

Selective Inhibitors of Fibroblast Activation Protein (FAP) with a (4-Quinolinoyl)-glycyl-2-cyanopyrrolidine Scaffold

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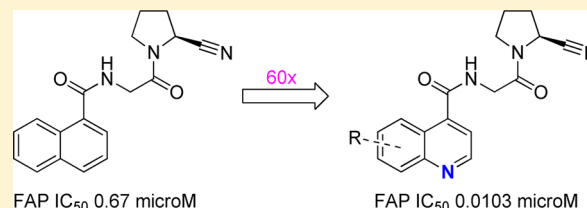
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S Supporting Information

ABSTRACT: Fibroblast activation protein (FAP) is a serine protease that is generally accepted to play an important role in tumor growth and other diseases involving tissue remodeling. Currently there are no FAP inhibitors with reported selectivity toward both the closely related dipeptidyl peptidases (DPPs) and prolyl oligopeptidase (PREP). We present the discovery of a new class of FAP inhibitors with a *N*-(4-quinolinoyl)-Gly-(2-cyanopyrrolidine) scaffold. We have explored the effects of substituting the quinoline ring and varying the position of its *sp*² hybridized nitrogen atom. The most promising inhibitors combined low nanomolar FAP inhibition and high selectivity indices (>10³) with respect to both the DPPs and PREP. Preliminary experiments on a representative inhibitor demonstrate that plasma stability, kinetic solubility, and log *D* of this class of compounds can be expected to be satisfactory.

KEYWORDS: Fibroblast activation protein (FAP), dipeptidyl peptidase IV (DPPIV), prolyl oligopeptidase (PREP), seprase



Fibroblast activation protein (FAP, FAP- α , seprase) is a type II transmembrane serine protease, belonging to the prolyl oligopeptidase family. This family comprises serine proteases that cleave peptides preferentially after proline residues. Other important members of this family that are expressed in the human proteome are prolyl oligopeptidase (PREP) and the dipeptidyl peptidases (DPPs): DPPIV, DPPII, and DPP8/9.¹

FAP expression is seen on activated stromal fibroblasts and pericytes of 90% of common human epithelial tumors examined.^{2,3} It has been established that FAP expression promotes tumorigenesis in mouse models and that FAP inhibition can attenuate tumor growth.^{4,5} FAP is also highly expressed in lesions characterized by activated stromal tissue such as present in cirrhosis, fibrotic diseases, osteoarthritis and rheumatoid arthritis, keloidosis, and in healing wounds.^{6–11}

FAP has been demonstrated to possess both dipeptidyl peptidase and endopeptidase activity, catalyzed by the same active site. Several studies have identified in vitro substrates for FAP. Peptides found to be substrates of FAP's dipeptidyl peptidase activity include Neuropeptide Y, B-type natriuretic peptide, substance P, and peptide YY. Analogously, α 2-antiplasmin, type I collagen, and gelatin were found to behave as in vitro substrates of the endopeptidase activity of FAP.^{12–14}

Nonetheless, the relevance of these findings under in vivo conditions has to be confirmed.

Currently no inhibitors with low nanomolar FAP affinity and selectivity toward both PREP and the DPPs have been reported. Most research effort in the domain of FAP-inhibitor discovery to date has been centered around pyrrolidine-2-boronic acid derivatives.^{15,16} These compounds in general also display significant affinity for one or several DPPs.^{15,16} The most representative of this class, Val-*boro*Pro (Talabostat, PT-100) **1** has reached phase II clinical trials (Table 1). It was evaluated as a therapeutic drug for, among others, metastatic kidney cancer, pancreatic adenocarcinoma, nonsmall cell lung cancer, and chronic lymphocytic leukemia (Figure 1). While talabostat in several of these trials was able to induce clinical response, safety concerns potentially related to the drug's lack of selectivity led to its withdrawal from further development.^{17,18}

More recently, we and the group of Jiaang have focused on 2-cyanopyrrolidine derivatives for FAP inhibitor discovery.^{19,20} The 2-cyanopyrrolidine fragment has been successfully ex-

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Table 1. IC₅₀s for Reference Compounds 1 and 2

Nr	IC ₅₀ (μM)				
	FAP	DPP IV	DPP9	DPP II	PREP
1	0.07 ± 0.01	0.022 ± 0.001	ND ^a	0.086 ± 0.007	0.98 ± 0.06
2	0.37 ± 0.002	0.0020 ± 0.0002	>100	>100	>100

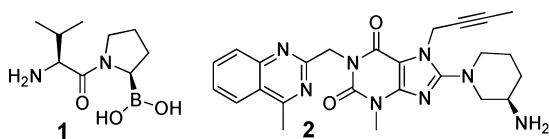
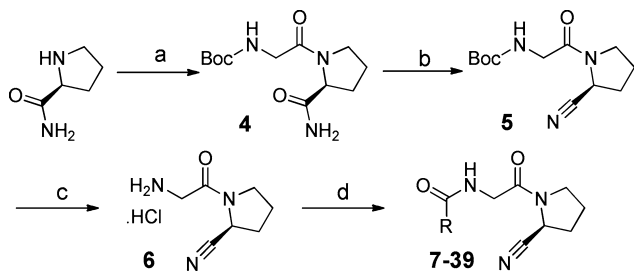
^aNot determined.

Figure 1. Structure of reference inhibitors used in this study: Val-boroPro (1) and linagliptin (2).

plored before for discovery of DPP inhibitors, exemplified, for example, by the marketed DPP IV inhibitors vildagliptin and saxagliptin.^{21–24} The work presented here deals with a group of 2-cyanopyrrolidines that represent the first examples of inhibitors combining low nanomolar FAP affinity and significant selectivity with respect to both PREP and the DPPs. Reference inhibitors used in this study are 1 and the FDA-approved, clinically used DPP IV inhibitor linagliptin (Tradgenta) 2 (Table 1).²⁵ The latter compound is structurally distinct from 1 but has also been described to possess significant FAP affinity.

Previous results pointed out that the *N*-acyl-glycyl-(2-cyanopyrrolidine) scaffold has significant potential to deliver FAP inhibitors with good selectivity toward PREP and the dipeptidyl peptidases.¹⁹ The most potent compound identified (*N*-(1-naphthoyl)-Gly-(2-cyanopyrrolidine), 3) displayed high nanomolar FAP-affinity.^{19,20} For further optimization of this compound's potency, we proposed further exploration of the P3 area, containing the naphthyl group. A docking study furthermore indicated that the 1-naphthoyl residue could be involved in a cation– π interaction with the guanidine side chain of FAP's Arg123. A set of analogues potentially capable of corroborating this hypothesis and providing additional SAR information in the parts of chemical space surrounding the 1-naphthoyl group were prepared.

A general pathway for the synthesis of the target compounds 3–39 is displayed in Scheme 1. Commercially available prolinamide was coupled to *N*-Boc-glycine giving *N*-Boc-glycine-prolinamide 4 and subsequently dehydrated with

Scheme 1. Synthesis of Target Compounds 3–39^a

^aReagents and conditions: (a) Boc-glycine, HATU, DIPEA, DMF-DCM, rt, 71%; (b) trifluoroacetic anhydride, pyridine, THF, 0 °C, 92%; (c) (i) TFA, MeCN, 0 °C, 24h; (ii) HCl, diethylether, 84%; (d) HATU, DIPEA, R-COOH or 1-chloro-*N,N*,2-trimethylprop-1-en-1-amine, RCOOH, TEA or RCOCl, TEA, 20–76%.

