

BAY-9835: Discovery of the First Orally Bioavailable ADAMTS7 Inhibitor

Daniel Meibom,* Pierre Wasnaire, Kristin Beyer, Andreas Broehl, Yolanda Cancho-Grande, Nadine Elowe, Kerstin Henninger, Sarah Johannes, Natalia Jungmann, Tanja Krainz, Niels Lindner, Stefanie Maassen, Bryan MacDonald, Denis Menshykau, Joachim Mittendorf, Guzman Sanchez, Martina Schaefer, Eric Stefan, Afra Torge, Yi Xing, and Dmitry Zubov



Cite This: *J. Med. Chem.* 2024, 67, 2907–2940



Read Online

ACCESS |



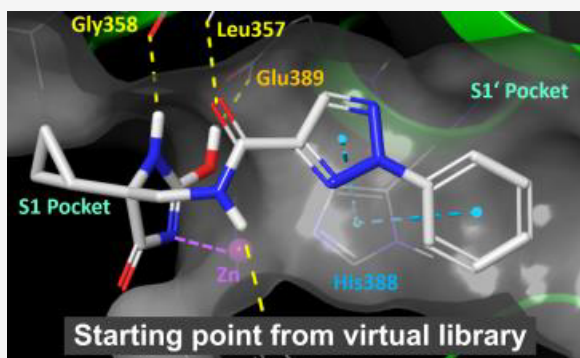
Metrics & More



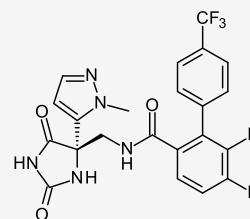
Article Recommendations



Supporting Information



Structure guided
optimization of
selectivity



BAY-9835

ADAMTS7-IC₅₀ 6 nM

ADAMTS4/5 > 1120x, ADAMTS12 5x

ADAM8/10/17 > 370x, MMP2/12/14/15 > 895x

Cl_{rat} 0.6 L/kg/h, F_{rat} 96%

ABSTRACT: The matrix metalloprotease ADAMTS7 has been identified by multiple genome-wide association studies as being involved in the development of coronary artery disease. Subsequent research revealed the proteolytic function of the enzyme to be relevant for atherogenesis and restenosis after vessel injury. Based on a publicly known dual ADAMTS4/ADAMTS5 inhibitor, we have in silico designed an ADAMTS7 inhibitor of the catalytic domain, which served as a starting point for an optimization campaign. Initially our inhibitors suffered from low selectivity vs MMP12. An X-ray cocrystal structure inspired us to exploit amino acid differences in the binding site of MMP12 and ADAMTS7 to improve selectivity. Further optimization composed of employing 5-membered heteroaromatic groups as hydantoin substituents to become more potent on ADAMTS7. Finally, fine-tuning of DMPK properties yielded BAY-9835, the first orally bioavailable ADAMTS7 inhibitor. Further optimization to improve selectivity vs ADAMTS12 seems possible, and a respective starting point could be identified.

INTRODUCTION

Atherosclerosis remains a leading cause of death worldwide¹ despite widespread statin use and more recent advances like PCSK9 targeting.² Unsurprisingly, much effort is spent to identify new targets involved in atherogenesis to meet unmet medical needs.³ Genome-wide association studies to reveal the genetics of coronary artery disease (CAD) have yielded until now undrugged targets that might play a role in CAD^{4,5} and potentially beyond. Among those targets is ADAMTS7 (a disintegrin and metalloproteinase with thrombospondin type 1 repeats 7)⁶ with the leading single nucleotide polymorphism (SNP) rs3825807^{7,8} resulting in a serine to proline switch in the prodomain of the enzyme. The wild-type serine containing ADAMTS7 could be related to higher cardiovascular (CV) mortality⁹ and other secondary CV events^{10,11} compared to the low-frequency mutant proline variant. Furthermore, high ADAMTS7 levels in atherosclerotic lesions correlated with an increased risk for CV outcomes.¹² On a cellular level, it was

found that reduced proteolytic function of the proline variant was associated with less vascular smooth muscle (VSMC) and endothelial cell (EC) migration.^{13–17} Because VSMC and EC migration is an important component of atherogenesis and neointima generation after vascular injury,^{18,19} it seems likely that ADAMTS7 is involved in the formation of lipid as well as nonlipid derived stenoses. This hypothesis was further substantiated when it was found that knockout of ADAMTS7 reduced atherosclerosis in mice with a hyperlipidemic phenotype and neointima proliferation as compared to wild-type controls.^{20,21} According to a recent study, loss of the

Received: November 1, 2023

Revised: December 20, 2023

Accepted: December 22, 2023

Published: February 13, 2024



catalytic function of ADAMTS7 protects against atherogenesis via a phenotypic switch of VSMCs, suggesting that the catalytic domain should be an attractive target for therapeutic intervention^{22,23} to treat coronary artery disease as well as conditions associated with intima hyperplasia. Consequently, ADAMTS7 antibodies raised against the ADAMTS7 catalytic domain protected against stent-induced restenosis in a preclinical swine model.^{24,25} Furthermore, high ADAMTS7 levels were identified in heart tissue²⁰ and injured vessels,¹⁸ which is in line with the CAD protection conferred by the low risk allele and the intimal thickening induced by the high risk allele as detailed above. Collectively, the data suggest that ADAMTS7 inhibition might be a new way to treat CAD and restenosis.²⁶

ADAMTS7 belongs to a family of 19 secreted zinc metalloproteases^{27,28} with proteolytic activity against extracellular substrates^{15,29,30} including matrix proteins.³¹ The basic ADAMTS structure comprises a pro-domain, a catalytic domain, and an ancillary domain containing thrombospondin repeats which determine substrate specificity and localization.³² To the best of our knowledge, selective small molecule inhibitors targeting the catalytic domain of ADAMTS7 are unknown to date. According to a pharmacophoric model³³ comparing the catalytic site of ADAMTSs, selectivity against the majority of ADAMTSs should be possible. However, a recently published hydroxamate-based ADAMTS7 inhibitor³⁴ still shows strong inhibition of ADAMTS4 and ADAMTS5 in addition to its activity on other metalloproteases. We herein report the identification of BAY-9835, the first dual ADAMTS7/ADAMTS12 antagonist selective against a broader range of metalloproteases and with good oral bioavailability.

RESULTS AND DISCUSSION

Identification of a Suitable Starting Point. One of our lead finding strategies consisted of characterizing published ADAMTS inhibitors in our newly established ADAMTS7 assay^{35–37} containing the human catalytic domain. To the best of our knowledge, such an assay was not available elsewhere until recently.^{34,38} By using this approach, we hoped to identify so far undetected ADAMTS7 inhibitors. When we resynthesized and tested a published dual ADAMTS4/ADAMTS5 inhibitor,^{39,40} we were pleased to see strong potency vs ADAMTS7 (Figure 1). The structure of the ADAMTS5 inhibitor GLPG1972⁴¹ was not publicly known during the project and the same strategy could not be applied in this case.

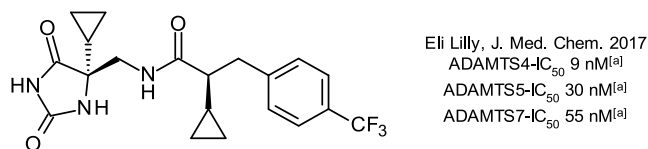


Figure 1. Published dual ADAMTS4/ADAMTS5 inhibitor (Compound 7 in *J. Med. Chem.* 2017, 5933) shows activity vs ADAMTS7. [a] See Supporting Information for details on IC₅₀ measurements.

Because no X-ray structure of ADAMTS7 is currently available, a homology model based on an ADAMTS4 template (PDB-ID: 4WKI) was generated and docking of the hydantoin-based compound revealed potential key interactions (Figure 2). The imine nitrogen from the hydantoin might bind to the catalytic zinc. The OH from the 5-membered ring could

make an H-bond to Glu389, while the NH from the hydantoin presumably employs an H-bond to Gly358. A hydrophobic contact in the S1 pocket is proposed for the cyclopropyl group attached to the heterocycle, and the amide NH potentially interacts with Pro417. Furthermore, docking suggests the carbonyl from the amide to form H-bonds with Gly358 and Leu357.

To build on these *in silico* identified key interactions, the cyclopropyl-methylamino substituted hydantoin was kept constant, and a virtual amide library with all acids/acid chlorides available on stock resulting in 6025 compounds was enumerated and docked. Taking docking score and visual examination of the docked poses into account, 62 amides were synthesized. Only one compound had an ADAMTS7-IC₅₀ below 100 nM, simultaneously showed reasonable selectivity vs ADAMTS4 and ADAMTS5 (Figure 3), and was thus characterized in more detail.

Inhibitor 1 revealed a clean profile against 77 off-targets (see Supporting Information), no activity on several serine proteases (FIIa, FXa, FXIa, TPA, Trypsin, Plasmin, Kallikrein), and medium to good selectivity vs MMP2, MMP15, and ADAM17. However, strong potency on ADAMTS12 and MMP12 was observed. In pharmacokinetic studies, the compound showed low clearance (Cl_{bl, rat} 0.7 L/kg/h), high oral bioavailability (F_{rat} 74%), and low protein binding (fu_{rat} 15%). Reactive metabolites identified on the phenyl ring after incubation with hepatocytes (see Supporting Information) were deemed manageable, e.g., by introduction of substituents in the para position. Taking potency, selectivity, PK, and physicochemistry (MW 340 g/mol, logD_{7.5} 1.8, HBD 3, HBA 5) into account, 1 was regarded as a good starting point for an optimization campaign.

Optimization of Selectivity vs MMP12. Given the strong binding of 1 to ADAMTS12 and MMP12, we first concentrated on synthesizing more selective ADAMTS7 inhibitors. The sequence identity of the ligand binding site between ADAMTS7 and ADAMTS12 is high^{42,43} (83%, 6 Å around the ligand) while low for ADAMTS7 and MMP12 (31%, 6 Å around the ligand). Therefore, we initially focused on selectivity vs MMP12 as the supposedly easier goal. As the docking pose of 1 into ADAMTS7 indicated potential π -stacking of the phenyltriazole with the enzyme (Figure 4) in addition to the above-suggested key interactions of the substituted hydantoin, more aryltriazole carboxamides were explored (Table 1) thus leaving the already established contacts with the enzyme potentially untouched.

In a first optimization round, we evaluated substituted 6-membered aromatic residues attached to the triazole which should not only provide more information on potency vs ADAMTS7 and selectivity vs MMP12 but also on how the respective moiety is metabolized (Table 1).

Compared to 1, *p*-fluoro (2) substitution improved potency and in addition also gave a compound that was no longer reactively metabolized. Further improvement of potency was achieved with a meta-fluoro (3) instead of a para-fluoro substituent. An ortho-fluoro substituent (4) significantly reduced potency by potentially impacting the dihedral angle between the triazole and the phenyl. To probe spatial and lipophilicity requirements, compounds 5–7 were synthesized. 5 was equipotent to the para-fluoro derivative 2, while the meta-chloro compound 6 was slightly less potent than its meta-fluoro counterpart 3. The sterically more demanding para-tolyl derivative 7 had a higher IC₅₀ than 5 but was still below 50

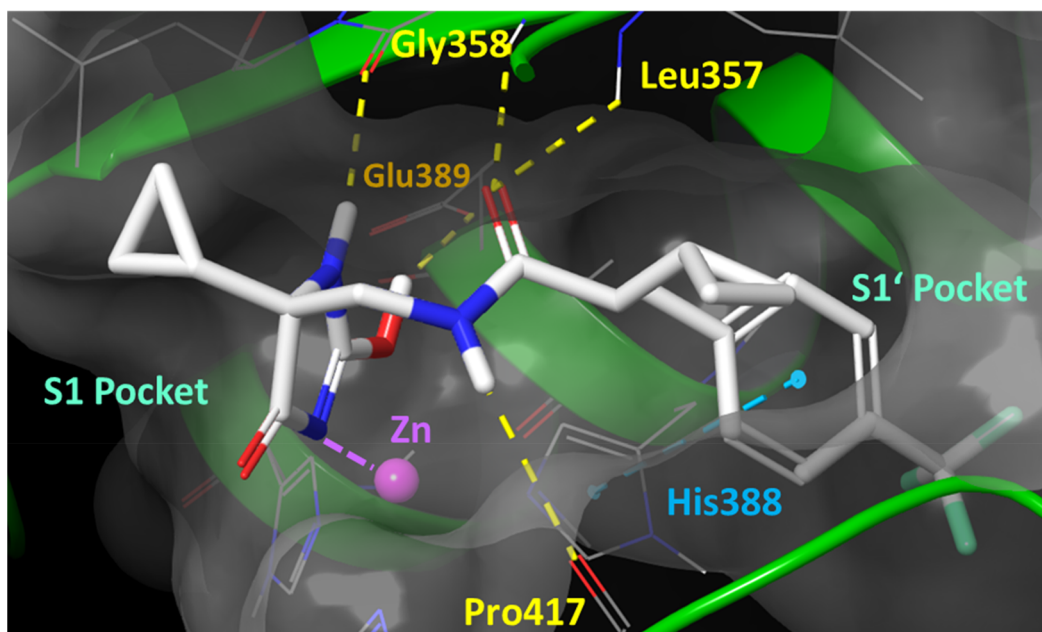
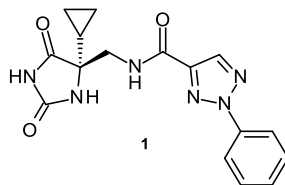


Figure 2. Binding pose of the compound from Figure 1 in an ADAMTS7 homology model based on the ADAMTS4 template with PDB-ID 4WKI. The inhibitor is depicted with gray carbon atoms, blue nitrogens, red oxygens, and light green fluorines. Glu-389, Gly-358, Leu-357, Pro-417, and His-388 are potentially directly interacting with the ligand. Hydrogen bonds are shown as dashed yellow lines. The putative interaction with the catalytic zinc atom is depicted as dashed purple and the π -stacking as dashed light blue line. The cyclopropyl and the substituted phenyl of the ligand might be buried within the S1 or the S1' pocket, respectively. The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).



| IC ₅₀ [nM] ^[a] | ADAMTS4 ^[b] | ADAMTS5 ^[b] | ADAMTS12 ^[b] | ADAM17 ^[b] | MMP2 ^[b] | MMP12 ^[b] | MMP15 ^[b] |
|--------------------------------------|------------------------|------------------------|-------------------------|-----------------------|---------------------|----------------------|----------------------|
| 61 | 24x | 84x | 0.2x | 27x | 76x | 0.2x | 331x |

Figure 3. Metalloprotease panel for compound 1, the starting point for the optimization campaign. [a] IC₅₀ vs ADAMTS7 enzyme, see Supporting Information. [b] Selectivity factors were calculated by dividing the IC₅₀ of a given metalloprotease by the ADAMTS7-IC₅₀, see Supporting Information for details on IC₅₀ measurements.

nM. The fluoropyridine **8** was clearly less active than the fluorophenyl **2** which might again be attributable to changes in the dihedral angle between the two aromatic rings of our inhibitors. On one hand, compounds **1–3** and **5–7** made us confident that it should be possible to identify additional potent compounds. On the other hand, we were worried that none of the inhibitors from Table 1 showed any significant difference in selectivity vs MMP12. An X-ray cocrystal structure of **3** in MMP12 overlaid with our ADAMTS7 homology model gave us first hints on how to obtain inhibitors more selective vs MMP12 (Figure 5).

Two amino acid differences between both enzymes were observed in proximity of two angstrom around the ligand. Near our 6-membered aromatic groups, Leu419 from ADAMTS7 is exchanged for Tyr240 in MMP12, while close to the cyclopropyl, Thr356 (ADAMTS7) is exchanged for Ile180 (MMP12). We first concentrated on exploiting the leucine/tyrosine exchange. Being encouraged by the overall good potency seen for the inhibitors from Table 1, we started

making broader variations in the amide portion like allowing more conformational flexibility or changing the exit vector between the two aromatic groups to potentially clash with Tyr240. Table 2 summarizes our most important findings.

Exchanging the phenyl of inhibitor **1** by an isothiazole (**9**) led to a drop in potency without impacting selectivity vs MMP12, as expected. Increasing three-dimensionality by attaching thiazoles (**10**, **11**) and a pyrazole (**12**) benzylically to the triazole further reduced potency but simultaneously increased selectivity. Changing the triazole of **1** by the pyrazole of **13** was detrimental to the ADAMTS7-IC₅₀. Attaching a benzylic group to the pyrazole (**14**) again further reduced potency and improved selectivity. Overall, the benzylic subseries never reached double digit nanomolar potency on ADAMTS7 and was thus abandoned despite being more selective than the potent examples from Table 1. To scan different exit vectors for the terminal phenyl groups typically present in our inhibitors, we synthesized compounds **15–17**. Compared to **7**, exchanging the triazole for a phenyl ring (**15**)

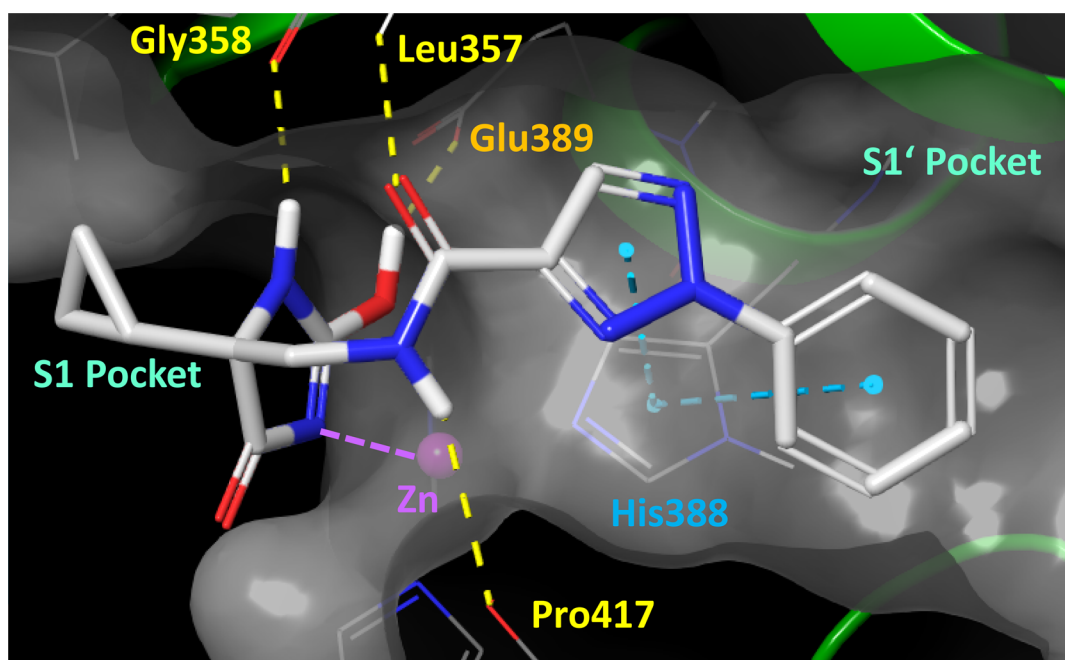


Figure 4. Binding pose of **1** in an ADAMTS7 homology model based on the ADAMTS4 template with PDB-ID 4WKI. The inhibitor is depicted with gray carbon atoms, blue nitrogens, and red oxygens. Glu-389, Gly-358, Leu-357, Pro-417, and His-388 might directly interact with the ligand. Hydrogen bonds are shown as dashed yellow lines. The potential interaction with the catalytic zinc atom is depicted as dashed purple and the π -stacking as dashed light blue lines. The cyclopropyl and the substituted phenyl of the ligand are presumably buried within the S1 or the S1' pocket, respectively. The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).

significantly reduced activity vs ADAMTS7 while increasing activity on MMP12. The meta tolyl substituted derivative **16** was even less potent vs ADAMTS7. However, with the ortho biphenyl containing hydantoin **17**, we had roughly regained the ADAMTS7-potency of **7** while improving MMP12-selectivity by a factor of 124 compared to **7**. Based on docking analysis of **17** in ADAMTS7 and MMP12, we hypothesize that the enhanced selectivity arises from increased negative steric interactions between the ortho biphenyl and the MMP12 tyrosine residue (Figure 6). Further characterization of **17** revealed that, compared to **1**, selectivity had increased not only vs MMP12 but also vs other metalloproteases (Table 3).

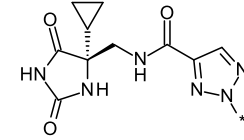
Unfortunately, this significant step forward was accompanied by a drop in metabolic stability. In vivo PK parameters of **17** ($Cl_{bl, rat}$ 1.8 L/kg/h, F_{rat} 54%) were inferior to those of **1** ($Cl_{bl, rat}$ 0.7 L/kg/h, F_{rat} 74%) which could be traced back to quick oxidation of the methyl group. Exchanging the CH_3 to H, F, or CN led to reduced activity vs ADAMTS7 of only 200 to 300 nM. However, the CF_3 -analogue **18** (Table 4) was only slightly less potent than **17** while showing very good PK parameters ($Cl_{bl, rat}$ 0.1 L/kg/h, F_{rat} 86%) and thus served as a starting point to further improve selectivity vs MMP12 by exploiting the Thr356 (ADAMTS7) Ile180 (MMP12) exchange mentioned above. In addition, increasing the size of the P1 substituent might improve the IC_{50} vs ADAMTS7 by addressing a subpocket in S1 (Figure 7).

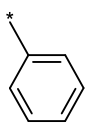
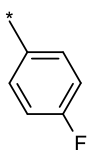
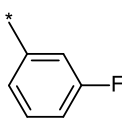
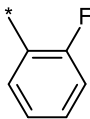
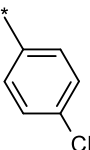
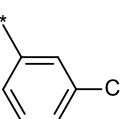
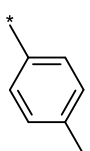
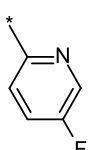
Several aliphatic residues on the hydantoin, including substituted cyclopropyls, were screened with modest success regarding potency vs ADAMTS7 (data not shown). From aromatic groups tested, 6-membered rings lacked potency as well, while 5-membered heteroaromatic moieties yielded much better results (Table 4).

Thiazole **19** was more potent vs ADAMTS7 and selective vs MMP12 than the cyclopropyl derivative **18**. The distomer of

19 and all other subsequently tested distomers were inactive vs ADAMTS7. The absolute stereochemistry of eutomers has not been elucidated up to this point. However, it can be assumed that the same stereochemistry is present for the eutomers as for cyclopropyl substituted hydantoins mentioned above. The α -methylsubstituted thiazole **20** yielded an ADAMTS7 inhibitor with further improved potency and selectivity. As reported by Durham et al.,^{44,45} for hydantoin-based dual ADAMTS4/ADAMTS5 inhibitors, the nature, and the substitution pattern of 5-membered heteroaromatic groups, can impact target enzyme potency and metalloprotease selectivity. This might be due to changes in the dihedral angle between the respective aromatic residue and the hydantoin and/or because of electronic differences among said moieties. Comparing **19** and **20**, we speculate that the methyl group of **20** impacts the dihedral angle resulting in a more favorable interaction with ADAMTS7. Strongly enlarging the substituent on the thiazole α to the attachment point of the hydantoin and simultaneously changing the electronics as in **21** significantly reduces potency vs ADAMTS7. The 1,3-dimethylthiazole **22** was slightly less potent and selective than **20**. Inhibitor **20** was abandoned after reactive metabolites were identified on the thiazole (see Supporting Information). The methylimidazole in **23** was the most potent hydantoin substituent that we could identify. However, suboptimal metabolic stability of **23** in vivo ($Cl_{bl, rat}$ 1.5 L/kg/h) and low exposure after oral administration ($AUC_{n, rat}$ 0.6 kg h/L) prompted us to continue our exploration. The low exposure seen for **23** might in part be attributable to polarity ($clogD_{7.5}$ 2.0) and basicity (calculated pK_a 4.4). We therefore focused on less polar and less basic pyrazoles like **24** ($clogD_{7.5}$ 2.2, calculated pK_a 1.0) as a next step. Compared to **23**, pyrazole substituted hydantoin **24** was slightly less potent vs ADAMTS7 and less selective vs MMP12. However, in vivo PK ($Cl_{bl, rat}$ 1.0 L/kg/h) as well as CYP3A4

Table 1. Initial SAR Exploration



| Compound | R | IC ₅₀ [nM] ^[a] | MMP12 ^[b] |
|----------|---|--------------------------------------|----------------------|
| 1 |  | 61 | 0.2 |
| 2 |  | 26 | 0.1 |
| 3 |  | 13 | 1.1 |
| 4 |  | 180 | 0.4 |
| 5 |  | 26 | 0.8 |
| 6 |  | 21 | 0.1 |
| 7 |  | 47 | 0.1 |
| 8 |  | 115 | 0.6 |

^aIC₅₀ vs ADAMTS7. ^bSelectivity factor vs MMP12 obtained by dividing the MMP12-IC₅₀ by the ADAMTS7-IC₅₀; see [Supporting Information](#) for details on IC₅₀ determination.

induction threshold concentration (**23**: 0.6 μ M, **24**: 16.6 μ M) had improved. Overall in vivo clearance depending on hydantoin substituents was increasing as follows: cyclopropyl < pyrazole < thiazole < imidazole. The pyrazole moiety therefore offered a good trade-off between potency/selectivity and PK properties. Enlarging the substituent on the pyrazole nitrogen from methyl to ethyl (**25**) decreased potency and

selectivity. As for **21**, incorporation of a sterically demanding CF₃-group (**26**) was detrimental to potency and selectivity. The 1,5-dimethylpyrazole **27** was synthesized to compare the impact of two methyl groups adjacent to each other with two methyl groups being further apart (**22**) on the SAR. Given the high IC₅₀ vs ADAMTS7 and the activity on MMP12, **27** was discarded. Further, α -methyl containing 5-membered hetero-aromatic groups like isoxazoles, an oxadiazole, regioisomeric thiazoles, an isothiazole, and a thiadiazole was screened (data not shown).³⁶ Taking ADAMTS7 potency, MMP12 selectivity, and DMPK into account, the methylpyrazole of inhibitor **24** was the best P1 substituent. In addition to weak binding to MMP12, activity of **24** on other metalloprotease off-targets was low as well ([Table 5](#)).

Retrospective docking calculations and WaterMap analysis of **24** within our ADAMTS7 homology model suggest placement of the *N*-methyl from the pyrazole in an S1 subpocket thereby potentially displacing an unstable water molecule ([Figure 8](#)). This interaction with the enzyme is not present in inhibitor **18** and might explain the improved potency vs ADAMTS7 of **24**. We further hypothesize that the methyl group binds close to or even clashes with Ile180 in MMP12, thus contributing to the selectivity of **24** (see [Supporting Information](#)).

Fine-tuning of DMPK Properties. From the *o*-biphenyl group, only the CF₃-phenyl residue had been optimized to a certain extent (see above) but not the “lower” phenyl ring. Furthermore, metabolite identification studies had shown that the amide substituted phenyl ring can be metabolized reactively by epoxidation and GSH conjugation (e.g., **18**, **23**, see [Supporting Information](#)). We therefore experimented with halogenations of the “lower” phenyl ring to further improve DMPK while hopefully retaining the already good ADAMTS7 potency and metalloprotease selectivity of **24** ([Table 6](#)).

The fluoro derivative **28** showed improved potency and in vivo PK properties compared to **24**. The regioisomers **29**, **30**, and **31** were less potent vs ADAMTS7 and were not further pursued. Unexpectedly, the combination of the two fluorines from **28** and **29** yielded an ADAMTS7 inhibitor (**32**, BAY-9835) that was similarly potent and metabolically stable than the monofluoro compound **28**. Obviously, the fluoro atom para to the amide in **32** was only having subtle effects on potency and PK in this case. The positive results of the additional substituent ortho to the “upper” phenyl ring (**28**) prompted us to evaluate more substituents in this position. However, neither the chloro (**33**) nor the methoxy (**34**) derivative were as potent as **28**. As a next step, we therefore kept the fluoro atom from **28** and evaluated more combinations analogous to **32**. We were first intrigued by the gain in potency of the fluorochloro derivative **35** and the almost neutral effect on the ADAMTS7 IC₅₀ of the additional CF₃-group in **36**. Unfortunately, the metalloprotease panel for **35** and **36** showed that employing substituents larger than fluorine para to the amide has led to reduced selectivities (data not shown). According to [Table 6](#), ADAMTS7 inhibitors **28** and **32** had the best profile. The metalloprotease panel showed very good results for both compounds as well and could not be used for differentiation. Furthermore, no reactive metabolites could be detected from hepatocyte incubations of **28** and **32**. When the slightly lower clearance seen for **32** in rats was also found in mice and dogs (**28**: Cl_{bl, mouse} 1.29 L/kg/h, Cl_{bl, dog} 0.08 L/kg/h; **32**: [Table 9](#)), we chose **32** (BAY-9835) as our best compound. According to [Table 7](#), **32** is exceptionally

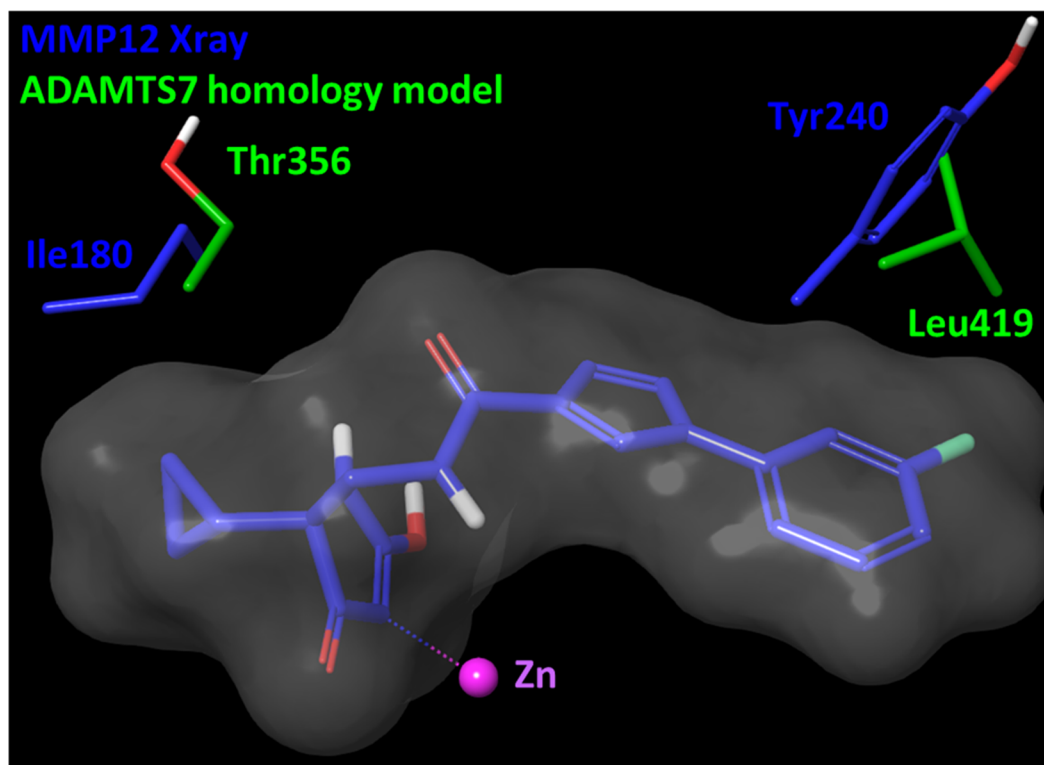


Figure 5. X-ray structure of **3** in cocrystal with MMP12 (atomic coordinates have been deposited under PDB entry 8RIJ) overlaid with an ADAMTS7 homology model based on the ADAMTS4 template with PDB-ID 4WKI. Amino acid differences between MMP12 and ADAMTS7 two angstrom around the ligand are indicated (other amino acids are hidden). MMP12 amino acids are shown in blue, ADAMTS7 amino acids in green. The interaction of the catalytic zinc with the ligand is depicted as a dotted line. The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).

selective vs a range of metalloproteases, except for ADAMTS12. Thus, **32** (BAY-9835) can be considered being a dual ADAMTS7/ADAMTS12 inhibitor. For clarity, we emphasize that all compounds discussed up to this point where an IC_{50} vs ADAMTS12 has been measured (**1**, **2**, **19**, **20**, **22–25**, **27–30**, **32–36**) showed strong activity on ADAMTS12. Further below, options will be discussed on how it might be possible to achieve selectivity vs ADAMTS12 within the lead series.

