

Extended Structure–Activity Relationship and Pharmacokinetic Investigation of (4-Quinolinoyl)glycyl-2-cyanopyrrolidine Inhibitors of Fibroblast Activation Protein (FAP)

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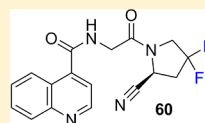
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S Supporting Information

ABSTRACT: Fibroblast activation protein (FAP) is a serine protease related to dipeptidyl peptidase IV (DPPIV). It has been convincingly linked to multiple disease states involving remodeling of the extracellular matrix. FAP inhibition is investigated as a therapeutic option for several of these diseases, with most attention

so far devoted to oncology applications. We previously discovered the *N*-4-quinolinoyl-Gly-(2*S*)-cyanoPro scaffold as a possible entry to highly potent and selective FAP inhibitors. In the present study, we explore in detail the structure–activity relationship around this core scaffold. We report extensively optimized compounds that display low nanomolar inhibitory potency and high selectivity against the related dipeptidyl peptidases (DPPs) DPPIV, DPP9, DPPII, and prolyl oligopeptidase (PREP). The log *D* values, plasma stabilities, and microsomal stabilities of selected compounds were found to be highly satisfactory. Pharmacokinetic evaluation in mice of selected inhibitors demonstrated high oral bioavailability, plasma half-life, and the potential to selectively and completely inhibit FAP *in vivo*.



IC₅₀(FAP) = 3.2 nM
IC₅₀(PREP) = 1.8 μM
-Oral bioavailability in rats: 51%
-Circulating half-life: 3.2 hours
-Potent, long-lasting *in vivo* FAP-inhibition

■ INTRODUCTION

Fibroblast activation protein (FAP, FAP-α, seprase) belongs to the prolyl oligopeptidase family S9, which consists of serine proteases that cleave peptide substrates preferentially after proline residues. Other members of this family include the dipeptidyl peptidases (DPPs: DPPIV, DPP8, DPP9) and prolyl oligopeptidase (PREP, POP).¹ FAP has been linked to multiple disease states involving remodeling of the extracellular matrix such as hepatic and pulmonary fibrosis, keloid formation, rheumatoid arthritis, and osteoarthritis.^{2–7} FAP is also highly expressed on activated fibroblasts in over 90% of common human epithelial tumors.^{8,9} It has been demonstrated in syngeneic mouse models that FAP activity promotes tumorigenesis and that FAP inhibition attenuates tumor growth.^{10–12} The enzyme is furthermore expressed only transiently during wound healing and is essentially absent in normal adult tissues and in nonmalignant tumors.¹³ These appealing characteristics of FAP account for its ongoing evaluation as a drug target. Both immunotherapy and small-molecule based approaches have so far been reported, most of them focusing on applications in the oncology domain (*vide infra*).

FAP possesses both dipeptidyl peptidase and endopeptidase activity, catalyzed by the same active center. This is in contrast with the DPPs, possessing only the former activity type, and

PREP, which is an enzyme of strict endopeptidase capability.^{14,15} While designing out DPP affinity in FAP inhibitors is relatively straightforward, obtaining inhibitors possessing selectivity for FAP over PREP is considered to be far more challenging. This is, among others, illustrated by the significant overlap between *in vitro* processable substrate sequences for FAP and PREP and the fact that numerous reported FAP inhibitors have limited or no selectivity with respect to PREP.¹⁶ Given that recent findings indicate that PREP deficient mice have impaired spatial learning, memory, and neuronal development, FAP over PREP selectivity could nonetheless be an important feature for inhibitors.^{17,18} The availability of such compounds can already have significant impact when applied as tool compounds to study the role of FAP in pathophysiology. In some cancer types for example, FAP and PREP are simultaneously overexpressed by cell types that are part of the metastatic tumor microenvironments, and there are currently no reports available that quantitatively discriminate between the potential contributions of both proteolytic activities to disease progression.¹⁷

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Several of the relevant FAP inhibitors reported in the literature were used as reference compounds in this study. Val-boroPro **1** (talabostat, PT-100) is a nonselective boronic acid inhibitor that reached phase II clinical trials for several cancer types before it was withdrawn, apparently because of both safety and efficacy reasons (Figure 1 and Table 1).^{19–21}

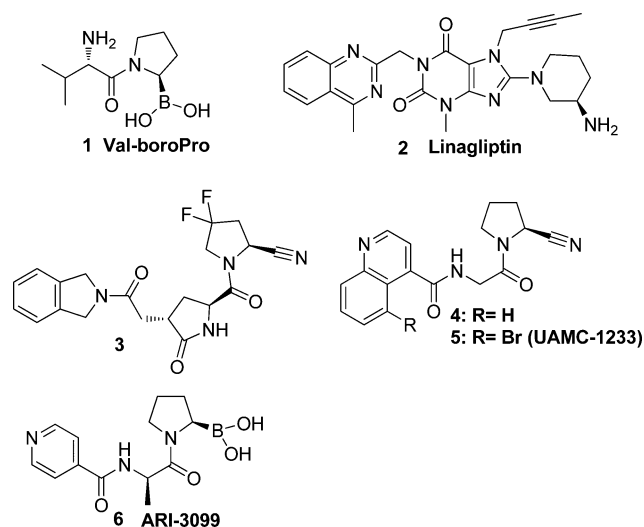


Figure 1. Relevant FAP inhibitors used as references in this study.

Linagliptin **2** has received clinical approval as a DPP-4 inhibitor but also displays substantial FAP affinity.²² Compound **3** is a representative of a series of pyroglutamyl(2-cyanopyrrolidine) derivatives reported by Jiaang et al.²³ Compounds **4** and **5** are part of the quinolinoylglycyl(2-cyanopyrrolidine) class of FAP inhibitors reported recently by our group.²⁴ Although no PREP-assay results were published by Jiaang, we report here that compound **3** does have highly satisfactory FAP over PREP selectivity. Therefore, Jiaang's and our molecules represent the only two reported inhibitor chemotypes with potential for full FAP selectivity across the panel of related enzymes evaluated. Jiaang's compounds nonetheless were reported to have poor pharmacokinetic (PK) behavior in mice. The corresponding PK data for selected representatives of our own molecules are reported in this manuscript. In addition, Bachovchin et al. have also recently reported the D-Ala-boroPro based FAP inhibitor **6**.²⁴ This compound's approximate 40-fold selectivity toward PREP (determined under our assay conditions) is remarkable for a boronic acid, certainly since a recently published pseudo peptide boronic acid inhibitor with the same D-Ala-boroPro scaffold but with an acetyl-Arg-2-(2-(2-aminoethoxy)ethoxy)-

acetamide group as the N-substituent instead of a 4-pyridinoyl group was found not to be selective toward PREP.²⁵ Nonetheless, no in vivo PK data have so far been published for **6**.²⁶

In our earlier report on FAP inhibitors related to **4**, we have investigated the quinolinoyl moiety's substitution pattern and the influence of the position of the heterocyclic N-atom on FAP affinity and selectivity. No heterocyclic scaffolds other than quinoline and isoquinoline were so far built in at this position. Evaluating the impact of other azaheteroaromatic rings was therefore considered a first goal of this study with which we aimed to significantly expand our current SAR knowledge for this part of the molecule. Second, SAR for the P2 glycine residue also remained underexplored, as no fragments other than glycine have been introduced at this position. Several P2-modified analogues were therefore made. Furthermore, these molecules were deemed of additional interest to anticipate on potential sensitivity of the P2 glycine amide bonds to unspecific proteases in vivo. Third, we sought to further optimize the P1 cyanopyrrolidine residue of our inhibitors. This was done by applying predictive SAR data that we derived earlier for inhibitors containing a P1-(2-cyanopyrrolidine) moiety.^{24,27} In the P1-modified series, we also investigated the importance of the warhead on our molecules and the influence on inhibitory potency of changing the carbonitrile function for other electrophilic warhead types (boronic acid, chloromethyl ketone). Fourth, in vitro PK and cellular toxicity data are reported for five optimized inhibitors. On the basis of these data, we selected four compounds for in vivo PK studies and in vivo inhibition experiments in mice.

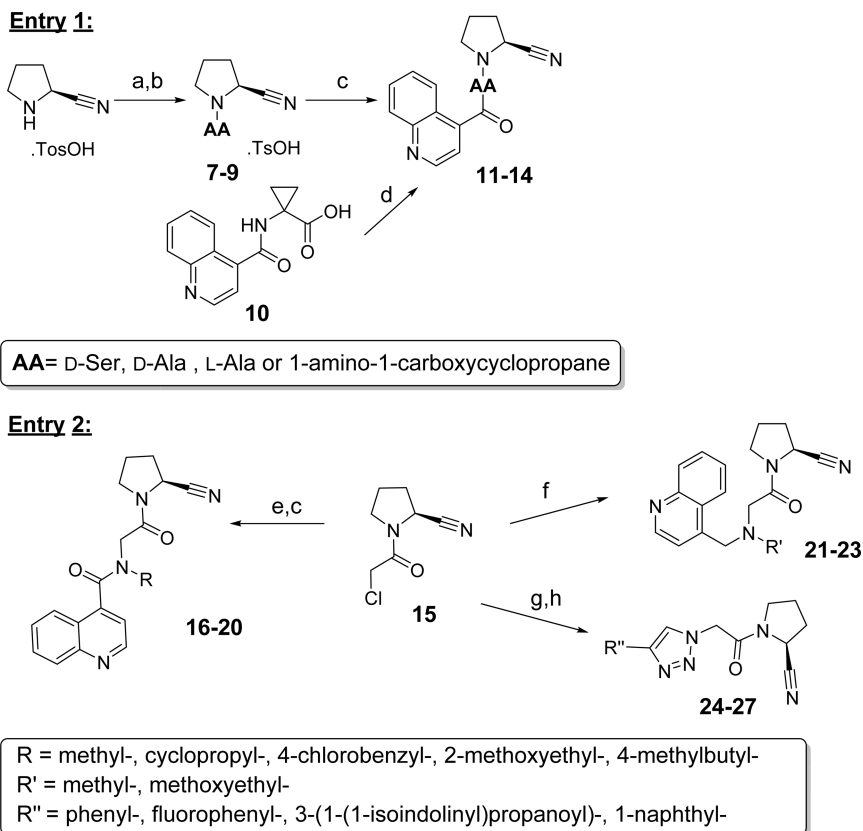
CHEMISTRY

A total of around 60 novel inhibitors were synthesized for this study. All compounds were prepared following the general strategies in Schemes 1 and 2, in which target compounds are clustered according to the modification type they contain, relative to reference compound **4**. The preparation of inhibitors containing an adapted P2 group (**9–11** and **16–27**) is summarized in Scheme 1. Three types of modifications were included in this series: (1) attachment of a side chain to the α -methylene group of the P2 moiety, (2) introduction of N-substituents, and (3) replacement of the P2–P3 amide bond by a reduced analogue or a peptidomimetic triazole ring. For the preparation of the α -methylene substituted series (Scheme 1, entry 1), the corresponding, commercially available Boc-protected P2-amino acids were coupled to 2-cyanopyrrolidine. The Boc group of the coupled products was then removed using tosylic acid in acetonitrile under conditions we identified as optimal to avoid Ritter-type addition of the intermediate *tert*-

Table 1. IC₅₀ of Reference FAP Inhibitors

compd	IC ₅₀ (μM) ^a					SI (FAP/PREP) ^b
	FAP	PREP	DPP-IV	DPP-9	DPP-2	
1	0.066 ± 0.011	0.98 ± 0.06	0.022 ± 0.001	ND ^c	0.086 ± 0.007	14.8
2	0.37 ± 0.002	>100	0.0020 ± 0.0002	>100	>100	>250
3	0.017 ± 0.001	>100	>100	>100	>100	5882.4
4	0.0103 ± 0.0004	0.86 ± 0.07	>100	>100	>100	83.5
5	0.011 ± 0.0004	>50	>100	>100	>100	>4500
6	0.025 ± 0.001	0.99 ± 0.04	>100	>50	>100	39.6

^aDetermined under our own assay conditions. ^bSI stands for "selectivity Index" (calculated as [IC₅₀(PREP)/IC₅₀(FAP)]). ^cND stands for "not determined".

Scheme 1. Synthesis of P2-Modified FAP Inhibitors 11–14 and 16–27^a

^aReagents and conditions: (a) HATU, *N*-Boc-AA-OH, DIPEA, 75–80%; (b) TsOH 1.4 equiv, MeCN, 24 h, 84–86%; (c) 4-quinolinoyl chloride hydrochloride, DIPEA, 25–86%; (d) (2*S*)-(2-cyanopyrrolidine) tosylate, HATU, DIPEA, 64%; (e) K₂CO₃, RNH₂, 13–52%; (f) K₂CO₃, quinolin-4-ylmethanamine, 25–49%; (g) NaN₃, DMF; (h) CuI, THF, 17–50%.

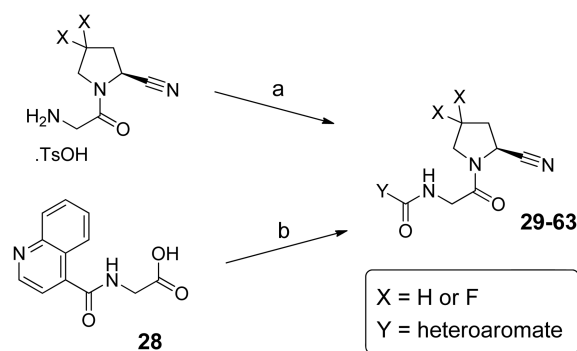
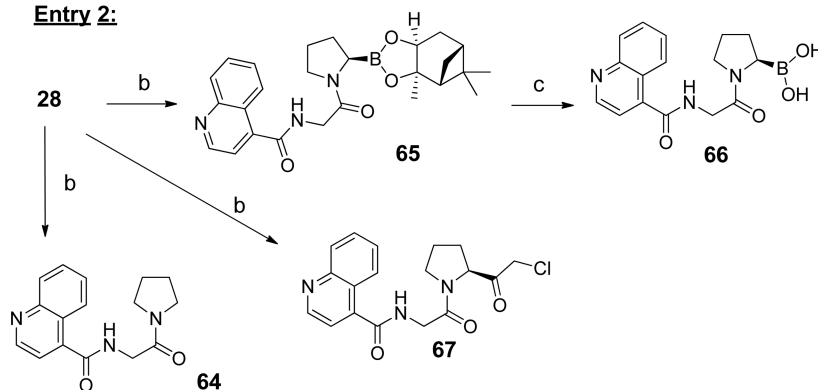
butyl cation to the nitrile group of products 7–9. These products were coupled with 4-quinolinoyl chloride hydrochloride to yield final compounds 11–13. The 2-cyanopyrrolidine starting material was obtained as described earlier.²³ For molecules involving the introduction of N-substituents or P2–P3 amide group modifications, (S)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **15** was used as a central intermediate²⁸ (Scheme 1, entry 2). Reaction of **15** with primary amines and subsequent coupling with 4-quinolinoyl chloride hydrochloride yielded compounds 16–20. To obtain target compounds **21** and **22** with a reduced P2–P3 amide group, the chloroacetyl group of **15** was substituted with N-substituted (4-quinolinyl)methylamines. Finally, the peptidomimetic triazole containing compounds were made by nucleophilic substitution of **15**'s chloro group with sodium azide and subsequent Cu(I)-catalyzed 1,3-dipolar addition with the desired alkynes to give products 24–27.

Scheme 2 describes the preparation of P3- and P1-modified analogues. Two strategies were followed for all reported compounds bearing a 2-cyanopyrrolidine derivative in P1 (29–63) (Scheme 2, entry 1). For molecules designed to mainly generate SAR data for the P3 position, diverse heteroarylcarboxylates were coupled to a specific glycyl-(2-cyanopyrrolidine) derivative using standard peptide coupling reagents. Alternatively, for a number of compounds designed mainly to render P1 SAR information, *N*-(4-quinolinoyl)glycine **28** was coupled to a desired 2-cyanopyrrolidine derivative. The 2-cyanopyrrolidine, its 4*S*-fluoro, 4*R*-fluoro, and 4,4-difluoro congeners and all glycine adducts of these molecules were

prepared as reported earlier or as described in the Supporting Information.²⁹ Heteroarylcarboxylates were acquired commercially or, in the case of substituted 4-quinolinoyl derivatives, synthesized using the Sandmeyer isatin synthesis followed by the Pfitzinger reaction and decarboxylation.^{30,31} Detailed procedures for all intermediates and compounds in this report are given in the Supporting Information. The boronate, the chloromethyl ketone, and unsubstituted pyrrolidine derivatives were synthesized in an analogous manner (Scheme 2, entry 2). Again, *N*-(4-quinolinoyl)glycine **28** was coupled to the desired pyrrolidine derivative using standard peptide coupling techniques. Both the required 2-boronyl- and chloromethylacylpyrrolidine derivatives were obtained as described in the literature.^{32,33} To obtain boronic acid final compound **66**, its pinanediol ester precursor **65** was deprotected using phenylboronic acid.

RESULTS AND DISCUSSION

All synthesized compounds were evaluated as inhibitors of FAP, DPPIV, DPP9, DPP11, and PREP.²⁷ No separate screening experiments were carried out on DPP8. On the basis of the enzyme's close homology with DPP9 and the outcome of earlier directed studies, potencies toward both enzymes can be expected to be comparable with a high degree of confidence. Table 2 summarizes the assay results of the P2-modified analogues. In a first set of compounds (11–14), the P2-glycine residue of **4** was replaced by D-Ser and related D-Ala, L-Ala, and 1-amino-1-carboxycyclopropane moieties. The choice for the

Scheme 2. General Synthetic Strategies for the Synthesis of P1 and/or P3 Modified Target Compounds^a**Entry 1:****Entry 2:**

^aReagents and conditions: (a) YCOOH, HATU, DIPEA, 3 h, 43–67%, or YCOOH, HOBT, EDC, DIPEA, 16 h, 84–86%, or YCOOH, $(\text{CH}_3)_2\text{C}=\text{C}(\text{Cl})\text{N}(\text{CH}_3)_2$, DIPEA, 25–70%, or quinoline-4-carbonyl chloride hydrochloride, DIPEA, 55–59%; (b) pyrrolidine derivative, HOBT, EDC, DIPEA, 16 h, 65–86%; (c) phenylboronic acid, MTBE– H_2O , 79%.

first two residues was based on an extensive screening of fluorogenic peptide substrates by Edosada et al., demonstrating that FAP's endopeptidase capability is strictly limited to peptides with a P2-Gly, D-Ser, or D-Ala residue.³⁴ As an additional illustration, a P2-D-Ala residue present in Bachovchin's boronic acid FAP-inhibitor **6** also demonstrated low nanomolar FAP affinity and good selectivity toward PREP.²⁶ When introduced as a replacement for compound **4**'s glycine residue however (**11** and **12**), the FAP affinities observed dropped 600- and 300-fold, respectively. FAP inhibition was completely abolished by introducing the closely related amino acids L-Ala and 1-amino-1-carboxycyclopropane at P2. While these data underscore the importance of a P2-glycine in our inhibitors, the lack of affinity of **13** and **14** also serves as confirmation that Edosada's original findings on substrates translate similarly for inhibitors. Finally, L-Ala containing analogue **13** has a PREP potency that stands out among the four congeneric inhibitors discussed.

Moving further with our SAR exploration of the P2 position, we decided to synthesize analogues with an N-alkylated and/or a reduced P2–P3 amide bond. Taking into account that N-alkylation of amide bonds in peptides is a well-known way of reducing susceptibility to (non-FAP-related) proteolytic activity, both modification types could increase the metabolic stability of this potentially labile part of the inhibitors' basic structure. In the N-alkylated amide series (compounds **16**–**20**), substituents of varying size and electronic properties were tested. All these interventions were found to lead to a nearly complete loss of FAP potency, even in the case of the smallest

methyl substituent. Remarkably, PREP affinity was affected to a significantly smaller extent, bringing on additional evidence that the 4-quinolinyl substituent of **4** is involved in a specific interaction with FAP's active center that significantly adds to target affinity but is disrupted by small structural changes. Similar conclusions were drawn from the assay results of the reduced analogues: tertiary amines **21**–**23** also had strongly reduced FAP inhibition. It is, however, impossible to state to what extent the increased conformational freedom of the quinolinylmethyl substituent or the lack of a conjugated, electron withdrawing carbonyl function contributes to these findings. Furthermore, a basic amine functionality is present in these molecules. Apparently this feature does not allow picking up additional potency from salt-bridge formation with FAP's Glu203 and Glu204 residues, the interaction responsible for FAP's recognition of dipeptide substrates. Neither does it seem to be involved in a significant interaction with the other DPPs' homologous GluGlu motifs, as reflected by the assay results for these enzymes. On the basis of the obtained results with the set of inhibitors **16**–**23**, we did not prepare any additional analogues in this series. Fitting in the same framework of metabolic P2–P3 amide stabilization, molecules (**24**–**27**) were then prepared in which this amide group is replaced by an isosteric, 4-substituted 1,2,3-triazole ring. The 4-alkynyl derivatized quinolines required to obtain triazole analogues of **4** were, however, found not to be readily accessible. This led us to prepare analogues of older FAP inhibitors that we reported: N-acylated glycyl(2-cyanopyrrolidines) with a P3-benzoyl or naphthoyl substituent. The compounds obtained were again

Table 2. IC₅₀ Data of Inhibitors with a Modified P2 Residue

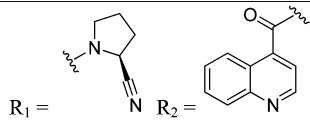
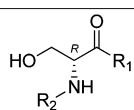
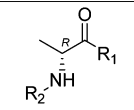
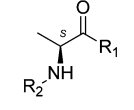
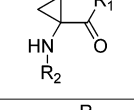
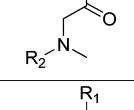
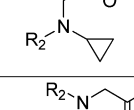
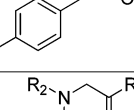
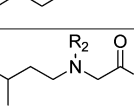
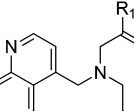
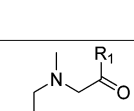
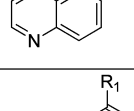
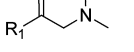
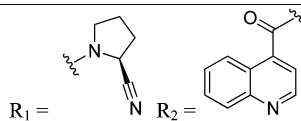
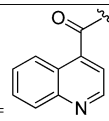
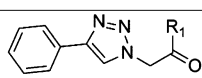
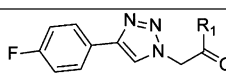
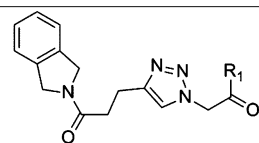
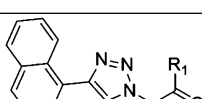
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Nr	Structure	IC ₅₀ (μM)					SI (FAP/PREP) ^a
		FAP	PREP	DPPIV	DPP9	DPPII	
11		6 ± 0.2	>100	>100	>100	>100	16.7
12		3.4 ± 0.1	5.8 ± 0.6	>100	>100	>100	1.7
13		>100	0.044 ± 0.004	>100	>100	>100	< 0.005
14		>100	46 ± 2	>100	>100	>100	0.46
16		37 ± 2	25 ± 2	>100	>25	>100	0.7
17		>100	2.6 ± 0.3	>100	>100	>100	0.03
18		>12.5	9 ± 2	>100	>100	>100	0.7
19		>100	6.3 ± 0.2	>100	>100	>100	< 0.06
20		>100	2 ± 0.2	>100	>100	>100	< 0.02
21		15.5 ± 0.7	>50	>100	>100	>100	> 3.5
22		28.1 ± 0.9	>25	>100	>100	>100	> 0.8
23		28 ± 1	67 ± 6	>100	>100	>100	2.4

Table 2. continued

		<div></div> $R_1 =$  $R_2 =$ 					
Nr	Structure	IC ₅₀ (μM)					SI (FAP/PREP) ^a
		FAP	PREP	DPPIV	DPP9	DPPII	
24		28.0 ± 1.1	>100	>100	>10	>100	> 3.5
25		19.3 ± 1.2	>100	>100	>100	>100	> 5
26		>100	>100	>100	>100	>100	-
27		>12.5	9 ± 1	>100	>100	>100	<0.7

^aSI stands for “selectivity index” (calculated as [IC₅₀(PREP)/IC₅₀(FAP)]).

found to have reduced inhibitory affinity, with phenyl analogue **24** possessing around 8-fold less potency compared to the benzoyl amide. Naphthyl-containing **27** is characterized by an even higher affinity loss compared to its 1-naphthoyl substituted parent compound that possesses FAP submicromolar potency. Not satisfied by these results, we concluded that the 4-substituted-1,2,3-triazole is not a good amide isostere at the glycine P2 position.

Investigations of the P3 region of inhibitors related to **4** was aimed at replacing its quinoline ring with various other azaheterocycles. Our earlier studies, during which we had already investigated quinoline and isoquinoline isomers, clearly demonstrated the position of the azaheteroaromatic nitrogen to be crucial, with appreciable FAP affinity only present in case of the 4-quinolinoyl isomer of compound **4**. In all compounds reported here, we therefore limited the selection of heterocycles to systems that have a azaheteroatom topology for at least one nitrogen that is comparable to that of the 4-quinolinoyl ring nitrogen. Table 3 summarizes the data obtained for a number of (substituted) five- and six-membered heteroaromatic containing analogues of **4**. The closely related pyridine **29** was found to have a 6-fold reduced FAP potency compared to **4**. In spite of its lower absolute affinity, **29** has the highest ligand efficiency (0.38) of all compounds we hitherto prepared. Introduction of substituents in 2-position of the pyridine ring as in compounds **30–35** resulted in a further decrease of inhibitory potency, but no additional steric or electronic determinants of FAP potency could be discerned here: all compounds from this subset seem to have inhibitory potential within the same order of magnitude. Additionally, an analogue containing an extra methylene linker (**36**) and a reduced P3-heteroaromatic ring system (piperidine **37**) were prepared. Similarly, these molecules did not demonstrate better FAP affinities than **29** and also convey the hypothesis that high FAP-affinity-conferring P3 substituents are mainly present in limited parts of heteroaromatic chemical space.