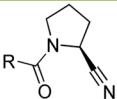
trifluoroacetic anhydride to yield the corresponding nitrile 5. The nitrile was deprotected in acetonitrile to give hydrochloric acid salt 6, which was then coupled with a carboxylic acid or acyl chloride to give final compounds.

All compounds synthesized in this study were evaluated as inhibitors of FAP, DPP IV, DPP9, DPP II, and PREP using a chromogenic substrate assay.¹⁹ It is worthwhile to stipulate that DPP9 potencies reported can reasonably be expected to also be indicative for inhibitor affinities toward the highly homologous DPP8.^{26,27}

The results summarized in Table 2 show that the *N*-(4-quinolinoyl) substituted compound 7 has about 60 times more FAP-affinity than the initial *N*-(1-naphthoyl) based 'hit' 3. Its potency as an inhibitor of FAP also clearly stands out among the other compounds in Table 2. The presence of other azaheteroaromatic substituents, as, for example, in the *N*-(indolyl-3-acyl) containing 8 and its regio-isomeric congener 9, leads to a drastic drop in FAP-affinity. The same affinity trend was observed for compounds 10–12: in these molecules, the electron density in the naphthyl ring can reasonably be expected to be higher than in compound 3. Directly inspired by the docking study, these molecules were designed to gain affinity from a stronger cation– π interaction with FAP's Arg123. Further deviation from 'hit' structure 3 was investigated with compounds 13 and 14 in which the acylated glycine amine function was changed for an oxygen atom. This, however, was also found to be detrimental for FAP potency. Likewise and in line with results obtained earlier, introduction of a sulfonamide function replacing the P3 acyl group in 15 decreases potency very significantly. The ((5-cyanopyridin-2-yl)amino)acetamide 16 and 3-hydroxyadamantane-1-carboxamide 17 are nonbasic analogues of the *N*-substituted glycyl-(2-cyanopyrrolidine) DPPIV inhibitors NVP-DPP 728 and vildagliptin, respectively.²⁴ By introducing an amide group instead of the parent structures' basic P2-amine function that is critical for DPP IV interaction, we hoped to decrease DPP IV potency, while retaining affinity for the phylogenetically very closely related FAP. Only moderate inhibition of the latter was observed however. Finally, an analogue of compound 18 with a P1-pyrrolidine-2-boronate residue instead of a 2-cyanopyrrolidine group has been described as a low nanomolar FAP inhibitor.²⁸ Nitrile 18, however, loses almost all FAP inhibition when compared to its boronate analogue.

In terms of selectivity, all inhibitors in Table 2 display very limited to no affinity for the DPPs. As mentioned earlier, this can be rationalized by the absence of a basic P2 amine function known to be engaged in salt bridging with two acidic glutamate residues in the active center of the DPPs. The corresponding Glu203 and Glu204 in FAP have a different orientation to accommodate endopeptidase substrates, and thus also allow acylated P2-amines to enter FAP's active center.¹⁵ As reported earlier, selectivity of *N*-acylated inhibitors with respect to the endopeptidase PREP is far less evident. Nonetheless, a comparison of initial 'hit' 3 ($SI_{[FAP/PREP]} = 5.3$) and our new

Table 2. Enzymatic Evaluation Data for Compounds 3–18



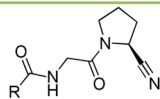
Nr	R	FAP	IC ₅₀ (μM)			
			DPP IV	DPP 9	DPP II	PREP
3		0.67 ± 0.04	>100	>100	>100	3.3 ± 0.2
7		0.010 ± 0.000 ^{a,b}	>100	>100	>100	0.86 ± 0.07
8		3.6 ± 0.2	>100	>25	>100	13.2 ± 0.4
9		15.4 ± 0.4	>100	>100	>100	>100
10		14.0 ± 0.7	>100	>100	>100	7.7 ± 0.5
11		9.4 ± 0.5	>100	>100	>100	5.6 ± 0.5
12		8.5 ± 0.3	>100	>100	>100	0.60 ± 0.06
13		>12.5	>100	>100	>100	33 ± 2
14		>12.5	>100	>100	>100	>50
15		17.6 ± 0.5	>100	>100	>100	8.3 ± 0.5
16		11.8 ± 0.4	>100	>100	>100	>100
17		2.1 ± 0.1	>10	>10	>10	2.4 ± 0.3
18		42 ± 2	>10	>10	>10	39 ± 3

^aFAP-affinity (K_i) was determined to be 3.0 ± 0.4 nM. ^bThe mean result of three separate measurements.

'lead' 7 ($SI_{[FAP/PREP]} = 85$) indicates that selectively optimizing for FAP affinity is possible.

Next, we turned our attention to the effect of substituting the *N*-(4-quinolinyl) ring in 7 (Table 3). Overall, none of the evaluated substituents was able to improve FAP potency significantly, with substituents in the 2- and 3-position of the quinoline ring as in compounds 19–24, having a clearly negative effect on this parameter. Identifying the factors that cause this effect is not evident from these six analogues, but both steric (19, 22, and 23) and electronic (20, 21, and 24, with an increased electron density in the pyridine ring compared to 7) factors seem to be contributive. Remarkably, PREP-affinity is affected in a more intricate manner by these substitution types: the negative effect of 3-hydroxylation in 20 and 24 significantly contrasts with the influence of a 2-hydroxyl functionality in 21. Introducing a chlorine or bromine

Table 3. Effect of Substituents on the 4-Quinolinoyl Residue



Nr	R	FAP	IC ₅₀ (μM)			PRE P
			DPP IV	DPP 9	DPP II	
19		0.67 ± 0.02	>100	>100	>100	0.64 ± 0.04
20		5.9 ± 0.4	>100	>100	>100	53.6 ± 1.3
21		1<IC ₅₀ <10	>100	>100	>100	0.35 ± 0.02
22		>10	>100	>100	>100	1 ± 0.1
23		0.77 ± 0.06	>100	>100	>100	18.5± 1.4
24		0.16 ± 0.01	>100	>100	>100	38 ± 2
25		0.0099 ± 0.0003	>100	>100	>100	16.8 ± 0.5
26		0.011 ± 0.0004 ^a	>100	>100	>100	>50
27		0.014 ± 0.001	>100	>100	>100	0.84 ± 0.07
28		0.012 ± 0.001	>100	>100	>100	0.71 ± 0.05
29		0.0103 ± 0.0007	>100	>100	>100	0.61 ± 0.06
30		0.0092 ± 0.0005	>100	>100	>100	6.1 ± 0.4
31		0.0071 ± 0.0003	>100	>100	>100	1.4 ± 0.08
32		0.0062 ± 0.0004	>100	>100	>100	1.2 ± 0.1
33		0.19 ± 0.01	>100	>100	>100	0.35 ± 0.03
34		0.37 ± 0.02	>100	>100	>100	0.36 ± 0.02

^aFAP-affinity (K_i) was determined to be 4.1 ± 0.6 nM.

substituent at the 5-position of the quinoline ring such as in analogues 25 and 26 has limited effect on FAP potency. Most remarkably, the selectivity with respect to PREP of these inhibitors sharply increases ($SI_{[FAP/PREP]} > 1000$), rendering

them the most favorable affinity/selectivity profile of all FAP-inhibitors we had identified so far. Functionalizing the quinoline ring's 6-position as in compounds **27–30** does not generally lead to marked differences in the affinity and selectivity profiles of the corresponding inhibitors compared to 'lead' **7**. The most notable compound in this subseries of molecules is the 6-methoxyquinoline **30**, which displays 10-fold less PREP affinity than its congeners. The 7-substituted chloroquinoline **31** and 7-bromoquinoline **32** again do not display relevantly changed FAP affinity when compared to **7**, but they do have moderately increased selectivity toward PREP. Finally, 8-chloroquinoline **33** and 8-bromoquinoline **34** were found to possess significantly reduced FAP potency, possibly due to steric effects. These modifications, however, have little effect on the PREP inhibitory potential of the corresponding molecules.