Synthesis of BAY-9835. For a detailed characterization, gram amounts of BAY-9835 (**32**) were prepared according to [Scheme 2](#) where hydantoin **41** is one of the starting materials. The synthesis of **41** is described in [Scheme 1](#) and began with a 3 + 2 cycloaddition⁴⁶ between ethyl isocyanoacetate and the commercially available pyrazole carboxylic acid **37** toward **38**. Acidic hydrolysis and decarboxylation converted **38** to the α -aminoketone **39**. BOC-protection yielded **40**, the starting material for the subsequent Bucherer Bergs reaction leading to **41**. For us, this sequence proved to be robust and was also used for other hydantoins like, e.g., those shown in [Table 4](#).

Racemic **41** was separated into its enantiomers by supercritical fluid chromatography ([Scheme 2](#)). The absolute configuration of the enantiomerically pure hydantoin **42** is likely as shown and should be identical to the stereo-configuration of the final product (see below). Removal of the BOC-group yielded **43**, one of the two amide coupling partners. Acid **46** was prepared by Suzuki reaction of commercial **44** and **45**. Finally, **32** (BAY-9835) was obtained by amide formation. The synthesis detailed in [Schemes 1](#) and [2](#)

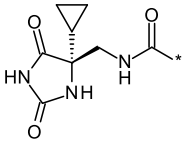
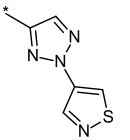
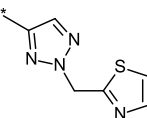
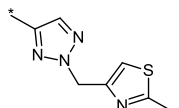
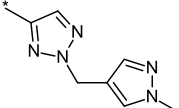
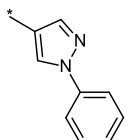
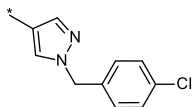
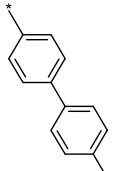
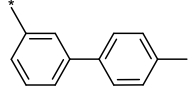
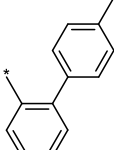
was used to prepare close to 2 g of **32** but was also followed to prepare 20 g (see [Supporting Information](#)).

Recrystallization of **32** from boiling ethanol gave crystalline material. Single crystal X-ray analysis (see [Supporting Information](#)) proved the structure of **32** to be as depicted in [Scheme 2](#).

Characterization of BAY-9835 (32**).** Solubility of crystalline **32** in buffered water at pH 7 is 135 mg/L (at 25 °C, after 24 h). Solubility in FaSSIF is 297 mg/L and in FeSSIF 513 mg/L (both at 37 °C, after 24 h). In the aqueous vehicle Solutol HS 15/ethanol/water 40/10/50 (v/v/v), solubility was much higher (30 g/L, crystalline material), enabling high exposures in a repeat-dose toxicological study in rats (see below). No instabilities of BAY-9835 (**32**) were observed in aqueous solutions at pH 1, pH 7, and pH 10 for 24 h at 37 °C. A PEG-based liquid service formulation could be stabilized by addition of an antioxidant (0.02% BHA) over a time period of 13 weeks at ambient and accelerated conditions. No instabilities were observed in a tablet blend after 13 weeks at two conditions (see [Supporting Information](#)). Further physicochemical characterization placed **32** well within traditional Rule of Five compliant chemical space ([Table 8](#)).

To be prepared for potential animal pharmacodynamic studies,^{47–49} we measured potencies of **32** vs mouse (mIC_{50} 8 nM) and rat (rIC_{50} 27 nM) ADAMTS7 enzymes. Furthermore, activity against several cysteine proteases (Calpain 1, Caspase 3, Cathepsin B, Cathepsin S, MALT1) was evaluated. As for the metalloprotease panel in [Table 7](#), again high selectivity factors were recorded (>16666 \times , see [Supporting Information](#)). Screening 77 off-targets unrelated to proteases

Table 2. Exploration of Increased Conformational Freedom and Exit Vectors

|  | | | |
|---|---|--------------------------------------|----------------------|
| Compound | R | IC ₅₀ [nM] ^[a] | MMP12 ^[b] |
| 9 |  | 230 | 0.2 |
| 10 |  | 910 | 4.5 |
| 11 |  | 930 | 33.8 |
| 12 |  | 850 | 29.4 |
| 13 |  | 129 | 0.03 |
| 14 |  | 280 | 7.4 |
| 15 |  | 580 | 4.7*10 ⁻⁴ |
| 16 |  | 2300 | n.d. |
| 17 |  | 60 | 12.4 |

^aIC₅₀ vs ADAMTS7. ^bSelectivity factor vs MMP12 obtained by dividing the MMP12-IC₅₀ by the ADAMTS7-IC₅₀; see [Supporting Information](#) for details on IC₅₀ determination.

for activity of **32** (see [Supporting Information](#)) showed no hit, underscoring the selectivity of BAY-9835 for ADAMTS7 and ADAMTS12.

Investigation of metabolites formed in hepatocytes of rats, dogs, and humans revealed no reactive pathways and no species-specific metabolites (see [Supporting Information](#)). In all three species, hydroxylation as well as traces of *N*-demethylation on the pyrazole was observed after an incubation time of 4 h. Blood clearance of BAY-9835 in hepatocytes was low (mouse/rat/dog/human 0.32/0.17/0.06/10⁻⁴ L/h/kg). Furthermore, high metabolic stability in an incubation with human microsomes (Cl_{bl} 10⁻⁴ L/h/kg) was observed and no degradation was found over 24 h when stirring our best compound in human plasma. Protein binding for **32** is low to moderate in several species including humans (fraction unbound: mouse 11%, rat 5%, dog 5%, human 15%). No inhibition of CYP1A2, 2C8, 2C9, 2D6, and 3A4 enzymes was detected up to 20 μM, and no induction of CYP1A2 was seen up to 61 μM while the CYP3A4 induction threshold was determined to 18 μM. No indication for genotoxicity (Ames, micronucleus test *in vitro*) or cytotoxicity was found for our best compound (see [Supporting Information](#)). Furthermore, cardiac ion channels (hERG, hNav1.5, hCav1.2) were not inhibited up to a concentration of 10 μM of **32**. The *in vivo* pharmacokinetic behavior was evaluated by iv and oral administration to mice, rats, and dogs. In all three species, clearance is low, particularly in dogs resulting in long terminal half-life. Bioavailability is high in all three species ([Table 9](#)).

In an exploratory two-week toxicological study in rats, **32** was well tolerated and the NOAEL was 50 mg/kg body weight administered once daily orally by gavage in Solutol HS 15/ethanol/water 40/10/50 (v/v/v). A recent publication on ADAMTS7^{-/-} ADAMTS12^{-/-} double knock out mice reported heterotopic ossification in tendons after the animals had reached four months of age.⁵⁰ We therefore included histopathological investigations of tendons and ligaments in our toxicological study. However, no indications for heterotopic ossification nor any other changes were observed in these tissues.

32 has not been tested in *in vivo* pharmacodynamic studies. Given potency, selectivity, protein binding, solubility, tolerability, and PK, an evaluation of ADAMTS7/ADAMTS12-mediated pharmacology should be feasible with BAY-9835 (**32**).

Toward ADAMTS7 Inhibitors with Selectivity vs ADAMTS12. ADAMTS12 is involved in various pathological processes including inflammation, cancer, and arthritis.⁴² Depending on the context, ADAMTS12 can have a protective or a deleterious role. For example, in a model of colitis, pancreatitis, and sepsis, knockout of ADAMTS12 resulted in aggravated inflammation.⁵¹ Conversely, elevated ADAMTS12 levels in the synovial fluid of osteoarthritis patients⁵² paired with increased COMP degradation⁵³ suggest a protective role for inhibition of ADAMTS12 in cartilage turnover. A complex situation is present in cancer where studies have found that ADAMTS12 can have an anti-^{54–58} or pro-tumoral^{59–64} effect. In conclusion, the consequences of blocking the catalytic function of ADAMTS12 are not entirely clear. To study ADAMTS7 mediated biology not overlaid by potential ADAMTS12 effects, selective ADAMTS7 inhibition is nevertheless desirable.

The high sequence identity of the ligand binding site between ADAMTS7 and ADAMTS12^{42,43} (83%, 6 Å around

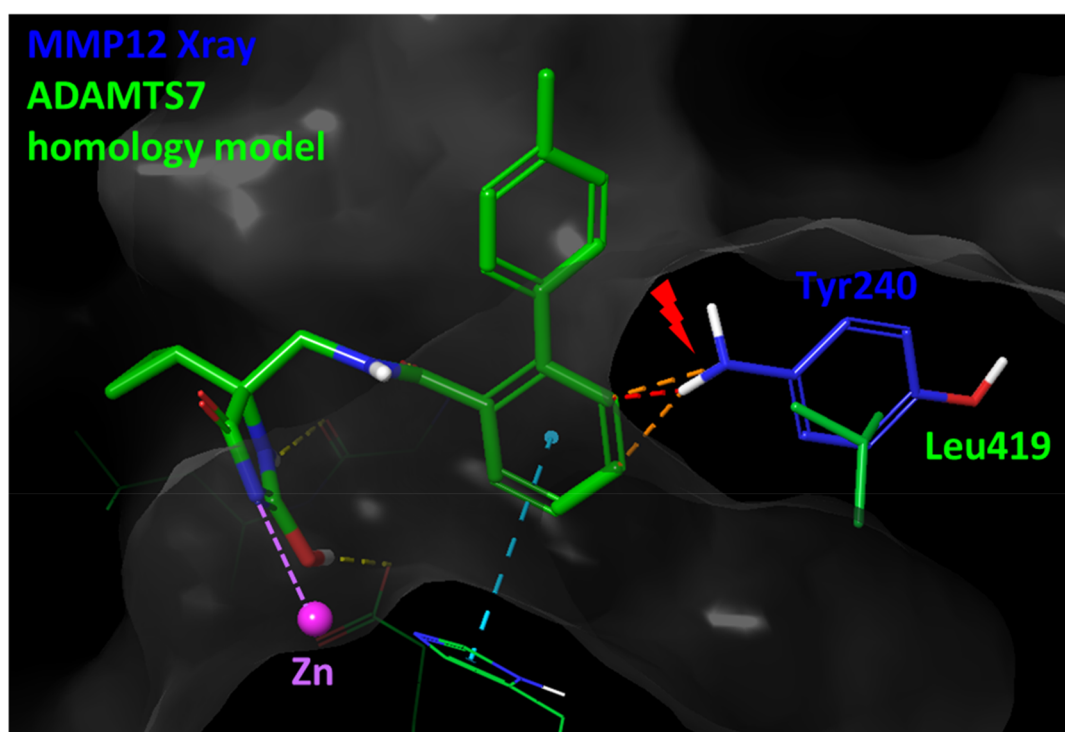


Figure 6. Docking of 17 in an ADAMTS7 homology model (based on the ADAMTS4 template with PDB-ID 4WKI) overlaid with the MMP12 X-ray obtained with 3 (atomic coordinates have been deposited under PDB entry 8RIJ). Relevant amino acid differences between MMP12 and ADAMTS7 two angstrom around the ligand are indicated (other amino acids are hidden). Tyr240 from MMP12 amino acids is shown in blue, Leu419 from ADAMTS7 in green. The proposed interaction of the catalytic zinc with the ligand is depicted as a dashed purple line, hydrogen bonds are shown as dashed yellow lines, and π -stacking as dashed light blue line. Steric repulsions are indicated as dashed red and orange lines. The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).

Table 3. Metalloprotease Panel for Compound 17

| IC ₅₀ [nM] ^a | ADAMTS4 ^b | ADAMTS5 ^b | ADAMTS12 ^b | ADAM17 ^b | MMP2 ^b | MMP12 ^b | MMP15 ^b |
|------------------------------------|----------------------|----------------------|-----------------------|---------------------|-------------------|--------------------|--------------------|
| 60 | 115× | 213× | n.d. | 826× | 759× | 12× | 872× |

^aIC₅₀ vs ADAMTS7 enzyme, see [Supporting Information](#). ^bSelectivity factors were calculated by dividing the IC₅₀ of a given metalloprotease by the ADAMTS7-IC₅₀, see [Supporting Information](#) for details on IC₅₀ measurements. n.d. = not determined.

the ligand) makes identification of ADAMTS7 selective inhibitors challenging. However, potentially exploitable differences between both enzymes can be found within a flexible loop region. Docking of 32 in our ADAMTS7 homology model superimposed with an ADAMTS12 homology model indicated that amino acid exchanges within the S1 pocket might be addressable with an additional substituent on the 5-membered heteroaromatic group (Figure 9).

The project team had the opportunity to briefly evaluate this idea on thiazoles and pyrazoles. The most important findings of the incomplete SAR are summarized in Table 10. As we had been successful with α -methyl substituted 5-membered heteroaryl moieties regarding metalloprotease selectivity, we kept this motif and chose a large second substituent to reach the loop region. We opted for 6-membered aromatic groups instead of chains to limit conformational freedom in the respective area of the ligand. This strategy has had some success. Compared to 32, inhibitors 47–50 showed slightly improved selectivity factors vs ADAMTS12.

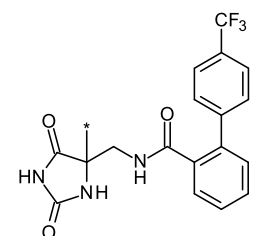
We were pleased to see that the additional substituent had no negative effect on selectivities vs metalloproteases routinely tested in the program. For compound 50, the selectivity profile was for most proteases even better than for 32 (Table 11).

Although we report here with hydantoin 50 to the best of our knowledge the first small molecule ADAMTS7 inhibitor with some selectivity vs ADAMTS12, more work is needed to come to a truly ADAMTS7-selective compound.

CONCLUSIONS

Based on docking of a published dual ADAMTS4/ADAMTS5 inhibitor⁴⁰ into an ADAMTS7 homology model, we have designed and subsequently synthesized the ADAMTS7 inhibitor 1 as a starting point for an optimization campaign. Initially our compounds lacked selectivity, e.g., vs MMP12. An X-ray cocrystal structure of the early lead 3 in MMP12 provided hints on how to become more selective. The ortho-biphenylamide 17 marked a breakthrough for us as it was the first ADAMTS7 inhibitor with some selectivity vs MMP12, potentially due to negative steric interactions imposed by the biphenyl moiety. A significant improvement of potency vs ADAMTS7 and thereby selectivity factors was achieved by exchanging the cyclopropyl substituent of the hydantoin by 5-membered heteroaromatic residues like in 24. Finally, substitution patterns on the ortho-biphenyl group were screened to prevent reactive metabolism. With BAY-9835 (32), we have identified a potent ADAMTS7 inhibitor which is very selective against a range of off-targets and metal-

Table 4. Toward Improved P1 Substituents




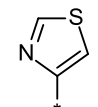
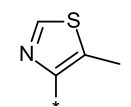
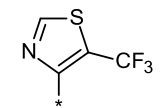
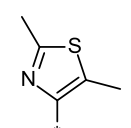
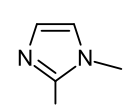
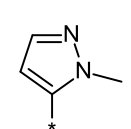
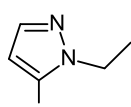
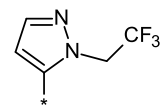
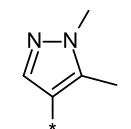
| Compound | R | IC ₅₀ [nM] ^[a] | MMP12 ^[b] | Cl _{bl, rat} [L/kg/h] ^[c] |
|-----------------------|---|--------------------------------------|----------------------|---|
| 18 _R (abs) |  | 99 | 11 | 0.1 |
| 19 _{eut} |  | 14 | 67 | 1.4 |
| 20 _{eut} |  | 6 | 188 | 1.2 |
| 21 _{eut} |  | 72 | 31 | n.d. |
| 22 _{eut} |  | 15 | 36 | n.d. |
| 23 _{eut} |  | 3 | 450 | 1.5 |
| 24 _S (abs) |  | 12 | 270 | 1.0 |
| 25 _{eut} |  | 18 | 96 | 1.0 |
| 26 _{eut} |  | 60 | 29 | n.d. |
| 27 _{eut} |  | 46 | 18 | n.d. |

Table 4. continued

^aIC₅₀ vs ADAMTS7. ^bSelectivity factor vs MMP12 obtained by dividing the MMP12 IC₅₀ by the ADAMTS7 IC₅₀. ^cBlood clearance after iv administration to rats; see [Supporting Information](#) for details on IC₅₀ determination; R (abs): absolute stereochemistry R; eut: eutomer; S (abs): absolute stereochemistry S.

loproteases except for ADAMTS12. In preclinical species, BAY-9835 showed low clearance, high oral bioavailability, and good tolerability in a 2-week toxicology study in rats. To the best of our knowledge, BAY-9835 represents the first orally bioavailable ADAMTS7 inhibitor without pan-metalloprotease activity.

First results on how to achieve selectivity vs ADAMTS12 within the lead series were discussed here as well. The phenyl substituted pyrazole derivative **50** potentially exploits differences within a flexible loop region of ADAMTS7 and ADAMTS12, thus leading to a more selective compound. Hydantoin **50** might be a starting point toward further improved ADAMTS7 inhibitors.

EXPERIMENTAL SECTION

Chemistry - General. All commercial reagents and catalysts were used as provided by the commercial supplier without purification. Solvents for synthesis, extraction, and chromatography were of reagent grade and used as received. Moisture-sensitive reactions were carried out under an atmosphere of argon, and anhydrous solvents were used as provided by the commercial supplier. Reaction progress was monitored by HPLC, LC-MS, or thin-layer chromatography. Crude products were immediately purified using preparative reversed-phase HPLC methodology with UV detection or flash chromatography on silica gel (see [Supporting Information](#)). The fractions obtained were concentrated in vacuo to remove organic volatiles. Unless otherwise indicated, all compounds have greater than 95% purity. ¹H NMR and ¹³C NMR spectra were recorded in solvents indicated below at RT with Bruker Avance spectrometers or Bruker Ultrashield AV300 operating at 300, 400, or 600 MHz for ¹H NMR and at 126 MHz for ¹³C NMR. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as an internal standard. The descriptions of the coupling patterns of ¹H NMR signals are based on the optical appearance of the signals and do not necessarily reflect the physically correct interpretation. In general, the chemical shift information refers to the center of the signal. In the case of multiplets, intervals are given. Spin multiplicities are reported as s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. The following mass spectrometry methods were used. LC-MS-Method 1–8: See [Supporting Information](#) for details. Single mass analysis (HRMS): Instrument: Waters Time of Flight System (ToF), Electrospray Ionization (ESI). Optical rotation was measured using the device Anton Paar Polarimeter MCP200: Specific rotation [α] (depending on wavelength, temperature, optical pathway, solvent, and concentration).

Preparation of 1 and Respective Starting Materials. Ethyl (2E)-3-oxo-2-(phenylhydrazono)propanoate. Sodium nitrite (12.05 g, 174.6 mmol) dissolved in 200 mL of water was added dropwise to a cold solution of aniline (15.92 mL, 174.6 mmol) in 40 mL of conc. HCl and 200 mL of water. The mixture was stirred for 5 min. Then the mixture was added dropwise to a cold solution of ethyl (2E)-3-(dimethylamino)acrylate (25 g, 174.8 mmol) and potassium acetate (25.7 g, 174.6 mmol) in 400 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 38.4 g (100% purity, 99% yield) of the title compound was obtained. LC-MS (Method 8): Rt = 0.935 min; MS (ESIpos): m/z = 221 [M + H]⁺.

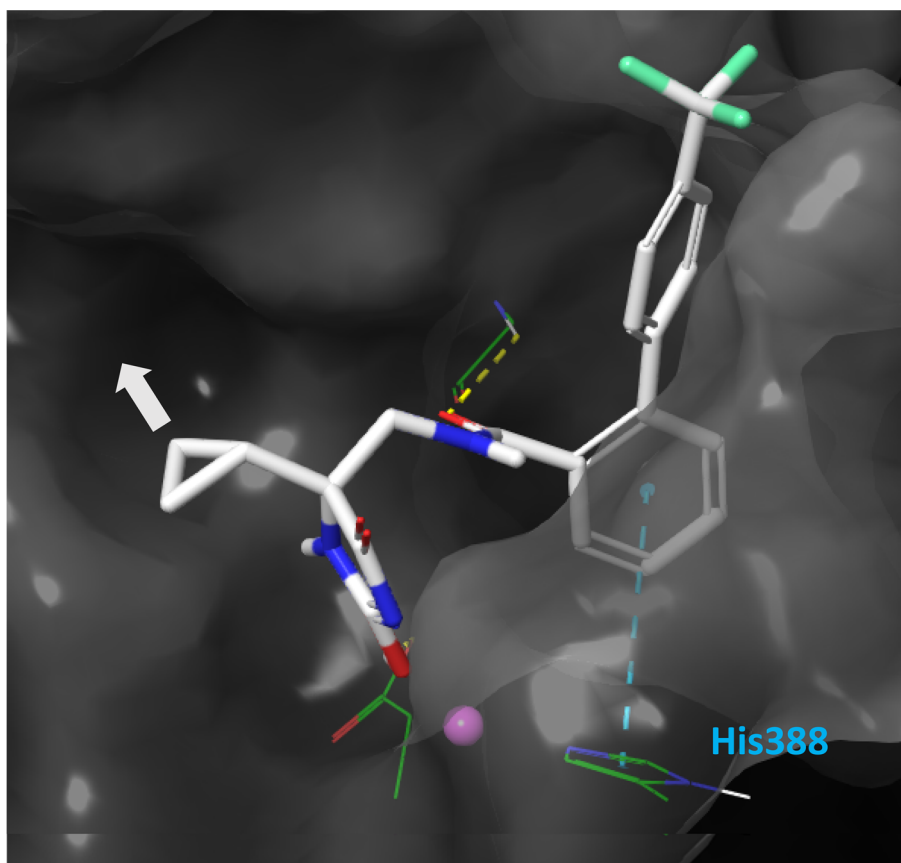


Figure 7. Docking of **18** in an ADAMTS7 homology model (based on the ADAMTS4 template with PDB-ID 4WKI) and indication of an S1 subpocket with a white arrow. The ligand is depicted with gray carbon atoms, blue nitrogens, red oxygens, and light green fluorines. The catalytic zinc is shown as a purple ball. Potential hydrogen bonds are depicted as dashed yellow lines and π -stacking as dashed light blue line. The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).

Table 5. Metalloprotease Panel for Compound 24

| IC ₅₀ [nM] ^a | ADAMTS4 ^b | ADAMTS5 ^b | ADAMTS12 ^b | ADAM17 ^b | MMP2 ^b | MMP12 ^b | MMP15 ^b |
|------------------------------------|----------------------|----------------------|-----------------------|---------------------|-------------------|--------------------|--------------------|
| 12 | 1435× | 3223× | 1× | 303× | 8319× | 270× | 6394× |

^aIC₅₀ vs ADAMTS7 enzyme, see [Supporting Information](#); ^bSelectivity factors were calculated by dividing the IC₅₀ of a given metalloprotease by the ADAMTS7-IC₅₀, see [Supporting Information](#) for details on IC₅₀ measurements.

Ethyl (2E,3E)-3-(hydroxyimino)-2-(phenylhydrazono)-propanoate. Ethyl (2E)-3-oxo-2-(phenylhydrazono)propanoate (38.4 g, 174.36 mmol), hydroxylamine hydrochloride (14.54 g, 209.24 mmol), and potassium acetate (42.78 g, 435.91 mmol) were dissolved in 400 mL of ethanol. The mixture was stirred at 78 °C for 30 min. The precipitated solid was filtered off, washed with water, and dried in vacuo. 41 g of the title compound was obtained. LC–MS (Method 8): Rt = 0.834 min; MS (ESIpos): m/z = 236 [M + H]⁺.

Ethyl 2-phenyl-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-3-(hydroxyimino)-2-(phenylhydrazono)propanoate (41 g, 174.29 mmol) was dissolved in 500 mL of acetic anhydride. The mixture was stirred at 140 °C for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. The residue was purified by flash chromatography eluting with heptane/dichloromethane. 17.44 g (100% purity, 46% yield) of the title compound was obtained. LC–MS (Method 8): Rt = 1.037 min; MS (ESIpos): m/z = 218 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 8.59 (s, 1H), 8.06 (d, 2H), 7.62 (t, 2H), 7.54 (t, 1H), 4.38 (q, 2H), 1.35 (t, 3H).

2-Phenyl-2H-1,2,3-triazole-4-carboxylic acid. Lithium hydroxide monohydrate (2.13 g, 50.64 mmol) was added to a solution of ethyl 2-phenyl-2H-1,2,3-triazole-4-carboxylate (11 g, 50.64 mmol) in 100 mL of THF and 50 mL of water. The mixture was stirred at room

temperature overnight. The reaction mixture was adjusted to pH 4 with 2 N HCl and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (eluent: from dichloromethane/methanol 9:1 to dichloromethane/methanol 8:2). Product containing samples were united and the solvents were evaporated. 8.46 g (100% purity, 88% yield) of the title compound was obtained. LC–MS (Method 8): Rt = 2.373 min; MS (ESIpos): m/z = 190 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 8.04–7.99 (m, 3H), 7.56 (t, 2H), 7.40 (t, 1H).

N-[[[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-2-phenyl-2H-1,2,3-triazole-4-carboxamide (1). 2-Phenyl-2H-1,2,3-triazole-4-carboxylic acid (92 mg, 0.49 mmol) was dissolved in 10 mL of dichloromethane. DIPEA (240 μ L, 1.36 mmol), EDC·HCl (121 mg, 0.63 mmol), and 1-hydroxybenzotriazole (97 mg, 0.63 mmol) were added, and the mixture was stirred at room temperature. After 5 min, (SR)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (100 mg, 0.49 mmol) was added and the mixture was stirred at room temperature for 2 d. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 51 mg (100% purity, 31% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.25 min;

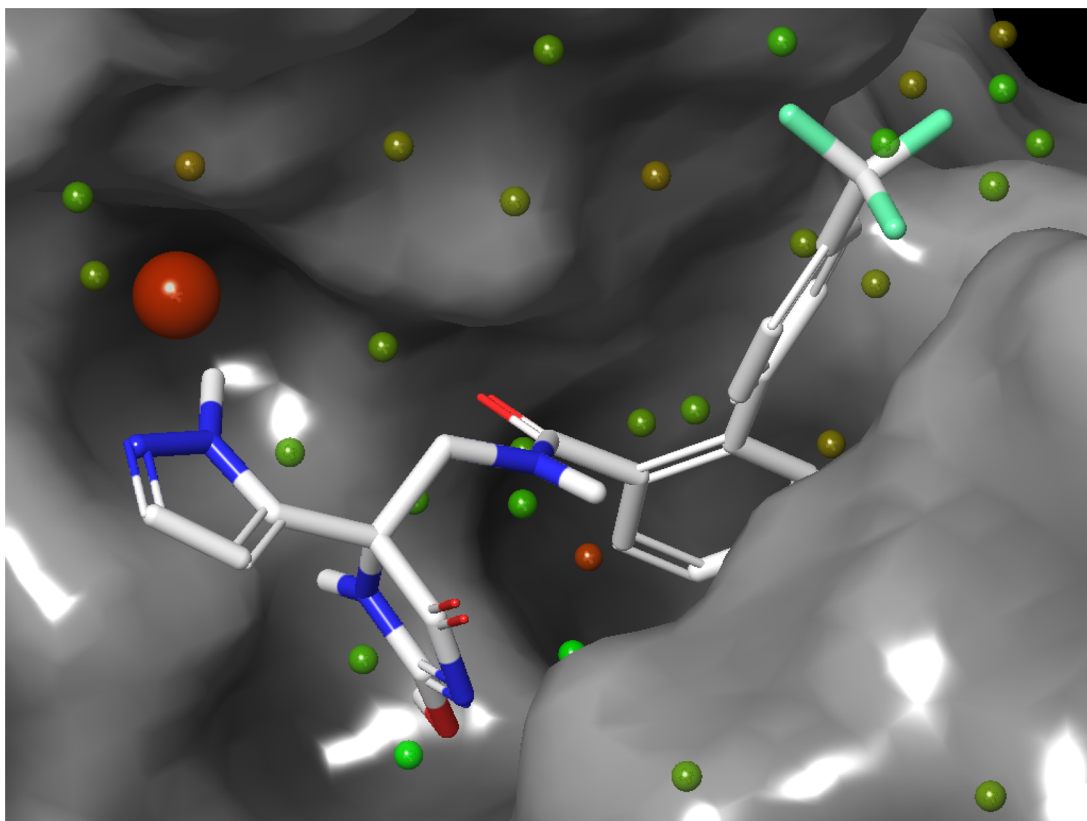


Figure 8. Docking of **24** in an ADAMTS7 homology model (based on the ADAMTS4 template with PDB-ID 4WK1) and WaterMap analysis. The ligand is depicted with gray carbon atoms, blue nitrogens, red oxygens, and light green fluorines. Water molecules predicted for the complex are shown as spheres colored by their predicted free energies (G) (green: favorable G; red: unfavorable G). The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).

MS (ESIpos): $m/z = 341$ $[M + H]^+$. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ [ppm] = 10.66 (s, 1H), 8.49–8.43 (m, 2H), 8.07 (dd, $J = 8.55$ Hz, 2H), 7.66–7.59 (m, 3H), 7.53–7.48 (m, 1H), 3.78–3.65 (m, 2H), 1.22–1.14 (m, 1H), 0.49–0.41 (m, 2H), 0.38–0.31 (m, 1H), 0.18–0.11 (m, 1H).

Preparation of 2 and Respective Starting Materials. Ethyl (2E)-2-[(4-fluorophenyl)hydrazono]-3-oxopropanoate. 4-Fluoroaniline (5.12 mL, 54 mmol) was dissolved in 60 mL of water and 12 mL of conc. hydrochloric acid. The mixture was cooled to 0 °C. At this temperature, a solution of sodium nitrite (3.73 g, 54 mmol) in 30 mL of water was added and the mixture was stirred at 0 °C for 5 min. This solution was added to a solution of ethyl (2E)-3-(dimethylamino)acrylate (8.54 mL, 59.4 mmol) and potassium acetate (7.95 g, 81 mmol) in 90 mL of ethanol. The mixture was stirred at room temperature. After 10 min, the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 5.3 g (90% purity, 37% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.82$ min; MS (ESIpos): $m/z = 239$ $[M + H]^+$.