Identification of these other potentially interesting parts of chemical space was then attempted with compounds **38–44**. These contain heteroaromatic P3 substituents that are more distant from the pyridine- or quinoline-based systems that we so far discovered to be optimal. Pyridazine (in inhibitor **38**) was the only azine with more than one nitrogen heteroatom that we evaluated. Already, this compound had a 3-fold decreased FAP potency relative to **29**. This finding withheld us from investing effort in preparing target molecules with a triazine isomer at P3. Conversely, we shifted attention to more electron-rich, five-membered azaheterocycles. Pyrrole was not selected for this subset because of stability reasons, but a suitable (vide supra) imidazole derivative and two triazole isomers were included (compounds **39–41**). These molecules, together with the homologated **42** and the thia- and oxazole derivatives **43** and **44**, all performed significantly less than **29** in the FAP inhibition assay. Finally, mutation of the pyridine ring into an aniline was found not to be beneficial for FAP affinity either: the aniline **45** and its trifluoroacetylated precursor **46** have IC₅₀ values in the low micromolar range, more than 100-fold less than pyridine **29**. Taken altogether, the data in Table 3 were not compelling enough to immediately continue further structural exploration of monocyclic heteroaromatic P3 rings: FAP potencies observed in this series were not able to compete with reference **4**. In the case of the highly efficiently binding inhibitor **29**, further optimization did not seem obvious. In addition, the FAP/PREP selectivity indices observed, so far not mentioned in the framework of this series, were generally very satisfactory for the pyridine subset but again not significantly better than the optimal quinolines described earlier. It deserves highlighting however that as reported by Poplawski et al., introduction of a P3–P4-pyridinoyl substituent has been used to obtain a series of highly potent, boronate-based FAP inhibitors represented by reference compound **6**.²⁶

For the reasons cited above, we then turned attention to bicyclic P3 heteroaromatics with at least one nitrogen atom that

Table 3. IC₅₀ Data of Five- and Six-Membered P3 Heterocycles

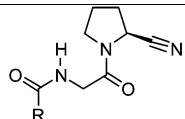
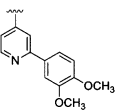
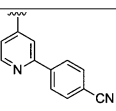
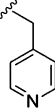
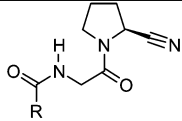
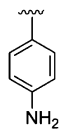
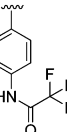
							
Nr	R =	IC ₅₀ (μM)					SI (FAP/PREP) ^a
		FAP	PREP	DPPIV	DPP9	DPPII	
29		0.063± 0.003	11.3 ± 1.2	>100	>100	>100	179.4
30		0.76 ± 0.04	6.8 ± 0.4	>100	>100	>100	8.9
31		0.126± 0.009	7.3 ± 0.5	>100	>100	>100	57.9
32		0.138± 0.006	33.7 ± 1.6	>100	>100	>100	244.2
33		0.29 ± 0.02	14.2 ± 1.0	>100	>25	>100	49
34		0.22 ± 0.02	11.8 ± 0.6	>100	>100	>100	53.6
35		0.27 ± 0.02	29.1 ± 2.4	>100	>100	>100	107.7
36		3.3 ± 0.1	2.0 ± 0.2	>100	>100	>100	0.6
37		0.75 ± 0.07	5.9 ± 0.2	>100	>100	>100	7.9
38		0.164± 0.003	39 ± 2	>100	>100	>100	237.8
39		1.37 ± 0.04	6.6 ± 0.3	>100	>100	>100	4.8
40		5.7 ± 0.2	>100	>100	>100	>100	> 17.5
41		22.4 ± 1.1	>100	>100	>100	>100	> 4.5

Table 3. continued

							
Nr	R =	IC ₅₀ (μM)					SI (FAP/PREP) ^a
		FAP	PREP	DPPIV	DPP9	DPPII	
42		7.2 ± 0.3	22 ± 2	>100	>100	>100	3.1
43		2.5 ± 0.1	7.2 ± 0.5	>100	>100	>100	2.9
44		10.2 ± 0.5	13.7 ± 1.2	>100	>100	>100	1.3
45		10.3 ± 0.8	48 ± 4	>100	>100	>100	4.7
46		7.1 ± 0.3	5.6 ± 0.5	>100	>100	>100	0.8

^aSI stands for “selectivity index” (calculated as [IC₅₀(PREP)/IC₅₀(FAP)]).

is equivalent to the azaheteroatom in quinoline **4** (Table 4). With the set of inhibitors **47–51**, 2,3-annulated pyridines were evaluated. Pyrrolopyridine **47** was found to be a nanomolar FAP inhibitor but still almost 5-fold less potent than **4**. Selectivity indices with respect to PREP were largely comparable for both (SI_[FAP/PREP] values were calculated to be ~50 and 80 for **47** and **4**, respectively). Substituted imidazopyridines **48** and **49** and certainly triazolopyridine **50** were significantly less potent as FAP inhibitors than **4**, suggesting that introduction of additional nitrogen atoms in the annulated five-membered heterocycle leads to lower target affinities. A similar effect seems to be in place when a six-membered heterocycle is annulated with pyridine (1,7-naphthyridine **51**). The IC₅₀ value of this azaquinoline derivative is roughly 3 times higher than that of quinoline **4**; nonetheless, selectivity toward PREP is slightly better than that of the reference.

Since a P3-quinoline residue out of all the heterocycles investigated still proved to be optimal, we decided to return to this scaffold and investigate the effect of hitherto unexplored substitution patterns at its 5 and 7 positions. Our preliminary data had already indicated that introduction of 5- or 7-chloro and bromo substituents resulted in low nanomolar FAP inhibitors with optimal selectivity toward PREP (SI_[FAP/PREP] of up to 10³). Similar FAP potencies were obtained here with the methyl-substituted analogues **52** and **53**, of which the 5-substituted congener clearly had the best FAP/PREP selectivity seen so far (SI_[FAP/PREP] = 2 × 10³). Introduction of bulkier

phenyl-based groups (compounds **54–56**) seems less desirable at both the 5 and 7 positions: FAP potencies drop close to 1 order of magnitude. Because of the relatively small size of the methoxy substituent, one would furthermore expect the 5-methoxylated inhibitor **57** to have potencies in the same range as **5** or **53**. Surprisingly however, potency was reduced more than 1000-fold, again indicating that the supposed specific interaction of the quinolinoyl system with FAP's active center can be critically disturbed by small structural changes. Additional investigation of this finding was undertaken during the optimization of the P1 position (vide infra, Table 5).

As part of an earlier study, we had shown that in the glycyl(2-cyanopyrrolidine) series, FAP's S1 pocket preferentially accommodates 2-cyanopyrrolidine and a limited number of derivatives with 4-substituents of minimal steric bulk, e.g., a 4S-fluoride. Similarly, (2S,4S)-2-cyano-4-fluoropyrrolidine and its 4,4-difluorinated analogue were also used earlier as P1 residues by Jiaang in FAP inhibitors related to **6**. Depending on the target, these residues have also been investigated with considerable success in compounds targeting DPPIV and DPP8/9. The same strategy was applied to the inhibitor series studied here.²⁷ Complementarily, we also prepared the (2S,4R)-2-cyano-4-fluoropyridine diastereomer and introduced it as a P1 residue. Supposedly relating to its more elaborate synthesis involving a chirality inversion step on the 4-position of starting material *trans*-4-hydroxyproline, this isomer so far had not been investigated in FAP or related DPP inhibitors. To allow efficient assessment of the effect of introducing each of

Table 4. IC₅₀ Data of Bicyclic P3 Heterocycles

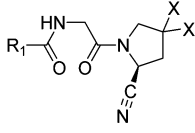
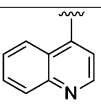
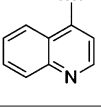
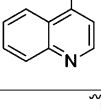
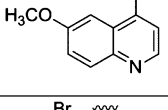
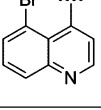
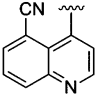
Nr.	R	IC ₅₀ (μM)					SI (FAP/PREP) ^a
		FAP	PREP	DPPIV	DPP9	DPPII	
47		0.040 ± 0.002	2.7 ± 0.2	>100	>100	>100	67.5
48		<0.2 ^b	>100	>100	>100	>100	> 200
49		<0.2	>100	>100	>100	>100	> 200
50		3.2 ± 0.1	24 ± 1	>100	>100	>100	7.5
51		0.028 ± 0.001	3.4 ± 0.2	>100	>100	>100	121.4
52		0.0069 ± 0.0003	0.50 ± 0.03	>100	>100	>100	72.5
53		0.0043 ± 0.0001	9.1 ± 0.6	>100	>50	>100	2116.3
54		0.070 ± 0.009	19.4 ± 0.7	>100	>50	>100	277.1
55		0.059 ± 0.008	2.03 ± 0.04	>100	20.8 ± 1.8	>100	34.4
56		0.064 ± 0.002	8.7 ± 0.5	>100	>50	>100	135.9
57		16.6 ± 0.6	1.28 ± 0.06	>100	>100	>100	0.08

^aSI stands for “selectivity index” (calculated as [IC₅₀(PREP)/IC₅₀(FAP)]). ^bSolubility problems upon dilution of compounds in aqueous buffer, resulting in poor reducibility of inhibition curves.

the three mono- and difluorinated P1 isomers, direct analogues of reference compound **4** were prepared (**58–60**). Grossly in line with expectations, the 4S-fluoropyrrolidine **58** displayed around 3-fold higher FAP affinity than the parent compound. PREP-affinity however had also increased slightly more than

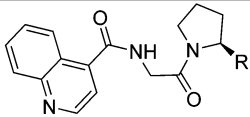
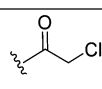
proportionally with FAP-potency. This is consistent with our earlier mentioned report that included 4S-fluorinated (2-cyano)pyrrolidine inhibitors.²⁷ Most remarkably, the 4R-fluoropyrrolidine isomer **59** has lost FAP affinity compared to **4** and **58**, amounting to up to 3 orders of magnitude. Making

Table 5. IC₅₀ Data of Inhibitors with a Modified P1 Residue

								
Nr	R ₁	X	IC ₅₀ (μM)					SI (FAP/PPREP)
			FAP	PPREP	DPPIV	DPP9	DPP2	
58		H,F (S)	0.0033± 0.0002	0.29 ± 0.01	≥100	>50	>100	87.8
59		H,F (R)	1.0 ± 0.1	>100	>100	>100	>100	>100
60		F,F	0.0032± 0.0004	>1.8 ± 0.2	>100	>12.5	>100	562.5
61		F,F	0.0085± 0.0009	8.3 ± 0.7	19±1.3	27.2±0.8	>100	976.4
62		F,F	9.0 ± 0.5	9.3 ± 0.4	>100	≥25	>50	1.0
63		F,F	7.2 ± 0.3	16.8 ± 0.7	>100	>25	>50	2.3

^aSI stands for “selectivity index” (calculated as [IC₅₀(PPREP)/IC₅₀(FAP)]).

Table 6. Influence of the Warhead on IC₅₀

							
Nr	R	IC ₅₀ (μM)					SI (FAP/PREP) ^a
		FAP	PREP	DPPIV	DPP9	DPP2	
64	-H	5.1 ± 0.2	>50	>100	>100	>100	>10
66	-B(OH) ₂	0.0037 ± 0.0002	0.0112 ± 0.0004	>50	15.8 ± 0.8	>100	3.0
67		0.177 ± 0.005	0.78 ± 0.003	>100	>100	>100	4.4

^aSI stands for “selectivity index” (calculated as [IC₅₀(PPREP)/IC₅₀(FAP)]).

steric factors less likely to be accountable for this effect, the corresponding 4,4-difluorinated analogue of **4** (UAMC-1110, vide infra) was one of the most potent FAP inhibitors identified to date. Seminal studies by Raines et al. have shown that 4S-fluoroproline derivatives possess a hyperconjugatively stabilized endo-puckered ring conformation, while in 4R-fluoroprolines an exo-puckered ring conformer is more favored. This difference, which should also be present with 2-cyanopyrroli-

dine derivatives, could be contributing to the large potency differences observed between **58** and **59**.³⁵ Analogously, 4,4-difluoroproline derivatives have been demonstrated to have endo- and exo-puckered conformations of comparable energy, similar to unsubstituted prolines.³⁶ Although this would imply that the slightly higher FAP affinity of **60** compared to **4** cannot be rationalized using conformational arguments, increased hydrophobicity resulting from difluorination might be in play

Table 7. In Vitro Pharmacokinetic Properties of Selected FAP Inhibitors^a

parameter	4	5	68	60	61
kinetic solubility (μM)	>200	>200	>200	>200	>200
log <i>D</i>	0.5	0.7	0.8	1	nd
plasma stability (% unchanged at 6 h) (mouse/rat/human)	100/90/nd	90/nd/100	nd/100/80	85/95/nd	nd
microsomal stability (% unchanged at 6 h) (mouse/rat)	nd	75/nd	90/nd	90/94/nd	nd
cytotoxicity (MRC-5 cells) (μM)	>64	>64	>64	>64	>64

^and stands for “not determined”.

Table 8. In Vivo PK Properties of Selected FAP Inhibitors in Rats

	<i>T</i> _{max} (h)	<i>C</i> _{max} ($\mu\text{g/mL}$)	<i>T</i> _{1/2} (h)	AUC ($\mu\text{g}\cdot\text{h/mL}$)	Cl (mL/min)	<i>V</i> _d (L)	relative bioavailability (%)
5 (iv) ^a		6.5	1.73	6.1	11.7	1.75	52
5 (po) ^b	0.33	5.6	1.7	13.4	23.0	3.5	
60 (iv) ^a		11.8	1.74	23.4	2.83	0.43	74
60 (po) ^b	0.33	14.6	3.4	76.7	1.55	0.34	
61 (iv) ^a		8.5	1.40	11.1	6.5	0.77	79
61 (po) ^b	0.75	14.7	1.22	39.39	8.2	0.86	

^aCompound was formulated in PEG₂₀₀ and administered via single intravenous injection at 5 mg/kg. ^bCompound was formulated in PEG₂₀₀ and administered per os (gavage) at 20 mg/kg.

here. Noteworthy, **60** was also found to have better FAP/PREP selectivity and a very proficient ligand efficiency of 0.34, which is significantly higher than the corresponding value calculated for **4** (0.27).

To further expand the P1-4,4-difluorinated subset, compounds **61**–**63** were synthesized. These differ by their P3-quinoline residues' substitution patterns. The 6-methoxyquinoline **61** is the difluorinated analogue of an inhibitor that we reported in our foregoing study.²³ Both the low nanomolar FAP potency and thousandfold FAP/PREP selectivity of these two molecules are highly comparable. Compounds **62** and **63** were specifically included to extract additional information on the SAR of the P3-quinoline's 5-position (vide supra). Surprisingly, **62** and **63** were found to be micromolar FAP inhibitors. Although the 5-methoxylated compound **57** (Table 5) had a similar profile, drawing the general conclusion that introduction of substituents at the 5-position reduces FAP affinity, would oversee reference molecule **5**. The latter all but compares to its difluorinated counterpart **62**, but together with its 5-chlorinated analogue that we also published earlier, it belongs to the set of most potent and selective molecules discovered.²⁹ More subtle hypotheses related to the compound's binding kinetics might therefore be required to tentatively explain the observed behavior. It is clear that invoking only the steric or electronic parameters of the quinolone substituents is not sufficient to rationalize the assay data of all 5-substituted molecules we prepared.

Finally, we investigated how either omitting or changing the warhead function to a boronic acid would impact FAP potency and selectivity of our molecules. We also selected the well-known chloromethyl ketone warhead to verify whether modification to irreversible inhibitors could be possible. All molecules prepared were analogues of reference **4** (Table 6). First, building in an unsubstituted pyrrolidine residue at P1 in **64** was found to give a 3 log reduction in potency compared to reference compound **4**. Qualitatively, this finding is in line with earlier reports by our own group and by Jiaang et al. and underscores the very considerable contribution to inhibitor affinity from the warhead function. Compared to the mentioned examples though, the residual micromolar affinity of **64** still is significant and one of the best potencies reported

for FAP inhibitors without a warhead group. Next, the boronic acid warhead, present in many of the older series of (mostly nonselective) FAP inhibitors, was evaluated in **66**. This molecule was found to be highly potent but also considerably less selective toward PREP when compared to its nitrile-based counterpart **4** ((*SI*_[FAP/Prep] of 3 vs ~85, respectively). Evaluation data of Bachovchin et al.'s most promising boronate **6** (that under our conditions had *SI*_[FAP/Prep] = 40) together with the PREP affinities of our own inhibitors **12** and **13** nonetheless suggest that this selectivity could be improved by selecting a D-Ala P2 group. Finally, the enzymatic assay data for chloromethyl ketone **67** show this compound to be 17 times less potent than nitrile **4**. Detailed kinetic analysis also revealed this molecule not to bind irreversibly to the target enzyme.

Taking into account the number of low nanomolar FAP inhibitors with high FAP/PREP selectivity that we had discovered as part of this and the foregoing study, four of the most promising compounds were then selected for which in vitro pharmacokinetic parameters were determined (log *D*_{7.4}, kinetic solubility, stabilities in mouse, rat, and/or human plasma) (Table 7). Compound **4**, for which we reported these parameters earlier, was used as a reference. In general, no large differences between individual inhibitors were observed for the evaluated parameters. Compounds with a P1-(2-cyano-4,4-difluoropyrrolidine) residue were found to behave similarly to the nonfluorinated molecules in the series (**4**, **5**, and **46** vs **60** and **61**). The log *D*_{7.4} values determined varied between 0.5 and 1. Kinetic solubility in all cases was >200 μM , and plasma stabilities were satisfactory throughout the assay results with half-lives exceeding 6 h for all inhibitors and in all media tested. For compounds **5**, **60**, and **61**, stability in the presence of mouse and/or rat hepatic microsomes was also tested. Gratifyingly, these molecules were found not to be subject of fast oxidative metabolism and, again, displayed half-lives of more than 6 h. Finally, cytotoxicity was evaluated on MRC-5 cells. At the highest concentration measured (64 μM), no signs of cellular toxicity could be observed for the compounds in Table 7. Additional cytotoxicity data for other compounds in this report can be retrieved from the Supporting Information. These are comparable with the data shown here, although

compound **57** did show slight toxicity at concentrations greater than 24 μ M.

The *in vivo* PK parameters were then determined for inhibitors **4**, **5**, **60**, and **61** in rats. Six male rats were treated for each inhibitor tested, three of which received the compound via a single intravenous (iv) administration at 5 mg/kg. The other three animals were dosed per os (po) at 20 mg/kg. Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h after administration. Inhibitor concentrations were determined using UPLC–MS/MS, and pharmacokinetic parameters were calculated using standard algorithms. Notably, all rats subjected to **4** were found to die within 6 h. No signs of toxicity were observed during the observation period or upon autopsy in any of the other animals treated with compounds **5**, **60**, and **61**, and parameters for these molecules are summarized in Table 8. Taking into account the high degree of structural similarity between the four compounds evaluated, we do not have a clear view on possible factors that could explain the singular toxicity to rats of **4**.

All inhibitors evaluated displayed significant, roughly comparable oral bioavailabilities (50–79%) and reasonable elimination half-lives (1.5–3 h). These data were complemented with experiments in which we followed up the extent of plasma FAP inhibition in the inhibitor-treated rats. A soluble form of the enzyme is present in plasma, where it was originally characterized as α_2 -antiplasmin cleaving enzyme (APCE).²⁵ In brief, residual FAP activity was determined in the same plasma samples that were collected for the determination of inhibitor concentrations after iv and po administration. FAP activity was measured using the commercially available substrate Z-Gly-Pro-AMC under conditions that excluded interference from soluble PREP activity.³⁷ It deserves mentioning that for the *ex vivo* assay plasma samples are diluted 100-fold, potentially leading to underestimation of the amount of FAP that is blocked *in vivo*. At each of the investigated time points (5 min to 24 h) after iv or oral administration of compound **60**, the residual FAP activity measured *ex vivo* was less than 15% when compared with vehicle controls. Also after oral administration of compounds **5** and **61**, more than 85% inhibition of FAP activity was observed for all time points. Whereas at the 4 h time point after iv administration of the latter two compounds, the measured % inhibition still reached more than 75%. The FAP activity measured at the 24 h time point was around 50% compared to vehicle controls. All *ex vivo* FAP measurements point to a complete or near complete and prolonged inhibition of plasma FAP activity *in vivo* after single doses of compounds **5**, **60**, and **61**. The *in vivo* observations corroborate the *in vivo* pK parameters $C_{\max}$ and $T_{1/2}$ and the *in vitro* stability testing. To check whether slow, tight binding might be contributing to maintaining long-term FAP inhibition, a kinetic and mechanistic investigation was performed for compound **60**, as this compound resulted in the most extensive and prolonged inhibition of FAP in the PK studies. No tight binding behavior was observed, and the inhibitor proved to bind reversibly to FAP. We therefore conclude that the longer *in vivo* half-life of **60** (compared to **5** and **61**) is the main contributor to the prolonged *ex vivo* inhibition of FAP activity observed for this compound. On the basis of the high degree of structural similarity between **5**, **60**, and **61**, we assume comparable kinetic behavior for these molecules. A more extended *in vivo* study using several, especially lower doses of these compounds is needed to confirm this hypothesis.

CONCLUSION

In this study we have built a thorough structure–activity relationship around the 4-quinolinoyl-Gly-cyanoPro scaffold. First, replacing the P2 glycine residue with a limited set of other amino acids was found to decrease FAP potency significantly for each alternative residue evaluated. Adding substituents to the P2–P3 amide bond or changing it with a triazole peptide bond isostere abolished almost all FAP affinity. Second, an extended series of azaheterocycles was evaluated at the P3 position in order to identify alternatives for the quinoline group. Of all moieties evaluated, only pyridines resulted in inhibitors with appreciable FAP affinity, albeit in absolute terms still less potent than their quinoline-bearing analogues. Furthermore, we extended our investigation of the quinoline ring's substitution pattern. We confirmed with more potent inhibitors our earlier preliminary observation that substituents at the 5-position of the quinoline moiety can improve selectivity toward PREP while maintaining FAP affinity. Nonetheless, notable outliers of this hypothesis were identified as well (*vide infra*). Third, the P1 position was investigated. We found that either 4S-fluoro or 4,4-difluoro substitution of the 2-cyanopyrrolidine ring can be used to optimize FAP affinity and selectivity with respect to PREP. Introduction of a 4R-fluoro substituent on the other hand was found to give rise to far less potent compounds, a feature that we tentatively rationalized using stereoelectronic arguments. Furthermore, we found that the combined presence of a 5-substituted quinolinoyl residue in P3 and a 2-cyano-4,4-difluoropyrrolidine in P1 unexpectedly leads to drastic FAP affinity loss. Although we do not have a clear explanation, this finding suggests that FAP imposes strict structural requirements on its inhibitors that seem related to the possibility of covalent bond formation between the enzyme and the nitrile warhead present in these molecules. Investigating this feature in depth, we prepared compounds in which the nitrile warhead was either omitted or replaced by a boronate or chloromethyl ketone. The presence of a warhead in the studied series of molecules was shown to be indispensable for high affinity, and a nitrile group was found to be preferable above the boronate function, mainly in terms of inhibitor selectivity. The chloromethyl ketone function, although of potential interest for the synthesis of activity-based probes for FAP, did not lead to irreversible inhibition or to high target affinity, again suggesting defected interaction with the enzyme's catalytic serine.

A selection was made among the most promising FAP inhibitors discovered so far in the studied series. *In vitro* PK and toxicity parameters were determined for these, generally predicting potential for satisfactory *in vivo* behavior. Four of these compounds were then submitted to *in vivo* PK analysis in rats. The corresponding data show that all four compounds tested had good oral bioavailability and half-lives. However, some questions remain with respect to compound **4**. We were surprised to see that all rats treated with this compound died within 6 h after compound administration, while the rats treated with the other, structurally highly similar inhibitors did not show signs of toxicity under an identical dosing regimen. Related to this, we do not have indications of a likely off-target of **4**. It is also highly speculative to suggest that the toxicity mechanism of **4** could be related to that of *allo*-Ile-isoindoline, a DPP8/9 inhibitor for which a putative off-target effect has been invoked to rationalize *in vivo* toxicity in rats and dogs.³⁸

Summarizing, we have identified a series of highly potent FAP inhibitors with promising pharmacokinetic behavior. We believe that our selective, *in vivo* active inhibitors are currently best placed among all published compounds to help elucidate the function of FAP in different animal models of disease and to allow its continuing validation as a drug target.

■ EXPERIMENTAL SECTION

Unless otherwise stated, laboratory reagent grade solvents were used. Reagents were obtained from Sigma-Aldrich, Acros Organics, Apollo Scientific, Manchester Organics, or Fluorochem and were used without further purification unless otherwise indicated. Characterization of all compounds was done with ^1H NMR and mass spectrometry. ^1H NMR spectra were recorded on a 400 MHz Bruker Avance III Nanobay spectrometer with Ultrashield. Chemical shifts are in ppm, and coupling constants are in hertz (Hz). Minor rotamers of the amide bond, which were less than 10% of the major rotamer, are not reported in the NMR data. ES mass spectra were obtained from an Esquire 3000plus ion trap mass spectrometer from Bruker Daltonics. Purity was verified using two different LC/MS systems, and purities of all final products were found to be >95%. Water (A) and MeCN (B) were used as eluents. LC–MS spectra were recorded on an Agilent 1100 series HPLC system using a Alltech Prevail C18 column (2.1 mm $\times$ 50 mm, 3 μm) coupled with an Esquire 3000plus as MS detector, and a “method A” 5–100% B, 20 min gradient was used with a flow rate of 0.2 mL/min. Formic acid, 0.1%, was added to solvents A and B. UPLC involved the following: Waters Acquity H-class UPLC system coupled to a Waters TQD ESI mass spectrometer and Waters TUV detector. A Waters Acquity UPLC BEH C18 1.7 μm , 2.1 mm $\times$ 50 mm column was used. Solvent A consisted of water with 0.1% formic acid. Solvent B consisted of acetonitrile with 0.1% formic acid. Method I involved the following: 0.15 min 95% A, 5% B, then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25 min (0.350 mL/min), 95% B, 5% A. The wavelength for UV detection was 254 nm. Method II involved the following: flow 0.4 mL/min, 0.25 min 95% A, 5% B, then in 4.75 min to 95% B, 5% A, then 0.25 min 95% B, 5% A, followed by 0.75 min 95% A, 5% B. The wavelength for UV detection was 214 nm. Where necessary, flash purification was performed on a Biotage ISOLERA One flash system equipped with an internal variable dual-wavelength diode array detector (200–400 nm). For normal phase purifications SNAP cartridges (10–340 g, flow rate of 10–100 mL/min) were used, and reversed phase purifications were done making use of KP-C18 containing cartridges. Dry sample loading was done by self-packing samplet cartridges using silica and Celite 545, respectively, for normal and reversed phase purifications. Gradients used varied for each purification. However, typical gradients used for normal phase were 30 min gradient of 0–50% EtOAc in hexane to 100% hexane or 0–5% methanol in DCM to 20% methanol in DCM. For reversed phase a gradient of 5% MeCN in water to 50% MeCN in water was used. HRMS involved the following: The dry samples were dissolved in 1 mL of methanol and diluted $1/100$ in MeCN/ H_2O , 0.1% formic acid. Then 10 μL of each sample was injected using the CapLC system (Waters, Manchester, U.K.) and electrosprayed through the Nanomate (Advion, Ithaca, NY) nanoelectrospray source. The Nanomate was operated in positive ion mode at an electrospray potential of 1.5 kV. Samples were injected with an interval of 3 min. Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, U.K.). The mass spectrometer was calibrated prior to use with a 0.2% H_3PO_4 solution. The spectra were lock-mass-corrected using the known mass of the nearest H_3PO_4 cluster or the phthalate background ions. The Waters Acquity UPLC system coupled to a Waters TQD ESI mass spectrometer was also used for LC/MS/MS measurements. Enzymes used in the IC_{50} measurements were purified from human or animal sources (human seminal plasma for DPPIV and DPPII, bovine testes for DPP9) or were isolated from recombinant sources (recombinant murine FAP from human HEK293 kidney cells; recombinant human PREP from *E. coli*). Substrates used in the IC_{50} measurements were Ala-Pro-*p*-nitroanilide (2 mM) at pH 7.4 for FAP assays and Z-Gly-Pro-*p*-nitroanilide (0.25 mM) at pH 7.5

for PREP assays. DPPIV assays used Gly-Pro-*p*-nitroanilide (100 μM) at pH 8.3. DPPII assays were carried out using Lys-Ala-*p*-nitroanilide (1 mM) at pH 5.5, and DPP9 assays used Ala-Pro-*p*-nitroanilide (300 μM) at pH 7.4. The substrate concentrations were chosen around the K_m value obtained under the assay conditions used. Mouse and rat plasma came from Innovative Research. The turbidity in the kinetic solubility experiments was measured using the UV/vis spectrophotometer Synergy MX, Biotek with Gen5. MRC-SSV2 cells (human embryonic diploid fibroblasts) were obtained from Sigma and cultured in MEM + Earl's salts medium, supplemented with L-glutamine, NaHCO_3 , and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 $^\circ\text{C}$ under an atmosphere of 5% CO_2 . Male Wistar rats (body weight of ~ 250 g) were acquired from Janvier (France).

The following section comprises the synthetic procedures and analytical data for all final compounds reported in this manuscript. The corresponding data for synthesis intermediates en route to final compounds can be found in the Supporting Information. Synthesis procedures that were used in the preparation of several final products are summarized here as “General Procedures”.