We subsequently studied the effect of varying the position of the sp^2 hybridized nitrogen atom of the quinoline ring (Table 4). We considered these investigations highly instrumental for

Table 4. Effect of Nitrogen Position in the (Iso-)Quinolinoyl Ring

Nr	R	IC ₅₀ (μM)				
		FAP	DPP IV	DPP 9	DPP II	PREP
35		4.8 ± 0.2	>100	>100	>100	0.61 ± 0.07
7		0.0103 ± 0.0004	>100	>100	>100	0.86 ± 0.07
36		0.42 ± 0.04	>100	>100	>100	3.4 ± 0.2
37		0.107 ± 0.008	>100	>100	>100	2.4 ± 0.1
38		0.158 ± 0.004	>100	>100	>100	11.7 ± 0.8
39		2.17 ± 0.09	>100	>100	>100	8.2 ± 0.8

understanding the striking difference in FAP affinity between 'hit' **3** and 'lead' **7**. If inhibitor affinity would depend significantly on the ring nitrogen's position, this could be indicative for a specific interaction with the enzyme, other than the cation- π complexation we originally hypothesized. On the basis of our docking study, the favorable contribution to binding of the latter would be less dependent on the nature of the (iso-)quinolinoyl isomer selected.

With FAP-affinities spanning almost 3 orders of magnitude, evaluation results of compounds **35–39** nonetheless reveal a pivotal importance of the nitrogen's position. Of all the positional isomers synthesized, the 4-quinolinoyl ring of 'lead' **7** clearly displays the best results and takes in a singular position within this series. The 4-isoquinolinoyl and 8-quinolinoyl

derivatives (**35** and **39**, respectively) are characterized by very low FAP-affinity, even when compared to 'hit' **3**.

In conclusion, we have identified the *N*-(4-quinolinoyl)-glycyl-(2-cyanopyrrolidine) scaffold as highly promising for discovery of FAP-inhibitors. It is the first scaffold type demonstrated to have potential for rendering compounds that combine low nanomolar FAP-affinity and a selectivity index $>10^3$ with respect to both the DPPs and PREP. Our SAR investigations so far have mainly focused on the P3 part of the scaffold but already allow to conclude that the *N*-(4-quinolinoyl) residue is a critical element in determining FAP-affinity. As such, it was found to hold a privileged position within the series of different quinolinoyl and isoquinolinoyl isomers evaluated. Furthermore, we have shown that introduction of substituents at the 5-position of the quinolinoyl ring system is a possible means of maximizing inhibitor selectivity with respect to PREP. So far, however, we have not been able to identify the eventual specific interactions between FAP and the 4-quinolinoyl ring that could underlie the observed affinities.

To this end, we are currently expanding our SAR-data set by investigating additional modification types in the P3, P2, and P1 region of these molecules. In addition, preclinical ADME and in vivo pharmacokinetic parameters of the optimal molecules will be determined. Inhibitor **7** has a kinetic solubility of >200 μM and a log *D* of 0.51 at pH 7.4. Furthermore, **7** was stable for >24 h both in PBS buffer at pH 7.4 and in rat plasma. Furthermore, **26**, which together with inhibitor **25** shares low-nanomolar FAP-affinity and a FAP/PREP selectivity index $>10^3$, has a kinetic solubility of >200 μM and a log *D* of 0.7 at pH 7.4. This molecule was stable for 24 h in PBS and human plasma and 80% stable after 24 h in mouse plasma. It displayed mouse microsomal stability of 70% over 24 h.

■ ASSOCIATED CONTENT

● Supporting Information

Analytical data and experimental procedures for synthetic preparation, enzymatic evaluation, and physicochemical characterization of compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

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

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

FAP, fibroblast activation protein; PREP, prolyl oligopeptidase; DPPII, dipeptidyl peptidase II; DPPIV, dipeptidyl peptidase IV; DPP9, dipeptidyl peptidase 9; HATU, 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIPEA, *N,N*-diisopropylethylamine; SI, selectivity index

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Supporting information

Selective inhibitors of fibroblast activation protein (FAP) with a (4-quinolinoyl)-glycyl-2-cyanopyrrolidine scaffold.

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1) Chemistry: General Experimental Notes

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 mentioned. Characterization of all compounds was done with ^1H NMR and mass spectrometry. ^1H NMR spectra were recorded on a 400 M Hz 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 iontrap mass spectrometer from Bruker Daltonics. Purity was verified using two diverse HPLC systems using, respectively, a mass and UV-detector. 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 (2.1×50 mm, $3 \mu\text{m}$) coupled with an Esquire 3000plus as MS detector and a 'method A' 5–100% B, 20 min gradient was used with a flow rate from 0.2 mL/min. Formic acid 0.1% was added to solvents A and B. Waters acquity UPLC system coupled to a waters TQD ESI mass spectrometer and waters TUV detector. A waters acquity UPLC BEH C18 $1.7 \mu\text{m}$ 2.1×50 mm column was used. Solvent A: water with 0.1% formic acid, solvent B: acetonitrile with 0.1% formic acid. Method I: 0.15min 95% A, 5% B then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25min (0.350mL/min), 95% B, 5% A. The wavelength for UV detection was 254nm. Method II: flow 0.4mL/min, 0.25min 95% A, 5% B, then in 4.75 min to 95%B, 5% A, then 0.25min 95% B, 5% A, followed by 0.75min 95%A, 5%B. The wavelength for UV detection was 214nm. Where necessary flash purification was performed on a Biotage[®] ISOLERA One flash system equipped with a internal variable dual-wavelength diode array detector(200-400nm). For normal phase purifications SNAP cartridges (10-340g; flow rate 10mL/min.-100mL/min.) were used, reversed phase purifications were done making use of KP-C18 containing cartridges. Dry sample loading was done by self packing samplet[®] cartridges using silica or Celite 545 respectively for normal and reversed phase purifications. Gradients used varied by purification. However typical gradients used for normal phase were 30min. gradient of 0-50% EtOAc in hexane to 100 hexane or 0-5% methanol in DCM to 20%

methanol in DCM and for reversed phase a gradient of 10% MeCN in water to 50% MeCN in water. HRMS: The dry samples were dissolved in 1ml methanol and diluted 1/100 in MeCN/H₂O 0.1%formic acid. 10µl of each sample was injected using the CapLC system (Waters, Manchester, UK) 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 minutes Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, UK). The MS was calibrated prior to use with a 0.2% H₃PO₄ solution. The spectra were lock mass corrected using the know mass of the nearest H₃PO₄ 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. Mouse and rat plasma came from Innovative Research. The turbidity was measured using UV/VIS-spectrophotometer Synergy® MX, Biotek with Gen5™

2) Synthesis of compounds

(S)-tert-Butyl 2-(2-carbamoylpyrrolidin-1-yl)-2-oxoethylcarbamate (4)

2-(3H-[1,2,3]Triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (17,84g, 46,9mmol) was dissolved in 15mL DMF and slowly added to a solution of 2-(tert-butoxycarbonylamino)acetic acid (8.22 g, 46.9 mmol) and N-ethyl-N-isopropylpropan-2-amine (15.55 ml, 94 mmol) in 40mL DCM. After 5min a solution of (S)-pyrrolidine-2-carboxamide (5.1 g, 44.7 mmol) in DCM was added. After 3h stirring at room temperature some isopropanol and dichloromethane were added, the mixture was washed with 1N HCl in water (2x), once with saturated sodium bicarbonate and brine, dried over sodium sulfate, filtered and evaporated. The residue was recrystallized from ethyl acetate to give compound **4**. (8.61g, 71%)

