.14 (d, J=7.1 Hz, 1H), 4.23 (s, 2H), 3.87 (s, 2H), 3.38–3.24 (m, 5H), 2.48 (s, 3H), 2.08 (s, 3H), 1.50 ppm (s, 9H); UHPLC/MS: *m/z*: 514.6 [*M*+H]$^+$; HPLC (method A): t_R=4.32 min (purity: 97.6%).

Step 2: Hydrolysis of ({3-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,3,4]oxadiazol-2-yl]-benzyl}methylamino)acetic acid *tert*-butyl ester was performed following the procedure described for compound **28** (step 2), affording compound **30** as a white powder (88 mg, 68%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.36–8.24 (m, 2H), 8.24–8.17 (m, 1H), 8.12 (dd, J=7.9, 1.9 Hz, 1H), 7.82–7.63 (m, 2H), 7.46–7.23 (m, 4H), 7.15 (d, J=7.2 Hz, 1H), 4.30 (s, 2H), 4.26–4.09 (m, 2H), 3.88 (s, 2H), 3.24 (s, 3H), 2.68 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 458.5 [*M*+H]$^+$, 456.6 [*M*−H]$^-$; HPLC (method A): t_R=3.63 min (purity: 99.5%).

{3-[5-(2-Methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,3,4]thiadiazol-2-yl]-benzyl}methylamino)acetic acid, hydrochloride salt (31): *Step 1*: Compound **78** (200 mg, 0.38 mmol) was dissolved in dry THF (8.00 mL) and Lawesson's reagent (160 mg, 0.39 mmol) was added in one portion. The resulting suspension was then heated at 120 °C for 30 min under microwave irradiation (full power, 400 W). The reaction mixture was passed through a column of neutral alu-

mina (15 g), eluting with EtOAc. The solvents were evaporated in vacuo. The crude product was purified by flash chromatography (heptane/EtOAc, 90:10→40:60) affording ({3-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,3,4]thiadiazol-2-yl]-benzyl}methylamino)-acetic acid *tert*-butyl ester as a yellow oil (100 mg, 52%): ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.16 (dd, J=1.9, 0.7 Hz, 1H), 8.09–7.91 (m, 3H), 7.61–7.51 (m, 1H), 7.51–7.41 (m, 1H), 7.36–7.21 (m, 4H), 7.19–7.09 (m, 1H), 4.23–4.19 (m, 2H), 3.78 (s, 2H), 3.32 (s, 3H), 3.23 (s, 2H), 2.41 (s, 3H), 2.10 (s, 3H), 1.54–1.44 ppm (m, 9H); UHPLC/MS: *m/z*: 530.6 $[M+H]^+$; HPLC (method A): t_R=4.48 min (purity: 100.0%).

Step 2: Hydrolysis of ({3-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,3,4]thiadiazol-2-yl]-benzyl}methylamino)acetic acid *tert*-butyl ester was performed following the procedure described for compound **28** (step 2), affording compound **31** as a white powder (76 mg, 79%): ^{1}H NMR (300 MHz, $[D_6]$DMSO): δ=8.32 (t, J=1.7 Hz, 1H), 8.23–8.09 (m, 2H), 8.01 (dd, J=7.9, 2.0 Hz, 1H), 7.81–7.64 (m, 2H), 7.41–7.23 (m, 4H), 7.18–7.08 (m, 1H), 4.49 (s, 2H), 4.31–4.04 (m, 4H), 3.24 (s, 3H), 2.82 (s, 3H), 2.05 ppm (s, 3H); UHPLC/MS: *m/z*: 474.5 $[M+H]^+$, 472.5 $[M-H]^-$; HPLC (method A): t_R=3.80 min (purity: 99.8%).

***N*-(3-{3-[2-(Methoxymethyl)-2′-methylbiphenyl-4-yl]-1H-1,2,4-triazol-5-yl}benzyl)-*N*-methylglycine, hydrochloride salt (32)**: *Step 1*: Using the procedure described for the preparation of **52 f** (step 1), starting from 3-(bromomethyl)benzonitrile (5.9 g, 30.3 mmol) and sarcosine *tert*-butyl ester hydrochloride (5.0 g, 27.5 mmol), *tert*-butyl *N*-(3-cyanobenzyl)-*N*-methylglycinate was isolated as a brown oil (7 g, 98%): HPLC (method A): t_R=2.60 min (purity: 90.5%).