General Procedure A (Formation of the P2–P3 Amide Bond). The carboxylic acid (0.065 g, 0.377 mmol) was dissolved in 3 mL of DMF, and *N*-ethyl-*N*-isopropylpropan-2-amine (0.210 mL, 1.168 mmol) and HATU (0.121 g, 0.377 mmol) were added. After 15 min a solution of (S)-1-(2-aminoacetyl)-pyrrolidine-2-carbonitrile trifluoroacetate or tosylate (0.085 g, 0.377 mmol) in DMF was added. The mixture was stirred for 3–4 h at room temperature. The volatiles were evaporated. The residue was dissolved in ethyl acetate and washed with 1 N citric acid, saturated sodium bicarbonate, and brine. The solution was dried over sodium sulfate, filtered, and evaporated. It was purified using column chromatography using DCM–methanol.

General Procedure B (Deprotection of Boc Groups). Tosylic acid (1.4 equiv) was added to a cold (0 $^\circ\text{C}$) solution of the Boc-protected product (1 equiv) in acetonitrile (0.4 M) at 0 $^\circ\text{C}$. After 30 min the mixture was allowed to warm to room temperature and stirred for 24 h. The volatiles were evaporated and the mixture was washed with cold ethyl acetate to remove *N*-*tert*-butylacetamide. The white precipitate formed is the product.

General Procedure C (Formation of the P2–P3 Amide Bond of Compounds with a P3-(4-Quinoloyl) Residue). Quinoline-4-carbonyl chloride hydrochloride (0.102 g, 0.448 mmol) was added to a solution of the amine (0.448 mmol) and DIPEA (0.217 mL, 1.242 mmol) in DCM and stirred until completion as followed with LC–MS (usually 2–3 h). On completion of the reaction, it was diluted with DCM–isopropanol (4:1), washed with saturated aqueous NaHCO_3 solution (10 mL), 0.1 N aqueous citric acid solution (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated and purified with flash chromatography.

General Procedure D (Preparation of Final Products with an *N*-Alkylated P2–P3 Amide Bond). (S)-1-(2-Chloroacetyl)-pyrrolidine-2-carbonitrile 15 (0.400 g, 2.317 mmol, 1 equiv) in THF (0.5M) was added dropwise to a 0.3 M ice–water cooled mixture of primary amine and K_2CO_3 (0.961 g, 6.95 mmol, 3 equiv) in THF. The mixture was stirred for 2 h at 0 $^\circ\text{C}$, then another 3 days at room temperature. After filtration of K_2CO_3 and evaporation of volatiles, the concentrate was dissolved in 30 mL of DCM and washed with saturated sodium bicarbonate (3 $\times$), brine, dried over Na_2SO_4 , filtered, and concentrated to give the secondary amine as an oily substance. If necessary the product was purified using silica gel column chromatography with 0.1 N ammonia as an additive. Quinoline-4-carbonyl chloride hydrochloride (0.102 g, 0.448 mmol) was added to a solution of the secondary amine and DIPEA (0.217 mL, 1.242 mmol) in DCM and stirred until completion as followed with LC–MS (usually 2–3 h). On completion of the reaction, it was diluted with DCM–isopropanol (4:1) and washed with saturated aqueous NaHCO_3 solution (10 mL), 0.1 N aqueous citric acid solution (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated and purified with flash chromatography using a DCM–methanol gradient and/or preparative HPLC.

General Procedure E (Preparation of Final Products with a P2–P3 Triazole Isostere). Sodium azide (0.863 g, 13.27 mmol) was added to a solution of (S)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **15** (1.27 g, 7.4 mmol) in DMF (25 mL) and stirred for 24 h. After evaporation of the volatiles, the residue was dissolved in ethyl acetate and washed with water and brine. Purification was done using column chromatography with a hexane–ethyl acetate gradient (20–35%) to give (1.1 g, 83%) white crystals of (S)-1-(2-azidoacetyl)pyrrolidine-2-carbonitrile. ¹H NMR (400 MHz, CDCl₃) (4:1 mixture of trans–cis rotamers) δ 4.80 (d, J = 5.8 Hz, 0.8H), 4.63 (dd, J = 12.8, 6.4 Hz, 0.2H), 3.93 (d, J = 14.5 Hz, 1H), 3.86 (d, J = 16.0 Hz, 1H), 3.64–3.54 (m, 1H), 3.47–3.38 (m, 1H), 2.38–2.02 (m, 4H); MS (ESI) *m/z* 180.2 [M + H]⁺. The alkyne (0.096 mL, 0.837 mmol) was added to a mixture of (S)-1-(2-azidoacetyl)pyrrolidine-2-carbonitrile (0.075 g, 0.419 mmol) and copper(I) iodide (7.97 mg, 0.042 mmol) in THF (volume, 4 mL) with one drop of triethylamine. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then filtered, the filtrate evaporated and the residue chromatographed, yielding the title compound as a slightly yellowish transparent colorless oil.

General Procedure F (Formation of the P2–P3 Amide Bond). The carboxylic acid (0.092 g, 0.814 mmol) was dispersed in dry dioxane (3 mL) in a round-bottom flask with nitrogen. To this 1-chloro-*N,N,N*,2-trimethylprop-1-en-1-amine (0.151 mL, 1.139 mmol) was added, and the mixture was stirred for 30 min at room temperature. The starting material dissolved over time. Then (S)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile hydrochloride (0.247 g, 1.302 mmol) with *N*-ethyl-*N*-isopropylpropan-2-amine (0.307 mL, 1.709 mmol) was added, and the mixture was stirred for 2 h, evaporated to dryness, and redissolved in ethyl acetate. This was followed by washing with 0.1 N citric acid and saturated sodium bicarbonate and brine. After drying over sodium sulfate, filtration, and evaporation, the product was purified using column chromatography.

General Procedure G (Formation of the P2–P3 Amide Bond). To a solution containing the carboxylic acid (0.058 g, 0.333 mmol) and hydroxybenzotriazole (0.056 g, 0.366 mmol) in 1,4-dioxane (3 mL) was added a solution of EDC (0.070 g, 0.366 mmol) in DCM (2 mL). The mixture was stirred for 10 min at room temperature. To the resulting solution were added the appropriate amine (S)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (0.130 g, 0.400 mmol) and DIPEA (0.122 mL, 0.699 mmol) in DCM (4 mL), with stirring. After 2 h or when the reaction was complete according to HPLC, the reaction mixture was diluted with DCM and washed with saturated aqueous NaHCO₃ solution (10 mL), 0.1 N aqueous citric acid solution (10 mL), and brine (10 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated and purified with flash chromatography.

General procedures A–G were used for the preparation of all final compounds present in the publication.

(S)-1-((R)-2-Amino-3-hydroxypropanoyl)pyrrolidine-2-carbonitrile 4-Methylbenzenesulfonate (7). *tert*-Butyl (R)-3-(*tert*-butyldimethylsilyloxy)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxopropan-2-ylcarbamate was prepared according to general procedure A using (S)-pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate and (R)-2-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsilyloxy)propanoic acid. ¹H NMR (400 MHz, CDCl₃) δ 5.31–5.22 (d, J = 8.4 Hz, 1H), 4.72–4.66 (m, 1H), 4.66–4.56 (td, J = 8.9, 5.3 Hz, 1H), 3.86–3.70 (m, 3H), 3.69–3.61 (t, J = 9.1 Hz, 1H), 2.36–2.07 (m, 4H), 1.50–1.37 (s, 9H), 0.88–0.78 (s, 9H), 0.03–0.02 (s, 3H), 0.015–0.00 (s, 3H); UPLC I (ESI) *t*_R 2.25 min, *m/z* 398.67 [M + H]⁺ (96%); 0.260g, 65%. Then Boc deprotection was done using general procedure B. ¹H NMR (400 MHz, D₂O) δ 7.73–7.66 (m, 2H), 7.40–7.35 (m, 2H), 4.84–4.81 (m, 1H), 4.43 (dd, J = 5.5, 4.3 Hz, 1H), 4.02–3.91 (m, 1H), 3.99 (dd, J = 12.6, 4.3 Hz, 1H), 3.94 (dd, J = 12.6, 5.6 Hz, 1H), 3.62 (dt, J = 9.8, 7.9 Hz, 1H), 2.41 (s, 3H), 2.39–2.16 (m, 4H); white powder, 0.220 g, 95%.

(S)-1-((R)-2-Aminopropanoyl)pyrrolidine-2-carbonitrile 4-Methylbenzenesulfonate (8). *tert*-Butyl (R)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxopropan-2-ylcarbamate was made using general procedure A and purified by column chromatography with a gradient DCM

to DCM–MeOH (95:5), yielding white crystals. UPLC I (ESI) *t*_R 1.44 min, *m/z* 268.5 [M + H]⁺ (96%); 0.47g, 83%. Then the Boc deprotection was done using general procedure B. ¹H NMR (400 MHz, D₂O) δ 7.73–7.65 (d, J = 8.0 Hz, 3H), 7.40–7.34 (d, J = 7.9 Hz, 3H), 4.79–4.75 (m, 1H), 4.39–4.29 (q, J = 7.1 Hz, 1H), 3.83–3.73 (dt, J = 10.6, 5.7 Hz, 1H), 3.60–3.49 (q, J = 8.8 Hz, 1H), 2.45–2.36 (s, 3H), 2.37–2.12 (m, 4H), 1.54–1.44 (dd, J = 7.1 Hz, 3H); UPLC I (ESI) *t*_R 0.25 min, *m/z* 168.4 [M + H]⁺ (95%); 0.5 g, 84%.

(S)-1-((S)-2-Aminopropanoyl)pyrrolidine-2-carbonitrile 4-Methylbenzenesulfonate (9). *tert*-Butyl (S)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxopropan-2-ylcarbamate was made using general procedure A and purified by column chromatography with a gradient DCM to DCM–MeOH (95:5). ¹H NMR (400 MHz, CDCl₃) δ 5.26 (d, J = 7.9 Hz, 1H), 4.78 (dd, J = 6.8, 3.7 Hz, 1H), 4.47–4.37 (m, 1H), 3.77–3.58 (m, 2H), 2.33–2.12 (m, 4H), 1.42 (s, J = 6.3 Hz, 9H), 1.35 (d, J = 6.9 Hz, 3H); UPLC I (ESI) *t*_R 1.45 min, *m/z* 268.5 [M + H]⁺ (96%); 0.46 g, 81%. Then Boc deprotection was done using general procedure B, yielding a white powder. ¹H NMR (400 MHz, D₂O) δ 7.73–7.66 (d, J = 7.9, 2H), 7.41–7.34 (d, J = 7.9 Hz, 2H), 4.87–4.80 (dd, J = 7.5, 5.2 Hz, 1H), 4.38–4.29 (q, J = 7.1 Hz, 1H), 3.75–3.59 (m, 2H), 2.43–2.38 (s, 3H), 2.37–2.09 (m, 4H), 1.58–1.51 (d, J = 7.1 Hz, 3H); UPLC I (ESI) *t*_R 0.19 min, *m/z* 168.4 [M + H]⁺ (97%); 0.43 g, 77%. Optical purity was confirmed with Marfey's reagent.

Methyl 1-(Quinoline-4-carboxamido)cyclopropanecarboxylate (10). The title compound was prepared using general procedure C starting from 1-aminocyclopropanecarboxylic acid, producing a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 8.92 (d, J = 4.3 Hz, 1H), 8.33 (dd, J = 8.5, 0.8 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.77 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.63 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.44 (d, J = 4.3 Hz, 1H), 6.66 (s, 1H), 3.81–3.76 (m, 3H), 1.76–1.72 (m, 2H), 1.38 (dd, J = 8.1, 4.9 Hz, 2H); UPLC I (ESI) *t*_R 1.14 min, *m/z* 271.6 [M + H]⁺ (93%).

***N*-((R)-1-((S)-2-Cyanopyrrolidin-1-yl)-3-hydroxy-1-oxopropan-2-yl)quinoline-4-carboxamide (11).** The title product was prepared using general procedure C, yielding a white powder. ¹H NMR (400 MHz, CDCl₃) *cis/trans* amide conformers (3/10) δ 8.91 (d, J = 4.35 Hz, 0.3H), 8.89 (d, J = 4.33 Hz, 1H), 8.19 (dd, J = 1.29, 8.69 Hz, 1H), 8.19–8.16 (m, 0.3H), 8.13 (d, J = 8.75 Hz, 0.3H), 8.11 (dd, J = 1.09, 8.75 Hz, 1H), 7.77–7.74 (m, 0.3H), 7.73 (ddd, J = 1.39, 6.87, 8.40 Hz, 1H), 7.63–7.59 (d, J = 1.35 Hz, 0.3H), 7.58 (ddd, J = 1.30, 6.82, 8.36 Hz, 1H), 7.51 (d, J = 7.87 Hz, 1H), 7.46 (d, J = 4.31 Hz, 1.3H), 7.43 (d, J = 7.26 Hz, 0.3H), 5.62 (dd, J = 2.08, 7.73 Hz, 0.3H), 5.11 (dt, J = 4.58, 7.87 Hz, 1H), 4.94 (dt, J = 3.82, 7.23 Hz, 0.3H), 4.60–4.55 (m, 1H), 4.15 (dd, J = 3.43, 11.74 Hz, 0.3H), 4.02 (dd, J = 4.18, 11.36 Hz, 1.3H), 3.97–3.89 (m, 2.3H), 3.73–3.62 (m, 2H), 3.59–3.50 (m, 0.6H), 2.46–2.10 (m, 5.2H); major conformer, ¹³C NMR (101 MHz, CDCl₃) δ 169.42, 167.55, 149.86, 148.70, 140.63, 130.31, 129.98, 128.14, 125.17, 124.37, 119.00, 117.90, 77.36, 63.36, 53.19, 47.01, 30.08, 25.24; minor conformer, ¹³C NMR (101 MHz, CDCl₃) δ 170.61, 168.09, 149.86, 148.76, 140.21, 130.34, 130.10, 128.18, 125.02, 124.37, 119.07, 117.90, 77.36, 62.35, 52.11, 46.65, 32.30, 23.27; UPLC I (ESI) *t*_R 1.10 min, *m/z* 339.6 [M + H]⁺ (95%); LC–MS (A) (ESI) *t*_R 10.5 min, *m/z* 339.0 [M + H]⁺ (95%); 42 mg, 40%.

***N*-((R)-1-((S)-2-Cyanopyrrolidin-1-yl)-1-oxopropan-2-yl)-quinoline-4-carboxamide (12).** The title product was prepared using general procedure C. Major conformer, ¹H NMR (400 MHz, CDCl₃) δ 8.94 (d, J = 4.31 Hz, 1H), 8.24 (dd, J = 1.16, 8.99 Hz, 1H), 8.16–8.11 (m, 1H), 7.76 (ddd, J = 1.41, 6.93, 8.38 Hz, 1H), 7.62 (ddd, J = 1.36, 6.84, 8.32 Hz, 1H), 7.49 (d, J = 4.29 Hz, 1H), 7.19 (d, J = 7.55 Hz, 1H), 5.04 (qd, J = 2.10, 6.79 Hz, 1H), 4.68 (dd, J = 2.00, 8.01 Hz, 1H), 4.00–3.91 (m, 1H), 3.59–3.51 (m, 1H), 2.49–2.10 (m, 4H), 1.52 (d, J = 6.83 Hz, 3H); minor conformer, ¹H NMR (400 MHz, CDCl₃) δ 8.93 (d, J = 4.30 Hz, 1H), 8.20 (dd, J = 1.15, 8.41 Hz, 1H), 8.16–8.11 (m, 1H), 7.76 (ddd, J = 1.41, 6.93, 8.38 Hz, 1H), 7.62 (ddd, J = 1.36, 6.84, 8.32 Hz, 1H), 7.46 (d, J = 4.29 Hz, 1H), 6.93 (d, J = 7.24 Hz, 1H), 5.38 (dd, J = 2.16, 7.68 Hz, 1H), 5.01–4.95 (m, 1H), 4.00–3.91 (m, 1H), 3.68–3.61 (m, 1H), 2.49–2.10 (m, 4H), 1.62 (d, J = 6.87 Hz, 3H); major conformer, ¹³C NMR (101 MHz, CDCl₃) δ 171.10, 166.71, 149.92, 148.79, 141.13, 130.17, 130.02, 127.96, 125.29,

124.50, 118.86, 117.97, 47.67, 47.06, 46.59, 30.15, 25.23, 18.31; minor conformer, ^{13}C NMR (101 MHz, CDCl_3) δ 171.07, 166.63, 149.88, 148.76, 141.09, 130.23, 130.07, 128.03, 125.12, 124.43, 118.91, 117.97, 47.56, 47.06, 46.59, 29.82, 23.23, 17.93; UPLC I (ESI) t_{R} 1.18 min, m/z 323.6 $[\text{M} + \text{H}]^+$ (96%); LC-MS (A) (ESI) t_{R} 10.2 min, m/z 323.0 $[\text{M} + \text{H}]^+$ (97%); 48 mg, 50%.

***N*-(*S*)-1-((*S*)-2-Cyanopyrrolidin-1-yl)-1-oxopropan-2-yl)-quinoline-4-carboxamide (13).** The title product was prepared using general procedure C to give a white powder. ^1H NMR (400 MHz, CDCl_3) δ 8.91–8.88 (d, J = 4.3 Hz, 1H), 8.26–8.20 (dd, J = 8.7, 1.5 Hz, 1H), 8.15–8.09 (dt, J = 8.5, 1.0 Hz, 1H), 7.78–7.71 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.63–7.56 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.46–7.43 (d, J = 4.2 Hz, 1H), 7.43–7.41 (s, 1H), 5.23–5.16 (p, J = 7.1 Hz, 0.05H), 5.05–4.93 (p, J = 7.1 Hz, 0.95H), 4.86–4.84 (d, J = 7.5 Hz, 0.05H), 4.74–4.67 (m, 0.95H), 3.88–3.78 (qd, J = 7.0, 3.1 Hz, 1H), 3.76–3.68 (m, 1H), 2.36–2.16 (m, 4H), 1.56 (d, J = 6.96 Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.35, 166.89, 149.79, 148.57, 141.08, 130.03, 129.84, 127.76, 125.21, 124.39, 118.85, 118.09, 47.39, 46.65, 46.52, 29.78, 25.34, 17.89; UPLC I (ESI) t_{R} 1.20 min, m/z 323.6 $[\text{M} + \text{H}]^+$ (96%); LC-MS (A) (ESI) t_{R} 10.5 min, m/z 323.0 $[\text{M} + \text{H}]^+$ (97%); 53 mg, 59%.

***S*)-*N*-(1-(2-Cyanopyrrolidine-1-carbonyl)cyclopropyl)-quinoline-4-carboxamide (14).** Methyl 1-(quinoline-4-carboxamido)cyclopropanecarboxylate was prepared using general procedure C starting from methyl 1-aminocyclopropanecarboxylate. ^1H NMR (400 MHz, CDCl_3) δ 8.92 (d, J = 4.3 Hz, 1H), 8.33 (dd, J = 8.5, 0.8 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.77 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.63 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.44 (d, J = 4.3 Hz, 1H), 6.66 (s, 1H), 3.81–3.76 (m, 3H), 1.76–1.72 (m, 2H), 1.38 (dd, J = 8.1, 4.9 Hz, 2H); UPLC I (ESI) t_{R} 1.14 min, m/z 271.6 $[\text{M} + \text{H}]^+$ (93%). Then methyl 1-(quinoline-4-carboxamido)cyclopropanecarboxylate (0.130 g, 0.481 mmol) was added to a solution of KOH (0.033 g, 0.495 mmol) in methanol (1 mL). The mixture was stirred for 2 h and evaporated till dryness. The resulting potassium 1-(quinoline-4-carboxamido)cyclopropanecarboxylate (0.140 g, 0.476 mmol, 99% yield) was used without further purification in the next step. Compound 14 was made according to general procedure A with cyanoproline tosylate and methyl 1-(quinoline-4-carboxamido)-cyclopropanecarboxylate, giving a colorless oil. ^1H NMR (400 MHz, MeOD) δ 8.92 (d, J = 4.41 Hz, 1H), 8.17 (d, J = 7.27 Hz, 1H), 8.09 (d, J = 8.42 Hz, 1H), 7.83 (ddd, J = 1.42, 6.78, 8.37 Hz, 1H), 7.68 (ddd, J = 1.21, 6.92, 8.30 Hz, 1H), 7.59 (d, J = 4.51 Hz, 1H), 3.74 (dq, J = 6.88, 19.58 Hz, 2H), 3.35 (s, 1H), 2.38–2.15 (m, 2H), 2.11 (ddt, J = 6.08, 14.42, 17.60 Hz, 2H), 1.57 (s, 2H), 1.24 (d, J = 2.75 Hz, 2H); ^{13}C NMR (101 MHz, MeOD) δ 171.74, 170.51, 151.01, 149.19, 142.80, 131.61, 129.88, 129.18, 126.26, 125.85, 120.22, 119.89, 55.81, 43.79, 37.07, 30.80, 26.56, 15.48, 15.38; LC-MS (A) (ESI) t_{R} 10.4 min, m/z 335.0 $[\text{M} + \text{H}]^+$ (96%); UPLC I (ESI) t_{R} 1.15 min, m/z 335.6 $[\text{M} + \text{H}]^+$ (96%); 76 mg, 47%.

***S*)-1-(2-Chloroacetyl)pyrrolidine-2-carbonitrile (15).** The title compound was made as described by Singh et al.²⁸

***S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-*N*-methylquinoline-4-carboxamide (16).** The title product was prepared using general procedure D. ^1H NMR (CDCl_3 , 400 MHz) (cis/trans amide rotamer, 1/3) δ 8.98 (dd, J = 4.2, 2.5 Hz, 1H), 8.20–8.09 (m, 1.75H), 7.88 (dd, J = 8.2, 1.1 Hz, 0.25H), 7.78 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.67 (ddd, J = 8.3, 6.9, 1.3 Hz, 0.75H), 7.60 (ddd, J = 8.3, 7.0, 1.3 Hz, 0.25H), 7.38 (d, J = 4.4 Hz, 0.75H), 7.33 (d, J = 4.3, 0.25H), 4.94–4.86 (m, 1H), 3.84–3.69 (m, 1H), 3.70–3.59 (m, 1H), 2.98 (d, J = 11.5 Hz, 1H), 2.95 (s, 3H), 2.94 (d, J = 11.4 Hz, 1H), 2.44–2.09 (m, 4H); UPLC (ESI) t_{R} 1.05, m/z 323.50 $[\text{M} + \text{H}]^+$; LC-MS (A) (ESI) t_{R} 10.4 min, m/z 323.1 $[\text{M} + \text{H}]^+$ (97%); yellowish oil, 0.022 g, 13%.

***S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-*N*-cyclopropylquinoline-4-carboxamide (17).** The title product was prepared using general procedure D. ^1H NMR (CDCl_3 , 400 MHz) (cis/trans amide rotamer, 1/9) δ 8.95 (d, J = 4.4 Hz, 1H), 8.25–8.09 (m, 2H), 7.74 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.62 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H), 7.44 (d, J = 4.7, 1H), 4.91–4.87 (m, 0.1H), 4.85 (dd, J = 7.6, 2.5 Hz, 0.9H), 4.66 (d, J = 16.1 Hz, 1H), 4.05 (d, J = 16.1 Hz, 1H), 3.74–3.65 (m, 1H), 3.64–3.55 (m, 1H), 2.89–2.78 (m, 1H), 2.42–2.06 (m,

4H), 0.62–0.37 (m, 3H), 0.31–0.20 (m, 1H); UPLC (ESI) t_{R} 1.26, m/z 349.40 $[\text{M} + \text{H}]^+$; LC-MS (A) (ESI) t_{R} 11.7 min, m/z 349.1 $[\text{M} + \text{H}]^+$ (98%); yellowish oil, 0.050 g, 18%.

***S*)-*N*-(4-Chlorobenzyl)-*N*-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (18).** The title product was prepared using general procedure D. ^1H NMR (CDCl_3 , 400 MHz) (cis/trans amide rotamer, 1/4) δ 8.98–8.94 (m, 0.2H), 8.93 (d, J = 4.35, 0.8H), 8.35 (br s, 1H), 8.18 (dd, J = 16.44, 3.43 Hz, 0.2H), 8.13 (d, J = 8.36 Hz, 0.8H), 7.91–7.83 (m, 0.2H), 7.78 (ddd, J = 8.30, 6.69, 1.44 Hz, 0.8H), 7.69 (ddd, J = 8.23, 6.92, 1.29 Hz, 0.8H), 7.64–7.58 (m, 0.2H), 7.43 (d, J = 3.70, 0.8H), 7.41–7.36 (m, 0.2H), 7.34 (q, J = 6.69, 5.04 Hz, 1.6H), 7.33–7.27 (m, J = 6.69, 5.04 Hz, 0.4H), 7.13–7.03 (m, 2H), 4.94–4.72 (m, 2H), 5.37–5.33 (m, 0.8H), 5.47–5.43 (m, 0.2H), 4.43 (d, J = 3.14 Hz, 2H), 3.75 (dt, J = 11.03, 5.33 Hz, 0.4H), 3.67–3.43 (d, J = 26.63 Hz, 1.6H), 2.41–2.06 (m, 3.6H), 2.40–2.05 (m, 0.4H); UPLC (ESI) t_{R} 1.69, m/z 433.30 $[\text{M} + \text{H}]^+$; LC-MS (A) (ESI) t_{R} 15.6 min, m/z 433.3 $[\text{M} + \text{H}]^+$ (98%); yellowish oil, 0.030 g, 18%.

***S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-*N*-(2-methoxyethyl)quinoline-4-carboxamide (19).** The title product was prepared using general procedure D. ^1H NMR (CDCl_3 , 400 MHz) (cis/trans mixture of amide rotamers) δ 8.96 (d, J = 4.4, 1H), 8.34–8.29 (m, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.78 (td, J = 7.9, 7.4, 1.6 Hz, 1H), 7.68 (s, 1H), 7.44–7.38 (m, 1H), 4.97–4.85 (m, 1H), 4.07 (m, 1H), 3.70 (m, 2H), 3.47–3.30 (m, 5H), 3.27 (s, 3H), 2.41–2.11 (m, 4H); UPLC (ESI) t_{R} 1.21, m/z 367.30 $[\text{M} + \text{H}]^+$; LC-MS (A) (ESI) t_{R} 11.2 min, m/z 367.1 $[\text{M} + \text{H}]^+$ (99%); yellowish oil, 0.049 g, 28%.

***S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-*N*-isopentylquinoline-4-carboxamide (20).** The title product was prepared using general procedure D. ^1H NMR (400 MHz, CDCl_3) (cis/trans amide rotamer mixture) δ 8.94 (dd, J = 4.2, 2.5 Hz, 1H), 8.13 (dd, J = 8.6, 1.3 Hz, 1H), 8.25 (br s, 1H), 7.76 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H), 7.66 (t, J = 7.6, 1H), 7.33 (br s, 1H), 4.92–4.68 (m, 1.5H), 4.50–4.10 (m, 0.5H), 3.84–3.54 (m, 3H), 3.21–3.06 (m, 2H), 2.42–2.05 (m, 4H), 1.45–1.15 (m, 3H), 0.55 (d, J = 6.3 Hz, 6H); LC-MS (A) (ESI) t_{R} 14.5 min, m/z 379.1 $[\text{M} + \text{H}]^+$ (99%); yellowish oil, 0.056 g, 33%.