¹H NMR (400 MHz, DMSO-*d*₆) δ 6.83 (br s, 1H), 5.54 (br s, 1H), 5.43 (br s, 1H), 4.18 (dd, *J* = 8.8, 2.8 Hz, 1H), 3.74 (d, *J* = 5.7 Hz, 2H), 3.55 - 3.49 (m, 1H), 3.45 - 3.38 (m, 1H), 2.22 - 1.72 (m, 4H), 1.38 (s, 9H)

MS (ESI) *m/z* 294.2 [M + Na]⁺

(S)-tert-Butyl 2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamate (5)

(S)-tert-Butyl 2-(2-carbamoylpyrrolidin-1-yl)-2-oxoethylcarbamate 4 (8.4 g, 31.0 mmol) was dissolved in dry THF at 0°C. Then pyridine (17.53 ml, 217 mmol) was added, followed by dropwise addition of the solution of trifluoroacetic anhydride (5.03 ml, 35.6 mmol) in 10 mL of DCM. After stirring for 90min, the volatiles were evaporated and the mixture was redissolved in ethyl acetate and subsequently washed with 2 M solution of aqueous hydrochloric acid (3 x 50 mL). The acidic water phase was extracted with ethyl acetate (2x). The aqueous layer, was discarded and the combined organic layers were washed successively with a saturated aqueous solution of sodium bicarbonate (50 mL) and brine. After drying over sodium sulfate, the almost colorless organic layer was evaporated under reduced pressure, yielding the crude whitish crystals that were used without further purification.

¹H NMR (400 MHz, CDCl₃) (9/1 mixture of *trans/cis* amide rotamers) δ 5.40 (s, 1H), 4.83 – 4.69 (m, 0.9H), 4.69 – 4.62 (m, 0.1H), 4.14 (dd, *J* = 16.8, 6.0 Hz, 0.1H), 3.99 (dd, *J* = 17.5, 5.2 Hz, 0.9H), 3.93 - 3.89 (m, 0.1H), 3.88 (dd, *J* = 17.4, 4.2 Hz, 0.9H), 3.64 – 3.55 (m, 1H), 3.47 – 3.39 (m, 1H), 2.36 – 2.06 (m, 4H), 1.45 (s, 9H); MS (ESI) *m/z* 276.1[M + Na]⁺; (7.2g, 92%)

(S)-1-(2-Aminoacetyl)pyrrolidine-2-carbonitrile 4-methylbenzenesulfonate (6)

To an acetonitrile solution containing (S)-tert-butyl 2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamate (0.490 g, 1,934 mmol) was added 4-methylbenzenesulfonic acid hydrate (0,552 g, 2,90 mmol) at room temperature. After 24hours, was removed in vacuo and then the resulting white precipitation was dissolved in 500 mL of diethylether. Within 1 min a solid precipitated out and the solution was cooled in an ice-water bath after stirring for 15min, the solid was collected via vacuum filtration. The collected solid was rinsed with cold (-20 °C) EtOAc (20 mL) and then pumped on under high vacuum overnight yielding (0.52g, 84%)

¹H NMR (400 MHz, DMSO-*d*₆) (9/1 mixture of *trans/cis* amide rotamers) δ 8.13 (s, 3H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 5.16 (dd, *J* = 7.8, 2.0 Hz, 0.1H), 4.83 (dd, *J* = 6.7, 4.6 Hz, 0.9H), 3.91 (dt, *J* = 17.0, 5.8 Hz,

1H), 3.83 (dt, $J = 16.7, 5.7$ Hz, 1H), 3.58 - 3.51 (m, 1H), 3.47 - 3.37 (m, 1H), 2.29 (s, 3H), 2.21 - 2.15 (m, 2H), 2.08 - 2.00 (m, 2H). MS (ESI) m/z 154.1 $[M + H]^+$

General procedure 1: TBTU coupling

The carboxylic acid (0.065 g, 0.377 mmol) was dissolved in 3 ml of DMF, *N*-ethyl-*N*-isopropylpropan-2-amine (0.210 ml, 1.168 mmol), HOBT (0.058 g, 0.377 mmol) and O-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) (0.121g, 0.377mmol) were added. After 15 min a solution of (*S*)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile trifluoroacetate (0.085g, 0.377mmol) or an equivalent amount of (*S*)-1-(2-aminoacetyl)-4,4-difluoropyrrolidine-2-carbonitrile tosylate in DMF was added. The mixture was stirred for 4 hours at room temperature. The volatiles were evaporated, the residue was dissolved in ethyl acetate and washed with 1N citric acid, saturated sodium bicarbonate and brine. The solution was dried over sodium sulfate, filtrated and evaporated. It was purified using column chromatography using DCM-methanol.

The following compounds we synthesized using General procedure 1:

(*S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1-naphthamide (3)

^1H NMR (400 MHz, CDCl_3) δ 8.37 (dd, $J = 8.2, 0.9$ Hz, 1H), 7.95 (d, $J = 8.3$ Hz, 1H), 7.90 - 7.85 (m, 1H), 7.72 (dd, $J = 7.1, 1.2$ Hz, 1H), 7.60 - 7.52 (m, 2H), 7.48 (dd, $J = 8.2, 7.1$ Hz, 1H), 6.98 (s, $J = 6.0$ Hz, 1H), 4.81 (d, $J = 5.7$ Hz, 1H), 4.45 (dd, $J = 17.9, 4.9$ Hz, 1H), 4.28 (dd, $J = 17.9, 3.9$ Hz, 1H), 3.79 - 3.67 (m, 1H), 3.62 - 3.50 (m, 1H), 2.44 - 2.12 (m, 4H); LC-MS(A) R_t 5.2 min, m/z 308.1 $[M+H]^+$ (95%); (50 mg, 44%)

(*S*)-*N*-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (7)

^1H NMR (400 MHz, CDCl_3) (8.5/1.5 mixture of *trans/cis* amide rotamers) δ 8.95 (d, $J = 4.3$ Hz, 1H), 8.28 (dd, $J = 8.07, 0.79$ Hz, 1H), 8.15 (d, $J = 8.49$ Hz, 1H), 7.77 (d tr, $J = 7.6, 1.4$ Hz, 1H), 7.62 (d tr, $J = 7.7, 1.3$ Hz, 1H), 7.49 (d, $J = 6$ Hz, 1H), 7.14 (br s, 1H), 4.78 - 4.75 (m, 1H), 4.60 (dd, $J = 17.3, 5.4$ Hz, 0.15H), 4.43 (dd, $J = 18.0, 5.0$ Hz, 0.85H), 4.33 (dd, $J = 17.3, 4.3$ Hz, 0.15H), 4.27 (dd, $J = 17.6, 4.1$ Hz, 0.85H), 3.74 - 3.68 (m, 1H), 3.58 - 3.50 (m,

1H), 2.38 - 2.12 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 167.52, 167.06, 149.97, 148.85, 140.98, 130.20, 130.08, 127.98, 125.35, 124.50, 118.98, 117.93, 46.83, 45.76, 42.59, 30.05, 25.21; UPLC I (ESI) R_t 1.10 min, m/z 309.6 [M+H]⁺ (99%); LC-MS (A) R_t 9.7 min, m/z 309.0 [M+H]⁺ (98%); HRMS calcd for: C₁₇H₁₇N₄O₂ [M + H]⁺, 309.1352; found, 309.1351; (66 mg, 62%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1H-indole-3-carboxamide (8)