Ethyl (2E,3E)-2-[(4-fluorophenyl)hydrazono]-3-(hydroxyimino)propanoate. Ethyl (2E)-2-[(4-fluorophenyl)hydrazono]-3-oxopropanoate (5.3 g, 90% purity, 20.02 mmol), hydroxylamine hydrochloride (1.67 g, 24.03 mmol), and potassium acetate (4.91 g, 50.06 mmol) were dissolved in 67 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 3.7 g (100% purity, 73% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.75$ min; MS (ESIpos): $m/z = 254$ $[M + H]^+$.

Ethyl 2-(4-fluorophenyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-2-[(4-fluorophenyl)hydrazono]-3-(hydroxyimino)propanoate (3.7 g, 14.61 mmol) was dissolved in 54 mL of acetic anhydride. The mixture was stirred at 60 °C for 1.5 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 1.4 g (100% purity, 41% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.94$ min; MS (ESIpos): $m/z = 236$ $[M + H]^+$.

2-(4-Fluorophenyl)-2H-1,2,3-triazole-4-carboxylic acid. Ethyl 2-(4-fluorophenyl)-2H-1,2,3-triazole-4-carboxylate (1.4 g, 5.95 mmol) was dissolved in 43 mL of THF. Lithium hydroxide solution (5.95 mL, 1N, 5.95 mmol) was added and the mixture was stirred at room temperature for 1 h. The THF was removed on a rotary evaporator and the aqueous residue was acidified with 2 N HCl. The precipitated solid was filtered off, washed with water, and dried in vacuo. 1.4 g (100% purity, 114% yield) of the title compound was obtained. LC–MS (Method 1): $R_t = 0.71$ min; MS (ESIpos): $m/z = 208$ $[M + H]^+$. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ [ppm] = 8.21 (s, 1H), 8.08–7.98 (m, 2H), 7.42 (t, $J = 8.8$ Hz, 2H).

N-[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl)methyl]-2-(4-fluorophenyl)-2H-1,2,3-triazole-4-carboxamide (2). 2-(4-Fluorophenyl)-2H-1,2,3-triazole-4-carboxylic acid (1.01 g) was dissolved in 100 mL of dichloromethane. DIPEA (2.37 mL, 13.62 mmol), EDC·HCl (1.21 g, 6.32 mmol), and 1-hydroxybenzotriazole hydrate (968 mg, 6.32 mmol) were added and the mixture was stirred at room temperature. After 5 min (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (1 g, 4.86 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate and washed with water and brine. The

Table 6. Evaluation of Substituents on “Lower” Phenyl Ring

| Compound | R | IC ₅₀ [nM] ^[a] | Cl _{bl, rat} [L/kg/h] ^[b] | Compound | R | IC ₅₀ [nM] ^[a] | Cl _{bl, rat} [L/kg/h] ^[b] |
|----------|---|--------------------------------------|---|----------|---|--------------------------------------|---|
| 28 | | 5 | 0.7 | 33 | | 16 | n.d. |
| 29 | | 19 | 0.9 | 34 | | 29 | n.d. |
| 30 | | 19 | 0.6 | 35 | | 3 | n.d. |
| 31 | | 79 | n.d. | 36 | | 10 | n.d. |
| 32 | | 6 | 0.6 | | | | |

^aIC₅₀ vs ADAMTS7. ^bBlood clearance after iv administration to rats; see [Supporting Information](#) for details on IC₅₀ and clearance determination.

Table 7. Metalloprotease Panel for BAY-9835 (32)

| IC ₅₀ [nM] ^a | ADAMTS4 ^b | ADAMTS5 ^b | ADAMTS12 ^b | ADAM8 ^b | ADAM10 ^b | ADAM17 ^b | MMP2 ^b | MMP12 ^b | MMP14 ^b | MMP15 ^b |
|------------------------------------|----------------------|----------------------|-----------------------|--------------------|---------------------|---------------------|-------------------|--------------------|--------------------|--------------------|
| 6 | 1121× | 1654× | 5× | 375× | 5467× | 962× | 17316× | 896× | >16667× | 13079× |

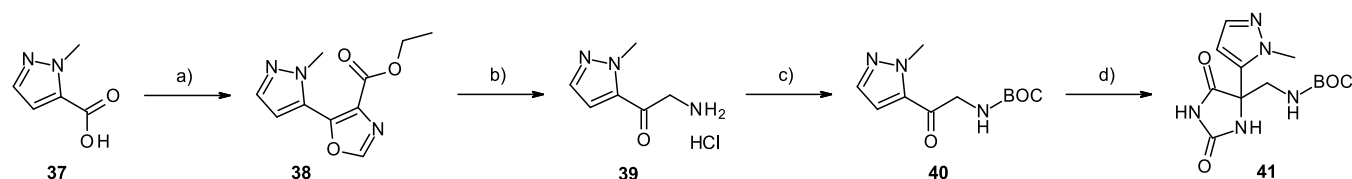
^aIC₅₀ vs ADAMTS7 enzyme, see [Supporting Information](#). ^bSelectivity factors were calculated by dividing the IC₅₀ of a given metalloprotease by the ADAMTS7-IC₅₀, see [Supporting Information](#) for details on IC₅₀ measurements.

combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. The residue was stirred in acetonitrile. The precipitate was filtered off, washed with acetonitrile, and dried in vacuo. 374 mg (100% purity, 21% yield) of the title compound was obtained. LC-MS (Method 2): Rt = 1.32 min; MS (ESIpos): *m/z* = 359 [M + H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.66 (s, 1H), 8.52–8.44

(m, 2H), 8.12–8.05 (m, 2H), 7.62 (s, 1H), 7.48 (t, *J* = 8.80 Hz, 2H), 3.79–3.61 (m, 2H), 1.21–1.13 (m, 1H), 0.48–0.40 (m, 2H), 0.39–0.30 (m, 1H), 0.18–0.09 (m, 1H).

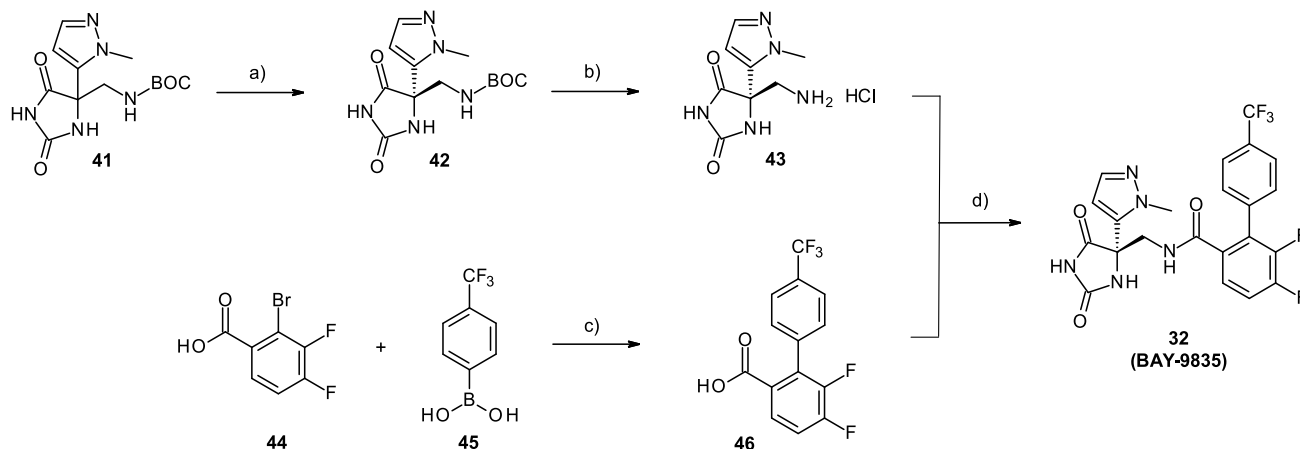
Preparation of 3 and Respective Starting Materials. Ethyl (2E)-2-[(3-fluorophenyl)hydrazono]-3-oxopropanoate. Sodium nitrite (964 mg, 13.97 mmol) dissolved in 5 mL of water was added dropwise to a cold solution of 3-fluoroaniline (1.35 mL, 13.97 mmol) in 2 mL of conc. HCl and 15 mL of water and stirred for 5 min. Then the solution was added dropwise to a cold solution of ethyl (2E)-3-

Scheme 1. Synthesis of Hydantoin 41 Needed for Preparation of BAY-9835 (32)



Reagents and conditions: (a) CDI, ethyl isocyanoacetate, LiHMDS, THF, 0 °C → r.t., 4 h, 69%; (b) 6 N HCl aq., 100 °C, 2 h, 86%; (c) (BOC)₂O, TEA, DCM, r.t., 1.5 h, 88%; (d) KCN, (NH₄)₂CO₃, MeOH, 80 °C, 2 d, 81%. aq. = aqueous, (BOC)₂O = di(tert-butyl)carbonate, CDI = carbonyl diimidazole, d = days, DCM = dichloromethane, h = hour(s), LiHMDS = lithium bis(trimethylsilyl)amide, MeOH = methanol, N = normal, r.t. = room temperature, TEA = triethylamine, THF = tetrahydrofuran.

Scheme 2. Synthesis of BAY-9835 (32)



Reagents and conditions: (a) chiral separation, CO₂, MeOH, 40 °C, 99.5% ee, 38%; (b) HCl in dioxane 1 M, DCM, r.t., 3 h, quant.; (c) dichlorobis(triphenylphosphin)palladium(II), XPhos, K₃PO₄, dioxane, H₂O, 80 °C, 3 h, 84%; (d) T3P, DIPEA, ACN, r.t., overnight, 45%. ACN = acetonitrile, DCM = dichloromethane, DIPEA = diisopropylethylamine, ee = enantiomeric excess, MeOH = methanol, quant. = quantitative conversion, r.t. = room temperature, T3P = propanephosphonic acid anhydride, XPhos = dicyclohexyl[2',4',6'-tris(propan-2-yl)[1,1'-biphenyl]-2-yl]phosphane.

Table 8. Physicochemical Descriptors for BAY-9835 (32)

| MW [g/mol] | log D ^a | HBD ^b | HBA ^c | NROTB ^d | tPSA [Å ²] ^e | BEI ^f | LLE ^g |
|---------------|--------------------|------------------|------------------|--------------------|--|------------------|------------------|
| 493 | 2.3 | 3 | 4 | 6 | 105 | 19.4 | 5.9 |

^aMeasured at pH 7.5 (see Supporting Information). ^bNumber of H-bond donors. ^cNumber of H-bond acceptors. ^dNumber of rotatable bonds. ^eTopological polar surface area. ^fBinding efficiency index $-\log(\text{IC}_{50})/\text{MW} \times 1000$. ^gLipophilic ligand efficiency $-\log(\text{IC}_{50}) - \log \text{D}_{7.5}$.

(dimethylamino)acrylate (2 g, 13.97 mmol) and potassium acetate (2.06 g, 20.95 mmol) in 40 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers

were dried over magnesium sulfate, filtered, and evaporated. 3.2 g (100% purity, 96% yield) of the title compound was obtained. LC-MS (Method 8): Rt = 0.92 min; MS (ESIpos): m/z = 239 [M + H]⁺.

Ethyl (2E,3E)-2-[(3-fluorophenyl)hydrazono]-3-(hydroxyimino)propanoate. Ethyl (2E)-2-[(3-fluorophenyl)hydrazono]-3-oxopropanoate (3.2 g, 13.43 mmol), hydroxylamine hydrochloride (933 mg, 13.43 mmol), and potassium acetate (3.3 g, 33.58 mmol) were dissolved in 40 mL of ethanol. The mixture was stirred at 78 °C for 30 min. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 3.39 g of the title compound was obtained. LC-MS (Method 8): Rt = 0.900 min; MS (ESIpos): m/z = 254 [M + H]⁺.

Ethyl 2-(3-fluorophenyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-2-[(3-fluorophenyl)hydrazono]-3-(hydroxyimino)-

Table 9. In Vivo Pharmacokinetic Profile of BAY-9835 (32)^a

| Species | AUC _{norm, i.v.} ^b [kg L ⁻¹ h] | CL _b ^c [L h ⁻¹ kg ⁻¹] | V _{ss} ^d [L kg ⁻¹] | t _{1/2, i.v.} ^e [h] | AUC _{norm, p.o.} ^f [kg L ⁻¹ h] | T _{max, p.o.} ^g [h] | t _{1/2, p.o.} ^h [h] | F ⁱ [%] |
|---------|---|--|--|---|---|---|---|-----------------------|
| Mouse | 1.5 | 1.10 | 1.6 | 2.1 | 2.0 | 1 | 2.5 | complete ^j |
| Rat | 3.1 | 0.55 | 1.2 | 3.6 | 3.0 | 2 | 3.7 | 96 |
| Dog | 88 | 0.02 | 0.4 | 22 | 68 | 5 | 25 | 77 |

^aSummary of pharmacokinetic parameters derived with noncompartmental analysis (NCA). For iv studies, 0.3 mg kg⁻¹ dose in either plasma 99%/DMSO 1% (mouse, rat) or water 50%/PEG400 40%/EtOH 10% (dog) were administered. For p.o. studies, 1.0 mg/kg solutions in water 50%/PEG400 40%/EtOH 10% were administered. ^bNormalized area under the curve after i.v. administration. ^cBlood clearance. ^dVolume of distribution at steady state. ^eTerminal half-life after i.v. administration. ^fNormalized area under the curve after p.o. administration. ^gMedian time to reach maximum concentration after p.o. administration. ^hTerminal half-life after p.o. administration. ⁱBioavailability after p.o. application; see Supporting Information for details. ^jNumerical value of bioavailability estimated with NCA is 132%.

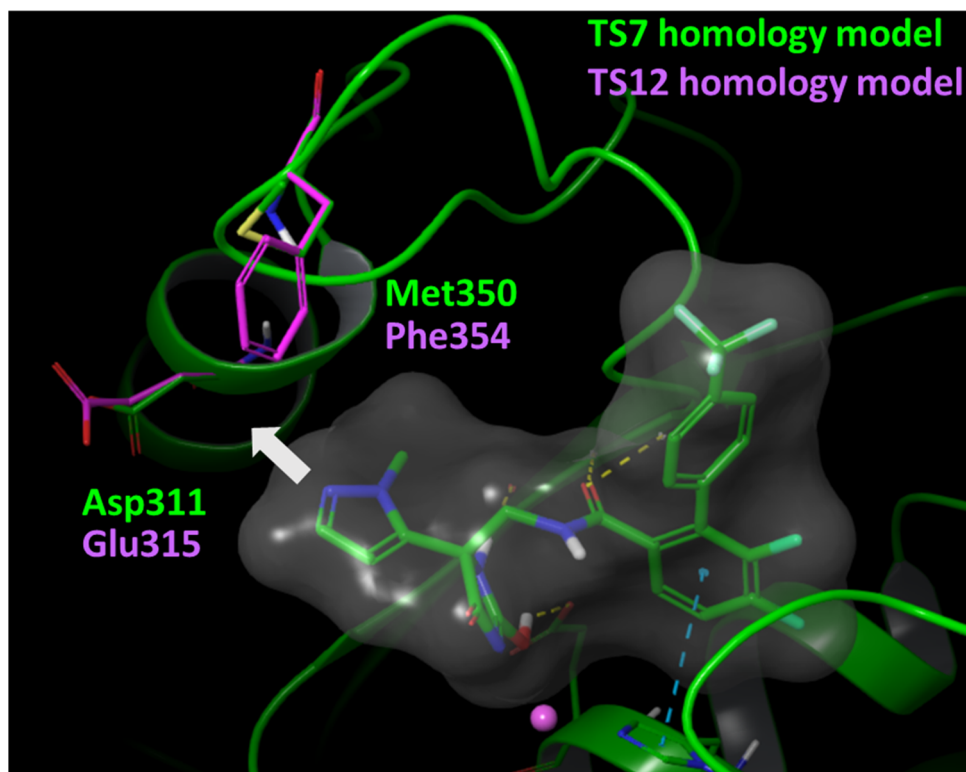


Figure 9. Docking of **32** in an ADAMTS7 homology model (based on the ADAMTS4 template with PDB-ID 4WKI) overlaid with an ADAMTS12 homology model (based on the ADAMTS4 template with PDB-ID 4WKI). Amino acid differences between ADAMTS7 and ADAMTS12 within a flexible loop region of both enzymes are indicated (other amino acids are hidden). Asp311 and Met350 from ADAMTS7 are shown in green, Glu315 and Phe354 from ADAMTS12 in purple. The catalytic zinc is depicted as a purple ball, putative hydrogen bonds are shown as dashed yellow lines, and π -stacking as dashed light blue line. The white arrow indicates where to potentially place an additional substituent on 5-membered heteroaromatic groups. The figure has been prepared with Maestro (Version 13.0.135 Schrödinger, LLC).

propanoate (3.3 g, 13.03 mmol) was dissolved in 30 mL of acetic anhydride. The mixture was stirred at 140 °C for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. The residue was purified by flash chromatography eluting with heptane/dichloromethane. 17.44 g (100% purity, 46% yield) of the title compound was obtained. LC–MS (Method 8): R_t = 1.004 min; MS (ESIpos): m/z = 236 [$M + H$] $^+$. 1H NMR (300 MHz, DMSO- d_6) δ [ppm] = 8.63 (s, 1H), 7.93 (d, 1H), 7.86 (d, 1H), 7.67 (q, 1H), 7.41–7.35 (m, 1H), 4.39 (q, 2H), 1.34 (t, 3H).

2-(3-Fluorophenyl)-2H-1,2,3-triazole-4-carboxylic acid. Lithium hydroxide monohydrate (107 mg, 2.55 mmol) was added to a solution of ethyl 2-(3-fluorophenyl)-2H-1,2,3-triazole-4-carboxylate (300 mg, 1.28 mmol) in 4 mL of THF and 2 mL of water. The mixture was stirred at room temperature for 2 h. The reaction mixture was adjusted to pH 4 with 2 N HCl and the solvent was removed under reduced pressure. 260 mg (100% purity, 98% yield) of the title compound was obtained. LC–MS (Method 8): R_t = 0.667 min; MS (ESIpos): m/z = 208 [$M + H$] $^+$.

N-[[[(4R)-4-Cyclopropyl-2,5-dioxoimidazolidin-4-yl]methyl]-2-(3-fluorophenyl)-2H-1,2,3-triazole-4-carboxamide (3). 2-(3-Fluorophenyl)-2H-1,2,3-triazole-4-carboxylic acid (92 mg, 0.44 mmol) was dissolved in 2 mL of DMF. DIPEA (220 μ L, 1.24 mmol), EDC * HCl (110 mg, 0.58 mmol), and 1-hydroxybenzotriazole monohydrate (88 mg, 0.58 mmol) were added and the mixture was stirred at room temperature. After 5 min (5R)-5-(aminomethyl)-5-cyclopropyl-imidazolidine-2,4-dione hydrochloride⁴⁰ (100 mg, 0.49 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was taken up in ethyl acetate and washed with 1 N HCl and sodium bicarbonate solution. The combined organic layers were dried over magnesium sulfate and concentrated. The residue was precipitated by adding diethyl ether and heptane. The

precipitated solid was filtered off and dried in vacuo. 70 mg (97% purity, 41% yield) of the title compound was obtained. LC–MS (Method 7): R_t = 2.496 min; MS (ESIpos): m/z = 359 [$M + H$] $^+$. 1H NMR (300 MHz, DMSO- d_6) δ [ppm] = 8.55–8.50 (m, 2H), 7.94–7.86 (m, 2H), 7.71–7.64 (m, 1H), 7.39–7.29 (m, 2H), 3.73–3.58 (m, 2H), 1.18–1.09 (m, 1H), 0.43–0.15 (m, 4H).

Preparation of 4 and Respective Starting Materials. Ethyl (2E)-2-[(2-fluorophenyl)hydrazono]-3-oxopropanoate. Sodium nitrite (310 mg, 4.50 mmol) dissolved in 2.5 mL of water was added dropwise to a cold solution of 2-fluoroaniline (0.43 mL, 4.50 mmol) in 1 mL of conc. HCl and 7.5 mL of water and stirred for 5 min. Then the solution was added dropwise to a cold solution of ethyl (2E)-3-(dimethylamino)acrylate (0.71 mL, 4.95 mmol) and potassium acetate (662 mg, 6.75 mmol) in 7.5 mL of ethanol. The mixture was stirred at room temperature. After 10 min the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. 1.01 g (84% purity, 79% yield) of the title compound was obtained. LC–MS (Method 2): R_t = 1.92 min; MS (ESIpos): m/z = 239 [$M + H$] $^+$.

Ethyl (2E,3E)-2-[(2-fluorophenyl)hydrazono]-3-(hydroxyimino)-propanoate. Ethyl (2E)-2-[(2-fluorophenyl)hydrazono]-3-oxopropanoate (1.01 g, 84%, purity 3.55 mmol), hydroxylamine hydrochloride (296 mg, 4.26 mmol), and potassium acetate (870 mg, 8.87 mmol) were dissolved in 10 mL of ethanol. The mixture was stirred at 80 °C for 30 min. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. 1.1 g (81% purity, 99% yield) of the title compound was obtained. LC–MS (Method 2): R_t = 1.79 min; MS (ESIpos): m/z = 254 [$M + H$] $^+$.

Ethyl 2-(2-fluorophenyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-2-[(2-fluorophenyl)hydrazono]-3-(hydroxyimino)-propanoate (1.1 g, 3.50 mmol) was dissolved in 11 mL of acetic

Table 10. SAR of Disubstituted P1 Substituents

| Compound | R | IC ₅₀ [nM] ^[a] | ADAMTS12 ^[b] |
|-------------------|---|--------------------------------------|-------------------------|
| | | | |
| 47 _{eut} | | 47 | 10 |
| 48 _{eut} | | 9 | 14 |
| 49 _{eut} | | 8 | 14 |
| 50 _{eut} | | 2 | 26 |

^aIC₅₀ vs ADAMTS7. ^bSelectivity factor vs ADAMTS12 obtained by dividing the ADAMTS12 IC₅₀ by the ADAMTS7 IC₅₀; see [Supporting Information](#) for details on IC₅₀ determination; eut: eutomer.

anhydride. The mixture was stirred at 140 °C for 30 min. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 594 mg (98% purity, 71% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.76 min; MS (ESIpos): *m/z* = 236 [M + H]⁺.

2-(2-Fluorophenyl)-2H-1,2,3-triazole-4-carboxylic acid. Ethyl 2-(2-fluorophenyl)-2H-1,2,3-triazole-4-carboxylate (594 mg, 98% purity, 2.48 mmol) was dissolved in 18 mL of THF. Lithium hydroxide solution (2.53 mL, 1N, 2.53 mmol) was added and the mixture was stirred at room temperature for 45 min. The reaction mixture was acidified with 2 N HCl and purified by column chromatography.

Product containing samples were united and the solvents were evaporated. 383 mg (98% purity, 72% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.25 min; MS (ESIpos): *m/z* = 208 [M + H]⁺.

N-[[[(4R)-4-Cyclopropyl-2,5-dioxoimidazolidin-4-yl]methyl]-2-(2-fluorophenyl)-2H-1,2,3-triazole-4-carboxamide (4). 2-(2-Fluorophenyl)-2H-1,2,3-triazole-4-carboxylic acid (50.3 mg, 0.24 mmol) was dissolved in 5 mL of dichloromethane. DIPEA (120 μL, 0.68 mmol), EDC·HCl (60.6 mg, 0.32 mmol), and 1-hydroxybenzotriazole hydrate (48.4 mg, 0.32 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (50 mg, 0.24 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. The residue was further purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 25 mg (100% purity, 29% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.16 min; MS (ESIpos): *m/z* = 359 [M + H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.65 (s, 1H), 8.52 (s, 1H), 8.46 (t, *J* = 6.3 Hz, 1H), 7.88 (td, *J* = 7.82 Hz, 1H), 7.67–7.54 (m, 3H), 7.46 (td, *J* = 7.67 Hz, 1H), 3.75–3.65 (m, 2H), 1.19–1.11 (m, 1H), 0.46–0.40 (m, 2H), 0.37–0.31 (m, 1H), 0.16–0.10 (m, 1H).

Preparation of 5 and Respective Starting Materials. Ethyl (2E)-2-[(4-chlorophenyl)hydrazono]-3-oxopropanoate. Sodium nitrite (482 mg, 6.98 mmol) dissolved in 1.5 mL of water was added dropwise to a cold solution of 4-chloroaniline (891 mg, 6.98 mmol) in 1.5 mL of conc. HCl and 10 mL of water and stirred for 5 min. Then the solution was added dropwise to a cold solution of ethyl (2E)-3-(dimethylamino)acrylate (1.0 g, 6.98 mmol) and potassium acetate (1.03 g, 10.48 mmol) in 14 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was extracted between water and dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 1.58 g of the title compound was obtained. LC–MS (Method 8): Rt = 0.914 min; MS (ESIpos): *m/z* = 255 [M + H]⁺.

Ethyl (2E,3E)-2-[(4-chlorophenyl)hydrazono]-3-(hydroxyimino)propanoate. Ethyl (2E)-2-[(4-chlorophenyl)hydrazono]-3-oxopropanoate (1.58 g), hydroxylamine hydrochloride (517 mg, 7.45 mmol), and potassium acetate (1.52 g, 15.51 mmol) were dissolved in 40 mL of ethanol. The mixture was stirred at 78 °C for 30 min. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. 1.42 g of the title compound was obtained. LC–MS (Method 8): Rt = 0.889 min; MS (ESIpos): *m/z* = 270 [M + H]⁺.

Ethyl 2-(4-chlorophenyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-2-[(4-chlorophenyl)hydrazono]-3-(hydroxyimino)propanoate (1.42 g) was dissolved in 15 mL of acetic anhydride. The mixture was stirred at 140 °C for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. The residue was purified by flash chromatography eluting with heptane/dichloromethane. Product containing samples were united and the solvents were evaporated. 561 mg (91% purity) of the title compound was obtained. LC–MS (Method 8): Rt = 0.986 min; MS (ESIpos): *m/z* = 252 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 8.62 (s, 1H), 8.08 (d, 2H), 7.68 (d, 2H), 4.39 (q, 2H), 1.35 (t, 3H).

Table 11. Metalloprotease Panel for Compound 50

| IC ₅₀ [nM] ^a | ADAMTS4 ^b | ADAMTS5 ^b | ADAMTS12 ^b | ADAM17 ^b | MMP2 ^b | MMP12 ^b | MMP15 ^b |
|------------------------------------|----------------------|----------------------|-----------------------|---------------------|-------------------|--------------------|--------------------|
| 2 | >20833× | 12708× | 26× | 863× | n.d. | 1446× | 41667× |

^aIC₅₀ vs ADAMTS7 enzyme, see [Supporting Information](#). ^bSelectivity factors were calculated by dividing the IC₅₀ of a given metalloprotease by the ADAMTS7-IC₅₀, see [Supporting Information](#) for details on IC₅₀ measurements. n.d. = not determined.

2-(4-Chlorophenyl)-2H-1,2,3-triazole-4-carboxylic acid. Lithium hydroxide monohydrate (112 mg, 2.68 mmol) was added to a solution of ethyl 2-(4-chlorophenyl)-2H-1,2,3-triazole-4-carboxylate (561 mg, 91% purity, 2.03 mmol) in 6 mL of THF and 3 mL of water. The mixture was stirred at room temperature for 1 h. The reaction mixture was adjusted to pH 4 with 2 N HCl and the solvent was removed under reduced pressure. 490 mg (82% purity, 89% yield) of the title compound was obtained. LC–MS (Method 8): Rt = 0.704 min; MS (ESIpos): m/z = 224 [M + H]⁺.

2-(4-Chlorophenyl)-N-[[[(4R)-4-cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-2H-1,2,3-triazole-4-carboxamide (5). 2-(4-Chlorophenyl)-2H-1,2,3-triazole-4-carboxylic acid (82 mg, 82% purity, 0.30 mmol) was dissolved in 2 mL of DMF. Triethylamine (150 μ L, 1.09 mmol), EDC*HCl (105 mg, 0.55 mmol), and 1-hydroxybenzotriazole monohydrate (74 mg, 0.55 mmol) were added and the mixture was stirred at room temperature. After 5 min (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (75 mg, 0.37 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate. The solution was washed with 2 N HCl with sat. aqueous sodium bicarbonate solution. The combined organic layers were dried over magnesium sulfate and concentrated. The crude product was taken up in a small amount of dichloromethane then heptane was added and the precipitate formed was collected by filtration and dried in vacuo. 70 mg (99% purity, 62% yield) of the title compound was obtained. LC–MS (Method 7): Rt = 2.731 min; MS (ESIpos): m/z = 375 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 10.64 (s, 1H), 8.54–8.49 (m, 2H), 8.08 (d, 2H), 7.70 (d, 2H), 7.63 (s, 1H), 3.78–3.62 (m, 2H), 1.19–1.15 (m, 1H), 0.48–0.32 (m, 3H), 0.15–0.12 (m, 1H).

Preparation of 6 and Respective Starting Materials. Ethyl (2E)-2-[(3-chlorophenyl)hydrazono]-3-oxopropanoate. Sodium nitrite (482 mg, 6.98 mmol) dissolved in 1.5 mL of water was added dropwise to a cold solution of 3-chloroaniline (891 mg, 6.98 mmol) in 1.5 mL of conc. HCl and 10 mL of water and stirred for 5 min. Then the solution was added dropwise to a cold solution of ethyl (2E)-3-(dimethylamino)acrylate (1.0 g, 6.98 mmol) and potassium acetate (1.03 g, 10.48 mmol) in 14 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was extracted between water and dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 1.64 g of the title compound was obtained. LC–MS (Method 8): Rt = 0.919 min; MS (ESIpos): m/z = 255 [M + H]⁺.

Ethyl (2E,3E)-2-[(3-chlorophenyl)hydrazono]-3-(hydroxyimino)-propanoate. Ethyl (2E)-2-[(3-chlorophenyl)hydrazono]-3-oxopropanoate (1.64 g), hydroxylamine hydrochloride (537 mg, 7.73 mmol), and potassium acetate (1.58 g, 16.10 mmol) were dissolved in 40 mL of ethanol. The mixture was stirred at 78 °C for 30 min. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 1.22 g of the title compound was obtained. LC–MS (Method 8): Rt = 0.992 min; MS (ESIpos): m/z = 252 [M + H]⁺.

Ethyl 2-(3-chlorophenyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-2-[(3-chlorophenyl)hydrazono]-3-(hydroxyimino)-propanoate (1.22 g) was dissolved in 15 mL of acetic anhydride. The mixture was stirred at 140 °C for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. The residue was purified by flash chromatography eluting with heptane/dichloromethane. Product containing samples were united and the solvents were evaporated. 472 mg (93% purity) of the title compound was obtained. LC–MS (Method 8): Rt = 0.992 min; MS (ESIpos): m/z = 252 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 8.64 (s, 1H), 8.07–8.04 (m, 2H), 7.69–7.59 (m, 2H), 4.40 (q, 2H), 1.35 (t, 3H).