***S*)-1-(2-((2-Methoxyethyl)(quinolin-4-ylmethyl)amino)acetyl)pyrrolidine-2-carbonitrile (21).** A solution of (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile 11 (0.272 g, 1.576 mmol) in 2.5 mL of THF was added dropwise over 0.5 h into an ice–water cooled mixture of the appropriate 2-methoxy-*N*-(quinolin-4-ylmethyl)ethanamine S10 (0.150 g, 0.694 mmol) and potassium carbonate (0.392 g, 2.84 mmol) in 5 mL of THF. The resulting mixture was stirred at ice–water temperature for 2 h and then at room temperature for 1–3 days. The resulting mixture was then filtered to remove K_2CO_3 , concentrated, and then partitioned between DCM and saturated aqueous NaHCO_3 . The aqueous layer was then washed with DCM (2 $\times$), and the combined organic layers were washed with water, dried over Na_2SO_4 , and concentrated in vacuo. Purification was done using column chromatography with a DCM–MeOH gradient to yield a yellowish oil. ^1H NMR (CDCl_3 , 400 MHz) (cis/trans amide rotamer, 1/4) δ 8.84 (d, J = 4.4 Hz, 0.2H), 8.83 (d, J = 4.3 Hz, 0.8H), 8.26 (dd, J = 8.4 Hz, J' = 0.7 Hz, 0.8H), 8.09 (dd, J = 8.5 Hz, J' = 0.7 Hz, 0.8H), 8.1 (dd, J = 8.5 Hz, J' = 0.7 Hz, 0.2H), 8.18 (dd, J = 8.7 Hz, J' = 1.0 Hz, 0.2H), 7.68 (ddd, J = 8.4 Hz, J' = 6.8 Hz, J'' = 1.4 Hz, 1H), 7.54 (ddd, J = 8.3 Hz, J'' = 6.8 Hz, J' = 1.3 Hz, 1H), 7.45 (dd, J = 4.4 Hz, J' = 0.9 Hz, 0.8H), 7.41 (d, J = 4.3 Hz, 0.2H), 5.20 (dd, J = 8.0 Hz, J' = 1.6 Hz, 0.2H), 4.70–4.57 (m, 0.8H), 4.38 (d, J = 14.0 Hz, 0.8H), 4.30 (d, J = 14.2 Hz, 0.8H), 4.24 (d, J = 14.1 Hz, 0.2H), 4.16 (d, J = 14.2 Hz, 0.2H), 3.71 (d, J = 14.4 Hz, 0.4H), 3.66 (td, J = 7.7 Hz, J' = 3.9 Hz, 0.4H), 3.53 (t, J = 5.2 Hz, 1.6H), 3.50–3.44 (m, 0.2H), 3.39 (d, J = 15.0 Hz, 1.6H), 3.32 (s, 3H), 3.38–3.31 (m, 0.8H), 3.27–3.11 (m, 1H), 2.99 (dt, J = 5.3 Hz, J' = 2.4 Hz, 1.6H), 2.81 (ddd, J = 13.9 Hz, J' = 5.5 Hz, J'' = 3.2 Hz, 0.4H), 2.25–1.89 (m, 4H); UPLC (ESI) t_{R} 1.18, m/z 353.50 $[\text{M} + \text{H}]^+$; LC-MS (A) (ESI) t_{R} 10.3 min, m/z 353.1 $[\text{M} + \text{H}]^+$ (97%); 0.055 g, 25%.

***S*)-1-(2-(Methyl(quinolin-4-ylmethyl)amino)acetyl)pyrrolidine-2-carbonitrile (22).** The title compound was prepared in a similar manner as compound 21 starting from *N*-methyl-1-

(quinolin-4-yl)methanamine (**S9**). Purification was done using column chromatography with a DCM–MeOH gradient with an ammonia additive to yield a yellowish oil. ^1H NMR (CDCl_3 , 400 MHz) (cis/trans amide rotamer, 1/3) δ 8.79 (s, 0.25H), 8.77 (d, J = 4.3 Hz, 0.75H), 8.17 (dd, 0.75H), 8.13–8.09 (m, 0.25H), 8.06 (d, J = 8.5 Hz, 0.25H), 8.03 (d, J = 8.5 Hz, 0.75H), 7.62 (ddd, J = 8.4, 6.8, 1.4 Hz, 1H), 7.49 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 7.32 (d, J = 4.4, 0.75H), 7.29 (d, J = 4.3, 0.25H), 4.78 (dd, J = 7.8, 1.8 Hz, 0.25H), 4.63–4.52 (m, 0.75H), 4.07 (d, J = 13.4 Hz, 0.75H), 3.98 (d, J = 11.4 Hz, 0.75H), 3.96 (d, J = 13.6 Hz, 0.25H), 3.92 (d, J = 13.6 Hz, 0.25H), 3.50–3.37 (m, 1H), 3.20 (d, J = 1.5 Hz, 2H), 3.24–3.09 (m, 1H), 2.38 (s, 0.75H), 2.35 (s, 2.25H), 2.22–1.59 (m, 4H); UPLC (ESI) t_R 0.57, m/z 309.50 $[\text{M} + \text{H}]^+$; LC–MS (A) (ESI) t_R 8.8 min, m/z 309.1 $[\text{M} + \text{H}]^+$ (97%); 0.131 g, 48.8%.

(2S,2'S)-1,1'-(2,2'-(Methylazanediyl)bis(acetyl))bis(pyrrolidine-2-carbonitrile) (23). The title product was prepared using general procedure D, but only 0.5 equiv of 33% methylamine in EtOH was used as the primary amine. ^1H NMR (400 MHz, CDCl_3) (mixture of trans/cis rotamers) δ 5.69 (d, J = 7.3 Hz, 0.4H), 4.80–4.62 (m, 1.6H), 3.72–3.66 (m, 1H), 3.62–3.56 (m, 1H), 3.53–3.44 (m, J = 13.7, 8.1 Hz, 3H), 3.43–3.36 (m, J = 14.6, 3.3 Hz, 3H), 2.54 (s, 1.5H), 2.42 (s, J = 7.7 Hz, 1.5H), 2.35–1.99 (m, 8H); UPLC (ESI) t_R 0.29, m/z 304.50 $[\text{M} + \text{H}]^+$; LC–MS (A) (ESI) t_R 2.2 min, m/z 304.1 $[\text{M} + \text{H}]^+$ (98%); yellowish oil, 0.028 g, 13%.

(S)-1-(2-(4-Phenyl-1H-1,2,3-triazol-1-yl)acetyl)pyrrolidine-2-carbonitrile (24). The title product was prepared using general procedure E. ^1H NMR (400 MHz, CDCl_3) δ 8.1 (s, 1H), 7.85–7.74 (m, 2H), 7.53–7.40 (m, 3H), 5.35 (d, J = 16.5 Hz, 1H), 5.12 (d, J = 16.5 Hz, 1H), 4.79–4.73 (m, 1H), 3.81–3.72 (m, 1H), 3.66–3.58 (m, 1H), 2.40–2.13 (m, 4H); LC–MS (A) (ESI) t_R 12.8 min, m/z 282.3 $[\text{M} + \text{H}]^+$ (96%); white powder, 54 mg, 62%.

(S)-1-(2-(4-(4-Fluorophenyl)-1H-1,2,3-triazol-1-yl)acetyl)pyrrolidine-2-carbonitrile (25). The title product was prepared using general procedure E. ^1H NMR (400 MHz, CDCl_3) δ 8.04 (s, 1H), 7.84–7.75 (m, 2H), 7.09 (t, J = 8.84 Hz, 2H), 5.35 (d, J = 16.38 Hz, 1H), 5.14 (d, J = 16.40 Hz, 1H), 4.79–4.73 (m, 1H), 3.81–3.72 (m, 1H), 3.66–3.58 (m, 1H), 2.40–2.13 (m, 4H); LC–MS (A) (ESI) t_R 13.5 min, m/z 300.0 $[\text{M} + \text{H}]^+$ (97%); white powder, 39 mg, 37%.

(S)-1-(2-(4-(3-(Isoindolin-2-yl)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)acetyl)pyrrolidine-2-carbonitrile (26). The title product was prepared using general procedure E. ^1H NMR (400 MHz, CDCl_3) δ 7.65 (s, 1H), 7.32–7.22 (m, 4H), 5.22 (d, J = 16.33 Hz, 1H), 5.08 (d, J = 16.33 Hz, 1H), 4.79 (d, J = 5.59 Hz, 4H), 4.74–4.70 (m, 1H), 3.76–3.68 (m, 1H), 3.62–3.53 (m, 1H), 3.16 (t, J = 7.19 Hz, 2H), 2.84 (t, J = 7.19 Hz, 2H), 2.36–2.16 (m, 4H); LC–MS (A) (ESI) t_R 12.9 min, m/z 379.1 $[\text{M} + \text{H}]^+$ (97%); white powder, 36 mg, 23%.

(S)-1-(2-(4-(Naphthalen-1-yl)-1H-1,2,3-triazol-1-yl)acetyl)pyrrolidine-2-carbonitrile (27). The title product was prepared using general procedure E. ^1H NMR (400 MHz, CDCl_3) δ 8.43–8.34 (m, 1H), 8.10 (s, 1H), 7.94–7.87 (m, 2H), 7.76 (dd, J = 1.23, 7.14 Hz, 1H), 7.57–7.49 (m, 3H), 5.43 (d, J = 16.32 Hz, 1H), 5.23 (d, J = 16.30 Hz, 1H), 4.79 (dd, J = 2.60, 7.46 Hz, 1H), 3.84–3.74 (m, 1H), 3.72–3.61 (m, 1H), 2.40–2.14 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 164.05, 147.28, 133.89, 131.10, 129.05, 128.46, 127.76, 127.40, 126.77, 126.05, 125.37, 125.35, 124.41, 117.74, 51.55, 46.97, 46.28, 29.87, 25.24; LC–MS (A) (ESI) t_R 15.0 min, m/z 332.1 $[\text{M} + \text{H}]^+$ (95%); white powder, 140 mg, 63.1%.

2-(Quinoline-4-carboxamido)acetic Acid (28). Ethyl 2-(Quinoline-4-carboxamido)acetate. To a solution of quinoline-4-carboxyl chloride hydrochloride (5 g, 21.9 mmol) in DCM were added a solution of ethyl 2-aminoacetate hydrochloride (3.06 g, 21.92 mmol) and triethylamine (9.72 mL, 70.2 mmol) in DCM. The mixture was stirred for 4 h at room temperature. It was washed with 1 N citric acid. The precipitate that formed was the product (5.66 g, 90%). UPLC I (ESI) t_R 1.20 min, m/z 259.6 $[\text{M} + \text{H}]^+$ (96%). Then sodium hydroxide (21.30 mL, 21.30 mmol) was added to a mixture of ethyl 2-(quinoline-4-carboxamido)acetate (5.5 g, 21.30 mmol) in MeOH (10 mL) and water (10.00 mL). The mixture was stirred for 16 h, evaporated, redissolved in water, washed with DCM, acidified, and the precipitate was 2-(quinoline-4-carboxamido)acetic acid: white powder,

4g, 82%. ^1H NMR (sodium salt) (400 MHz, D_2O) δ 8.94 (d, J = 4.5 Hz, 1H), 8.23 (d, J = 7.9 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 7.90 (t, J = 7.1 Hz, 1H), 7.75 (t, J = 7.2 Hz, 1H), 7.71 (d, J = 4.5 Hz, 1H), 4.07 (s, 2H).

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-isonicotinamide (29). The title product was prepared using general procedure A. ^1H NMR (400 MHz, CDCl_3): δ 8.63 (d, J = 6 Hz, 2H), 7.99 (br s, 1H), 7.61 (d, J = 6 Hz, 2H), 4.76–4.72 (m, 1H), 4.53–4.46 (m, 1H), 4.12–4.05 (m, 1H), 3.74–3.66 (m, 1H), 3.54–3.45 (m, 1H), 2.36–2.13 (m, 4H); MS (ESI) m/z 259.1 $[\text{M} + \text{H}]^+$; LC–MS (A) t_R 3.7 min, m/z 259.1 $[\text{M} + \text{H}]^+$ (96%); yellowish oil, 24 mg, 16%.

(S)-2-Chloro-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-isonicotinamide (30). The title product was prepared using general procedure F. ^1H NMR (400 MHz, CDCl_3) (mixture of cis/trans amide rotamers, 1/9) δ 8.51 (dd, J = 5.1, 0.6 Hz, 1H), 7.72 (dd, J = 1.5, 0.7 Hz, 1H), 7.57 (dd, J = 5.1, 1.5 Hz, 1H), 7.44 (s, 1H), 4.81–4.75 (m, 0.9H), 4.68 (d, J = 5.7 Hz, 0.1H), 4.44 (dd, J = 17.6, 5.0 Hz, 0.1H), 4.35 (dd, J = 17.9, 5.0 Hz, 0.9H), 4.30–4.22 (m, 0.1H), 4.16 (dd, J = 17.9, 3.7 Hz, 0.9H), 3.74–3.66 (m, 1H), 3.55–3.46 (m, 1H), 2.42–2.14 (m, 4H); UPLC I (ESI) t_R 1.20 min, 293.3 m/z $[\text{M} + \text{H}]^+$ (96%); white powder, 53 mg, 57%.

(S)-2-Bromo-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-isonicotinamide (31). The title product was prepared using general procedure A. ^1H NMR (400 MHz, CDCl_3) (mixture of cis/trans amide rotamers, 1/9) δ 8.50 (dd, J = 5.1, 0.6 Hz, 1H), 7.88 (dd, J = 1.4, 0.7 Hz, 1H), 7.62 (dd, J = 5.1, 1.5 Hz, 1H), 7.36 (s, 1H), 4.80–4.76 (m, 0.9H), 4.68 (d, J = 6.2 Hz, 0.1H), 4.43 (dd, J = 17.6, 5.2 Hz, 0.1H), 4.33 (dd, J = 17.9, 4.8 Hz, 0.9H), 4.29–4.23 (m, 0.1H), 4.17 (dd, J = 17.9, 3.7 Hz, 0.9H), 3.73–3.65 (m, 1H), 3.58–3.45 (m, 1H), 2.42–2.17 (m, 4H); UPLC I (ESI) t_R 1.23 min, m/z 337.5, 339.6 $[\text{M} + \text{H}]^+$ (96%); colorless oil, 36 mg, 41%.

(S)-2-Amino-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-isonicotinamide (32). (S)-tert-Butyl 4-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-2-ylcarbamate was prepared using general procedure A using potassium 2-(tert-butoxycarbonylamino)-isonicotinate to give a Boc-protected intermediate. Then TFA (0.827 mL, 10.74 mmol) was slowly added to a solution of (S)-tert-butyl 4-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamoyl)pyridin-2-ylcarbamate (0.537 mmol) in acetonitrile (2 mL) at 0 °C. After 1 h, the mixture was allowed to warm to room temperature and was stirred for 24 h. The volatiles were evaporated. The products were purified by reversed phase flash column using a acetonitrile–water gradient or converted into the HCl salt by stirring for 15 min in 1 N HCl in ether, followed by decantation of the ether and washing with cold ethyl acetate. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) (9/1 mixture of trans/cis amide rotamers) δ 9.13 (t, J = 5.87 Hz, 1H), 8.06 (d, J = 6.40 Hz, 1H), 7.95 (s, 2H), 7.26 (s, 1H), 7.10 (dd, J = 1.56, 6.44 Hz, 1H), 5.24 (dd, J = 2.26, 7.39 Hz, 0.1H) 4.76 (dd, J = 3.74, 7.38 Hz, 0.9H), 4.30 (dd, J = 5.74, 16.59 Hz, 0.2H), 4.20–4.02 (m, 1.8H), 3.70 (ddd, J = 3.88, 7.60, 9.51 Hz, 1H), 3.55–3.48 (m, 1H), 2.23–1.96 (m, 4H); UPLC I (ESI) t_R 0.45 min, m/z 274.6 $[\text{M} + \text{H}]^+$ (98%); LC–MS (A) t_R 2.2 min, m/z 274.0 $[\text{M} + \text{H}]^+$ (97%); yellowish oil, 40 mg, 60%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-phenylisonicotinamide (33). The title product was prepared using general procedure A using 2-phenylisonicotinic acid **S11**. ^1H NMR (400 MHz, CDCl_3) (9/1 mixture of trans/cis amide rotamers) δ 8.83–8.79 (dd, J = 5.0, 0.9 Hz, 1H), 8.13–8.10 (dd, J = 1.6, 0.9 Hz, 1H), 8.07–8.02 (m, 2H), 7.60–7.56 (dd, J = 5.0, 1.6 Hz, 1H), 7.53–7.42 (m, 3H), 7.41–7.36 (s, 1H), 4.83–4.77 (dd, J = 8.0, 2.4 Hz, 0.9H), 4.75–4.71 (d, J = 8.3 Hz, 0.1H), 4.53–4.46 (dd, J = 17.6, 5.2 Hz, 0.1H), 4.40–4.31 (dd, J = 18.0, 4.7 Hz, 0.9H), 4.26–4.17 (dd, J = 18.0, 3.7 Hz, 1H), 3.75–3.67 (ddd, J = 14.9, 8.4, 4.0 Hz, 1H), 3.58–3.49 (td, J = 8.6, 7.6, 5.9 Hz, 1H), 2.43–2.15 (m, 4H); UPLC I (ESI) t_R 1.45 min, m/z 335.6 $[\text{M} + \text{H}]^+$ (98%); LC–MS (A) t_R 1.32min, 335.6 m/z $[\text{M} + \text{H}]^+$ (99%); white powder, 0.037 g, 52%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(3,4-dimethoxyphenyl)isonicotinamide (34). The title product was prepared using general procedure A using 2-(3,4-dimethoxyphenyl)-isonicotinic acid **S12**. ^1H NMR (400 MHz, CDCl_3) (9/1 mixture of trans/cis amide rotamers) δ 8.82–8.69 (dd, J = 5.0, 0.9 Hz, 1H),

8.11–8.00 (s, 1H), 7.73–7.66 (d, J = 2.1 Hz, 1H), 7.61–7.57 (dd, J = 8.4, 2.1 Hz, 1H), 7.52–7.48 (dd, J = 5.1, 1.6 Hz, 1H), 7.40–7.33 (t, J = 3.9 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 4.83–4.76 (dd, J = 8.1, 2.8 Hz, 0.9H), 4.74–4.72 (d, J = 8.1 Hz, 0.1H), 4.52–4.46 (dd, J = 17.3, 5.1 Hz, 0.1H), 4.36 (dd, J = 18.0, 4.7 Hz, 0.9H), 4.21 (dd, J = 18.0, 3.7 Hz, 1H), 4.03–3.99 (s, 3H), 3.97–3.92 (s, 3H), 3.78–3.68 (m, 1H), 3.60–3.50 (m, 1H), 2.42–2.16 (m, 4H); UPLC I (ESI) t_R 1.34 min, m/z 395.5 $[M + H]^+$ (100%); LC–MS (A) t_R 12.6 min, m/z 395.1 $[M + H]^+$ (99%); white powder, 36 mg, 43%.

(S)-2-(4-Cyanophenyl)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)isonicotinamide (35). The title product was prepared using general procedure A using 2-(4-cyanophenyl)isonicotinic acid **S13**. ^1H NMR (400 MHz, $CDCl_3$) (9/1 mixture of trans/cis amide rotamers) δ 8.76 (d, J = 5.0 Hz, 1H), 8.16–8.05 (m, 3H), 7.81–7.72 (m, 3H), 7.61 (dd, J = 5.0, 1.5 Hz, 1H), 4.84–4.74 (m, 0.9H), 4.73–4.71 (dd, J = 9.6, 1.9 Hz, 0.1H), 4.53–4.43 (dd, J = 17.8, 5.7 Hz, 1H), 4.34–4.28 (dd, J = 17.4, 3.6 Hz, 0.1H), 4.23–4.13 (dd, J = 17.8, 3.5 Hz, 0.9H), 3.81–3.70 (m, 1H), 3.58–3.51 (m, 1H), 2.42–2.18 (m, 4H); UPLC I (ESI) t_R 1.44 min, m/z 360.6 $[M + H]^+$ (100%); LC–MS (A) t_R 13.5 min, m/z 360.0 $[M + H]^+$ (99%); white powder, 45 mg, 52%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(pyridin-4-yl)acetamide (36). The title product was prepared using general procedure A. ^1H NMR (400 MHz, MeOD) δ 8.49–8.44 (d, J = 4.5 Hz, J' = 1.6 Hz, 2H), 7.46–7.41 (d, J = 4.5 Hz, J' = 1.6 Hz, 2H), 5.05–5.02 (m, 0.14H), 4.79–4.74 (t, J = 5.4 Hz, 0.86H), 4.26–4.16 (m, 0.2H), 4.12–4.00 (m, 1.8H), 3.73–3.66 (m, 3H), 3.57–3.49 (dt, J = 9.6, 7.6 Hz, 1H), 2.28–2.12 (m, 4H); ^{13}C NMR (101 MHz, MeOD) δ 172.54, 169.57, 150.0, 147.3, 126.35, 119.51, 47.99, 46.90, 42.90, 42.50, 30.97, 26.12; UPLC I (ESI) t_R 0.28 min, m/z 273.6 $[M + H]^+$ (97%); LC–MS (A) t_R 1.5 min, m/z 273.0 $[M + H]^+$ (97%); colorless oil, 72 mg, 66%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)piperidine-4-carboxamide (37). *Step 1.* 1-(*tert*-Butoxycarbonyl)piperidine-4-carboxylic Acid. The piperidine-4-carboxylic acid (1 g, 7.74 mmol) was added to a stirred solution of guanidine hydrochloride (0.111 g, 1.161 mmol) and di-*tert*-butyl dicarbonate (4.22 g, 19.36 mmol) in EtOH (7 mL) at 35–40 °C. The mixture was stirred overnight. Then the ethanol was evaporated and the residue was dissolved in DCM and filtered to separate the catalyst. The filtrate was evaporated and washed with hexane to yield a white powder (1.5g, 84%). ^1H NMR (400 MHz, MeOD) δ 4.02–3.94 (td, J = 4.0, 1.3 Hz, 2H), 2.96–2.83 (br s, 2H), 2.55–2.43 (tt, J = 11.0, 4.0 Hz, 1H), 1.94–1.83 (dq, J = 13.9, 3.6 Hz, 2H), 1.61–1.47 (m, 2H), 1.52–1.38 (s, 9H); MS (ESI) m/z 230.4 $[M + H]^+$.

Step 2: (S)-*tert*-Butyl 4-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethylcarbamoyl)piperidine-1-carboxylate. The title product was prepared using general procedure A using 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid to give the product (0.1 g, 63%). UPLC I (ESI) t_R 1.47 min, m/z 365.6 $[M + H]^+$ (90%). Then (S)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)piperidine-4-carboxamide (**40**) was made using general procedure B. ^1H NMR (400 MHz, MeOD) (9/1 mixture of trans/cis amide rotamers) δ 5.07–5.03 (m, 0.1H), 4.80–4.72 (dd, J = 6.1, 4.6 Hz, 0.1H), 4.12–3.99 (d, J = 17.1 Hz, 1H), 4.04–3.92 (d, J = 17.1 Hz, 1H), 3.77–3.63 (m, 1H), 3.61–3.48 (dt, J = 9.5, 7.5 Hz, 1H), 3.15–3.09 (t, J = 3.4 Hz, 1H), 3.12–3.06 (t, J = 3.3 Hz, 1H), 2.73–2.58 (td, J = 12.5, 2.9 Hz, 2H), 2.54–2.39 (tt, J = 11.6, 3.8 Hz, 1H), 2.31–2.19 (m, 2H), 2.22–2.11 (m, 2H), 1.89–1.77 (m, 2H), 1.76–1.58 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 178.10, 169.80, 119.55, 47.9, 46.90, 46.11, 43.66, 42.70, 42.48, 30.97, 29.85, 26.13, 23.76; UPLC I (ESI) t_R 0.26 min, m/z 265.5 $[M + H]^+$ (98%); yellowish oil, 40 mg, 70%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)pyridazine-4-carboxamide (38). The title product was prepared using general procedure F. Major conformer (4:1 mixture of trans/cis amide rotamers), ^1H NMR (400 MHz, $CDCl_3$) δ 9.51 (dd, J = 1.24, 2.31 Hz, 1H), 9.24 (dd, J = 1.24, 5.31 Hz, 1H), 8.50 (dd, J = 3.60, 6.74 Hz, 1H), 7.81 (dd, J = 2.33, 5.30 Hz, 1H), 4.72 (dd, J = 3.04, 7.21 Hz, 1H), 4.54 (dd, J = 6.78, 17.41 Hz, 1H), 4.10 (dd, J = 3.62, 17.41 Hz, 1H), 3.71 (ddd, J = 3.41, 7.40, 10.39 Hz, 1H), 3.58–3.47 (m, 1H), 2.48–2.05

