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

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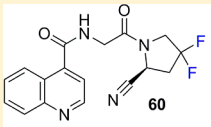
Supporting Information

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.



IC_{50}(FAP)= 3.2 nM
IC_{50}(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 an 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]

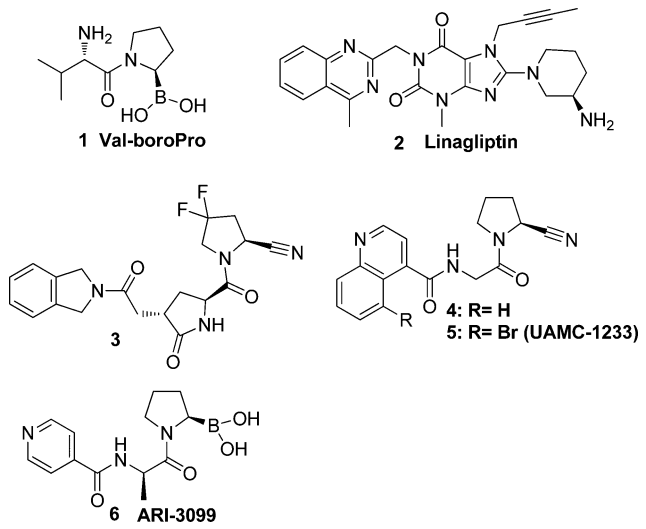

Figure 1. Relevant FAP inhibitors used as references in this study.

Linagliptin **2** has received clinical approval as a DPPIV 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. IC_{50} of Reference FAP Inhibitors

	IC_{50} (μM)[a]					
compd	FAP	PREP	DPPIV	DPP9	DPP2	SI (FAP/PREP)[b]
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

[a]Determined under our own assay conditions. [b]SI stands for "selectivity Index" (calculated as [IC_{50}(PREP)/IC_{50}(FAP)]). [c]ND stands for "not determined".

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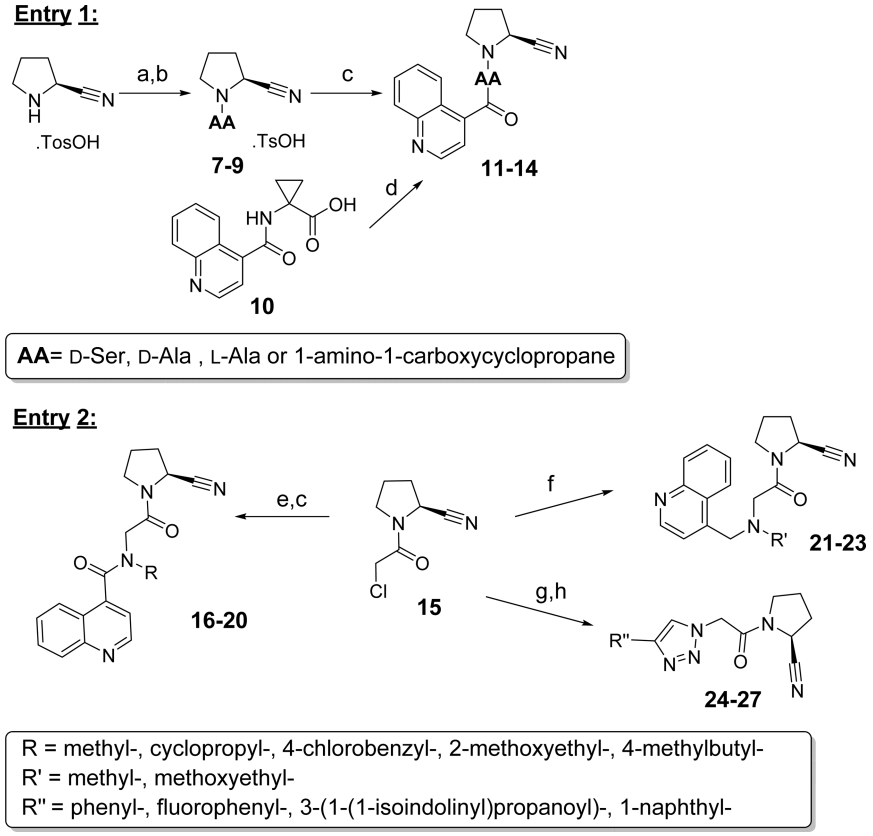
Scheme 1. Synthesis of P2-Modified FAP Inhibitors 11−14 and 16−27[a]



[a]Reagents 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) K_2CO_3, RNH_2, 13−52%; (f) K_2CO_3, quinolin-4-ylmethanamine, 25−49%; (g) NaN_3, 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 intermediate[28] (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, DPPII, 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

