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Discovery of a Potent Thiazolidine Free Fatty Acid Receptor 2 Agonist with Favorable Pharmacokinetic Properties

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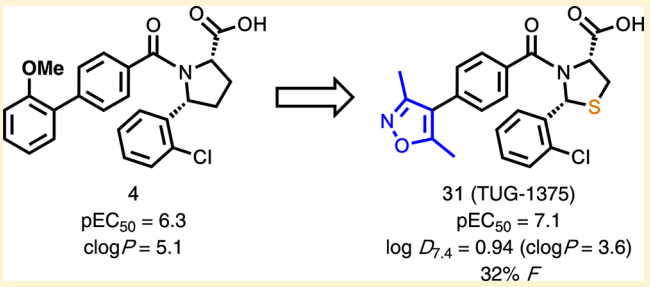
Supporting Information

ABSTRACT: Free fatty acid receptor 2 (FFA2/GPR43) is a receptor for short-chain fatty acids reported to be involved in regulation of metabolism, appetite, fat accumulation, and inflammatory responses and is a potential target for treatment of various inflammatory and metabolic diseases. By bioisosteric replacement of the central pyrrolidine core of a previously disclosed FFA2 agonist with a synthetically more tractable thiazolidine, we were able to rapidly synthesize and screen analogues modified at both the 2- and 3-positions on the thiazolidine core. Herein, we report SAR exploration of thiazolidine FFA2 agonists and the identification of **31** (TUG-1375), a compound with significantly increased potency (7-fold in a cAMP assay) and reduced lipophilicity (50-fold reduced clog*P*) relative to the pyrrolidine lead structure. The compound has high solubility, high chemical, microsomal, and hepatocyte stability, and favorable pharmacokinetic properties and was confirmed to induce human neutrophil mobilization and to inhibit lipolysis in murine adipocytes.



INTRODUCTION

The short-chain free fatty acid receptor FFA2 (formerly GPR43) is a G-protein-coupled receptor that is activated by physiological concentrations of short-chain fatty acids (SCFAs), primarily acetate and propionate.[1−5] FFA2 is expressed in a variety of tissues including immune cells,[3] adipocytes,[6] pancreatic β-cells,[2] and enteroendocrine L-cells.[7] Colonic fermentation of dietary fiber produces large amounts of SCFAs, and results indicate that an interplay between FFA2 and dietary fiber via colonic fermentation to produce SCFAs is implicated in promoting a healthy composition of microorganisms in the gut.[8] Fiber-rich diets and SCFAs can counteract colitis,[9,10] and exacerbated inflammation in colitis models has been reported for FFA2-knockout mice.[11] However, others have found that FFA2-knockout mice exhibited reduced inflammatory responses in colitis models,[12] and it is currently unclear if antagonists or agonists are preferable for the treatment of intestinal inflammation. So far, the antagonist GLPG0974 (**1**, Chart 1) is the only FFA2 modulator that has undergone clinical trials but unfortunately failed to meet end points against ulcerative colitis.[13]

Dietary fiber is also known to exert positive effects in diabetic subjects.[14] Acetate has been shown to potentiate glucose-stimulated insulin secretion from β-cells in an FFA2-dependent manner,[15] and SCFAs were able to promote secretion of the incretin glucagon-like peptide-1 (GLP-1) from colonic cultures through FFA2[16] and both GLP-1 and the appetite regulating peptide YY (PYY) in vivo,[17] indicating that activation of FFA2 could have beneficial effects on type 2 diabetes (T2D). Interestingly and in contrast, loss of FFA2 and FFA3 was found to improve insulin secretion and glucose tolerance.[18] Moreover, SCFA-mediated activation of FFA2 has been found to suppress adipose insulin signaling, leading to reduced fat accumulation in adipose tissue, and to promote GLP-1 secretion in the gut.[19] Thus, both agonists and antagonists of FFA2 are considered to be potential therapeutics

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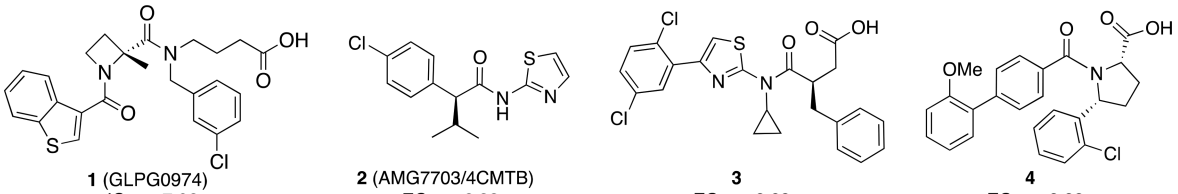
Chart 1. FFA2 Modulators[a]



[a]clogP values are calculated using ChemBioDraw v16.0.1.4. pIC_{50} for **1** and pEC_{50} for **2** and **3** are previously reported.[28,30,32]

for treatment of T2D and obesity.[5,19−25] Several FFA2 agonists have been disclosed, including an allosteric agonist series from Amgen represented by **2** (known as AMG7703 or 4-CMTB) and an orthosteric agonist series originating from Euroscreen represented by **3**.[20,26−30] A recent study found that agonist **4**, also originating from Euroscreen,[31] caused only marginal GLP-1 secretion but led to significant PYY mucosal responses, inhibited insulin-promoted fat accumulation and intestinal functions, and suppressed food intake, supporting FFA2 as a possible antiobesity target.[21]

The FFA2 agonists disclosed hitherto have relatively high lipophilicity, which is associated with several undesired properties,[33−35] and only moderate potency (e.g., see Chart 1). Furthermore, the allosteric agonist **2** has solubility issues and has been reported to give results that differ from orthosteric FFA2 agonists,[15] and the orthosteric agonist **3** was found to have stability issues.[36] We therefore wished to explore **4**, an FFA2 agonist reported to have properties useful for in vivo studies.[21] Initially, we sought to explore the SAR around the pyrrolidine scaffold of **4**; however, the synthetic route to obtain this class of FFA2 agonists is rather lengthy and precludes efficient exploration of parts of the molecule. We therefore opted for replacing the central pyrrolidine by a thiazolidine. This replacement strategy has previously been employed for HIV protease inhibitors.[37] Herein, we report SAR investigations of substituted thiazolidine FFA2 agonists and the discovery of an FFA2 agonist with improved potency, reduced lipophilicity, and favorable physicochemical and pharmacokinetic properties.

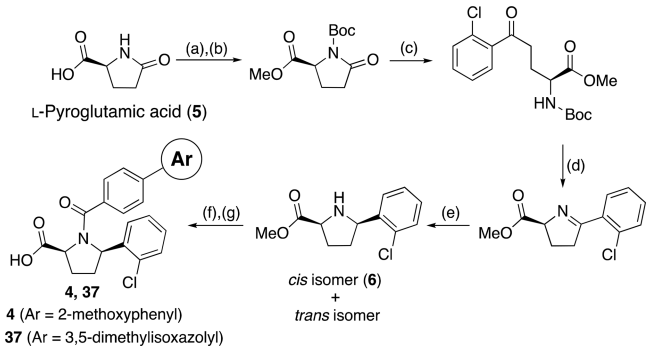
RESULTS AND DISCUSSION

Synthesis. Pyrrolidine ligands were synthesized in seven linear steps from L-pyroglutamic acid (**5**, Scheme 1) essentially as previously described by Euroscreen,[31] with the modifications that the ring opening by aryllithium was allowed to reach room temperature and that the amide coupling with pyrrolidine **6** was performed with bis(tetramethylene)-fluoroformamidinium hexafluorophosphate (BTFFH).[36]

Thiazolidine ligands were synthesized in three steps by condensation of (*R*)-Cys-OMe hydrochloride with benzaldehydes via **7a−j** (Scheme 2). In contrast to the pyrrolidine route, this strategy allowed rapid access also to variations on the 2-position of the thiazolidine scaffold in a single step from readily available starting materials[38] and formation of the desired (2*R*,4*R*)-thiazolidine amide as the major product in the next step.[39]

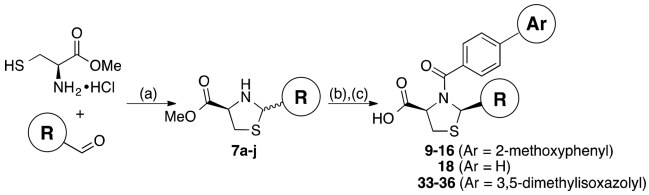
Condensation of (*R*)-Cys-OMe hydrochloride with aldehydes produced mixtures of C(2) epimeric methyl (4*R*)-2-arylthiazolidine-4-carboxylates (**7a−j**).[38] To obtain the (2*R*,4*R*)-configuration, **7a−j** were treated with Et_3N and subsequently reacted with the relevant acid chlorides to give

Scheme 1. Synthesis of Pyrrolidines 4 and 37 from L-Pyroglutamic Acid[a]



[a]Reagents and conditions: (a) $SOCl_2$, MeOH, rt, 70%; (b) Boc_2O, DMAP, DCM, rt, 4 h, 98%; (c) (i) 1-iodo-2-chlorobenzene, *n*-BuLi, THF, 20 min at −78 °C → rt; (ii) sat. aq NH_4Cl, 67%; (d) TFA, DCM, rt, 98%; (e) $NaBH_3CN$, AcOH (cat.), MeOH, rt, 71% (*cis*/*trans* 2:1, 49%/22%); (f) carboxylic acid, BTFFH, DIPEA, DCM, 80 °C, 8 h, 75%; (g) LiOH (aq), THF, rt, 42−93%.

Scheme 2. Synthesis of (2*R*,4*R*)-*N*-Acylthiazolidine-4-carboxylic Acids from (*R*)-Cys-OMe[a]



[a]Reagents and conditions: (a) H_2O/EtOH 1:1, $KHCO_3$, rt, 4−24 h, 63−81%; (b) benzoyl chlorides, Et_3N, −10 °C → rt, THF, 1−14 h, 21−83%; (c) LiI, EtOAc, 80 °C, 24−92%.

the desired (2*R*,4*R*)-products.[39] Finally, demethylation using LiI in EtOAc afforded the corresponding (2*R*,4*R*)-*N*-acylthiazolidine-4-carboxylic acids.[39]

A second set of analogues aimed at exploring the *N*-benzoyl part of the ligands was synthesized from central intermediates **8a,b** by Suzuki−Miyaura or Sonogashira cross-coupling followed by demethylation, affording the desired biarylic compounds in low to good yields (Scheme 3).

The crystal structure of **14** confirms unambiguously the (2*R*,4*R*)-configuration of thiazolidine substituents (Figure 1). A relatively short intramolecular hydrogen bond of 2.598(2) Å is found between the carboxylic acid and pyridine group. The crystal packing (Supporting Information, Figure S1) shows no significant intermolecular interactions stronger than van de Waals forces. The intramolecular hydrogen bond is a likely reason for why crystals suitable for structural information were

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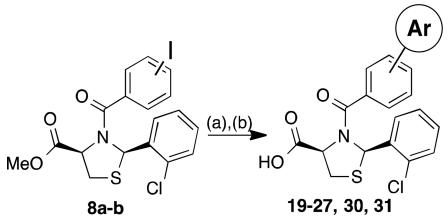
Scheme 3. Synthesis of Biaryl Analogues via Coupling Reactions[a]



[a]Reagents and conditions: (a) $ArB(OH)_2$ or ArBPin, $Pd(PPh_3)_4$, MeOH, toluene, 6–27 h, 20–86%; or ArCCH, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 4 h, rt, 76%; (b) LiI, EtOAc, 80 °C, 37–78%.

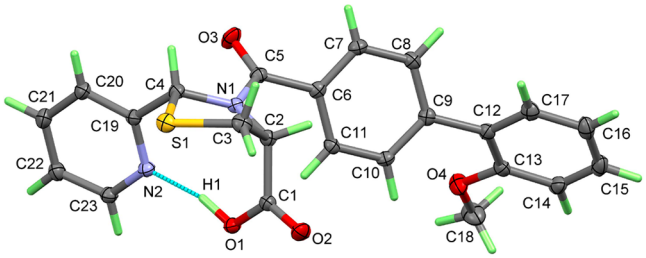


Figure 1. Perspective view of **14** drawn with 50% probability ellipsoids.

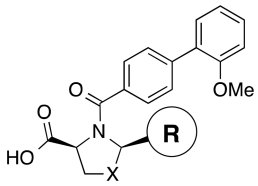
obtained for this compound but for none of the others. At physiological pH with the carboxylic acid ionized, the pyridyl hydrogen bond will be absent and the overall conformation is expected to be different. It is therefore not possible to draw conclusions regarding the configuration around the amide in the active conformation of **14**. In fact, rather than the (*Z*)-configuration apparent in the crystal structure of **14**, computer minimizations indicate a general preference for the (*E*)-configuration around the amide in the compound series.

Biological Testing and SAR Analysis. Compounds were tested on the human (h)FFA2 receptor using both a bioluminescence resonance energy transfer (BRET)-based *β*-arrestin-2 interaction assay and a cAMP inhibition assay. The former assay is an upstream signaling assay measuring BRET arising from agonist-mediated recruitment of *β*-arrestin-2 to FFA2, and the latter assay evaluates inhibition of forskolin-induced production of cAMP facilitated by coupling of FFA2 to $G\alpha_{i/o}$ proteins.[40]

The FFA2 agonist activity of **4** was confirmed in both assays. Compound **4** has previously been reported with an EC_{50} of 81 nM.[21] We obtained somewhat lower potencies of 1.2 *μ*M in the *β*-arrestin-2 assay and 0.53 *μ*M in the cAMP assay. We were pleased to find that the thiazolidine analog **9** had only moderately decreased potency in both the BRET and the cAMP assay (ΔpEC_{50} = −0.67 and −0.31, respectively) relative to pyrrolidine **4** (Table 1). The easy access to substituted thiazolidines enabled a rapid screen of variations in the 2-position of the thiazolidine scaffold for 2-methoxy-1,1′-biphenyl derivatives (Table 1).

Compared to 2-chlorophenyl (**9**), 3-chlorophenyl (**10**) or 4-chlorophenyl (**11**) led to a decrease in potencies in the *β*-arrestin-2 assay, whereas **11** retained potency in the cAMP assay. Thus, the 3-position appeared to be disfavored and 4-position substitution may be a route to G-protein-biased ligands. Substituting the 2-chloro by bromo (**12**) or hydrogen (**13**) retained potency in both assays.

Table 1. SAR Investigations at the Thiazolidine 2-Position



cmpd	X	R	hFFA2 β-arrestin pEC_{50} (E_{max})[a]	hFFA2 cAMP pEC_{50} (E_{max})[b]	clog*P*[c]	LLE[d]
4	CH_2		5.91 ± 0.21 (127)	6.28 ± 0.19 (96)	5.07	0.84/1.21
9	S		5.60 ± 0.10 (116)	5.61 ± 0.06 (96)	5.07	0.53/0.54
10	S		4.91 ± 0.29 (93)	5.15 ± 0.19 (106)	5.07	-0.16/0.08
11	S		4.73 ± 0.29 (136)	5.55 ± 0.19 (80)	5.07	-0.34/0.48
12	S		5.65 ± 0.18 (108)	5.45 ± 0.11 (90)	5.22	0.43/0.23
13	S		5.53 ± 0.16 (116)	6.03 ± 0.15 (88)	4.36	1.17/1.67
14	S		4.83 ± 0.09 (124)	5.87 ± 0.10 (75)	2.86	1.97/3.01
15	S		4.74 ± 0.38 (68)	5.66 ± 0.19 (70)	3.53	1.21/2.13
16	S		~4[e]	nr[f]	4.50	-
17	O		nr[f]	nr[f]	4.12	-

[a]BRET-based *β*-arrestin-2 recruitment assay. Efficacy (E_{max}) is relative to propionate. [b]cAMP FFA2 assay. Efficacy (E_{max}) is relative to propionate. [c]clogP values were calculated using ChemBioDraw v16.0.1.4. [d]BRET/cAMP LLE values (LLE = pEC_{50} − clogP). [e]50% activation at 100 *μ*M. [f]No response up to 30 *μ*M.

Installation of 2-pyridyl (**14**) or 2-furyl (**15**) onto the thiazolidine scaffold eroded potency in the *β*-arrestin-2 assay compared to the phenyl derivatives but mostly preserved potency in the cAMP assay, again suggesting a route to G-protein-biased agonists. Since the goal was both to increase potency and decrease lipophilicity, values for ligand-lipophilicity efficiency (LLE)[33] based on calculated lipophilicity of the neutral compounds (clogP) were also used to guide the optimization. The hydrophilic pyridyl led to a marked improvement in LLE for **14** compared to all previous compounds including **4** but insufficient potency. The introduction of a *tert*-butyl group in the 2-position of the thiazolidine scaffold resulted in a compound (**16**) that only caused 50% receptor activation at 100 *μ*M. Replacing sulfur with oxygen on **16** led to a completely inactive compound (**17**); hence, the oxazolidine scaffold was not explored further.