(m, 4H); minor conformer, ^1H NMR (400 MHz, $CDCl_3$) δ 9.56 (dd, J = 1.26, 2.38 Hz, 1H), 9.34 (dd, J = 1.25, 5.28 Hz, 1H), 8.05 (t, J = 4.70 Hz, 1H), 7.87 (dd, J = 2.36, 5.28 Hz, 1H), 4.85 (dd, J = 2.12, 7.69 Hz, 1H), 4.47 (dd, J = 5.30, 17.11 Hz, 1H), 4.29 (dd, J = 4.40, 17.09 Hz, 1H), 3.71 (ddd, J = 3.41, 7.40, 10.39 Hz, 1H), 3.58–3.47 (m, 1H), 2.48–2.05 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.07, 163.44, 151.93, 148.47, 130.64, 123.93, 118.16, 46.98, 45.97, 42.34, 29.82, 25.22; LC–MS (A) (ESI) t_R 5.0 min, m/z 259.9 $[M + H]^+$ (96%); white crystals, 134 mg, 65%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1-methyl-1H-imidazole-5-carboxamide (39). The title product was prepared using general procedure A. ^1H NMR (400 MHz, $CDCl_3$) (9/1 mixture of trans/cis amide rotamers) δ 7.57–7.51 (s, 1H), 7.51–7.44 (s, 1H), 7.08–7.02 (d, J = 5.6 Hz, 1H), 4.81–4.75 (m, 0.9H), 4.72–4.68 (dd, J = 7.8, 1.9 Hz, 0.1H), 4.43–4.34 (dd, J = 17.3, 5.2 Hz, 0.1H), 4.33–4.24 (dd, J = 17.7, 5.2 Hz, 0.9H), 4.23–4.15 (m, 0.1H), 4.13–4.05 (dd, J = 17.7, 3.9 Hz, 0.9H), 3.97–3.84 (s, 3H), 3.71–3.64 (ddd, J = 9.4, 7.0, 3.0 Hz, 1H), 3.54–3.44 (td, J = 9.0, 8.4, 6.3 Hz, 1H), 2.40–2.12 (m, 4H); UPLC I (ESI) t_R 0.29 min, m/z 262.6 $[M + H]^+$ (98%); LC–MS (A) t_R 1.5 min, m/z 262.0 $[M + H]^+$ (97%); colorless oil, 46 mg, 38%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1H-1,2,3-triazole-5-carboxamide (40). The title product was prepared using general procedure A. ^1H NMR (400 MHz, DMSO- d_6) (9/1 mixture of trans/cis amide rotamers) δ 15.53 (s, 1H), 8.58–8.44 (s, 1H), 8.39 (s, 1H), 5.28–5.22 (m, 0.1H), 4.77 (dd, J = 3.76, 7.32 Hz, 1H), 4.30 (dd, J = 5.61, 16.77 Hz, 0.2H), 4.10 (d, J = 5.76 Hz, 2H), 3.68 (ddd, J = 4.04, 7.68, 9.39 Hz, 1H), 3.50 (td, J = 6.84, 9.09 Hz, 1H), 2.31–1.88 (m, 4H); UPLC I (ESI) t_R 0.72 min, m/z 249.6 $[M + H]^+$ (96%); LC–MS (A) t_R 4.0 min, m/z 249.0 $[M + H]^+$ (96%); colorless oil, 34 mg, 40%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1H-1,2,4-triazole-3-carboxamide (41). The title product was prepared using general procedure A. ^1H NMR (400 MHz, D_2O) (9/1 mixture of trans/cis amide rotamers) δ 8.56 (s, 1H), 5.12 (dd, J = 2.05, 7.76 Hz, 0.1H), 4.85–4.81 (m, 0.9H), 4.43 (d, J = 2.63 Hz, 0.2H), 4.32 (s, 2H), 3.79 (ddd, J = 4.53, 7.02, 9.82 Hz, 1H), 3.63 (dt, J = 7.65, 9.63 Hz, 1H), 2.48–2.10 (m, 4H); UPLC I (ESI) t_R 0.77 min, m/z 271.5 $[M + Na]^+$ (97%); LC–MS (A) t_R 2.8 min, m/z 249.0 $[M + H]^+$ (97%); yellowish oil, 90 mg, 55%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(1H-imidazol-4-yl)acetamide (42). The title product was prepared using general procedure A. ^1H NMR (400 MHz, MeOD) δ 7.68–7.63 (s, 1H), 7.05–6.99 (s, 1H), 4.78–4.73 (t, J = 5.3 Hz, 1H), 4.10–3.98 (m, 2H), 3.71–3.64 (ddd, J = 9.7, 6.4, 4.7 Hz, 1H), 3.63–3.58 (s, 2H), 3.56–3.47 (dt, J = 9.5, 7.5 Hz, 1H), 2.36–2.06 (m, 4H); UPLC I (ESI) t_R 0.29 min, m/z 262.6 $[M + H]^+$ (99%); LC–MS (A) t_R 1.2 min, m/z 262.0 $[M + H]^+$ (96%); yellowish oil, 72 mg, 66%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-4-methylthiazole-5-carboxamide (43). The title product was prepared using general procedure A. ^1H NMR (400 MHz, $CDCl_3$) (9/1 mixture of trans/cis amide rotamers) δ 8.71–8.67 (s, 1H), 7.13–7.04 (d, J = 7.3 Hz, 1H), 4.79–4.74 (d, J = 7.7 Hz, 0.9H), 4.74–4.71 (d, J = 7.7 Hz, 0.1H), 4.47–4.40 (dd, J = 17.4, 5.1 Hz, 0.1H), 4.38–4.27 (dd, J = 17.8, 5.1 Hz, 0.9H), 4.26–4.18 (dd, J = 17.4, 3.5 Hz, 0.1H), 4.16–4.05 (dd, J = 18.0, 3.4 Hz, 0.9H), 3.71–3.64 (ddd, J = 9.3, 7.2, 3.3 Hz, 1H), 3.54–3.44 (m, 1H), 2.76–2.73 (s, 3H), 2.38–2.14 (m, 4H); UPLC I (ESI) t_R 1.08 min, m/z 279.6 $[M + H]^+$ (100%); LC–MS (A) t_R 9.3 min, m/z 279.0 $[M + H]^+$ (99%); whitish powder, 57 mg, 39%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-4-methyloxazole-5-carboxamide (44). The title product was prepared using general procedure A. ^1H NMR (400 MHz, $CDCl_3$) (9/1 mixture of trans/cis amide rotamers) δ 7.83–7.75 (s, 1H), 7.19–7.12 (s, 1H), 4.83–4.76 (dd, J = 8.0, 2.6 Hz, 0.9H), 4.83–4.74 (d, 8.0 Hz, 0.1H), 4.46–4.39 (dd, J = 17.3, 5.6 Hz, 0.1H), 4.31–4.22 (dd, J = 17.9, 4.9 Hz, 0.9H), 4.22–4.17 (m, 0.1H), 4.16–4.09 (dd, J = 17.9, 4.0 Hz, 0.9H), 3.72–3.64 (ddd, J = 9.5, 7.1, 2.8 Hz, 1H), 3.56–3.43 (m, 1H), 2.58–2.45 (s, 3H), 2.40–2.18 (m, 4H); UPLC I (ESI) t_R 1.04 min, m/z 263.4 $[M + H]^+$ (96%); whitish powder, 34 mg, 36%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-4-(2,2,2-trifluoroacetamido)benzamide (46) and (S)-4-Amino-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)benzamide 2,2,2-Trifluoroacetate (45). TFA (0.228 mL, 2.95 mmol) was added to a solution of (S)-tert-butyl 4-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamoyl)-phenylcarbamate S22 (0.055 g, 0.148 mmol) in acetonitrile (2 mL) at 0 °C. The mixture was allowed to warm to room temperature slowly and was stirred for 24 h. The volatiles were evaporated and the products were purified by reversed phase flash column using a acetonitrile–water gradient to yield (S)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-4-(2,2,2-trifluoroacetamido)benzamide (whitish crystals, 0.02 g, 0.054 mmol, 36% yield) and (S)-4-amino-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)benzamide 2,2,2-trifluoroacetate (colorless oil, 0.03 g, 0.078 mmol, 52% yield).

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-4-(2,2,2-trifluoroacetamido)benzamide (45). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.21 (t, *J* = 5.9 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 6.55 (d, *J* = 8.6 Hz, 2H), 5.64 (s, 2H), 4.75 (dd, *J* = 7.3, 3.7 Hz, 1H), 4.07 (dd, *J* = 16.8, 5.6 Hz, 1H), 3.98 (dd, *J* = 16.8, 5.5 Hz, 1H), 3.72–3.64 (m, 1H), 3.54–3.44 (m, 1H), 2.22–1.95 (m, 4H); LC–MS (A) (ESI) *t*_R 6.5 min, *m/z* 273.0 [M + H]⁺ (97%).

(S)-4-Amino-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-benzamide 2,2,2-Trifluoroacetate (46). ¹H NMR (400 MHz, CDCl₃) (mixture of cis/trans amide rotamers, 1/5) δ 8.09 (s, 1H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.64 (d, *J* = 8.7 Hz, 2H), 7.27 (s, 1H), 4.81–4.75 (m, 0.8H), 4.76–4.72 (m, 0.2H), 4.52–4.43 (m, 0.2H), 4.35 (dd, *J* = 17.9, 5.0 Hz, 0.8H), 4.27–4.21 (m, 0.2H), 4.16 (dd, *J* = 17.9, 3.7 Hz, 0.8H), 3.76–3.66 (m, 1H), 3.55–3.47 (m, 1H), 2.41–2.18 (m, 4H); LC–MS (A) (ESI) *t*_R 12.4 min, *m/z* 369.0 [M + H]⁺ (97%).

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1H-pyrrrole-[2,3-*b*]pyridine-4-carboxamide (47). The title product was prepared using general procedure A. ¹H NMR (400 MHz, DMSO-*d*₆) (9/1 mixture of trans/cis amide rotamers) δ 11.88 (s, 1H), 8.68 (t, *J* = 5.69 Hz, 1H), 8.33 (d, *J* = 4.91 Hz, 1H), 7.61 (dd, *J* = 2.51, 3.44 Hz, 1H), 7.42 (d, *J* = 4.92 Hz, 1H), 6.86 (dd, *J* = 1.86, 3.43 Hz, 1H), 5.29 (d, *J* = 7.22 Hz, 0.1H), 4.80 (dd, *J* = 3.62, 7.39 Hz, 0.9H), 4.38 (dd, *J* = 5.71, 16.63 Hz, 0.1H), 4.22 (dd, *J* = 6.09, 16.95 Hz, 0.9H), 4.14 (dd, *J* = 5.47, 16.93 Hz, 1H), 3.73 (ddd, *J* = 3.72, 7.57, 9.60 Hz, 1H), 3.54 (td, *J* = 6.79, 8.95 Hz, 1H), 2.34–2.01 (m, 4H); UPLC I (ESI) *t*_R 1.08 min, *m/z* 298.6 [M + H]⁺ (96%); LC–MS (A) (ESI) *t*_R 9.2 min, *m/z* 298.0 [M + H]⁺ (97%); colorless oil, 50 mg, 35%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(4-fluorophenyl)-3H-imidazo[4,5-*b*]pyridine-7-carboxamide (48). The title product was prepared using general procedure A using 2-(4-fluorophenyl)-3H-imidazo[4,5-*b*]pyridine-7-carboxylic acid S26. ¹H NMR (400 MHz, DMSO-*d*₆) (9/1 mixture of trans/cis amide rotamers) δ 14.02 (s, 1H), 10.09 (s, 1H), 8.55–8.41 (m, 3H), 7.75 (d, *J* = 5.13 Hz, 1H), 7.49 (t, *J* = 8.60 Hz, 2H), 5.29 (dd, *J* = 2.78, 7.47 Hz, 0.1H), 4.88 (dd, *J* = 3.96, 6.56 Hz, 0.9H), 4.56 (d, *J* = 4.53 Hz, 0.1H), 4.45 (dd, *J* = 5.16, 17.77 Hz, 0.9H), 4.36 (dd, *J* = 4.92, 17.84 Hz, 1H), 3.79–3.69 (m, 1H), 3.55 (q, *J* = 8.30 Hz, 1H), 2.36–1.94 (m, 4H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 167.34, 166.85, 163.21, 162.70, 152.85, 144.21, 129.72, 129.63, 127.99, 125.40, 119.28, 116.58, 116.42, 116.21, 46.36, 45.35, 42.40, 29.57, 24.80; UPLC I (ESI) *t*_R 1.52 min, *m/z* 393.5 [M + H]⁺ (96%); LC–MS (A) (ESI) *t*_R 14.7 min, *m/z* 393.0 [M + H]⁺ (97%); white powder, 53 mg, 58%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(4-methoxyphenyl)-3H-imidazo[4,5-*b*]pyridine-7-carboxamide (49). The title product was prepared using general procedure A using 2-(4-fluorophenyl)-3H-imidazo[4,5-*b*]pyridine-7-carboxylic acid S28. ¹H NMR (400 MHz, DMSO-*d*₆) (9/1 mixture of trans/cis amide rotamers) δ 10.15 (s, 1H), 8.44 (d, *J* = 5.02 Hz, 1H), 8.37 (d, *J* = 8.74 Hz, 2H), 7.72 (d, *J* = 4.99 Hz, 1H), 7.17 (d, *J* = 8.78 Hz, 2H), 5.32–5.26 (m, 0.1H), 4.89 (dd, *J* = 4.13, 6.70 Hz, 0.9H), 4.58 (dd, *J* = 4.45, 17.77 Hz, 0.1H), 4.45 (dd, *J* = 4.92, 17.95 Hz, 0.9H), 4.36 (dd, *J* = 4.78, 17.81 Hz, 1H), 3.88 (s, 3H), 3.74 (ddd, *J* = 3.71, 7.64, 11.08 Hz, 1H), 3.55 (td, *J* = 6.92, 9.11 Hz, 1H), 2.23–1.96 (m, 4H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 170.68, 166.32, 162.77, 161.25, 153.30, 149.94, 142.98, 132.23, 128.45, 120.49, 118.63, 115.75, 114.05, 54.98, 45.82, 44.80, 41.88, 29.03, 24.26; UPLC I (ESI) *t*_R 1.50 min, *m/z*

405.6 [M + H]⁺ (96%); LC–MS (A) (ESI) *t*_R 14.6 min, *m/z* 405.0 [M + H]⁺ (97%); white powder, 67 mg, 75%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)[1,2,4]triazolo-[1,5-*a*]pyrimidine-7-carboxamide (50). The title product was prepared using general procedure A. ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.59 (d, *J* = 6.96 Hz, 1H), 9.28 (s, 1H), 8.86 (s, 1H), 7.87 (d, *J* = 6.95 Hz, 1H), 4.79 (dd, *J* = 3.72, 7.35 Hz, 1H), 4.18 (d, *J* = 5.11 Hz, 2H), 3.76–3.67 (m, 1H), 3.52 (q, *J* = 8.33 Hz, 1H), 2.36–1.97 (m, 4H); UPLC I (ESI) *t*_R 1.02 min, *m/z* 300.6 [M + H]⁺ (96%); LC–MS (A) (ESI) *t*_R 9.0 min, *m/z* 300.0 [M + H]⁺ (97%); white powder, 54 mg, 57%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1,7-naphthyridine-4-carboxamide (51). The title product was prepared using general procedure G, using 1,7-naphthyridine-4-carboxylic acid S23. A 5:1 mixture of trans/cis amide rotamers, major rotamer, ¹H NMR (400 MHz, CDCl₃) δ 9.44 (d, *J* = 1.02 Hz, 1H), 8.97 (d, *J* = 4.29 Hz, 1H), 8.59 (d, *J* = 5.90 Hz, 1H), 8.12 (dd, *J* = 0.98, 5.86 Hz, 1H), 7.72 (d, *J* = 4.34 Hz, 1H), 7.64–7.57 (m, 1H), 4.76–4.69 (m, 1H), 4.41 (dd, *J* = 5.58, 17.60 Hz, 1H), 4.19 (dd, *J* = 4.00, 17.59 Hz, 1H), 3.69 (ddd, *J* = 3.37, 6.58, 11.84 Hz, 1H), 3.58–3.46 (m, 1H), 2.39–2.07 (m, 4H); minor rotamer, ¹H NMR (400 MHz, CDCl₃) δ 9.48 (d, *J* = 0.94 Hz, 1H), 9.02 (d, *J* = 4.29 Hz, 1H), 8.61 (d, *J* = 4.26 Hz, 1H), 8.10 (d, 5.86 Hz, 1H), 7.70 (d, *J* = 4.12 Hz, 1H), 7.44 (t, *J* = 4.78 Hz, 1H), 4.79 (dd, *J* = 2.04, 7.77 Hz, 1H), 4.54 (dd, *J* = 5.44, 17.23 Hz, 1H), 4.29 (dd, *J* = 3.98, 17.27 Hz, 1H), 3.69 (ddd, *J* = 3.37, 6.58, 11.84 Hz, 1H), 3.58–3.46 (m, 1H), 2.39–2.07 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 167.24, 166.00, 154.45, 151.46, 144.79, 143.45, 139.40, 127.89, 122.74, 118.00, 117.76, 46.82, 45.79, 42.45, 29.91, 25.16; UPLC I (ESI) *t*_R 1.03 min, *m/z* 310.7 [M + H]⁺ (96%); LC–MS (A) *t*_R 9.0 min, *m/z* 309.9 [M + H]⁺ (96%); white powder, 58 mg, 57%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-7-methylquinoline-4-carboxamide (52). The title product was prepared using general procedure A, using 7-methylquinoline-4-carboxylic acid S31. ¹H NMR (400 MHz, CDCl₃) δ 8.89 (d, *J* = 4.36 Hz, 1H), 8.17 (d, *J* = 8.64 Hz, 1H), 7.92–7.88 (m, 1H), 7.47–7.41 (m, 2H), 7.16 (t, *J* = 4.64 Hz, 1H), 4.79–4.73 (m, 1H), 4.58 (dd, *J* = 5.36, 17.37 Hz, 0.1H), 4.40 (dd, *J* = 5.05, 17.76 Hz, 0.9H), 4.31 (dd, *J* = 3.70, 17.23 Hz, 0.1H), 4.24 (dd, *J* = 3.98, 17.77 Hz, 0.9H), 3.70 (ddd, *J* = 2.85, 6.84, 10.97 Hz, 1H), 3.58–3.49 (m, 1H), 2.57 (s, 3H), 2.39–2.17 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 167.66, 167.14, 149.91, 149.10, 140.66, 140.60, 130.21, 128.98, 124.96, 122.52, 118.18, 117.96, 46.81, 45.75, 42.56, 30.03, 25.20, 21.93; UPLC I (ESI) *t*_R 1.14 min, *m/z* 323.5 [M + H]⁺ (98%); LC–MS (A) *t*_R 10.1 min, *m/z* 323.0 [M + H]⁺ (97%); white powder, 55 mg, 56%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-5-methylquinoline-4-carboxamide (53). The title product was prepared using general procedure G, using 5-methylquinoline-4-carboxylic acid S30. ¹H NMR (400 MHz, CDCl₃) δ 8.93–8.84 (m, 1H), 8.04–7.96 (m, 1H), 7.62 (dd, *J* = 7.06, 8.49 Hz, 1H), 7.38 (dt, *J* = 1.20, 7.04 Hz, 1H), 7.35 (d, *J* = 4.24 Hz, 1H), 7.06 (t, *J* = 4.50 Hz, 1H), 4.75 (dd, *J* = 1.99, 7.79 Hz, 0.1H), 4.72–4.67 (m, 0.9H), 4.56 (dd, *J* = 5.53, 17.38 Hz, 0.1H), 4.37 (dd, *J* = 4.95, 17.82 Hz, 0.9H), 4.32 (dd, *J* = 5.53, 17.38 Hz, 0.1H), 4.23 (dd, *J* = 3.95, 17.81 Hz, 0.9H), 3.70 (ddd, *J* = 2.77, 7.09, 11.25 Hz, 1H), 3.52 (dt, *J* = 7.63, 10.69 Hz, 1H), 2.65 (s, 3H), 2.42–2.09 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 170.38, 166.83, 149.68, 149.13, 141.90, 134.49, 130.19, 129.73, 128.76, 123.43, 119.96, 117.89, 46.78, 45.74, 42.61, 30.05, 25.19, 21.31; UPLC I (ESI) *t*_R 1.07 min, *m/z* 323.5 [M + H]⁺ (99%); LC–MS (A) *t*_R 10.0 min, *m/z* 323.1 [M + H]⁺ (98%); white powder, 72 mg, 47%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-7-phenylquinoline-4-carboxamide (54). The title product was prepared using general procedure G, using 7-phenylquinoline-4-carboxylic acid S24. ¹H NMR (400 MHz, CDCl₃) δ 8.96 (d, *J* = 4.35 Hz, 1H), 8.38–8.33 (m, 2H), 7.89 (dd, *J* = 2.01, 8.66 Hz, 1H), 7.79–7.71 (m, 2H), 7.55–7.46 (m, 3H), 7.47–7.37 (m, 1H), 7.22 (t, *J* = 4.58 Hz, 1H), 7.12 (t, *J* = 4.28 Hz, 0.1H), 4.77 (dd, *J* = 2.41, 7.67 Hz, 1H), 4.60 (dd, *J* = 5.54, 17.33 Hz, 0.1H), 4.43 (dd, *J* = 5.07, 17.75 Hz, 0.9H), 4.34 (dd, *J* = 3.51, 17.28 Hz, 0.1H), 4.26 (dd, *J* = 4.02, 17.77 Hz, 0.9H), 3.72 (ddd, *J* = 2.95, 7.09, 9.42 Hz, 1H), 3.59–3.50 (m, 1H), 2.51–2.12 (m, 4H);

^{13}C NMR (101 MHz, CDCl_3) δ 167.35, 166.97, 150.32, 149.12, 142.68, 140.57, 139.78, 129.07, 128.16, 127.50, 127.47, 127.39, 125.73, 123.49, 118.69, 117.83, 46.72, 45.65, 42.48, 29.94, 25.10; LC-MS (A) (ESI) t_{R} 14.6 min, m/z 385.1 $[\text{M} + \text{H}]^+$ (95%); white powder, 86 mg, 74%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-7-(phenylamino)quinoline-4-carboxamide (55). The title product was prepared using general procedure G, using 7-(phenylamino)-quinoline-4-carboxylic acid **S25**. ^1H NMR (400 MHz, CDCl_3) δ 8.67 (d, J = 4.43 Hz, 1H), 8.07 (d, J = 9.10 Hz, 1H), 7.57 (d, J = 2.39 Hz, 1H), 7.42 (t, J = 4.78 Hz, 1H), 7.33–7.27 (m, 2H), 7.20 (ddd, J = 1.60, 2.58, 8.15 Hz, 4H), 7.02 (tt, J = 1.21, 7.35 Hz, 1H), 6.53 (s, 1H), 4.75 (dd, J = 1.94, 7.81 Hz, 0.1H), 4.72–4.66 (m, 0.9H), 4.50 (dd, J = 5.62, 17.21 Hz, 0.1H), 4.33 (dd, J = 5.36, 17.59 Hz, 0.9H), 4.24 (dd, J = 3.96, 17.21 Hz, 0.1H), 4.13 (dd, J = 4.18, 17.59 Hz, 0.9H), 3.62 (ddd, J = 3.23, 7.04, 9.02 Hz, 1H), 3.44 (td, J = 4.49, 8.52, 9.05 Hz, 1H), 2.32–2.08 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.88, 167.29, 150.41, 150.23, 145.40, 141.28, 140.57, 129.56, 126.41, 122.81, 120.71, 119.91, 119.07, 118.12, 115.97, 110.03, 46.77, 45.72, 42.42, 29.93, 25.16; LC-MS (A) (ESI) t_{R} 11.8 min, m/z 400.0 $[\text{M} + \text{H}]^+$ (98%); white powder, 78 mg, 73%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-5-phenylquinoline-4-carboxamide (56). The title product was prepared using general procedure G, using 5-phenylquinoline-4-carboxylic acid **S32**. ^1H NMR (400 MHz, CDCl_3) (mixture of 1:9 cis/trans amide rotamer) major rotamer, δ 8.98 (d, J = 4.21 Hz, 1H), 8.20 (dd, J = 1.30, 8.46 Hz, 1H), 7.80 (dd, J = 7.14, 8.47 Hz, 1H), 7.52 (dd, J = 1.35, 7.16 Hz, 1H), 7.49–7.46 (m, 1H), 7.43 (s, 1H), 7.33 (dd, J = 8.72, 11.73 Hz, 2H), 7.05 (s, 1H), 6.49–6.43 (m, 2H), 4.77–4.71 (m, 1H), 4.43 (dd, J = 4.80, 17.69 Hz, 1H), 4.29 (dd, J = 4.01, 17.76 Hz, 1H), 3.44 (ddd, J = 3.19, 7.76, 10.27 Hz, 1H), 3.32–3.10 (m, 1H), 2.44–2.10 (m, 4H); minor rotamer, ^1H NMR (400 MHz, CDCl_3) δ 8.95 (d, J = 4.26 Hz, 1H), 8.16 (dd, J = 1.24, 8.46 Hz, 1H), 7.60 (dd, J = 7.55, 8.47 Hz, 1H), 7.55 (d, J = 4.33 Hz, 1H), 7.49 (d, J = 4.27 Hz, 1H), 7.43 (s, 1H), 7.33 (dd, J = 8.72, 11.73 Hz, 2H), 6.94 (s, 1H), 6.49–6.43 (m, 2H), 4.79 (d, J = 5.81 Hz, 1H), 4.43 (dd, J = 4.80, 17.69 Hz, 1H), 4.29 (dd, J = 4.01, 17.76 Hz, 1H), 3.76–3.66 (m, 1H), 3.62–3.50 (m, 1H), 2.44–2.10 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.91, 166.54, 149.70, 141.36, 139.43, 133.70, 131.05, 129.81, 129.32, 128.11, 127.02, 122.45, 121.11, 118.86, 117.70, 100.00, 46.55, 45.37, 42.20, 29.98, 24.97; LC-MS (A) (ESI) t_{R} 13.3 min, m/z 385.1 $[\text{M} + \text{H}]^+$ (96%); colorless oil, 52 mg, 84%.

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-5-methoxyquinoline-4-carboxamide (57). The title product was prepared using general procedure G, using 5-methoxyquinoline-4-carboxylic acid **S33**. ^1H NMR (400 MHz, CDCl_3) (9:1 mixture of trans/cis amide rotamers) δ 9.01 (d, J = 5.51 Hz, 1H), 8.70 (dd, J = 0.83, 8.64 Hz, 1H), 8.21 (d, J = 8.64 Hz, 1H), 7.74 (dt, J = 0.92, 8.66 Hz, 1H), 7.66 (dd, J = 7.62, 8.60 Hz, 1H), 6.92 (dd, J = 1.01, 7.73 Hz, 1H), 4.88 (d, J = 6.04 Hz, 0.1H), 4.85–4.81 (m, 0.9H), 4.58 (dd, J = 6.21, 17.03 Hz, 0.1H), 4.42 (dd, J = 5.53, 17.72 Hz, 0.9H), 4.34–4.27 (m, 0.1H), 4.25 (dd, J = 4.34, 17.75 Hz, 0.9H), 4.02 (s, 3H), 3.78–3.68 (m, 1H), 3.63–3.49 (m, 1H), 2.41–2.17 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.50, 164.82, 155.19, 149.17, 147.28, 132.81, 130.53, 122.20, 121.83, 118.15, 118.00, 105.71, 56.01, 46.80, 45.81, 42.34, 30.08, 25.29; UPLC I (ESI) t_{R} 1.61 min, m/z 339.6 $[\text{M} + \text{H}]^+$ (97%); LC-MS (A) t_{R} 14.0 min, m/z 339.1 $[\text{M} + \text{H}]^+$ (97%); white crystals, 63 mg, 64%.

N-(2-(2S,4S)-2-Cyano-4-fluoropyrrolidin-1-yl)-2-oxoethyl-quinoline-4-carboxamide (58). The title product was prepared using general procedure G with 2-(quinoline-4-carboxamido)acetic acid and **S16**. ^1H NMR (400 MHz, CDCl_3) (1:9 mixture of cis/trans amide rotamers) δ 8.87 (d, J = 4.45 Hz, 1H), 8.23 (ddd, J = 0.66, 1.36, 8.60 Hz, 1H), 8.13–8.09 (m, 1H), 7.76–7.69 (m, 1H), 7.60–7.54 (m, 1H), 7.48 (d, J = 4.33 Hz, 1H), 7.41–7.35 (m, 1H), 5.43 (dt, J = 3.29, 51.00 Hz, 0.9H), 5.34 (dt, J = 3.38, 51.11 Hz, 0.1H), 5.04 (d, J = 8.96 Hz, 0.1H), 4.92 (d, J = 9.29 Hz, 0.9H), 4.62 (dd, J = 5.79, 17.21 Hz, 0.1H), 4.37 (dd, J = 5.64, 17.48 Hz, 0.9H), 4.29 (dd, J = 4.02, 17.22 Hz, 0.1H), 4.17 (dd, J = 4.19, 17.49 Hz, 0.9H), 3.96 (ddd, J = 1.21, 11.79, 22.38 Hz, 1H), 3.76 (ddd, J = 3.62, 12.05, 36.05 Hz, 1H), 2.66

(t, J = 14.69 Hz, 1H), 2.35 (dddd, J = 3.51, 9.44, 14.83, 40.62 Hz, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.53, 167.30, 149.58, 148.15, 141.17, 130.28, 129.49, 127.94, 125.24, 124.35, 118.90, 117.24, 92.83, 91.03, 52.46, 52.22, 45.09, 42.34, 36.09, 35.88; UPLC I (ESI) t_{R} 1.11 min, m/z 327.6 $[\text{M} + \text{H}]^+$ (98%); LC-MS (A) t_{R} 9.5 min, m/z 327.0 $[\text{M} + \text{H}]^+$ (98%); white crystals, 95 mg, 60%.

N-(2-(2S,4R)-2-Cyano-4-fluoropyrrolidin-1-yl)-2-oxoethyl-quinoline-4-carboxamide (59). The title product was prepared using general procedure G with 2-(quinoline-4-carboxamido)acetic acid and **S15**. ^1H NMR (400 MHz, CDCl_3) (1:9 mixture of cis/trans amide rotamers) δ 8.94 (d, J = 4.39 Hz, 1H), 8.31–8.24 (m, 1H), 8.17 (dd, J = 1.16, 8.63 Hz, 1H), 7.77 (ddd, J = 1.41, 6.87, 8.45 Hz, 1H), 7.62 (ddd, J = 1.24, 6.92, 8.32 Hz, 1H), 7.56 (d, J = 4.33 Hz, 1H), 7.35 (s, 0.9H), 7.12 (s, 0.1H), 5.41–5.25 (m, 1H), 4.93 (t, J = 8.15 Hz, 0.1H), 4.80 (dd, J = 7.86, 9.23 Hz, 0.9H), 4.63 (dd, J = 5.23, 17.31 Hz, 0.1H), 4.43 (dd, J = 4.62, 17.54 Hz, 0.9H), 4.28 (dd, J = 4.24, 17.50 Hz, 1H), 3.96 (dd, J = 12.24, 20.03 Hz, 1H), 3.82 (ddd, J = 3.13, 12.30, 34.74 Hz, 0.9H), 3.60 (dd, J = 12.96, 33.60 Hz, 0.1H), 2.91–2.76 (m, 1H), 2.49 (dddd, J = 3.91, 9.27, 14.76, 37.98 Hz, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.44, 167.24, 149.32, 147.91, 141.58, 130.68, 129.38, 128.29, 125.45, 124.60, 119.03, 117.18, 91.70, 89.88, 52.52, 52.29, 45.29, 42.74, 36.63, 36.41, 3; UPLC I (ESI) t_{R} 1.09 min, m/z 327.6 $[\text{M} + \text{H}]^+$ (99%); LC-MS (A) t_{R} 9.1 min, m/z 327.0 $[\text{M} + \text{H}]^+$ (98%); white crystals, 86 mg, 63%.