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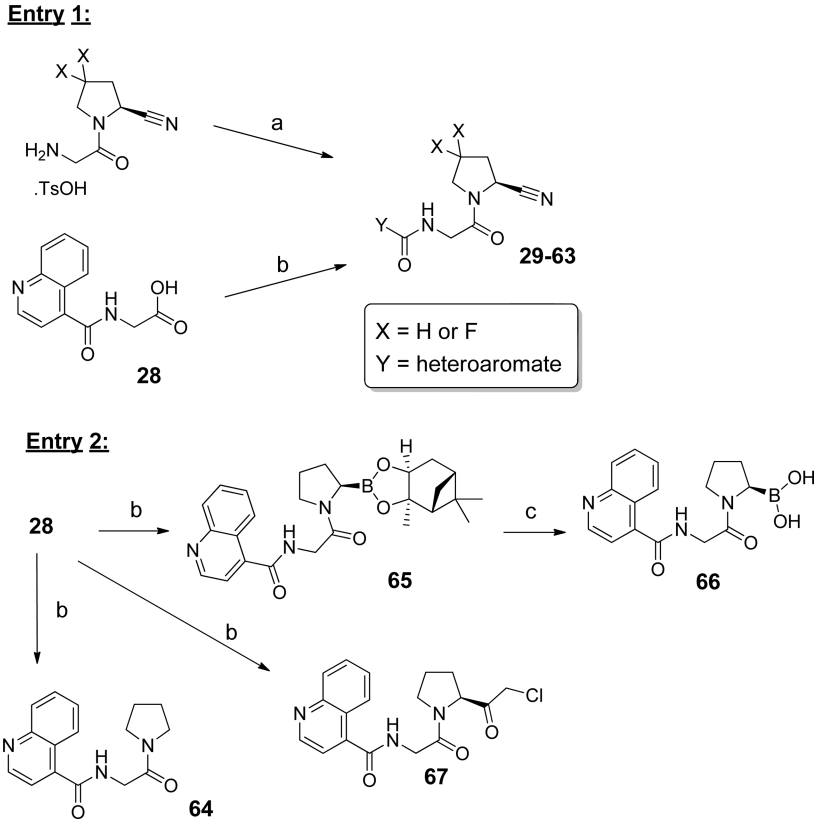
Scheme 2. General Synthetic Strategies for the Synthesis of P1 and/or P3 Modified Target Compounds[a]



[a]Reagents and conditions: (a) YCOOH, HATU, DIPEA, 3 h, 43−67%, or YCOOH, HOBT, EDC, DIPEA, 16 h, 84−86%, or YCOOH, $(CH_3)_2C{=}C(Cl)N(CH_3)_2$, 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-quinolinoyl 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

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Table 2. IC_{50} Data of Inhibitors with a Modified P2 Residue

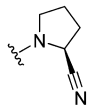
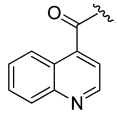
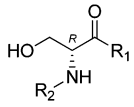
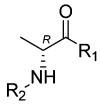
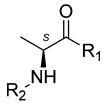
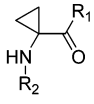
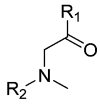
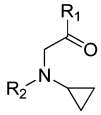
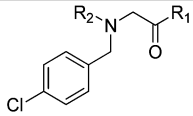
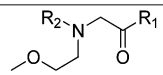
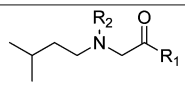
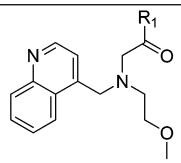
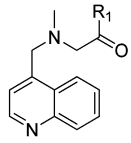
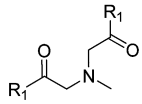
		R_1 =  R_2 = 					
Nr	Structure	IC_{50} (μM)					SI (FAP/PREP)[a]
		FAP	PREP	DPPIV	DPP9	DPPII	
11		6 ± 0.2	>100	>100	>100	>100	16.7
12		3.4 ± 0.1	5.8 ± 0.6	>100	>100	>100	1.7
13		>100	0.044 ± 0.004	>100	>100	>100	< 0.005
14		>100	46 ± 2	>100	>100	>100	0.46
16		37 ± 2	25 ± 2	>100	>25	>100	0.7
17		>100	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

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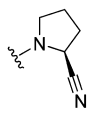
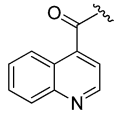
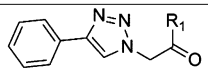
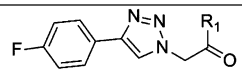
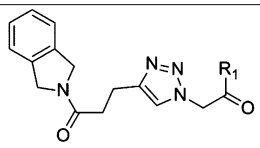
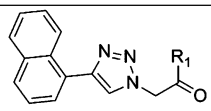
Table 2. continued

		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

[a]SI stands for "selectivity index" (calculated as [IC_{50}(PREP)/IC_{50}(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 IC_{50} 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

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Table 3. IC_{50} Data of Five- and Six-Membered P3 Heterocycles