We next directed attention to the *N*-acyl substituent. As evident from **18** (Table 2), the biphenyl system is important for activity, as removing the terminal ring leads to complete loss of activity. Removing only the 2′-methoxy (**19**) also resulted in a pronounced loss of potency in both assays, as did moving the terminal phenyl to the 3-position (**20**). The 2′-fluoro-3′-methoxy (**21**) and the 4′-methoxy (**22**) analogues both led to decreased potency in both assays relative to the 2′-methoxy analogue **9**. Compared to **19**, installation of either acetyl (**23**) or nitrile (**24**) in the 4-position markedly boosted

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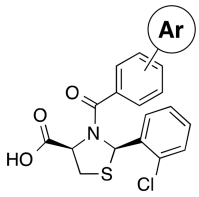
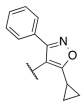
Table 2. SAR Investigation of the *N*-Acyl Substituent



cmpd	Ar (3/4-position)	hFFA2		clog*P*[c]	LLE[d]
		β-arrestin-2 pEC_{50} (E_{max})[a]	cAMP pEC_{50} (E_{max})[b]		
18	(4) 	nr[e]	nr[e]	3.82	-
19	(4) 	5.09 ± 0.05 (146)	5.00 ± 0.20 (93)	5.71	-0.62/-0.71
20	(3) 	4.59 ± 0.13 (141)	5.40 ± 0.04 (91)	5.71	-1.12/-0.31
21	(4) 	5.32 ± 0.23 (122)	5.04 ± 0.18 (82)	5.57	-0.25/-0.53
22	(4) 	4.66 ± 0.16 (174)	5.35 ± 0.15 (87)	5.63	-0.97/-0.31
23	(4) 	6.09 ± 0.04 (103)	5.68 ± 0.07 (90)	5.15	0.94/0.53
24	(4) 	5.89 ± 0.08 (95)	5.89 ± 0.13 (95)	5.15	0.74/0.74
25	(4) 	5.14 ± 0.24 (143)	6.04 ± 0.16 (74)	6.18	-1.04/-0.14
26	(4) 	5.36 ± 0.12 (146)	5.40 ± 0.19 (82)	5.91	-0.55/-0.51
27	(4) 	5.04 ± 0.17 (134[f])	5.32 ± 0.15 (89)	6.07	-1.03/-0.75
28	(4) 	5.22 ± 0.12 (130)	5.68 ± 0.09 (83)	6.46	-1.24/-0.78
29	(4) 	4.80 ± 0.35 (99)	5.65 ± 0.09 (89)	6.38	-1.58/-0.73
30	(4) 	6.01 ± 0.04 (103)	6.67 ± 0.12 (77)	4.04	1.97/2.63
31	(4) 	6.10 ± 0.07 (99)	7.11 ± 0.08 (86)	3.63	2.47/3.48
32	(4) 	4.68 ± 0.11 (108)	-	5.60	-0.92/-

[a]BRET-based β arrestin-2 recruitment assay. Efficacy (E_{max}) is relative to propionate. [b]cAMP FFA2 assay. Efficacy (E_{max}) is relative to propionate. [c]clogP values were calculated using ChemBioDraw v16.0.1.4. [d]LLE = pEC_{50}(β-arrestin/cAMP) − clogP. [e]No response up to 30 μM. [f]Average response at highest concentration relative to propionate.

potency in both β-arresin-2 and cAMP assays, indicating that certain hydrogen bond accepting groups are favored in this region.

Replacing the 2′-methoxy (**9**) with 2′-chloro (**25**) or 2′-methyl (**26**) resulted in a marked potency boost in the cAMP assay for **25**, whereas 2′-methoxyphenyl remained the preferred ring system in the β-arrestin-2 assay (**9** > **25** ∼ **26**).

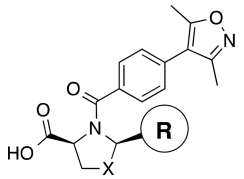
Extension of the biphenyl **19** by insertion of acetylene between the rings (**28**) caused a minor loss of potency in the cAMP assay but no changes in the β-arrestin-2 assay. In a parallel manner, alkyne extension of 2-methoxyphenyl (**9**) into **29** reduced potency in the β-arrestin-2 assay but preserved activity in the cAMP assay.

Overall, 4-biphenyl compounds with the terminal phenyl ring carrying lipophilic (**25**, **26**) or moderately hydrophilic (**9**) substituents in the *ortho*-position showed higher potencies than a nonsubstituted phenyl analogue such as **19** or **20**, indicating that a nonplanar biphenyl conformation is favored for optimal binding of this compound class.

With this and the encouraging results from **23** and **24** in mind, we wished to explore biaryl systems with *ortho*-substituted polar heterocycles as the terminal ring. Satisfyingly, installation of 1-methyl-1*H*-pyrazolyl produced a significantly improved compound (**30**), whereas introduction of 3,5-dimethylisoxazolyl (**31**) appeared as the most potent compound of the series with a pEC_{50} of 7.11 in the cAMP assay and an LLE increased by 2.3 relative to the initial pyrrolidine **4**. Replacement of the methyl groups of **31** by phenyl and cyclopropyl (**32**) resulted in a 40-fold lower potency in the β-arrestin-2 assay, indicating a limit to the acceptable steric bulk in this region.

Having identified 4-(3,5-dimethylisoxazolyl)benzoyl as a preferred N-substituent, we directed the attention back to the 2-substituent of the thiazolidine scaffold to screen for improved substituents in a second iteration (Table 3). This effort showed again that the 2-chloro (**31**) was clearly favored

Table 3. Variations in the 2-Position and Pyrrolidine Analogue with the Optimized Acyl Part



cmpd	X	R	hFFA2		clog*P*[c]	LLE[d]
			β-arrestin-2 pEC_{50} (E_{max})[a]	cAMP pEC_{50} (E_{max})[b]		
33	S		5.64 ± 0.04 (79)	5.84 ± 0.08 (94)	2.92	2.72/2.92
34	S		5.99 ± 0.21 (73)	5.46 ± 0.12 (49)	3.19	2.80/2.27
35	S		5.09 ± 0.11 (79)	5.78 ± 0.04 (83)	2.84	2.25/2.94
36	S		4.45 ± 0.04 (102)	nr[e]	1.42	3.03/-
37	CH_2		5.97 ± 0.08 (90)	6.82 ± 0.07 (85)	3.63	2.34/3.19

[a]BRET-based β arrestin-2 recruitment assay. Efficacy (E_{max}) is relative to propionate. [b]cAMP FFA2 assay. Efficacy (E_{max}) is relative to propionate. [c]clogP values were calculated using ChemBioDraw v16.0.1.4. [d]BRET/cAMP LLE values (LLE = pEC_{50} − clogP). [e]No response up to 30 μM.

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over a naked phenyl (**33**). The same was true for 2-ethynyl (**34**) and 2-methoxy (**35**). Furthermore, increasing the polarity by installation of 2-pyridyl (**36**) resulted in a weakly potent agonist in the β-arrestin-2 assay, whereas **36** was devoid of any activity when evaluated in the cAMP assay. Since pyridyl analog **14** was a reasonably potent compound in both β-arrestin-2 and cAMP assays, the observation that **36** lost activity is likely explained by this compound being too polar to effectively reach the lipophilic binding site of FFA2.

As the initial idea was to use the thiazolidine as a bioisostere for pyrrolidine in the optimization of the compound series, the corresponding pyrrolidine **37** of thiazolidine **31** was synthesized and tested (Table 3). Although pyrrolidine **37** displayed appreciable FFA2 activity, its potency was lower than that of **31**.

The occasionally diverging results in the cAMP and β-arrestin-2 assays illustrate that potencies in the functional assays do not directly reflect receptor affinity. To better understand the relationship between affinity and functional activity, we proceeded with further examination of **31** and selected analogs in an FFA2 competition binding assay using a radiolabeled version of antagonist **1** (Table 4). It has previously been confirmed that **1** and radiotracer [^{3}H]-**1** are orthosteric ligands and fully displaceable by propionate and the synthetic orthosteric agonist **3**.[32,41] Pyrrolidines **4** and **37** and thiazolidines **9**, **13**, **19**, and **31** were also all confirmed to fully displace [^{3}H]-**1**. Parent pyrrolidine **4** exhibited a moderate binding affinity, and the corresponding thiazolidine (**9**) was found to be a slightly stronger binder, in contrast to the slightly lower potency observed in both functional assays. Upon comparing **9** with the analogue lacking the 2-chloro substituent of the 2-aryl (**13**) or lacking the 2′-methoxy (**19**) substituent at the biphenyl system, it becomes clear that both these features contribute significantly to the affinity (ΔpK_i = 0.55 and 0.46, respectively). The most potent thiazolidine (**31**) was indeed found to also exhibit the highest binding affinity within this selection. The corresponding pyrrolidine (**37**) displayed a significantly weaker binding affinity when compared to **31** (ΔpK_i = −0.29), which correlated well with the observed differences in functional potency between these two compounds. Hence, for isoxazoles **31** and **37**, the thiazolidine central scaffold boosted both affinity and receptor activation compared to the pyrrolidine. Replacement of the 2-methoxyphenyl (**9**) by the more polar 3,5-dimethylisoxazole (**31**) not only gave rise to the more potent thiazolidine-based agonist (**31**) but also reduced lipophilicity resulting in a marked improvement in LLE over **4** (ΔLLE = 2.27), and **31** indeed exhibits the highest LLE of all compounds in the study.

Table 4. Binding Affinities of Compounds for FFA2

compd	pK_i[a]
4	6.32 ± 0.02
9	6.47 ± 0.06
13	5.92 ± 0.08
19	6.01 ± 0.02
31	6.69 ± 0.03
37	6.40 ± 0.06

[a]Binding affinities of agonists determined using [^{3}H]-**1** in radioligand binding assays.

Molecular Modeling. To better understand the interaction of **31** with FFA2, the compound was docked in a previously established homology model of the receptor.[32] The lowest energy pose of **31** is depicted in Figure 2. Compound **31** was found to effectively engage Arg180 and Arg255 via ionic and hydrogen bonding interactions, an observation that is in agreement with previous reports that both residues are known to be vital for receptor binding and activation.[30,41] Simultaneously, the carboxylate carbonyl also interacted with the conserved Tyr238 through hydrogen bonding. The 2-chlorophenyl moiety situated *cis* to the carboxylate was observed to interact with Phe89 via displaced $\pi-\pi$ interactions, where the chloro substituent appears to orient the phenyl for

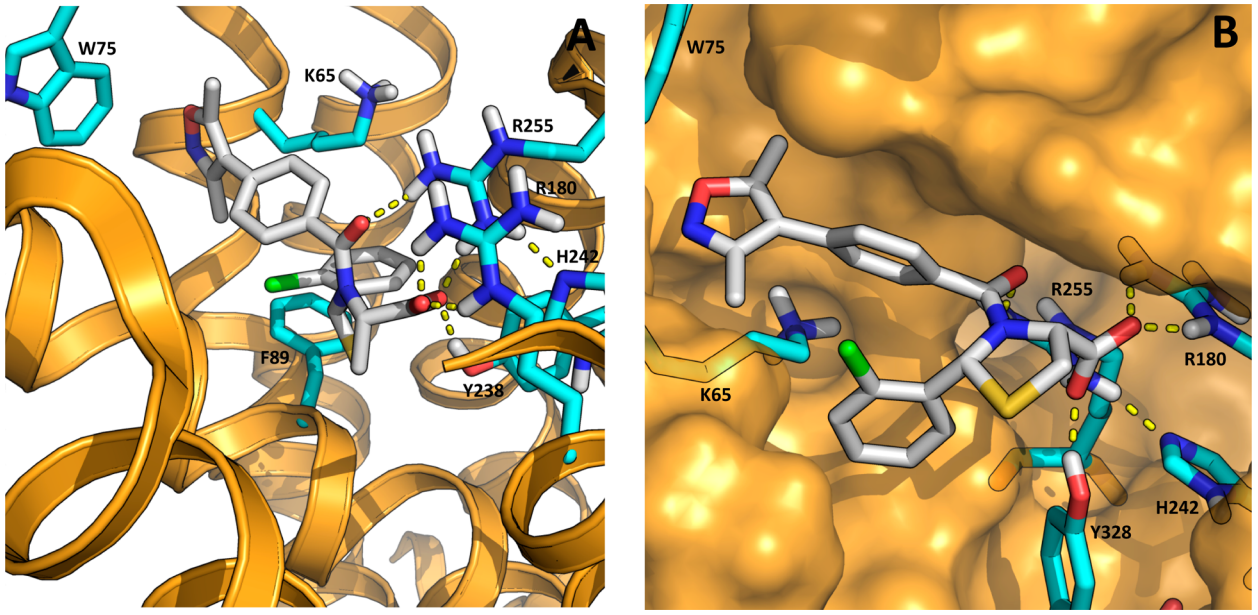

Figure 2. Lowest energy pose of **31** (gray) docked into hFFA2 (gold) with strongly interacting residues shown (cyan) and hydrogen bonds highlighted (yellow stipples). (A) Receptor as ribbon viewed from the angle of helix 4 and 5. (B) Same pose with receptor as surface viewed from the angle of helix 3 and 4.

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optimal contact. Furthermore, the dimethylisoxazole ring formed π−cation interactions with Lys65 and edge-to-face π−π interactions with Trp75 (Supporting Information, Figure S2). The shown pose also reflects higher energy poses. No alternative low-energy pose was identified.

Overall, groups of **31** found in the SAR analysis to be crucial for binding, such as 2-chlorophenyl and dimethylisoxazole, were confirmed by docking to contribute to the ligand receptor interaction, further supporting the proposed binding pose of **31** in hFFA2. Notably, both the carboxylate and the amide carbonyl of **31** were oriented toward the two arginine residues and Arg255 interacted directly with both groups through hydrogen bonds (Figure 2).

Characterization of 31. Since **31** showed the highest potency and LLE based on calculated lipophilicity in both functional assays and the binding assay, it was decided to characterize its properties in further detail. Thus, **31** was tested and confirmed to be active on the murine FFA2 orthologue (pEC_{50} = 6.44 ± 0.13 in the cAMP assay). The compound was found to be inactive up to 100 μM concentration at the closely related SCFA receptor FFA3 (formerly GPR41) and evoked no significant agonist or antagonist response in the long-chain fatty acid receptors FFA1 (GPR40) and FFA4 (GPR120) or in nuclear receptors PPARα, PPARγ, PPARδ, LXRα, and LXRβ at 10 μM (see the Supporting Information).

Previous FFA2 ligands are generally, apart from SCFAs, lipophilic compounds. In contrast, experimental lipophilicity of **31** (Table 5) was found to be in the lower part of the optimal range,[34] giving an LLE of 6.2 based on the cAMP assay and measured lipophilicity. The compound also showed high aqueous solubility and chemical stability (Table 5). Recovery after incubation with PBS at pH 7.4 or with fasted-state simulated gastric or intestinal fluid (FaSSGF/FaSSIF) was close to quantitative, indicating excellent chemical stability. Investigation of metabolic stability of **31** in mouse liver microsomes also resulted in a high recovery.

Table 5. Physicochemical Properties

assay	31
log *D* (*n*-octanol/PBS, pH 7.4)	0.94
aqueous solubility (PBS, pH 7.4)	182 μM
chemical stability	
PBS, 37 °C, 3 weeks	97.6%
FaSSIF, 2 h	95%
FaSSGF, 2 h	100%
metabolic stability (MLM, remaining, 1 h)	82%

Hepatocyte stability is a better approximation to the in vivo properties than liver microsomes since mitochondrial metabolism and conjugation processes also take place. Incubation of **31** with primary mouse hepatocytes gave a recovery of 72% after 2 h and low intrinsic clearance (<8 (μL/min)/10^6 cells), indicating high metabolic stability in mouse hepatocytes (Supporting Information, Figure S3 and Table S6).

The pharmacokinetic profile of **31** was investigated in mice to establish its properties as a tool for in vivo studies, revealing a satisfactory half-life of 2 h and a moderate to low clearance of 20.3 mL min^{-1} kg^{-1} in mice (Table 6). Oral dosing of 10 mg/kg resulted in a maximal plasma concentration of 6.5 μM after 15 min and a bioavailability of 32%. Furthermore, ip administration of **31** at 5 mg/kg in mice and analysis of plasma concentrations indicated an $AUC_{0-\infty}$ of 60 500 (ng/mL)·min a half-life of 82 min and thus a favorable profile relative to **4** ($AUC_{0-\infty}$ ≈ 25 000 (ng/mL)·min, $t_{1/2}$ ≈ 50 min).[21]

Table 6. Pharmacokinetic Properties in Mice[a]

parameters	31
Intravenous Administration	
$t_{1/2}$	138 min
$AUC_{0-\infty}$	246 000 ng min mL^{-1}
V_d	4050 mL kg^{-1}
CL_{total}	20.3 mL min^{-1} kg^{-1}
Oral Administration	
t_{max}	15 min
C_{max}	2880 ng mL^{-1}
$AUC_{0-\infty}$	156 000 ng min mL^{-1}
F	32%

[a]Administered at 10 mg/kg po and 5 mg/kg iv in Balb/c mice.