Step 2: In a microwave vial, K_2CO_3 (767 mg, 5.6 mmol) was added to a solution of intermediate **77** (300 mg, 1.1 mmol) and *tert*-butyl *N*-(3-*c*yanobenzyl)-*N*-methylglycinate (578 mg, 2.2 mmol) in *n*BuOH (6 mL). The resulting mixture was heated at 200 °C for 2 h under microwave irradiation (full power, 400 W). Water (5 mL) was added, and the solution was directly purified by preparative HPLC (see general comments for details). Pure fractions were isolated, mixed and concentrated. The residue was dissolved in CH_3CN (5 mL) and 2.5 M HCl in Et_2O (5 mL) was added. The mixture was stirred overnight at RT. The solvents were removed, and the solid was suspended in Et_2O (2 mL) and filtered affording compound **32** as an orange powder (80 mg, 15%): ^{1}H NMR (300 MHz, $[D_6]$DMSO): δ=8.22 (s, 1H), 8.17–8.13 (m, 2H), 8.00 (dd, J=1.7, 8.0 Hz, 1H), 7.66–7.58 (m, 2H), 7.31–7.21 (m, 4H), 7.07 (d, J=6.8 Hz, 1H), 4.41 (br s, 2H), 4.13 (s, 2H), 4.07 (s, 2H), 3.16 (s, 3H), 2.81 (s, 3H), 1.98 ppm (s, 3H); UHPLC/MS: *m/z*: 457.2 $[M+H]^+$, 455.3 $[M-H]^-$; HPLC (method A): t_R=3.81 min (purity: 96.2%).

***N*-(2-Fluoro-4-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (33)**: Compound **33** was prepared following the procedure described for the preparation of compound **28**, replacing amidoxime **52 k** with **52 l**. It was isolated as a white powder (172 mg, 74%): ^{1}H NMR (300 MHz, $[D_6]$DMSO): δ=8.34 (d, J=1.4 Hz, 1H), 8.18 (dd, J=7.9, 1.7 Hz, 1H), 8.08 (dd, J=7.9, 1.3 Hz, 1H), 7.99 (dd, J=10.2, 1.3 Hz, 1H), 7.91 (t, J=7.7 Hz, 1H), 7.44 (d, J=7.9 Hz, 1H), 7.39–7.27 (m, 3H), 7.15 (d, J=7.0 Hz, 1H), 4.50 (s, 2H), 4.26–4.11 (m, 4H), 3.26 (s, 3H), 2.81 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 474.2 $[M-H]^-$, 476.0 $[M+H]^+$; HPLC (method A): t_R=4.06 min (purity: 99.6%).

2-((3-Fluoro-4-(5-(2-(methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)benzyl)(methyl)amino)acetic acid, hydrochloride salt (34): *Step 1*: Using the procedure for compound **28**, replacing amidoxime **52 k** with **52 m**, (3-fluoro-4-(5-(2-(methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol was isolated as a white solid (80 mg, 65%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.43 (d, J=1.8 Hz, 1H), 8.24–8.14 (m, 2H), 7.36–7.21 (m, 6H), 7.13 (d, J=7.5 Hz, 1H), 4.81 (d, J=5.7 Hz, 2H), 4.23 (t, J=13.4 Hz, 2H), 3.32 (s, 3H), 2.08 (s, 3H), 1.90 ppm (t, J=6.0 Hz, 1H); LC/MS: *m/z*: 405 $[M+H]^+$; HPLC (method C): t_R=4.25 min (purity: 99.1%).

Step 2: (3-fluoro-4-(5-(2-(methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)methanol (80 mg, 0.2 mmol) was dissolved in dioxane (5 mL) and MnO_2 (200 mg, 2.32 mmol) was added. The mixture was heated at 70 °C overnight, and then the solvent was removed in vacuo. The residue was triturated with a mixture of petroleum ether and Et_2O to give 4-(5-(2-(methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)-3-fluorobenzaldehyde as an off-white solid (55.9 mg, 69%). This intermediate was dissolved in a mixture of MeOH (3 mL), CH_2Cl_2 (3 mL) and acetic acid (75 μL), and the solution was treated with $NaBH_3CN$ (9.5 mg, 0.15 mmol) and 2-(methylamino)acetic acid (21 mg, 0.28 mmol). The mixture was stirred at RT overnight, and then the solvent was then removed in vacuo. The residue was purified by preparative HPLC (see general comments for details), treated with 4 M HCl in dioxane (3 mL) and triturated with CH_3CN, affording compound **34** as a white solid (55.4 mg, 78%): ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ=8.31 (d, J=1.8 Hz, 1H), 8.24 (t, J=7.7 Hz, 1H), 8.18 (dd, J=7.9, 1.9 Hz, 1H), 7.66 (d, J=11.3 Hz, 1H), 7.60 (dd, J=8.1, 1.6 Hz, 1H), 7.43 (d, J=7.9 Hz, 1H), 7.37–7.26 (m, 3H), 7.13 (d, J=7.4 Hz, 1H), 4.42 (s, 2H), 4.24–4.12 (m, 2H), 4.08 (s, 2H), 3.23 (s, 3H), 2.81 ppm (s, 3H), 2.02 (s, 3H); LC/MS: *m/z*: 476 $[M+H]^+$; HPLC (method C): t_R=3.08 min (purity: 99.4%).