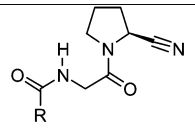
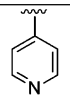
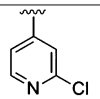
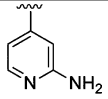
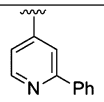
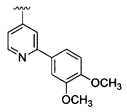
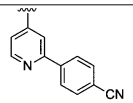
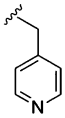
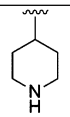
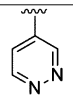
							
Nr	R =	IC_{50} (μM)					SI (FAP/PREP)[a]
		FAP	PREP	DPPIV	DPP9	DPPII	
29		0.063± 0.003	11.3 ± 1.2	>100	>100	>100	179.4
30		0.76 ± 0.04	6.8 ± 0.4	>100	>100	>100	8.9
31		0.126± 0.009	7.3 ± 0.5	>100	>100	>100	57.9
32		0.138± 0.006	33.7 ± 1.6	>100	>100	>100	244.2
33		0.29 ± 0.02	14.2 ± 1.0	>100	>25	>100	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

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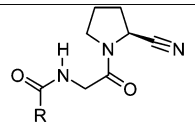
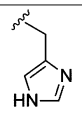
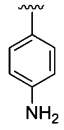
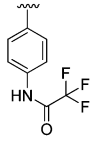
Table 3. continued

							
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

[a]SI stands for "selectivity index" (calculated as [IC_{50}(PREP)/IC_{50}(FAP)]).

is equivalent to the azaheteroatom in quinoline **4** (Table 4). With the set of inhibitors **47**–**51**, 2,3-annelated 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 annelated five-membered heterocycle leads to lower target affinities. A similar effect seems to be in place when a six-membered heterocycle is annelated with pyridine (1,7-naphthyridine **51**). The IC_{50} 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 10^3). 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 × 10^3). 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 4*S*-fluoride. Similarly, (2*S*,4*S*)-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 (2*S*,4*R*)-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

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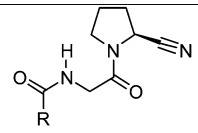
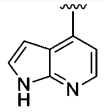
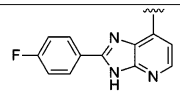
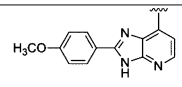
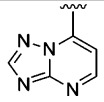
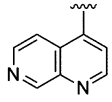
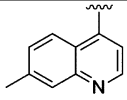
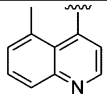
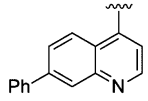
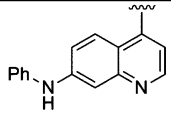
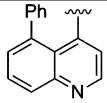
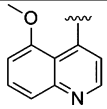
Table 4. IC_{50} Data of Bicyclic P3 Heterocycles

							
		IC_{50} (μM)					SI (FAP/PREP)[a]
Nr.	R	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

[a]SI stands for "selectivity index" (calculated as [IC_{50}(PREP)/IC_{50}(FAP)]). [b]Solubility 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 4*S*-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 4*S*-fluorinated (2-cyano)pyrrolidine inhibitors.[27] Most remarkably, the 4*R*-fluoropyrrolidine isomer **59** has lost FAP affinity compared to **4** and **58**, amounting to up to 3 orders of magnitude. Making

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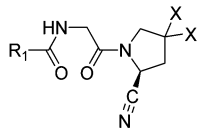
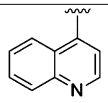
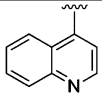
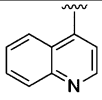
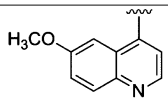
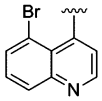
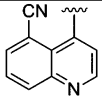
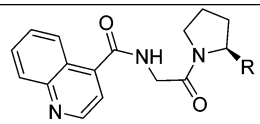
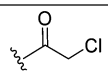
Table 5. IC_{50} Data of Inhibitors with a Modified P1 Residue

								
Nr	R_1	X	IC_{50} (μM)					**SI (FAP/PREP)[a]**
			FAP	PREP	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

[a]SI stands for "selectivity index" (calculated as [IC_{50}(PREP)/IC_{50}(FAP)]).

Table 6. Influence of the Warhead on IC_{50}

							
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

[a]SI stands for "selectivity index" (calculated as [IC_{50}(PREP)/IC_{50}(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 4*S*-fluoroproline derivatives possess a hyperconjugatively stabilized endo-puckered ring conformation, while in 4*R*-fluoroprolines an exo-puckered ring conformer is more favored. This difference, which should also be present with 2-cyanopyrrolidine 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

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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 D	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

[a]nd stands for "not determined".