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.64 (br s, 1H), 8.23 - 8.07 (m, 3H), 7.22 - 7.13 (m, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 4.81 (dd, *J* = 7.6, 3.5 Hz, 1H), 4.18 (dd, *J* = 17.0, 5.6 Hz, 1H), 4.09 (dd, *J* = 17.0, 5.5 Hz, 1H), 3.63 - 3.53 (m, 1H), 3.78 - 3.70 (m, 1H), 2.35 - 2.03 (m, 4H); LC-MS(A) R_t 11.7 min, m/z 297.0 [M+H]⁺ (97%); (54 mg, 36%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1H-indole-2-carboxamide (9)

¹H NMR (400 MHz, CDCl₃) δ 9.45 (br s, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.80 (br s, 1H), 7.24 - 7.12 (m, 2H), 7.09 - 7.00 (m, 1H), 6.96 (s, 1H), 4.69 - 4.63 (m, 1H), 4.45 (dd, *J* = 17.4, 6.4 Hz, 1H), 4.04 (dd, *J* = 17.3, 5.5 Hz, 1H), 3.58 - 3.51 (m, 1H), 3.32 - 3.22 (m, 1H), 2.25 - 1.95 (m, 4H); LC-MS(A) R_t 13.1 min, m/z 297.0 [M+H]⁺ (98%); (120 mg, 47%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(naphthalen-2-yloxy)acetamide (10)

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.86 (br s, 1H), 7.77 (d, *J* = 2.5 Hz, 1H), 7.80 (s, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.46 (dt, *J* = 6.7, 1.3 Hz, 1H), 7.38 (dt, *J* = 6.9, 1.2 Hz, 1H), 7.23 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.07 (d, *J* = 2.3 Hz, 1H), 4.76 - 4.71 (m, 1H), 4.53 (d (AB), *J* = 14.8 Hz, 1H), 4.60 (d (AB), *J* = 14.8 Hz, 1H), 4.30 (dd, *J* = 17.6, 5.8 Hz, 1H), 4.02 (dd, *J* = 17.6, 4.0 Hz, 1H), 3.86 (dd, *J* = 17.6, 4.0 Hz, 1H), 3.58 - 3.47 (m, 1H), 3.42 - 3.32 (m, 1H), 2.27 - 2.06 (m, 4H); LC-MS(A) R_t 14.9 min, m/z 338.0 [M+H]⁺ (98%); (49 mg, 33%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(naphthalen-1-yloxy)acetamide (11)

¹H NMR (400 MHz, CDCl₃) δ 8.34 - 8.28 (m, 1H), 7.85 - 7.79 (m, 1H), 7.73 (br s, 1H), 7.58 - 7.46 (m, 3H), 7.34 (t, *J* = 8.0, 1H), 6.75 (d, *J* = 7 Hz, 1H), 4.77 - 4.60 (m, 3H), 4.19 (dd, *J* = 17.6, 5.2 Hz, 1H), 4.07 (dd, *J* = 17.6, 4.4 Hz, 1H), 3.66 - 3.57 (m, 1H), 3.50 - 3.36 (m, 1H), 2.33 - 2.06 (m, 4H); LC-MS(A) R_t 13.9 min, m/z 338.0 [M+H]⁺ (95%); (59 mg, 54%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(naphthalen-1-yl)acetamide (12)

¹H NMR (400 MHz, CDCl₃) δ 7.98 (m, 1H), 7.87 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.82 (dd, *J* = 6.8, 2.5 Hz, 1H), 7.56 - 7.48 (m, 2H), 7.47 - 7.43 (m, 2H), 6.37 (br s, 1H), 4.62 (dd, *J* = 8.0, 2.3 Hz, 1H), 4.07 (s, 2H), 4.02 (dd, *J* = 17.8, 5.0 Hz, 1H), 3.86 (dd, *J* = 17.6, 4.0 Hz, 1H), 3.58 - 3.47 (m, 1H), 3.42 - 3.32 (m, 1H), 2.27 - 2.06 (m, 4H); LC-MS(A) R_t 14.0 min, m/z 322.1 [M+H]⁺ (97%); (30 mg, 23%)

(S)-1-(2-(Naphthalen-1-yloxy)acetyl)pyrrolidine-2-carbonitrile (13)

¹H NMR (400 MHz, CDCl₃) δ 8.30 - 8.25 (m, 1H), 7.85 - 7.80 (m, 1H), 7.55 - 7.47 (m, 3H), 7.37 (t, *J* = 8.1 Hz, 1H), 6.84 (d, *J* = 7.5 Hz, 1H), 5.02 - 4.83 (m, 1H), 4.78 (d, *J* = 4.5 Hz, 2H), 3.87 - 3.76 (m, 1H), 3.67 - 3.55 (m, 1H), 2.33 - 2.05 (m, 4H); LC-MS(A) R_t 15.8 min, m/z 281.1 [M+H]⁺ (96%); (100 mg, 37%)

(S)-1-(2-(Naphthalen-2-yloxy)acetyl)pyrrolidine-2-carbonitrile (14)

¹H NMR (400 MHz, CDCl₃) δ 7.81 - 7.72 (m, 3H), 7.48 - 7.43 (m, 1H), 7.40 - 7.35 (m, 1H), 7.23 - 7.15 (m, 2H), 5.02 - 4.83 (m, 1H), 4.78 (d, *J* = 4.5 Hz, 2H), 3.87 - 3.76 (m, 1H), 3.67 - 3.55 (m, 1H), 2.33 - 2.05 (m, 4H); LC-MS(A) R_t 15.5 min, m/z 281.0 [M+H]⁺ (98%); (120 mg, 45%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)naphthalene-1-sulfonamide (15)

¹H NMR (400 MHz, CDCl₃) δ 8.68 (dd, *J* = 8.6, 0.8 Hz, 1H), 8.25 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.08 (d, *J* = 8.3, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.61 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.7 (ddd, *J* = 8.6, 6.9, 1.4 Hz, 1H), 5.89 (br s, 1H), 7.54 (dd, *J* = 8.2, 7.3 Hz, 1H), 4.60 - 4.57 (m, 1H), 3.80 (d, *J* = 16.4 Hz, 1H), 3.68 (d, *J* = 16.4 Hz, 1H), 3.47 - 3.38 (m, 1H),

3.33 - 3.23 (m, 1H), 2.25 - 2.00 (m, 4H); ^{13}C NMR (CDCl_3 , 100MHz) δ 25.0, 29.9, 44.8, 45.5, 46.7, 117.8, 124.1, 124.6, 127.2, 128.4, 128.8, 129.0, 129.5, 134.2, 134.5, 134.7, 166.2; LC-MS(A) R_t 14.3 min, m/z 344.0 $[\text{M}+\text{H}]^+$ (99%); (75 mg, 52%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-methylquinoline-4-carboxamide (19)