(S)-N-(2-(2-Cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-quinoline-4-carboxamide (60). The title product was prepared using general procedure A. ^1H NMR (400 MHz, CDCl_3) (8.5/1.5 mixture of trans/cis amide rotamers) δ 8.96–8.86 (m, 1H), 8.23 (t, J = 9.99 Hz, 1H), 8.12 (d, J = 8.42 Hz, 1H), 7.74 (t, J = 7.69 Hz, 1H), 7.60 (dd, J = 11.22, 4.11 Hz, 1H), 7.49 (dd, J = 10.11, 4.30 Hz, 1H), 7.30 (s, 1H), 5.15 (d, J = 9 Hz, 0.15H), 4.99–4.92 (m, 0.85H), 4.70 (dd, J = 17.4, 5.7 Hz, 0.15H), 4.39 (dd, J = 17.4, 5.6 Hz, 0.85H), 4.33 (dd, J = 17.4, 4.3 Hz, 0.15H), 4.21 (dd, J = 17.4, 4.2 Hz, 0.85H), 4.07–3.91 (m, 2H), 2.83–2.72 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.68, 167.40, 149.93, 148.82, 140.67, 130.30, 130.11, 128.08, 125.24, 124.41, 118.93, 116.09, 52.54, 52.22, 51.90, 44.44, 42.42, 37.78, 37.53, 37.28, 31.07; MS (ESI) m/z 345.0 $[\text{M} + 1]^+$; LC-MS (A) t_{R} 10.8 min, m/z 345.0 $[\text{M} + \text{H}]^+$ (98%); HRMS calcd for $\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}_2\text{F}_2$ $[\text{M} + \text{H}]^+$, 345.1163; found, 345.1172; off-white powder, 350 mg, 67%.

(S)-N-(2-(2-Cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-6-methoxyquinoline-4-carboxamide (61). The title product was prepared using general procedure A. ^1H NMR (400 MHz, CDCl_3) (9/1 mixture of trans/cis amide rotamers) δ 8.63 (d, J = 4.43 Hz, 1H), 7.90 (d, J = 9.19 Hz, 1H), 7.54 (d, J = 2.75 Hz, 1H), 7.47–7.40 (br s, 1H), 7.35 (d, J = 4.40 Hz, 1H), 7.31 (dd, J = 2.78, 9.24 Hz, 1H), 5.19–5.12 (m, 0.1H), 4.91 (dd, J = 4.58, 8.37 Hz, 0.9H), 4.52 (d, J = 5.77 Hz, 0.1H), 4.30 (dd, J = 5.71, 17.35 Hz, 0.9H), 4.12 (d, J = 4.73, 17.33 Hz, 1H), 4.08–3.86 (m, 2H), 3.86 (s, 3H), 2.83–2.65 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 168.18, 167.71, 158.77, 147.05, 144.85, 139.04, 131.13, 127.88, 125.54, 123.14, 119.15, 116.40, 102.85, 55.83, 52.05 (t, J = 32.25 Hz), 44.41, 42.20, 37.26 (t, J = 25.29 Hz); UPLC I (ESI) t_{R} 1.28 min, m/z 375.6 $[\text{M} + \text{H}]^+$ (99%); LC-MS (A) (ESI) t_{R} 11.6 min, m/z 374.9 $[\text{M} + \text{H}]^+$ (98%); white powder, 61 mg, 33%.

(S)-5-Bromo-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (62). The title product was prepared using general procedure A. ^1H NMR (400 MHz, CDCl_3) (cis/trans mixture of amide rotamers, 1:9) δ 9.10 (dd, J = 4.46, 6.43 Hz, 0.9H), 8.88 (s, 0.1H), 8.41 (d, J = 8.76 Hz, 1H), 8.05 (d, J = 8.73 Hz, 1H), 7.96 (d, J = 8.55 Hz, 1H), 7.84 (dd, J = 1.06, 7.58 Hz, 1H), 7.55 (dd, J = 7.47, 8.49 Hz, 1H), 5.34 (d, J = 8.83 Hz, 0.1H), 5.03 (dd, J = 5.05, 8.22 Hz, 0.9H), 4.64 (dd, J = 6.13, 17.08 Hz, 0.1H), 4.56 (dd, J = 6.74, 17.53 Hz, 0.9H), 4.30 (dd, J = 4.51, 17.10 Hz, 0.1H), 4.17 (dd, J = 4.17, 17.48 Hz, 0.9H), 4.14–4.05 (m, 1H), 4.04–3.95 (m, 1H), 2.84–2.73 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 168.40, 164.31, 149.25, 146.80, 136.27, 131.65, 130.18, 129.81, 128.24, 121.69, 119.40, 116.80, 53.55, 51.98 (t, 32.1 Hz, 1C), 44.42, 41.96, 37.19 (t, 25.4 Hz, 1C); UPLC I (ESI) t_{R} 1.83 min, m/z 423.5, 425.3 $[\text{M} + \text{H}]^+$ (96%); LC-MS (A) t_{R} 15.8 min, m/z 265.9, 267.9 (95%); white powder, 89 mg, 54%.

(S)-5-Cyano-N-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (63). To a round-bottom flask were added (S)-5-bromo-N-((2-cyano-4,4-difluoropyrrolidin-1-yl)-methyl)quinoline-4-carboxamide **65** (0.085 g, 0.215 mmol), zinc cyanide (0.015 g, 0.129 mmol) (caution, highly toxic), 10% palladium on carbon (0.023 g, 0.022 mmol), 1,1'-bis(diphenylphosphino)-ferrocene (4.82 mg, 8.60 μ mol), and DMAC (volume, 2 mL). The resulting slurry was sparged with subsurface nitrogen for 10 min, and zinc(II) formate dihydrate (6.2 mg, 0.032 mmol) was added to the reaction mixture. The reaction mixture was again sparged with subsurface nitrogen for 10 min and was heated under nitrogen to 110 °C for 2 h. The reaction mixture was diluted with 10 mL of EtOAc. The resulting slurry was filtered, and the cake was rinsed with EtOAc (2 mL). The product was isolated by washing the filtrate with water (2 $\times$ 10 mL) and 5% NH_4OH (1 $\times$ 10 mL). The organic layer was dried with Na_2SO_4 . The volatile was removed in vacuo to give a residue, which was further purified by silica gel chromatography (heptanes/EtOAc) to provide the product (white powder, 40 mg, 50%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 9.20 (t, J = 5.73 Hz, 1H), 8.73 (d, J = 8.75 Hz, 1H), 8.49 (d, J = 8.53 Hz, 1H), 8.44–8.37 (m, 2H), 8.05 (dd, J = 7.08, 8.62 Hz, 1H), 5.13 (dd, J = 2.65, 9.28 Hz, 1H), 4.37–4.27 (m, 1H), 4.27 (d, J = 5.92 Hz, 2H), 4.14 (dt, J = 10.32, 21.10 Hz, 1H), 3.00–2.73 (m, 2H); UPLC II (ESI) t_R 3.24 min, m/z 370.6 $[\text{M} + \text{H}]^+$ (95%).

N-(2-Oxo-2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (64). The title product was prepared using general procedure A using 2-amino-1-(pyrrolidin-1-yl)ethanone hydrochloride **S34**. The product was purified using column chromatography with a DCM–MeOH gradient, followed by recrystallization from methanol. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.99 (d, J = 4.30 Hz, 1H), 8.92 (t, J = 5.90 Hz, 1H), 8.41 (d, 8.16 Hz, 1H), 8.09 (d, J = 8.32 Hz, 1H), 7.82 (ddd, J = 1.45, 6.88, 8.44 Hz, 1H), 7.68 (ddd, J = 1.31, 6.84, 8.25 Hz, 1H), 7.55 (d, J = 4.27 Hz, 1H), 4.17–4.07 (m, 2H), 3.51 (t, J = 6.75 Hz, 2H), 3.37 (t, J = 6.89 Hz, 2H), 1.94 (h, J = 6.12, 6.65 Hz, 2H), 1.87–1.75 (m, 2H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 167.55, 166.67, 150.69, 148.38, 142.83, 130.28, 129.66, 127.63, 126.45, 124.74, 119.44, 46.16, 45.46, 42.13, 26.16, 24.22; UPLC I (ESI) t_R 1.11 min, m/z 284.7 $[\text{M} + \text{H}]^+$ (98%); LC–MS (A) t_R 7.8 min, m/z 284.1 $[\text{M} + \text{H}]^+$ (98%); white powder, 0.187 g, 73%.

N-(2-Oxo-2-((2R)-2-((3aS,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (65). (2R)-2-((3aS,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidine hydrochloride³³ (0.190 g, 0.665 mmol) was added to a mixture of 2-(quinoline-4-carboxamido)acetic acid (0.153 g, 0.665 mmol), HATU (0.253 g, 0.665 mmol), and DIPEA (0.360 mL, 2.062 mmol) in dichloromethane (5 mL). The mixture was stirred for 3 h and washed with 0.5 N citric acid, saturated sodium bicarbonate, and brine, followed by filtration and evaporation. Purification was done using column chromatography DCM–MeOH, 0–6% MeOH. ^1H NMR (400 MHz, CDCl_3) δ 8.97 (d, J = 4.36 Hz, 1H), 8.30 (ddd, J = 0.72, 1.48, 8.50 Hz, 1H), 8.17 (dd, J = 1.25, 8.28 Hz, 1H), 7.77 (ddd, J = 1.44, 6.92, 8.47 Hz, 1H), 7.62 (ddd, J = 1.29, 6.94, 8.30 Hz, 1H), 7.53 (d, J = 4.34 Hz, 1H), 7.19 (t, J = 4.10 Hz, 1H), 4.32–4.27 (m, 3H), 3.61–3.39 (m, 2H), 3.21 (dd, J = 6.93, 10.00 Hz, 1H), 2.50–2.25 (m, 1H), 2.25–1.95 (m, 5H), 1.94–1.78 (m, 3H), 1.41 (s, 3H), 1.28–1.24 (m, 4H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 167.01, 165.44, 149.56, 130.42, 129.57, 128.03, 125.63, 124.73, 119.02, 86.32, 78.05, 77.36, 53.57, 51.35, 45.86, 42.35, 39.61, 38.75, 38.35, 35.62, 28.59, 27.53, 27.34, 27.20, 26.41, 24.15; colorless oil, 0.270 g, 88%.

(R)-1-(2-(Quinoline-4-carboxamido)acetyl)pyrrolidin-2-ylboronic Acid (66). To a stirred solution of the N-(2-oxo-2-((2R)-2-((3aS,7aR)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide **69** (0.27 g, 0.585 mmol) in water (8 mL) at pH 3 (adjusting as necessary with 2 N aqueous HCl) were added phenylboronic acid (0.143 g, 1.17 mmol) and methyl *tert*-butyl ether (7 mL). The mixture was stirred for 2 days (pH 4). Water and the organic layer were separated. The water layer was adjusted to pH 3, and the water layer

was extracted with a mixture 4:1 of DCM–isopropanol. The DCM layer was dried over sodium sulfate, filtered, evaporated, and further purified using column chromatography (DCM–MeOH, 0–10% MeOH) to yield a clear oil of 0.119 g (79%) of (R)-1-(2-(quinoline-4-carboxamido)acetyl)pyrrolidin-2-ylboronic acid. ^1H NMR (400 MHz, D_2O) δ 8.92–8.84 (m, 1H), 8.15 (ddd, J = 0.64, 1.43, 8.58 Hz, 1H), 8.09–8.00 (m, 1H), 7.84 (ddd, J = 1.41, 6.92, 8.47 Hz, 1H), 7.70 (ddd, J = 1.18, 6.94, 8.29 Hz, 1H), 7.64 (d, J = 4.46 Hz, 1H), 4.32–4.27 (m, 2H), 3.67 (ddd, J = 3.33, 8.37, 10.14 Hz, 1H), 3.55 (ddd, J = 6.54, 8.44, 10.90 Hz, 1H), 3.12 (dd, J = 7.01, 10.34 Hz, 1H), 2.13 (tdd, J = 3.42, 7.02, 9.03 Hz, 2H), 2.08–1.87 (m, 1H), 1.82–1.68 (m, 1H); ^{13}C NMR (101 MHz, D_2O) δ 170.63, 168.11, 150.41, 147.54, 142.15, 131.50, 128.92, 128.64, 125.66, 124.61, 119.99, 48.94 (^{13}C -B splitting), 47.22, 42.32, 27.48, 27.43; UPLC I (ESI) t_R 1.01 min, m/z 328.6 $[\text{M} + \text{H}]^+$ (99%); LC–MS (A) t_R 9.3 min, m/z 328.0 $[\text{M} + \text{H}]^+$ (98%); HRMS calcd for $\text{C}_{16}\text{H}_{19}\text{BN}_3\text{O}_4$ $[\text{M} + \text{H}]^+$, 328.1469; found, 328.1469.

(S)-N-(2-(2-(2-Chloroacetyl)pyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (67). 4 M HCl (10.90 mL, 43.6 mmol) in dioxane was added to (S)-*tert*-butyl 2-(2-chloroacetyl)pyrrolidine-1-carboxylate (1.2 g, 4.84 mmol) **S35** and stirred for 1 h. Then the volatiles were evaporated and the residue was washed with ether to give 0.9 g of (S)-2-chloro-1-(pyrrolidin-2-yl)ethanone hydrochloride. The product was used in the next reaction without further purification.

To a solution containing 2-(quinoline-4-carboxamido)acetic acid (0.180 g, 0.782 mmol) and HOBt (0.132 g, 0.860 mmol) in 1,4-dioxane (5 mL) was added a solution of EDC (0.165 g, 0.860 mmol) in DCM (5 mL). The mixture was stirred for 30 min at 0 °C. To the resulting solution was added the appropriate amine (S)-2-chloro-1-(pyrrolidin-2-yl)ethanone hydrochloride (0.144 g, 0.782 mmol) and DIPEA (0.150 mL, 0.860 mmol) in DCM (4 mL), with stirring. After 2 h (on completion of the reaction), the reaction mixture was diluted with DCM and washed with saturated aqueous NaHCO_3 solution (10 mL), 0.1 N aqueous citric acid solution (10 mL), and brine (10 mL). The combined organic layers were dried over MgSO_4 , filtered, concentrated, and purified with flash chromatography using DCM–MeOH with 0–5% methanol (slightly yellowish oil, 0.120 g, 43%). ^1H NMR (400 MHz, CDCl_3) δ 8.96 (d, J = 4.38 Hz, 1H), 8.29 (dd, J = 1.36, 8.17 Hz, 1H), 8.19 (d, J = 8.17 Hz, 1H), 7.78 (ddd, J = 1.41, 6.99, 8.50 Hz, 1H), 7.64 (ddd, J = 1.22, 6.91, 8.30 Hz, 1H), 7.54 (d, J = 4.32 Hz, 1H), 7.02 (s, 1H), 4.83 (dd, J = 5.10, 8.35 Hz, 1H), 4.43 (dd, J = 4.78, 17.74 Hz, 1H), 4.33 (d, J = 15.29 Hz, 1H), 4.35–4.25 (m, 1H), 4.23 (d, J = 15.33 Hz, 1H), 3.71 (ddd, J = 5.79, 7.27, 9.78 Hz, 1H), 3.63 (dt, J = 6.68, 9.75 Hz, 1H), 2.35–1.97 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 200.81, 167.32, 166.61, 149.78, 148.58, 141.31, 130.25, 129.78, 127.98, 125.42, 124.54, 118.95, 62.52, 47.19, 46.54, 42.41, 28.81, 25.16; UPLC I (ESI) t_R 1.25 min, m/z 360.6 $[\text{M} + \text{H}]^+$ (95%); LC–MS (A) t_R 11.0 min, m/z 360.1 $[\text{M} + \text{H}]^+$ (98%).

■ ASSOCIATED CONTENT

● Supporting Information

Detailed synthetic procedures and analytical data for intermediates, protocols of the enzymatic assays used, and procedures and extended data series for in vitro and in vivo pharmacokinetic analyses. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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■ ABBREVIATIONS USED

FAP, fibroblast activation protein; PREP, prolyl oligopeptidase; DPPIV, dipeptidyl peptidase IV; DPPII, dipeptidyl peptidase II; DPP8, dipeptidyl peptidase 8; DPP9, dipeptidyl peptidase 9

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Supporting information for:

Extended structure-activity relationship and pharmacokinetic evaluation of (4-quinolinoyl)-glycyl-2-cyanopyrrolidine inhibitors of fibroblast activation protein (FAP)

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I. Synthetic procedures and analytical data for intermediates

This part contains detailed procedures and analytical information for the intermediates specified below, the synthesis of which was not included in the manuscript text or described earlier in literature:

I.1 (S)-4,4-difluoro-1-glycylpyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S8). This compound is used as a building block for the synthesis of final compounds **60-63**. The further synthesis of these final products is given in **Scheme 2** of the manuscript.

I.2 N-methyl-1-(quinolin-4-yl)methanamine (S9) and 2-methoxy-N-(quinolin-4-ylmethyl)ethanamine (S10). These intermediates were used as building blocks for the synthesis of final compounds **21** and **22**. The further synthesis of these final products is given in **Scheme 1** of the manuscript.

I.3 2-Phenylisonicotinic acid (S11), 2-(3,4-dimethoxyphenyl)isonicotinic acid (S12) and 2-(4-cyanophenyl)isonicotinic acid (S13). These intermediates were used in the synthesis of final compounds **33-35**. The further synthesis of these final products is given in **Scheme 1** of the manuscript.

I.4 (2S,4R)-4-fluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S15) and (2S,4S)-4-fluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S16). As described in the manuscript, these compounds were originally synthesized by us using literature procedures (reference 29 of the manuscript). An optimized protocol that renders substantially higher yields is described here. These intermediates were used in the synthesis of final compounds **58** and **59**. The further synthesis of these final products is given in **Scheme 2** of the manuscript.

I.5 2-(4-Fluorophenyl)-7-methyl-3H-imidazo[4,5-b]pyridine (S17), 2-(4-fluorophenyl)-3H-imidazo[4,5-b]pyridine-7-carboxylic acid (S18), 2-(4-methoxyphenyl)-7-methyl-3H-imidazo[4,5-b]pyridine (S19) and 2-(4-methoxyphenyl)-3H-imidazo[4,5-b]pyridine-7-carboxylic acid (S20). Intermediates S17 and S18 were used as building blocks for final compound **48**. Intermediates S19 and S20 were used for the synthesis of final compound **49**. The further synthesis of these final products is given in **Scheme 1** of the manuscript.

I.6 4-Bromo-1,7-naphthyridine (S21), 1,7-naphthyridine-4-carbonitrile (S22) and 1,7-naphthyridine-4-carboxylic acid (S23). These intermediates were used in the synthesis of final compound **51**. The further synthesis of this final product is given in **Scheme 1** of the manuscript.

I.7 7-Phenylquinoline-4-carboxylic acid (S24) and 7-(phenylamino)quinoline-4-carboxylic acid (S25). These intermediates were used in the synthesis of final compounds **54** and **55**. The further synthesis of these final products is given in **Scheme 1** of the manuscript.

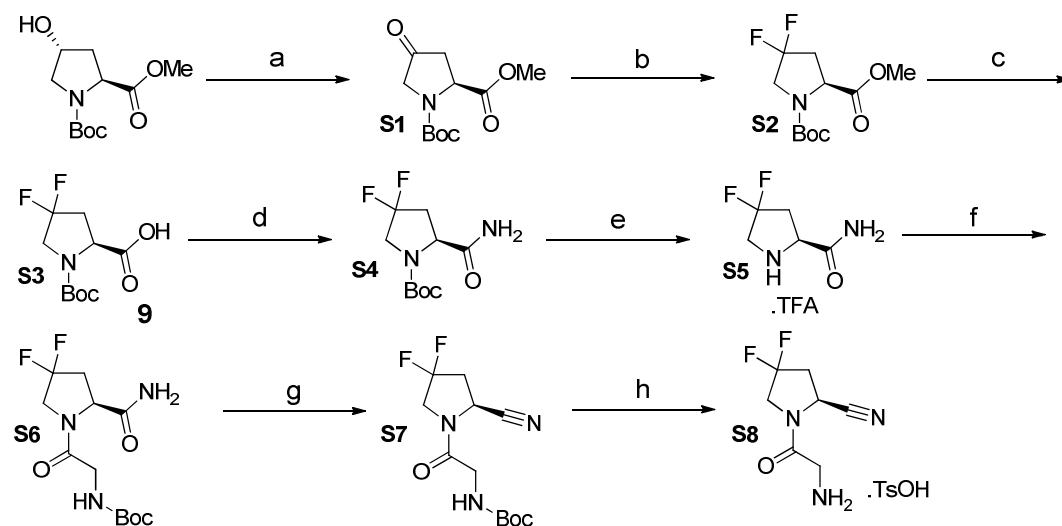
I.8 4-Methylisatin (S26), 6-methylisatin (S27), 5-methylquinoline-2,4-dicarboxylic acid (S28), 7-methylquinoline-2,4-dicarboxylic acid (S29), 5-methylquinoline-4-carboxylic acid (S30) and 7-methylquinoline-4-carboxylic acid (S31). These intermediates were used in the synthesis of final compounds **52** and **53**. The further synthesis of these final products is given in **Scheme 1** of the manuscript.

I.9 5-Phenylquinoline-4-carboxylic acid (S32) and 5-methoxyquinoline-4-carboxylic acid (S33). These intermediates were used in the synthesis of final compounds **56** and **57**. The further synthesis of these final products is given in **Scheme 1** of the manuscript.

I.10 2-Amino-1-(pyrrolidin-1-yl)ethanone hydrochloride (S34) and (*S*)-*tert*-butyl 2-(2-chloroacetyl)pyrrolidine-1-carboxylate (S35). These intermediates were used in the synthesis of final compounds **64** and **67**. The further synthesis of these final products is given in **Scheme 2** of the manuscript.

I.1 (*S*)-4,4-difluoro-1-glycylpyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S8)

Scheme S1: synthesis of (*S*)-4,4-difluoro-1-glycylpyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S8)



^aa) TEMPO (0.02 eq), 1,3,5-Trichloro-1,3,5-triazine-2,4,6-trione, DCM, 1 h, 0°C, 74%; b) DAST, DCM, 16 h, 61%; c) KOH, MeOH, 16 h, 86%; d) DCC, HONSu, NH₃, DCM, 2 h, 72%; e) TFA, DCM, 1 h, 90%; f) HATU, Boc-Gly-OH, DIPEA, DCM, 3 h, 77%; g) TFAA, pyridine, THF, -15°C, 70%; h) TsOH, MeCN, 24 h, 90%.

(S)-1-tert-butyl 2-methyl 4-oxopyrrolidine-1,2-dicarboxylate (S1)

1,3,5-Trichloro-1,3,5-triazinane-2,4,6-trione (3.78 g, 16.27 mmol) was added to a cooled (0°C) solution of (S)-1-tert-butyl 2-methyl 4-oxopyrrolidine-1,2-dicarboxylate (3.8 g, 15.49 mmol) in DCM (25 ml), followed by the addition of catalytic TEMPO (0.024 g, 0.155 mmol). After 5 min the mixture was allowed to reach room temperature, stirred for another 30 minutes and filtrated over Celite. The organic layer was washed with 20 ml saturated potassium carbonate solution, sodium thiosulfate, brine, dried over anhydrous sodium sulfate, filtrated and evaporated. The crude compound **1** was used without further purification.

¹H NMR (400 M Hz, CDCl₃): δ 4.77 (dd, J = 36.8, 8 Hz, 1H), 3.88 (br s, 2H), 3.75 (s, 3H), 2.90 (s, 1H), 2.57 (dd, J = 18.8, 2.4, 1H), 1.46 (s, 9H); MS (ESI) m/z 376.2 [M + MeOH + H]⁺; (2.77 g, 74%)

(S)-1-tert-butyl 2-methyl 4,4-difluoropyrrolidine-1,2-dicarboxylate (S2)

A solution of the (S)-1-tert-butyl 2-methyl 4-oxopyrrolidine-1,2-dicarboxylate (0.23 g, 0.946 mmol), obtained from step 1, in DCM (3 ml), in a 25-ml flask equipped under nitrogen and stirring bar, was treated with a solution of diethylaminosulfur trifluoride (0.197 ml, 1.607 mmol) in DCM (2 ml) at room temperature. Ethanol (0.011 ml, 0.189 mmol) was added and the mixture was stirred for 18h at room temperature. The solution was poured into saturated sodium bicarbonate and after CO₂ evolution ceased it was extracted into DCM (3 x 15 ml), dried (Na₂SO₄), filtered, and evaporated in vacuo. Chromatography on silica gel in DCM afforded a yellowish oil.

¹H NMR (400 MHz, CDCl₃): δ 4.55 - 4.45 (m, 1H), 3.90 - 3.60 (m, 2H), 3.75 (s, 3H), 2.81 - 2.61 (m, 1H), 2.45 (dq, J = 13.6, 5.2 Hz, 1H), 1.44 (br s, 9H), MS (ESI) m/z 266.1 [M + H]⁺; (0.150 g, 61%)

(S)-1-(tert-butoxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylic acid (S3)

(S)-1-tert-butyl 2-methyl 4,4-difluoropyrrolidine-1,2-dicarboxylate (1.51 g, 5.69 mmol), obtained from step 2, was dissolved in 6 ml of 1M potassium hydroxide solution. The solution was stirred overnight. The mixture was washed with ether, acidified, extracted with ethyl acetate, washed with brine, dried over sodium sulfate, filtered and evaporated to yield slightly brownish crystals. The crude mixture was used without further purification.

¹H NMR (400 MHz, DMSO-*d*₆): δ 13.0 (br s, 1H), 4.37 - 4.31 (m, 1H), 3.77 - 3.65 (m, 2H), 2.98 - 2.78 (m, 1H), 2.48 - 2.32 (m, 1H), 1.38 (s, 9H); UPLC I R_t 1.51 min, m/z 252.5 [M+H]⁺ (90%); (1.23 g, 86%)

(S)-tert-butyl 2-carbamoyl-4,4-difluoropyrrolidine-1-carboxylate (S4)

[S4]

In a 50 ml round-bottomed flask (S)-1-(*tert*-butoxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylic acid (1.6 g, 6.37 mmol), obtained from step 4, was dissolved in 10 ml of dichloromethane at 15°C. Then 1-hydroxypyrrolidine-2,5-dione (0.806 g, 7.0 mmol) was added. To the formed suspension *N,N'*-dicyclohexylcarbodiimide (1.445 g, 7.0 mmol) was added at vigorous stirring. In a few seconds a cloudy white suspension forms. The mixture was allowed to reach RT and stirred for 30 min, followed by the addition of 7*N* ammonia in methanol (2.0 ml, 14 mmol) and stirring for another 20 min. Before evaporation of volatile components 5 g of Celite was added to the flask. Cold ethyl acetate was added to the residue and filtered over Celite. The filtrate was washed with saturated sodium bicarbonate. The formed slightly yellowish crystals were used without further purification.

¹H NMR (400 MHz, CDCl₃): δ 6.77 (br s, 1H), 5.54 (br s, 1H), 4.52 (br s, 1H), 4.00 - 3.80 (m, 1H), 3.78 - 3.56 (m, 1H), 3.02 - 2.82 (m, 1H), 2.77 - 2.44 (m, 1H), 1.45 (s, 9H); LC-MS (A) R_t 1.34 min, m/z 251.5 [M+H]⁺ (89%); MS (ESI) m/z 251.2 [M + H]⁺; (1.15 g, 72%)

(S)-4,4-difluoropyrrolidine-2-carboxamide trifluoroacetate (S5)

9.54 ml of trifluoroacetic acid was added to a solution of (S)-*tert*-butyl 2-carbamoyl-4,4-difluoropyrrolidine-1-carboxylate (1.25 g, 5 mmol) in 10 ml of dichloromethane. The solution was stirred for 1 h before evaporation. The residue was washed with ether to yield white crystals.