Agonist **3** has been shown to affect inflammatory responses in human neutrophils via FFA2 by induction of intracellular calcium and superoxide production.[42] Propionate is known to exert beneficial anti-inflammatory effects,[9] and the elimination of FFA2 from mice has previously been shown to cause increased inflammation in tissue models.[11] Although the FFA2 antagonist **1** failed to meet the end point in clinical trials with ulcerative colitis patients, the compound was found to modulate neutrophil recruitment,[43] and the therapeutic potential of FFA2 modulation in inflammatory disease is still not clear, especially since neutrophil recruitment may have both beneficial and detrimental effects.[44] Hence, **31** and propionate were examined for their ability to promote human neutrophil migration, as it is already established that antagonists, such as **1**, can inhibit chemotaxis in vitro and that acetate can induce chemotaxis in mouse neutrophils.[45,46] Compound **31** at 1 μM significantly induced migration of human neutrophils at a level comparable to propionate (C3) at a physiologically relevant concentration,[47] an effect that was more than doubled at 10 μM (Figure 3).

SCFAs have been shown to inhibit lipolysis in adipocytes mediated through FFA2 via the Gα_i pathway.[48] In this study we demonstrate that thiazolidine **31**, in particular when evaluated in cAMP assays, is a potent agonist of hFFA2 with

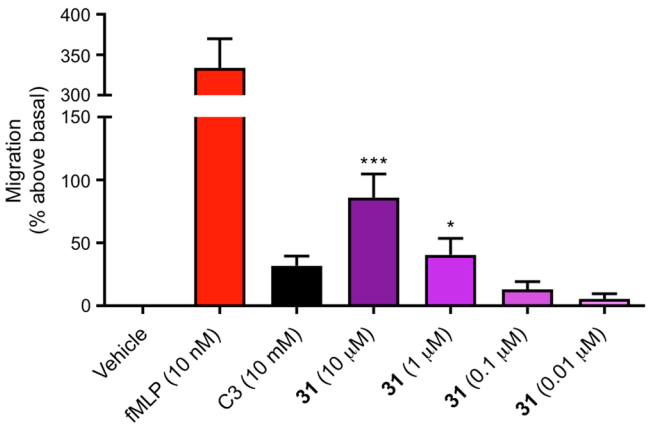

Figure 3. Ability of propionate (C3) and **31** to induce neutrophil migration relative to the highly potent chemotactic factor fMLP (n = 4−6): (*) $p < 0.05$, (***) $p < 0.01$; one-way ANOVA followed by Dunnett post hoc test.

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retained activity at mFFA2. Therefore, **31** was evaluated for its ability to inhibit isoproterenol-induced lipolysis in murine adipocytes, a process known to be $G\alpha_i$ dependent.[48] Even though **31** is slightly less potent on murine FFA2, **31** was, as predicted, 50-fold more potent than propionate in inhibiting lipolysis in isoproterenol-stimulated adipocytes (Figure 4).

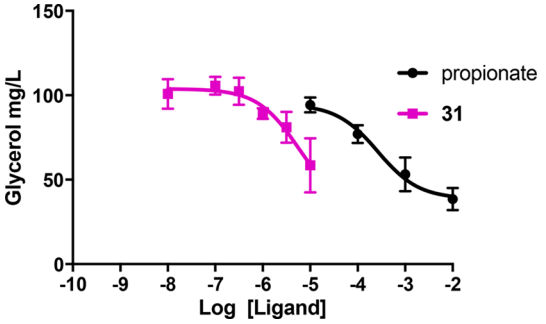

Figure 4. Inhibition of isoproterenol-induced lipolysis by propionate (pEC_{50} = 3.6 ± 0.4) and **31** (pEC_{50} = 5.3 ± 0.6) in murine adipocytes (n = 3–4).

CONCLUSION

Substitution of the central pyrrolidine of a published FFA2 agonist by a synthetically more tractable thiazolidine and rapid screening of substituents at this scaffold led to the discovery of the potent and selective FFA2 agonist **31**, a compound with lipophilicity in the lower part of the optimal range and therefore also high LLE. The compound furthermore has excellent solubility, high chemical, microsomal, and hepatocyte stability, and favorable pharmacokinetic properties. Compound **31** was able to induce migration of human neutrophils and to inhibit lipolysis in murine adipocytes. Collectively, these properties render **31** an interesting tool compound for further exploration of FFA2 as a potential therapeutic target for treatment of inflammatory and metabolic diseases.

EXPERIMENTAL SECTION

Synthesis. All commercial starting materials and solvents were used without further purification unless otherwise stated. THF was freshly distilled from sodium/benzophenone. MeOH was freshly distilled from Mg. DCM was freshly distilled and stored over 4 Å sieves. TLC was performed on TLC silica gel 60 F254 plates (Merck) and visualized at 254 nm or by staining with ninhydrin, $KMnO_4$, or $FeCl_3$ stains. Petroleum ether (PE) refers to alkanes with bp 60–80 °C. Water used in reactions was demineralized, and water used for freeze-drying was filtered Milli-Q. Purification by flash chromatography was carried out using silica gel 60 (0.040–0.063 mm, Merck). Test compounds suspended in Milli-Q water (1 mL/10 mg, 3 drops MeCN) were lyophilized on a Heto Drywinner freeze-dryer. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 101 MHz, respectively, on a Bruker Avance III 400 at 300 K. Spectra were calibrated relative to residual solvent peaks: ^{1}H NMR ($CDCl_3$), 7.26 ppm; ^{13}C NMR ($CDCl_3$), 77.16 ppm; ^{1}H NMR (DMSO-d_6), 2.50 ppm; ^{13}C NMR (DMSO-d_6), 39.52 ppm; ^{1}H NMR (CD_3OD), 3.31 ppm; ^{13}C NMR (CD_3OD), 49.00 ppm; ^{1}H NMR (CD_2Cl_2), 5.32 ppm; ^{13}C NMR (CD_2Cl_2), 53.84 ppm. Rotamer chemical shift values in ^{1}H NMR are marked by "*" and have been assigned where possible. Rotamer peaks in ^{1}H NMR are labeled by an integral equal to that of its main partner. ^{1}H and ^{13}C spectra of **31** were obtained at 600 and 151 MHz, respectively, on an Agilent NMRS 600 at the indicated temperature (Supporting Information). High-resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II (ESI). Specific optical rotation was recorded on Anton Paar MCP 100 polarimeter. Purity was determined by HPLC and confirmed by inspection of NMR spectra (^{1}H and ^{13}C NMR). HPLC analysis was performed using a Dionex 120 C18 column (5 μm, 4.6 mm × 150 mm); flow, 1 mL/min; 10% MeCN in water (0–1 min), 10–100% MeCN in water (1–10 min), 100% MeCN (11–15 min), with both solvents containing 0.1% formic acid as modifier; UV detection at 254 nm. All test compounds were of ≥ 95% purity. None of the test compounds contain substructures associated with pan-assay interfering activities (PAINS)[49] by inspection or by screening for PAINS or aggregators at http://zinc15.docking.org/patterns/home.

Methyl (4*R*)-2-(2-Chlorophenyl)thiazolidine-4-carboxylate (7a). L-Cysteine methyl ester hydrochloride (1.51 g, 8.80 mmol) was dissolved in water (6.6 mL), and potassium bicarbonate (879 mg, 8.78 mmol) was added followed by a solution of 2-chlorobenzaldehyde (2.49 g, 18.1 mmol) in EtOH (6.6 mL). The reaction was stirred at rt for 12 h, whereafter the reaction mixture was diluted with water and extracted with DCM (×3). The combined organic phases were washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (EtOAc/PE, 1:5) to give **7a** as a light yellow oil (1.68 g, 75% yield, 40% minor isomer and 60% major isomer): R_f = 0.22 (EtOAc/PE, 1:5); ^{1}H NMR for minor isomer (400 MHz, $CDCl_3$) δ 7.81 (dd, J = 7.6, 1.8 Hz, 1H), 7.50–7.24 (m, 6H) [minor and major], 6.04 (br s, 1H), 4.14–4.06 (m, 1H), 3.90 (s, 3H), 3.56 (dd, J = 10.3, 6.9 Hz, 1H), 3.24–3.14 (m, 2H) [minor and major], 2.83 (br s, 1H); ^{1}H NMR for major isomer (400 MHz, $CDCl_3$) δ 7.66 (dd, J = 7.7, 1.7 Hz, 1H), 7.50–7.23 (m, 6H) [minor and major], 6.18 (s, 1H), 4.34 (app t, J = 6.6 Hz, 1H), 3.91 (s, 3H), 3.43 (dd, J = 10.6, 6.5 Hz, 1H), 3.24–3.16 (m, 2H) [minor and major], 3.15 (br s, 1H); ^{13}C NMR for mixture of diastereomers (101 MHz, $CDCl_3$) δ 172.2, 171.7, 140.2, 136.0, 133.9, 133.1, 130.0, 129.9, 129.8, 128.7, 128.3, 127.5, 127.0, 126.7, 68.5, 67.4, 65.6, 65.1, 52.8, 52.7, 39.1, 37.6; HRMS (ESI) calcd for $C_{11}H_{12}ClNNaO_2S$ ($M + Na^+$), 280.0169; found, 280.0162.

Methyl (4*R*)-2-(3-Chlorophenyl)thiazolidine-4-carboxylate (7b). The title compound was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (119 mg, 1.16 mmol) and 3-chlorobenzaldehyde (328 mg, 2.33 mmol) and obtained after flash chromatography (EtOAc/PE, 1:5) as a light yellow oil (214 mg, 71% yield, 44% minor isomer and 56% major isomer): R_f = 0.16 (EtOAc/PE, 1:5); ^{1}H NMR for minor isomer (400 MHz, $CDCl_3$) δ 7.50–7.48 (m, 1H), 7.41–7.28 (m, 4H) [minor and major], 7.25–7.20 (m, 2H) [minor and major], 5.78 (s, 1H), 4.12 (app t, J = 6.6 Hz, 1H), 3.79 (s, 3H), 3.37 (dd, J = 10.6, 7.0 Hz, 1H), 3.18–3.06 (m, 2H) [minor and major], 2.92 (br s, 1H); ^{1}H NMR for major isomer (400 MHz, $CDCl_3$) δ 7.54–7.52 (m, 1H), 7.41–7.28 (m, 4H) [minor and major], 7.25–7.20 (m, 2H) [minor and major], 5.50 (br s, 1H), 4.01–3.93 (m, 1H), 3.80 (s, 3H), 3.45 (dd, J = 10.3, 7.1 Hz, 1H), 3.18–3.06 (m, 2H) [minor and major], 2.62 (br s, 1H); ^{13}C NMR for mixture of diastereomers (101 MHz, $CDCl_3$) δ 172.1, 171.5, 143.9, 140.4, 134.6, 134.4, 130.0, 129.74, 129.0, 128.0, 127.8, 127.1, 125.9, 125.3, 71.8, 69.9, 65.6, 64.2, 52.7, 52.7, 39.2, 38.2; HRMS (ESI) calcd for $C_{11}H_{13}ClNO_2S$ ($M + H^+$), 258.0350; found, 258.0356.

Methyl (4*R*)-2-(4-Chlorophenyl)thiazolidine-4-carboxylate (7c). The title compound was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (200 mg, 1.17 mmol) and 4-chlorobenzaldehyde (328 mg, 2.33 mmol) and obtained after flash chromatography (EtOAc/PE, 1:5) as a light yellow oil (208 mg, 69% yield, 42% minor isomer and 58% major isomer): R_f = 0.19 (EtOAc/PE, 1:5); ^{1}H NMR for minor isomer (400 MHz, $CDCl_3$) δ 7.36–7.25 (m, 4H), 5.77 (s, 1H), 4.13 (t, J = 6.5 Hz, 1H), 3.78 (s, 3H), 3.36 (dd, J = 10.6, 7.0 Hz, 1H), 3.16 (dd, J = 10.6, 6.1 Hz, 1H), 2.67 (br s, 2H) [minor and major]; ^{1}H NMR for major isomer (400 MHz, $CDCl_3$) δ 7.48–7.39 (m, 4H), 5.51 (s, 1H), 3.97 (dd, J = 8.8, 7.2 Hz, 1H), 3.45 (dd, J = 10.3, 7.1 Hz, 1H), 3.79 (s, 3H), 3.10 (dd, J = 10.3, 9.0 Hz, 1H), 2.67 (br s, 2H) [minor and major]; ^{13}C NMR for mixture of diastereomers (101 MHz, $CDCl_3$) δ 172.1, 171.5, 140.1, 136.8, 134.5, 133.6, 128.9, 128.9, 128.5, 128.4, 71.8, 70.0, 65.5, 64.2, 52.7, 52.6, 39.2, 38.1; HRMS (ESI) calcd for $C_{11}H_{13}ClNO_2S$ ($M + H^+$), 258.0350; found, 258.0361.

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Methyl (*R*)-2-(Pyridin-2-yl)thiazolidine-4-carboxylate (7e). The title compound was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (200 mg, 1.16 mmol) and picolinaldehyde (187 mg, 1.75 mmol) and obtained after flash chromatography (EtOAc/PE, 1:1) as a clear oil (165 mg, 63% yield, 45% minor isomer and 55% major isomer): R_f = 0.18 (EtOAc/PE, 1:1); ^{1}H NMR for minor isomer (400 MHz, $CDCl_3$) δ 8.57 (d, J = 4.8 Hz, 1H), 7.72−7.61 (m, 2H) [minor and major], 7.35−7.27 (m, 2H) [minor and major], 7.26−7.17 (m, 2H) [minor and major], 5.85 (s, 1H), 4.56 (dd, J = 6.4, 4.6 Hz, 1H), 3.80 (s, 3H), 3.63 (br s, 2H) [minor and major], 3.41−3.31 (m, 2H); ^{1}H NMR for major isomer (400 MHz, $CDCl_3$) δ 8.63 (d, J = 4.8 Hz, 1H), 7.72−7.61 (m, 2H) [minor and major], 7.35−7.27 (m, 2H) [minor and major], 7.26−7.17 (m, 2H) [minor and major], 5.66 (s, 1H), 4.04 (dd, J = 9.7, 6.7 Hz, 1H), 3.83 (s, 3H), 3.63 (br s, 2H) [minor and major], 3.45 (dd, J = 10.1, 6.7 Hz, 1H), 3.09 (t, J = 9.9 Hz, 1H); ^{13}C NMR for mixture of diastereomers (101 MHz, $CDCl_3$) δ 172.2, 171.2, 158.7, 156.8, 149.9, 149.6, 136.8, 136.8, 123.4, 123.0, 122.1, 121.6, 71.6, 71.1, 66.3, 65.6, 52.6 [minor and major], 39.4, 38.7; HRMS (ESI) calcd for $C_{10}H_{12}N_2NaO_2S$ (M + Na^+) 247.0512, found 247.0524.

Methyl (4*R*)-2-(2-Methoxyphenyl)thiazolidine-4-carboxylate (7f). The title compound was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (254 mg, 1.48 mmol) and 2-methoxybenzaldehyde (199 mg, 1.46 mmol) and obtained after flash chromatography (EtOAc/PE, 1:4) as a clear oil (297 mg, 81% yield, 40% minor isomer and 60% major isomer): R_f = 0.21 (EtOAc/PE, 1:4); ^{1}H NMR for minor isomer (400 MHz, $CDCl_3$) δ 7.42 (dd, J = 7.6, 1.5 Hz, 1H), 7.25−7.19 (m, 1H), 7.00−6.92 (m, 2H), 5.99 (br s, 1H), 4.25 (app t, J = 6.6 Hz, 1H), 3.85 (s, 3H), 3.79 (s, 6H) [minor and major], 3.29 (dd, J = 10.4, 6.6 Hz, 1H), 3.06 (br s, 2H) [minor and major], 3.12−3.00 (m, 2H) [minor and major]; ^{1}H NMR for major isomer (400 MHz, $CDCl_3$) δ 7.49 (dd, J = 7.6, 1.6 Hz, 1H), 7.32−7.26 (m, 1H), 6.92−6.83 (m, 2H), 5.83 (s, 1H), 3.98−3.91 (m, 1H), 3.86 (s, 3H), 3.79 (s, 6H) [minor and major], 3.42 (dd, J = 10.1, 6.8 Hz, 1H), 3.06 (br s, 2H) [minor and major], 3.12−3.00 (m, 2H) [minor and major]; ^{13}C NMR for mixture of diastereomers (101 MHz, $CDCl_3$) δ 172.3, 171.8, 157.4, 156.8, 130.1, 129.7, 128.7, 127.8, 126.2, 125.8, 120.9, 120.5, 111.2, 110.7, 67.7, 66.0, 65.8, 64.9, 55.7, 55.5, 52.51, 52.49, 39.0, 37.6; HRMS (ESI) calcd for $C_{12}H_{16}NO_3S$ (M + H^+) 254.0845, found 254.0855.