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

	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC (μg·h/mL)	Cl (mL/min)	V_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	

[a]Compound was formulated in PEG_{200} and administered via single intravenous injection at 5 mg/kg. [b]Compound was formulated in PEG_{200} 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 metabolization 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

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

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 p*K* 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 preliminar 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 4*S*-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 4*R*-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 ^{1}H NMR and mass spectrometry. ^{1}H 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 × 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 × 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-5SV2 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 °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 °C) solution of the Boc-protected product (1 equiv) in acetonitrile (0.4 M) at 0 °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-Quinolinoyl) 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 °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×), 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.

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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. ^{1}H NMR (400 MHz, $CDCl_3$) (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*,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 $NaHCO_3$ 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 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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. ^{1}H NMR (400 MHz, D_2O) 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. ^{1}H NMR (400 MHz, D_2O) 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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. ^{1}H NMR (400 MHz, D_2O) 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. ^{1}H 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 $[M + H]^+$ (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. ^{1}H NMR (400 MHz, $CDCl_3$) 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, ^{13}C NMR (101 MHz, $CDCl_3$) 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, ^{13}C NMR (101 MHz, $CDCl_3$) 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, ^{1}H NMR (400 MHz, $CDCl_3$) 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, ^{1}H NMR (400 MHz, $CDCl_3$) 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, ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.10, 166.71, 149.92, 148.79, 141.13, 130.17, 130.02, 127.96, 125.29,

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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 $[M + H]^+$ (96%); LC−MS (A) (ESI) t_R 10.2 min, m/z 323.0 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (96%); LC−MS (A) (ESI) t_R 10.5 min, m/z 323.0 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (96%); UPLC I (ESI) t_R 1.15 min, m/z 335.6 $[M + H]^+$ (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. ^{1}H 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) I t_R 1.05, m/z 323.50 $[M + H]^+$; LC−MS (A) (ESI) t_R 10.4 min, m/z 323.1 $[M + H]^+$ (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. ^{1}H 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) I t_R 1.26, m/z 349.40 $[M + H]^+$; LC−MS (A) (ESI) t_R 11.7 min, m/z 349.1 $[M + H]^+$ (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. ^{1}H 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) I t_R 1.69, m/z 433.30 $[M + H]^+$; LC−MS (A) (ESI) t_R 15.6 min, m/z 433.3 $[M + H]^+$ (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. ^{1}H 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) I t_R 1.21, m/z 367.30 $[M + H]^+$; LC−MS (A) (ESI) t_R 11.2 min, m/z 367.1 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (99%); yellowish oil, 0.056 g, 33%.

(_S_)-1-(2-((2-Methoxyethyl)(quinolin-4-ylmethyl)amino)-acetyl)pyrrolidine-2-carbonitril (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×), 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. ^{1}H 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) I t_R 1.18, m/z 353.50 $[M + H]^+$; LC−MS (A) (ESI) t_R 10.3 min, m/z 353.1 $[M + H]^+$ (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. ^{1}H 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) I t_R 0.57, m/z 309.50 $[M + H]^+$; LC−MS (A) (ESI) t_R 8.8 min, m/z 309.1 $[M + H]^+$ (97%); 0.131 g, 48.8%.

(2*S*,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. ^{1}H 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) I t_R 0.29, m/z 304.50 $[M + H]^+$; LC−MS (A) (ESI) t_R 2.2 min, m/z 304.1 $[M + H]^+$ (98%); yellowish oil, 0.028 g, 13%.

(*S*)-1-(2-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)acetyl)pyrrolidine-2-carbonitrile (24). The title product was prepared using general procedure E. ^{1}H 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 $[M + H]^+$ (96%); white powder, 54 mg, 62%.

(*S*)-1-(2-(4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)acetyl)-pyrrolidine-2-carbonitrile (25). The title product was prepared using general procedure E. ^{1}H 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 $[M + H]^+$ (97%); white powder, 39 mg, 37%.

(*S*)-1-(2-(4-(3-(Isoindolin-2-yl)-3-oxopropyl)-1*H*-1,2,3-triazol-1-yl)acetyl)pyrrolidine-2-carbonitrile (26). The title product was prepared using general procedure E. ^{1}H 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 $[M + H]^+$ (97%); white powder, 36 mg, 23%.

(*S*)-1-(2-(4-(Naphthalen-1-yl)-1*H*-1,2,3-triazol-1-yl)acetyl)-pyrrolidine-2-carbonitrile (27). The title product was prepared using general procedure E. ^{1}H 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 $[M + H]^+$ (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-carbonyl 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 $[M + H]^+$ (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%. ^{1}H 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. ^{1}H 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 $[M + H]^+$; LC−MS (A) t_R 3.7 min, m/z 259.1 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (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. ^{1}H NMR (400 MHz, 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 $[M + H]^+$ (98%); LC−MS (A) t_R 2.2 min, m/z 274.0 $[M + H]^+$ (97%); yellowish oil, 40 mg, 60%.