2-((4-(5-(2-(Methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)-2-(trifluoromethyl)benzyl)(methyl)amino)acetic acid (35): To a solution of carboxylic acid **56 a** (0.112 g, 0.31 mmol) and amidoxime **52 n** (0.067 mg, 0.26 mmol) in CH_3CN (2 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.069 g, 0.36 mmol). The reaction mixture was stirred at RT for 18 h. Pyridine (2 mL) was added to the mixture, and the reaction was heated at 150 °C for 30 min under microwave irradiation (full power, 400 W). The reaction was repeated at the same scale and combined for work up. The solvent was removed in vacuo, and the residue was redissolved in CH_2Cl_2 (15 mL). The mixture was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (heptane/EtOAc, 90:10→40:60). The crude material was treated with a 4 N HCl in dioxane (4 mL), and the resulting mixture was stirred at 70 °C for 18 h. The solvent was evaporated in vacuo, and the residue was purified by preparative HPLC (see general comments for details), affording compound **35** as an orange oil (85 mg, 65%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.98 (br s, 1H), 8.50 (s, 1H), 8.43–8.34 (m, 2H), 8.14 (d, J=8.0 Hz, 2H), 7.34–7.21 (m, 4H), 7.12 (d, J=7.4 Hz, 1H), 4.26–4.18 (m, 4H), 3.56 (s, 2H), 3.32 (s, 3H), 2.62 (s, 3H), 2.07 ppm (s, 3H); LC/MS: *m/z*: 526 $[M+H]^+$; HPLC (method C): t_R=3.74 min (purity: 99.4%).

2-((4-(5-(2-(Methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)-3-(trifluoromethyl)benzyl)(methyl)amino)acetic acid (36): Compound **36** was prepared following the procedure described for the preparation of compound **35**, replacing amidoxime **52 n** with **52 o**. It was isolated as a pale yellow oil (77 mg, 60%): ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ=8.31 (d, J=1.8 Hz, 1H), 8.14 (dd, J=7.9, 1.9 Hz, 1H), 8.00 (s, 1H), 7.95 (d, J=7.9 Hz, 1H), 7.85 (d, J=8.0 Hz, 1H), 7.39 (d, J=7.9 Hz, 1H), 7.35–7.25 (m, 3H), 7.12 (d, J=7.4 Hz, 1H), 4.27–4.11 (m, 2H), 3.90 (s, 2H), 3.34 (s, 2H), 3.23 (s,

3H), 2.35 (s, 3H), 2.03 ppm (s, 3H); LC/MS: *m/z*: 526 $[M+H]^+$; HPLC (method C): $t_R = 3.28$ min (purity: 99.8%).

2-(((5-(5-(2-(Methoxymethyl)-2′-methylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl)pyridin-2-yl)methyl)(methyl)amino)acetic acid (37): *Step 1*: Using the procedure described for the preparation of compound **35** (step 1), replacing amidoxime **52n** with **52p**, and following purification by flash chromatography (iso-hexane/EtOAc, 1:1), *tert*-butyl *N*-[(5-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}pyridin-2-yl)methyl]-*N*-methylglycinate was isolated as an off-white solid (550 mg, 42%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 9.35–9.34 (m, 1H), 8.46–8.43 (m, 2H), 8.19–8.17 (m, 1H), 7.71–7.68 (m, 1H), 7.36–7.28 (m, 4H), 7.14–7.12 (m, 1H), 4.27–4.20 (m, 2H), 3.95 (s, 2H), 3.33 (s, 3H), 3.21 (s, 2H), 2.46 (s, 3H), 2.05 (s, 3H), 1.50 ppm (s, 9H).

Step 2: *tert*-Butyl *N*-[(5-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}pyridin-2-yl)methyl]-*N*-methylglycinate (0.550 g, 1.07 mmol) was dissolved in 4 M HCl in dioxane (15 mL), and the reaction mixture was stirred at RT for 2 h and then at 80 °C for 1 h. The reaction mixture was allowed to cool and the suspension was filtered. The solid was washed with Et_2O (10 mL) and dried in vacuo. The crude material was triturated with CH_2Cl_2 to afford compound **37** as an off-white solid (0.420 g, 74%): ^{1}H NMR (400 MHz, [D$_6$]DMSO/D_2O): $\delta = 9.31$ (s, 1H), 8.57 (dd, $J = 8.2$, 2.3 Hz, 1H), 8.30 (d, $J = 2.3$ Hz, 1H), 8.19–8.16 (m, 1H), 7.77 (d, $J = 7.6$ Hz, 1H), 7.42–7.26 (m, 4H), 7.11–7.10 (m, 1H), 4.65 (s, 2H), 4.26–4.12 (m, 4H), 3.21 (s, 3H), 2.92 (s, 3H), 2.00 ppm (s, 3H); LC/MS: *m/z*: 459 $[M+H]^+$; HPLC (method C): $t_R = 8.60$ min (purity: 98.5%).

({2-Fluoro-3-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-benzyl}methylamino)acetic acid, hydrochloride salt (38): Compound **38** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56a** and amidoxime **52q**. It was isolated as a white solid (140 mg, 86%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.34$ (d, $J =$ 1.7 Hz, 1H), 8.32–8.24 (m, 1H), 8.18 (dd, $J = 7.9$, 1.9 Hz, 1H), 7.94–7.85 (m, 1H), 7.57 (t, $J = 7.8$ Hz, 1H), 7.45 (d, $J = 7.9$ Hz, 1H), 7.40–7.25 (m, 3H), 7.15 (d, $J = 7.2$ Hz, 1H), 4.52 (s, 2H), 4.31–4.03 (m, 4H), 3.26 (s, 3H), 2.81 (s, 3H), 2.04 ppm (s, 3H); LC/MS: *m/z*: 476.2 $[M+H]^+$, 474.3 $[M-H]^-$; HPLC (method A): $t_R = 3.96$ min (purity: 97.6%).