Methyl (4*R*)-2-Phenylthiazolidine-4-carboxylate (7g). The title compound was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (327 mg, 1.91 mmol) and benzaldehyde (288 μL, 2.83 mmol) and obtained after flash chromatography (EtOAc/PE, 1:4) as a clear oil (340 mg, 80% yield, 37% minor isomer and 63% major isomer): R_f = 0.32 (EtOAc/PE, 1:4); ^{1}H NMR for minor isomer (400 MHz, $CDCl_3$) δ 7.49−7.46 (m, 2H), 7.39−7.22 (m, 6H) [minor and major], 5.81 (s, 1H), 4.20 (app t, J = 6.1 Hz, 1H), 3.77 (s, 3H), 3.37 (dd, J = 10.6, 7.1 Hz, 1H), 3.19 (dd, J = 10.6, 5.8 Hz, 1H), 2.84 (br s, 1H); ^{1}H NMR for major isomer (400 MHz, $CDCl_3$) δ 7.54−7.49 (m, 2H), 7.39−7.22 (m, 6H) [minor and major], 5.55 (s, 1H), 4.02−3.93 (m, 1H), 3.78 (s, 3H), 3.45 (dd, J = 10.3, 7.1 Hz, 1H), 3.10 (dd, J = 10.1, 9.2 Hz, 1H), 2.66 (br s, 1H); ^{13}C NMR for mixture of diastereomers (101 MHz, $CDCl_3$) δ 172.2, 171.6, 141.2, 138.3, 128.73, 128.72, 128.5, 127.9, 127.5, 127.0, 72.7, 70.9, 65.6, 64.4, 52.6, 52.5, 39.3, 38.2; HRMS (ESI) calcd for $C_{11}H_{13}NNaO_2S$ (M + Na^+) 246.0559, found 246.0565.

Methyl (2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-iodobenzoyl)-thiazolidine-4-carboxylate (8a). A predried vial with **7a** (926 mg, 3.59 mmol) in THF (7.4 mL, anhydrous) was cooled to −10 °C and to it was added triethylamine (1.25 mL, 8.97 mmol) dropwise. After stirring for 15 min at rt the solution was evaporated, the triethylammonium salt was redissolved in THF (11 mL), and the solution was cooled to −10 °C. 4-Iodobenzoyl chloride (1.05 g, 3.95 mmol) dissolved in THF (15 mL, anhydrous) was added dropwise, and the reaction mixture was allowed to reach rt. After stirring at rt for 4 h the solvent was evaporated off, to the crude residue was added water (18 mL), and the mixture was acidified with 1 M HCl (pH 2−3) and extracted with EtOAc (×3). The organic phases were washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (EtOAc/PE, 1:3) to give central intermediate **8a** as a white foam (1.43 g, 82% yield): R_f = 0.21 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 6.4 Hz, 1H), 7.64−7.47 (m, 2H), 7.40−7.31 (m, 2H), 7.30−7.22 (m, 1H), 7.07−6.91 (m, 2H), 6.17 (br s, 1H), 5.07 (app s, 1H), 3.86 (s, 3H), 3.45−3.32 (m, 1H), 3.20 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 169.9, 138.9, 137.3, 134.1, 131.4, 129.8, 129.2, 128.5, 127.5, 127.3, 97.6, 65.8, 65.5, 52.8, 31.1; HRMS (ESI) calcd for $C_{18}H_{16}ClINO_3S$ (M + H^+) 487.9579, found 487.9573. $[\alpha]^{20}_D$ +45° (c 1.0, DCM).

Methyl (2*R*,4*R*)-2-(2-Chlorophenyl)-3-(3-iodobenzoyl)-thiazolidine-4-carboxylate (8b). The title compound was prepared as described for **8a** using **7a** (300 mg, 0.16 mmol) and 3-iodobenzoyl chloride (347 mg, 0.13 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a colorless oil (237 mg, 42% yield): R_f = 0.26 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, J = 7.1 Hz, 1H), 7.68 (d, J = 7.3 Hz, 1H), 7.57−7.48 (m, 1H), 7.40 (t, J = 7.2 Hz, 1H), 7.35 (dd, J = 7.9, 1.3 Hz, 1H), 7.29 (d, J = 7.3 Hz, 1H), 7.24−7.17 (m, 1H), 7.01−6.91 (m, 1H), 6.15 (s, 1H), 5.13−4.96 (m, 1H), 3.89 (s, 3H), 3.47−3.32 (m, 1H), 3.32−3.15 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 169.3, 139.7, 139.1, 136.6, 136.2, 131.6, 130.0, 129.4, 127.8, 127.6, 126.1, 93.8, 65.8, 65.6, 53.0, 31.4; HRMS (ESI) calcd for $C_{18}H_{15}ClINNaO_3S$ (M + Na^+) 509.9398, found 509.9393.

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(2′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (9). *Step 1:* The methyl ester of the title compound **9a** was prepared as described for **8a** using **7a** (100 mg, 0.39 mmol) and 2′-methoxybiphenyl-4-carbonyl chloride (107 mg, 0.44 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear viscous oil (141 mg, 77% yield): R_f = 0.20 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 7.0 Hz, 1H), 7.49−7.19 (m, 9H), 7.07−6.91 (m, 2H), 6.32 (br s, 1H), 5.18−5.08 (m, 1H), 3.89 (br s, 3H), 3.76 (s, 3H), 3.45−3.34 (m, 1H), 3.27−3.18 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.8, 156.5, 141.4, 139.5, 133.0, 131.7, 130.7, 129.9, 129.6, 129.4, 129.3, 129.1, 127.9, 127.4, 126.9, 121.0, 111.5, 66.1, 65.9, 55.6, 52.8, 31.3; HRMS (ESI) calcd for $C_{25}H_{23}ClNO_4S$ (M + H^+) 468.1031, found 468.1028.

Step 2: Methyl ester **9a** (40 mg, 86 μmol) and LiI (46 mg, 0.34 mmol) were dissolved in degassed EtOAc (120 μL), the vial was capped, flushed with argon (×3), and the reaction stirred at 80 °C in the dark. To the reaction mixture were added water and 1 M HCl (1:1), and extraction was with EtOAc (×3). The combined organic phases were washed with $NaHSO_3$, water, and brine. The organic layers were dried over Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (EtOAc/PE, 2:1 [1% AcOH]) to give the title compound **9** after freeze-drying as an amorphous solid (20 mg, 51% yield): R_f = 0.13 (EtOAc/PE, 2:1 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 6.1 Hz, 1H), 7.45−7.40 (m, 2H), 7.39−7.29 (m, 5H), 7.29−7.21 (m, 2H), 7.05−6.93 (m, 2H), 6.37 (br s, 1H), 5.28−5.16 (m, 1H), 3.77 (s, 3H), 3.50−3.35 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 172.6, 156.6, 141.9, 138.9, 132.4, 131.7, 130.8, 130.1, 129.6, 129.5, 129.5, 129.4, 127.6, 127.0, 121.1, 111.6, 66.6, 66.1, 55.7, 31.0; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_4S$ (M + Na^+) 476.0694, found 476.0680. HPLC: t_R = 12.28 min, 98.4%. $[\alpha]^{20}_D$ +13° (c 0.25, DCM).

(2*R*,4*R*)-2-(3-Chlorophenyl)-3-(2′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (10). *Step 1:* The methyl ester of the title compound **10a** was prepared as described for **8a** using **7b** (100 mg, 0.39 mmol) and 2′-methoxybiphenyl-4-carbonyl chloride (105 mg, 0.43 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear viscous oil (152 mg, 83% yield): R_f = 0.18 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71−7.64 (m, 1H), 7.57 (d, J = 5.8 Hz, 1H), 7.48−7.41 (m, 4H), 7.35−7.22 (m, 4H), 7.06−6.92 (m, 2H), 6.15 (br s, 1H), 5.17 (app s, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.47−3.14 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.0, 170.5, 156.5, 143.5, 141.4, 134.4, 133.5, 130.8, 129.8, 129.6, 129.5, 129.4, 128.2, 127.2, 126.9, 125.2, 121.0, 111.4, 67.8, 65.2, 55.6, 52.9, 32.0; HRMS (ESI) calcd for $C_{25}H_{22}ClNNaO_4S$ (M + Na^+) 490.0850, found 490.0830.

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Step 2: The title compound **10** was prepared as described for **9** using methyl ester **10a** (100 mg, 0.21 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (65 mg, 67% yield): R_f = 0.16 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61−7.39 (m, 5H), 7.37−7.30 (m, 2H), 7.29−7.22 (m, 3H), 7.05−6.92 (m, 2H), 6.68 (br s, 1H), 6.16 (br s, 1H), 5.23 (app s, 1H), 3.78 (s, 3H), 3.49−3.35 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.1, 156.5, 142.8, 141.9, 134.7, 132.7, 130.9, 130.0, 129.8, 129.5, 129.4, 128.5, 127.0, 124.9, 121.1, 111.5, 68.3, 65.0, 55.7, 31.6; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_4S$ (M + Na^+) 476.0694, found 476.0687. HPLC: t_R = 12.10 min, 97.6%. $[\alpha]^{20}_D$ +23° (*c* 1.0, DCM).

(2*R*,4*R*)-2-(4-Chlorophenyl)-3-(2′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (11). *Step 1:* The methyl ester of the title compound **11a** was prepared as described for **8a** using **7c** (99 mg, 0.39 mmol) and 2′-methoxybiphenyl-4-carbonyl chloride (107 mg, 0.44 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear viscous oil (131 mg, 73% yield): R_f = 0.18 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63−7.57 (m, 2H), 7.47−7.42 (m, 4H), 7.36−7.20 (m, 4H), 7.09−6.92 (m, 2H), 6.17 (br s, 1H), 5.15 (app s, 1H), 3.84 (br s, 3H), 3.79 (s, 3H), 3.44−3.14 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.6, 156.5, 141.3, 140.1, 133.8, 133.6, 130.8, 129.6, 129.5, 129.4, 128.7, 128.5, 126.9, 121.0, 111.4, 67.7, 65.2, 55.6, 52.9, 32.0; HRMS (ESI) calcd for $C_{25}H_{23}ClNO_4S$ (M + H^+) 468.1031, found 468.1009.

Step 2: The title compound **11** was prepared as described for **9** using methyl ester **11a** (44 mg, 94 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (56 mg, 24% yield): R_f = 0.17 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52−7.18 (m, 10H), 7.09−6.91 (m, 2H), 6.18 (br s, 1H), 5.22 (app s, 1H), 3.79 (s, 3H), 3.48−3.33 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 172.0, 156.5, 141.9, 139.2, 134.2, 132.7, 130.8, 129.8, 129.5, 129.4, 128.9, 128.2, 127.0, 121.1, 111.5, 68.4, 65.2, 55.7, 31.5; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_4S$ (M + Na^+) 476.0694, found 476.0688. HPLC: t_R = 12.17 min, 100%. $[\alpha]^{20}_D$ +31° (*c* 1.0, DCM).

(2*R*,4*R*)-2-(2-Bromophenyl)-3-(2′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (12). *Step 1: Methyl (2R,4R)-2-(2-Bromophenyl)-3-(2′-methoxybiphenyl-4-carbonyl)-thiazolidine-4-carboxylate (**7i**).* The thiazolidine was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (201 mg, 1.17 mmol) and 2-bromobenzaldehyde (431 mg, 2.33 mmol). Crude methyl (4*R*)-2-(2-bromophenyl)thiazolidine-4-carboxylate (**7i**) was used without further purification in the next step.

*Step 2: Methyl (2R,4R)-2-(2-Bromophenyl)-3-(2′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylate (**12a**).* Using the crude **7i** (95 mg) and 2′-methoxybiphenyl-4-carbonyl chloride (77 mg, 0.31 mmol), the title compound was prepared as described for **8a** and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear viscous oil (31 mg, 20% yield): R_f = 0.18 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, *J* = 7.4 Hz, 1H), 7.55 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.47−7.36 (m, 4H), 7.34−7.28 (m, 2H), 7.25−7.16 (m, 2H), 7.06−6.92 (m, 2H), 6.27 (br s, 1H), 5.17−5.07 (m, 1H), 3.90 (br s, 3H), 3.76 (s, 3H), 3.41−3.33 (m, 1H), 3.28−3.18 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 170.8, 156.6, 141.5, 133.3, 130.8, 129.7, 129.5, 129.43, 129.35, 128.2, 128.1, 127.0, 121.1, 111.6, 68.5, 66.3, 55.7, 52.9, 31.2; HRMS (ESI) calcd for $C_{25}H_{23}BrNO_4S$ (M + H^+) 512.0526, found 512.0520.

Step 3: The title compound **12** was prepared as described for **9** using methyl ester **12a** (31 mg, 61 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [2% AcOH]) and subsequent freeze-drying as a beige amorphous solid (16 mg, 54% yield): R_f = 0.23 (EtOAc/PE, 1:1 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 4.4 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.41−7.33 (m, 3H), 7.33−7.27 (m, 2H), 7.25−7.23 (m, 1H), 7.20−7.09 (m, 2H), 7.03−6.89 (m, 2H), 6.25 (br s, 1H), 5.20−5.13 (m, 1H), 3.72 (s, 3H), 3.40−3.31 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 172.6, 156.6, 141.7, 140.6, 133.3, 132.6, 130.8, 129.6, 129.5, 129.4, 128.3, 128.0, 127.0, 121.8, 121.0, 111.6, 69.2, 67.0, 55.7, 30.9; HRMS (ESI) calcd for $C_{24}H_{21}BrNO_4S$ (M + H^+) 498.0369, found 498.0345. HPLC: t_R = 12.36 min, 99.8%. $[\alpha]^{20}_D$ +17° (*c* 0.3, DCM).

(2*R*,4*R*)-3-(2′-Methoxybiphenyl-4-carbonyl)-2-phenylthiazolidine-4-carboxylic Acid (13). *Step 1: Methyl (2R,4R)-2-Phenylthiazolidine-4-carboxylate (**7g**).* The thiazolidine was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (201 mg, 1.17 mmol) and benzaldehyde (247 mg, 2.33 mmol). Crude methyl (4*R*)-2-phenylthiazolidine-4-carboxylate (**7g**) was used without further purification in the next step.

*Step 2: Methyl (2R,4R)-3-(2′-Methoxybiphenyl-4-carbonyl)-2-phenylthiazolidine-4-carboxylate (**13a**).* Using crude **7g** (105 mg) and 2′-methoxybiphenyl-4-carbonyl chloride (128 mg, 0.52 mmol), the title compound was prepared as described for **8a** and obtained after flash chromatography (EtOAc/PE, 1:3) as a white foam (146 mg, 72% yield): R_f = 0.18 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71−7.56 (m, 2H), 7.47−7.18 (m, 9H), 7.04−6.91 (m, 2H), 6.19 (br s, 1H), 5.17 (app s, 1H), 3.83 (br s, 3H), 3.76 (s, 3H), 3.44−3.16 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 170.6, 156.5, 141.6, 141.2, 133.6, 130.8, 129.6, 129.5, 129.3, 128.5, 127.9, 127.0, 126.8, 121.0, 111.4, 68.5, 65.2, 55.6, 52.8, 31.9; HRMS (ESI) calcd for $C_{25}H_{24}NO_4S$ (M + H^+) 434.1421, found 434.1412.

Step 3: The title compound **13** was prepared as described for **9** using methyl ester **13a** (100 mg, 0.23 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (67 mg, 69% yield): R_f = 0.23 (EtOAc/PE, 1:1 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (br s, 1H), 7.46−7.39 (m, 6H), 7.35−7.29 (m, 3H), 7.28−7.21 (m, 2H), 7.02−6.90 (m, 2H), 6.22 (br s, 1H), 5.24 (app s, 1H), 3.76 (s, 3H), 3.47−3.34 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 172.3, 156.5, 141.7, 140.7, 132.8, 130.8, 129.6, 129.4, 128.7, 128.2, 127.0, 126.6, 121.0, 111.5, 69.1, 65.0, 55.6, 31.4; HRMS (ESI) calcd for $C_{24}H_{22}NO_4S$ (M + H^+) 420.1264, found 420.1259. HPLC: t_R = 11.70 min, 97.6%. $[\alpha]^{20}_D$ +22° (*c* 1.0, DCM).