2-(3-Chlorophenyl)-2H-1,2,3-triazole-4-carboxylic acid. Lithium hydroxide monohydrate (94 mg, 2.68 mmol) was added to a solution of ethyl 2-(3-chlorophenyl)-2H-1,2,3-triazole-4-carboxylate (472 mg, 93% purity, 1.75 mmol) in 6 mL of THF and 3 mL of water. The mixture was stirred at room temperature for 1 h. The reaction mixture

was adjusted to pH 4 with 2 N HCl and the solvent was removed under reduced pressure. 410 mg (89% purity, 93% yield) of the title compound was obtained. LC–MS (Method 8): Rt = 0.697 min; MS (ESIpos): m/z = 224 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 8.56 (s, 1H), 8.06–8.03 (m, 2H), 7.68–7.58 (m, 2H).

2-(3-Chlorophenyl)-N-[[[(4R)-4-cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-2H-1,2,3-triazole-4-carboxamide (6). 2-(3-Chlorophenyl)-2H-1,2,3-triazole-4-carboxylic acid (82 mg, 89% purity, 0.33 mmol) was dissolved in 2 mL of DMF. Triethylamine (150 μ L, 1.09 mmol), EDC*HCl (105 mg, 0.55 mmol), and 1-hydroxybenzotriazole monohydrate (74 mg, 0.55 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (75 mg, 0.37 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate. The mixture was washed with 2 N HCl and then with sat. aqueous sodium bicarbonate solution. The combined organic layers were dried over magnesium sulfate and concentrated. The crude product was taken up in a small amount of dichloromethane then heptane was added and the precipitate formed was collected by filtration and dried in vacuo. 90 mg (99% purity, 72% yield) of the title compound was obtained. LC–MS (Method 7): Rt = 2.756 min; MS (ESIpos): m/z = 375 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 10.66 (s, 1H), 8.57 (t, 1H), 8.51 (s, 1H), 8.12 (s, 1H), 8.05 (d, 1H), 7.69–7.56 (m, 3H), 3.79–3.61 (m, 2H), 1.21–1.12 (m, 1H), 0.48–0.32 (m, 3H), 0.16–0.12 (m, 1H).

Preparation of 7 and Respective Starting Materials. Ethyl (2E)-2-[(4-methylphenyl)hydrazono]-3-oxopropanoate. Sodium nitrite (482 mg, 6.98 mmol) dissolved in 10 mL of water was added dropwise to a cold solution of 4-methylaniline (748 mg, 6.98 mmol) in 3 mL of conc. HCl and 5 mL of water and stirred for 5 min. Then the solution was added dropwise to a cold solution of ethyl (2E)-3-(dimethylamino)acrylate (1.0 g, 6.98 mmol) and potassium acetate (1.03 g, 10.48 mmol) in 15 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was extracted between water and ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 1.19 g (100% purity, 73% yield) of the title compound was obtained. LC–MS (Method 8): Rt = 0.903 min; MS (ESIpos): m/z = 235 [M + H]⁺.

Ethyl (2E,3E)-3-(hydroxyimino)-2-[(4-methylphenyl)hydrazono]-propanoate. Ethyl (2E)-2-[(4-methylphenyl)hydrazono]-3-oxopropanoate (1.19 g, 5.08 mmol), hydroxylamine hydrochloride (424 mg, 6.10 mmol), and potassium acetate (1.25 g, 12.70 mmol) were dissolved in 30 mL of ethanol. The mixture was stirred at 78 °C for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. 1.19 g of the title compound was obtained. LC–MS (Method 8): Rt = 0.858 min; MS (ESIpos): m/z = 250 [M + H]⁺.

Ethyl 2-(4-methylphenyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-3-(hydroxyimino)-2-[(4-methylphenyl)hydrazono]-propanoate (1.19 g) was dissolved in 20 mL of acetic anhydride. The mixture was stirred at 140 °C for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered, and evaporated. The residue was purified by flash chromatography eluting with heptane/dichloromethane. Product containing samples were united and the solvents were evaporated. 250 mg (100% purity) of the title compound was obtained. LC–MS (Method 8): Rt = 0.955 min; MS (ESIpos): m/z = 232 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ [ppm] = 8.56 (s, 1H), 7.94 (d, 2H), 7.41 (d, 2H), 4.38 (q, 2H), 2.39 (s, 3H), 1.34 (t, 3H).

2-(4-Methylphenyl)-2H-1,2,3-triazole-4-carboxylic acid. Lithium hydroxide monohydrate (46 mg, 1.09 mmol) was added to a solution of ethyl 2-(4-methylphenyl)-2H-1,2,3-triazole-4-carboxylate (210 mg, 0.91 mmol) in 4 mL of THF and 2 mL of water. The mixture was stirred at room temperature for 2 h. The reaction mixture was adjusted to pH 4 with 2 N HCl and the solvent was removed under reduced pressure. 180 mg (100% purity, 98% yield) of the title compound was obtained. LC–MS (Method 8): Rt = 0.670 min; MS

(ESIpos): $m/z = 204$ $[M + H]^+$. 1H NMR (300 MHz, DMSO- d_6) δ [ppm] = 8.13 (s, 1H), 7.90 (d, 2H), 7.37 (d, 2H), 2.37 (s, 3H).

N-[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl-2-(4-methylphenyl)-2H-1,2,3-triazole-4-carboxamide (7). 2-(4-Methylphenyl)-2H-1,2,3-triazole-4-carboxylic acid (74 mg, 0.37 mmol) was dissolved in 2 mL of DMF. Triethylamine (150 μ L, 1.09 mmol), EDC \cdot HCl (105 mg, 0.55 mmol), and 1-hydroxybenzotriazole monohydrate (74 mg, 0.55 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (75 mg, 0.37 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in dichloromethane. The mixture was washed with 2 N HCl and then with sat. aqueous sodium bicarbonate solution. The combined organic layers were dried over magnesium sulfate and concentrated. The crude product was purified by flash chromatography eluting with dichloromethane/methanol. Product containing samples were united and the solvents were evaporated. 70 mg (100% purity, 56% yield) of the title compound was obtained. LC–MS (Method 7): Rt = 2.642 min; MS (ESIpos): $m/z = 355$ $[M + H]^+$. 1H NMR (300 MHz, DMSO- d_6) δ [ppm] = 10.66 (s, 1H), 8.45–8.43 (m, 2H), 7.95 (d, 2H), 7.63 (s, 1H), 7.42 (d, 2H), 3.77–3.71 (m, 2H), 3.33 (s, 3H), 1.19–1.14 (m, 1H), 0.48–0.32 (m, 3H), 0.13–0.12 (m, 1H).

Preparation of 8 and Respective Starting Materials. 5-Fluoro-2-hydrazinopyridine. 2,5-Difluoropyridine (1.0 g, 8.69 mmol) was dissolved in hydrazine hydrate (4.23 mL, 86.89 mmol). The mixture was stirred at 120 °C for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The crude product (984 mg) was used in the next step without further purification. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 7.95 (d, $J = 3.12$ Hz, 1H), 7.44–7.40 (m, 1H), 7.36 (s, 1H), 6.73 (dd, $J = 9.17$ Hz, 1H), 4.09 (s, 2H).

N-[(1E,2Z)-2-[(5-Fluoropyridin-2-yl)hydrazono]propylidene]hydroxylamine. Crude 5-fluoro-2-hydrazinopyridine (984 mg) was dissolved in 35 mL of ethanol. 1-(Hydroxyimino)acetone (809 mg, 9.29 mmol) was added and the mixture was stirred at 80 °C for 2 h. The solvent was removed on a rotary evaporator and the residue was taken up in *n*-pentane. The precipitate was filtered off, washed with *n*-pentane, and dried in vacuo. 1.46 g (76% yield, 79% purity) of the title compound was obtained. LC–MS (Method 2): Rt = 1.11 min; MS (ESIpos): $m/z = 197$ $[M + H]^+$.

5-Fluoro-2-(4-methyl-2H-1,2,3-triazol-2-yl)pyridine. *N-[(1E,2Z)-2-[(5-Fluoropyridin-2-yl)hydrazono]propylidene]hydroxylamine* (1.14 g, 79% purity, 4.59 mmol) was dissolved in 23 mL of acetic anhydride. The mixture was stirred at 130 °C for 3 h. The solvent was removed on a rotary evaporator and the crude product (1.45 g) was used in the next step without further purification. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 8.53 (m, 1H), 8.03–7.98 (m, 2H), 7.95 (s, 1H), 2.37 (s, 3H).

2-(5-Fluoropyridin-2-yl)-2H-1,2,3-triazole-4-carboxylic acid. Crude 5-fluoro-2-(4-methyl-2H-1,2,3-triazol-2-yl)pyridine (895 mg) was dissolved in 30 mL of sulfuric acid (66% in water). Sodium dichromate dihydrate (7.48 g, 25.12 mmol) was added and the mixture was stirred at 80 °C for 1 h. The reaction mixture was diluted with ice water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. 285 mg (100% purity) of the title compound was obtained. LC–MS (Method 2): Rt = 0.73 min; MS (ESIpos): $m/z = 209$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 8.71–8.51 (m, 2H), 8.20–8.02 (m, 2H).

N-[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl-2-(5-fluoropyridin-2-yl)-2H-1,2,3-triazole-4-carboxamide (8). 2-(5-Fluoropyridin-2-yl)-2H-1,2,3-triazole-4-carboxylic acid (50.6 mg, 0.24 mmol) was dissolved in 5 mL of dichloromethane. DIPEA (120 μ L, 0.68 mmol), EDC \cdot HCl (60.6 mg, 0.32 mmol), and 1-hydroxybenzotriazole hydrate (48.4 mg, 0.32 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰

(50 mg, 0.24 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 19 mg (95% purity, 21% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.64 min; MS (ESIpos): $m/z = 359$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 10.64 (s, 1H), 8.66 (s, 1H), 8.56 (t, $J = 6.24$ Hz, 1H), 8.54 (s, 1H), 8.16–8.08 (m, 2H), 7.62 (s, 1H), 3.74–3.68 (m, 2H), 1.19–1.14 (m, 1H), 0.45–0.42 (m, 2H), 0.36–0.32 (m, 1H), 0.16–0.09 (m, 1H).

Preparation of 9 and Respective Starting Materials. Ethyl (2E)-3-oxo-2-(1,2-thiazol-4-ylhydrazono)propanoate. Sodium nitrite (126 mg, 1.83 mmol) dissolved in 3 mL of water was added dropwise to a cold solution of 1,2-thiazol-4-amine hydrochloride (250 mg, 41.83 mmol) in 0.41 mL of conc. HCl and 3 mL of water and the mixture was stirred for 5 min. Then the solution was added dropwise to a cold solution of ethyl (2E)-3-(dimethylamino)acrylate (0.29 mL, 2.01 mmol) and potassium acetate (269 mg, 2.75 mmol) in 3 mL of ethanol. The mixture was stirred at room temperature. After 30 min the reaction mixture was diluted with water. The precipitated solid was filtered off, washed with water and dried in vacuo. 170 mg (100% purity, 41% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.33 min; MS (ESIpos): $m/z = 228$ $[M + H]^+$.

Ethyl (2E,3E)-3-(hydroxyimino)-2-(1,2-thiazol-4-ylhydrazono)propanoate. Ethyl (2E)-3-oxo-2-(1,2-thiazol-4-ylhydrazono)propanoate (170 mg, 0.75 mmol), hydroxylamine hydrochloride (62 mg, 0.90 mmol), and potassium acetate (184 mg, 1.87 mmol) were dissolved in 7 mL of ethanol. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and the precipitated solid was filtered off, washed with water, and dried in vacuo. 80 mg (100% purity, 44% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.45 min; MS (ESIpos): $m/z = 243$ $[M + H]^+$.

Ethyl 2-(1,2-thiazol-4-yl)-2H-1,2,3-triazole-4-carboxylate. Ethyl (2E,3E)-3-(hydroxyimino)-2-(1,2-thiazol-4-ylhydrazono)propanoate (80 mg, 0.33 mmol) was dissolved in 2 mL of acetic anhydride. The mixture was stirred at 140 °C for 30 min. The crude product was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 60 mg (100% purity, 81% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.78 min; MS (ESIpos): $m/z = 225$ $[M + H]^+$.

2-(1,2-Thiazol-4-yl)-2H-1,2,3-triazole-4-carboxylic acid. Ethyl 2-(1,2-thiazol-4-yl)-2H-1,2,3-triazole-4-carboxylate (60 mg, 0.27 mmol) was dissolved in 2.5 mL of THF. Lithium hydroxide solution (0.27 mL, 1 N, 0.27 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified with 2 N HCl and the precipitated solid was filtered off, washed with water, and dried in vacuo. 45 mg (100% purity, 86% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.41 min; MS (ESIpos): $m/z = 197$ $[M + H]^+$.

N-[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl-2-(1,2-thiazol-4-yl)-2H-1,2,3-triazole-4-carboxamide (9). 2-(1,2-Thiazol-4-yl)-2H-1,2,3-triazole-4-carboxylic acid (45 mg, 0.23 mmol) was dissolved in 5 mL of dichloromethane. DIPEA (110 μ L, 0.64 mmol), EDC \cdot HCl (57 mg, 0.30 mmol), and 1-hydroxybenzotriazole hydrate (46 mg, 0.30 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5R)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (47 mg, 0.23 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 54 mg (100% purity, 68% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.96 min; MS (ESIpos): $m/z = 348$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 10.65 (s, 1H), 9.44 (s, 1H), 9.10 (s, 1H), 8.52–8.49 (m, 2H), 7.62 (s, 1H), 3.77–3.63 (m, 2H), 1.20–1.13 (m, 1H), 0.45 (q, 2H), 0.38–0.31 (m, 1H), 0.17–0.11 (m, 1H).

Preparation of 10 and Respective Starting Materials. Ethyl 2-[(1,3-thiazol-2-ylmethyl)-2H-1,2,3-triazole-4-carboxylate. Ethyl 2H-1,2,3-triazole-4-carboxylate (200 mg, 1.42 mmol) was dissolved in 6 mL of DMF. 2-(Chloromethyl)-1,3-thiazole (208 mg, 1.56 mmol) and potassium carbonate (490 mg, 3.54 mmol) were added and the mixture was stirred at room temperature overnight. The reaction mixture was filtered and the filtrate was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 135 mg (97% purity, 39% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.28 min; MS (ESIpos): m/z = 239 $[M + H]^+$. 1H NMR (500 MHz, DMSO- d_6) δ [ppm] = 8.36 (s, 1H), 7.83–7.82 (m, 1H), 7.81–7.79 (m, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 4H).

2-[(1,3-Thiazol-2-ylmethyl)-2H-1,2,3-triazole-4-carboxylic acid. Ethyl 2-[(1,3-thiazol-2-ylmethyl)-2H-1,2,3-triazole-4-carboxylate (135 mg, 0.55 mmol) was dissolved in 4 mL of THF. Lithium hydroxide solution (0.57 mL, 1 N, 0.57 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was acidified with 2 N HCl and the solvent was removed at a rotary evaporator. The crude product was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 56 mg (100% purity, 48% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.59 min; MS (ESIpos): m/z = 211 $[M + H]^+$.

N-[[[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-2-(1,3-thiazol-2-ylmethyl)-2H-1,2,3-triazole-4-carboxamide (10). 2-[(1,3-Thiazol-2-ylmethyl)-2H-1,2,3-triazole-4-carboxylic acid (51 mg, 0.24 mmol) was dissolved in 5 mL of dichloromethane. DIPEA (120 μ L, 0.68 mmol), EDC*HCl (61 mg, 0.32 mmol), and 1-hydroxybenzotriazole hydrate (48 mg, 0.32 mmol) were added and the mixture was stirred at room temperature. After 5 min, (SR)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (50 mg, 0.24 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator, and the residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 53 mg (100% purity, 60% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.81 min; MS (ESIpos): m/z = 362 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 10.61 (s, 1H), 8.28–8.23 (m, 2H), 7.82–7.79 (m, 2H), 7.56 (s, 1H), 6.11 (s, 2H), 3.65 (d, J = 6.48 Hz, 2H), 1.16–1.10 (m, 1H), 0.43–0.38 (m, 2H), 0.35–0.28 (m, 1H), 0.14–0.08 (m, 1H).

Preparation of 11 and Respective Starting Materials. Ethyl 2-[(2-methyl-1,3-thiazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylate. Ethyl 2H-1,2,3-triazole-4-carboxylate (200 mg, 1.42 mmol) was dissolved in 6 mL of DMF. 4-(Chloromethyl)-2-methyl-1,3-thiazole hydrochloride (287 mg, 1.56 mmol) and potassium carbonate (490 mg, 3.54 mmol) were added and the mixture was stirred at room temperature overnight. The reaction mixture was filtered, and the filtrate was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 97 mg (85% purity, 23% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.37 min; MS (ESIpos): m/z = 253 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 8.28 (s, 1H), 7.53 (s, 1H), 5.76 (s, 2H), 4.28–4.35 (m, 2H), 2.60 (s, 3H), 1.20–1.36 (m, 3H).

2-[(2-Methyl-1,3-thiazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylic acid. Ethyl 2-[(2-methyl-1,3-thiazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylate (97 mg, 85% purity, 0.32 mmol) was dissolved in 3 mL of THF. Lithium hydroxide solution (0.38 mL, 1 N, 0.38 mmol) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was acidified with 2 N HCl and the solvent was removed at a rotary evaporator. The crude product was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 51 mg (97% purity, 69% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.72 min; MS (ESIpos): m/z = 225 $[M + H]^+$.

N-[[[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-2-[(2-methyl-1,3-thiazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxamide

(11). 2-[(2-Methyl-1,3-thiazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylic acid (51 mg, 97% purity, 0.22 mmol) was dissolved in 5 mL of dichloromethane. DIPEA (110 μ L, 0.64 mmol), EDC*HCl (57 mg, 0.30 mmol), and 1-hydroxybenzotriazole hydrate (45 mg, 0.30 mmol) were added and the mixture was stirred at room temperature. After 5 min, (SR)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (47 mg, 0.23 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 40 mg (100% purity, 48% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.54 min; MS (ESIpos): m/z = 375 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 10.62 (s, 1H), 8.22–8.16 (m, 2H), 7.56 (s, 1H), 7.49 (s, 1H), 5.73 (s, 2H), 3.65 (d, J = 6.36 Hz, 2H), 2.61 (s, 3H), 1.15–1.10 (m, 1H), 0.43–0.38 (m, 2H), 0.34–0.30 (m, 1H), 0.15–0.08 (m, 1H).

Preparation of 12 and Respective Starting Materials. Ethyl 2-[(1-methyl-1H-pyrazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylate. Ethyl 2H-1,2,3-triazole-4-carboxylate (300 mg, 2.13 mmol) was dissolved in 9 mL of DMF. 4-(Chloromethyl)-1-methyl-1H-pyrazole (306 mg, 2.34 mmol) and potassium carbonate (735 mg, 5.31 mmol) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was filtered, and the filtrate was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 62 mg (79% purity, 10% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.13 min; MS (ESIpos): m/z = 236 $[M + H]^+$.

2-[(1-Methyl-1H-pyrazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylic acid. Ethyl 2-[(1-methyl-1H-pyrazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylate (60 mg, 77% purity, 0.20 mmol) was dissolved in 3 mL of THF. Lithium hydroxide solution (0.31 mL, 1 N, 0.31 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was acidified with 2 N HCl and the solvent was removed at a rotary evaporator. The crude product was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 37 mg (100% purity, 89% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.56 min; MS (ESIpos): m/z = 208 $[M + H]^+$.

N-[[[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-2-[(1-methyl-1H-pyrazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxamide (12). 2-[(1-Methyl-1H-pyrazol-4-yl)methyl]-2H-1,2,3-triazole-4-carboxylic acid (37 mg, 0.18 mmol) was dissolved in 3.5 mL of dichloromethane. DIPEA (90 μ L, 0.50 mmol), EDC*HCl (45 mg, 0.23 mmol), and 1-hydroxybenzotriazole hydrate (36 mg, 0.23 mmol) were added and the mixture was stirred at room temperature. After 5 min, (SR)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (37 mg, 0.18 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed on a rotary evaporator and the residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 19 mg (100% purity, 30% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.45 min; MS (ESIpos): m/z = 359 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 10.62 (s, 1H), 8.17–8.12 (m, 2H), 7.75 (s, 1H), 7.55 (s, 1H), 7.45 (s, 1H), 5.54 (s, 2H), 3.80 (s, 3H), 3.65 (d, J = 6.36 Hz, 2H), 1.16–1.09 (m, 1H), 0.43–0.38 (m, 2H), 0.37–0.30 (m, 1H), 0.15–0.08 (m, 1H).

Preparation of 13. N-[[[(4R)-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-1-phenyl-1H-pyrazole-4-carboxamide (13). 1-Phenyl-1H-pyrazole-4-carboxylic acid (37 mg, 0.20 mmol) was dissolved in 1 mL of DMF. DIPEA (95 μ L, 0.55 mmol), EDC*HCl (48 mg, 0.25 mmol), and 1-hydroxybenzotriazole hydrate (39 mg, 0.25 mmol) were added and the mixture was stirred at room temperature. After 5 min, (SR)-5-(aminomethyl)-5-cyclopropylimidazolidine-2,4-dione hydrochloride⁴⁰ (40 mg, 0.20 mmol) was added and the mixture was

stirred at room temperature overnight. The reaction mixture was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. 55 mg (100% purity, 81% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.11 min; MS (ESIpos): *m/z* = 340 [*M* + *H*]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 10.62 (s, 1H), 8.95 (s, 1H), 8.22 (t, *J* = 6.33 Hz, 1H), 8.15 (s, 1H), 7.84 (d, *J* = 7.7 Hz, 2H), 7.55–7.52 (m, 3H), 7.34 (m, 1H), 3.80 (dd, *J* = 13.85 Hz, 1H), 3.50 (dd, *J* = 13.85 Hz, 1H), 1.15 (t, *J* = 8.21 Hz, 1H), 0.51–0.41 (m, 2H), 0.37–0.32 (m, 1H), 0.15–0.11 (m, 1H).

Preparation of 14. 1-(4-Chlorobenzyl)-*N*-[[4*R*]-4-cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-1*H*-pyrazole-4-carboxamide (14). 1-(4-Chlorobenzyl)-1*H*-pyrazole-4-carboxylic acid (58 mg, 0.24 mmol) was dissolved in 3 mL of DMF. DIPEA (120 μL, 0.68 mmol), EDC*HCl (61 mg, 0.32 mmol), and 1-hydroxybenzotriazole hydrate (48 mg, 0.32 mmol) were added and the mixture was stirred at room temperature. After 5 min (5*R*)-5-(aminomethyl)-5-cyclopropyl-imidazolidine-2,4-dione hydrochloride⁴⁰ (50 mg, 0.24 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 58 mg (100% purity, 62% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.25 min; MS (ESIpos): *m/z* = 388 [*M* + *H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.55 (s, 1H), 8.26 (s, 1H), 8.04 (t, *J* = 6.24 Hz, 1H), 7.88 (d, *J* = 0.61 Hz, 1H), 7.44–7.41 (m, 3H), 7.28 (d, *J* = 8.56 Hz, 2H), 5.35 (s, 2H), 3.71 (dd, *J* = 13.75 Hz, 1H), 3.45 (dd, *J* = 13.75 Hz, 1H), 1.13–1.07 (m, 1H), 0.47–0.38 (m, 2H), 0.35–0.28 (m, 1H), 0.14–0.09 (m, 1H).

Preparation of 15. *N*-[[4*R*]-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-4'-methyl[biphenyl]-4-carboxamide (15). 4'-Methyl[biphenyl]-4-carboxylic acid (41 mg, 0.20 mmol) was dissolved in 1 mL of DMF. DIPEA (95 μL, 0.55 mmol), EDC*HCl (48 mg, 0.25 mmol), and 1-hydroxybenzotriazole hydrate (39 mg, 0.25 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5*R*)-5-(aminomethyl)-5-cyclopropyl-imidazolidine-2,4-dione hydrochloride⁴⁰ (40 mg, 0.20 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and the precipitated solid was filtered off, washed with water, and dried in vacuo. 50 mg (100% purity, 70% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.59 min; MS (ESIpos): *m/z* = 364 [*M* + *H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.62 (s, 1H), 8.42 (t, *J* = 6.24 Hz, 1H), 7.88 (d, *J* = 8.44 Hz, 2H), 7.74 (d, *J* = 8.44 Hz, 2H), 7.63 (d, *J* = 8.19 Hz, 2H), 7.53 (s, 1H), 7.30 (d, *J* = 7.95 Hz, 2H), 3.83–3.78 (m, 1H), 3.59–3.55 (m, 1H), 2.36 (s, 3H), 1.17–1.14 (m, 1H), 0.49–0.40 (m, 2H), 0.36–0.31 (m, 1H), 0.16–0.10 (m, 1H).

Preparation of 16. *N*-[[4*R*]-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-4'-methyl[biphenyl]-3-carboxamide (16). 4'-Methyl[biphenyl]-3-carboxylic acid (41 mg, 0.20 mmol) was dissolved in 1 mL of DMF. DIPEA (95 μL, 0.55 mmol), EDC*HCl (48 mg, 0.25 mmol), and 1-hydroxybenzotriazole hydrate (39 mg, 0.25 mmol) were added and the mixture was stirred at room temperature. After 5 min (5*R*)-5-(aminomethyl)-5-cyclopropyl-imidazolidine-2,4-dione hydrochloride⁴⁰ (40 mg, 0.20 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 48 mg (100% purity, 68% yield) of the title compound was obtained. LC–MS (Method 5): *R*_t = 1.13 min; MS (ESIpos): *m/z* = 364 [*M* + *H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.63 (s, 1H), 8.54 (t, *J* = 6.17 Hz, 1H), 8.05 (s, 1H), 7.78 (dd, *J* = 15.47 Hz, 2H), 7.62 (d, *J* = 8.19 Hz, 2H), 7.55–7.52 (m, 2H), 7.31 (d, *J* = 8.07 Hz, 2H), 3.82 (dd, *J* = 13.51 Hz, 1H), 3.56 (dd, *J* = 13.63 Hz, 1H), 2.36 (s, 3H), 1.20–1.13 (m, 1H), 0.52–0.40 (m, 2H), 0.37–0.30 (m, 1H), 0.16–0.10 (m, 1H).

Preparation of 17. *N*-[[4*R*]-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-4'-methyl[biphenyl]-2-carboxamide (17). 4'-Methyl[biphenyl]-2-carboxylic acid (41 mg, 0.20 mmol) was dissolved in 1 mL of DMF. DIPEA (95 μL, 0.55 mmol), EDC*HCl

(48 mg, 0.25 mmol), and 1-hydroxybenzotriazole hydrate (39 mg, 0.25 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5*R*)-5-(aminomethyl)-5-cyclopropyl-imidazolidine-2,4-dione hydrochloride⁴⁰ (40 mg, 0.20 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and the precipitated solid was filtered off, washed with water and dried in vacuo. 44 mg (100% purity, 62% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.46 min; MS (ESIpos): *m/z* = 364 [*M* + *H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.62 (s, 1H), 8.31 (t, *J* = 6.11 Hz, 1H), 7.50–7.46 (m, 2H), 7.40–7.34 (m, 3H), 7.26 (m, 2H), 7.19 (m, 2H), 3.51–3.45 (m, 2H), 2.32 (s, 3H), 1.09–1.00 (m, 1H), 0.47–0.36 (m, 2H), 0.34–0.28 (m, 1H), 0.15–0.09 (m, 1H).

Preparation of 18 and Respective Starting Materials. Methyl 4'-(trifluoromethyl)[biphenyl]-2-carboxylate. Under an argon atmosphere, methyl 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-benzoate (200 mg, 0.76 mmol) and 1-bromo-4-(trifluoromethyl)-benzene (214 mg, 0.95 mmol) were suspended in 3.5 mL of DME. Sodium carbonate solution (1.91 mL, 2 M, 3.82 mmol) and tetrakis(triphenylphosphine) palladium (0) (44 mg, 0.04 mmol) were added, and the mixture was stirred at 80 °C overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 123 mg (100% purity, 57% yield) of the title compound was obtained. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 7.85 (dd, *J* = 7.79 Hz, 1H), 7.78 (d, *J* = 8.07 Hz, 2H), 7.68 (td, *J* = 7.61 Hz, 1H), 7.56 (td, *J* = 7.61 Hz, 1H), 7.52 (d, *J* = 8.07 Hz, 2H), 7.47 (dd, *J* = 7.7 Hz, 1H), 3.61 (s, 3H).

4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid. Methyl 4'-(trifluoromethyl)[biphenyl]-2-carboxylate (120 mg, 0.43 mmol) was dissolved in 1.5 mL of THF and 0.3 mL of methanol. Lithium hydroxide solution (1.07 mL, 2 N, 2.14 mmol) was added and the mixture was stirred at 60 °C overnight. The reaction mixture was acidified with 2 N HCl and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 97 mg (100% purity, 85% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.87 min; MS (ESIpos): *m/z* = 265 [*M* + *H*]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ [ppm] = 12.88 (br s, 1H), 7.83 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 2H), 7.60–7.66 (m, 1H), 7.49–7.57 (m, 3H), 7.42 (dd, *J* = 7.6, 0.9 Hz, 1H).

N-[[4*R*]-4-Cyclopropyl-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (18). 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (52 mg, 0.20 mmol) was dissolved in 2 mL of DMF. DIPEA (95 μL, 0.55 mmol), EDC*HCl (48 mg, 0.25 mmol), and 1-hydroxybenzotriazole hydrate (39 mg, 0.25 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5*R*)-5-(aminomethyl)-5-cyclopropyl-imidazolidine-2,4-dione hydrochloride⁴⁰ (40 mg, 0.20 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 61 mg (99% purity, 74% yield) of the title compound was obtained. LC–MS (Method 1): *R*_t = 0.87 min; MS (ESIpos): *m/z* = 418 [*M* + *H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 10.64 (s, 1H), 8.55 (t, *J* = 6.11 Hz, 1H), 7.76 (d, *J* = 8.07 Hz, 2H), 7.57–7.53 (m, 4H), 7.47–7.43 (m, 3H), 3.53–3.50 (m, 2H), 1.11–1.04 (m, 1H), 0.50–0.42 (m, 1H), 0.41–0.37 (m, 1H), 0.36–0.30 (m, 1H), 0.17–0.11 (m, 1H).

Preparation of 19 and Respective Starting Materials. Ethyl 5-(1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate. 1,3-Thiazole-4-carboxylic acid (5 g, 38.72 mmol) was dissolved in 60 mL of THF. CDI (7.5 g, 46.46 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At

this temperature, first a solution of ethyl isocyanoacetate (4.66 mL, 42.59 mmol) in 60 mL THF and finally a LiHMDS solution (38.72 mL, 1 M in THF, 38.72 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred overnight. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 5.58 g (100% purity, 64% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.10 min; MS (ESIpos): m/z = 225 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 9.27 (d, J = 2.0 Hz, 1H), 8.73 (d, J = 2.0 Hz, 1H), 8.57 (s, 1H), 4.33 (q, J = 7.0 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H).