^1H NMR (400 MHz, CDCl_3) (8.5/1.5 mixture of *trans/cis* amide rotamers) δ 8.95 (d, J = 4.3 Hz, 1H), 8.28 (br d, J = 8.3 Hz, 1H), 8.06 (br d, J = 8.0 Hz, 1H), 7.74 (d tr, J = 7.6, 1.4 Hz, 1H), 7.55 (tr, J = 7.7 Hz, 1H), 7.43 (s, 1H), 7.02 (br s, 1H), 4.83 - 4.78 (m, 0.85H), 4.76 (d, J = 9 Hz, 0.15H), 4.60 (dd, J = 18.0, 5.4 Hz, 0.15H), 4.42 (dd, J = 18.0, 5.1 Hz, 0.85H), 4.33 (dd, J = 18.0, 4.3 Hz, 0.15H), 4.26 (dd, J = 18.0, 4.1 Hz, 0.85H), 3.74 - 3.68 (m, 1H), 3.58 - 3.53 (m, 1H), 2.78 (s, 3H), 2.41 - 2.23 (m, 4H); LC-MS (A) R_t 8.5 min, m/z 323.1 $[\text{M}+\text{H}]^+$ (95%); (40 mg, 43%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-3-hydroxy-2-methylquinoline-4-carboxamide hydrochloride (20)

^1H NMR (400 MHz, MeOD) δ 8.30 (d, J = 7.5 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.98 - 7.84 (m, 2H), 4.90 - 4.86 (m, 1H), 4.44 (d, J = 5.8 Hz, 2H), 3.88 - 3.79 (m, 1H), 3.68 - 3.64 (m, 1H), 2.95 (s, 3H), 2.39 - 2.19 (m, 4H); UPLC I (ESI) R_t 1.11 min, m/z 339.6 $[\text{M}+\text{H}]^+$ (95%); LC-MS (A) R_t 9.4 min, m/z 339.0 $[\text{M}+\text{H}]^+$ (96%); (35mg, 21%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-3-hydroxyquinoline-4-carboxamide (21)

^1H NMR (400 MHz, CDCl_3) (8.5/1.5 mixture of *trans/cis* amide rotamers) δ 8.77 (d, J = 3.51 Hz, 1H), 8.24 - 8.17 (m, 1H), 8.10 (dd, J = 8.0, 1.5 Hz, 1H), 7.64 (ddd, J = 8.47, 7.06, 1.58 Hz, 1H), 7.59 (ddd, J = 8.28, 6.92, 1.48 Hz, 1H), 7.41 (s, 1H), 4.82 (d, J = 7.01 Hz, 0.85H), 4.77 - 4.71 (m, 0.15H), 4.47 (dd, J = 17.1, 4.8 Hz, 0.15H), 4.47 (dd, J = 17.2, 4.8 Hz, 0.85H), 4.42 - 4.36 (m, 0.15H), 4.28 (dd, J = 17.84, 3.76 Hz, 0.85H), 3.78 - 3.69 (m, 1H), 3.57 (m, 1H), 2.43 - 2.20 (m, 4H); UPLC I (ESI) R_t 1.19 min, m/z 325.6 $[\text{M}+\text{H}]^+$ (96%); LC-MS (A) R_t 10.1 min, m/z 325.0 $[\text{M}+\text{H}]^+$ (97%); (50mg, 36%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-2-(4-methoxyphenyl)quinoline-4-carboxamide (22)

¹H NMR (400 MHz, CDCl₃) (9/1 mixture of *trans/cis* amide rotamers) δ 8.24 (d, *J* = 7.6 Hz, 1H), 8.20 – 8.14 (m, 3H), 7.97 (s, 1H), 7.75 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.57 (ddd, *J* = 8.3, 6.9, 1.3 Hz, 1H), 7.12 - 7.00 (m, 3H), 4.82 (d, *J* = 5.3 Hz, 0.9H), 4.78-4.76 (m, 0.1H), 4.64 (dd, *J* = 17.4, 5.4 Hz, 0.1H), 4.46 (dd, *J* = 17.9, 4.9 Hz, 0.9H), 4.42-4.38 (m, 0.1H), 4.31 (dd, *J* = 17.9, 3.9 Hz, 0.9H), 3.78 - 3.71 (m, 1H), 3.62 - 3.53 (m, 1H), 2.43 – 2.17 (m, 4H); UPLC I (ESI) R_t 1.65 min, m/z 415.7 [M+H]⁺ (97%); LC-MS (A) R_t 14.8 min, m/z 415.1 [M+H]⁺ (97%); (65mg, 56%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-1,2,3,4-tetrahydroacridine-9-carboxamide (23)

¹H NMR (400 MHz, MeOD) (9/1 mixture of *trans/cis* amide rotamers) δ 9.40 (br s, 1H), 8.51 (d, *J* = 6.99 Hz, 1H), 8.21 - 8.04 (m, 2H), 7.94 (ddt, *J* = 9.85, 7.40, 2.50 Hz, 1H), 5.18 - 5.14 (m, 0.1H), 4.88 - 4.84 (m, 0.9H), 4.42 (s, 2H), 3.83 (dt, *J* = 5.28, 9.41 Hz, 1H), 3.74 - 3.61 (m, 1H), 3.43 - 3.36 (m, 2H), 3.13 (d, *J* = 11.34 Hz, 2H), 2.46 - 2.19 (m, 4H), 2.19 - 1.94 (m, 4H); UPLC I (ESI) R_t 1.13 min, m/z 363.7 [M+H]⁺ (98%); LC-MS (A) R_t 13.7 min, m/z 363.0 [M+H]⁺ (97%); (51mg, 55%)

(S)-5-Chloro-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (25)

¹H NMR (400 MHz, CDCl₃) δ 8.97 (d, *J* = 4.27 Hz, 1H), 8.12 (t, *J* = 4.86 Hz, 1H), 7.68 (dd, *J* = 4.89, 0.86 Hz, 2H), 7.47 (d, *J* = 4.27 Hz, 1H), 6.89 (s, 1H), 4.79 (d, *J* = 6.74 Hz, 1H), 4.32 (br d, *J* = 20 Hz, 2H), 3.77 - 3.67 (m, 1H), 3.60 - 3.51 (m, 1H), 2.41 - 2.20 (m, 4H); UPLC I (ESI) R_t 1.26 min, m/z 343.6 [M+H]⁺ (96%); LC-MS (A) R_t 11.6 min, m/z 343.1 [M+H]⁺ (96%); (58mg, 42%)

(S)-5-Bromo-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (26)

¹H NMR (400 MHz, CDCl₃) δ 8.95 (d, *J* = 4.24 Hz, 1H), 8.16 (dd, *J* = 8.51, 1.25 Hz, 1H), 7.90 (dd, *J* = 7.51, 1.33 Hz, 1H), 7.60 (dd, *J* = 8.47, 7.51 Hz, 1H), 7.49 (d, *J* = 4.25 Hz, 1H), 6.97 (s, 1H), 4.81 - 4.74 (m, 1H), 4.34 (s, 2H), 3.72 (ddd, *J* = 11.35, 6.78, 2.48 Hz, 1H), 3.59 - 3.50 (m, 1H), 2.43 - 2.13 (m, 4H); UPLC I (ESI) R_t 1.29 min, m/z 387.5,

389.5 [M+H]⁺ (96%); LC-MS (A) R_t 11.9 min, m/z 387.1, 388.9 [M+H]⁺ (96%); HRMS calcd for: C₁₇H₁₆N₄O₂Br [M + H]⁺, 387.0457; found, 387.0448; (39mg, 42%)

(S)-6-Chloro-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (27)

¹H NMR (400 MHz, CDCl₃) δ 9.00 (d, *J* = 4.34 Hz, 1H), 8.36 (d, *J* = 2.23 Hz, 1H), 8.12 (d, *J* = 8.98 Hz, 1H), 7.74 (dd, *J* = 9.00, 2.33 Hz, 1H), 7.59 (d, *J* = 4.32 Hz, 1H), 7.07 (br s, 1H), 4.83 (m, 1H), 4.45 (dd, *J* = 17.8, 4.76 Hz, 1H), 4.31 (dd, *J* = 17.8, 4.2 Hz, 1H), 3.75 (m, 1H), 3.59 (m, 1H), 2.44 - 2.24 (m, 4H); UPLC I (ESI) R_t 1.41 min, m/z 343.5 [M+H]⁺ (95%); LC-MS (A) R_t 13.2 min, m/z 343.0 [M+H]⁺ (95%); (21mg, 20%).)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-6-(trifluoromethoxy)quinoline-4-carboxamide (28)