***N*-(2-Fluoro-5-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (39)**: Compound **39** was prepared following the procedure described for the preparation of compound **8**, using carboxylic acid **56a** and amidoxime **52r**. The solvents were evaporated to afford compound **39** as a white powder (512 mg, 67%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.46$ (dd, $J = 7.0$, 2.1 Hz, 1H), 8.33 (d, $J =$ 1.5 Hz, 1H), 8.30–8.26 (m, 1H), 8.17 (dd, $J = 7.9$, 1.9 Hz, 1H), 7.59 (t, $J = 9.2$ Hz, 1H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.38–7.27 (m, 3H), 7.15 (d, $J = 7.0$ Hz, 1H), 4.55–4.51 (m, 2H), 4.26–4.16 (m, 4H), 3.26 (s, 3H), 2.83 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 474.3 $[M-H]^-$, 476.2 $[M+H]^+$; HPLC (method A): $t_R = 4.54$ min (purity: 98.4%).

***N*-(3-Fluoro-5-{5-[2-(methoxymethyl)-2′-methylbiphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (40)**: Compound **40** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56a** and amidoxime **52s**. It was isolated as a white solid (153 mg, 76%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.34$ (d, $J = 1.8$ Hz, 1H), 8.21–8.12 (m, 2H), 8.03–7.93 (m, 1H), 7.78–7.68 (m, 1H), 7.44 (d, $J = 8.0$ Hz, 1H), 7.38–7.25 (m, 3H), 7.14 (d, $J = 7.3$ Hz, 1H), 4.48 (s, 2H), 4.31–4.08 (m, 2H), 4.10 (s, 2H), 3.25 (s, 3H), 2.81 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 474.3 $[M-H]^-$, 476.2 $[M+H]^+$; HPLC (method A): $t_R = 4.16$ min (purity: 99.5%); Anal. calcd for $C_{27}H_{26}N_3O_4F{\cdot}HCl$: C 63.34, H 5.32, N 8.21, Cl 6.92, found: C 62.95, H 5.24, N 8.23, Cl 6.67.

({4-Fluoro-3-[5-(2-methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-benzyl}methylamino)acetic acid (41): Compound **41** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56a** and amidoxime **52t**. It was isolated as a white solid (185 mg, 87%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.39$ (dd, $J = 6.8$, 2.3 Hz, 1H), 8.34 (d, $J = 1.7$ Hz, 1H), 8.18 (dd, $J = 7.9$, 1.9 Hz, 1H), 7.89–7.78 (m, 1H), 7.62 (dd, $J = 10.5$, 8.5 Hz, 1H), 7.46 (d, $J = 8.0$ Hz, 1H), 7.41–7.25 (m, 3H), 7.20–7.10 (m, 1H), 4.46 (s, 2H), 4.30–4.14 (m, 2H), 4.09 (s, 2H), 3.26 (s, 3H), 2.80 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 474.2 $[M-H]^-$, 476.0 $[M+H]^+$; HPLC (method A): $t_R = 3.98$ min (purity: 98.0%).

({3-[5-(2-Methoxymethyl-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-5-trifluoromethylbenzyl}methylamino)acetic acid (42): Compound **42** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56a** and amidoxime **52u**. It was isolated as a white solid (225 mg, 83%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.62$ (s, 1H), 8.48–8.32 (m, 2H), 8.21 (dt, $J = 4.1$, 2.0 Hz, 2H), 7.45 (d, $J = 7.9$ Hz, 1H), 7.41–7.23 (m, 3H), 7.15 (d, $J = 7.2$ Hz, 1H), 4.53 (s, 2H), 4.32–4.14 (m, 2H), 4.08 (s, 2H), 3.26 (s, 3H), 2.79 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 524.1 $[M-H]^-$, 526.0 $[M+H]^+$; HPLC (method A): $t_R = 5.03$ min (purity: 99.5%).

({4-[5-(2-Ethoxy-2′-methyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-benzyl}methylamino)acetic acid (43): Compound **43** was prepared following the procedure described for the preparation of compound **28**, using carboxylic acid **56c** and amidoxime **52f**. It was isolated as a white solid (157 mg, 83%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.21$ (d, $J = 8.3$ Hz, 2H), 7.86 (dd, $J = 7.9$, 1.4 Hz, 1H), 7.82–7.76 (m, 3H), 7.41 (d, $J = 7.8$ Hz, 1H), 7.33–7.23 (m, 3H), 7.17 (d, $J = 6.8$ Hz, 1H), 4.44 (s, 2H), 4.19 (q, $J = 7.0$ Hz, 2H), 4.08 (s, 2H), 2.80 (s, 3H), 2.12 (s, 3H), 1.25 ppm (t, $J = 6.9$ Hz, 3H); UHPLC/MS: *m/z*: 456.1 $[M-H]^-$, 458.0 $[M+H]^+$; HPLC (method A): $t_R = 4.17$ min (purity: 99.4%).