2-Amino-1-(1,3-thiazol-4-yl)ethanone hydrochloride. Ethyl 5-(1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate (5.58 g, 24.88 mmol) was stirred at 100 °C in 19 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The residue was treated with dichloromethane and a little methanol. The solid was filtered off and dried in vacuo. 5.9 g (86% purity, 114% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.46 min; MS (ESIpos): m/z = 143 $[M - Cl]^+$.

tert-Butyl [2-oxo-2-(1,3-thiazol-4-yl)ethyl]carbamate. 2-Amino-1-(1,3-thiazol-4-yl)ethanone hydrochloride (5.9 g, 86% purity, 28.52 mmol) was dissolved in 113 mL of dichloromethane. Di-tert-butyl dicarbonate (7.21 mL, 31.37 mmol) and triethylamine (11.93 mL, 85.56 mmol) were added, and the mixture was stirred at room temperature for 1.5 h. The solvent was evaporated, and the residue was extracted between water and ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 6.95 g (90% purity, 91% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.71 min; MS (ESIpos): m/z = 243 $[M + H]^+$.

Ent-tert-butyl {[2,5-dioxo-4-(1,3-thiazol-4-yl)imidazolidin-4-yl]methyl}carbamate. In a microwave vial, tert-butyl [2-oxo-2-(1,3-thiazol-4-yl)ethyl]carbamate (6.95 g, 90% purity, 25.88 mmol) was dissolved in 40 mL of methanol. Potassium cyanide (6.7 g, 103.50 mmol) and ammonium carbonate (9.9 g, 103.50 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C overnight. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude racemate (12 g) was separated on chiral phase using the following preparative HPLC method: Machine: THAR SFC-Super Chrom Prep 200; Column: Chiralpak AD-H 5 μ m, 250 \times 25 mm²; Eluent: CO₂/i-propanol 70:30; Flow: 100 mL/min; Backpressure: 115 bar; Temperature eluent: 38 °C; Temperature cyclone: 40 °C; Pressure cyclone: 24 bar; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 2.63 g (100% purity, 32% yield) of the title compound was obtained. Chiral HPLC (Column: Chiralpak AD-H 3 μ m 100 \times 4.6 mm²; solvent: 80% CO₂/20% i-propanol; BPR pressure: 130 bar; BPR temperature: 60 °C; Column temperature: 40 °C; flow: 3 mL/min; UV-detection: 210 nm): Rt = 1.581 min, 100% ee. LC–MS (Method 2): Rt = 0.96 min; MS (ESIpos): m/z = 311 $[M - H]^+$.

Ent-5-(aminomethyl)-5-(1,3-thiazol-4-yl)imidazolidine-2,4-dione hydrochloride. Ent-tert-butyl {[2,5-dioxo-4-(1,3-thiazol-4-yl)-imidazolidin-4-yl]methyl}carbamate (2.62 g, 8.42 mmol) was dissolved in 50 mL of dichloromethane. Hydrochloric acid in 1,4-dioxan (10.53 mL, 4 N, 42.10 mmol) was added and the mixture was stirred at room temperature overnight. The solvents were evaporated. The residue was dissolved in water/acetonitrile and lyophilized. 2.43 g (100% purity, 116% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.24 min; MS (ESIpos): m/z = 213 $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.17 (s, 1H), 9.18 (d, J = 1.8 Hz, 1H), 8.59 (s, 1H), 8.49 (br s, 3H), 3.19–3.76 (m, 2H).

Ent-N-[[2,5-dioxo-4-(1,3-thiazol-4-yl)imidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (19). 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (107 mg, 0.40 mmol) was dissolved in 2.5 mL of DMF. DIPEA (0.35 mL, 2.01 mmol), EDC*HCl (100 mg, 0.52 mmol), and 1-hydroxybenzotriazole hydrate (80 mg, 0.52 mmol) were added and the mixture was stirred at room

temperature. After 5 min, ent-5-(aminomethyl)-5-(1,3-thiazol-4-yl)imidazolidine-2,4-dione hydrochloride (100 mg, 0.40 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Further purification by preparative HPLC was needed. Product containing samples were united and the solvents were evaporated. 82 mg (100% purity, 44% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.154 min; MS (ESIpos): m/z = 461 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 10.85 (br s, 1H), 9.12 (d, J = 1.96 Hz, 1H), 8.66 (t, J = 6.16 Hz, 1H), 8.25 (s, 1H), 7.78–7.75 (m, 3H), 7.57–7.54 (m, 3H), 7.48–7.42 (m, 3H), 4.00–3.89 (m, 2H).

Preparation of 20 and Respective Starting Materials. Ethyl 5-(5-methyl-1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate. 5-Methyl-1,3-thiazole-4-carboxylic acid (940 mg, 6.57 mmol) was dissolved in 10 mL of THF. CDI (1.28 g, 7.88 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocyanoacetate (0.79 mL, 7.22 mmol) in 10 mL THF and finally a LiHMDS solution (6.57 mL, 1 M in THF, 6.57 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 2 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 1.13 g (100% purity, 72% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.15 min; MS (ESIpos): m/z = 239 $[M + H]^+$.

2-Amino-1-(5-methyl-1,3-thiazol-4-yl)ethanone hydrochloride. Ethyl 5-(5-methyl-1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate (1.13 g, 4.74 mmol) was stirred at 100 °C in 25 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The residue was treated with dichloromethane/methanol 20:1. The solid was filtered off and dried in vacuo. 730 mg (98% purity, 79% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.80 min; MS (ESIpos): m/z = 156 $[M - Cl]^+$.

tert-Butyl [2-(5-methyl-1,3-thiazol-4-yl)-2-oxoethyl]carbamate. 2-Amino-1-(5-methyl-1,3-thiazol-4-yl)ethanone hydrochloride (730 mg, 98% purity, 3.71 mmol) was dissolved in 15 mL of dichloromethane. Di-tert-butyl dicarbonate (0.96 mL, 4.17 mmol) and triethylamine (1.58 mL, 11.37 mmol) were added, and the mixture was stirred at room temperature for 2 h. The solvent was evaporated, and the residue was extracted between water and ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 980 mg (100% purity, 100% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.57 min; MS (ESIpos): m/z = 257 $[M + H]^+$.

Rac-tert-butyl {[4-(5-methyl-1,3-thiazol-4-yl)-2,5-dioxoimidazolidin-4-yl]methyl}carbamate. In a microwave vial, tert-butyl [2-(5-methyl-1,3-thiazol-4-yl)-2-oxoethyl]carbamate (980 mg, 25.88 mmol) was dissolved in 7 mL of methanol. Potassium cyanide (996 mg, 15.29 mmol) and ammonium carbonate (1.47 g, 15.29 mmol) were added. The vial was sealed, and the mixture was stirred at 40 °C for 2 d. More potassium cyanide (996 mg, 15.29 mmol) and ammonium carbonate (1.47 g, 15.29 mmol) were added, and the reaction mixture was stirred at 40 °C for 3 d. The reaction mixture was concentrated. The residue was poured on Isolute and purified by column chromatography. Product containing samples were united and the solvents were evaporated. 766 mg (100% purity, 61% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.09 min; MS (ESIpos): m/z = 325 $[M - H]^+$.

Rac-5-(aminomethyl)-5-(5-methyl-1,3-thiazol-4-yl)-imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl {[4-(5-methyl-1,3-thiazol-4-yl)-2,5-dioxoimidazolidin-4-yl]methyl}carbamate (766 mg, 2.35 mmol) was dissolved in 9 mL of dichloromethane. Hydrochloric acid in 1,4-dioxan (2.93 mL, 4 N, 11.74 mmol) was added and the mixture was stirred at room temperature for 30 min. The precipitated solid was filtered off, washed with dichloromethane, and dried in vacuo. 640 mg (86% purity, 89% yield) of the title

compound was obtained. LC–MS (Method 4): R_t = 0.25 min; MS (ESIpos): m/z = 227 $[M - Cl]^+$.

Rac-N-([4-(5-methyl-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl)-4'-(trifluoromethyl)[biphenyl]-2-carboxamide. 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (129 mg, 0.49 mmol) was dissolved in 10 mL of dichloromethane. DIPEA (0.24 mL, 1.36 mmol), EDC·HCl (121 mg, 0.63 mmol), and 1-hydroxybenzotriazole hydrate (97 mg, 0.63 mmol) were added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 56 mg (100% purity, 24% yield) of the title compound was obtained. LC–MS (Method 2): R_t = 1.63 min; MS (ESIpos): m/z = 475 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 11.04 (s, 1H), 8.85 (s, 1H), 8.62 (t, J = 6.2 Hz, 1H), 8.26 (s, 1H), 7.77 (d, J = 8.2 Hz, 2H), 7.63–7.51 (m, 3H), 7.50–7.41 (m, 3H), 4.08 (dd, J = 6.2, 2.2 Hz, 2H), 2.36 (s, 3H).

Ent-N-([4-(5-methyl-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl)-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (20). Enantiomeric separation of 52 mg rac-N-([4-(5-methyl-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl)-4'-(trifluoromethyl)-[biphenyl]-2-carboxamide was done using the following preparative chiral HPLC method: Machine: HPLC-Agilent prep 100; Column: Diacel Chiralpak ID 5 μ m, 20 \times 250 mm; Eluent: 30% n-Heptan and 70% i-propanol; Flow: 20 mL/min; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 16 mg (100% purity, 31% yield) of the title compound was obtained. Chiral HPLC (Column: Diacel ID 3 μ m 50 \times 4.6 mm 2 ; solvent: *n*-heptane/*i*-propanol 1:1; flow: 1 mL/min; UV-detection: 220 nm): R_t = 1.581 min, 100% ee. LC–MS (Method 2): R_t = 1.63 min; MS (ESIpos): m/z = 475 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 11.04 (s, 1H), 8.85 (s, 1H), 8.62 (t, J = 6.24 Hz, 1H), 8.26 (s, 1H), 7.77 (d, J = 8.31 Hz, 2H), 7.59–7.53 (m, 3H), 7.49–7.42 (m, 3H), 4.12–4.04 (m, 2H), 2.36 (s, 3H).

Preparation of 21 and Respective Starting Materials. Ethyl 5-[5-(trifluoromethyl)-1,3-thiazol-4-yl]-1,3-oxazole-4-carboxylate. 5-(Trifluoromethyl)-1,3-thiazole-4-carboxylic acid (2.0 g, 10.15 mmol) was dissolved in 15 mL of THF. CDI (1.97 g, 12.17 mmol) was added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocyanoacetate (1.22 mL, 11.16 mmol) in 15 mL THF and finally a LiHMDS solution (10.15 mL, 1 M in THF, 10.15 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 2 d. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 1.43 g (100% purity, 48% yield) of the title compound was obtained. LC–MS (Method 2): R_t = 1.62 min; MS (ESIpos): m/z = 293 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 9.57 (s, 1H), 8.78 (s, 1H), 4.18 (q, J = 7.1 Hz, 2H), 1.10 (t, J = 7.2 Hz, 3H).

2-Amino-1-[5-(trifluoromethyl)-1,3-thiazol-4-yl]ethanone hydrochloride. Ethyl 5-[5-(trifluoromethyl)-1,3-thiazol-4-yl]-1,3-oxazole-4-carboxylate (1.43 g, 4.89 mmol) was stirred at 100 °C in 200 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated, and the residue was dried in vacuo. 1.25 g (95% purity, 98% yield) of the title compound was obtained. LC–MS (Method 4): R_t = 1.00 min; MS (ESIpos): m/z = 211 $[M - Cl]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 9.52 (s, 1H), 8.41 (br s, 3H), 4.57 (s, 2H).

tert-Butyl {2-oxo-2-[5-(trifluoromethyl)-1,3-thiazol-4-yl]ethyl}carbamate. 2-Amino-1-[5-(trifluoromethyl)-1,3-thiazol-4-yl]ethanone hydrochloride (1.25 g, 98% purity, 4.97 mmol) was dissolved in 23 mL of dichloromethane. Di-tert-butyl dicarbonate (1.22 g, 5.58 mmol) and triethylamine (3.53 mL, 25.34 mmol) were

added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 1.66 g (68% purity, 73% yield) of the title compound was obtained. LC–MS (Method 4): R_t = 1.58 min; MS (ESIpos): m/z = 309 $[M - H]^+$.

Rac-tert-butyl{[2,5-dioxo-4-[5-(trifluoromethyl)-1,3-thiazol-4-yl]imidazolidin-4-yl]methyl}carbamate. In a microwave vial, tert-butyl {2-oxo-2-[5-(trifluoromethyl)-1,3-thiazol-4-yl]ethyl}carbamate (1.6 g, 68% purity, 3.51 mmol) was dissolved in 9.2 mL of water and 3.7 mL of ethanol. Potassium cyanide (1.34 g, 20.62 mmol) and ammonium carbonate (1.98 g, 20.62 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C overnight. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude product was purified by preparative HPLC. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 485 mg (100% purity, 36% yield) of the title compound was obtained. LC–MS (Method 4): R_t = 0.76 min; MS (ESIpos): m/z = 379 $[M - H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 11.37–10.51 (m, 1H), 9.32 (s, 1H), 7.78–7.13 (m, 1H), 6.71 (br s, 1H), 3.97–3.68 (m, 2H), 1.36 (s, 9H).

Rac-5-(aminomethyl)-5-[5-(trifluoromethyl)-1,3-thiazol-4-yl]imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl{[2,5-dioxo-4-[5-(trifluoromethyl)-1,3-thiazol-4-yl]imidazolidin-4-yl]methyl}carbamate (485 mg, 1.28 mmol) was dissolved in 20 mL of dichloromethane. Hydrochloric acid in 1,4-dioxan (1.59 mL, 4 N, 6.38 mmol) was added and the mixture was stirred at room temperature overnight. The solvents were removed, and the residue was dried in vacuo. 413 mg (95% purity, 97% yield) of the title compound was obtained. LC–MS (Method 4): R_t = 0.29 min; MS (ESIpos): m/z = 281 $[M - Cl]^+$.

Ent-N-([2,5-dioxo-4-[5-(trifluoromethyl)-1,3-thiazol-4-yl]imidazolidin-4-yl]methyl)-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (21). 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (110 mg, 0.34 mmol) was dissolved in 2.1 mL of DMF. DIPEA (0.3 mL, 1.72 mmol), EDC·HCl (86 mg, 0.45 mmol), and 1-hydroxybenzotriazole hydrate (68 mg, 0.45 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-[5-(trifluoromethyl)-1,3-thiazol-4-yl]imidazolidine-2,4-dione hydrochloride (109 mg, 0.34 mmol) was added and the mixture was stirred at room temperature for 2 d. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 108 mg (85% purity) of the racemate was obtained. Enantiomeric separation of the racemate was done using the following preparative chiral HPLC method: Machine: HPLC-Agilent prep 100; Column: Diacel Chiralpak ID 5 μ m, 20 \times 250 mm 2 ; Eluent: 50% n-Heptan and 50% i-propanol; Flow: 20 mL/min; UV-detection: 220 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 31 mg (100% purity, 17% yield) of the title compound was obtained. Chiral HPLC (Column: Diacel ID 3 μ m 50 \times 4.6 mm 2 ; solvent: *n*-heptane/*i*-propanol 1:1; flow: 1 mL/min; UV-detection: 220 nm): R_t = 1.633 min, 100% ee. LC–MS (Method 2): R_t = 1.73 min; MS (ESIpos): m/z = 529 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.13 (s, 1H), 9.83 (s, 1H), 8.65 (t, J = 6.33 Hz, 1H), 8.32 (s, 1H), 7.77 (d, J = 8.07 Hz, 2H), 7.58–7.55 (m, 3H), 7.50–7.42 (m, 3H), 4.26 (dd, J = 13.76 Hz, 1H), 4.03 (dd, J = 13.66 Hz, 1H).

Preparation of 22 and Respective Starting Materials. Ethyl 5-(2,5-dimethyl-1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate. 2,5-Dimethyl-1,3-thiazole-4-carboxylic acid (885 mg, 5.63 mmol) was dissolved in 10 mL of THF. CDI (1.1 g, 6.76 mmol) was added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocyanoacetate (0.68 mL, 6.19 mmol) in 10 mL THF and then a LiHMDS solution (5.63 mL, 1 M in THF, 5.63 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred overnight. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The

combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 811 mg (100% purity, 57% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.31$ min; MS (ESIpos): $m/z = 253$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.59 (s, 1H), 4.23 (q, $J = 7.0$ Hz, 2H), 2.63 (s, 3H), 2.33 (s, 3H), 1.20 (t, $J = 7.0$ Hz, 3H).

tert-Butyl [2-(2,5-dimethyl-1,3-thiazol-4-yl)-2-oxoethyl]-carbamate. Ethyl 5-(2,5-dimethyl-1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate (811 mg, 3.22 mmol) was stirred at 100 °C in 17 mL of hydrochloric acid (6 N in water). After 3 h, the solvent was evaporated, and the residue was dried in vacuo. The residue was dissolved in 18 mL of dichloromethane. Di-tert-butyl dicarbonate (771 mg, 3.53 mmol) and triethylamine (4.47 mL, 32.10 mmol) were added, and the mixture was stirred at room temperature for 2 h. The solvent was evaporated, and the residue was extracted between water and ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 775 mg (82% purity, 73% yield) of the title compound was obtained. LC–MS (Method 1): $R_t = 0.91$ min; MS (ESIpos): $m/z = 271$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 6.94 (br t, $J = 5.9$ Hz, 1H), 4.35 (d, $J = 5.9$ Hz, 2H), 2.67 (s, 3H), 2.63 (s, 3H), 1.40 (s, 9H).

Rac-tert-butyl[4-(2,5-dimethyl-1,3-thiazol-4-yl)-2,5-dioxoimidazolidin-4-yl]methyl]carbamate. In a microwave vial, tert-butyl [2-(2,5-dimethyl-1,3-thiazol-4-yl)-2-oxoethyl]carbamate (773 mg, 82% purity, 2.35 mmol) was dissolved in 5 mL of methanol. Potassium cyanide (745 mg, 11.44 mmol) and ammonium carbonate (1.65 g, 17.16 mmol) were added. The vial was sealed, and the mixture was stirred at 60 °C overnight. More ammonium carbonate (550 mg, 5.72 mmol) was added, and the mixture was stirred at 60 °C. After 8 h, further ammonium carbonate (550 mg, 5.72 mmol) and potassium cyanide (93 mg, 1.43 mmol) were added, and the mixture was stirred at 60 °C for 8 h. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude product was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 484 mg (100% purity, 60% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.22$ min; MS (ESIpos): $m/z = 341$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 10.84 (br s, 1H), 8.06 (s, 1H), 6.74 (br s, 1H), 3.92–3.61 (m, 2H), 2.54 (s, 3H), 2.26 (s, 3H), 1.37 (s, 9H).

Rac-5-(aminomethyl)-5-(2,5-dimethyl-1,3-thiazol-4-yl)-imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl[4-(2,5-dimethyl-1,3-thiazol-4-yl)-2,5-dioxoimidazolidin-4-yl]methyl]carbamate (480 mg, 1.41 mmol) was dissolved in 5.5 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (1.76 mL, 4 N, 7.05 mmol) was added and the mixture was stirred at room temperature for 2 h. The precipitated solid was filtered off, washed with dichloromethane, and dried in vacuo. 339 mg (100% purity, 87% yield) of the title compound was obtained. LC–MS (Method 4): $R_t = 0.27$ min; MS (ESIpos): $m/z = 241$ $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.31 (s, 1H), 8.60 (br s, 1H), 8.31 (br s, 3H), 3.66–3.49 (m, 2H), 2.58 (s, 3H), 2.30 (s, 3H).

Ent-N-[4-(2,5-dimethyl-1,3-thiazol-4-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (22). 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (35 mg, 0.13 mmol) was dissolved in 0.65 mL of DMF. DIPEA (0.09 mL, 0.54 mmol), EDC*HCl (27 mg, 0.14 mmol), and 1-hydroxybenzotriazole hydrate (22 mg, 0.14 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-(2,5-dimethyl-1,3-thiazol-4-yl)imidazolidine-2,4-dione hydrochloride (30 mg, 0.11 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 32 mg (100% purity) of the racemate was obtained. Enantiomeric separation was done using the following preparative chiral HPLC method: Column: Diacel Chiralpak IC 5 μ m, 20 \times 250 mm; Eluent: 60% *n*-heptane and 40% *i*-propanol; Flow: 20 mL/min; UV-detection: 220 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was

lyophilized. 14 mg (100% purity, 26% yield) of the title compound was obtained. Chiral HPLC (Column: Diacel Chiralpak IC 5 μ m 250 \times 4.6 mm²; solvent: 40% *i*-hexane and 60% *i*-propanol; flow: 1 mL/min; UV-detection: 220 nm): $R_t = 6.142$ min, 100% ee. LC–MS (Method 2): $R_t = 1.73$ min; MS (ESIpos): $m/z = 489$ $[M + H]^+$. 1H NMR (500 MHz, DMSO- d_6) δ [ppm] = 10.99 (br s, 1H), 8.60 (t, $J = 6.31$ Hz, 1H), 8.22 (s, 1H), 7.77 (d, $J = 8.28$ Hz, 2H), 7.59–7.54 (m, 3H), 7.49–7.41 (m, 3H), 4.09–3.98 (m, 2H), 2.87 (s, 3H).

Preparation of 23 and Respective Starting Materials. tert-Butyl [2-(1-methyl-1H-imidazol-2-yl)-2-oxoethyl]carbamate. Under an argon atmosphere, 1-methylimidazole (6.12 g, 74.52 mmol) was dissolved in 50 mL of THF. The solution was cooled to –78 °C. At this temperature, an *n*-BuLi -solution (32.79 mL, 2.5 M in hexane, 81.97 mmol) was slowly added. The mixture was stirred at –78 °C for 30 min. Then a solution of tert-butyl [2-(methoxy(methyl)amino)-2-oxoethyl]carbamate (17.89 g, 81.97 mmol) in 100 mL of THF was added slowly. The mixture was stirred at –78 °C for further 2 h and at room temperature for 10 min. The reaction mixture was quenched with 60 mL of 1 M HCl, then mixed with 60 mL of brine and 60 mL saturated sodium carbonate solution, and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. The crude product was suspended in water and refluxed for 10 min. After cooling, the precipitate was filtered off and dried in vacuo. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 5.44 g (100% purity, 31% yield) of the title compound was obtained. LC–MS (Method 1): $R_t = 0.67$ min; MS (ESIpos): $m/z = 283$ $[M - H]^+$. 1H NMR (500 MHz, DMSO- d_6) δ [ppm] = 7.52 (s, 1H), 7.12 (s, 1H), 6.97 (br t, $J = 5.8$ Hz, 1H), 4.41 (d, $J = 6.0$ Hz, 2H), 3.92 (s, 3H), 1.40 (s, 9H).

Ent-tert-butyl[4-(1-methyl-1H-imidazol-2-yl)-2,5-dioxoimidazolidin-4-yl]methyl]carbamate. In a microwave vial, tert-butyl [2-(1-methyl-1H-imidazol-2-yl)-2-oxoethyl]carbamate (4.96 g, 20.73 mmol) was dissolved in 12 mL of water and 30 mL of ethanol. Potassium cyanide (5.4 g, 82.92 mmol) and ammonium carbonate (7.97 g, 82.92 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C for 2 d. Water was added to the reaction mixture and the ethanol was removed on a rotary evaporator. Then the solid was filtered off and the filtrate was lyophilized. 7.3 g of the crude racemate was obtained. Enantiomeric separation was done using the following preparative chiral HPLC method: Machine: THAR SFC-Super Chrom Prep 200; Column: Chiralpak AD-H 5 μ m, 250 \times 25 mm²; Eluent: CO₂/*i*-propanol 78:22; Flow: 125 mL/min; Backpressure: 135 bar; Temperature eluent: 38 °C; Temperature cyclone: 40 °C; Pressure cyclone: 24 bar; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 2 g (100% purity, 31% yield) of the title compound was obtained. Chiral HPLC (Column: SFC AD; solvent: 80% CO₂ and 20% methanol; flow: 3 mL/min; UV-detection: 210 nm): $R_t = 1.702$ min, 100% ee. LC–MS (Method 4): $R_t = 0.46$ min; MS (ESIpos): $m/z = 308$ $[M - H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.27–10.91 (m, 1H), 8.16 (br s, 1H), 7.20 (s, 1H), 6.85 (d, $J = 1.1$ Hz, 1H), 6.83–6.75 (m, 1H), 3.83 (br s, 2H), 3.49 (s, 3H), 1.37 (s, 9H).

Ent-5-(aminomethyl)-5-(1-methyl-1H-imidazol-2-yl)-imidazolidine-2,4-dione hydrochloride. Ent-tert-butyl[4-(1-methyl-1H-imidazol-2-yl)-2,5-dioxoimidazolidin-4-yl]methyl]carbamate (2 g, 6.46 mmol) was dissolved in 46 mL of methanol. Hydrochloric acid in 1,4-dioxane (1.76 mL, 4 N, 7.05 mmol) was added at 0 °C and the mixture was stirred at room temperature overnight. The precipitated solid was filtered off, washed with methanol, and dried in vacuo. 410 mg (89% purity, 23% yield) of the title compound was obtained. The filtrate was concentrated, and the residue dried in vacuo. 1.23 g (91% purity, 70% yield) of the title compound was obtained. LC–MS (Method 4): $R_t = 0.23$ min; MS (ESIpos): $m/z = 210$ $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.74 (s, 1H), 9.12 (br s, 1H), 8.69 (br s, 3H), 7.55 (br s, 1H), 7.34 (br s, 1H), 3.79 (br s, 3H), 3.74 (br s, 2H).

Ent-N-[4-(1-methyl-1H-imidazol-2-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (23). 4'-

(Trifluoromethyl)[biphenyl]-2-carboxylic acid (54 mg, 0.20 mmol) was dissolved in 4 mL of DMF. DIPEA (0.10 mL, 0.57 mmol), EDC*HCl (51 mg, 0.26 mmol), and 1-hydroxybenzotriazole hydrate (41 mg, 0.26 mmol) were added and the mixture was stirred at room temperature. After 5 min, ent-5-(aminomethyl)-5-(1-methyl-1H-imidazol-2-yl)imidazolidine-2,4-dione hydrochloride (50 mg, 91% purity, 0.18 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 52 mg (99% purity, 63% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.41 min; MS (ESIpos): m/z = 458 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.23 (br s, 1H), 8.58 (t, J = 6.36 Hz, 1H), 8.33 (s, 1H), 7.77 (d, J = 8.19 Hz, 2H), 7.59–7.54 (m, 3H), 7.50–7.44 (m, 3H), 7.21 (d, J = 1.1 Hz, 1H), 6.85 (d, J = 1.22 Hz, 1H), 4.14–3.99 (m, 2H), 3.52 (s, 3H).

Preparation of 24 and Respective Starting Materials. Ethyl 5-(1-methyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate (**38**). Described below under Preparation of BAY-9835 (32) and Respective Starting Materials.

tert-Butyl [2-(1-methyl-1H-pyrazol-5-yl)-2-oxoethyl]carbamate (40). Described below under Preparation of BAY-9835 (32) and Respective Starting Materials.

Rac-tert-butyl{[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl}carbamate (41). Described below under Preparation of BAY-9835 (32) and Respective Starting Materials.

tert-Butyl{[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl}carbamate (42). Described below under Preparation of BAY-9835 (32) and Respective Starting Materials.

(5S)-5-(Aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride (43). Described below under Preparation of BAY-9835 (32) and Respective Starting Materials.

N-[[4-(1-Methyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (24). 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (94 mg, 0.35 mmol) was dissolved in 2 mL of DMF. DIPEA (0.31 mL, 1.77 mmol), EDC*HCl (88 mg, 0.46 mmol), and 1-hydroxybenzotriazole hydrate (71 mg, 0.46 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5S)-5-(aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (**43**) (100 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 63 mg (100% purity, 39% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.56 min; MS (ESIpos): m/z = 458 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 11.23 (br s, 1H), 8.72 (t, J = 6.3 Hz, 1H), 8.37 (s, 1H), 7.76 (d, J = 8.19 Hz, 2H), 7.58–7.43 (m, 6H), 7.38 (d, J = 1.96 Hz, 1H), 6.47 (d, J = 1.96 Hz, 1H), 3.98–3.86 (m, 2H), 3.80 (s, 3H).

Preparation of 25 and Respective Starting Materials. Ethyl 5-(1-ethyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate. 1-Ethyl-1H-pyrazole-5-carboxylic acid (4 g, 28.54 mmol) was dissolved in 50 mL of THF. CDI (5.55 g, 34.25 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocynoacetate (3.43 mL, 31.40 mmol) in 30 mL THF and then a LiHMDS solution (28.54 mL, 1 M in THF, 28.54 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred overnight. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 4.4 g (100% purity, 66% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.68 min; MS (ESIpos): m/z = 236 $[M + H]^+$.

2-Amino-1-(1-ethyl-1H-pyrazol-5-yl)ethanone hydrochloride. Ethyl 5-(1-ethyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate (4.4 g, 18.70 mmol) was stirred at 100 °C in 135 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The crude

product (4.7 g) was used in the next step without purification and analytics.

tert-Butyl [2-(1-ethyl-1H-pyrazol-5-yl)-2-oxoethyl]carbamate. 2-Amino-1-(1-ethyl-1H-pyrazol-5-yl)ethanone hydrochloride (4.7 g) was dissolved in 98 mL of dichloromethane. Di-tert-butyl dicarbonate (5.95 g, 27.26 mmol) and triethylamine (10.36 mL, 74.35 mmol) were added, and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator. The residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 5.6 g (76% purity) of the title compound was obtained. LC–MS (Method 2): Rt = 1.55 min; MS (ESIpos): m/z = 254 $[M + H]^+$.

Rac-tert-butyl{[4-(1-ethyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl}carbamate. Divided in three microwave vials: tert-butyl [2-(1-ethyl-1H-pyrazol-5-yl)-2-oxoethyl]carbamate (5.6 g, 76% purity, 16.8 mmol) was dissolved in 60 mL of methanol. Potassium cyanide (5.76 g, 88.43 mmol) and ammonium carbonate (4.73 g, 96.09 mmol) were added. The vials were sealed, and the mixture was stirred at 60 °C overnight. The salts were filtered off and rinsed with methanol and the filtrate was concentrated. The residue was purified by preparative HPLC. Product containing samples were united and evaporated. 920 mg (80% purity, 14% yield) of the title compound was obtained. LC–MS (Method 5): Rt = 0.79 min; MS (ESIpos): m/z = 322 $[M - H]^+$.

Rac-5-(aminomethyl)-5-(1-ethyl-1H-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl{[4-(1-ethyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl}carbamate (918 mg, 80% purity, 2.27 mmol) was dissolved in 13.4 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (3.55 mL, 4 N, 14.42 mmol) was added and the mixture was stirred at room temperature overnight. The solvents were evaporated, and the residue was dried in vacuo. 1.15 g (89% purity) of the title compound was obtained. LC–MS (Method 4): Rt = 0.23 min; MS (ESIpos): m/z = 224 $[M - Cl]^+$.

Rac-N-[[4-(1-ethyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide. 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (1.06 g, 3.99 mmol) was dissolved in 4 mL of DMF. DIPEA (2.08 mL, 11.96 mmol), EDC*HCl (993 mg, 5.18 mmol), and 1-hydroxybenzotriazole hydrate (793 mg, 5.18 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-(1-ethyl-1H-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (1.15 g, 89% purity, 3.95 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 500 mg (100% purity, 27% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.59 min; MS (ESIpos): m/z = 472 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 11.26 (s, 1H), 8.77 (t, J = 6.2 Hz, 1H), 8.44 (s, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.38–7.61 (m, 7H), 6.46 (d, J = 2.0 Hz, 1H), 4.04 (dd, J = 7.2, 1.9 Hz, 2H), 3.97–3.85 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H).