¹H NMR (400 MHz, DMSO-*d*₆): δ 10.01 (br s, 2H), 8.07 (s, 1H), 7.82 (s, 1H), 4.46 (t, J = 8.59 Hz, 1H), 3.71 (dd, J = 19.54, 7.48 Hz, 2H), 3.00 - 2.86 (m, 1H), 2.61 - 2.42 (m, 1H); MS (ESI) m/z 155.2 [M + H]⁺; (0.84 g, 90%)

(S)-*tert*-butyl 2-(2-carbamoyl-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamate (S6)

HATU (12.47 g, 32.8 mmol) was dissolved in 20 ml DMF and added to a solution of 2-(*tert*-butoxycarbonylamino)acetic acid (5.75 g, 32.8 mmol), and DIPEA (5.43 ml, 32.65 mmol) in 30 ml DCM. After 10 min a solution of (S)-4,4-difluoropyrrolidine-2-carboxamide hydrochloride (5.1 g, 27.3 mmol) and DIPEA (9.1 ml, 54.4 mmol) in 40 ml DCM was added. After 3 h the cloudy mixture was filtered of. The filtrate was cooled and filtrated again. The combined residues were washed with DCM and water and recrystallized from ethyl acetate.

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.73 - 7.13 (m, 2H), 6.89 (br tr, J = 5.6 Hz, 1H), 4.46 (dd, J = 9.72, 4.13 Hz, 1H), 4.09 (dd, J = 25.40, 12.76 Hz, 1H), 3.96 (dd, J = 24.58, 11.16 Hz, 1H), 3.81 (dd, J = 17.2, 5.68 Hz, 1H), 3.71 (dd, J = 17.2, 5.32 Hz, 1H), 2.78 - 2.65 (m, 1H), 2.43 - 2.30 (m, 1H), 1.39 (s, 9H); LC-MS (A) R_t 1.28 min, m/z 308.5 [M+H]⁺ (94%); MS (ESI) m/z 308.1 [M + H]⁺; (8.39 g, 77%)

[S5]

(S)-tert-butyl 2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamate (S7)

In a 50 ml round-bottomed flask (S)-tert-butyl 2-(2-carbamoyl-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamate (0.720 g, 2.343 mmol) was dissolved in dry THF at -15°C. Then pyridine was added, followed by the dropwise addition of the solution of 2,2,2-trifluoroacetic anhydride (0.094 ml, 0.664 mmol) in 5 ml of DCM after the complete addition, the mixture was allowed to reach RT. The reaction mixture was stirred for 90 minutes. The reaction mixture was washed with 1 M solution of aqueous solution of hydrochloric acid. Then the organic layer was washed three times with saturated sodium bicarbonate, brine, dried over sodium sulfate and evaporated. The crude mixture was purified using column chromatography (hexane-ethyl acetate 2-3) yielding a yellowish oil.

¹H NMR (400 MHz, CDCl₃) δ 5.29 (br s, 1H), 5.00 - 4.94 (m, 1H), 4.04 - 3.80 (m, 4H), 2.83 - 2.70 (m, 2H), 1.45 (s, 9H); LC-MS (A) R_t 1.52 min, m/z 290.6 [M+H]⁺ (94%); MS (ESI) m/z 290.1 [M+H]⁺; (0.678 g, 70%)

1.2 N-methyl-1-(quinolin-4-yl)methanamine (S9) and 2-methoxy-N-(quinolin-4-ylmethyl)ethanamine (S10).

(S)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S8)

p-Toluenesulfonic acid monohydrate (3.59 g, 18.87 mmol) was added to a cooled (0°C) solution of (S)-tert-butyl 2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethylcarbamate (3.9 g, 13.48 mmol) in 35 ml of acetonitrile. The mixture was slowly warmed to room temperature and stirred for 24 h. The residue was evaporated to dryness and washed with cold ether and cold ethyl acetate and dried again to give off-white crystals.

¹H NMR (400 MHz, D₂O): (5/1 mixture of *trans/cis* amide rotamers) δ 7.47 (d, *J* = 7.8 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 5.37 (d, *J* = 8.4 Hz, 0.2H) 5.17 (dd, *J* = 8.4, 4.1 Hz, 0.8H), 4.24 - 4.03 (m, 2H), 4.01 (s, 2H), 3.05-2.84 (m, 2H), 2.29 (s, 3H) MS (ESI) m/z: 190.2 [M+H]⁺; (4.3 g, 90%)

N-methyl-1-(quinolin-4-yl)methanamine (S9)

Quinoline-4-carbaldehyde (0.5 g, 3.18 mmol) was dissolved in 30 ml of dry EtOH and added to methanamine (0.618 ml, 15.91 mmol) also dissolved in 30 ml of dry EtOH. A few drops of acetic acid were added and the resulting solution was stirred for 2 h, then sodium borohydride (0.554 g, 14.63 mmol) was added portionwise. After 2 h at room temperature, the solvent was evaporated to dryness. The resulting residue was treated with water and repeatedly extracted with

dichloromethane (3 × 50 ml). The organic phase was then dried with anhydrous sodium sulfate, and the solvent was evaporated to yield the free amine as a yellowish oil. (0.320 g, 58%)

¹H NMR (CDCl₃, 400 MHz) δ 8.60 (d, *J* = 4.47 Hz, 1H), 7.92 (dd, *J* = 7.7, 0.7 Hz, 1H), 7.77 (dd, *J* = 8.5, 1.3 Hz, 1H), 7.45 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.29 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 7.12 (d, *J* = 4.3 Hz, 1H), 3.87 (s, 2H), 3.28 (s, 3H), 2.26 (br s, 1H); UPLC (ESI) I R_t 0.22, *m/z* 173.3 [M+H]⁺ (94%);

2-methoxy-*N*-(quinolin-4-ylmethyl)ethanamine (S10)

The title compound was made in a similar manner as **S9**.

¹H NMR (CDCl₃, 400 MHz) δ 8.74 (d, *J* = 4.39, 1H), 8.03 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.58 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.93 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.43 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 7.33 (d, *J* = 4.4 Hz, 1H), 4.14 (s, 2H), 3.44 (t, *J* = 5.1, 2H), 3.26 (s, 3H), 2.79 (t, *J* = 5.1 Hz, 2H), 2.26 (br s, 1H); UPLC (ESI) I R_t 0.20, *m/z* 217.3 [M+H]⁺ (95%); (0.210 g, 43%)

1.3 2-Phenylisonicotinic acid (S11), 2-(3,4-dimethoxyphenyl)isonicotinic acid (S12) and 2-(4-cyanophenyl)isonicotinic acid (S13).

2-phenylisonicotinic acid (S11)

The title compound was prepared in a similar manner as **S13**.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.62 (br s, 1H), 8.93 – 8.79 (dd, *J* = 5.1, 0.8 Hz, 1H), 8.35 – 8.23 (t, *J* = 1.2 Hz, 1H), 8.19 – 8.05 (m, 2H), 7.86 – 7.71 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.60 – 7.44 (m, 3H); UPLC I (ESI) R_t 1.35 min, *m/z* 200.5 [M+H]⁺ (100%); (0.1 g, 48%)

2-(3,4-dimethoxyphenyl)isonicotinic acid (S12)

The title compound was prepared in a similar manner as **S13**.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.73 (s, 1H), 8.80 (dd, *J* = 5.0, 0.9 Hz, 1H), 8.25 (t, *J* = 1.2 Hz, 1H), 7.70 (ddd, *J* = 10.5, 5.5, 2.1 Hz, 3H), 7.08 (d, *J* = 8.4 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H); UPLC I (ESI) R_t 1.21 min, *m/z* 260.5 [M+H]⁺ (96%); (0.13 g, 49%)

2-(4-cyanophenyl)isonicotinic acid (S13)

2-bromoisonicotinic acid (0.210 g, 1.040 mmol) was dissolved in DME (8 ml) under nitrogen and the solvent was degassed in the ultrasonic bath under nitrogen. Tetrakis(triphenylphosphine)palladium(0) (0.060 g, 0.052 mmol) was added, the resulting reaction mixture was stirred for 15min. Degassed aqueous potassium carbonate (4.16 ml, 8.32 mmol) and 4-cyanophenylboronic acid (0.206 g, 1.403 mmol) were added subsequently. The resulting reaction

mixture was refluxed at 95°C for 18 h and then cooled to rt. After filtration over Celite and flushing the filter with DME and 5% sodium bicarbonate solution, the reaction mixture was acidified to pH 3-4 the white precipitate was filtered off, washed with water and recrystallized from 2-methoxyethanol to give a white powder. (0.180 g, 73%)

^1H NMR (400 MHz, DMSO- d_6) δ 13.92 (br s, 1H), 8.92 (d, J = 4.8 Hz, 1H), 8.41 (t, J = 1.1 Hz, 1H), 8.34 (d, J = 8.4 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.87 (dd, J = 5.2, 1.3 Hz, 1H); UPLC I (ESI) R_t 1.45 min, m/z 225.4 $[\text{M}+\text{H}]^+$ (98%).

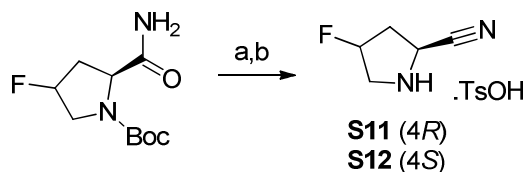
1.4 (2S,4R)-4-fluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S15) and (2S,4S)-4-fluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S16).

(S)-tert-butyl 4-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamoyl)phenylcarbamate (S14)

The target compound was made according to general procedure A.

^1H NMR (400 MHz, DMSO- d_6) (trans-cis mixture of rotamers) δ 9.63 (s, 1H), 8.56 (t, J = 5.7 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.53 (d, J = 8.8 Hz, 2H), 5.26 (d, J = 4.9 Hz, 0.1H), 4.75 (dd, J = 7.4, 3.6 Hz, 0.9H), 4.28 - 4.17 (m, 0.2H), 4.11 (dd, J = 16.9, 6.1 Hz, 0.9H), 4.03 (dd, J = 16.9, 5.5 Hz, 0.9H), 3.74 - 3.65 (m, 1H), 3.64 - 3.56 (m, 1H), 2.28 - 1.94 (m, 4H), 1.48 (s, 9H); UPLC I (ESI) R_t 1.54 min, m/z 373.6 $[\text{M}+\text{H}]^+$ (96%); (0.075 g, 67%)

Scheme S2. Fluoroproline synthesis



a) TFAA, pyridine, THF, -15°C, (70-80%); b) TsOH, MeCN, 80-86%

(2S,4R)-4-fluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S15)

TFAA (0.154 ml, 1.089 mmol) was added to a solution of pyridine (0.561 ml, 6.93 mmol) and (2S,4R)-tert-butyl 2-carbamoyl-4-fluoropyrrolidine-1-carboxylate (0.230 g, 0.990 mmol) in THF (5 ml) at 0°C. The mixture was stirred for 1 h, followed by evaporation, washing (3x) with 1 N HCl, sodium bicarbonate and brine. To give white crystals, which were used in the next reaction without further purification. (0.150 g, 70%)

p-Toluenesulfonic acid monohydrate (0.124 g, 0.653 mmol) was added to a cold (0°C) solution of (2S,4R)-tert-butyl 2-cyano-4-fluoropyrrolidine-1-carboxylate (0.1 g, 0.467 mmol) in acetonitrile (about 0.4 M) the mixture was stirred at 0°C for 30 min and was then allowed to warm to rt and

stirred for 24 h. The volatiles were evaporated and the residue washed with ethyl acetate to yield whitish crystals.

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.48 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 5.59 - 5.41 (m, 1H), 4.87 (dd, *J* = 10.3, 7.2 Hz, 1H), 3.60 (dd, *J* = 2.1, 1.1 Hz, 1H), 3.57 - 3.47 (m, *J* = 3.3 Hz, 1H), 2.79 - 2.67 (m, 1H), 2.58-2.50 (m, 1H), 2.44 (ddd, *J* = 14.6, 10.3, 4.2 Hz, 1H), 2.30 (s, 3H); UPLC I (ESI) *R*_t 0.20 min, *m/z* 115.4 [M+H]⁺ (98%)

(2S,4S)-4-fluoropyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (S16)

The title compound was obtained as a white powder in a manner similar to the preparation of **S15**

¹H NMR (400 MHz, MeOD) δ 7.71 (d, *J* = 8.2 Hz, 2H), 7.24 (d, *J* = 7.9 Hz, 2H), 5.62 - 5.44 (m, 1H), 4.97 - 4.91 (m, 1H), 3.83 - 3.72 (m, 1H), 3.60 - 3.46 (m, 1H), 2.84 - 2.72 (m, 1H), 2.72 - 2.65 (m, 1H), 2.37 (s, 3H). UPLC I (ESI) *R*_t 0.19 min, *m/z* 115.4 [M+H]⁺ (93%); (0.460 g, 86%)

1.5 2-(4-Fluorophenyl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridine (S17), 2-(4-fluorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylic acid (S18), 2-(4-methoxyphenyl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridine (S19) and 2-(4-methoxyphenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylic acid (S20).

2-(4-fluorophenyl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridine (S17) ³

A solution of 4-methyl-3-nitro-pyridin-2-ylamine (0.350 g, 2.286 mmol) and 4-fluorobenzaldehyde (0.240 ml, 2.286 mmol) in EtOH (4 ml) was treated with 1 M aq hydrosulfite (6.86 ml, 6.86 mmol). After heating the reaction mixture at 70°C for 24 h in a closed vial, it was cooled to rt and treated dropwise with 5 eq NH₄OH (2 ml). A precipitate was immediately formed which was then filtered, washed with water (2 x 15 ml) and dried under reduced pressure to afford the desired product in satisfactory purity. (0.140 g, 28%)

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.46 (s, 1H), 8.34 – 8.24 (m, 2H), 8.17 (d, *J* = 5.3 Hz, 1H), 7.48 – 7.36 (m, 2H), 7.07 (d, *J* = 4.9 Hz, 1H), 2.60 (s, 3H); UPLC I (ESI) *R*_t 1.21 min, *m/z* 228.5 [M+H]⁺ (94%)

2-(4-fluorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylic acid (S18)

A mixture of 2-(4-fluorophenyl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridine (0.130 g, 0.572 mmol) and selenium dioxide (0.248 g, 2.231 mmol) in pyridine (15 ml) was heated at 110°C for 3 h. The hot reaction mixture was filtered through diatomaceous earth, washed with hot pyridine (10 ml) and methanol (20 ml), and the filtrate was concentrated and dried. The crude residue was dissolved in methanol (20 ml) followed by the dropwise addition of thionyl chloride (0.418 ml, 5.72 mmol) at room temperature. The reaction mixture was stirred at reflux for 5 h, cooled, concentrated,

suspended in ice-water (10 ml) and filtered. The pH of the filtrate was adjusted to 7 using conc. NH_4OH followed by extraction with DCM-iPrOH 4-1 (3 x 20 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to provide the desired product as brown-yellow solid (0.13 g, 68% yield) A mixture of methyl 2-(4-fluorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylate (0.130 g, 0.479 mmol) and lithium hydroxide (0.023 g, 0.959 mmol) was suspended in THF-water 1-1 (2 ml) and the reaction mixture was heated at 60°C for 18 h. The volatiles were evaporated, the residue redissolved in water and the pH adjusted to 3-4. The precipitate was filtered and washed with water to give white crystals.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.93 - 12.72 (s, 1H), 8.54 - 8.48 (d, J = 5.0 Hz, 1H), 8.47 - 8.38 (dd, J = 8.8, 5.0 Hz, 2H), 7.65 - 7.62 (d, J = 5.0 Hz, 1H), 7.46 - 7.39 (t, J = 8.8 Hz, 2H); UPLC I (ESI) R_t 1.19 min, m/z 258.5 $[\text{M}+\text{H}]^+$ (96%); (0.1 g, 81%)

2-(4-methoxyphenyl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridine (S19)

Was prepared in a similar manner as compound **S17**

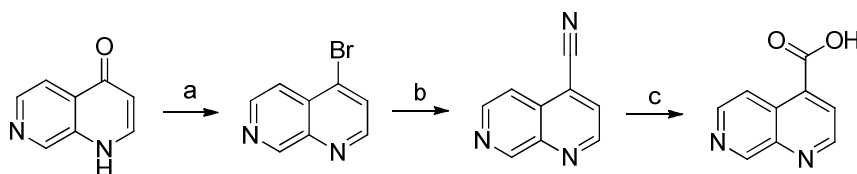
^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.27 – 8.08 (m, 3H), 7.50-7.45 (m, 1H), 7.12 (d, J = 8.2 Hz, 2H), 3.85 (s, J = 12.2 Hz, 3H), 2.59 (s, J = 8.4 Hz, 3H); UPLC I (ESI) R_t 1.20 min, m/z 240.4 $[\text{M}+\text{H}]^+$ (91%); (0.203, 32%)

2-(4-methoxyphenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylic acid (S20)

Was prepared in a similar manner as compound **S18**

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 14.07 – 13.65 (m, 1H), 12.69 (s, 1H), 8.46 (s, 1H), 8.33 (d, J = 8.8 Hz, 2H), 7.59 (d, J = 5.0 Hz, 1H), 7.13 (d, J = 8.9 Hz, 2H), 3.86 (s, 3H); UPLC I (ESI) R_t 1.17 min, m/z 270.5 $[\text{M}+\text{H}]^+$ (99%);

Scheme S3



a) PBr_3 , DMF, 3 h, 63%; b) $\text{Zn}(\text{CN})_2$, Pd/C, $\text{Zn}^{++}(\text{COO})_2$, dppf, 110°C, 3 h, 72%:
c) NaOH, $\text{H}_2\text{O}/\text{EtOH}$, reflux, 94%

1,7 4-Bromo-1,7-naphthyridine (S21), 1,7-naphthyridine-4-carbonitrile (S22) and 1,7-naphthyridine-4-carboxylic acid (S23).

4-bromo-1,7-naphthyridine (S21)

A solution of 1,7-naphthyridin-4(1*H*)-one⁴ (0.552 g, 3.78 mmol) in DMF (5 ml) was treated with tribromophosphine (0.461 ml, 4.91 mmol) After 2 hours the mixture was added to water and

[S10]

neutralized with saturated aqueous sodium bicarbonate solution. Filtration and drying afforded a yellow solid (0.5 g, 63%).

^1H NMR (400 MHz, DMSO- d_6) δ 9.45 (d, J = 0.89 Hz, 1H), 8.91 (d, J = 4.64 Hz, 1H), 8.78 (d, J = 5.77 Hz, 1H), 8.23 (d, J = 4.63 Hz, 1H), 8.00 (dd, J = 0.89, 5.78 Hz, 1H); UPLC (ESI) | R_t 1.30, m/z 209.4, 211.4 $[\text{M}+\text{H}]^+$ (94%);

1,7-naphthyridine-4-carbonitrile (S22)

The cyanation procedure as described in Yu et al was used.⁵ To a round bottom flask was added 4-bromo-1,7-naphthyridine (0.3 g, 1.435 mmol), zinc cyanide (0.101 g, 0.861 mmol), 10 wt % palladium on carbon (0.153 g, 0.144 mmol), 1,1'-Bis(diphenylphosphino)ferrocene (0.032 g, 0.057 mmol) and DMAC (2 ml). The resulting slurry was sparged with sub-surface argon for 10 min, and zinc(II) formate dihydrate (0.041 g, 0.215 mmol) was added to the reaction mixture. The reaction mixture was again sparged with sub-surface argon for 10 min and was heated under argon to 100-110°C for 3 h. The reaction mixture was diluted with 10 ml of EtOAc. The resulting slurry was filtered and the cake was rinsed with EtOAc (2 ml). The product was isolated by washing the filtrate with water (2 $\times$ 10 ml) and 5% NH_4OH (1 $\times$ 10 ml). The organic layer was dried with Na_2SO_4 . The volatiles were removed in vacuo to give a residue, which was further purified by silica gel chromatography (EtOAc/heptane) to provide the product. (0.16 g, 72%)

^1H NMR (400 MHz, CDCl_3) δ 9.63 (s, 1H), 9.17 (d, J = 4.30 Hz, 1H), 8.83 (d, J = 5.75 Hz, 1H), 7.99 (dd, J = 0.97, 5.71 Hz, 1H), 7.94 (d, J = 4.31 Hz, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 154.97, 151.13, 146.15, 142.88, 129.02, 128.66, 118.17, 117.00, 114.35; UPLC (ESI) | R_t 1.09, m/z 156.5 $[\text{M}+\text{H}]^+$ (94%);

1,7-naphthyridine-4-carboxylic acid (S23)

A mixture of 1,7-naphthyridine-4-carbonitrile (0.09 g, 0.580 mmol), NaOH (0.048 g, 1.19 mmol) 1 ml of ethanol and 1 ml of water was heated to reflux for five hours. The reaction mixture was cooled to room temperature, and concentrated under reduced pressure. 2 M hydrochloric acid was added so that pH of the resultant residue became about 3, which was concentrated under reduced pressure again. UPLC (ESI) | R_t 0.31, m/z 175.8 $[\text{M}+\text{H}]^+$ (94%); (0.095 g, 94%)

7-phenylquinoline-4-carboxylic acid (S24)

A mixture of methyl 7-bromoquinoline-4-carboxylate⁶ (0.15 g, 0.564 mmol), phenylboronic acid (0.103 g, 0.846 mmol), tetrakis(triphenylphosphine)palladium(0) (0.033 g, 0.028 mmol), K_3PO_4 (0.598 g, 2.82 mmol), in 2 ml H_2O and 2 ml dioxane was stirred at 60°C for 1.5 h. EtOAc and water were

added then the organics were extracted with H₂O. The water was separated, acidified and the white precipitate was filtered to give 7-phenylquinoline-4-carboxylic acid (0.110 g, 0.441 mmol, 78 % yield)

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.07 (d, J = 4.36 Hz, 1H), 8.81 (d, J = 8.91 Hz, 1H), 8.37 (d, J = 1.80 Hz, 1H), 8.09 (dd, J = 2.01, 8.94 Hz, 1H), 7.93 (d, J = 4.36 Hz, 1H), 7.92 - 7.88 (m, 2H), 7.67 - 7.59 (m, 1H), 7.60 - 7.51 (m, 2H); UPLC (ESI) | R_t 1.34, m/z 250.5 [M+H]⁺ (94%);

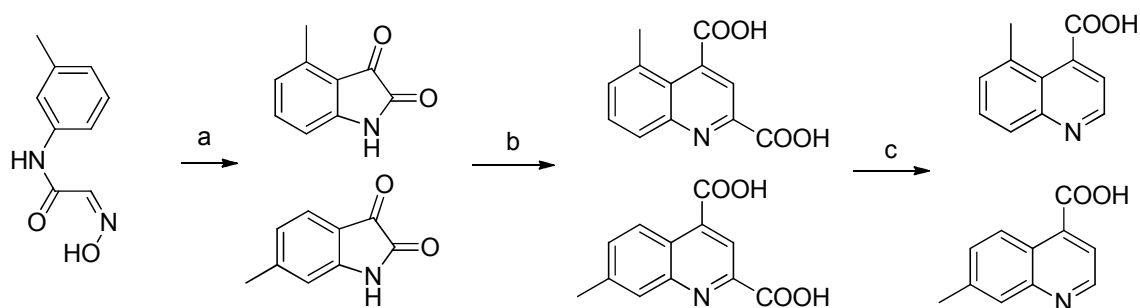
7-(phenylamino)quinoline-4-carboxylic acid (S25)

A mixture of methyl 7-(phenylamino)quinoline-4-carboxylate (0.150 g, 0.539 mmol), NaOH (0.043 g, 1.078 mmol) in MeOH/H₂O (5 ml) (1/1) was stirred for 16 h at rt. the volatiles were evaporated, water was added and acidified to pH 2. This yielded a red precipitate, which was filtered to give 7-(phenylamino)quinoline-4-carboxylic acid (0.120 g, 84 % yield)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.82 - 8.79 (m, 2H), 8.54 (d, J = 9.25 Hz, 1H), 7.57 (t, J = 3.38 Hz, 2H), 7.43 (dd, J = 2.49, 9.27 Hz, 1H), 7.39 - 7.33 (m, 2H), 7.31 - 7.26 (m, 2H), 7.00 (tt, J = 1.23, 7.21 Hz, 1H); UPLC | (ESI) R_t 1.12 min, m/z 265.6 [M+H]⁺ (96%).

1.8 4-methylisatin (S26), 6-methylisatin (S27), 5-methylquinoline-2,4-dicarboxylic acid (S28), 7-methylquinoline-2,4-dicarboxylic acid (S29), 5-methylquinoline-4-carboxylic acid (S30) and 7-methylquinoline-4-carboxylic acid (S31).

Scheme S4



a) MsOH, 55°C; b) NaOH, sodium pyruvate, H₂O, reflux; c) H₂O, pressure tube, 210°C

4-methylisatin (S26) and 6-methylisatin (S27)

The intermediate isonitrosoacetanilide was added in small portions, with stirring, to a 100 ml Erlenmeyer flask containing 5.15 ml concentrated methanesulfonic acid (1.06 ml, 16.3 mmol), which had been heated to 55°C. The temperature of the reaction mixture was maintained below 70°C during this addition. After all the isonitroso had been added, the dark-colored solution was heated at

80°C for an additional 10 min and then cooled to room temperature, poured onto 25 ml of crushed ice, and allowed to stand for 30 min. The precipitate was collected by filtration, washing three times with water, and dried under vacuum to yield a mixture of 4-and 6-methylisatin that was of sufficient purity to be used directly in the next step. (6-methylisatin, 40%, 1.02 g, 4-methylisatin 30%, 0.77 g)

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.98 (s, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 6.88 – 6.82 (t, *J* = 7.7 Hz, 1H), 6.69 (d, *J* = 7.8 Hz, 1H), 2.34 (d, *J* = 2.4 Hz, 3H). UPLC I (ESI) *R*_t 1.30 min, *m/z* 162.5 [M+H]⁺

6-methylisatin

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.98 (s, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 6.88 – 6.82 (m, 1H), 6.70 (d, *J* = 2.5 Hz, 1H), 2.42 (s, 3H). UPLC I (ESI) *R*_t 1.32 min, *m/z* 162.5 [M+H]⁺

5-methylquinoline-2,4-dicarboxylic acid (S28) and 7-methylquinoline-2,4-dicarboxylic acid (S29)

An aqueous solution of NaOH (72.6 ml, 17.57 g) was added to 6-methylindoline-2,3-dione (5.9 g, 36.6 mmol), and sodium 2-oxopropanoate (4.83 g, 43.9 mmol) was added. The mixture was refluxed for 4 h, cooled to rt and the products were precipitated by adjusting the pH to 2 with 2N HCl. The precipitate was filtered and purified using a Biotage KP-C18 flash column with a water-acetonitrile gradient with 0.1% formic acid as an additive.