***N*-(4-{5-[2-(Methoxymethyl)-2′-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (44)**: Compound **44** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56b** and amidoxime **52f**. It was isolated as an off-white solid (353 mg, 73%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.32$ (d, $J =$ 1.7 Hz, 1H), 8.26–8.12 (m, 3H), 7.96–7.87 (m, 1H), 7.86–7.64 (m, 4H), 7.45 (dd, $J = 12.4$, 7.9 Hz, 2H), 4.49 (s, 2H), 4.28–4.02 (m, 4H), 3.24 (s, 3H), 2.82 ppm (s, 3H); UHPLC/MS: *m/z*: 512.0 $[M+H]^+$, 510.0 $[M-H]^-$; HPLC (method A): $t_R = 4.04$ min (purity: 98.8%).

***N*-Methyl-*N*-(4-{5-[2-methyl-2′-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)glycine, hydrochloride salt (45)**: Compound **45** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56h** and amidoxime **52f**. It was isolated as a white solid (433 mg, 75%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 8.25$–8.16 (m, 3H), 8.12–8.05 (m, 1H), 7.91 (d, $J = 7.2$ Hz, 1H), 7.84–7.76 (m, 3H), 7.74–7.65 (m, 1H), 7.47–7.37 (m, 2H), 4.47 (s, 2H), 4.11 (s, 2H), 2.81 (s, 3H), 2.12 ppm (s, 3H); UHPLC/MS: *m/z*: 480.1 $[M-H]^-$, 482.0 $[M+H]^+$; HPLC (method A): $t_R = 4.18$ min (purity: 99.8%); Anal. calcd for $C_{26}H_{22}N_3O_3F_3{\cdot}HCl$: C 60.29, H 4.48, N 8.11, Cl 6.85, found: C 60.14, H 4.36, N 8.12, Cl 6.81.

***N*-{4-[5-(2,2′-Dimethylbiphenyl-4-yl)-1,2,4-oxadiazol-3-yl]benzyl}-*N*-methylglycine, hydrochloride salt (46)**: Compound **46** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56 d** and amidoxime **52 f**. It was isolated as a white solid (187 mg, 44%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.20–8.11 (m, 3H), 8.07–8.02 (m, 1H), 7.80 (d, J = 7.8 Hz, 2H), 7.41–7.26 (m, 4H), 7.13–7.10 (m, 1H), 4.48 (br s, 2H), 4.11 (br s, 2H), 2.81 (s, 3H), 2.14 (s, 3H), 2.04 ppm (s, 3H); UHPLC/MS: *m/z*: 428.3 $[M+H]^+$, 426.4 $[M-H]^-$; HPLC (method A): t_R = 4.14 min (purity: 100%).

***N*-Methyl-*N*-(4-{5-[2′-methyl-2-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)glycine, hydrochloride salt (47)**: Compound **47** was prepared following the procedure described for the preparation of compound **28**, using carboxylic acid **56 f** and amidoxime **52 f**. It was isolated as a white solid (172 mg, 85%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.55 (s, 1H), 8.51 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 8.1 Hz, 2H), 7.80 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 7.9 Hz, 1H), 7.42–7.26 (m, 3H), 7.17 (d, J = 7.3 Hz, 1H), 4.46 (s, 2H), 4.10 (s, 2H), 2.80 (s, 3H), 2.03 ppm (s, 3H); UHPLC/MS: *m/z*: 480.1 $[M-H]^-$, 482.0 $[M+H]^+$; HPLC (method A): t_R = 4.76 min (purity: 100.0%).

***N*-(2-Fluoro-4-{5-[2′-methyl-2-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)-*N*-methylglycine, hydrochloride salt (48)**: Compound **48** was prepared following the procedure described for the preparation of compound **28**, using carboxylic acid **56 f** and amidoxime **52 l**. It was isolated as a white solid (192 mg, 86%): m.p.: 183 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.56 (d, J = 1.3 Hz, 1H), 8.51 (dd, J = 7.9, 1.5 Hz, 1H), 8.09 (dd, J = 8.0, 1.5 Hz, 1H), 8.02 (dd, J = 10.2, 1.3 Hz, 1H), 7.91 (t, J = 7.7 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.42–7.26 (m, 3H), 7.17 (d, J = 7.5 Hz, 1H), 4.50 (s, 2H), 4.13 (s, 2H), 2.81 (s, 3H), 2.03 ppm (s, 3H); UHPLC/MS: *m/z*: 498.1 $[M-H]^-$, 500.0 $[M+H]^+$; HPLC (method A): t_R = 4.35 min (purity: 99.9%); Anal. calcd for $C_{26}H_{21}N_3O_3F_4$·HCl·H_2O: C 56.38, H 4.37, N 7.59, Cl 6.40, found: C 56.18, H 4.29, N 7.51, Cl 6.12.