Ent-N-[[4-(1-ethyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (25). Enantiomeric separation of 500 mg of rac-N-[[4-(1-ethyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide was done using the following preparative chiral HPLC method: Machine: ABSYS SFC; Column: Chiralpak AD-H 5 μ m, 250 \times 25 mm²; Eluent: CO₂/Methanol 80%: 20%; Flow: 140 mL/min; Backpressure: 100 bar; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator, and the residue was lyophilized. 95 mg (100% purity, 19% yield) of the title compound was obtained. Chiral HPLC (Column: IC-5 250 \times 4.6 mm²; solvent: 70% CO₂/30% methanol; flow: 3 mL/min; UV-detection: 210 nm): Rt = 2.903 min, 98.3% ee. LC–MS (Method 2): Rt = 1.60 min; MS (ESIpos): m/z = 472 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.24 (br s, 1H), 8.74 (t, J = 6.36 Hz, 1H), 8.41 (s, 1H), 7.75 (d, J = 8.22 Hz, 2H), 7.58–7.53 (m, 3H), 7.48 (m, 1H), 7.45–7.43 (m, 3H), 6.45 (d, J = 1.96 Hz, 1H), 4.09–4.02 (m, 2H), 3.95–3.86 (m, 2H), 1.31 (t, J = 7.24 Hz, 3H).

Preparation of 26 and Respective Starting Materials. Ethyl 5-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]-1,3-oxazole-4-carboxylate. 1-(2,2,2-Trifluoroethyl)-1H-pyrazole-5-carboxylic acid (450 mg, 2.32 mmol) was dissolved in 5 mL of THF. CDI (451 mg, 2.78 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocynoacetate (280 μ L, 2.55 mmol) in 5 mL of THF and then a LiHMDS solution (2.32 mL, 1 M in THF, 2.32 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 2 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 590 mg (98% purity, 87% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.51 min; MS (ESIpos): m/z = 290 [M + H]⁺.

2-Amino-1-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]ethanone hydrochloride. Ethyl 5-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]-1,3-oxazole-4-carboxylate (590 mg 98% purity, 1.99 mmol) was stirred at 100 °C in 10.75 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The crude product (511 mg) was used in the next step without purification and analytics. LC–MS (Method 2): Rt = 1.78 min; MS (ESIpos): m/z = 308 [M + H]⁺.

tert-Butyl {2-oxo-2-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]ethyl}carbamate. 2-Amino-1-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]ethanone hydrochloride (511 mg) was dissolved in 8.3 mL of dichloromethane. Di-tert-butyl dicarbonate (504 mg, 2.31 mmol) and triethylamine (0.88 mL, 6.29 mmol) were added, and the mixture was stirred at room temperature for 2 h. The solvent was removed on a rotary evaporator. The residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 689 mg (100% purity) of the title compound was obtained.

Rac-tert-butyl [(2,5-dioxo-4-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidin-4-yl)methyl]carbamate. In a microwave vial, tert-butyl {2-oxo-2-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]ethyl}carbamate (689 mg, 2.24 mmol) was dissolved in 5 mL of methanol. Potassium cyanide (730 mg, 11.21 mmol) and ammonium carbonate (1.08 g, 11.21 mmol) were added. The vial was sealed, and the mixture was stirred at 50 °C for 48 h. The salts were filtered off and rinsed with methanol and the filtrate was concentrated. The residue was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 195 mg (100% purity, 23% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.21 min; MS (ESIpos): m/z = 378 [M + H]⁺.

Rac-5-(aminomethyl)-5-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl [(2,5-dioxo-4-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidin-4-yl)methyl]carbamate (195 mg, 0.52 mmol) was dissolved in 2 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (0.65 mL, 4 N, 2.58 mmol) was added and the mixture was stirred at room temperature for 3 h. The solvents were evaporated, and the residue was dried in vacuo. 155 mg (96% purity, 92% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.23 min; MS (ESIpos): m/z = 277 [M – Cl]⁺.

Rac-N-[(2,5-dioxo-4-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidin-4-yl)methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide. 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (102 mg, 0.38 mmol) was dissolved in 10 mL of dichloromethane. DIPEA (0.19 mL, 1.07 mmol), EDC*HCl (95 mg, 0.50 mmol), and 1-hydroxybenzotriazole hydrate (76 mg, 0.50 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidine-2,4-dione hydrochloride (120 mg, 96% purity, 0.36 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 39 mg (100% purity, 20% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.71 min; MS (ESIpos): m/z = 526 [M + H]⁺.

¹H NMR (400 MHz, DMSO-d₆) δ [ppm] = 11.30 (s, 1H), 8.82 (t, J = 6.2 Hz, 1H), 8.47 (s, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.38–7.64 (m, 7H), 6.59 (d, J = 2.0 Hz, 1H), 5.16 (q, J = 8.8 Hz, 2H), 3.85–3.96 (m, 1H), 3.74–3.84 (m, 1H).

Ent-N-[(2,5-dioxo-4-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidin-4-yl)methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (26). Enantiomeric separation of 35 mg of rac-N-[(2,5-dioxo-4-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidin-4-yl)methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide was done using the following preparative chiral HPLC method: Column: Daicel Chiralcel OX-H 250 \times 20 mm²; Eluent: 50% *n*-heptane/50% *i*-propanol; Flow: 20 mL/min; UV-detection: 220 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 12 mg (100% purity, 34% yield) of the title compound was obtained. Chiral HPLC (Column: Daicel OX-3 3 μ m, 50 \times 4.6 mm²; solvent: 50% *n*-heptane/50% *i*-propanol; flow: 1 mL/min; UV-detection: 220 nm): Rt = 2.483 min, 99.5% ee. LC–MS (Method 2): Rt = 1.71 min; MS (ESIpos): m/z = 526 [M + H]⁺. ¹H NMR (600 MHz, DMSO-d₆) δ [ppm] = 11.28 (s, 1H), 8.82 (t, J = 6.33 Hz, 1H), 8.44 (s, 1H), 7.75 (d, J = 8.25 Hz, 2H), 7.60–7.41 (m, 7H), 6.59 (d, J = 1.83 Hz, 1H), 5.17–5.13 (m, 2H), 3.9 (dd, J = 13.94 Hz, 1H), 3.79 (dd, J = 13.48 Hz, 1H).

Preparation of 27 and Respective Starting Materials. Ethyl 5-(1,5-dimethyl-1H-pyrazol-4-yl)-1,3-oxazole-4-carboxylate. 1,5-Dimethyl-1H-pyrazole-4-carboxylic acid (1.0 g, 7.14 mmol) was dissolved in 11 mL of THF. CDI (1.39 g, 8.56 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocynoacetate (0.86 mL, 7.85 mmol) in 11 mL of THF and then a LiHMDS solution (7.14 mL, 1 M in THF, 7.14 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 1 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 1.53 g (75% purity, 69% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 1.05 min; MS (ESIpos): m/z = 236 [M + H]⁺.

2-Amino-1-(1,5-dimethyl-1H-pyrazol-4-yl)ethanone hydrochloride. Ethyl 5-(1,5-dimethyl-1H-pyrazol-4-yl)-1,3-oxazole-4-carboxylate (1.53 g 75% purity, 4.89 mmol) was stirred at 100 °C in 22 mL of hydrochloric acid (6 N in water). After 1 h, the solvent was evaporated. The residue was treated with dichloromethane/methanol 20:1. The solid was filtered off and dried in vacuo. 762 mg (97% purity, 80% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.64 min; MS (ESIpos): m/z = 153 [M – Cl]⁺.

tert-Butyl [2-(1,5-dimethyl-1H-pyrazol-4-yl)-2-oxoethyl]carbamate. 2-Amino-1-(1,5-dimethyl-1H-pyrazol-4-yl)ethanone hydrochloride (762 mg, 97% purity, 3.78 mmol) was dissolved in 15 mL of dichloromethane. Di-tert-butyl dicarbonate (965 mg, 4.42 mmol) and triethylamine (1.68 mL, 12.05 mmol) were added, and the mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator. The residue was taken up in ethyl acetate and washed with water and brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. 980 mg (100% purity, 102% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 1.22 min; MS (ESIpos): m/z = 254 [M + H]⁺.

Rac-tert-butyl[(4-(1,5-dimethyl-1H-pyrazol-4-yl)-2,5-dioximidazolidin-4-yl)methyl]carbamate. In a microwave vial, tert-butyl [2-(1,5-dimethyl-1H-pyrazol-4-yl)-2-oxoethyl]carbamate (980 mg, 3.87 mmol) was dissolved in 10 mL of methanol. Potassium cyanide (1.26 g, 19.34 mmol) and ammonium carbonate (1.86 g, 19.34 mmol) were added. The vial was sealed, and the mixture was stirred at 60 °C for 4 d. The salts were filtered off and rinsed with methanol and the filtrate was concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 281 mg (100% purity, 22% yield) of the title compound

was obtained. LC–MS (Method 1): $R_t = 0.54$ min; MS (ESIpos): $m/z = 324$ $[M + H]^+$.

Rac-5-(aminomethyl)-5-(1,5-dimethyl-1H-pyrazol-4-yl)-imidazolidine-2,4-dione hydrochloride. Rac-*tert*-butyl[4-(1,5-dimethyl-1H-pyrazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]-carbamate (281 mg, 0.87 mmol) was dissolved in 4.5 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (1.09 mL, 4 N, 4.35 mmol) was added and the mixture was stirred at room temperature for 4 h. The precipitated solid was filtered off, washed with dichloromethane, and dried in vacuo. 237 mg (97% purity, 102% yield) of the title compound was obtained. LC–MS (Method 4): $R_t = 0.24$ min; MS (ESIpos): $m/z = 223$ $[M - Cl]^+$.

Rac-N-[[4-(1,5-dimethyl-1H-pyrazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide. 4'-(Trifluoromethyl)[biphenyl]-2-carboxylic acid (103 mg, 0.39 mmol) was dissolved in 2.5 mL of DMF. DIPEA (0.20 mL, 1.16 mmol), EDC·HCl (96 mg, 0.50 mmol), and 1-hydroxybenzotriazole hydrate (77 mg, 0.50 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-(1,5-dimethyl-1H-pyrazol-4-yl)imidazolidine-2,4-dione hydrochloride (100 mg, 97% purity, 0.38 mmol) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 91 mg (100% purity, 51% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.48$ min; MS (ESIpos): $m/z = 472$ $[M + H]^+$.

Ent-N-[[4-(1,5-dimethyl-1H-pyrazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (27). Enantiomeric separation of 85 mg of rac-N-({2,5-dioxo-4-[1-(2,2,2-trifluoroethyl)-1H-pyrazol-5-yl]imidazolidin-4-yl}methyl)-4'-(trifluoromethyl)[biphenyl]-2-carboxamide was done using the following preparative chiral HPLC method: Column: Daicel Chiralcel IC 5 μ m 250 \times 20 mm²; Eluent: 40% *n*-heptane/60% *i*-propanol; Flow: 15 mL/min; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator, and the residue was lyophilized. 16 mg (97% purity, 18% yield) of the title compound was obtained. Chiral HPLC (Column: Daicel IC-3 3 μ m, 50 \times 4.6 mm²; solvent: 50% *n*-heptane/50% *i*-propanol; flow: 1 mL/min; UV-detection: 220 nm): $R_t = 1.787$ min, 100% ee. LC–MS (Method 2): $R_t = 1.48$ min; MS (ESIpos): $m/z = 472$ $[M + H]^+$. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 10.86 (s, 1H), 8.60 (t, *J* = 6.24 Hz, 1H), 8.13 (s, 1H), 7.75 (d, *J* = 8.07 Hz, 2H), 7.56–7.54 (m, 3H), 7.48–7.40 (m, 3H), 7.37 (s, 1H), 3.79–3.77 (m, 2H), 3.70 (s, 3H), 2.25 (s, 3H).

Preparation of 28 and Respective Starting Materials. 6-Fluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid. [4-(Trifluoromethyl)phenyl]boronic acid (2.0 g, 10.53 mmol) and 2-bromo-3-fluorobenzoic acid (1.54 g, 7.02 mmol) were treated with 50 mL of 1,4-dioxane. Potassium phosphate solution (14.04 mL, 1.5 M in water, 21.06 mmol) was added to the mixture and the mixture was flushed with argon for 20 min. Then dichloropalladium-triphenylphosphine (1:2) (493 mg, 0.70 mmol) and dicyclohexyl(2',4',6'-triisopropyl[biphenyl]-2-yl)phosphine (335 mg, 0.70 mmol) were added and the mixture was stirred at 80 °C overnight. The reaction mixture was filtered over Celite and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 1.2 g (64% purity, 26% yield) of the title compound was obtained. LC–MS (Method 1): $R_t = 0.97$ min; MS (ESIneg): $m/z = 283$ $[M - H]^+$.

6-Fluoro-N-[[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (28). 6-Fluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (463 mg, 64% purity, 1.04 mmol) was dissolved in 5 mL of DMF. DIPEA (0.71 mL, 4.07 mmol), EDC·HCl (203 mg, 1.06 mmol), and 1-hydroxybenzotriazole hydrate (162 mg, 1.06 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5S)-5-(aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (43) (200 mg) was added and the mixture was stirred at room temperature for 4 h. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the

solvents were lyophilized. Further purification by column chromatography was performed. Product containing samples were united, the solvents were evaporated, and the residue was dissolved in water/acetonitrile and lyophilized. 80 mg (100% purity, 21% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.52$ min; MS (ESIpos): $m/z = 476$ $[M + H]^+$. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.27 (s, 1H), 8.80 (t, *J* = 6.26 Hz, 1H), 8.40 (s, 1H), 7.78 (d, *J* = 8.22 Hz, 2H), 7.55 (td, *J* = 7.87 Hz, 1H), 7.51 (d, *J* = 8.02 Hz, 2H), 7.45 (m, 1H), 7.37 (d, *J* = 1.76 Hz, 1H), 7.27 (d, *J* = 7.43 Hz, 1H), 6.46 (d, *J* = 1.76 Hz, 1H), 3.90–3.81 (m, 2H), 3.77 (s, 3H).

Preparation of 29. 5-Fluoro-N-[[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)-[biphenyl]-2-carboxamide (29). 5-Fluoro-4'-(trifluoromethyl)-[biphenyl]-2-carboxylic acid (116 mg, 0.41 mmol) was dissolved in 2 mL of DMF. DIPEA (0.20 mL, 1.14 mmol), EDC·HCl (101 mg, 0.53 mmol), and 1-hydroxybenzotriazole hydrate (81 mg, 0.53 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5S)-5-(aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride (43) (100 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 61 mg (100% purity, 32% yield) of the title compound was obtained. LC–MS (Method 4): $R_t = 0.85$ min; MS (ESIpos): $m/z = 476$ $[M + H]^+$. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.23 (s, 1H), 8.77 (t, *J* = 6.36 Hz, 1H), 8.40 (d, *J* = 0.98 Hz, 1H), 7.77 (d, *J* = 8.02 Hz, 2H), 7.55 (d, *J* = 8.02 Hz, 2H), 7.50–7.47 (m, 1H), 7.38–7.32 (m, 3H), 6.47 (d, *J* = 1.96 Hz, 1H), 3.98–3.94 (m, 1H), 3.89–3.85 (m, 1H), 3.79 (s, 3H).

Preparation of 30. 4-Fluoro-N-[[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)-[biphenyl]-2-carboxamide (30). 4-Fluoro-4'-(trifluoromethyl)-[biphenyl]-2-carboxylic acid (194 mg, 0.68 mmol) was dissolved in 3.5 mL of DMF. DIPEA (0.50 mL, 2.85 mmol), EDC·HCl (142 mg, 0.74 mmol), and 1-hydroxybenzotriazole hydrate (113 mg, 0.74 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5S)-5-(aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride (43) (140 mg) was added and the mixture was stirred at room temperature for 2 d. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 55 mg (100% purity, 17% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.60$ min; MS (ESIpos): $m/z = 476$ $[M + H]^+$. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.18 (s, 1H), 8.88 (t, *J* = 6.36 Hz, 1H), 8.43 (s, 1H), 7.75 (d, *J* = 8.22 Hz, 2H), 7.52–7.48 (m, 3H), 7.44–7.41 (m, 1H), 7.39 (d, *J* = 1.96 Hz, 1H), 7.24 (dd, *J* = 2.74 Hz, 1H), 6.48 (d, *J* = 1.96 Hz, 1H), 3.98–3.94 (m, 1H), 3.89–3.86 (m, 1H), 3.79 (s, 3H).

Preparation of 31. 3-Fluoro-N-[[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)-[biphenyl]-2-carboxamide (31). 3-Fluoro-4'-(trifluoromethyl)-[biphenyl]-2-carboxylic acid (139 mg, 0.49 mmol) was dissolved in 3 mL of DMF. DIPEA (0.36 mL, 2.03 mmol), EDC·HCl (101 mg, 0.53 mmol), and 1-hydroxybenzotriazole hydrate (81 mg, 0.53 mmol) were added and the mixture was stirred at room temperature. After 5 min, (5S)-5-(aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride (43) (100 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 94 mg (100% purity, 50% yield) of the title compound was obtained. LC–MS (Method 1): $R_t = 0.78$ min; MS (ESIpos): $m/z = 476$ $[M + H]^+$. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.13–10.81 (m, 1H), 9.00 (t, *J* = 6.16 Hz, 1H), 8.35 (br s, 1H), 7.79 (d, *J* = 8.22 Hz, 2H), 7.60 (d, *J* = 8.02 Hz, 2H), 7.58–7.54 (m, 1H), 7.36–7.32 (m, 2H), 7.28 (dd, *J* = 7.73 Hz, 1H), 6.43 (d, *J* = 1.96 Hz, 1H), 4.00–3.96 (m, 1H), 3.87–3.84 (m, 1H), 3.79 (s, 3H).

Preparation of BAY-9835 (32) and Respective Starting Materials. Ethyl 5-(1-methyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate (38). 1-Methyl-1H-pyrazole-5-carboxylic acid (12.6 g, 100.0 mmol) was

dissolved in 150 mL of THF. CDI (19.5 g, 120.0 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocynoacetate (12.02 mL, 110.0 mmol) in 50 mL of THF and then a LiHMDS solution (100 mL, 1 M in THF, 100.0 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 1 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (Machine: Biotage Isolera; column: Biotage SNAP Ultra 340 g; eluent: Cy/EE: 12% EE → 100% EE; flow: 200 mL/min). Product containing samples were united and the solvents were evaporated. 15.16 g (100% purity, 69% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.07 min; MS (ESIpos): m/z = 222 [M + H]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 8.68 (s, 1H), 7.59 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 4.26 (q, J = 7.0 Hz, 2H), 3.88 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H).

2-Amino-1-(1-methyl-1H-pyrazol-5-yl)ethanone hydrochloride (39). Ethyl 5-(1-methyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate (38) (8.05 g, 38.85 mmol) was stirred at 100 °C in 135 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The residue was treated with dichloromethane/methanol 20:1. The solid was filtered off and dried in vacuo. 8.36 g (71% purity, 86% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.60 min; MS (ESIpos): m/z = 139 [M – Cl]⁺.

tert-Butyl [2-(1-methyl-1H-pyrazol-5-yl)-2-oxoethyl]carbamate (40). 2-Amino-1-(1-methyl-1H-pyrazol-5-yl)ethanone hydrochloride (39) (13.34 g, 71% purity, 53.93 mmol) was dissolved in 200 mL of dichloromethane. Di-*tert*-butyl dicarbonate (18.24 g, 83.56 mmol) and triethylamine (31.76 mL, 227.88 mmol) were added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was washed with water and brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (Machine: Biotage Isolera; column: Biotage SNAP Ultra 100 g; eluent: Cy/EE: 12% EE → 100% EE; flow: 100 mL/min). Product containing samples were united and the solvents were evaporated. 11.33 g (100% purity, 88% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.38 min; MS (ESIpos): m/z = 240 [M + H]⁺.

Rac-tert-butyl[[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate (41). Divided in five microwave vials (30 mL volume vials): *tert*-butyl [2-(1-methyl-1H-pyrazol-5-yl)-2-oxoethyl]carbamate (40) (10.0 g, 85% purity, 35.52 mmol) was dissolved in 60 mL of methanol. Potassium cyanide (10.89 g, 167.17 mmol) and ammonium carbonate (16.06 g, 167.17 mmol) were added. The vials were sealed, and the mixture was stirred at 80 °C for 2 d. The contents of the vials were pooled, the salts were filtered off and rinsed with methanol, and the filtrate was concentrated. The residue was purified by column chromatography (Machine: Biotage Isolera; column: Biotage SNAP Ultra 100 g; eluent: DCM/MeOH: 5% MeOH → 30% MeOH; flow: 100 mL/min). Product containing samples were united and the solvents were evaporated. 8.9 g (100% purity, 81% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.95 min; MS (ESIpos): m/z = 310 [M – H]⁺.

tert-Butyl[[4-(4S)-4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate (42). Enantiomeric separation of 8.9 g of rac-*tert*-butyl[[4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate (41) was done using a preparative chiral HPLC method (see Supporting Information for details). Product containing samples were united, the solvents were evaporated with a rotary evaporator, and the residue was lyophilized. 3.34 g (100% purity, 38% yield) of the title compound was obtained. Chiral HPLC (Column: Chiralpak AD-H 3 μ m 100 \times 4.6 mm²; solvent: 85% CO₂/15% methanol; flow: 3 mL/min; UV-detection: 210 nm): Rt = 1.618 min, 99.5% ee. LC–MS (Method 1): Rt = 0.53 min; MS (ESIpos): m/z = 310 [M + H]⁺.

(5S)-5-(Aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride (1:1) (43). *tert*-Butyl[[4-(4S)-

4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate (42) (5.0 g, 16.1 mmol) was dissolved in 75 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (20.21 mL, 4 N, 80.82 mmol) was added and the mixture was stirred at room temperature overnight. The precipitated solid was filtered off, washed with dichloromethane, and dried in vacuo. 6.56 g (100% purity) of the crude title compound was obtained and used in the next step without further purification. LC–MS (Method 4): Rt = 0.23 min; MS (ESIpos): m/z = 209 [M – Cl]⁺.

5,6-Difluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (46). [4-(Trifluoromethyl)phenyl]boronic acid (45) (10.82 g, 56.96 mmol) and 2-bromo-3,4-difluorobenzoic acid (44) (9.00 g, 37.97 mmol) were treated with 233.5 mL of 1,4-dioxane. Potassium phosphate solution (75.95 mL, 1.5 M in water, 113.92 mmol) was added to the mixture and the mixture was flushed with argon for 10 min. Then dichloropalladium-triphenylphosphine (1:2) (493 mg, 0.70 mmol) and dicyclohexyl(2',4',6'-triisopropyl[biphenyl]-2-yl)-phosphine (2.67 g, 3.80 mmol) were added and the mixture was stirred at 80 °C for 3 h and at room temperature overnight. The reaction mixture was filtered over Celite and concentrated. The residue was dissolved in ethyl acetate and washed with 1 N HCl. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (Machine: Biotage Isolera; column: Biotage SNAP Ultra 100 g; eluent: DCM/MeOH: 1% MeOH → 8% MeOH; flow: 100 mL/min). Product containing samples were united and the solvents were evaporated. 9.7 g (99% purity, 84% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 0.97 min; MS (ESIneg): m/z = 301 [M – H]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 12.74–13.37 (m, 1H), 7.77–7.84 (m, 3H), 7.54–7.68 (m, 3H).

5,6-Difluoro-N-[[4-(4S)-4-(1-methyl-1H-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (32). 5,6-Difluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (46) (2.44 g, 8.08 mmol) was dissolved in 76 mL of acetonitrile. 2,4,6-Tripropyl-1,3,5,2,4,6-trioxatriphosphinane 2,4,6-trioxide-solution (6.26 mL, 50% in ethyl acetate, 10.51 mmol) and DIEA (5.63 mL, 32.33 mmol) were added, and the solution was stirred at room temperature. After 15 min. (5S)-5-(aminomethyl)-5-(1-methyl-1H-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (43) (6.56 g, crude) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated. The residue was dissolved in ethyl acetate and washed with water, saturated sodium bicarbonate-solution, and brine. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (Machine: Biotage Isolera; column: Biotage SNAP Ultra 340 g; eluent: DCM/MeOH: 2% MeOH → 20% MeOH; flow: 200 mL/min). Product containing samples were united and the solvents were evaporated. The residue was dissolved in acetonitrile/water and lyophilized. 1.79 g (100% purity, 45% yield) of the title compound was obtained. LC–MS (Method 1): Rt = 0.87 min; MS (ESIpos): m/z = 494 [M + H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 11.26 (s, 1H), 8.82 (t, J = 6.3 Hz, 1H), 8.41 (s, 1H), 7.81 (d, J = 8.07 Hz, 2H), 7.63 (dt, J = 9.87 Hz, 1H), 7.54 (d, J = 8.19 Hz, 2H), 7.37 (d, J = 1.96 Hz, 1H), 7.31–7.28 (m, 1H), 6.46 (d, J = 1.96 Hz, 1H), 3.91–3.79 (m, 2H), 3.76 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ [ppm] = 172.98, 167.06, 156.38, 151.37, 149.39, 147.77, 145.81, 137.24, 136.74, 136.34, 134.00, 130.06, 127.40, 127.36, 125.09, 124.24, 123.04, 116.70, 106.94, 63.39, 43.26, 37.59. HRMS: m/z [M + H]⁺ calculated for C₂₂H₁₇N₅O₃F₅: 494.1252, found: 494.1250. [α]_D²⁰ = +34.45° (in methanol, 20.0 °C, 589 nm). Recrystallization from boiling ethanol yielded crystalline material which was used for X-ray structure elucidation and determination of solubility (see Supporting Information).

Preparation of 33 and Respective Starting Materials. 6-Chloro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid. [4-(Trifluoromethyl)phenyl]boronic acid (45) (200 mg, 1.05 mmol) and 2-bromo-3-chlorobenzoic acid (165 mg, 0.70 mmol) were treated with 5 mL of 1,4-dioxane. Potassium phosphate solution (1.40 mL, 1.5 M in water, 2.11 mmol) was added to the mixture and the mixture was flushed with argon for 20 min. Then dichloropalladium-

triphenylphosphine (1:2) (49 mg, 0.07 mmol) and dicyclohexyl-(2',4',6'-triisopropyl[biphenyl]-2-yl)phosphine (33 mg, 0.07 mmol) were added and the mixture was stirred at 80 °C overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 51 mg (63% purity, 10% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.97 min; MS (ESI[−]): *m/z* = 299 [*M* − *H*]⁺.

6-Chloro-*N*-[[[(4*S*)-4-(1-methyl-1*H*-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (33). 6-Chloro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (51 mg, 63% purity, 0.11 mmol) and (5*S*)-5-(aminomethyl)-5-(1-methyl-1*H*-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (43) (42 mg) were dissolved 1.5 mL of DMF. DIPEA (0.15 mL, 0.85 mmol), EDC*HCl (42 mg, 0.22 mmol), and 1-hydroxybenzotriazole hydrate (34 mg, 0.22 mmol) were added and the mixture was stirred at room temperature for 4 h. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 33 mg (100% purity, 61% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.62 min; MS (ESI⁺): *m/z* = 492 [*M* + *H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm] = 11.26 (s, 1H), 8.73 (t, *J* = 6.3 Hz, 1H), 8.37 (d, *J* = 0.98 Hz, 1H), 7.77 (d, *J* = 8.19 Hz, 2H), 7.69 (dd, *J* = 8.07 Hz, 1H), 7.52 (t, *J* = 7.89 Hz, 1H), 7.44 (d, *J* = 7.21 Hz, 2H), 7.36–7.34 (m, 2H), 6.44 (d, *J* = 1.96 Hz, 1H), 3.78–3.75 (m, 5H).

Preparation of 34 and Respective Starting Materials. 6-Methoxy-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid. [4-(Trifluoromethyl)phenyl]boronic acid (45) (411 mg, 2.16 mmol) and 2-bromo-3-methoxybenzoic acid (400 mg, 1.73 mmol) were treated with 9 mL of 1,2-dimethoxyethane. Sodium carbonate solution (4.33 mL, 2 M in water, 8.66 mmol) was added to the mixture and the mixture was flushed with argon for 20 min. Then Tetrakis(triphenylphosphine)palladium(0) (100 mg, 0.09 mmol) was added and the mixture was stirred at 80 °C overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 33 mg (100% purity, 5% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.80 min; MS (ESI⁺): *m/z* = 297 [*M* − *H*]⁺.

6-Methoxy-*N*-[[[(4*S*)-4-(1-methyl-1*H*-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (34). 6-Methoxy-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (33 mg, 0.11 mmol) and (5*S*)-5-(aminomethyl)-5-(1-methyl-1*H*-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (43) (27 mg) were dissolved 1.0 mL of DMF. DIPEA (0.10 mL, 0.56 mmol), EDC*HCl (28 mg, 0.14 mmol), and 1-hydroxybenzotriazole hydrate (22 mg, 0.14 mmol) were added and the mixture was stirred at room temperature for 4 h. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. The residue was further purified by column chromatography. Product containing samples were united and the solvents were evaporated. The residue was dissolved in acetonitrile/water and lyophilized. 15 mg (100% purity, 28% yield) of the title compound was obtained. LC–MS (Method 2): *R*_t = 1.51 min; MS (ESI⁺): *m/z* = 488 [*M* + *H*]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.12 (br s, 1H), 8.52 (t, *J* = 6.26 Hz, 1H), 8.25 (s, 1H), 7.68 (d, *J* = 8.22 Hz, 2H), 7.44 (t, *J* = 7.92 Hz, 1H), 7.39 (d, *J* = 8.02 Hz, 2H), 7.35 (d, *J* = 1.96 Hz, 1H), 7.22 (d, *J* = 8.22 Hz, 1H), 6.99 (d, *J* = 7.63 Hz, 1H), 6.41 (d, *J* = 1.96 Hz, 1H), 3.84–3.80 (m, 1H), 3.77–3.73 (m, 4H), 3.71 (s, 3H).

Preparation of 35 and Respective Starting Materials. 5-Chloro-6-fluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid. [4-(Trifluoromethyl)phenyl]boronic acid (45) (300 mg, 1.58 mmol) and 2-bromo-4-chloro-3-fluorobenzoic acid (267 mg, 1.05 mmol) were treated with 7.5 mL of 1,4-dioxane. Potassium phosphate solution (2.11 mL, 1.5 M in water, 3.16 mmol) was added to the mixture and the mixture was flushed with argon for 20 min. Then dichloropalladium-triphenylphosphine (1:2) (74 mg, 0.11 mmol) and dicyclohexyl-(2',4',6'-triisopropyl[biphenyl]-2-yl)phosphine (50 mg, 0.11 mmol) were added and the mixture was stirred at 80 °C overnight. The reaction mixture was purified by column chromatog-

raphy. Product containing samples were united and the solvents were evaporated. 75 mg (90% purity, 13% yield) of the title compound was obtained. LC–MS (Method 1): *R*_t = 1.04 min; MS (ESI[−]): *m/z* = 317 [*M* − *H*]⁺.