(*S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-phenyl-isonicotinamide (33). The title product was prepared using general procedure A using 2-phenylisonicotinic acid **S11**. ^{1}H NMR (400 M Hz, $CDCl_3$) (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 $[M + H]^+$ (98%); LC−MS (A) t_R 1.32min, 335.6 m/z $[M + H]^+$ (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**. ^{1}H 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),

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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**. ^{1}H 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. ^{1}H 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%). ^{1}H 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. ^{1}H 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), ^{1}H 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, ^{1}H 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-1*H*-imidazole-5-carboxamide (39). The title product was prepared using general procedure A. ^{1}H 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)-1*H*-1,2,3-triazole-5-carboxamide (40). The title product was prepared using general procedure A. ^{1}H 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)-1*H*-1,2,4-triazole-3-carboxamide (41). The title product was prepared using general procedure A. ^{1}H 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-(1*H*-imidazol-4-yl)acetamide (42). The title product was prepared using general procedure A. ^{1}H 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. ^{1}H 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. ^{1}H 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%.

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(*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). ^{1}H 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). ^{1}H NMR (400 MHz, $CDCl_3$) (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)-1*H*-pyrrolo-[2,3-*b*]pyridine-4-carboxamide (47). The title product was prepared using general procedure A. ^{1}H 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)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide (48). The title product was prepared using general procedure A using 2-(4-fluorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylic acid **S26**. ^{1}H 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); ^{13}C 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)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxamide (49). The title product was prepared using general procedure A using 2-(4-fluorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-7-carboxylic acid **S28**. ^{1}H 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); ^{13}C 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. ^{1}H 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, ^{1}H NMR (400 MHz, $CDCl_3$) 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, ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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**. ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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**. ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (101 MHz, $CDCl_3$) 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**. ^{1}H NMR (400 MHz, $CDCl_3$) 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 $[M + H]^+$ (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**. ^{1}H 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 $[M + H]^+$ (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**. ^{1}H 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); mionor rotamer, ^{1}H 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 $[M + H]^+$ (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**. ^{1}H 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 $[M + H]^+$ (97%); LC−MS (A) t_R 14.0 min, m/z 339.1 $[M + H]^+$ (97%); white crystals, 63 mg, 64%.

***N*-(2-((2*S*,4*S*)-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**. ^{1}H 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 1.11min, m/z 327.6 $[M + H]^+$ (98%); LC−MS (A) t_R 9.5 min, m/z 327.0 $[M + H]^+$ (98%); white crystals, 95 mg, 60%.

***N*-(2-((2*S*,4*R*)-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**. ^{1}H 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 1.09min, m/z 327.6 $[M + H]^+$ (99%); LC−MS (A) t_R 9.1 min, m/z 327.0 $[M + H]^+$ (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. ^{1}H 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 $[M + 1]^+$; LC−MS (A) t_R 10.8 min, m/z 345.0 $[M + H]^+$ (98%); HRMS calcd for $C_{17}H_{15}N_4O_2F_2$ $[M + H]^+$, 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. ^{1}H 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 (dd, 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 $[M + H]^+$ (99%); LC−MS (A) (ESI) t_R 11.6 min, m/z 374.9 $[M + H]^+$ (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. ^{1}H 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 $[M + H]^+$ (96%); LC−MS (A) t_R 15.8 min, m/z 265.9, 267.9 (95%); white powder, 89 mg, 54%.

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(*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 μmol), 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 × 10 mL) and 5% NH_4OH (1 × 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%). ^{1}H NMR (400 MHz, 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 $[M + H]^+$ (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. ^{1}H NMR (400 MHz, 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, 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 1.11min, m/z 284.7 $[M + H]^+$ (98%); LC−MS (A) t_R 7.8 min, m/z 284.1 $[M + H]^+$ (98%); white powder, 0.187 g, 73%.

***N*-(2-Oxo-2-((2*R*)-2-((3a*S*,7a*R*)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)-ethyl)quinoline-4-carboxamide (65).** (2*R*)-2-((3a*S*,7a*R*)-3a,5,5-Trimethylhexahydro-4,6-methanobenzo[*d*][1,3,2]dioxaborol-2-yl)-pyrrolidine hydrochloride[33] (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. ^{1}H 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-((2*R*)-2-((3aS,7a*R*)-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. ^{1}H 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 $[M + H]^+$ (99%); LC−MS (A) t_R 9.3min, m/z 328.0 $[M + H]^+$ (98%); HRMS calcd for $C_{16}H_{19}BN_3O_4$ $[M + H]^+$, 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%). ^{1}H 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 1.25min, m/z 360.6 $[M + H]^+$ (95%); LC−MS (A) t_R 11.0 min, m/z 360.1 $[M + H]^+$ (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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enzymatic assays and the determination of in vitro and in vivo pharmacokinetic parameters.