***N*-Methyl-*N*-(3-{5-[2′-methyl-2-(trifluoromethyl)biphenyl-4-yl]-1,2,4-oxadiazol-3-yl}benzyl)glycine (49)**: Compound **49** was prepared following the procedure described for the preparation of compound **8**, using carboxylic acid **56 f** and amidoxime **52 i**. It was isolated as a white solid (321 mg, 90%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.55 (s, 1H), 8.50 (d, J = 8.0 Hz, 1H), 8.36 (s, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.78–7.63 (m, 2H), 7.42–7.12 (m, 3H), 7.17 (d, J = 7.4 Hz, 1H), 4.51 (s, 2H), 4.12 (s, 2H), 2.81 (s, 3H), 2.02 ppm (s, 3H); HPLC (method A): t_R = 4.20 min (purity: 99.48%); Anal. calcd for $C_{26}H_{22}O_3F_3N_3$·HCl: C 60.29, H 4.48, N 8.11, found: C 60.08, H 4.42, N 7.91.

{3-[5-(2′-Methyl-2-trifluoromethyl-biphenyl-4-yl)-[1,2,4]oxadiazol-3-yl]-benzyloxy}-acetic acid (50): Compound **50** was prepared following the procedure described for the preparation of compound **3**, using carboxylic acid **56 f** and amidoxime **52 d**. It was isolated as a white solid (140 mg, 65%): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.78 (br s, 1H), 8.55 (d, J = 1.4 Hz, 1H), 8.51 (dd, J = 7.8, 1.6 Hz, 1H), 8.14 (s, 1H), 8.11–8.05 (m, 1H), 7.66 (d, J = 7.9 Hz, 1H), 7.63 (s, 1H), 7.61 (s, 1H), 7.42–7.26 (m, 3H), 7.18 (d, J = 7.5 Hz, 1H), 4.69 (s, 2H), 4.15 (s, 2H), 2.03 ppm (s, 3H); UHPLC/MS: *m/z*: 467.1 $[M-H]^-$, 468.9 $[M+NH_4]^+$; HPLC (method A): t_R = 5.45 min (purity: 100%).

In vitro assays

Binding assays: Clonal Chinese hamster ovary (CHO) K1 cells expressing high levels of S1P$_{1,3,4,5}$ receptors were isolated, and crude membranes from these cells were prepared for use in ligand binding studies by nitrogen cavitation. [^{33}P]Sphingosine-1-phosphate (3000 Ci mmol^{-1}; American Radiolabeled Chemicals, Inc.) was added to test compound in 2% final DMSO by competition. Crude membrane fractions and wheat germ agglutinin (WGA) scintillation proximity assay (SPA) beads (GE Healthcare) were added to give a final assay concentrations of 30 pM, 15 pM, 25 pM [^{33}P]sphingosine-1-phosphate (for S1P$_{1,3,5}$, respectively), 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 5 mM $MgCl_2$, 100 mM NaCl, 0.4% fatty acid-free bovine serum albumin (BSA), 1–5 μg/well of proteins and 100 μg/well of WGA SPA beads in 96-well format. Filter binding was performed in an assay mixture containing 50 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 100 mM NaCl, 0.4% fatty acid-free BSA, 6 μg/well of S1P$_4$ membranes and 100 pM [^{33}P]sphingosine-1-phosphate in 96-well plates. Binding was performed for 60 min and 90 min for S1P$_{1,3,5}$ and S1P$_4$, respectively, at RT on a shaker. Bound radioactivity was measured on a PerkinElmer 1450 MicroBeta counter for S1P$_{1,3,5}$, and the mixture was filtered by vacuum through a Whatman GF/C filter for S1P$_4$. The filter was washed, and radioactivity was then determined in scintillation mix cocktail on a PerkinElmer 1450 MicroBeta counter. Triplicate samples were averaged and normalized as percentage of inhibition relative to total binding (DMSO control wells) and non-specific binding (1000-fold excess of unlabeled S1P for S1P$_{1,3,5}$ and phytosphingosine phosphate for S1P$_4$). Ten-point dose–response curves allowed IC$_{50}$ determination for each compound. K_i values were calculated according to the Cheng–Prusoff equation. Each reported K_i value is the average of a minimum of two independent experiments, unless indicated otherwise.

Microsomal stability: The stability of the test compounds was studied in vitro in mouse and human liver microsomes. Microsomes (final concentration 0.5 mg mL^{-1}), 50 mM phosphate buffer (pH 7.4), NADPH (final concentration 1.5 mM) and compound (final concentration 1 μM) were added to the assay plate. The microsome suspension was added to initiate the reaction, and the plate was incubated at 37 °C. The reaction was stopped by addition of cold acetonitrile at the appropriate time points (t = 0, 5, 15 and 45 min). The samples were centrifuged at 4000 rpm for 30 min at 4 °C and analyzed by LC-MS/MS. The in vitro intrinsic clearance (Cl$_{int}$) was calculated from the rate of compound disappearance.