5-Chloro-6-fluoro-*N*-[[[(4*S*)-4-(1-methyl-1*H*-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (35). 5-Chloro-6-fluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (75 mg, 90% purity, 0.21 mmol) and (5*S*)-5-(aminomethyl)-5-(1-methyl-1*H*-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (43) (58 mg) were dissolved 1.5 mL of DMF. DIPEA (0.21 mL, 1.18 mmol), EDC*HCl (59 mg, 0.31 mmol), and 1-hydroxybenzotriazole hydrate (47 mg, 0.31 mmol) were added and the mixture was stirred at room temperature for 4 h. The reaction mixture was purified by preparative HPLC. Further purification by preparative HPLC was needed (2 times). Product containing samples were united and lyophilized. 19 mg (97% purity, 17% yield) of the title compound was obtained. LC–MS (Method 3): *R*_t = 2.80 min; MS (ESI⁺): *m/z* = 510 [*M* + *H*]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.24 (s, 1H), 8.80 (t, *J* = 6.16 Hz, 1H), 8.39 (s, 1H), 7.80 (d, *J* = 8.22 Hz, 2H), 7.77 (t, *J* = 7.63 Hz, 1H), 7.53 (d, *J* = 8.02 Hz, 2H), 7.36 (d, *J* = 1.96 Hz, 1H), 7.26 (d, *J* = 8.41 Hz, 1H), 6.44 (d, *J* = 1.76 Hz, 1H), 3.88–3.80 (m, 2H), 3.77 (s, 3H).

Preparation of 36 and Respective Starting Materials. 6-Fluoro-4',5-bis(trifluoromethyl)[biphenyl]-2-carboxylic acid. [4-(Trifluoromethyl)phenyl]boronic acid (45) (300 mg, 1.58 mmol) and 2-bromo-3-fluoro-4-(trifluoromethyl)benzoic acid (302 mg, 1.05 mmol) were treated with 7.5 mL of 1,4-dioxane. Potassium phosphate solution (2.11 mL, 1.5 M in water, 3.16 mmol) was added to the mixture and the mixture was flushed with argon for 20 min. Then dichloropalladium-triphenylphosphine (1:2) (74 mg, 0.11 mmol) and dicyclohexyl-(2',4',6'-triisopropyl[biphenyl]-2-yl)phosphine (50 mg, 0.11 mmol) were added and the mixture was stirred at 80 °C overnight. The reaction mixture was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 93 mg (87% purity, 15% yield) of the title compound was obtained. LC–MS (Method 1): *R*_t = 1.06 min; MS (ESI[−]): *m/z* = 351 [*M* − *H*]⁺.

6-Fluoro-*N*-[[[(4*S*)-4-(1-methyl-1*H*-pyrazol-5-yl)-2,5-dioximidazolidin-4-yl]methyl]-4',5-bis(trifluoromethyl)[biphenyl]-2-carboxamide (36). 6-Fluoro-4',5-bis(trifluoromethyl)[biphenyl]-2-carboxylic acid (90 mg, 87% purity, 0.23 mmol) and (5*S*)-5-(aminomethyl)-5-(1-methyl-1*H*-pyrazol-5-yl)imidazolidine-2,4-dione hydrochloride (43) (63 mg) were dissolved 1.5 mL of DMF. DIPEA (0.22 mL, 1.28 mmol), EDC*HCl (64 mg, 0.33 mmol), and 1-hydroxybenzotriazole hydrate (51 mg, 0.33 mmol) were added and the mixture was stirred at room temperature for 4 h. The reaction mixture was purified by preparative HPLC. Product containing samples were united and lyophilized. 30 mg (100% purity, 24% yield) of the title compound was obtained. LC–MS (Method 6): *R*_t = 0.95 min; MS (ESI⁺): *m/z* = 544 [*M* + *H*]⁺. ¹H NMR (600 MHz, DMSO-*d*₆) δ [ppm] = 11.19 (br s, 1H), 8.91 (t, *J* = 6.36 Hz, 1H), 8.43 (s, 1H), 7.97 (t, *J* = 7.43 Hz, 1H), 7.82 (d, *J* = 8.22 Hz, 2H), 7.57 (d, *J* = 8.22 Hz, 2H), 7.42 (d, *J* = 8.02 Hz, 1H), 7.37 (d, *J* = 1.96 Hz, 1H), 6.45 (d, *J* = 1.96 Hz, 1H), 3.85 (d, *J* = 6.46 Hz, 2H), 3.77 (s, 3H).

Preparation of 47 and Respective Starting Materials. Ethyl 5-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate. 5-Methyl-2-phenyl-1,3-thiazole-4-carboxylic acid (5 g, 22.80 mmol) was dissolved in 30 mL of THF. CDI (4.4 g, 27.36 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocyanacetate (2.74 mL, 25.08 mmol) in 30 mL of THF, then a LiHMDS solution (22.80 mL, 1 M in THF, 22.80 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 3 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 4.61 g (93% purity, 60% yield) of the title compound was obtained. LC–MS (Method 1): *R*_t = 0.96 min; MS

(ESIpos): $m/z = 315$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.67 (s, 1H), 7.92 (br dd, $J = 5.1, 1.3$ Hz, 2H), 7.41–7.61 (m, 3H), 4.27 (q, $J = 7.0$ Hz, 2H), 2.46 (s, 3H), 1.20 (t, $J = 7.1$ Hz, 3H).

2-Amino-1-(5-methyl-2-phenyl-1,3-thiazol-4-yl)ethanone hydrochloride. Ethyl 5-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-1,3-oxazole-4-carboxylate (4.61 g, 93% purity, 13.63 mmol) was stirred at 100 °C in 50 mL of hydrochloric acid (6 N in water). After 3 h, the solvent was evaporated. The residue was treated with dichloromethane and evaporated again. The residue was dried in vacuo. 3.85 g (96% purity, 101% yield) of the title compound was obtained. LC–MS (Method 4): $R_t = 1.49$ min; MS (ESIpos): $m/z = 233$ $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.47 (br s, 3H), 7.97 (dd, $J = 6.6, 2.9$ Hz, 2H), 7.47–7.61 (m, 3H), 4.51 (s, 2H), 2.83 (s, 3H).

tert-Butyl [2-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-2-oxoethyl]carbamate. 2-Amino-1-(5-methyl-2-phenyl-1,3-thiazol-4-yl)ethanone hydrochloride (3.8 g, 96% purity, 14.14 mmol) was dissolved in 50 mL of dichloromethane. Di-tert-butyl dicarbonate (4.63 g, 21.21 mmol) and DMAP (864 mg, 7.07 mmol) were added, and the mixture was stirred at room temperature overnight. Water was added to the mixture and the precipitated solid was filtered off and dried in vacuo. 2.86 g (89% purity, 56% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 2.29$ min; MS (ESIpos): $m/z = 333$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 7.89–7.98 (m, 2H), 7.49–7.56 (m, 3H), 6.99 (br t, $J = 5.8$ Hz, 1H), 4.51 (d, $J = 5.9$ Hz, 2H), 2.77 (s, 3H), 1.42 (s, 9H).

Rac-tert-butyl[4-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate. In a microwave vial, tert-butyl [2-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-2-oxoethyl]carbamate (2.86 g, 89% purity, 7.66 mmol) was dissolved in 30 mL of methanol. Potassium cyanide (2.24 g, 34.41 mmol) and ammonium carbonate (3.31 g, 34.41 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C overnight. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude product was purified by preparative HPLC. Product containing samples were united, the solvents were evaporated with a rotary evaporator, and the residue was lyophilized. 2.08 g (100% purity, 67% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.74$ min; MS (ESIpos): $m/z = 403$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 10.93 (br s, 1H), 8.13 (br s, 1H), 7.85–7.90 (m, 2H), 7.42–7.54 (m, 3H), 6.79 (br s, 1H), 3.89–4.02 (m, 2H), 2.38 (s, 3H), 1.38 (s, 9H).

Rac-5-(aminomethyl)-5-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl[4-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate (2.03 g, 5.04 mmol) was dissolved in 40 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (12.61 mL, 4 N, 50.44 mmol) was added and the mixture was stirred at room temperature overnight. The solvents were removed, and the residue was dried in vacuo. 1.98 g (98% purity) of the title compound was obtained. LC–MS (Method 2): $R_t = 0.85$ min; MS (ESIpos): $m/z = 303$ $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.40 (s, 1H), 8.80 (s, 1H), 8.49 (br s, 3H), 7.92 (dd, $J = 7.5, 1.7$ Hz, 2H), 7.44–7.55 (m, 3H), 3.71 (br s, 2H), 2.42 (s, 3H).

Ent-5,6-difluoro-N-[[4-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]-4'-(trifluoromethyl)biphenyl]-2-carboxamide (47). 5,6-Difluoro-4'-(trifluoromethyl)biphenyl-2-carboxylic acid (46) (107 mg, 0.35 mmol) was dissolved in 2.5 mL of DMF. DIPEA (0.26 mL, 1.48 mmol), EDC·HCl (74 mg, 0.38 mmol), and 1-hydroxybenzotriazole hydrate (59 mg, 0.38 mmol) were added and the mixture was stirred at room temperature. After 5 min rac-5-(aminomethyl)-5-(5-methyl-2-phenyl-1,3-thiazol-4-yl)-imidazolidine-2,4-dione hydrochloride (100 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 59 mg (100% purity) of the racemate was obtained. Enantiomeric separation of the racemate was done using the following preparative chiral HPLC method: Machine: SFC; Column: Diacel IG 5 μ m, 20 \times 250 mm²; Eluent: 85% CO₂ and 15% ethanol; Flow: 80 mL/min; UV-detection:

210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 8 mg (100% purity, 5% yield) of the title compound was obtained. Chiral HPLC (Column: Diacel Chiralpak IG-3; 3 μ m; 100 \times 4.6 mm²; solvent: 85% CO₂ and 15% ethanol; flow: 3 mL/min; UV-detection: 210 nm): $R_t = 6.186$ min, 100% ee. LC–MS (Method 2): $R_t = 2.12$ min; MS (ESIpos): $m/z = 587$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 10.98 (br s, 1H), 8.68 (t, $J = 6.05$ Hz, 1H), 8.30 (s, 1H), 7.86 (m, 2H), 7.82 (d, $J = 8.25$ Hz, 2H), 7.62–7.56 (m, 3H), 7.51–7.45 (m, 3H), 7.32–7.29 (m, 1H), 4.14–4.06 (m, 2H), 2.38 (s, 3H).

Preparation of 48 and Respective Starting Materials. Ethyl 5-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]-1,3-oxazole-4-carboxylate. 5-Methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carboxylic acid (1.9 g, 8.63 mmol) was dissolved in 30 mL of THF. CDI (1.68 g, 10.35 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocynoacetate (1.04 mL, 9.49 mmol) in 10 mL THF, then a LiHMDS solution (8.63 mL, 1 M in THF, 8.63 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred overnight. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 1.91 g (92% purity, 65% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.35$ min; MS (ESIpos): $m/z = 316$ $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 9.09–9.12 (m, 1H), 8.69 (dd, $J = 4.8, 1.5$ Hz, 1H), 8.66 (s, 1H), 8.26–8.30 (m, 1H), 7.56 (ddd, $J = 7.9, 4.8, 0.6$ Hz, 1H), 4.25 (q, $J = 7.0$ Hz, 2H), 2.49 (s, 3H), 1.19 (t, $J = 7.1$ Hz, 3H).

2-Amino-1-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]ethanone hydrochloride. Ethyl 5-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]-1,3-oxazole-4-carboxylate (1.91 g, 92% purity, 5.58 mmol) was stirred at 100 °C in 40 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The residue was treated with dichloromethane and evaporated again. The residue was dried in vacuo. 2.08 g (100% purity) of the title compound was obtained. LC–MS (Method 4): $R_t = 1.06$ min; MS (ESIpos): $m/z = 234$ $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 9.35 (s, 1H), 8.89 (dt, $J = 3.4, 1.7$ Hz, 1H), 8.75 (br dd, $J = 6.9, 5.6$ Hz, 1H), 8.60 (br s, 3H), 7.88–7.98 (m, 1H), 4.53 (q, $J = 5.5$ Hz, 2H), 2.87 (s, 3H).

tert-Butyl [2-(5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl)-2-oxoethyl]carbamate. 2-Amino-1-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]ethanone hydrochloride (2.08 g) was dissolved in 25 mL of dichloromethane. Di-tert-butyl dicarbonate (1.85 g, 8.48 mmol) and triethylamine (4.30 mL, 30.84 mmol) were added, and the mixture was stirred at 0 °C for 1 h. The solvent was removed, and the residue was dried in vacuo. 4.29 g (100% purity) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.77$ min; MS (ESIpos): $m/z = 334$ $[M + H]^+$.

Rac-tert-butyl[4-(5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate. In a microwave vial, tert-butyl [2-(5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl)-2-oxoethyl]carbamate (4.29 g, 12.87 mmol) was dissolved in 30 mL of methanol. Potassium cyanide (3.35 g, 51.47 mmol) and ammonium carbonate (4.95 g, 51.47 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C overnight. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude product was purified by preparative HPLC. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 1.97 g (100% purity, 38% yield) of the title compound was obtained. LC–MS (Method 2): $R_t = 1.21$ min; MS (ESIpos): $m/z = 404$ $[M + H]^+$.

Rac-5-(aminomethyl)-5-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl[4-(5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl)-2,5-dioximidazolidin-4-yl]methyl]carbamate (1.97 g, 4.88 mmol) was dissolved in 25 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (6.10 mL, 4 N, 24.41 mmol) was added and the mixture was stirred at room

temperature for 2 d. The solvents were removed, and the residue was dried in vacuo. 1.96 g (99% purity) of the title compound was obtained. LC–MS (Method 2): Rt = 0.47 min; MS (ESIpos): m/z = 304 $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.45 (s, 1H), 9.32 (s, 1H), 8.89 (s, 1H), 8.86–8.91 (m, 1H), 8.63 (br d, J = 7.2 Hz, 1H), 8.50 (br s, 3H), 7.76–7.88 (m, 1H), 3.63–3.83 (m, 2H), 2.47 (s, 3H).

Ent-5,6-difluoro-N-([4-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]-2,5-dioxoimidazolidin-4-yl]methyl)-4'-(trifluoromethyl)-[biphenyl]-2-carboxamide (48). 5,6-Difluoro-4'-(trifluoromethyl)-[biphenyl]-2-carboxylic acid (**46**) (107 mg, 0.35 mmol) was dissolved in 2.5 mL of DMF. DIPEA (0.26 mL, 1.48 mmol), EDC*HCl (74 mg, 0.38 mmol), and 1-hydroxybenzotriazole hydrate (59 mg, 0.38 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-[5-methyl-2-(pyridin-3-yl)-1,3-thiazol-4-yl]imidazolidine-2,4-dione hydrochloride (100 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 127 mg (100% purity) of the racemate was obtained. Enantiomeric separation of the racemate was done using the following preparative chiral HPLC method: Column: Daicel Chiralpak IF 5 μ m, 20 \times 250 mm; Eluent: 50% *n*-heptane and 50% ethanol; Flow: 20 mL/min; UV-detection: 220 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 54 mg (100% purity, 31% yield) of the title compound was obtained. Chiral HPLC (Column: Daicel Chiralpak IF-3; 3 μ m; 100 \times 4.6 mm²; solvent: 50% *n*-heptane and 50% ethanol; flow: 1 mL/min; UV-detection: 220 nm): Rt = 2.538 min, 100% ee. LC–MS (Method 2): Rt = 1.77 min; MS (ESIpos): m/z = 588 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 10.95 (br s, 1H), 9.06 (d, J = 2.02 Hz, 1H), 8.68 (t, J = 6.33 Hz, 1H), 8.65 (dd, J = 4.77 Hz, 1H), 8.34 (s, 1H), 8.23 (dt, J = 7.93 Hz, 1H), 7.82 (d, J = 8.25 Hz, 2H), 7.62–7.58 (m, 3H), 7.53–7.51 (m, 1H), 7.32–7.30 (m, 1H), 4.13–4.07 (m, 2H), 2.41 (s, 3H).

Preparation of 49 and Respective Starting Materials. Ethyl 5-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-1,3-oxazole-4-carboxylate. 4-Methyl-1-phenyl-1H-pyrazole-3-carboxylic acid (2.5 g, 12.36 mmol) was dissolved in 25 mL of THF. CDI (2.41 g, 14.84 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocynoacetate (1.49 mL, 13.60 mmol) in 5 mL THF, then a LiHMDS solution (12.36 mL, 1 M in THF, 12.36 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 3 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 2.14 g (100% purity, 58% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.78 min; MS (ESIpos): m/z = 298 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.63 (s, 1H), 8.49 (s, 1H), 7.85 (d, J = 7.7 Hz, 2H), 7.53 (t, J = 8.0 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 4.26 (q, J = 7.0 Hz, 2H), 2.10 (s, 3H), 1.21 (t, J = 7.2 Hz, 3H).

2-Amino-1-(4-methyl-1-phenyl-1H-pyrazol-3-yl)ethanone hydrochloride. Ethyl 5-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-1,3-oxazole-4-carboxylate (2.14 g, 7.20 mmol) was stirred at 100 °C in 20 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The residue was treated with dichloromethane and evaporated again. The residue was dried in vacuo. 1.85 g (94% purity, 96% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 1.33 min; MS (ESIpos): m/z = 216 $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.61 (br d, J = 3.5 Hz, 3H), 8.57 (s, 1H), 7.92 (d, J = 7.9 Hz, 2H), 7.57 (br t, J = 7.6 Hz, 2H), 7.36–7.46 (m, 1H), 4.46 (br s, 2H), 2.33 (s, 3H).

tert-Butyl [2-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-2-oxoethyl]-carbamate. 2-Amino-1-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-ethanone hydrochloride (1.75 g, 94% purity, 6.53 mmol) was dissolved in 8 mL of dichloromethane. Ditetert-butyl dicarbonate

(2.28 g, 10.43 mmol) and DMAP (425 mg, 3.48 mmol) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were lyophilized. 1.18 g (84% purity, 48% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 2.14 min; MS (ESIpos): m/z = 316 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.45 (s, 1H), 7.89 (d, J = 8.1 Hz, 2H), 7.55 (t, J = 8.0 Hz, 2H), 7.36–7.42 (m, 1H), 6.98 (br t, J = 5.7 Hz, 1H), 4.45 (br d, J = 5.7 Hz, 2H), 2.29 (s, 3H), 1.41 (s, 9H).

Rac-tert-butyl[4-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-2,5-dioxoimidazolidin-4-yl]methyl]carbamate. In a microwave vial, tert-butyl [2-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-2-oxoethyl]carbamate (1.18 g, 84% purity, 3.14 mmol) was dissolved in 15 mL of methanol. Potassium cyanide (1.46 g, 22.45 mmol) and ammonium carbonate (2.16 g, 22.45 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C overnight. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude product was purified by preparative HPLC. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 730 mg (100% purity, 60% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.61 min; MS (ESIpos): m/z = 386 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 10.28–11.41 (m, 1H), 8.26 (s, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.75 (br s, 1H), 7.47 (t, J = 7.9 Hz, 2H), 7.27 (t, J = 7.3 Hz, 1H), 6.61 (br s, 1H), 3.71–3.90 (m, 2H), 1.98 (s, 3H), 1.38 (s, 9H).

Rac-5-(aminomethyl)-5-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl[4-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-2,5-dioxoimidazolidin-4-yl]methyl]carbamate (730 mg, 1.89 mmol) was dissolved in 15 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (2.37 mL, 4 N, 9.47 mmol) was added and the mixture was stirred at room temperature overnight. The solvents were removed, and the residue was dried in vacuo. 629 mg (100% purity, 103% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 0.49 min; MS (ESIpos): m/z = 285 $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.39 (s, 1H), 8.69 (s, 1H), 8.47 (br s, 3H), 8.38 (s, 1H), 7.84 (d, J = 8.3 Hz, 2H), 7.50 (t, J = 7.8 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 3.64 (br s, 2H), 2.01 (s, 3H).

Ent-5,6-difluoro-N-([4-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-2,5-dioxoimidazolidin-4-yl]methyl)-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (49). 5,6-Difluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (**46**) (94 mg, 0.31 mmol) was dissolved in 2 mL of DMF. DIPEA (0.27 mL, 1.55 mmol), EDC*HCl (77 mg, 0.40 mmol), and 1-hydroxybenzotriazole hydrate (62 mg, 0.40 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-(4-methyl-1-phenyl-1H-pyrazol-3-yl)-imidazolidine-2,4-dione hydrochloride (100 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 91 mg (100% purity) of the racemate was obtained. Enantiomeric separation of the racemate was done using the following preparative chiral HPLC method: Machine: SFC; Column: Maisch Diacel OJ-H 5 μ m, 25 \times 250 mm²; Eluent: 90% CO₂ and 10% methanol; Flow: 80 mL/min; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 40 mg (100% purity, 22% yield) of the title compound was obtained. Chiral HPLC (Column: Diacel Chiralcel OJ-3; 3 μ m; 100 \times 4.6 mm²; solvent: 90% CO₂ and 10% methanol; flow: 3 mL/min; UV-detection: 210 nm): Rt = 1.812 min, 100% ee. LC–MS (Method 6): Rt = 1.06 min; MS (ESIpos): m/z = 570 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.00 (br s, 1H), 8.65 (t, J = 5.87 Hz, 1H), 8.30 (s, 1H), 8.23 (s, 1H), 7.82 (d, J = 8.07 Hz, 2H), 7.76 (d, J = 8.25 Hz, 2H), 7.62–7.58 (m, 3H), 7.48 (t, J = 7.7 Hz, 2H), 7.31–7.27 (m, 2H), 4.08–3.98 (m, 2H), 1.99 (s, 3H).

Preparation of 50 and Respective Starting Materials. Ethyl 5-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate. 1-Methyl-3-phenyl-1H-pyrazole-5-carboxylic acid (5.0 g, 24.73 mmol)

was dissolved in 30 mL of THF. CDI (4.81 g, 29.67 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to 0 °C. At this temperature, first a solution of ethyl isocyanacetate (2.97 mL, 27.20 mmol) in 10 mL of THF, then a LiHMDS solution (24.73 mL, 1 M in THF, 24.73 mmol) was added dropwise. The reaction mixture was slowly allowed to warm to room temperature and stirred for 3 h. The solvent was evaporated. The residue was extracted between water and ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography. Product containing samples were united and the solvents were evaporated. 4.53 g (95% purity, 59% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.78 min; MS (ESIpos): m/z = 298 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 8.71 (d, J = 0.5 Hz, 1H), 7.83 (d, J = 8.1 Hz, 2H), 7.44 (t, J = 7.6 Hz, 2H), 7.32–7.36 (m, 1H), 7.30 (s, 1H), 4.29 (q, J = 7.2 Hz, 2H), 3.93 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H).

2-Amino-1-(1-methyl-3-phenyl-1H-pyrazol-5-yl)ethanone hydrochloride. Ethyl 5-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-1,3-oxazole-4-carboxylate (4.53 g, 95% purity, 14.48 mmol) was stirred at 100 °C in 30 mL of hydrochloric acid (6 N in water). After 2 h, the solvent was evaporated. The residue was treated with dichloromethane and evaporated again. The residue was dried in vacuo. 4.2 g (60% purity, 69% yield) of the title compound was obtained. LC–MS (Method 4): Rt = 1.24 min; MS (ESIpos): m/z = 215 $[M - Cl]^+$.

tert-Butyl [2-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-2-oxoethyl]-carbamate. 2-Amino-1-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-ethanone hydrochloride (3.9 g, 60% purity, 9.29 mmol) was dissolved in 18 mL of DMF. Dittert-butyl dicarbonate (5.07 g, 23.24 mmol) and DMAP (946 mg, 7.75 mmol) were added, and the mixture was stirred at room temperature overnight. Further dittert-butyl dicarbonate (5.07 g, 23.24 mmol) and DMAP (946 mg, 7.75 mmol) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 255 mg (93% purity, 8% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 2.01 min; MS (ESIpos): m/z = 316 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 7.81–7.89 (m, 2H), 7.71 (s, 1H), 7.44 (t, J = 7.7 Hz, 2H), 7.30–7.39 (m, 1H), 7.15 (br t, J = 5.8 Hz, 1H), 4.34 (d, J = 5.9 Hz, 2H), 4.11 (s, 3H), 1.41 (s, 9H).

Rac-tert-butyl[4-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methylcarbamate. In a microwave vial, tert-butyl [2-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-2-oxoethyl]carbamate (255 mg, 93% purity, 0.75 mmol) was dissolved in 3 mL of methanol. Potassium cyanide (316 mg, 4.85 mmol) and ammonium carbonate (466 mg, 4.85 mmol) were added. The vial was sealed, and the mixture was stirred at 80 °C overnight. The salts were filtered off and rinsed with methanol. The filtrate was concentrated. The crude product was purified by preparative HPLC. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 112 mg (100% purity, 39% yield) of the title compound was obtained. LC–MS (Method 2): Rt = 1.51 min; MS (ESIpos): m/z = 386 $[M + H]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.07–11.18 (m, 1H), 8.21 (br s, 1H), 7.76 (br d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.25–7.32 (m, 1H), 7.09 (br s, 1H), 6.94 (s, 1H), 3.81 (s, 3H), 3.69–3.78 (m, 2H), 1.38 (s, 9H).

Rac-5-(aminomethyl)-5-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride. Rac-tert-butyl[4-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methylcarbamate (112 mg, 0.29 mmol) was dissolved in 2.5 mL of dichloromethane. Hydrochloric acid in 1,4-dioxane (0.36 mL, 4 N, 1.45 mmol) was added and the mixture was stirred at room temperature overnight. The solvents were removed, and the residue was dried in vacuo. 110 mg (100% purity) of the title compound was obtained. LC–MS (Method 2): Rt = 0.73 min; MS (ESIpos): m/z = 285 $[M - Cl]^+$. 1H NMR (600 MHz, DMSO- d_6) δ [ppm] = 11.56 (s, 1H), 8.80 (s, 1H), 8.51 (br s, 3H), 7.78 (d, J = 7.5 Hz, 2H), 7.41 (t, J

= 7.6 Hz, 2H), 7.28–7.34 (m, 1H), 7.03 (s, 1H), 3.88 (s, 2H), 3.57 (s, 3H).

Ent-5,6-difluoro-N-[[4-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-2,5-dioxoimidazolidin-4-yl]methyl]-4'-(trifluoromethyl)[biphenyl]-2-carboxamide (50). 5,6-Difluoro-4'-(trifluoromethyl)[biphenyl]-2-carboxylic acid (46) (62 mg, 0.21 mmol) was dissolved in 1.5 mL of DMF. DIPEA (0.15 mL, 0.86 mmol), EDC·HCl (43 mg, 0.22 mmol), and 1-hydroxybenzotriazole hydrate (34 mg, 0.22 mmol) were added and the mixture was stirred at room temperature. After 5 min, rac-5-(aminomethyl)-5-(1-methyl-3-phenyl-1H-pyrazol-5-yl)-imidazolidine-2,4-dione hydrochloride (55 mg) was added and the mixture was stirred at room temperature overnight. The reaction mixture was purified by preparative HPLC. Product containing samples were united and the solvents were evaporated. 37 mg (100% purity) of the racemate was obtained. Enantiomeric separation of the racemate was done using the following preparative chiral HPLC method: Machine: SFC; Column: Lux C2 (21.2 mm × 250 mm², 5 μ m); Eluent: 50% CO₂ and 50% methanol (0.2% NH₃); Flow: 50 mL/min; Backpressure: 100 bar; Column temperature: 40 °C; UV-detection: 210 nm. Product containing samples were united, the solvents were evaporated with a rotary evaporator and the residue was lyophilized. 11 mg (100% purity, 11% yield) of the title compound was obtained. Chiral HPLC (Lux C4 4.6 mm × 250 mm², 5 μ m; solvent: 75% CO₂ and 25% methanol (0.2% NH₃); flow: 4 mL/min; UV-detection: 210–400 nm): Rt = 2.328 min, 99.3% ee. LC–MS (Method 2): Rt = 1.92 min; MS (ESIpos): m/z = 570 $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6) δ [ppm] = 11.27 (br s, 1H), 8.95–8.83 (m, 1H), 8.50–8.25 (m, 1H), 7.79 (dd, J = 17.61 Hz, 4H), 7.67–7.59 (m, 1H), 7.55 (d, J = 8.19 Hz, 2H), 7.41–7.37 (m, 2H), 7.34–7.28 (m, 2H), 7.00 (s, 1H), 3.98–3.87 (m, 2H), 3.80 (s, 3H).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jmedchem.3c02036>.

Experimental procedures for all compounds, X-ray data, docking analysis of compound 24, physicochemical, pharmacokinetic and metabolism assays, in vitro pharmacology assays including data generated with these assays (PDF)

Molecular formular strings (CSV)

Compound from Figure 1 (Compound 7 in *J. Med. Chem.* 2017, 5933) docked into ADAMTS7 homology model (Figure 2) (PDB)

Compound 1 docked into ADAMTS7 homology model (Figure 4) (PDB)

Superimposed view of ADAMTS7 homology model (Figure 5) (PDB)

MMP12 X-ray cocrystallized with compound 3 (Figure 5) (PDB)

Compound 17 docked into ADAMTS7 homology model (Figure 6) (PDB)

Compound 17 overlaid with MMP12 X-ray structure (Figure 6) (PDB)

Compound 18 docked into ADAMTS7 homology model (Figure 7) (PDB)

Compound 24 docked into ADAMTS7 homology model (Figure 8) (PDB)

Compound 24 overlaid with watermap (Figure 8) (PDB)

Compound 32 docked into ADAMTS7 homology model (Figure 9) (PDB)

Compound 32 overlaid with an ADAMTS12 homology model (Figure 9) (PDB)

Compound 24 docked into ADAMTS7 homology model (Figure S6) (PDB)

Compound **24** overlaid with MMP12 X-ray structure (Figure S6) (PDB)

AUTHOR INFORMATION

Corresponding Author

Daniel Meibom – Bayer AG, 42113 Wuppertal, Germany;
orcid.org/0000-0003-4978-9842;
Email: daniel.meibom@bayer.com

Authors

Pierre Wasnaire – Bayer AG, 42113 Wuppertal, Germany
Kristin Beyer – Bayer AG, 42113 Wuppertal, Germany
Andreas Broehl – Bayer AG, 42113 Wuppertal, Germany
Yolanda Cancho-Grande – Bayer AG, 42113 Wuppertal, Germany
Nadine Elowe – Broad Institute, 02142 Cambridge, United States
Kerstin Henninger – Bayer AG, 42113 Wuppertal, Germany
Sarah Johannes – Bayer AG, 42113 Wuppertal, Germany
Natalia Jungmann – Bayer AG, 42113 Wuppertal, Germany
Tanja Krainz – Broad Institute, 02142 Cambridge, United States
Niels Lindner – Bayer AG, 42113 Wuppertal, Germany
Stefanie Maassen – Bayer AG, 42113 Wuppertal, Germany
Bryan MacDonald – Broad Institute, 02142 Cambridge, United States; orcid.org/0000-0002-2090-2066
Denis Menshykau – Bayer AG, 42113 Wuppertal, Germany
Joachim Mittendorf – Bayer AG, 42113 Wuppertal, Germany
Guzman Sanchez – Villapharma Research, 30320 Murcia, Spain
Martina Schaefer – Bayer AG, 13353 Berlin, Germany
Eric Stefan – Broad Institute, 02142 Cambridge, United States
Afra Torge – Bayer AG, 42113 Wuppertal, Germany
Yi Xing – Broad Institute, 02142 Cambridge, United States
Dmitry Zubov – Bayer AG, 42113 Wuppertal, Germany

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.jmedchem.3c02036>

Author Contributions

The manuscript was written with contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): The authors D.M., P.W., K.B., A.B., Y.C.G., K.H., S.J., N.J., N.L., S.M., D.M., J.M., M.S., A.T.-F., and D.Z. are or have been employees of Bayer AG. The author N.E. is an employee of the Broad Institute. The author T.K. has been an employee of the Broad Institute and is now employed by Evotec SE. The author B.MacD. has been an employee of the Broad Institute and is now employed by Verve Therapeutics. The author G.S. has been an employee of Villapharma and is now employed by Eurofins Villapharma Research. The author E.S. has been an employee of the Broad Institute and is now employed by Biogen. The author Y.X. has been an employee of the Broad Institute and is now employed by Seismic Therapeutic.