■ 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

■ REFERENCES

(1) Aertgeerts, K.; Levin, I.; Shi, L.; Snell, G. P.; Jennings, A.; Prasad, G. S.; Zhang, Y.; Kraus, M. L.; Salakian, S.; Sridhar, V.; Wijnands, R.; Tennant, M. G. Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein alpha. *J. Biol. Chem.* **2005**, *280*, 19441−19444.

(2) Acharya, P. S.; Zukas, A.; Chandan, V.; Katzenstein, A. L.; Pure, E. Fibroblast activation protein: a serine protease expressed at the remodeling interface in idiopathic pulmonary fibrosis. *Hum. Pathol.* **2006**, *37*, 352−360.

(3) (a) Wang, X. M.; Yao, T.-W.; Nadvi, N. A.; Osborne, B.; McCaughan, G. W.; Gorrell, M. D. Fibroblast activation protein and chronic liver disease. *Front. Biosci.* **2008**, *13*, 3168−3180. (b) Keene, F. M.; Nadvi, N. A.; Yao, T. W.; Gorrell, M. D. Neuropeptide Y, B-type natriuretic peptide, substance P and peptide YY are novel substrates of fibroblast activation protein-α. *FEBS J.* **2011**, *278* (8), 1316−1332. (c) Levy, M. T.; McCaughan, G. W.; Abbott, C. A.; Park, J. E.; Cunningham, A. M.; Muller, E.; Rettig, W. J.; Gorrell, M. D. Fibroblast activation protein: a cell surface dipeptidyl peptidase and gelatinase expressed by stellate cells at the tissue remodelling interface in human cirrhosis. *Hepatology* **1999**, *29*, 1768−1778.

(4) Dienus, K.; Bayat, A.; Gilmore, B. F.; Seifert, O. Increased expression of fibroblast activation protein-alpha in keloid fibroblasts: implications for development of a novel treatment option. *Arch. Dermatol. Res.* **2010**, *302*, 725−731.

(5) Milner, J. M.; Kevorkian, L.; Young, D. A.; Jones, D.; Wait, R.; Donell, S. T.; Barksby, E.; Patterson, A. M.; Middleton, J.; Cravatt, B. F.; Clark, I. M.; Rowan, A. D.; Cawston, T. E. Fibroblast activation protein alpha is expressed by chondrocytes following a pro-inflammatory stimulus and is elevated in osteoarthritis. *Arthritis Res. Ther.* **2006**, *8*, R23.

(6) Milner, J. M.; Patel, A.; Rowan, A. D. Emerging roles of serine proteinases in tissue turnover in arthritis. *Arthritis Rheum.* **2008**, *58*, 3644−3656.

(7) Brokopp, C. E.; Schoenauer, R.; Richards, P.; Bauer, S.; Lohmann, C.; Emmert, M. Y.; Weber, B.; Winnik, S.; Aikawa, E.; Graves, K.; Genoni, M.; Vogt, P.; Lüscher, T. F.; Renner, C.; Hoerstrup, S. P.; Matter, C. M. Fibroblast activation protein is induced by inflammation and degrades type I collagen in thin-cap fibroatheromata. *Eur. Heart J.* **2011**, *32*, 2713−2722.

(8) Garin-Chesa, P.; Old, L. J.; Rettig, W. J. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7235−7239.

(9) Scanlan, M. J.; Raj, B. K.; Calvo, B.; Garin-Chesa, P.; Sanz-Moncasi, M. P.; Healey, J. H.; Old, L. J.; Rettig, W. J. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5657−5661.

(10) Santos, A. M.; Jung, J.; Aziz, N.; Kissil, J. L.; Puré, E. Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. *J. Clin. Invest.* **2009**, *119*, 3613−3625.

(11) Cheng, J. D.; Valianou, M.; Canutescu, A. A.; Jaffe, E. K.; Lee, H.-O.; Wang, H.; Lai, J. H.; Bachovchin, W. W.; Weiner, L. M. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. *Mol. Cancer Ther.* **2005**, *4*, 351−360.

(12) Huang, Y.; Wang, S.; Kelly, T. Seprase promotes rapid tumor growth and increased microvessel density in a mouse model of human breast cancer. *Cancer Res.* **2004**, *64*, 2712−2716.

(13) O'Brien, P.; O'Connor, B. F. Seprase: an overview of an important matrix serine protease. *Biochim. Biophys. Acta* **2008**, *1784*, 1130−1145.

(14) Walter, R.; Shlank, H.; Glass, J. D.; Schwartz, I. L.; Kerenyi, T. D. Leucylglycinamide released from oxytocin by human uterine enzyme. *Science* **1971**, *173*, 827−829.

(15) García-Horsman, J. A.; Männistö, P. T.; Venäläinen, J. I. On the role of prolyl oligopeptidase in health and disease. *Neuropeptides* **2007**, *41*, 1−24.