¹H NMR (400 MHz, CDCl₃) (9/1 mixture of *trans/cis* amide rotamers) δ 9.11 (d, *J* = 4.6 Hz, 1H), 8.34 (d, *J* = 9.3 Hz, 1H), 8.28 (s, 1H), 7.75 (d, *J* = 4.6 Hz, 1H), 7.74 - 7.70 (m, 1H), 7.20 (br s, 1H), 4.81 (m, 0.9H), 4.74 (d, *J* = 8.8 Hz, 0.1H), 4.43 (dd, *J* = 17.9, 4.8 Hz, 1H), 4.30 (dd, *J* = 17.9, 3.9 Hz, 1H), 3.57 (m, 1H), 3.7 (m, 1H), 2.42 - 2.20 (m, 4H); UPLC I (ESI) R_t 1.57 min, m/z 393.5 [M+H]⁺ (99%); LC-MS (A) R_t 14.3 min, m/z 393.0 [M+H]⁺ (95%); (47mg, 47%.)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-6-fluoroquinoline-4-carboxamide (29)

¹H NMR (400 MHz, CDCl₃) (9/1 mixture of *trans/cis* amide rotamers) δ 8.95 (d, *J* = 4.36 Hz, 1H), 8.17 (dd, *J* = 9.25, 5.45 Hz, 1H), 8.01 (dd, *J* = 10.00, 2.81 Hz, 1H), 7.59 - 7.56 (m, 1H), 7.56 - 7.51 (m, 1H), 7.06 (br s, 1H), 4.81 (m, 0.9H), 4.73 (d, *J* = 8.8 Hz, 0.1H), 4.42 (dd, *J* = 17.86, 4.81 Hz, 1H), 4.27 (dd, *J* = 17.81, 3.90 Hz, 1H), 3.73 (m, 1H), 3.57 (m, 1H), 2.41 - 2.21 (m, 4H); UPLC I (ESI) R_t 1.29 min, m/z 327.6 [M+H]⁺ (99%); LC-MS (A) R_t 11.7 min, m/z 327.0 [M+H]⁺ (98%) (67mg, 72%).)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-6-methoxyquinoline-4-carboxamide (30)

¹H NMR (400 MHz, CDCl₃) δ 8.85 - 8.79 (d, *J* = 4.4 Hz, 1H), 8.07 - 8.01 (d, *J* = 9.2 Hz, 1H), 7.70 - 7.66 (d, *J* = 2.8 Hz, 1H), 7.53 - 7.49 (d, *J* = 4.4 Hz, 1H), 7.44 - 7.39 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.07 - 7.01 (s, 1H), 4.82 - 4.77 (m, 1H), 4.44 - 4.36 (dd, *J* = 17.9, 4.7 Hz, 1H), 4.36 - 4.28 (dd, *J* = 17.8, 4.2 Hz, 1H), 3.99 - 3.93 (s, 3H), 3.78 - 3.70 (m, 1H), 3.60 - 3.51 (m, 1H), 2.42 - 2.22 (m, 4H); UPLC I (ESI) R_t 1.18 min, m/z 339.7 [M+H]⁺ (95%); (32mg, 34%)

(S)-7-Chloro-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (31)

¹H NMR (400 MHz, DMSO-*d*₆) (9/1 mixture of *trans/cis* amide rotamers) δ 9.17 - 9.11 (t, *J* = 5.9 Hz, 1H), 9.07 - 9.02 (d, *J* = 4.3 Hz, 1H), 8.45 - 8.38 (d, *J* = 8.9 Hz, 1H), 8.21 - 8.13 (d, *J* = 2.2 Hz, 1H), 7.79 - 7.72 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.65 - 7.59 (d, *J* = 4.3 Hz, 1H), 5.30 - 5.28 (dd, *J* = 7.0, 2.4 Hz, 0.1H), 4.88 - 4.83 (dd, *J* = 7.3, 3.6 Hz, 0.9H), 4.42 - 4.36 (dd, *J* = 16.8, 5.8 Hz, 0.1H), 4.30 - 4.22 (dd, *J* = 17.0, 6.2 Hz, 0.9H), 4.22 - 4.14 (dd, *J* = 17.0, 5.7 Hz, 1H), 3.78 - 3.70 (ddd, *J* = 9.4, 7.6, 3.7 Hz, 1H), 3.59 - 3.50 (td, *J* = 9.0, 6.8 Hz, 1H), 2.34 - 1.92 (m, 4H); ¹³C NMR (101MHz, CDCl₃) δ 166.89, 166.76, 150.94, 149.21, 140.69, 136.16, 128.88 (2 carbon atoms), 126.78, 122.88, 118.92, 117.72, 46.75, 45.63, 42.50, 29.95, 25.09; UPLC I (ESI) R_t 1.40 min, m/z 343.6 [M+H]⁺ (99%); LC-MS (A) R_t 12.9 min, m/z 343.0 [M+H]⁺ (98%); (43mg, 62%)

(S)-7-Bromo-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (32)

¹H NMR (400 MHz, CDCl₃) (9/1 mixture of *trans/cis* amide rotamers) δ 8.97 - 8.93 (d, *J* = 4.3 Hz, 1H), 8.34 - 8.29 (d, *J* = 2.0 Hz, 1H), 7.71 - 7.65 (dd, *J* = 9.0, 2.0 Hz, 1H), 8.22 - 8.15 (d, *J* = 9.0 Hz, 1H), 7.55 - 7.51 (d, *J* = 4.4 Hz, 1H), 7.07 - 7.01 (m, 1H), 4.80 - 4.74 (m, 0.9H), 4.70 (d, *J* = 8.0 Hz, 0.1H), 4.58 - 4.52 (dd, *J* = 17.5, 5.3 Hz, 0.1H), 4.42 - 4.32 (dd, *J* = 17.8, 4.8 Hz, 1H), 4.34 - 4.28 (dd, *J* = 17.8, 4.1 Hz, 0.1H), 4.29 - 4.20 (dd, *J* = 17.8, 4.0 Hz, 1H), 3.74 - 3.65 (ddd, *J* = 9.8, 7.4, 3.0 Hz, 1H), 3.58 - 3.48 (m, 1H), 2.40 - 2.11 (m, 4H); ¹³C NMR (101 M Hz, CDCl₃) δ 166.84, 166.74, 150.88, 149.41, 140.77, 132.24, 131.39, 126.77, 124.46, 123.16, 119.05, 117.71, 46.75, 45.63,

42.50, 29.95, 25.09; UPLC I (ESI) R_t 1.44 min, m/z 386.4, 389.4 $[M+H]^+$ (99%); LC-MS (A) R_t 13.2 min, m/z 387.1, 388.9 $[M+H]^+$ (98%); (37mg, 34%)

(S)-8-Chloro-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (33)