Cytochrome P450 (CYP) inhibition: Seven human recombinant CYP isoenzymes were tested (1A2, 2C9, 2C19, 2C8, 2B6, 2D6 and 3A4). The aim of this method is to determine the concentration of a compound required to obtain 50% inhibition of the recombinant human CYP. The assay is based on the Promega P450-Glo screening system, which includes a luminogenic substrate, an NADPH-regenerating system and a luciferin detection reagent. CYP activity is measured by convertion of the substrate (e.g., luciferin-ME EGE for CYP 2B6) to luciferin EGE, which is further derivatised to D-luciferin (readout via light emission using luciferin detection reagent). The CYP membranes were prepared from baculovirus-infected insect cells and contain human CYP and CYP reductase. In order not to be depleted in NADPH cofactor during the course of the reaction, the assay includes a NADPH regeneration system. Test compounds were pre-incubated for 15 min at RT with the CYP enzyme (and appropriate cofactors), in the absence of substrate. Then the enzymatic reaction was initiated by the addition of the substrate, followed by 30 min of incubation at 37 °C. As controls, blank and neutral values were tested on each plate. In the neutral control, DMSO was added in place of the test compound; it defines the maximum achievable substrate conversion, reflected by the strongest luminescence signal. In the blank control, a membrane fraction devoid of CYP activity (control membranes prepared from wild-type bacu-

lovirus-infected cells), substitutes the membranes from CYP-expressing cells, consequently, no substrate conversion is observed. The amount of control membranes in blank controls was calculated to match the amount of membrane proteins present in the CYP-positive membranes used in the rest of the assay plate. The measured signal in blank wells describes the background value. The % inhibition was determined for each inhibitor concentration, and the corresponding IC_{50} value was calculated by nonlinear curve fitting. The IC_{50} value was determined in triplicate, using ten different concentrations of test compound. As an additional control, the IC_{50} value of a well-characterized CYP inhibitor (reference control) was determined for each isoform.

Protein binding assays: Protein binding of test compounds was determined by ultrafiltration using serum from various species (mouse, rat, human, etc.). Test compound (final concentration 5 μM) was incubated in triplicate at three different serum dilutions (1:2, 1:5 and 1:10) for 30 min at 37 °C using slight agitation. After incubation, the 96-well filter plates were centrifuged for 45 min at 3500 rpm and 37 °C. Filtrate samples (25 μL) were treated with EtOH (50 μL) and an internal standard solution (50 μL), and analyzed by LC-MS/MS. The fraction unbound was calculated from the drug concentrations in the filtrate samples.

hERG patchclamp assay:[36] Samples of test compounds and positive controls were prepared fresh daily by diluting stock solutions into HEPES-buffered physiological saline (HB-PS) solution (NaCl, 137 mM; KCl, 4.0 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1 mM; HEPES, 10 mM; glucose, 10 mM), and the pH was adjusted to 7.4 with aq NaOH (5 N). HB-PS was prepared weekly and refrigerated until use. Since previous results have shown that $\leq$0.3% DMSO does not affect channel current, all test and control solutions contained 0.3% DMSO. Each test compound formulation was sonicated at RT for at least 20 min to facilitate dissolution. A glass-lined 96-well compound plate was loaded with the appropriate amounts of test and control solutions, and placed in the plate slot of a QPatch HT system (Sophion Bioscience A/S, Denmark). In this study, hERG channels were expressed in a CHO cell line that lacks endogenous rapidly activating delayed rectifier (I_{Kr}) channel. CHO cells were stably transfected with hERG cDNA. Stable transfectants were selected by coexpression with an antibiotic-resistance gene incorporated into the expression plasmid. Selection pressure was maintained by including the selection antibiotic in the culture medium. Cells were cultured in nutrient mixture F-12 (F-12) supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin G sodium, 100 μg mL^{-1} streptomycin sulfate. Before testing, cells in culture dishes were washed twice with Hank's balanced salt solution, treated with accutase and resuspended in the CHO serum-free media (1–1.5 $\times 10^6$ cells mL^{-1}). Intracellular solution for whole-cell recordings consisted of potassium aspartate (130 mM), $MgCl_2$ (5 mM), ethylene glycol tetraacetic acid (EGTA) (5 mM), ATP (4 mM), HEPES (10 mM), and the pH was adjusted to 7.2 with aq KOH (5 N). An intracellular solution was loaded into the intracellular compartments of the QPlate. Cell suspension was pipetted into the extracellular compartments of the QPlate. After establishment of a whole-cell configuration, membrane currents were recorded using the QPatch HT system. Before digitization, the current records were low-pass filtered at one-fifth of the sampling frequency.

In vivo biological methods

Animal welfare: All experimental protocols involving animals were performed in accordance with international standards for animal experimentation, reviewed by an internal ethics committee at Merck Serono, and approved the General Health Direction of the Republic and Canton of Geneva, Animal Experimentation Domain (Republique et Canton de Genève, Direction Générale de la Santé, Domaine de l'expérimentation animale), under the license numbers 1040/3338/1-2R and 1040/3602/3. Mice were acquired from Elevage Janvier (Le Genest-Saint-Isle, France) and were acclimatized for at least one week prior to experimentation. During the acclimatization period and the entire length of the studies, mice were maintained at 12/12 h light/dark cycle and given food and water ad libitum.