(16) Jambunathan, K.; Watson, D. S.; Endsley, A. N.; Kodukula, K.; Galande, A. K. Comparative analysis of the substrate preferences of two post-proline cleaving endopeptidases, prolyl oligopeptidase and fibroblast activation protein α. *FEBS Lett.* **2012**, *586*, 2507−2512.

(17) Christiansen, V.; Jackson, K.; Lee, K. N.; Downs, T. D.; McKee, P. A. Targeting inhibition of fibroblast activation protein-alpha and prolyl oligopeptidase activities on cells common to metastatic tumor microenvironments. *Neoplasia* **2013**, *15*, 348−358.

(18) D'Agostino, G.; Kim, J. D.; Liu, Z.-W.; Jeong, J. K.; Suyama, S.; Calignano, A.; Gao, X.-B.; Schwartz, M.; Diano, S. Prolyl endopeptidase-deficient mice have reduced synaptic spine density in the CA1 region of the hippocampus, impaired LTP, and spatial learning and memory. *Cereb. Cortex* **2012**, *23*, 2007−2014.

(19) Eager, R. M.; Cunningham, C. C.; Senzer, N.; Richards, D. A.; Raju, R. N.; Jones, B.; Uprichard, M.; Nemunaitis, J. Phase II trial of talabostat and docetaxel in advanced non-small cell lung cancer. *Clin. Oncol.* **2009**, *21*, 464−472.

(20) Narra, K.; Mullins, S. R.; Lee, H.-O.; Strzemkowski-Brun, B.; Magalong, K.; Christiansen, V. J.; McKee, P. A.; Egleston, B.; Cohen, S. J.; Weiner, L. M.; Meropol, N. J.; Cheng, J. D. Phase II trial of single agent Val-boroPro (talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. *Cancer Biol. Ther.* **2007**, *6*, 1691−1699.

(21) Eager, R. M.; Cunningham, C. C.; Senzer, N. N.; Stephenson, J.; Anthony, S. P.; O'Day, S. J.; Frenette, G.; Pavlick, A. C.; Jones, B.; Uprichard, M.; Nemunaitis, J. Phase II assessment of talabostat and cisplatin in second-line stage IV melanoma. *BMC Cancer* **2009**, *9*, DOI: 10.1186/1471-2407-9-263.

(22) Thomas, L.; Eckhardt, M.; Langkopf, E.; Tadayyon, M.; Himmelsbach, F.; Mark, M. (*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356), a novel xanthine-based dipeptidyl peptidase 4 iinhibitor, has a superior potency and longer duration of action compared with other dipeptidyl peptidase-4 inhibitors. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 175−182.

(23) Tsai, T.-Y.; Yeh, T.-K.; Chen, X.; Hsu, T.; Jao, Y.-C.; Huang, C.-H.; Song, J.-S.; Huang, Y.-C.; Chien, C.-H.; Chiu, J.-H.; Yen, S.-C.; Tang, H.-K.; Chao, Y.-S.; Jiaang, W.-T. Substituted 4-carboxymethyl-pyroglutamic acid diamides as potent and selective inhibitors of fibroblast activation protein. *J. Med. Chem.* **2010**, *53*, 6572−6583.

(24) Jansen, K.; Heirbaut, L.; Cheng, J. D.; Joossens, J.; Ryabtsova, O.; Cos, P.; Maes, L.; Lambeir, A.-M.; De Meester, I.; Augustyns, K.; Van der Veken, P. Selective inhibitors of fibroblast activation protein (FAP) with a (4-quinolinoyl)-glycyl-2-cyanopyrrolidine scaffold. *ACS Med. Chem. Lett.* **2013**, *4*, 491−496.

(25) Lee, K. N.; Jackson, K. W.; Christiansen, V. J.; Dolence, E. K.; McKee, P. A. Enhancement of fibrinolysis by inhibiting enzymatic cleavage of precursor A2-antiplasmin. *J. Thromb. Haemostasis* **2011**, *9*, 987−996.

(26) Poplawski, S. E.; Lai, J. H.; Li, Y.; Jin, Z.; Liu, Y.; Wu, W.; Wu, Y.; Zhou, Y.; Sudmeier, J. L.; Sanford, D. G.; Bachovchin, W. W. Identification of selective and potent inhibitors of fibroblast activation protein and prolyl ooligopeptidase. *J. Med. Chem.* **2013**, *56*, 3467−3477.