5-methylquinoline-2,4-dicarboxylic acid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.08 (d, *J* = 8.0 Hz, 1H), 8.00 (s, 1H), 7.81 (dd, *J* = 8.4, 7.1 Hz, 1H), 7.64 (d, *J* = 7.0 Hz, 1H), 2.69 (s, 3H); UPLC I (ESI) *R*_t 0.35 min, *m/z* 232.5 [M+H]⁺

7-methylquinoline-2,4-dicarboxylic acid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.67 (d, *J* = 8.8 Hz, 1H), 8.39 (s, 1H), 8.01 (s, *J* = 6.8 Hz, 1H), 7.66 (dd, *J* = 8.8, 1.9 Hz, 1H), 2.55 (s, 3H); UPLC I (ESI) *R*_t 0.76 min, *m/z* 232.5 [M+H]⁺

5-methylquinoline-4-carboxylic acid (S30)

5-methylquinoline-2,4-dicarboxylic acid (0.8 g, 3.46 mmol) was dispersed in water and heated in a sealed pressure tube for 50 min at 208°C with stirring. After cooling the precipitate was collected and used without further purification.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.89 (d, *J* = 4.3 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.68 (dd, *J* = 8.4, 7.1 Hz, 1H), 7.49 – 7.44 (m, 2H), 2.65 (s, 3H); UPLC I (ESI) *R*_t 0.24 min, *m/z* 188.5 [M+H]⁺; (0.4g, 61%)

7-methylquinoline-4-carboxylic acid (S31)

Was prepared in a similar manner as 5-methylquinoline-4-carboxylic acid, but stirred for 90 min at 208°C in a sealed pressure tube.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.98 (d, J = 4.4 Hz, 1H), 8.62 – 8.57 (m, 1H), 7.92 – 7.89 (m, 1H), 7.85 (d, J = 4.4 Hz, 1H), 7.56 (dt, J = 8.5, 3.0 Hz, 1H), 2.54 (dd, J = 6.0, 1.0 Hz, 3H); UPLC I (ESI) R_t 0.34 min, m/z 188.5 $[\text{M}+\text{H}]^+$; (0.6 g, 90%)

1.9 5-Phenylquinoline-4-carboxylic acid (S32) and 5-methoxyquinoline-4-carboxylic acid (S33).

5-phenylquinoline-4-carboxylic acid (S32)

A mixture of 5-bromoquinoline-4-carboxylic acid⁶ (0.11 g, 0.436 mmol), phenylboronic acid (0.080 g, 0.655 mmol), Tetrakis(triphenylphosphine)palladium(0) (0.025 g, 0.022 mmol) and K_3PO_4 (0.463 g, 2.182 mmol) in 2 ml H_2O and 2 ml dioxane was stirred at 60°C for 1.5 h. EtOAc was added then the organics were washed with H_2O and brine then concentrated. The residue was suspended in cold EtOAc and the solids were collected by suction filtration, washed with cold EtOAc and dried to give 5-phenylquinoline-4-carboxylic acid (0.092 g, 0.369 mmol, 85 % yield) as a solid.

^1H NMR (400 MHz, CDCl_3) δ 9.13 (t, J = 5.52 Hz, 1H), 8.35 (dd, J = 20.9, 8.6 Hz, 1H), 8.15 (s, 1H), 7.94 (t, J = 7.8 Hz, 1H), 7.87 (dd, J = 11.1, 6.4 Hz, 1H), 7.77 (t, J = 7.7 Hz, 1H), 7.68 (dt, J = 4.5, 2.3 Hz, 1H), 7.54 - 7.41 (m, 2H), 7.19 (t, J = 7.57 Hz, 1H); UPLC I (ESI) R_t 1.11 min, m/z 250.5 $[\text{M}+\text{H}]^+$ (96%).

5-methoxyquinoline-4-carboxylic acid (S33)

A mixture of 5-bromoquinoline-4-carboxylic acid (0.1 g, 0.397 mmol), sodium methoxide (0.744 ml, 3.97 mmol), DMF (2 ml) and MeOH (5ml) was stirred and heated at 90°C under nitrogen. copperbromide (0.028 g, 0.198 mmol) was added and the reaction mixture was maintained at 90°C for 16 h, poured into water and washed with ether. The water layer was acidified, and extracted with ether (3x). The organic layers were washed with water and brine, dried (MgSO_4), filtered and the filtrate concentrated. (0.07 g, 87%)

^1H NMR (400 MHz, CDCl_3) δ 8.64 (d, J = 5.8 Hz, 1H), 7.65 - 7.60 (m, 1H), 7.57 (dd, J = 8.4, 1.3 Hz, 1H), 6.92 (dd, J = 7.8, 1.2 Hz, 1H), 6.87 (d, J = 5.8 Hz, 1H), 3.96 (s, 3H); UPLC I (ESI) R_t 1.09 min, m/z 204.5 $[\text{M}+\text{H}]^+$ (96%)

1.10 2-Amino-1-(pyrrolidin-1-yl)ethanone hydrochloride (S34) and (S)-tert-butyl 2-(2-chloroacetyl)pyrrolidine-1-carboxylate (S35).

2-amino-1-(pyrrolidin-1-yl)ethanone hydrochloride (S34)

4 N HCl in dioxane (3.29 ml, 13.14 mmol) was added to *tert*-butyl 2-oxo-2-(pyrrolidin-1-yl)ethylcarbamate (0.3 g, 1.314 mmol), the mixture was stirred for 16 h and evaporated, the residue was washed with ether to give white crystals. ¹H NMR (400 MHz, MeOD) δ 3.66 (s, 2H), 3.47 (m, 4H), 2.06 - 1.97 (m, 2H), 1.96 - 1.88 (m, 2H); UPLC I (ESI) R_t 0.29 min, m/z 129.5 [M+H]⁺ (95%); (0.186 g, 86%)

(S)-*tert*-Butyl 2-(2-chloroacetyl)pyrrolidine-1-carboxylate (S35)²³

A solution of lithium diisopropylamide (15.20 ml, 27.4 mmol) was added dropwise over 30 min to a solution of (S)-1-*tert*-butyl 2-methyl pyrrolidine-1,2-dicarboxylate (1.4 ml, 6.84 mmol) and methylene chloriodide (2.0 ml, 27.4 mmol) in THF (5.5 ml) at -78°C. The internal temperature of the reaction mixture was kept below -70°C during the addition and the reaction mixture was stirred for 10 min at -75°C. A solution of acetic acid (1.5 ml) in THF (10 ml) was added dropwise over 10 min, keeping the internal temperature below -65°C. After stirring an additional 10 min at -75°C., the reaction mixture was partitioned between ethyl acetate (75 ml) and brine (75 ml). The organic layer was washed with saturated NaHCO₃ solution (2 x 50 ml), 5% NaHSO₃ solution (2 x 50 ml) and brine (50 ml), dried (MgSO₄) and concentrated affording a dark yellow solid. The residue was chromatographed on 2.5 x 12 cm silica gel using 7 to 35% ethyl acetate/hexane affording 1.2 g, 70% of chloromethylketone as a yellow oil. ¹H NMR (400 MHz, CDCl₃) (cis/trans 1-1 mixture of carbamate rotamers) δ 4.52 (dd, J = 4.23, 8.52 Hz, 0.5H), 4.46 (dd, J = 4.52, 8.46 Hz, 0.5H), 4.36 - 4.26 (m, 1H), 4.22 (d, J = 3.52 Hz, 1H), 3.59 - 3.38 (m, 2H), 2.32 - 2.11 (m, 1H), 1.92 (m, 3H), 1.45 (s, 4.5H), 1.41 (s, 4.5H); UPLC I (ESI) R_t 1.74 min, m/z 192.5 [M+H-*tert*-Bu]⁺ (98%)

II. Enzymatic assay data

II.1 Enzyme purification and assay conditions for IC₅₀ determinations

Enzyme purification

-Recombinant murine FAP was purified from the cultured supernatant of HEK293 human embryonic kidney cell line as described elsewhere (Cheng, J. et al. *Cancer Res.* **2002**, 62, 4767-4722).

-Recombinant human PREP was expressed in and purified from E coli as previously described (Szeltner, Z.; Renner, V.; Polgar, L. *Prot. Sci.* **2000**, 9, 353-360).

-DPP IV and DPPII were purified from human seminal plasma as described previously. (De Meester, I.; Vanhoof, G.; Lambeir, A.; Scharpe, S. *J. Immun. Methods* **1996**, 189, 99-105 and Maes, M. B.; Lambeir,

[S15]

A. M.; Gilany, K.; Senten, K.; Van der Veken, P.; Leiting, B.; Augustyns, K.; Scharpe, S.; De Meester, I. Kinetic investigation of human dipeptidyl peptidase II (DPPII)-mediated hydrolysis of dipeptide derivatives and its identification as quiescent cell proline dipeptidase (QPP)/dipeptidyl peptidase 7 (DPP 7). *Biochem. J.* **2005**, 386, 315-324)

-Recombinant human DPP8 was expressed and purified as described. (Chen, Y. S.; Chien, C. H.; Goparaju, C. M.; Hsu, J. T.; Liang, P. H.; Chen, X. Purification and characterization of human prolyl dipeptidase DPP8 in SF9 insect cells. *Prot. Exp. Purif.* **2004**, 35, 142-146)

-DPP9 was purified from bovine testes as described by Dubois et al. (Dubois, V.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Creemers, J.; Chen, X.; Scharpe, S.; De Meester, I. Purification and characterization of dipeptidyl peptidase IV-like enzymes from bovine testes. *Front. Biosci.* **2008**, 13, 3558–3568)

IC₅₀-determination for FAP and PREP

Enzyme activities were determined kinetically in a final volume of 200 µl for 10 minutes at 37°C by measuring the initial velocities of pNA release (405 nm) from the substrate using a Spectramax plus microtiterplate reader (Molecular devices). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 µmol pNA from the substrate per minute under assay conditions. All measurements were carried out in duplicate. The IC₅₀ value was defined as the inhibitor concentration which caused a 50% decrease of the activity under assay conditions.

The chromogenic substrate Ala-Pro-*p*-nitroanilide (2 mmol/l) was used at pH 7.4 for FAP activity measurement. The substrate concentrations were chosen around the Km value obtained under the assay conditions used. Buffer compositions for the DPP assays were previously reported in the purification articles– vide supra. The FAP assay buffer consisted of 50 mM Tris pH7.4 containing 100 mmol/l NaCl and 0.1 mg/ml bovine serum albumin. The PREP activity was measured as described by Brandt et al. using the chromogenic substrate Z-Gly-Pro-*p*-nitroanilide (0.25 mmol/l) at pH 7.5 in the presence of 10 mmol/l DTT. (Brandt, I.; Gérard, M.; Sergeant, K.; Devreese, B.; Baekelandt, V.; Augustyns, K.; Scharpé, S.; Engelborghs, Y.; Lambeir, A.M. *Peptides* **2005**, 26, 2536-2546)

IC₅₀-determination for DPPII, DPPIV, and DPP 9

Initial rates were determined kinetically in a final volume of 200 µl for 10 minutes at 37°C by measuring the initial velocities of pNA release (405 nm) from the substrate using a Spectrafluor Plus reader (Tecan Benelux). The chromogenic substrate Gly-Pro-*p*-nitroanilide (100 µmol/l) was used at

pH 8.3 for DPP IV, Lys-Ala-*p*-nitroanilide (1 mmol/l) at pH 5.5 for DPP II and Ala-Pro-*p*-nitroanilide (300 µmol/l) at pH 7.4 for DPP9-activity measurement. The substrate concentrations were chosen around the K_m value obtained under the assay conditions used. Buffer compositions were reported before. (Dubois, V.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Creemers, J.; Chen, X.; Scharpe, S.; De Meester, I. Purification and characterization of dipeptidyl peptidase IV-like enzymes from bovine testes. *Front. Biosci.* **2008**, *13*, 3558–3568)

Test compounds were dissolved and diluted in DMSO (final concentration of DMSO during assay was 5% v/v). Inhibitors were pre-incubated with the enzyme for 15 min at 37 °C before starting by the addition of substrate. The concentrations of enzyme and inhibitor during the preincubation were the double of the final concentrations during the initial rate measurement. All measurements were carried out in duplicate. The initial evaluation of compounds was carried out at 100 µmol/l, or in case of solubility limits, the highest concentration possible. If v_i/v_o (initial velocity in presence of inhibitor/velocity in presence of DMSO) was < 0.5, an IC_{50} value was determined experimentally using at least 8 different concentrations of inhibitor. For those compounds with IC_{50} values below 5 µmol/l for one of the enzymes, the analysis was repeated using a new stock of compound. Generally, independent measurements of IC_{50} differed less than 20% from each other. The IC_{50} -value was defined as the inhibitor concentration, which caused a 50% decrease of the activity under assay conditions. The total inhibitor concentration is represented by I_o . IC_{50} -values were calculated with the GraFit software (GraFit Version 5, Leatherbarrow, R.J., Erithacus Software Ltd., Horley, U.K.) using eq (1).

$$\frac{v_i}{v_o} = \frac{1}{1 + \left(\frac{I_o}{IC_{50}} \right)^s} + background$$

eq. (1)

where s is the slope factor and background represents the estimated minimal v_i/v_o value. The errors given in the tables represent standard errors of the fit unless otherwise specified.

II.2 Ex vivo Enzyme activity assays

Protocol for determination of FAP-activity in plasma of inhibitor-treated rats:

FAP-activity in plasma was determined by means of an enzymatic end-point method using Z-Gly-Pro-AMC as a fluorogenic substrate. First, samples were diluted 101 times in a buffer containing 0.1 M

Tris buffer containing 400 mM sodium chloride, 50 mM salicylic acid, 1 mM sodium azide, 1 mM EDTA at pH 7.5. To 100 µl of diluted sample was added 10 µl of 4.6 mM Z-Gly-Pro-AMC in acetonitrile:water (40:60) and incubated during 15 minutes at 37°C. Sample and substrate blank tubes were also incubated. The enzymatic reaction was stopped by adding 0.5 ml of 1.5 M acetic acid. The fluorescence of the liberated AMC was measured as described above. Within and between-run CV was 1.9 and 3.9% respectively. Specificity of the method was assessed by use of specific FAP-inhibitors. Addition of specific PREP-inhibitors demonstrated the absence of interference by active PREP in this assay that did not contain DTT (PREP in plasma requires treatment with DTT in order to be active). The influence of dilution on the read-out of the protocol was determined as described in:

Mattheeussen, V.; Lambeir, A.M.; Jungraithmayr, W.; Gomez, N.; McEntee, K.; Van der Veken, P.; Scharpé, S.; Van der Veken, P. Method comparison of DPP IV activity assays and their application in biological samples containing reversible inhibitors. *Clinica Chimica Acta* **2012**, *412*, 456-462.

III. *In vivo* PK experiments

III.1.1 Animals

Male Wistar rats (BW~250 g; Janvier, France) were housed individually and randomly allocated to 9 groups of 3 animals each, based on body weight. One of these 9 groups served as a control (that was not treated with inhibitor). The animals were fasted overnight until 4 hours after dosing. Drinking water remained available *ad libitum* throughout the experiment.

III.1.2 Inhibitor formulation

The inhibitors were dissolved in PEG200 at 10 mg/mL. The same formulation was used both for oral and parenteral dosing. The test formulation was freshly prepared and kept at room temperature before administration.

III.1.3 Dosing and blood sampling frequency

Six male rats were treated for each inhibitor tested, three of which (one group, *vide supra*) received the compound via a single intravenous (IV, tail vein) administration at 5 mg/kg. The other three animals were dosed per os (PO) at 20 mg/kg. Blood samples were collected at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6h and 24 h post administration.

III.1.4 Blood and tissue sampling procedure and sample processing

Blood (300 µL) was collected on ice in Serum-Gel Clotting Activator 1.1 mL microtubes (Sarstedt) upon intracardial puncture. After centrifugation (10,000 g, 5 min), the collected serum was divided into 2 aliquots and stored at -80°C. Sample processing consisted of adding 4 parts of cold acetonitrile to 1 part of serum; the mixture was vortexed for 30 sec and centrifuged at 4°C for 5 minutes at 15,000 rpm. The supernatant was further diluted (1/20) in solvent (90/10 H₂O/ACN). At each sampling time point, 1 aliquot of serum was stored at -80°C for FAP activity measurements. The overall dilution factor of the samples was 100.

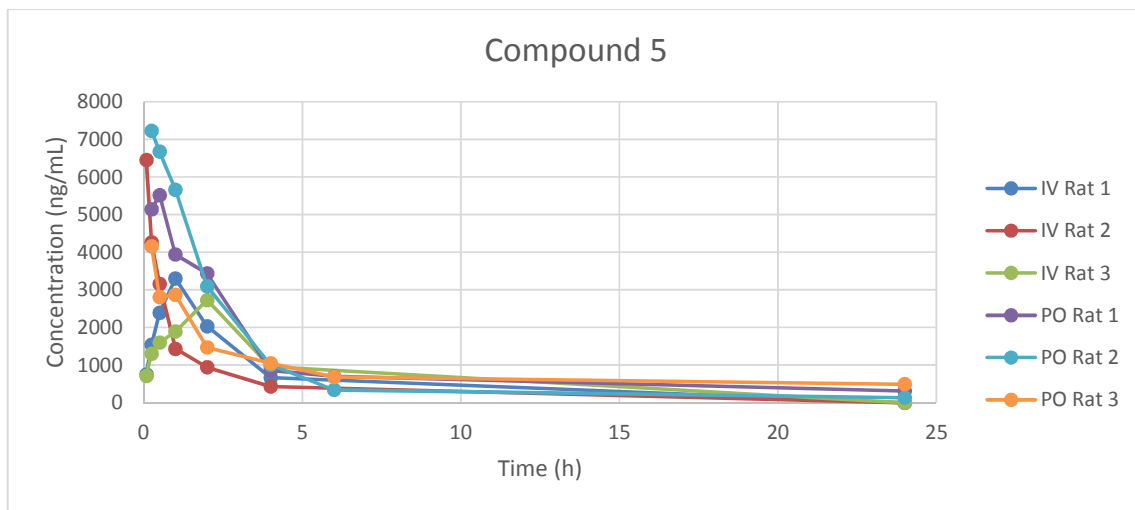
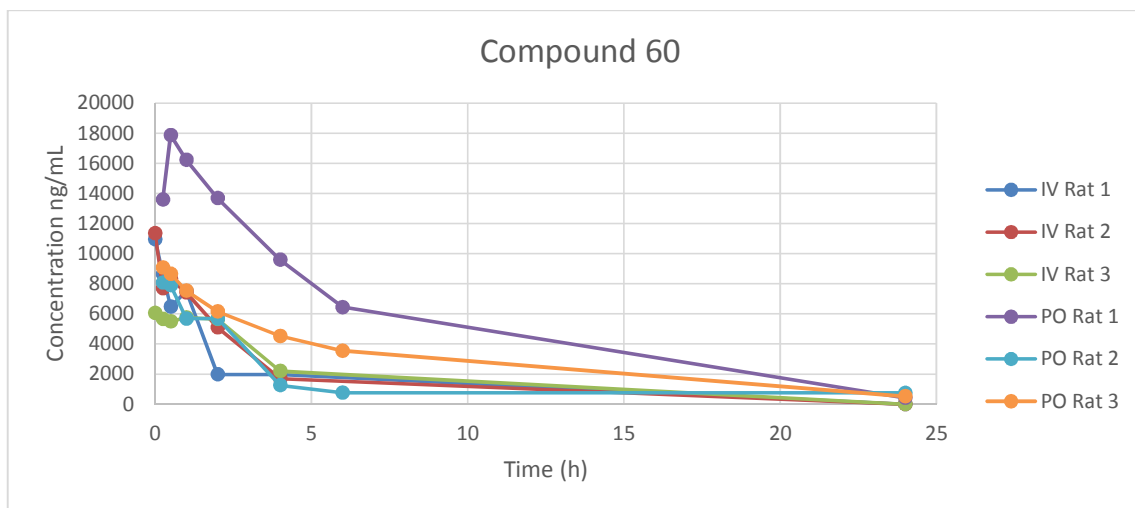
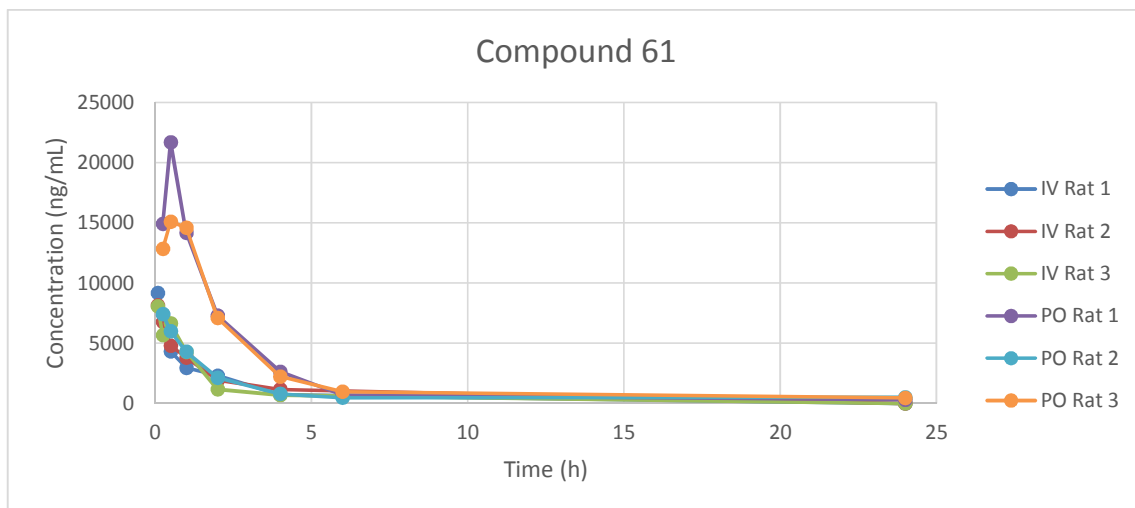
III.1.5 Determination of plasma levels using UPLC-MS.

The analysis of the serum was performed using a Waters Acquity H-class UPLC system coupled to a Waters TQD ESI mass spectrometer and a Waters TUV detector. A Waters Acquity UPLC BEH C18 1.7µm 2.1 x 50 mm column was used. The samples were analyzed in triplicate. A standard curve of six points was made to determine the measured concentrations.

The following parent and daughter peaks were used:

Compound Number	Parent ion	Daughter peak
4	309.14	185.11
5	389.104	171.15
60	345.14	185.08
61	375.13	159.06

Standard pharmacokinetic parameters were determined using the TopfitTM software: peak time (T_{max}), peak concentration (C_{max}), elimination half-life (T_{1/2}), systemic availability (AUC_{0-t}), volume of distribution (V_z) and clearance (Cl).



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IV. In vitro PK experiments

Kinetic solubility protocol:

A turbidimetric method was used. First a series of DMSO compound stock solutions was prepared (0.15-5 mM) An aliquot of 4µL stock solution was added to 196µL PBS buffer (pH 7.4). A series of concentrations were prepared (3.13-200 µM), includeing a blank on a microtiter plate. (2% DMSO – D6) The microtiter plate was shaken for 10 seconds and incubated for 2 hours at 37°C. When there was no turbidity measured at a given concentration, the sample was assumed to be dissolved.

Log D protocol

20 µL of a 10 mM DMSO stock solution of the compound was added to 990 µL saturated PBS (pH 7.4). To this mixture was 990 µL of octanol added. The mixture was shaken for 2 hours at 37°C, then at room temperature for 10minutes. 4 µL of octanol was added to 996 µL methanol and analyzed in triplicate. The PBS is injected directly without any further dilution and analyzed in triplicate. The experiment was done in duplicate. A standard curve of six points was made to fit the measured concentrations.

Plasma stability protocol

5 µl of a 10mM stock solution of the compound in DMSO was added to 995 µl of plasma to obtain a 50 µM final solution. The mixture was gently shaken for 6 h at 37°C. Aliquots of 100 µl were taken at various time points (0, 0.5h, 1 h, 2 h, 3 h, 6 h) and diluted with 400 µl of cold methanol (stored at 4°C). The suspension was centrifuged at 14000 rpm for 5 min. 50 µl of the supernatant was diluted with 950 µl of methanol and analyzed with LC/MS/MS. The samples were analyzed in triplicate. The experiment was done in duplicate. A standard curve of six points was made to fit the measured concentrations. Enalapril maleate was used as a control in mice, rats, and in human.

Metabolic stability protocol

Liver microsomes (20 mg protein/ml), NADPH regenerating system solutions A & B and 5 mM stock compound solution (100% DMSO) are prepared. The reaction mixture finally contains 713 µL purified water, 200 µL 0.5 M potassium phosphate pH 7.4, 50 µL NADPH regenerating system solution A (BD Biosciences Cat. No. 451220), 10 µL NADPH regenerating system solution B (BD Biosciences Cat. No. 451200) and 2 µL of the compound stock solution (10 µM final concentration). The reaction mixture is warmed to 37°C for 5 minutes in a water bath and the reaction is initiated by addition of 25 µL of liver microsomes (0.5 mg protein/ml final concentration). At different time points (0 min – 15 min –

30 min – 60 min – 120 min – 240 min – 360 min – 24h), 20 µL is withdrawn and 80 µL cold acetonitrile is added on ice for 10 minutes. Then the mixtures are centrifuged at 13 000 rpm for 5 min at 4°C. The supernatant is further diluted in 90% water/MeCN (1/25) to fall within the range of the LC/MS/MS analysis. At each time point, the compound is analyzed using LC/MS/MS with the same UPLC system as described above. A standard curve of six points was made to fit the measured concentrations. Verapamil was used as a positive control.

PBS buffer stability protocol

A µM solution of the compound in DMSO was prepared. 5 µL of this solution was added to a PBS buffer (pH 7.4) to give a final concentration of 1 µM. The mixture was stirred for 24h at 37°C. At different time points (0 min – 15 min – 30 min – 60 min – 120 min – 240 min – 360 min – 24h), 20 µL is withdrawn, diluted to 100 µL and analyzed using LC/MS/MS with the same UPLC system as described for the *in vivo* pK experiments. A standard curve of six points was made to fit the measured concentrations.

Detailed Cytotoxicity data

Assay protocol:

Cell culture:

MRC-5_{SV2} cells (human embryonic diploid fibroblasts) were obtained from Sigma and cultured in MEM + Earl's salts-medium, supplemented with L-glutamine, NaHCO₃ and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37°C under an atmosphere of 5% CO₂. All media, solvents, supplements, plates and reagents used in these experiments were also obtained from Sigma.

Compound solutions/dilutions:

Compound stock solutions were prepared in 100% DMSO at 20 mM. The compounds were serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%.

Compound assays:

The compounds were tested at 5 concentrations (64 - 16 - 4 - 1 and 0.25 μM). Assays were performed in sterile 96-well microtiter plates, each well containing 10 μL of the aqueous compound dilutions together with 190 μL of MRC-5_{SV2} inoculum (3×10^4 cells/ml). Cell growth was compared to untreated control wells (100% cell growth) and medium control wells (0% cell growth). After 3 days of incubation, cell viability was assessed fluorimetrically after addition of 50 μL resazurin solution (50 $\mu\text{g/mL}$ in PBS-buffer) per well. After 4 hours at 37°C, fluorescence was measured (λ_{ex} 550 nm, λ_{em} 590 nm). The results were obtained as % reduction in cell growth/viability compared to control wells, based on which an IC_{50} -value was determined. A compound was classified as non-toxic when the IC_{50} -value was higher than 30 μM . Between 10 and 30 μM , the compound was regarded as moderately toxic. When the IC_{50} -value was lower than 10 μM , the compound is classified as highly toxic. Cytotoxic reference compounds included were vinblastine and paclitaxel ($\text{IC}_{50} < 0.01 \mu\text{M}$).

Compound Nr	MRC-5, IC_{50} (μM)		Activity score MRC-5	Follow-up
UAMC-01063-4	> 64,00		< 1	non-toxic
UAMC-01109-29	> 64,00		< 1	non-toxic
UAMC-01110-60	> 64,00		< 1	non-toxic
UAMC-01233-5	> 64,00		< 1	non-toxic
UAMC-01241-52	> 64,00		< 1	non-toxic
UAMC-01285-61	> 64,00		< 1	non-toxic
UAMC-01532-66	> 64,00		< 1	non-toxic
UAMC-01530-57	28,84		= 2	slightly toxic

Screening panel		Date	Reference validation	
			Ref-A	IC_{50} (μM)
1	MRC-5	11/01/2013	Tamoxifen	10,73

V. References

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