Unbound fraction in the brain: Individual naïve mouse brains were harvested from female mice purchased from Janvier. Brains were homogenized in phosphate-buffered saline (PBS) (brain/PBS, 1:2 *w/v*) using a Precellys 24 tissue homogenizer. Homogenates were stored in aliquots at −20 °C until use. Membranes (3.5 kDa cut-off) were conditioned in deionized water for 60 min, followed by conditioning in water/EtOH (8:2) for 30 min, and rinsed in deionized water before use. On the donor side, diluted matrices were spiked with the test compound (~500 mg mL^{-1} for brain homogenate), and 150 μL aliquots ($n=6$ replicates for each test compound) were loaded into the upper-half-well of the equilibrium dialysis plate. On the acceptor side, PBS (150 μL) was loaded in the lower-half-well of the equilibrium dialysis plate. Dialysis versus PBS was carried out for 5 h at 37 °C in a temperature-controlled incubator using a microplate shaker. After 5 h, on the donor side, matrix aliquots (10 μL) were transferred to an Eppendorf tube containing 100 μL of 0.5 μg mL^{-1} of internal standard (IS) in CH_3CN. Fresh PBS was added to each eppendorf tube (50 μL) and vortex mixed. On the acceptor side, PBS aliquots (50 μL) were transferred to an Eppendorf tube containing 100 μL of 0.5 μg mL^{-1} of ISTD in CH_3CN. Naïve mouse brain was added to each Eppendorf tube (10 μL) and vortex mixed. Samples were then centrifuged for 15 min at 4000 rpm at 4 °C. Supernatant (100 μL) was recovered in vials and analyzed by LC-MS/MS. The unbound fraction was calculated as described by Kalvass and Maurer.[35]

Model of S1P receptor agonist-induced lymphopenia in mice: Female C57BL/6 mice (8 week old) received S1P receptor agonists by oral route using 0.5% carboxymethylcellulose (CMC)/0.25% Tween20 in water as a vehicle. Lymphopenia was measured at 24, 48 and 72 h after the administration of test compound. For that purpose, blood was sampled via intracardiac puncture under isoflurane anesthesia. Mice received an intraperitoneal (ip) injection of 100 IU kg^{-1} heparin (liquemin, Roche) 15 min prior to blood sampling. Total and differential blood cell counts were performed (including lymphocyte counts used for assessing lymphopenia) using a Beckman/Coulter counter (ACT5Diff AD32097). The quality of blood sampling was confirmed by assessing erythocytes and platelets counts. The PK profiles were determined from this experiment. After counting, the blood was centrifuged (6000 rpm, 10 min, 4 °C) and plasma (50 μL/mouse/time point) and brain sampling were performed at 2 h ($n=3$ mice) and 24, 48 h ($n=8$ mice each) post-dose at sacrifice. Lymph nodes were collected at 24, 48 and 72 h post-dose at sacrifice. Plasma, brain and lymph nodes were stored at −20 °C until analysis. For bioanalysis, samples were processed by protein precipitation and analyzed using LC-MS/MS.

MOG_{35-55}-induced experimental autoimmune encephalomyelitis (EAE) in mice: EAE was induced in 9–11 week-old female C57BL/6 mice by immunization against rat myelin oligodendrocyte glycoprotein peptide ($rMOG_{35-55}$). Each mouse received a dose of pertussis toxin (Alexis, cat# 630-003-C050, 300 ng/mouse in 200 μL PBS) administered ip and 100 μL of an emulsion containing $rMOG_{35-55}$ (200 μg/mouse; NeoMPS, cat# SC1272), *Mycobacterium tuberculosis*

(0.25 mg/mouse; DIFCO, cat# 231141) in complete Freund's adjuvant (DIFCO, cat# 263810) by subcutaneous injection into the back. For sham-immunization, the $rMOG_{35-55}$ solution used for the preparation of the emulsion was replaced by PBS. Two days later, the injection of Pertussis toxin was repeated. After EAE induction, mice were weighed daily, and the neurological impairment was quantified using a 15-point scale individually assessing the paralysis of the tail (0 = no motor impairment; 1 = flaccid extremity of the tail; 2 = completely flaccid tail), of the hind limbs (0 = no motor impairment; 1 = grid test positive, that is, mouse misplaces its steps when allowed to walk on the cage grid; 2 = flip test positive; 3 = mouse cannot hold on to the underside of the wire cage top for more than a few seconds using all paws; 4 = complete parapesis (paralysis of lower limbs), of the fore limbs (0 = no motor impairment, 1 = mild weakness of forelimbs; 2 = one forelimb is completely paralyzed; 3 = impossibility to move forward) and incontinence (0 = no incontinence; 1 = incontinence). The final score for each animal was determined by the addition of all the above-mentioned categories, therefore the maximum score for live animals was 10. Death due to disease (or moribund mice that need to be euthanized) was attributed a score of 15 for the day of the event. At day 14 post-immunization (14 d.p.i.), mice were homogeneously assigned into the different experimental groups according to the clinical score and the body weight loss. Only mice that developed clinical signs of disease were kept in the experiment. At this time point, that is, at 14 d.p.i., the treatment with the test compound or vehicle was initiated once daily five days per week. On the last day of the experiment, 24 hous after the last administration, mice were euthanized by CO_2 overdose, and the blood was harvested for leukocyte counts using a Beckman/Coulter counter (ACT5Diff AD32097) and plasma exposure determination.

Keywords: agonists · drug design · FTY-720 (fingolimod) · immunomodulators · sphingosine-1-phosphate receptors · structure–activity relationships

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[28] CCDC 1037201 and 1037202 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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