(2*R*,4*R*)-3-(2′-Methoxybiphenyl-4-carbonyl)-2-(pyridin-2-yl)-thiazolidine-4-carboxylic Acid (14). *Step 1:* The thiazolidine was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (150 mg, 0.87 mmol) and picolinaldehyde (187 mg, 1.75 mmol). Crude methyl (4*R*)-2-(pyridin-2-yl)thiazolidine-4-carboxylate (**7e**) was used without further purification in the next step.

*Step 2: Methyl (2R,4R)-3-(2′-Methoxybiphenyl-4-carbonyl)-2-(pyridin-2-yl)thiazolidine-4-carboxylate (**14a**).* Using crude **7e** (95 mg) and 2′-methoxybiphenyl-4-carbonyl chloride (116 mg, 0.47 mmol), methyl ester **14a** was prepared as described for **8a** and obtained after flash chromatography (EtOAc/PE, 1:2 → 1:1) as a clear viscous oil (77 mg, 42% yield): R_f = 0.35 (DCM with 2% MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 4.7 Hz, 1H), 8.15−7.99 (m, 1H), 7.80−7.71 (m, 1H), 7.44−7.37 (m, 3H), 7.37−7.27 (m, 2H), 7.26−7.16 (m, 2H), 7.05−6.91 (m, 2H), 6.18 (br s, 1H), 5.25 (app s, 1H), 3.85 (br s, 3H), 3.77 (s, 3H), 3.42−3.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) 171.1, 170.9, 156.5, 149.6, 141.4, 137.1, 133.4, 130.8, 129.6, 129.3, 126.9, 122.7, 121.0, 120.6, 111.4, 69.6, 65.2, 55.6, 52.8, 32.0; HRMS (ESI) calcd for $C_{24}H_{23}N_2O_4S$ (M + H^+) 435.1373, found 435.1395.

Step 3: The title compound **14** was prepared as described for **9** using methyl ester **14a** (75 mg, 0.17 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 2:1 → EtOAc [1% AcOH]) as brown crystals (53 mg, 73% yield): R_f = 0.18 (EtOAc/PE, 2:1 [1% AcOH]); dec 114−120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 4.8 Hz, 1H), 7.88 (br s, 1H), 7.61−7.49 (m, 4H), 7.45−7.39 (m, 1H), 7.37−7.30 (m, 1H), 7.29−7.13 (m, 2H), 7.07−6.95 (m, 2H), 6.63 (br s, 1H), 5.14 (app s, 1H), 3.79 (s, 3H), 3.58−3.41 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 170.1, 157.4, 156.5, 147.4, 141.7, 139.6, 133.2, 130.8, 129.9, 129.5, 129.3, 126.7, 124.3, 123.3, 121.0, 111.4, 67.6, 65.9, 55.6, 37.5; HRMS (ESI) calcd for $C_{23}H_{20}N_2NaO_4S$ (M + Na^+) 443.1036, found 443.1056. HPLC: t_R = 10.25 min, 99.5%. $[\alpha]^{20}_D$ +59° (*c* 1.0, DCM).

(2*R*,4*R*)-2-(Furan-2-yl)-3-(2′-methoxybiphenyl-4-carbonyl)-thiazolidine-4-carboxylic Acid (15). *Step 1: Methyl (2R,4R)-2-(Furan-2-yl)-3-thiazolidine-4-carboxylate (**7j**).* The thiazolidine was

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prepared as described for **7a** using L-cysteine methyl ester hydrochloride (150 mg, 0.87 mmol) and 2-furancarboxaldehyde (168 mg, 1.75 mmol). Crude methyl (4*R*)-2-(furan-2-yl)thiazolidine-4-carboxylate (**7j**) was used without further purification in the next step.

*Step 2: Methyl (2R,4R)-2-(Furan-2-yl)-3-(2′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylate (**15a**).* Using crude **7j** (91 mg) and 2′-methoxybiphenyl-4-carbonyl chloride (116 mg, 0.47 mmol), the title compound **15a** was prepared as described for **8a** and obtained after flash chromatography (EtOAc/PE, 1:3) as a brown viscous oil (46 mg, 25% yield): R_f = 0.21 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59−7.48 (m, 4H), 7.42−7.39 (m, 1H), 7.36−7.31 (m, 1H), 7.29 (dd, J = 7.5, 1.7 Hz, 1H), 7.05−7.00 (m, 2H), 6.87−6.78 (m, 1H), 6.37 (dd, J = 3.1, 1.8 Hz, 1H), 6.14 (br s, 1H), 5.25−5.08 (m, 1H), 3.80 (s, 6H), 3.48−3.34 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 156.5, 152.4, 142.8, 141.5, 133.2, 130.9, 129.7, 129.4, 127.2, 121.1, 111.5, 110.7, 109.4, 63.6, 62.7, 55.7, 52.9, 32.4; HRMS (ESI) calcd for $C_{23}H_{22}NO_5S$ (M + H^+) 424.1213, found 424.1222.

Step 3: The title compound **15** was prepared as described for **9** using methyl ester **15a** (43 mg, 0.10 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a whitish amorphous powder (33 mg, 80% yield): R_f = 0.18 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55−7.49 (m, 4H), 7.38 (d, J = 1.3 Hz, 1H), 7.36−7.30 (m, 1H), 7.28−7.24 (m, 1H), 7.06−6.93 (m, 2H), 6.70 (d, J = 2.8 Hz, 1H), 6.36−6.32 (m, 1H), 6.15 (br s, 1H), 5.22−5.12 (m, 1H), 3.78 (s, 3H), 3.53−3.36 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 171.7, 156.5, 151.8, 143.0, 141.9, 132.7, 130.9, 129.8, 129.47, 129.45, 127.2, 121.1, 111.5, 110.9, 109.3, 63.9, 63.0, 55.7, 31.9; HRMS (ESI) calcd for $C_{22}H_{19}NNaO_5S$ (M + Na^+) 432.0876, found 432.0877. HPLC: t_R = 11.84 min, 98.8%. $[\alpha]^{20}_D$ −1° (c 1.0, DCM).

(2*R*,4*R*)-2-(*tert*-Butyl)-3-(2′-methoxybiphenyl-4-carbonyl)-thiazolidine-4-carboxylic Acid (16). *Step 1:* The methyl ester of the title compound (**16a**) was prepared as described for **8a** using **7d** (62 mg, 0.31 mmol) and 2′-methoxybiphenyl-4-carbonyl chloride (84 mg, 0.34 mmol) and obtained after flash chromatography (DCM/PE, 1:1 → DCM → DCM/EtOAc 1:1) as a clear oil (63 mg, 50% yield): R_f = 0.34 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58−7.55 (m, 4H), 7.38−7.28 (m, 2H), 7.06−6.96 (m, 2H), 5.75 (br s, 1H), 4.93 (app s, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.36 (dd, J = 11.6, 5.9 Hz, 1H), 3.22−3.11 (m, 1H), 1.04 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 171.1, 156.5, 140.6, 135.0, 130.8, 129.7, 129.3, 126.8, 121.1, 111.5, 73.5, 66.0, 55.7, 52.6, 38.8, 33.7, 27.2; HRMS (ESI) calcd for $C_{23}H_{27}NNaO_4S$ (M + Na^+) 436.1530, found 436.1672.

Step 2: The title compound **16** was prepared as described for **9** using methyl ester **16a** (48 mg, 0.12 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:3 [1% AcOH]) to give a cloudy oil (28 mg, 60% yield): R_f = 0.23 (EtOAc/PE, 1:3 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67−7.53 (m, 4H), 7.39−7.30 (m, 2H), 7.08−6.98 (m, 2H), 5.47 (br s, 1H), 5.15−5.00 (m, 1H), 3.82 (s, 3H), 4.00−3.12 (m, 2H), 0.88 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 170.2, 155.6, 140.8, 132.0, 129.9, 129.0, 128.6, 128.3, 126.2, 120.1, 110.6, 74.1, 64.2, 54.7, 38.3, 31.1, 26.1; HRMS (ESI) calcd for $C_{22}H_{25}NNaO_4S$ (M + Na^+) 422.1397, found 422.1393. HPLC: t_R = 12.64 min, 99.8%. $[\alpha]^{20}_D$ −100° (c 0.5, DCM).

(2*R*,4*R*)-3-Benzoyl-2-(2-chlorophenyl)thiazolidine-4-carboxylic Acid (18). *Step 1:* The methyl ester of the title compound (**18a**) was prepared as described for **8a** using **7a** (156 mg, 0.60 mmol) and benzoyl chloride (79 μL, 0.68 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear oil (125 mg, 57% yield): R_f = 0.18 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 7.0 Hz, 1H), 7.45−7.13 (m, 8H), 6.20 (br s, 1H), 5.09 (app s, 1H), 3.86 (s, 3H), 3.42−3.30 (m, 1H), 3.27−3.15 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.6, 139.4, 134.9, 131.6, 130.8, 129.8, 129.1, 128.3, 127.7, 127.3, 126.9, 65.9, 65.7, 52.8, 31.3; HRMS (ESI) calcd for $C_{18}H_{16}ClNNaO_3S$ (M + Na^+) 384.0432, found 384.0447.

Step 2: The title compound **18** was prepared as described for **9** using methyl ester **18a** (114 mg, 0.32 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc [1% AcOH]) and subsequent freeze-drying as white crystals (59 mg, 53% yield): R_f = 0.18 (EtOAc [1% AcOH]); mp 166−172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.39 (br s, 1H), 8.14 (d, J = 7.3 Hz, 1H), 7.43−7.30 (m, 4H), 7.30−7.18 (m, 4H), 6.22 (br s, 1H), 5.29−5.08 (m, 1H), 3.48−3.39 (m, 1H), 3.38−3.28 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 172.2, 138.9, 134.5, 131.6, 131.2, 129.9, 129.3, 128.4, 127.6, 127.5, 127.1, 66.2, 66.0, 31.1; HRMS (ESI) calcd for $C_{17}H_{14}ClNNaO_3S$ (M + Na^+) 370.0275, found 370.0285. HPLC: t_R = 11.73 min, 95.2%. $[\alpha]^{20}_D$ +30° (c 1.0, DCM).

(2*R*,4*R*)-3-([1,1′-Biphenyl]-4-carbonyl)-2-(2-chlorophenyl)-thiazolidine-4-carboxylic Acid (19). *Step 1:* To a vial charged with **8a** (99 mg, 0.20 mmol) and phenylboronic acid (25 mg, 0.20 mmol) were added toluene (1.3 mL) and MeOH (0.7 mL). The reaction mixture was purged with argon (×5), and to it were added $Pd(PPh_3)_4$ (12 mg, 11 μmol) and aqueous Na_2CO_3 (170 μL, 4.0 M). The reaction was capped and stirred at 80 °C overnight under argon, then treated with water and extracted with EtOAc (×3), washed with brine, dried over Na_2SO_4, and concentrated in vacuo, and the methyl ester of the title compound (**19a**) was obtained after flash chromatography (EtOAc/PE, 1:3) as a viscous oil (58 mg, 65% yield): R_f = 0.24 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 7.0 Hz, 1H), 7.56−7.51 (m, 2H), 7.47−7.32 (m, 9H), 7.31−7.25 (m, 1H), 6.30 (br s, 1H), 5.21−5.09 (m, 1H), 3.90 (s, 3H), 3.43−3.36 (m, 1H), 3.28−3.18 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 170.8, 143.8, 140.0, 133.5, 131.7, 130.0, 129.2, 129.0, 128.1, 127.9, 127.8, 127.4, 127.2, 127.0, 66.1, 65.9, 52.9, 31.3; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_3S$ (M + Na^+) 460.0745, found 460.0725.

Step 2: The title compound **19** was prepared as described for **9** using methyl ester **19a** (57 mg, 0.13 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (41 mg, 75% yield): R_f = 0.16 (EtOAc/PE, 1:1 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 4.6 Hz, 1H), 7.74−7.63 (m, 1H), 7.54−7.50 (m, 2H), 7.50−7.39 (m, 4H), 7.39−7.32 (m, 4H), 7.30−7.20 (m, 1H), 6.33 (br s, 1H), 5.24 (app s, 1H), 3.49−3.35 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 172.3, 144.2, 139.9, 138.9, 133.0, 131.7, 130.1, 129.4, 129.0, 128.2, 127.8, 127.6, 127.3, 127.1, 66.5, 66.1, 31.0; HRMS (ESI) calcd for $C_{23}H_{19}ClNO_3S$ (M + H^+) 424.0769, found 424.0783. HPLC: t_R = 12.33 min, 97.8%. $[\alpha]^{20}_D$ +39° (c 1.0, DCM).

(2*R*,4*R*)-3-([1,1′-Biphenyl]-3-carbonyl)-2-(2-chlorophenyl)-thiazolidine-4-carboxylic Acid (20). *Step 1:* The methyl ester of the title compound (**20a**) was prepared as described for **19a** using **8b** (84 mg, 0.172 mmol) and phenylboronic acid (21 mg, 0.172 mmol) and obtained after flash chromatography (EtOAc/PE, 2:3) as a clear viscous oil (45 mg, 63% yield): R_f = 0.24 (EtOAc/PE, 2:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 6.6 Hz, 1H), 7.65−7.52 (m, 1H), 7.49−7.24 (m, 9H), 7.18−6.99 (m, 2H), 6.26 (br s, 1H), 5.21−5.07 (m, 1H), 3.90 (s, 3H), 3.47−3.33 (m, 1H), 3.30−3.14 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 170.7, 141.0, 139.8, 135.3, 131.8, 130.0, 129.5, 129.2, 129.1, 128.8, 128.0, 127.7, 127.6, 126.9, 126.2, 125.7, 66.0, 52.9, 31.3; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_3S$ (M + Na^+) 460.0745, found 460.0742.

Step 2: The title compound **20** was prepared as described for **9** using methyl ester **20a** (41 mg, 94 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (13 mg, 33% yield): R_f = 0.24 (EtOAc/PE, 1:1 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20−8.05 (m, 1H), 7.60 (d, J = 5.6 Hz, 1H), 7.46−7.23 (m, 9H), 7.18−7.00 (m, 2H), 6.26 (br s, 1H), 5.20 (app s, 1H), 3.49−3.26 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6 (br), 141.2, 139.7, 139.2, 134.9, 131.8, 130.1, 129.8, 129.3, 129.2, 128.9, 127.8, 126.9, 126.2, 125.8, 66.7, 66.1, 31.0; HRMS (ESI) calcd for $C_{23}H_{18}ClNNaO_3S$ (M + Na^+) 446.0588, found 446.0578. HPLC: t_R = 12.94 min, 97.3%. $[\alpha]^{20}_D$ −2° (c 0.1, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(2′-fluoro-3′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (21). *Step 1:* The methyl ester of the title compound (**21a**) was prepared as described for **19a** using **8a** (101 mg, 0.21 mmol) and (2-fluoro-3-methoxyphenyl)boronic acid (39 mg, 0.23 mmol) and obtained after flash chromatography (EtOAc/PE, 1:4) as a clear oil (77 mg,

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76% yield): R_f = 0.25 (EtOAc/PE, 1:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J = 6.7 Hz, 1H), 7.45−7.23 (m, 7H), 7.15−7.06 (m, 1H), 6.99−6.87 (m, 2H), 6.28 (br s, 1H), 5.13 (app s, 1H), 3.90 (s, 6H), 3.45−3.32 (m, 1H), 3.30−3.15 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.7, 150.9, 148.4 (d, J = 11.5 Hz), 139.4, 138.3, 134.0, 131.7, 129.9, 129.2, 129.0, 128.9, 128.8, 127.9, 127.4, 127.2, 124.1 (d, J = 4.3 Hz), 121.8, 112.9, 66.0, 65.8, 56.5, 52.9, 31.3; HRMS (ESI) calcd for $C_{25}H_{21}ClFNNaO_4S$ (M + Na^+) 508.0756, found 508.0767.