ACKNOWLEDGMENTS

We thank M. Drysdale, R. Hilgraf, V. Kaushik, and P. McCarren for their contributions during lead optimization.

ABBREVIATIONS

Å, angstrom; abs, absolute stereochemistry; ACN, acetonitrile; ADAM8, a disintegrin and metalloproteinase domain-containing protein 8; ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; ADAM17, tumor necrosis factor- α -converting enzyme; ADAMTS4, a disintegrin and metalloproteinase with thrombospondin type 1 repeats 4; ADAMTS5, a disintegrin and metalloproteinase with thrombospondin type 1 repeats 5; ADAMTS7, a disintegrin and metalloproteinase with thrombospondin type 1 repeats 7; ADAMTS12, a disintegrin and metalloproteinase with thrombospondin type 1 repeats 12; AG, Aktiengesellschaft; aq., aqueous; Asp, aspartate; AUC, area under the curve; BAH, butylated hydroxyanisole; BEI, binding efficiency index; BOC, *tert*-butoxycarbonyl protecting group; (BOC)₂O, di(*tert*-butyl)carbonate; br, broad (in NMR); CAD, coronary artery disease; CDI, carbonyl diimidazole; Cl, clearance; c log D, calculated descriptor for lipophilicity; CV, cardiovascular; Cy, cyclohexane; CYP, cytochrome P enzyme; d, doublet (in NMR); d, days (in reactions); DCM, dichloromethane; dd, doublet of doublets (in NMR); DIPEA, *N,N*-diisopropylethylamine; DMPK, drug metabolism pharmacokinetic; DMSO, dimethyl sulfoxide; EC, endothelial cell; ee, enantiomeric excess; EE, ethyl acetate; ESI, electrospray ionization; EtOH, ethanol; eut, eutomer; F, bioavailability; FaSSIF, fasted state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; FIIa, thrombin; fu, fraction unbound; FXa, factor Xa; g, gram (in units or reactions); g, gram; G, free energy; Glu, glutamate; Gly, glycine; h, hour (in unites or reactions); GSH, glutathione; HBA, number of H-bond acceptors; HBD, number of H-bond donors; hCa_v1.2, human L-type Ca channel; hERG, human Ether-a-go-go channel; His, histidine; hNa_v1.5, human voltage-gated sodium channel subunit; HPLC, high-performance liquid chromatography; HRMS, high resolution mass spectrometry; IC₅₀, concentration leading to 50% inhibition; Ile, isoleucine; iv, intravenous; kg, kilogram; L, liter; LC, liquid chromatography; Leu, leucine; LiHMDS, lithium bis(trimethylsilyl)amide; LLC, limited liability company; LLE, lipophilic ligand efficiency; log D, measured descriptor for lipophilicity; m, multiplet (in NMR); m, mouse; M, mass; M, molar (concentration); MeOH, methanol; Met, methionine; mg, milligram (in unites); MHz, megahertz; min, minute(s); mL, milliliters (in units); mm, millimeter; μ M, micromolar; MMP2, matrix metalloproteinase-2; MMP12, Matrix metalloproteinase-12; MMP14, Matrix metalloproteinase-14; MMP15, Matrix metalloproteinase-15; MS, mass spectrometry; MW, molecular weight; *m/z*, mass-to-charge ratio; N, normal; NCA, non-compartmental analysis; n.d., not determined; nm, nanometer; nM, nanomolar; NMR, nuclear magnetic resonance spectrometry; NOAEL, no observed adverse effect level; NROTB, number of rotatable bonds; PCSK9, proprotein convertase subtilisin/kexin type 9; PDB, Protein Data Bank; PEG, polyethylene glycol; Phe, phenylalanine; PK, pharmacokinetic; po, per os; ppm, parts per million (in NMR); Pro, proline; q, quartet (in NMR); quant., quantitative conversion; r, rat; Rt, retention time; r.t., room temperature; s, singlet (in NMR); SAR, structure activity relationship; SNP, single nucleotide

polymorphism; t, triplet (in NMR); t_{1/2}, terminal half-life; T3P, propanephosphonic acid anhydride; TEA, triethylamine; THF, tetrahydrofuran; Thr, threonine; T_{max}, median time to reach maximum concentration; TMS, tetramethylsilane; ToF, time-of-flight; TPA, tissue plasminogen activator; tPSA, topological polar surface area; Tyr, tyrosine; UV, ultraviolet; v, volume fraction; vs, versus; VSMC, vascular smooth muscle cell; V_{SS}, volume of distribution; XPhos, dicyclohexyl[2',4',6'-tris(propan-2-yl)[1,1'-biphenyl]-2-yl]phosphane.

REFERENCES

- (1) Libby, P.; Buring, J. E.; Badimon, L.; Hansson, G. K.; Deanfield, J.; Sommer Bittencourt, M.; Tokgözoğlu, L.; Lewis, E. F. Atherosclerosis. *Nat. Rev. Dis. Primers* **2019**, *5* (1), 56.
- (2) Ahamad, S.; Bhat, S. A. Recent update on the development of PCSK9 inhibitors for hypercholesterolemia treatment. *J. Med. Chem.* **2022**, *65*, 15513–15539.
- (3) Libby, P.; Everett, B. M. Novel antiatherosclerotic therapies. *Arterioscler. Thromb. Vasc. Biol.* **2019**, *39*, 539.
- (4) Khera, A. V.; Kathiresan, S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat. Rev. Genet.* **2017**, *18*, 331–344.
- (5) Kessler, T.; Schunkert, H. Coronary artery disease genetics enlightened by genome-wide association studies. *JACC Basic Transl. Sci.* **2021**, *6* (7), 610–623.
- (6) Reilly, M. P.; Li, M.; He, J.; Ferguson, J. F.; Stylianou, I. M.; Mehta, N. N.; Burnett, M. S.; Devaney, J. M.; Knouff, C. W.; Thompson, J. R.; Horne, B. D.; Stewart, A. F. R.; Assimes, T. L.; Wild, P. S.; Allayee, H.; Nitschke, P. L.; Patel, R. S.; Martinelli, N.; Girelli, D.; Quyyumi, A. A.; Anderson, J. L.; Erdmann, J.; Hall, A. S.; Schunkert, H.; Quertermous, T.; Blankenberg, S.; Hazen, S. L.; Roberts, R.; Kathiresan, S.; Samani, N. J.; Epstein, S. E.; Rader, D. J. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet* **2011**, *377* (9763), 383–92.
- (7) Schunkert, H.; König, I. R.; Kathiresan, S.; Reilly, M. P.; Assimes, T. L.; Holm, H.; Preuss, M.; Stewart, A. F. R.; Barbalic, M.; Gieger, C.; Absher, D.; Aherrahrou, Z.; Allayee, H.; Altshuler, D.; Anand, S. S.; Andersen, K.; Anderson, J. L.; Ardisson, D.; Ball, S. G.; Balmforth, A. J.; Barnes, T. A.; Becker, D. M.; Becker, L. C.; Berger, K.; Bis, J. C.; Boekholdt, S. M.; Boerwinkle, E.; Braund, P. S.; Brown, M. J.; Burnett, M. S.; Buysschaert, I.; Carlquist, J. F.; Chen, L.; Cichon, S.; Codd, V.; Davies, R. W.; Dedoussis, G.; Dehghan, A.; Demissie, S.; Devaney, J. M.; Diemert, P.; Do, R.; Doering, A.; Eifert, S.; Mokhtari, N. E. E.; Ellis, S. G.; Elosua, R.; Engert, J. C.; Epstein, S. E.; de Faire, U.; Fischer, M.; Folsom, A. R.; Freyer, J.; Gigante, B.; Girelli, D.; Gretarsdottir, S.; Gudnason, V.; Gulcher, J. R.; Halperin, E.; Hammond, N.; Hazen, S. L.; Hofman, A.; Horne, B. D.; Illig, T.; Iribarren, C.; Jones, G. T.; Jukema, J. W.; Kaiser, M. A.; Kaplan, L. M.; Kastelein, J. J. P.; Khaw, K.-T.; Knowles, J. W.; Kolovou, G.; Kong, A.; Laaksonen, R.; Lambrechts, D.; Leander, K.; Lettre, G.; Li, M.; Lieb, W.; Loley, C.; Lotery, A. J.; Mannucci, P. M.; Maouche, S.; Martinelli, N.; McKeown, P. P.; Meisinger, C.; Meitinger, T.; Melander, O.; Merlini, P. A.; Mooser, V.; Morgan, T.; Mühleisen, T. W.; Muhlestein, J. B.; Münzel, T.; Musunuru, K.; Nahrstaedt, J.; Nelson, C. P.; Nöthen, M. M.; Olivieri, O.; Patel, R. S.; Patterson, C. C.; Peters, A.; Peyvandi, F.; Qu, L.; Quyyumi, A. A.; Rader, D. J.; Rallidis, L. S.; Rice, C.; Rosendaal, F. R.; Rubin, D.; Salomaa, V.; Sampietro, M. L.; Sandhu, M. S.; Schadt, E.; Schäfer, A.; Schillert, A.; Schreiber, S.; Schrezenmeier, J.; Schwartz, S. M.; Siscovick, D. S.; Sivananthan, M.; Sivapalaratnam, S.; Smith, A.; Smith, T. B.; Snoep, J. D.; Soranzo, N.; Spertus, J. A.; Stark, K.; Stirrups, K.; Stoll, M.; Tang, W. H. W.; Tennstedt, S.; Thorgeirsson, G.; Thorleifsson, G.; Tomaszewski, M.; Uitterlinden, A. G.; van Rij, A. M.; Voight, B. F.; Wareham, N. J.; Wells, G. A.; Wichmann, H.-E.; Wild, P. S.; Willenborg, C.; Witteman, J. C. M.; Wright, B. J.; Ye, S.; Zeller, T.; Ziegler, A.; Cambien, F.; Goodall, A. H.; Cupples, L. A.; Quertermous, T.; März, W.; Hengstenberg, C.; Blankenberg, S.; Ouwehand, W. H.; Hall, A. S.; Deloukas, P.; Thompson, J. R.; Stefansson, K.; Roberts, R.; Thorsteinsdottir, U.; O'Donnell, C. J.; McPherson, R.; Erdmann, J. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat. Genet.* **2011**, *43*, 333–338.
- (8) Rabkin, S. W.; Koitsopoulos, P. G. Clinical genomics of the relationship between ADAMTS7 and coronary artery calcification and atherosclerosis. *J. Transl. Genet. Genom.* **2018**, *2*, 4.
- (9) Pereira, A.; Palma dos Reis, R.; Rodrigues, R.; Sousa, A. C.; Gomes, S.; Borges, S.; Ornelas, I.; Freitas, A. I.; Guerra, G.; Henriques, E.; Rodrigues, M.; Freitas, S.; Freitas, C.; Brehm, A.; Pereira, D.; Mendonça, M. I. Association of ADAMTS7 gene polymorphism with cardiovascular survival in coronary artery disease. *Physiol. Genomics* **2016**, *48*, 810–815.
- (10) Chan, K.; Pu, X.; Sandesara, P.; Poston, R. N.; Simpson, I. A.; Quyyumi, A. A.; Ye, S.; Patel, R. S. Genetic variation at the ADAMTS7 locus is associated with reduced severity of coronary artery disease. *J. Am. Heart Assoc.* **2017**, *6*, No. e006928.
- (11) Petrovic, D.; Nussdorfer, P.; Petrovic, D. The rs3825807 polymorphism of ADAMTS7 as a potential genetic marker for myocardial infarction in slovenian subjects with type 2 diabetes mellitus. *Genes* **2023**, *14*, 508.
- (12) Bengtsson, E.; Hultman, K.; Dunér, P.; Ascietto, G.; Almgren, P.; Orho-Melander, M.; Melander, O.; Nilsson, J.; Hultgårdh-Nilsson, A.; Gonçalves, I. ADAMTS-7 is associated with a high-risk plaque phenotype in human atherosclerosis. *Sci. Rep.* **2017**, *7*, 3753.
- (13) Pu, X.; Xiao, Q.; Kiehl, S.; Chan, K.; Ng, F. L.; Gor, S.; Poston, R. N.; Fang, C.; Patel, A.; Senner, E. C.; Shaw-Hawkins, S.; Willeit, J.; Liu, C.; Zhu, J.; Tucker, A. T.; Xu, Q.; Caulfield, M. J.; Ye, S. ADAMTS7 cleavage and vascular smooth muscle cell migration is affected by a coronary-artery-disease-associated variant. *Am. J. Hum. Genet.* **2013**, *92* (3), 366–74.
- (14) Zhang, L.; Yu, F.; Wang, L.; Zheng, J.; Du, Y.; Huang, Y.; Liu, B.; Wang, X.; Kong, W. ADAMTS-7 promotes vascular smooth muscle cells proliferation in vitro and in vivo. *Sci. China Life Sci.* **2015**, *58*, 674–681.
- (15) Kessler, T.; Zhang, L.; Liu, Z.; Yin, X.; Huang, Y.; Wang, Y.; Fu, Y.; Mayr, M.; Ge, Q.; Xu, Q.; Zhu, Y.; Wang, X.; Schmidt, K.; de Wit, C.; Erdmann, J.; Schunkert, H.; Aherrahrou, Z.; Kong, W. ADAMTS-7 inhibits re-endothelialization of injured arteries and promotes vascular remodeling through cleavage of thrombospondin-1. *Circulation* **2015**, *131*, 1191–1201.
- (16) Pu, X.; Chan, K.; Yang, W.; Xiao, Q.; Zhang, L.; Moore, A. D.; Liu, C.; Webb, T. R.; Caulfield, M. J.; Samani, N. J.; Zhu, J.; Ye, S. Effect of a coronary-heart-disease-associated variant of ADAMTS7 on endothelial cell angiogenesis. *Atherosclerosis* **2020**, *296*, 11–17.
- (17) Li, L.; Wang, S.; Wang, M.; Liu, G.; Yang, Z.; Wang, L. miR-654-5p suppresses migration and proliferation of vascular smooth muscle cells by targeting ADAMTS-7. *Cells Tissues Organs* **2022**, *212* (4), 285–292, DOI: 10.1159/000524677.
- (18) Wang, L.; Zheng, J.; Bai, X.; Liu, B.; Liu, C.; Xu, Q.; Zhu, Y.; Wang, N.; Kong, W.; Wang, X. ADAMTS-7 mediates vascular smooth muscle cell migration and neointima formation in balloon-injured rat arteries. *Circ. Res.* **2009**, *104* (5), 688–98.
- (19) Ren, W.; Liang, L.; Li, Y.; Wei, F.; Mu, N.; Zhang, L.; He, W.; Cao, Y.; Xiong, D.; Li, H. Upregulation of miR-423 improves autologous vein graft restenosis via targeting ADAMTS-7. *Int. J. Mol. Med.* **2019**, *45* (2), 532–542.
- (20) Bauer, R. C.; Tohyama, J.; Cui, J.; Cheng, L.; Yang, J.; Zhang, X.; Ou, K.; Paschos, G. K.; Zheng, X. L.; Parmacek, M. S.; Rader, D. J.; Reilly, M. P. Knockout of Adamts7, a novel coronary artery disease locus in humans, reduces atherosclerosis in mice. *Circulation* **2015**, *131* (13), 1202–1213.
- (21) Arroyo, A. G.; Andrés, V. ADAMTS7 in cardiovascular disease - From bedside to bench and back again? *Circulation* **2015**, *131* (13), 1156–9.
- (22) Mizoguchi, T.; MacDonald, B. T.; Bhandary, B.; Popp, N. R.; Laprise, D.; Arduini, A.; Lai, D.; Zhu, Q. M.; Xing, Y.; Kaushik, V. K.; Kathiresan, S.; Ellinor, P. T. Coronary disease association with

ADAMTS7 is due to protease activity. *Circ. Res.* **2021**, 129 (4), 458–470.

(23) Feinstein, M. J.; Thorp, E. B. ADAMTS7 knockdown in context. *Circ. Res.* **2021**, 129 (4), 471–473.

(24) Ma, Z.; Mao, C.; Chen, X.; Yang, S.; Qiu, Z.; Yu, B.; Jia, Y.; Wu, C.; Wang, Y.; Wang, Y.; Gu, R.; Yu, F.; Yin, Y.; Wang, X.; Xu, Q.; Liu, C.; Liao, Y.; Zheng, J.; Fu, Y.; Kong, W. Peptide vaccine against ADAMTS-7 ameliorates atherosclerosis and postinjury neointima hyperplasia. *Circulation* **2023**, 147, 728–742.

(25) Kessler, T.; Schunkert, H. Targeting ADAMTS-7: a vaccination against atherosclerosis and its complications? *Circulation* **2023**, 147, 743–745.

(26) Chung, A.; Reilly, M. P.; Bauer, R. C. ADAMTS7: a novel therapeutic target in atherosclerosis. *Curr. Atheroscler. Rep.* **2023**, 25, 447–455.

(27) Hanby, H. A.; Zheng, X. L. Biochemistry and physiological functions of ADAMTS7 metalloprotease. *Advances in Biochem.* **2013**, 1 (3), 43–50.

(28) Kelwick, R.; Desanlis, I.; Wheeler, G. N.; Edwards, D. R. The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. *Gen. Biol.* **2015**, 16, 113.

(29) Liu, C.; Kong, W.; Ilalov, K.; Yu, S.; Xu, K.; Prazak, L.; Fajardo, M.; Sehgal, B.; Di Cesare, P. E.; et al. ADAMTS-7: a metalloproteinase that directly binds to and degrades cartilage oligomeric matrix protein. *FASEB* **2006**, 20, 988.

(30) MacDonald, B. T.; Keshishian, H.; Mundorff, C. C.; Arduini, A.; Lai, D.; Bendinelli, K.; Popp, N. R.; Bhandary, B.; Clauser, K. R.; Specht, H.; Elowe, N. H.; Laprise, D.; Xing, Y.; Kaushik, V. K.; Carr, S. A.; Ellinor, P. T. TAILS identifies candidate substrates and biomarkers of ADAMTS7, a therapeutic protease target in coronary artery disease. *Mol. Cell Proteomics* **2022**, 21 (4), No. 100223.

(31) Bai, X.-H.; Wang, D.-W.; Kong, L.; Zhang, Y.; Luan, Y.; Kobayashi, T.; Kronenberg, H. M.; Yu, X.-P.; Liu, C. ADAMTS-7, a direct target of PTHrP, adversely regulates endochondral bone growth by associating with and inactivating GEP growth factor. *Mol. Cell. Biol.* **2009**, 29, 4201–4219.

(32) Mead, T. J.; Apte, S. S. ADAMTS proteins in human disorders. *Matrix Biol.* **2018**, 71–72, 225–239.

(33) Müller, M.; Kessler, T.; Schunkert, H.; Erdmann, J.; Tennstedt, S. Classification of ADAMTS binding sites: the first step toward selective ADAMTS7 inhibitors. *Biochem. Biophys. Res. Commun.* **2016**, 471, 380–385.

(34) Santamaria, S.; Buemi, F.; Nuti, E.; Cuffaro, D.; De Vita, E.; Tuccinardi, T.; Rossello, A.; Howell, S.; Mehmood, S.; Snijders, A. P.; de Groot, R. Development of a fluorogenic ADAMTS-7 substrate. *J. Enzyme Inhib. Med. Chem.* **2021**, 36, 2160–2169.

(35) Meibom, D.; Cancho Grande, Y.; Wasnaire, P.; Johannes, S. A. L.; Lindner, N.; Beyer, K.; Freudenberg, T.; Brockschneider, D.; Mathar, I.; Klar, J.; Zubov, D.; Menshykau, D.; Krainz, T.; Stefan, E.; MacDonald, B.; Xing, Y.; Elowe, N.; Sanchez, G. *Substituted hydantoinamides as ADAMTS7 antagonists*. Patent WO2021/094434 A1, 2021.

(36) Meibom, D.; Cancho Grande, Y.; Wasnaire, P.; Johannes, S. A. L.; Beyer, K.; Freudenberg, T.; Brockschneider, D.; Zubov, D.; Menshykau, D.; Krainz, T.; MacDonald, B.; Xing, Y.; Elowe, N.; Sanchez, G. *Substituted hydantoinamides as ADAMTS7 antagonists*. Patent WO2021/094436 A1, 2021.

(37) Zubov, D.; MacDonald, B.; Xing, Y.; Elowe, N.; Brockschneider, D. *Characterizing modulators of ADAMTS-7 and ADAMTS-12*. Patent WO2021/097043 A1, 2021.

(38) Sharifi, M. A.; Wierer, M.; Dang, T. A.; Milic, J.; Moggio, A.; Sachs, N.; Scheidt, M. V.; Hinterdobler, J.; Müller, P.; Werner, J.; Stiller, B.; Aherrahrou, Z.; Erdmann, J.; Zaliani, A.; Graettinger, M.; Reinshagen, J.; Gul, S.; Gribbon, P.; Maegdefessel, L.; Bernhagen, J.; Sager, H. B.; Mann, M.; Schunkert, H.; Kessler, T. ADAMTS-7 modulates atherosclerotic plaque formation by degradation of TIMP-1. *Circ. Res.* **2023**, 133, 674.

(39) Durham, T. B.; Marimuthu, J.; Wiley, M. R. *Aggrecanase inhibitors*. Patent WO2014/066151 A1, 2014.

(40) Durham, T. B.; Marimuthu, J.; Toth, J. L.; Liu, C.; Adams, L.; Mudra, D. R.; Swearingen, C.; Lin, C.; Chambers, M. G.; Thirunavukkarasu, K.; Wiley, M. R. A highly selective hydantoin inhibitor of aggrecanase-1 and aggrecanase-2 with a low projected human dose. *J. Med. Chem.* **2017**, 60, 5933–5939.

(41) Brebion, F.; Gosmini, R.; Deprez, P.; Varin, M.; Peixoto, C.; Alvey, L.; Jary, H.; Bienvenu, N.; Triballeau, N.; Blanque, R.; Cottreaux, C.; Christophe, T.; Vandervoort, N.; Mollat, P.; Touitou, R.; Leonard, P.; Ceuninck, F. d.; Botez, I.; Monjardet, A.; Aar, E. v. d.; Amantini, D. Discovery of GLPG1972/S201086, a potent, selective, and orally bioavailable ADAMTS-5 inhibitor for the treatment of osteoarthritis. *J. Med. Chem.* **2021**, 64, 2937–2952.

(42) Mohamedi, Y.; Fontanil, T.; Cal, S.; Cobo, T.; Obaya, Á. J. ADAMTS-12: functions and challenges for a complex metalloprotease. *Front. Mol. Biosci.* **2021**, 8, No. 686763.

(43) Mead, T. J.; Bhutada, S.; Martin, D. R.; Apte, S. S. Proteolysis: a key post-translational modification regulating proteoglycans. *Am. J. Physiol. Cell Physiol.* **2022**, 323 (3), C651–C665.

(44) Durham, T. B.; Klimkowski, V. J.; Rito, C. J.; Marimuthu, J.; Toth, J. L.; Liu, C.; Durbin, J. D.; Stout, S. L.; Adams, L.; Swearingen, C.; Lin, C.; Chambers, M. G.; Thirunavukkarasu, K.; Wiley, M. R. Identification of potent and selective hydantoin inhibitors of aggrecanase-1 and aggrecanase-2 that are efficacious in both chemical and surgical models of osteoarthritis. *J. Med. Chem.* **2014**, 57, 10476–10485.

(45) Wiley, M. R.; Durham, T. B.; Adams, L. A.; Chambers, M. G.; Lin, C.; Liu, C.; Marimuthu, J.; Mitchell, P. G.; Mudra, D. R.; Swearingen, C. A.; Toth, J. L.; Weller, J. M.; Thirunavukkarasu, K. Use of osmotic pumps to establish the pharmacokinetic–pharmacodynamic relationship and define desirable human performance characteristics for aggrecanase inhibitors. *J. Med. Chem.* **2016**, 59, 5810–5822.

(46) Efimov, I. V.; Kulikova, L. N.; Zhilyaev, D. I.; Voskressensky, L. G. Recent advances in the chemistry of isocyanides with activated methylene group. *Eur. J. Org. Chem.* **2020**, 2020, 7284–7303.

(47) Goloroush, P.; Yellon, D. M.; Davidson, S. M. Mouse models of atherosclerosis and their suitability for the study of myocardial infarction. *Basic Res. Cardiol.* **2020**, 115, 73.

(48) Tulis, D. A. Rat carotid artery balloon injury model. *Methods Mol. Med.* **2007**, 139, 1–30.

(49) Krishna, S. M.; Omer, S. M.; Golledge, J. Evaluation of the clinical relevance and limitations of current pre-clinical models of peripheral artery disease. *Clin. Sci. (Lond)* **2016**, 130, 127–150.

(50) Mead, T. J.; McCulloch, D. R.; Ho, J. C.; Du, Y.; Adams, S. M.; Birk, D. E.; Apte, S. S. The metalloproteinase-proteoglycans ADAMTS7 and ADAMTS12 provide an innate, tendon-specific protective mechanism against heterotopic ossification. *JCI Insight* **2018**, 3 (7), No. e92941.

(51) Moncada-Pazos, A.; Obaya, A. J.; Llamazares, M.; Heljasvaara, R.; Suárez, M. F.; Colado, E.; Noël, A.; Cal, S.; López-Otín, C. ADAMTS-12 metalloprotease is necessary for normal inflammatory response. *J. Biol. Chem.* **2012**, 287 (47), 39554–39563.

(52) Pérez-García, S.; Gutiérrez-Cañas, I.; Seoane, I. V.; Fernández, J.; Mellado, M.; Leceta, J.; Tío, L.; Villanueva-Romero, R.; Juarranz, Y.; Gomariz, R. P. Healthy and osteoarthritic synovial fibroblasts produce a disintegrin and metalloproteinase with thrombospondin motifs 4, 5, 7, and 12 - Induction by IL-1b and fibronectin and contribution to cartilage damage. *Am. J. Pathol.* **2016**, 186 (9), 2449.

(53) Pérez-García, S.; Carrión, M.; Villanueva-Romero, R.; Hermida-Gómez, T.; Fernández-Moreno, M.; Mellado, M.; Blanco, F. J.; Juarranz, Y.; Gomariz, R. P. Wnt and RUNX2 mediate cartilage breakdown by osteoarthritis synovial fibroblast-derived ADAMTS-7 and -12. *J. Cell. Mol. Med.* **2019**, 23, 3974–3983.

(54) El Hour, M.; Moncada-Pazos, A.; Blacher, S.; Masset, A.; Cal, S.; Berndt, S.; Detilleux, J.; Host, L.; Obaya, A. J.; Maillard, C.; Foidart, J. M.; Ectors, F.; Noel, A.; Lopez-Otin, C. Higher sensitivity of Adamts12-deficient mice to tumor growth and angiogenesis. *Oncogene* **2010**, 29, 3025–3032.

- (55) Llamazares, M.; Obaya, A. J.; Moncada-Pazos, A.; Heljasvaara, R.; Espada, J.; López-Otín, C.; Cal, S. The ADAMTS12 metalloproteinase exhibits antitumorigenic properties through modulation of the Ras-dependent ERK signalling pathway. *J. Cell Sci.* **2007**, *120*, 3544–3552.
- (56) Wang, D.; Zhu, T.; Zhang, F.-B.; He, C. Expression of ADAMTS12 in colorectal cancer-associated stroma prevents cancer development and is a good prognostic indicator of colorectal cancer. *Dig. Dis. Sci.* **2011**, *56*, 3281–3287.
- (57) Rabadán, R.; Mohamedi, Y.; Rubin, U.; Chu, T.; Alghalith, A. N.; Elliott, O.; Arnés, L.; Cal, S.; Obaya, A. J.; Levine, A. J.; Cámara, P. G. Identification of relevant genetic alterations in cancer using topological data analysis. *Nat. Commun.* **2020**, *11*, 3808.
- (58) Moncada-Pazos, A.; Obaya, A. J.; Fraga, M. F.; Vilorio, C. G.; Capellá, G.; Gausachs, M.; Esteller, M.; López-Otín, C.; Cal, S. The ADAMTS12 metalloproteinase gene is epigenetically silenced in tumor cells and transcriptionally activated in the stroma during progression of colon cancer. *J. Cell Sci.* **2009**, *122*, 2906–2913.
- (59) Li, C.; Luo, X.; Huang, B.; Wang, X.; Deng, Y.; Zhong, Z. ADAMTS12 acts as a cancer promoter in colorectal cancer via activating the Wnt/ β -catenin signaling pathway in vitro. *Ann. Transl. Med.* **2020**, *8* (6), 301.
- (60) Beristain, A. G.; Zhu, H.; Leung, P. C. K. Regulated expression of ADAMTS-12 in human trophoblastic cells: A role for ADAMTS-12 in epithelial cell invasion? *PLoS One* **2011**, *6* (4), No. e18473.
- (61) Mariani, A.; Wang, C.; Oberg, A. L.; Riska, S. M.; Torres, M.; Kumka, J.; Multinu, F.; Sagar, G.; Roy, D.; Jung, D.-B.; Zhang, Q.; Grassi, T.; Visscher, D. W.; Patel, V. P.; Jin, L.; Staub, J. K.; Cliby, W. A.; Weroha, S. J.; Kalli, K. R.; Hartmann, L. C.; Kaufmann, S. H.; Goode, E. L.; Shridhar, V. Genes associated with bowel metastases in ovarian cancer. *Gynecol. Oncol.* **2019**, *154*, 495–504.
- (62) Ho, T. H.; Serie, D. J.; Parasramka, M.; Cheville, J. C.; Bot, B. M.; Tan, W.; Wang, L.; Joseph, R. W.; Hilton, T.; Leibovich, B. C.; Parker, A. S.; Eckel-Passow, J. E. Differential gene expression profiling of matched primary renal cell carcinoma and metastases reveals upregulation of extracellular matrix genes. *Ann. Oncol.* **2017**, *28*, 604–610.
- (63) Liang, L.; Zhu, J.; Chen, G.; Qin, X.; Chen, J. Prognostic values for the mRNA expression of the ADAMTS family of genes in gastric cancer. *J. Oncol.* **2020**, *2020*, No. 9431560.
- (64) Li, X.; Xiao, X.; Chang, R.; Zhang, C. Comprehensive bioinformatics analysis identifies lncRNA HCG22 as a migration inhibitor in esophageal squamous cell carcinoma. *J. Cell. Biochem.* **2020**, *121*, 468–481.