(27) (a) Ryabtsova, O.; Jansen, K.; Van Goethem, S.; Joossens, J.; Cheng, J. D.; Lambeir, A.-M.; De Meester, I.; Augustyns, K.; Van der Veken, P. Acylated Gly-(2-cyano)pyrrolidines as inhibitors of fibroblast activation protein (FAP) and the issue of FAP/prolyl oligopeptidase (PREP)-selectivity. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3412−3417. (b) Van Goethem, S.; Van der Veken, P.; Dubois, V.;

Soroka, A.; Lambeir, A. M.; Chen, X.; Haemers, A.; Scharpe, S.; De Meester, I.; Augustyns, K. Inhibitors of dipeptidyl peptidase 8 and dipeptidyl peptidase 9. Part 2: Isoindoline containing inhibitors. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4159−4162. (c) Van der Veken, P.; Soroka, A.; Brandt, I.; Chen, Y. S.; Maes, M. B.; Lambeir, A. M.; Chen, X.; Haemers, A.; Scharpe, S.; Augustyns, K.; De Meester, I. Irreversible inhibition of dipeptidyl peptidase 8 by dipeptide-derived diaryl phosphonates. *J. Med. Chem.* **2007**, *50*, 5568−5570.

(28) Singh, S. K.; Manne, N.; Pal, M. Synthesis of (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile: a key intermediate for dipeptidyl peptidase IV inhibitors. *Beilstein J. Org. Chem.* **2008**, *4*, 20, DOI: 10.3762/bjoc.4.20.

(29) (a) Van der Veken, P.; De Meester, I.; Dubois, V.; Soroka, A.; Van Goethem, S.; Maes, M. B.; Brandt, I.; Lambeir, A. M.; Chen, X.; Haemers, A.; Scharpe, S.; Augustyns, K. Inhibitors of dipeptidyl peptidase 8 and dipeptidyl peptidase 9. Part 1: Identification of dipeptide derived leads. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4154−4158. (b) Fukushima, H.; Hiratate, A.; Takahishi, M.; Saito-Hori, M.; Munetomo, E.; Kitano, K.; Saito, H.; Takaoka, Y.; Yamamoto, K. Synthesis and structure−activity relationships of potent 1-(2-substituted-aminoacetyl)-4-fluoro-2-cyanopyrrolidine dipeptidyl-peptidase IV inhibitors. *Chem. Pharm. Bull.* **2008**, *56*, 1110−1117.

(30) Kaila, N.; Janz, K.; DeBernardo, S.; Bedard, P. W.; Camphausen, R. T.; Tam, S.; Tsao, D. H. H.; Keith, J. C.; Nickerson-Nutter, C.; Shilling, A.; Young-Sciame, R.; Wang, Q. Synthesis and biological evaluation of quinoline salicylic acids as P-selectin antagonists. *J. Med. Chem.* **2007**, *50*, 21−39.

(31) Zhu, H.; Yang, R. F.; Yun, L. H.; Li, J. Facile and efficient synthesis of quinoline-4-carboxylic acids under microwave irradiation. *Chin. Chem. Lett.* **2010**, *21*, 35−38.

(32) Chen, P.; Cheng, P. T. W.; Spergel, S. H.; Zahler, R.; Wang, X.; Thottathil, J.; Barrish, J. C.; Polniaszek, R. P. A practical method for the preparation of A′-chloroketones of *N*-carbamate protected-α-aminoacids. *Tetrahedron Lett.* **1997**, *38*, 3175−3178.

(33) Kelly, T. The efficient synthesis and simple resolution of a prolineboronate ester suitable for enzyme-inhibition studies. *Tetrahedron* **1993**, *49*, 1009−1016.

(34) Edosada, C. Y.; Quan, C.; Tran, T.; Pham, V.; Wiesmann, C.; Fairbrother, W.; Wolf, B. B. Peptide substrate profiling defines fibroblast activation protein as an endopeptidase of strict Gly(2)-Pro(1)-cleaving specificity. *FEBS Lett.* **2006**, *580*, 1581−1586.

(35) Pandey, A. K.; Naduthambi, D.; Thomas, K. M.; Zondlo, N. J. Proline editing: a general and practical approach to the synthesis of functionally and structurally diverse peptides. Analysis of steric versus stereoelectronic effects of 4-substituted prolines on conformation within peptides. *J. Am. Chem. Soc.* **2013**, *135*, 4333−4363.

(36) Shoulders, M. D.; Kamer, K. J.; Raines, R. T. Origin of the stability conferred upon collagen by fluorination. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3859−3862.

(37) Mattheeussen, V.; Lambeir, A. M.; Jungraithmayr, W.; Gomez, N.; McEntee, K.; Van der Veken, P.; Scharpé, S.; De Meester, I. Method comparison of DPP IV activity assays and their application in biological samples containing reversible inhibitors. *Clin. Chim. Acta* **2012**, *412*, 456−462.

(38) Van Goethem, S.; Mateeussen, V.; Joossens, J.; Lambeir, A. M.; Chen, X.; De Meester, I.; Haemers, A.; Augustyns, K.; Van der Veken, P. Structure−activity relationship studies on isoindoline inhibitors of dipeptidyl peptidases 8 and 8 (DPP8, DPP9): Is DPP8-selectivity attainable? *J. Med. Chem.* **2011**, *54*, 5737−5746.