Step 2: The title compound **21** was prepared as described for **9** using methyl ester **21a** (78 mg, 0.16 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (28 mg, 37% yield): R_f = 0.14 (EtOAc/PE, 1:2 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, J = 5.9 Hz, 1H), 7.44−7.30 (m, 6H), 7.27−7.21 (m, 1H), 7.10 (td, J = 8.1, 1.2 Hz, 1H), 6.96 (td, J = 8.1, 1.4 Hz, 1H), 6.93−6.89 (m, 1H), 6.32 (br s, 1H), 5.28−5.15 (m, 1H), 3.91 (s, 3H), 3.50−3.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.2, 150.0, 147.4 (d, J = 11.8 Hz), 137.7, 132.5, 130.7, 129.1, 128.4, 128.2, 127.8, 127.7, 126.6, 126.3, 123.2 (d, J = 3.6 Hz), 120.9, 112.1, 65.4, 65.0, 55.6, 30.0; HRMS (ESI) calcd for $C_{24}H_{19}ClFNNaO_4S$ (M + Na^+) 494.0600, found 494.0596. HPLC: t_R = 12.60 min, 99.4%. $[\alpha]^{20}_D$ +32° (c 0.4, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4′-methoxybiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (22). *Step 1:* The methyl ester of the title compound (**22a**) was prepared as described for **19a** using **8a** (96, 0.20 mmol) and (4-methoxyphenyl)boronic acid (31 mg, 0.21 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear viscous oil (42 mg, 46% yield): R_f = 0.26 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 7.2 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.42−7.38 (m, 3H), 7.38−7.27 (m, 4H), 6.98−6.92 (m, 2H), 6.29 (br s, 1H), 5.18−5.07 (m, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.43−3.35 (m, 1H), 3.28−3.18 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 170.8, 159.8, 143.4, 139.6, 132.8, 132.5, 131.7, 130.0, 129.2, 128.3, 127.8, 127.4, 126.4, 114.4, 66.1, 66.0, 55.5, 52.9, 31.3; HRMS (ESI) calcd for $C_{25}H_{22}ClNNaO_4S$ (M + Na^+) 490.0850, found 490.0861.

Step 2: The title compound **22** was prepared as described for **9** using methyl ester **22a** (41 mg, 87 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (29 mg, 73% yield): R_f = 0.32 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 5.8 Hz, 1H), 7.46−7.41 (m, 2H), 7.39−7.29 (m, 6H), 7.26−7.21 (m, 1H), 6.96−6.89 (m, 2H), 6.30 (br s, 1H), 5.26−5.13 (m, 1H), 3.83 (s, 3H), 3.44−3.31 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 172.4, 159.9, 143.7, 139.0, 132.3, 131.6, 130.1, 129.4, 128.4, 127.9, 127.6, 126.5, 114.5, 66.6, 66.3, 55.5, 30.9; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_4S$ (M + Na^+) 476.0694, found 476.0693. HPLC: t_R = 12.79 min, 100%. $[\alpha]^{20}_D$ +47° (c 0.4, DCM).

(2*R*,4*R*)-3-(4′-Acetylbiphenyl-4-carbonyl)-2-(2-chlorophenyl)thiazolidine-4-carboxylic Acid (23). *Step 1:* The methyl ester of the title compound (**23a**) was prepared as described for **19a** using **8a** (88 mg, 0.18 mmol) and (4-acetylphenyl)boronic acid (32 mg, 0.19 mmol) and obtained after flash chromatography (EtOAc/PE, 1:2) as a white solid (42 mg, 48% yield): R_f = 0.22 (EtOAc/PE, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 6.4 Hz, 1H), 8.03−7.98 (m, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.51−7.45 (m, 2H), 7.44−7.38 (m, 2H), 7.35 (dd, J = 7.9, 1.2 Hz, 2H), 7.29 (d, J = 7.2 Hz, 1H), 6.26 (br s, 1H), 5.20−5.06 (m, 1H), 3.90 (s, 3H), 3.45−3.36 (m, 1H), 3.29−3.18 (m, 1H), 2.62 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.7, 170.7, 144.5, 142.4, 139.4, 136.5, 134.4, 131.7, 130.0, 129.3, 129.1, 127.9, 127.5, 127.4, 127.2, 66.1, 65.9, 53.0, 31.3, 26.8; HRMS (ESI) calcd for $C_{26}H_{23}ClNO_4S$ (M + H^+) 480.1031, found 480.1054.

Step 2: The title compound **23** was prepared as described for **9** using methyl ester **23a** (42 mg, 86 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [2% AcOH]) and subsequent freeze-drying as a white solid (31 mg, 78% yield): R_f = 0.17 (EtOAc/PE, 1:1 [2% AcOH]); mp 202−208 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 5.0 Hz, 1H), 7.97 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 7.9 Hz, 2H), 7.47−7.39 (m, 2H), 7.39−7.29 (m, 4H), 7.29−7.21 (m, 1H), 6.26 (br s, 1H), 5.26−5.11 (m, 1H), 3.48−3.31 (m, 2H), 2.62 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.8, 172.7, 172.0, 144.3, 142.7, 139.0, 136.6, 134.0, 131.6, 130.1, 129.4, 129.1, 128.0, 127.6, 127.4, 127.3, 66.5, 31.0, 26.8; HRMS (ESI) calcd for $C_{25}H_{20}ClNNaO_4S$ (M + Na^+) 488.0694, found 488.0695. HPLC: t_R = 12.18 min, 95.7%. $[\alpha]^{20}_D$ +64° (c 0.4, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4′-cyanobiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (24). *Step 1:* The methyl ester of the title compound (**24a**) was prepared as described for **19a** using **8a** (81 mg, 0.17 mmol) and (4-cyanophenyl)boronic acid (26 mg, 0.18 mmol) and obtained after flash chromatography (EtOAc/PE, 2:7) as a white viscous oil (31 mg, 41% yield): R_f = 0.27 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 6.4 Hz, 1H), 7.74−7.70 (m, 2H), 7.64−7.60 (m, 2H), 7.49−7.33 (m, 6H), 7.32−7.28 (m, 1H), 6.24 (br s, 1H), 5.24−5.06 (m, 1H), 3.91 (s, 3H), 3.47−3.37 (m, 1H), 3.29−3.16 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.5, 144.5, 141.6, 139.4, 134.9, 132.8, 131.7, 130.0, 129.4, 128.0, 127.9, 127.5, 127.2, 118.8, 111.8, 66.0, 65.8, 53.0, 31.3; HRMS (ESI) calcd for $C_{25}H_{19}ClN_2NaO_3S$ (M + Na^+) 485.0697, found 485.0696.

Step 2: The title compound **24** was prepared as described for **9** using methyl ester **24a** (31 mg, 67 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (21 mg, 69% yield): R_f = 0.15 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 6.0 Hz, 1H), 7.73−7.67 (m, 2H), 7.61−7.55 (m, 2H), 7.46−7.31 (m, 6H), 7.29−7.21 (m, 1H), 6.25 (br s, 1H), 5.24−5.14 (m, 1H), 3.47−3.39 (m, 1H), 3.39−3.31 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 171.7, 144.3, 141.9, 138.9, 134.5, 132.8, 131.6, 130.1, 129.5, 128.1, 127.9, 127.6, 127.3, 118.7, 111.9, 66.4, 66.3, 31.0; HRMS (ESI) calcd for $C_{24}H_{17}ClN_2NaO_3S$ (M + Na^+) 471.0541, found 471.0554. HPLC: t_R = 12.30 min, 95.8%. $[\alpha]^{20}_D$ +59° (c 0.5, DCM).

(2*R*,4*R*)-3-(2′-Chlorobiphenyl-4-carbonyl)-2-(2-chlorophenyl)thiazolidine-4-carboxylic Acid (25). *Step 1:* The methyl ester of the title compound (**25a**) was prepared as described for **19a** using **8a** (98 mg, 0.20 mmol) and (2-chlorophenyl)boronic acid (34 mg, 0.22 mmol) and obtained after flash chromatography (EtOAc/PE, 1:5) as a viscous oil (80 mg, 84% yield): R_f = 0.16 (EtOAc/PE, 1:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J = 6.9 Hz, 1H), 7.60−7.12 (m, 11H), 6.30 (br s, 1H), 5.21−5.07 (m, 1H), 3.90 (s, 3H), 3.44−3.33 (m, 1H), 3.29−3.19 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.7, 141.9, 139.5, 139.3, 133.9, 132.4, 131.7, 131.2, 130.1, 129.9, 129.4, 129.2, 129.1, 127.9, 127.4, 127.0, 126.8, 65.9, 65.7, 52.9, 31.4; HRMS (ESI) calcd for $C_{24}H_{20}Cl_2NO_3S$ (M + H^+) 472.0535, found 472.0576.

Step 2: The title compound **25** was prepared as described for **9** using methyl ester **25a** (81 mg, 0.17 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (56 mg, 72% yield): R_f = 0.19 (EtOAc/PE, 1:2 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (br s, 1H), 8.08 (d, J = 5.4 Hz, 1H), 7.46−7.42 (m, 1H), 7.39−7.21 (m, 10H), 6.34 (br s, 1H), 5.31−5.16 (m, 1H), 3.66−3.29 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 172.2, 142.4, 139.4, 138.7, 133.5, 132.5, 131.7, 131.2, 130.2, 130.1, 129.6, 129.4, 129.2, 127.6, 127.3, 127.0, 126.9, 66.3, 66.0, 31.3; HRMS (ESI) calcd for $C_{23}H_{17}Cl_2NNaO_3S$ (M + Na^+) 480.0198, found 480.0186. HPLC: t_R = 13.33 min, 96.8%. $[\alpha]^{20}_D$ +73° (c 1.0, MeOH).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(2′-methylbiphenyl-4-carbonyl)thiazolidine-4-carboxylic Acid (26). *Step 1:* The methyl ester or the title compound (**26a**) was prepared as described for **19a** using **8a** (100 mg, 0.21 mmol) and *o*-tolylboronic acid (31 mg, 0.23 mmol) and obtained after flash chromatography (EtOAc/PE, 1:5) as a clear viscous oil (81 mg, 86% yield): R_f = 0.25 (EtOAc/PE, 1:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 7.3 Hz, 1H), 7.41−7.35 (m, 1H), 7.32−7.11 (m, 10H), 6.30 (br s, 1H), 5.14 (app s, 1H), 3.90 (s, 3H), 3.45−3.33 (m, 1H), 3.31−3.22 (m, 1H), 2.17 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.7, 144.6, 140.8, 139.4, 135.3,

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133.3, 131.6, 130.5, 129.8, 129.6, 129.2, 129.1, 127.9, 127.8, 127.4, 126.8, 125.9, 65.8, 65.7, 52.9, 31.5, 20.3; HRMS (ESI) calcd for $C_{25}H_{22}ClNNaO_3S$ ($M + Na^+$) 474.0901, found 474.0922.

Step 2: The title compound **26** was prepared as described for **9** using methyl ester **26a** (81 mg, 0.18 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:3 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (54 mg, 54% yield): R_f = 0.24 (EtOAc/PE, 1:3 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 7.2 Hz, 1H), 8.04 (br s, 1H), 7.56−6.99 (m, 11H), 6.34 (br s, 1H), 5.37−5.14 (m, 1H), 3.51−3.34 (m, 2H), 2.17 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 171.3, 144.0, 139.8, 137.9, 134.3, 131.8, 130.6, 129.5, 128.9, 128.6, 128.4, 128.3, 126.9, 126.7, 126.6, 125.9, 125.0, 65.2, 64.9, 30.2, 19.4; HRMS (ESI) calcd for $C_{24}H_{20}ClNNaO_3S$ ($M + Na^+$) 460.0745, found 460.0738. HPLC: t_R = 13.53 min, 97.2%. $[\alpha]^{20}_D$ +38° (c 1.0, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-(5-methylthiophen-2-yl)-benzoyl)thiazolidine-4-carboxylic Acid (27). *Step 1:* The methyl ester of the title compound (**27a**) was prepared as described for **19a** using **8a** (106 mg, 0.22 mmol) and 5-methylthiophene-2-boronic acid pinacol ester (51 mg, 0.23 mmol) and obtained after flash chromatography (EtOAc/PE, 1:5) as a red oil (78 mg, 78% yield): R_f = 0.20 (EtOAc/PE, 1:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J = 7.2 Hz, 1H), 7.43−7.35 (m, 5H), 7.32−7.28 (m, 2H), 7.11 (d, J = 3.5 Hz, 1H), 6.72−6.70 (m, 1H), 6.28 (br s, 1H), 5.17−5.04 (m, 1H), 3.88 (s, 3H), 3.41−3.33 (m, 1H), 3.25−3.16 (m, 1H), 2.48 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 141.0, 140.6, 139.4, 137.2, 132.8, 131.7, 130.0, 129.2, 128.0, 127.8, 127.4, 126.6, 125.0, 124.2, 66.1, 65.9, 52.9, 31.2, 15.6; HRMS (ESI) calcd for $C_{23}H_{21}ClNO_3S_2$ ($M + H^+$) 458.0646, found 458.0640.

Step 2: The title compound **27** was prepared as described for **9** using methyl ester **27a** (65 mg, 0.14 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (48 mg, 75% yield): R_f = 0.27 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 5.9 Hz, 1H), 7.79 (br s, 1H), 7.44−7.33 (m, 4H), 7.30−7.22 (m, 3H), 7.11 (d, J = 3.6 Hz, 1H), 6.74−6.68 (m, 1H), 6.31 (br s, 1H), 5.24−5.11 (m, 1H), 3.47−3.30 (m, 2H), 2.49 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 172.1, 141.2, 140.5, 138.9, 137.7, 132.3, 131.6, 130.2, 129.4, 128.1, 127.6, 127.5, 126.6, 125.1, 124.4, 66.6, 66.2, 30.9, 15.6; HRMS (ESI) calcd for $C_{22}H_{18}ClNNaO_3S_2$ ($M + Na^+$) 466.0309, found 466.0296. HPLC: t_R = 13.43 min, 98.6%. $[\alpha]^{20}_D$ +38° (c 1.0, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-(phenylethynyl)benzoyl)-thiazolidine-4-carboxylic Acid (28). *Step 1:* The methyl ester of the title compound (**28a**) was prepared as described for **8a** using **7a** (111 mg, 0.43 mmol) and 4-(phenylethynyl)benzoyl chloride (113 mg, 0.47 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as a clear viscous oil (127 mg, 65% yield): R_f = 0.31 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 7.0 Hz, 1H), 7.53−7.45 (m, 2H), 7.41−7.19 (m, 10H), 6.21 (br s, 1H), 5.10 (app s, 1H), 3.87 (s, 3H), 3.46−3.30 (m, 1H), 3.29−3.13 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 170.4, 139.2, 134.2, 133.1, 131.7, 131.4, 129.9, 129.2, 128.7, 128.4, 127.7, 127.4, 127.1, 126.0, 122.7, 91.6, 88.5, 65.9, 65.7, 52.8, 31.2; HRMS (ESI) calcd for $C_{26}H_{21}ClNO_3S$ ($M + H^+$) 462.0925, found 462.0938.

Step 2: The title compound **28** was prepared as described for **9** using methyl ester **28a** (83 mg, 0.18 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (67 mg, 83% yield): R_f = 0.25 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 7.3 Hz, 1H), 8.04−7.93 (m, 1H), 7.52−7.47 (m, 2H), 7.42−7.32 (m, 7H), 7.29−7.23 (m, 2H), 6.24 (br s, 1H), 5.23−5.15 (m, 1H), 3.50−3.42 (m, 1H), 3.38−3.30 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 171.6, 138.8, 133.7, 131.8, 131.6, 130.1, 129.4, 128.8, 128.5, 127.6, 127.5, 127.3, 126.5, 122.8, 91.9, 88.5, 66.3, 66.1, 31.0; HRMS (ESI) calcd for $C_{25}H_{18}ClNNaO_3S$ ($M + Na^+$) 470.0588, found 470.0597. HPLC: t_R = 13.58 min, 98.7%. $[\alpha]^{20}_D$ +61° (c 1.0, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-((2-methoxyphenyl)-ethynyl)benzoyl)thiazolidine-4-carboxylic Acid (29). *Step 1:* To a predried vial charged with **8a** (72 mg, 0.15 mmol) and 1-ethynyl-2-methoxybenzene (26 mg, 0.19 mmol) was added THF (1.2 mL, anhydrous). The reaction mixture was purged with argon (×3), and to it were added $Pd(PPh_3)_2Cl_2$ (6 mg, 9.0 μmol), CuI (4 mg, 22 μmol), and Et_3N (81 μL, 0.58 mmol). The reaction was capped and stirred at rt for 4 h under argon, whereafter the mixture was concentrated in vacuo. The methyl ester **29a** was obtained after flash chromatography (EtOAc/PE, 1:3) as a white oil (55 mg, 76% yield): R_f = 0.17 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 7.1 Hz, 1H), 7.46 (dd, J = 7.6, 1.5 Hz, 1H), 7.44−7.34 (m, 4H), 7.34−7.23 (m, 4H), 6.97−6.84 (m, 2H), 6.21 (br s, 1H), 5.10 (app s, 1H), 3.89 (s, 6H), 3.43−3.34 (m, 1H), 3.27−3.18 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 160.1, 139.4, 134.0, 133.8, 131.5, 130.3, 130.0, 129.3, 127.7, 127.4, 127.1, 126.5, 120.6, 112.0, 110.8, 92.6, 88.2, 66.1, 65.9, 55.9, 53.0, 31.2; HRMS (ESI) calcd for $C_{27}H_{22}ClNNaO_4S$ ($M + Na^+$) 514.0850, found 514.0766.

Step 2: The title compound **29** was prepared as described for **9** using methyl ester **29a** (55 mg, 0.11 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (39 mg, 72% yield): R_f = 0.23 (EtOAc/PE, 1:1 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 6.6 Hz, 1H), 7.50−7.21 (m, 9H), 6.98−6.86 (m, 2H), 6.25 (br s, 1H), 5.25−5.08 (m, 1H), 3.90 (s, 3H), 3.47−3.40 (m, 1H), 3.40−3.33 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 171.9, 160.2, 138.8, 133.8, 133.5, 131.6, 130.4, 130.2, 129.5, 128.0, 127.6, 127.5, 127.2, 126.9, 120.7, 112.0, 110.9, 92.5, 88.5, 66.4, 66.1, 56.0, 30.9; HRMS (ESI) calcd for $C_{26}H_{20}ClNNaO_4S$ ($M + Na^+$) 500.0694, found 500.0714. HPLC: t_R = 13.23 min, 96.2%. $[\alpha]^{20}_D$ +68° (c 1.0, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-(1-methyl-1*H*-pyrazol-5-yl)-benzoyl)thiazolidine-4-carboxylic Acid (30). *Step 1:* The methyl ester of the title compound (**30a**) was prepared as described for **19a** using **8a** (93 mg, 0.19 mmol) and 1-methyl-1*H*-pyrazole-5-boronic acid pinacol ester (41 mg, 0.19 mmol) and obtained after flash chromatography (EtOAc/PE, 1:1) as a clear viscous oil (17 mg, 20% yield): R_f = 0.17 (EtOAc/PE, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 6.7 Hz, 1H), 7.48 (d, J = 1.8 Hz, 1H), 7.43−7.21 (m, 7H), 6.28 (d, J = 1.5 Hz, 1H), 6.24 (br s, 1H), 5.18−5.06 (m, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 3.46−3.37 (m, 1H), 3.30−3.20 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.4, 142.5, 139.2, 138.7, 134.8, 133.1, 131.6, 130.0, 129.4, 128.6, 128.0, 127.54, 127.45, 106.5, 65.9, 65.6, 53.0, 37.6, 31.4; HRMS (ESI) calcd for $C_{22}H_{21}ClN_3O_3S$ ($M + H^+$) 442.0987, found 442.1007.

Step 2: The title compound **30** was prepared as described for **9** using methyl ester **30a** (17 mg, 39 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (12 mg, 71% yield): R_f = 0.14 (EtOAc [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 7.2 Hz, 1H), 7.55 (d, J = 1.9 Hz, 1H), 7.40−7.21 (m, 7H), 6.29 (d, J = 1.2 Hz, 1H), 6.25 (br s, 1H), 5.26−5.15 (m, 1H), 3.84 (s, 3H), 3.52−3.43 (m, 1H), 3.42−3.32 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 171.1, 142.8, 139.1, 138.5, 134.9, 132.9, 131.5, 129.9, 129.4, 128.7, 128.0, 127.6, 125.4, 106.6, 66.2, 66.1, 37.4, 31.4; HRMS (ESI) calcd for $C_{21}H_{19}ClN_3O_3S$ ($M + H^+$) 428.0830, found 428.0836. HPLC: t_R = 10.84 min, 98.1%. $[\alpha]^{20}_D$ +31° (c 0.2, DCM).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-(3,5-dimethylisoxazol-4-yl)benzoyl)thiazolidine-4-carboxylic Acid (31). *Step 2:* The methyl ester of the title compound (**31a**) was prepared as described for **19a** using **8a** (106 mg, 0.22 mmol) and 3,5-dimethylisoxazole-4-boronic acid pinacol ester (51 mg, 0.23 mmol) and obtained after flash chromatography (EtOAc/PE, 1:3) as white needles (63 mg, 63% yield): R_f = 0.14 (EtOAc/PE, 1:3); mp 146−148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 6.9 Hz, 1H), 7.44−7.36 (m, 1H), 7.36−7.22 (m, 4H), 7.19−7.01 (m, 2H), 6.25 (br s, 1H), 5.20−5.08 (m, 1H), 3.91 (s, 3H), 3.48−3.38 (m, 1H), 3.33−3.22 (m, 1H), 2.35 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 165.7, 158.4, 139.2, 134.0, 133.1, 131.5, 129.8, 129.3, 128.9, 128.0, 127.5, 115.9, 65.8, 65.5, 52.9, 31.5, 11.6, 10.7; HRMS (ESI) calcd for $C_{23}H_{21}ClN_2NaO_4S$ ($M + Na^+$) 479.0803, found 479.0812.

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Step 2: The title compound **31** was prepared as described for **9** using methyl ester **31a** (61 mg, 0.13 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:2 → EtOAc [2% AcOH]) and subsequent freeze-drying gave **31** as a powder (25 mg, 42% yield): R_f = 0.21 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (600 MHz, DMSO-d_6, 100 °C) δ 8.35 (dd, J = 7.8, 1.0 Hz, 1H), 7.42−7.34 (m, 3H), 7.33−7.29 (m, 1H), 7.29−7.26 (m, 3H), 6.41 (s, 1H), 5.02 (t, J = 6.5 Hz, 1H), 3.62 (dd, J = 12.0, 6.6 Hz, 1H), 3.30 (dd, J = 12.1, 6.4 Hz, 1H), 2.35 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6, 100 °C) δ 170.5, 168.9, 164.8, 157.3, 138.2, 134.6, 131.3, 130.6, 128.62, 128.55, 128.1, 127.7, 126.7, 126.3, 114.9, 64.9, 63.9, 32.1, 10.5, 9.5; HRMS (ESI) calcd for $C_{22}H_{19}ClN_2NaO_4S$ (M + Na$^+$) 465.0646, found 465.0627. HPLC: t_R = 11.65 min, 98.3%. $[\alpha]^{20}_D$ +72° (c 0.5, MeOH).

(2*R*,4*R*)-2-(2-Chlorophenyl)-3-(4-(5-cyclopropyl-3-phenylisoxazol-4-yl)benzoyl)thiazolidine-4-carboxylic Acid (32). *Step 1: Methyl (2R,4R)-2-(2-Chlorophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoyl)thiazolidine-4-carboxylate.* In a vial, a mixture of **8a** (120 mg, 0.25 mmol), bis(pinacolato)diboron (69 mg, 0.27 mmol), and potassium acetate (61 mg, 0.62 mmol) in DMF (0.35 mL) was purged with argon (×5), followed by the addition of 1,1′-bis(phenylphosphino)ferrocene palladium dichloride (9.0 mg, 12.30 μmol), and the mixture was again purged with argon (×5). The reaction was capped and stirred at 50 °C under argon until consumption of starting material (20 h). The reaction was treated with water and extracted with EtOAc (×5), washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*.

Step 2: 5-Cyclopropyl-4-iodo-3-phenylisoxazole. To (*Z*)-3-cyclopropyl-1-phenylprop-2-yn-1-one *O*-methyl oxime (69 mg, 0.35 mmol) dissolved in dry DCM (3.5 mL) was added IBr (143 mg, 0.69 mmol), and the reaction was stirred at rt until consumption of starting material. The reaction mixture was washed with saturated $Na_2S_2O_3$, extracted with DCM (×5), dried over Na_2SO_4, and concentrated in vacuo. The crude 5-cyclopropyl-4-iodo-3-phenylisoxazole was filtered through a plug of silica using PE (1% EtOAc) and used without further purification in the next step.

*Step 3: Methyl (2R,4R)-2-(2-Chlorophenyl)-3-(4-(5-cyclopropyl-3-phenylisoxazol-4-yl)benzoyl)thiazolidine-4-carboxylate (**32a**).* In a vial, to crude boronate from step 1 (100 mg) were added crude 5-cyclopropyl-4-iodo-3-phenylisoxazole (55 mg, 0.18 mmol) and XPhos Pd G4 precatalyst (3.8 mg, 4.4 μmol). The vial was capped and purged with argon (×5). Reactants were dissolved in THF (0.41 mL, degassed), after which K_3PO_4 (0.82 mL, 0.5 M, degassed) was added and the reaction was stirred at 80 °C for 5 h under argon. The reaction was treated with water and extracted with EtOAc (×5), washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The title compound was obtained after flash chromatography (EtOAc/PE, 1:3) as a cloudy viscous oil (25 mg, 26% yield): R_f = 0.20 (EtOAc/PE, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 7.3 Hz, 1H), 7.41−7.28 (m, 8H), 7.27−7.22 (m, 2H), 7.17−7.06 (m, 2H), 6.23 (s, 1H), 5.17−5.03 (m, 1H), 3.90 (s, 3H), 3.41 (dd, J = 6.4, 12.1 Hz, 1H), 3.29−3.19 (m, 1H), 1.98−1.89 (m, 1H), 1.20−1.12 (m, 2H), 1.06−0.97 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 170.6, 161.2, 139.4, 134.1, 133.3, 131.5, 129.9, 129.9, 129.6, 129.3, 128.8, 128.7, 128.5, 127.9, 127.5, 127.4, 114.4, 65.9, 65.8, 53.0, 31.4, 8.4, 7.8; HRMS (ESI) calcd for $C_{30}H_{25}ClN_2O_4S$ (M + H$^+$) 545.1296, found 545.1309.

Step 4: The title compound **32** was prepared as described for **9** using methyl ester **32a** (27 mg, 49 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (19 mg, 75% yield): R_f = 0.17 (EtOAc/PE, 1:1 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 6.8 Hz, 1H), 7.40−7.27 (m, 8H), 7.25−7.19 (m, 2H), 7.18−7.09 (m, 2H), 6.27 (s, 1H), 5.25−5.12 (m, 1H), 3.52−3.32 (m, 2H), 1.99−1.87 (m, 1H), 1.20−1.14 (m, 2H), 1.05−0.99 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 172.0, 171.0, 161.2, 138.9, 133.6, 131.5, 130.01, 129.95, 129.7, 129.5, 128.7, 128.5, 127.6, 127.4, 114.3, 66.3, 66.0, 31.2, 8.5, 7.8; HRMS (ESI) calcd for $C_{29}H_{23}ClN_2O_4S$ (M + Na$^+$) 553.0959, found 553.0965. HPLC: t_R = 13.58 min, 98.8%.

(2*R*,4*R*)-3-(4-(3,5-Dimethylisoxazol-4-yl)benzoyl)-2-phenylthiazolidine-4-carboxylic Acid (33). *Step 1:* The methyl ester of the title compound (**33a**) was prepared as described for **8a** using **7g** (55 mg, 0.25 mmol) and 4-(3,5-dimethylisoxazol-4-yl)benzoyl chloride (64 mg, 0.27 mmol) and obtained after flash chromatography (EtOAc/PE, 2:5) as a clear viscous oil (30 mg, 29% yield): R_f = 0.22 (EtOAc/PE, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65−7.54 (m, 2H), 7.45−7.36 (m, 2H), 7.36−7.30 (m, 2H), 7.30−7.24 (m, 1H), 7.19−7.05 (m, 2H), 6.09 (br s, 1H), 5.19 (app s, 1H), 3.86 (s, 3H), 3.50−3.23 (m, 2H), 2.36 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 170.4, 165.7, 158.5, 141.2, 134.7, 133.1, 128.9, 128.6, 128.1, 127.8, 126.9, 116.0, 68.3, 65.2, 52.9, 32.4, 11.7, 10.9; HRMS (ESI) calcd for $C_{23}H_{23}N_2O_4S$ (M + H$^+$) 423.1373, found 423.1381.

Step 2: The title compound **33** was prepared as described for **9** using methyl ester **33a** (30 mg, 71 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 1:1 [2% AcOH]) and subsequent freeze-drying as a white amorphous powder (18 mg, 61% yield): R_f = 0.14 (EtOAc/PE, 1:1 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56−7.34 (m, 4H), 7.33−7.20 (m, 3H), 7.19−7.08 (m, 2H), 6.12 (br s, 1H), 5.24 (app s, 1H), 3.50−3.37 (m, 2H), 2.36 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 171.9, 165.8, 158.5, 140.5, 134.1, 133.5, 129.0, 128.8, 128.3, 127.8, 126.7, 116.0, 68.9, 65.3, 31.7, 11.7, 10.9; HRMS (ESI) calcd for $C_{22}H_{21}N_2O_4S$ (M + H$^+$) 409.1217, found 409.1228. HPLC: t_R = 10.98 min, 99.9%. $[\alpha]^{20}_D$ +44° (c 0.3, DCM).

(2*R*,4*R*)-3-(4-(3,5-Dimethylisoxazol-4-yl)benzoyl)-2-(2-ethynylphenyl)thiazolidine-4-carboxylic Acid (34). *Step 1: Methyl (4R)-2-(2-Ethynylphenyl)thiazolidine-4-carboxylate (**7h**).* The thiazolidine was prepared as described for **7a** using L-cysteine methyl ester hydrochloride (352 mg, 2.05 mmol) and 2-ethynylbenzaldehyde (315 mg, 2.42 mmol). The crude was filtered through silica (EtOAc/PE, 1:3) and concentrated in vacuo to give the crude **7h**, which was used in the next step without further purification.

*Step 2: Methyl (2R,4R)-3-(4-(3,5-Dimethylisoxazol-4-yl)benzoyl)-2-(2-ethynylphenyl)thiazolidine-4-carboxylate (**34a**).* Using crude **7h** (69 mg) and 4-(3,5-dimethylisoxazol-4-yl)benzoyl chloride (72 mg, 0.31 mmol), the title compound was prepared as described for **8a** and obtained after flash chromatography (EtOAc/PE, 1:2) as a clear oil (26 mg, 21% yield): R_f = 0.26 (EtOAc/PE, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 7.8 Hz, 1H), 7.48−7.43 (m, 1H), 7.40 (d, J = 7.3 Hz, 1H), 7.36−7.20 (m, 3H), 7.17−6.98 (m, 2H), 6.38 (br s, 1H), 5.21−5.04 (m, 1H), 3.90 (s, 3H), 3.46−3.39 (m, 1H), 3.31 (s, 1H), 3.36−3.22 (m, 1H), 2.34 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.5, 165.7, 158.5, 144.4, 134.3, 133.1, 132.9, 129.6, 128.9, 127.9, 127.6, 126.4, 119.5, 116.1, 84.9, 80.6, 66.4, 65.7, 52.9, 31.8, 11.6, 10.8; HRMS (ESI) calcd for $C_{25}H_{23}N_2O_4S$ (M + H$^+$) 447.1373, found 447.1391.

Step 3: The title compound **34** was prepared as described for **9** using methyl ester **34a** (27 mg, 60 μmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 2:1 [1% AcOH]) and subsequent freeze-drying as a brownish amorphous powder (18 mg, 70% yield): R_f = 0.16 (EtOAc/PE, 3:1 [1% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.59 (br s, 1H), 8.03 (d, J = 6.6 Hz, 1H), 7.48−7.36 (m, 2H), 7.36−7.20 (m, 3H), 7.17−7.03 (m, 2H), 6.43 (br s, 1H), 5.27−5.19 (m, 1H), 3.53−3.41 (m, 2H), 3.30 (s, 1H), 2.35 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 171.8, 165.8, 158.5, 143.7, 133.8, 133.3, 133.2, 129.8, 129.1, 128.0, 127.6, 126.1, 119.5, 116.1, 85.0, 80.5, 66.9, 65.9, 31.5, 11.6, 10.8; HRMS (ESI) calcd for $C_{24}H_{20}N_2NaO_4S$ (M + Na$^+$) 455.1036, found 455.1058. HPLC: t_R = 11.25 min, 99.0%. $[\alpha]^{20}_D$ +84° (c 0.2, DCM).

(2*R*,4*R*)-3-(4-(3,5-Dimethylisoxazol-4-yl)benzoyl)-2-(2-methoxyphenyl)thiazolidine-4-carboxylic Acid (35). *Step 1:* The methyl ester of the title compound (**35a**) was prepared as described for **8a** using **7f** (69 mg, 0.27 mmol) and 4-(3,5-dimethylisoxazol-4-yl)benzoyl chloride (71 mg, 0.30 mmol) and obtained after flash chromatography (EtOAc/PE, 1:2) as a clear oil (91 mg, 74% yield): R_f = 0.26 (EtOAc/PE, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 6.0 Hz, 1H), 7.47−7.33 (m, 2H), 7.34−7.26 (m, 1H), 7.18−7.02 (m, 3H), 6.84 (d, J = 8.1 Hz, 1H), 6.26 (br s, 1H), 5.17−5.07 (m, 1H), 3.89 (br s, 3H), 3.73 (br s, 3H), 3.44−3.32

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(m, 1H), 3.29−3.18 (m, 1H), 2.35 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.5, 165.5, 158.3, 155.2, 134.3, 132.9, 130.5, 129.2, 128.6, 127.8, 126.9, 120.9, 115.9, 110.6, 65.6, 63.1, 55.5, 52.7, 31.5, 11.6, 10.7; HRMS (ESI) calcd for $C_{24}H_{25}N_2O_5S$ (M + H$^+$) 453.1479, found 453.1459.

Step 2: The title compound **35** was prepared as described for **9** using methyl ester **35a** (91 mg, 0.20 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc/PE, 3:1 [1% AcOH]) and subsequent freeze-drying as a white amorphous powder (41 mg, 46% yield): R_f = 0.12 (EtOAc/PE, 3:1 [1% AcOH]); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (br s, 1H), 8.11 (d, J = 4.8 Hz, 1H), 7.69−7.39 (m, 1H), 7.36−7.16 (m, 4H), 7.08−6.87 (m, 2H), 6.18 (br s, 1H), 4.98−4.88 (m, 1H), 3.91−3.73 (m, 1H), 3.62 (s, 3H), 3.33−3.05 (m, 1H), 2.36 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 169.3, 165.5, 157.9, 155.0, 134.5, 131.7, 129.8, 128.9, 128.3, 126.9, 126.6, 120.3, 115.2, 110.8, 64.9, 61.7, 55.5, 31.3, 11.2, 10.3; HRMS (ESI) calcd for $C_{23}H_{23}N_2O_5S$ (M + H$^+$) 439.1322, found 439.1318. HPLC: t_R = 11.11 min, 96.4%. $[\alpha]^{20}_D$ +89° (c 1.0, MeOH).

(2*R*,4*R*)-3-(4-(3,5-Dimethylisoxazol-4-yl)benzoyl)-2-(pyridin-2-yl)thiazolidine-4-carboxylic Acid (36). *Step 1:* The methyl ester of the title compound (**36a**) was prepared as described for **8a** using **7e** (70.0 mg, 0.31 mmol) and 4-(3,5-dimethylisoxazol-4-yl)benzoyl chloride (77 mg, 0.33 mmol) and obtained after flash chromatography (EtOAc/PE, 2:1) as a brownish viscous oil (95 mg, 73% yield): R_f = 0.21 (DCM [4% MeOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55−8.37 (m, 1H), 8.16−8.00 (m, 1H), 7.83−7.64 (m, 1H), 7.57−6.74 (m, 5H), 6.41* (br s, 1H^1), 6.07 (app m, 1H^1), 5.63−5.52* (m, 1H^2), 5.35−4.94 (m, 1H^2), 3.82 (br s, 3H^3), 3.64* (s, 3H^3), 3.52−3.16 (m, 2H), 2.31 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.4, 165.6, 158.3, 149.6, 149.4, 137.1, 134.3, 133.1, 128.9, 127.6, 122.7, 120.6, 115.8, 69.4, 65.0, 52.9, 52.8, 31.8, 11.6, 10.7; HRMS (ESI) calcd for $C_{22}H_{22}N_3O_4S$ (M + H$^+$) 424.1326, found 424.1317.

Step 2: The title compound **36** was prepared as described for **9** using methyl ester **36a** (51 mg, 0.12 mmol) and LiI (4 equiv) and obtained after flash chromatography (EtOAc [5% AcOH]) and subsequent freeze-drying as a brownish amorphous powder (32 mg, 65% yield): R_f = 0.15 (EtOAc [5% AcOH]); ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.61−8.30 (m, 2H), 7.99−7.68 (m, 2H), 7.46−7.11 (m, 4H), 6.54* (s, 1H), 6.11 (br s, 1H), 5.08 (app s, 1H), 4.86* (app s, 1H), 3.66−3.35 (m, 2H), 2.46−2.33 (m, 3H), 2.29−2.14 (m, 3H); ^{13}C NMR (101 MHz, MeOH-d_4) δ 176.0, 172.4, 167.4, 161.7, 160.8, 159.8, 149.2, 148.4, 139.7, 137.0, 136.5, 133.8, 130.2, 128.9, 128.4, 124.4, 123.6, 117.2, 70.3, 69.1, 36.5, 33.5, 11.4, 10.6; HRMS (ESI) calcd for $C_{21}H_{20}N_3O_4S$ (M + H$^+$) 410.1169, found 410.1153. HPLC: t_R = 8.86 min, 97.9%. $[\alpha]^{20}_D$ +116° (c 0.5, DCM).

Synthesis of (2*S*,5*R*)-5-(2-Chlorophenyl)-1-(4-(3,5-dimethylisoxazol-4-yl)benzoyl)pyrrolidine-2-carboxylic Acid (37). *Step 1: Methyl (2S,5R)-5-(2-Chlorophenyl)-1-(4-(3,5-dimethylisoxazol-4-yl)benzoyl)pyrrolidine-2-carboxylate (37a).* In a predried vial, to a suspension of 4-(3,5-dimethylisoxazol-4-yl)benzoic acid (24 mg, 0.11 mmol; see the Supporting Information) and BTFFH (40 mg, 0.13 mmol) in dry DCM (110 μL) under argon was added DIPEA (65 μL, 0.38 mmol), and the reaction was stirred for 2 h at rt. To the in situ generated acid fluoride was added **6** (20 mg, 83 μmol) dissolved in dry DCM (100 μL). The vial was sealed, and the reaction was heated to 80 °C and stirred overnight. The reaction mixture was cooled to rt, water was added, the aqueous phase was extracted with EtOAc (×5), and the combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to obtain **37** after flash chromatography (EtOAc/PE, 1:2) as a clear viscous oil (32 mg, 86%): R_f = 0.14 (EtOAc/PE, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 7.7 Hz, 1H), 8.16−8.08 (m, 1H)*, 7.65−7.54 (m, 2H)*, 7.34−7.24 (m, 3H), 7.19−7.09 (m, 2H), 7.02 (d, J = 7.9 Hz, 2H), 5.69−5.57 (m, 1H)*, 5.41−5.31 (m, 1H), 4.81 (t, J = 7.2 Hz, 1H), 4.58−4.50 (m, 1H)*, 3.89 (s, 3H), 3.71 (s, 3H)*, 2.47−2.35 (m, 2H), 2.32 (s, 3H), 2.18 (s, 3H), 2.17−2.09 (m, 1H), 2.03−1.95 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 170.7, 165.6, 158.6, 140.4, 135.0, 132.2, 131.7, 129.2, 128.7, 128.6, 127.6, 127.3, 116.2, 61.6, 61.2, 52.5, 34.4, 27.6, 11.5, 10.7; HRMS (ESI) calcd for $C_{24}H_{24}ClN_2O_4$ (M + H$^+$), 439.1419; found, 439.1420.

Step 2: (2S,5R)-5-(2-Chlorophenyl)-1-(4-(3,5-dimethylisoxazol-4-yl)-benzoyl)pyrrolidine-2-carboxylic Acid (37). Ester **37a** (16 mg, 37 μmol) was dissolved in freshly distilled THF (0.4 mL), $LiOH_{aq}$ (0.12 mL, 0.6 M) was added, and the reaction was run at rt until full conversion of starting material as indicated by HPLC. The reaction mixture was quenched with HCl (1 M) until pH 2, diluted with water, extracted with EtOAc (×5), and the organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound **37** as a cloudy viscous oil (15 mg, 93% yield): R_f = 0.23 (EtOAc/PE, 1:2 [2% AcOH]); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73−7.59 (m, 1H), 7.33−7.21 (m, 3H), 7.17−7.03 (m, 4H), 5.43 (t, J = 6.2 Hz, 1H), 4.97 (t, J = 6.5 Hz, 1H), 2.53−2.25 (m, 3H), 2.32 (s, 3H), 2.19 (s, 3H), 2.04−1.97 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 172.8, 165.7, 158.6, 139.5, 134.0, 132.9, 131.8, 129.4, 128.9, 128.1, 127.9, 127.3, 116.1, 62.6, 62.3, 34.2, 26.6, 11.6, 10.7; HRMS (ESI) calcd for $C_{23}H_{21}ClN_2NaO_4$ (M + Na$^+$) 447.1082, found 447.1066. HPLC: t_R = 11.32 min, 99.8%. $[\alpha]^{20}_D$ −33° (c 0.4, DCM).

Crystallography. Data for **14** were collected at 100(1) K on a Synergy, Dualflex, AtlasS2 diffractometer using Cu Kα radiation (λ = 1.541 84 Å) and the CrysAlis PRO 1.171.39.12b suite.[50] Using SHELXLE,[51] the structure was solved by dual space methods (SHELXT[52]) and refined on F^2 using all the reflections (SHELXL-2016[53]). All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters. Hydrogen atoms were inserted at calculated positions using a riding model except for the H1 of the carboxylate group, which was located in the difference map and its coordinates were refined. The absolute configuration was unambiguously established from the crystallographic data. Parameters for data collection and refinement are summarized in Table S1.

Molecular Modeling. A homology model of hFFA2 in complex with **1** was obtained as described previously.[32] The model was based on a crystal structure of hFFA1 (PDB code 4PHU).[54] As **31** fully displaces [^{3}H]-**1** at increasing concentrations in a radioligand binding assay (Table 4), the compound was docked into the binding site defined by **1**. Prior to docking, **31** was prepared (LigPrep, version 2.7, Schrödinger, LLC) using the OPLS-2005 force field.[55] Ionization states were generated using Epik at pH 7.0 ± 2.0 (Epik, version 2.5, Schrödinger, LLC). Induced-fit docking was performed using the IFD 2006 protocol (Glide version 5.9, Schrödinger, LLC; Prime version 3.2, Schrödinger, LLC). Ligand conformational sampling was executed using default settings; initial Glide docking was performed using standard settings; the maximum number of poses of **31** was restricted to 20. Redocking was executed for **31**-hFFA2 complexes within 30 kcal/mol of the lowest energy ligand−protein complex. Residues were refined within 5 Å of bound **31**.

In Vitro Assays. β-Arrestin-2 Interaction Assay. HEK293T cells were maintained in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 1× penicillin/streptomycin mixture (Sigma) at 37 °C and 5% CO_2. Cells were grown to 60% density and co-transfected at a 4:1 ratio with plasmids encoding an eYFP-tagged form of the receptor construct of interest and a β-arrestin-2 fused to Renilla luciferase using a polyethyleneimine-based transfection protocol.[56,57] Cells were transferred into white 96-well plates at 24 h post-transfection and incubated for 24 h at 37 °C. Immediately prior to conducting the assay, cells were washed, and the culture medium was replaced with Hanks' balanced salt solution. To quantify β-arrestin-2 recruitment to the receptor induced by FFA2 agonists, the Renilla luciferase substrate coelenterazine h (Nanolight Tech, Pinetop, CA) was added to a final concentration of 2.5 μM, and cells were incubated for 5 min at 37 °C. Next, varying concentrations of agonist were added, and cells were incubated for an additional 10 min at 37 °C. BRET resulting from receptor-β-arrestin-2 interaction was assessed by measuring the ratio of luminescence at 535 and 475 nm using a PHERAstar FS plate reader fitted with the BRET1 optic module (BMG Labtech, Aylesbury, U.K.). pEC_{50} values were determined with at least three independent replicates.

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cAMP Assay. All cAMP experiments were performed using Flp-In T-REx 293 cells modified to express receptors of interest in an inducible manner, which were maintained in DMEM without sodium pyruvate supplemented with 10% fetal bovine serum, 1× penicillin/streptomycin mixture, 5 μg/mL blasticidin, and 200 μg/mL hygromycin B.[58] Experiments were carried out using a homogeneous time-resolved FRET-based detection kit (Cis-Bio Bioassays, Codolet, France) in accordance with the manufacturer's protocol. Cells were plated at 2000 cells/well in low-volume white 384-well plates. The ability of compounds to inhibit 1 μM forskolin-induced cAMP production was quantified following a 30 min incubation of agonist compounds with cells, which were induced to express receptors of interest by a 16 h treatment with 100 ng/mL doxycycline. pEC_{50} values were determined with at least three independent replicates.

Radioligand Binding Assay. Radioligand competition binding experiments were performed as established previously.[41] The radioligand [^{3}H]-**1** at approximately K_d concentration was incubated with varying concentrations of unlabeled compounds and 5 μg of purified membranes isolated from Flp-In T-REx cells induced to express the receptor construct of interest. Nonspecific binding of the radioligand was determined in the presence of 10 μM CATPB.[30] After a 2 h incubation at 25 °C, bound [^{3}H]-**1** and free [^{3}H]-**1** were separated by rapid vacuum filtration and radioactivity was quantified by liquid scintillation spectrometry. To determine the affinity of unlabeled ligands in terms of K_i values, competition binding curves were fit to an inverse three-parameter sigmoidal one-site K_i value fit with radioligand affinity and concentration as constraints. K_i values were determined with $n = 6$ independent experiments for **31** and with $n = 3$ for all other compounds.

Isolation of Human Neutrophils and Migration Assay. Neutrophils were isolated from human whole blood as described previously.[59] After isolation, neutrophils were immediately resuspended in RPMI 1640 containing 0.5% fatty acid-free bovine serum albumin. Test compounds were prepared at the indicated concentrations in the same buffer and added at the bottom of a 96-well plate (Sigma-Aldrich). Inserts were then mounted to the plate, and neutrophils were added (3000 cells/μL). Cells were incubated at 37 °C for 1.5 h, and migrated cells were then collected and ATP content was assessed using ATPlite luminescence assay system (PerkinElmer) according to the manufacturer's instructions. Each experiment was performed with $n = 4–6$ independent replicates.

Derivation of Primary Mouse Adipocytes and Lipolysis Assay. Epididymal fat was collected from male mice, and isolation of preadipocytes was obtained as described previously.[59] Animals were cared for in accordance with national guidelines on animal experimentation. Preadipocytes were subsequently differentiated in DMEM medium (DMEM, 10% fetal bovine serum, 4 mM glutamine, 10 mM HEPES, 10 μg/mL insulin, 25 μg/mL sodium ascorbate, 10 μM rosiglitazone) for 8 days. After this, mature adipocytes were challenged with test compounds and glycerol production was quantified as previously reported.[59] Each experiment was performed with $n = 3–4$ independent replicates.

Chemical Stability. A 10 mM stock solution of **31** in DMSO was diluted in phosphate buffer (10 mM, pH = 7.4) obtaining 1.2 mL of 50 μM compound solution in phosphate buffer. The sample was shaken (650 rpm) at 37 °C, and samples were taken out at different time intervals during the 3-week experiment. The study was performed in duplicate, each parallel and with double injection. The stability was calculated based on peak area of the 0 point sample.

Stability in Simulated Gastric and Intestinal Fluids. FaSSIF and FaSSGF were prepared in accordance with the manufacturer's procedure. A 50 μM solution of **31** was prepared from FaSSIF/FaSSGF and a 10 mM stock solution of **31** in DMSO, and the samples were incubated in a thermomixer (37 °C, 650 rpm) for 0 and 120 min. Afterward, the samples were quenched with acetonitrile (+0.5% HCOOH), centrifuged for 10 min at 10 000 rpm and the supernatant was analyzed by HPLC. The stability was calculated based on peak area of the 0 point sample. The study was performed in duplicate. Average values are reported.

Microsomal Stability. Microsomal stability was studied in mouse liver microsomes (0.5 mg/mL) at a final test compound concentration of 1 μM, essentially as previously described.[60] In brief, prewarmed (37 °C) 0.1 M $PBS_{7.4}$ was added to 10 mM NADPH in PBS and test compound **31** (1 mM in DMSO). The samples were incubated for 5 min at 37 °C before addition of newly thawn microsomes. The samples were mixed by gentle vortexing and incubated for 1 h at 37 °C, 300 rpm in a thermomixer. Samples were quenched by addition of ice-cold MeOH/MeCN (1:1) and centrifuged for 5 min at 10 000*g*. The supernatant was transferred to HPLC vials and stored in the freezer until analysis by HPLC. The metabolic stability was calculated based on the 0 min sample. The experiment was performed in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b00855.

Synthetic procedures, collection and crystallographic data for **14**, and high- and low-temperature ^{1}H and ^{13}C NMR spectra of **31** (PDF)

Coordinates for crystal structure of **14** (PDB)

Molecular strings formula and some data (CSV)

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Notes

The authors declare no competing financial interest.

CCDC 1844850 contains 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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■ ABBREVIATIONS USED

BRET, bioluminescence resonance energy transfer; BTFFH, *N,N,N′,N′*-bis(tetramethylene)fluoroformamidinium hexafluorophosphate; DIPEA, *N,N*-diisopropylethylamine;

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FaSSGF, fasted state simulated gastric fluid; FFA1, free fatty acid receptor 1 (GPR40); FFA2, free fatty acid receptor 2 (GPR43); FFA3, free fatty acid receptor 3 (GPR41); LLE, lipophilic ligand efficiency; nr, no response; PE, petroleum ether; PYY, peptide tyrosine tyrosine; SCFA, short-chain fatty acid.

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