^1H NMR (400 MHz, $CDCl_3$) (9/1 mixture of *trans/cis* amide rotamers) δ 9.12 (d, J = 4.3 Hz, 1H), 8.24 (dd, J = 8.5, 1.2 Hz, 1H), 7.92 (dd, J = 7.5, 1.2 Hz, 1H), 7.62 (d, J = 4.3 Hz, 1H), 7.58 - 7.54 (m, 1H), 7.02 (br s, 1H), 4.81 (m, 0.9H), 4.69 (d, J = 8.8 Hz, 0.1H), 4.41 (dd, J = 17.8, 4.8 Hz, 1H), 4.29 (dd, J = 17.9, 3.9 Hz, 1H), 3.73 (m, 1H), 3.56 (m, 1H); UPLC I (ESI) R_t 1.36 min, m/z 343.8 $[M+H]^+$ (96%); LC-MS (A) R_t 12.3 min, m/z 343.0 $[M+H]^+$ (98%); (29mg, 28%)

(S)-8-Bromo-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-4-carboxamide (34)

^1H NMR (400 MHz, $CDCl_3$) (9/1 mixture of *trans/cis* amide rotamers) δ 9.14 (d, J = 4.29 Hz, 1H), 8.31 (dd, J = 8.47, 1.20 Hz, 1H), 8.16 (dd, J = 7.49, 1.21 Hz, 1H), 7.63 (d, J = 4.30 Hz, 1H), 7.52 (dd, J = 13.81, 6.28 Hz, 1H), 7.04 (br s, 1H), 4.83 (d, J = 5.15 Hz, 0.9H), 4.72 (d, J = 7.1 Hz, 0.1H), 4.44 (dd, J = 17.86, 4.80 Hz, 1H), 4.31 (dd, J = 17.85, 4.01 Hz, 1H), 3.75 (m, 1H), 3.59 (m, 1H), 2.45 - 2.16 (m, 4H); UPLC I (ESI) R_t 1.39 min, m/z 386.2 $[M+H]^+$ (99%); LC-MS (A) R_t 12.5 min, m/z 387.1, 388.9 $[M+H]^+$ (98%); (21mg, 36%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)isoquinoline-4-carboxamide (35)

^1H NMR (400 MHz, $CDCl_3$) δ 9.35 - 9.28 (d, J = 0.9 Hz, 1H), 8.79 - 8.73 (s, 1H), 8.44 - 8.39 (m, 1H), 8.06 - 8.00 (dt, J = 8.2, 1.0 Hz, 1H), 7.84 - 7.77 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 7.72 - 7.65 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H), 7.12 - 7.02 (s, 1H), 4.85 - 4.79 (m, 1H), 4.49 - 4.39 (dd, J = 17.8, 4.8 Hz, 1H), 4.34 - 4.25 (dd, J = 17.8, 4.0 Hz, 1H), 3.78 - 3.69 (ddd, J = 10.0, 7.1, 2.7 Hz, 1H), 3.62 - 3.53 (m, 1H), 2.41 - 2.19 (m, 4H); UPLC I (ESI) R_t 1.10 min, m/z 309.6 $[M+H]^+$ (97%); LC-MS (A) R_t 9.3 min, m/z 309.0 $[M+H]^+$ (98%); (30mg, 34%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-5-carboxamide (36)

¹H NMR (400 MHz, CDCl₃) δ 9.00 - 8.93 (dd, *J* = 4.1, 1.7 Hz, 1H), 8.85 - 8.76 (dt, *J* = 8.5, 1.3 Hz, 1H), 8.26 - 8.18 (m, 1H), 7.84 - 7.78 (dd, *J* = 7.2, 1.4 Hz, 1H), 7.74 - 7.67 (m, 1H), 7.50 - 7.45 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.10 - 7.01 (s, 0.9H), 7.01 - 6.98 (s, 0.1H), 4.83 - 4.75 (td, *J* = 7.9, 2.1 Hz, 1H), 4.46 - 4.37 (dd, *J* = 17.8, 4.9 Hz, 1H), 4.31 - 4.21 (dd, *J* = 17.8, 4.0 Hz, 1H), 3.77 - 3.68 (ddd, *J* = 9.6, 7.1, 2.8 Hz, 1H), 3.60 - 3.52 (td, *J* = 8.5, 6.1 Hz, 1H), 2.43 - 2.12 (m, 4H); UPLC I (ESI) R_t 0.94 min, *m/z* 309.6 [M+H]⁺ (99%); LC-MS (A) R_t 8.0 min, *m/z* 309.0 [M+H]⁺ (98%); (40mg, 39%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)isoquinoline-5-carboxamide (37)

¹H NMR (400 MHz, CDCl₃) δ 9.35 - 9.25 (d, *J* = 1.0 Hz, 1H), 8.65 - 8.58 (dd, *J* = 6.0, 1.6 Hz, 1H), 8.29 - 8.20 (dt, *J* = 6.0, 1.0 Hz, 1H), 8.12 - 8.04 (dt, *J* = 8.2, 1.1 Hz, 1H), 8.01 - 7.91 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.66 - 7.58 (dd, *J* = 8.3, 7.1 Hz, 1H), 7.11 - 7.00 (d, *J* = 5.2 Hz, 1H), 4.85 - 4.75 (m, 1H), 4.51 - 4.39 (dd, *J* = 17.8, 4.9 Hz, 1H), 4.32 - 4.22 (dd, *J* = 17.9, 3.9 Hz, 1H), 3.79 - 3.68 (m, 1H), 3.61 - 3.51 (m, 1H), 2.42 - 2.12 (m, 4H); UPLC I (ESI) R_t 0.86 min, *m/z* 309.7 [M+H]⁺ (96%); LC-MS (A) R_t 4.5 min, *m/z* 309.0 [M+H]⁺ (98%); (57mg, 56%)

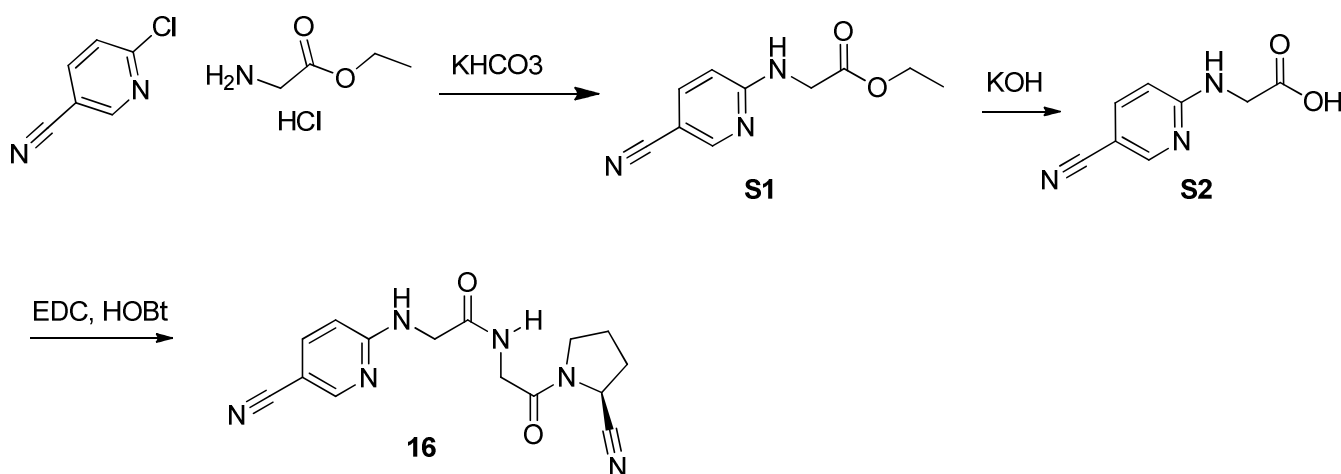
(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)isoquinoline-8-carboxamide (38)

¹H NMR (400 MHz, CDCl₃) (9/1 mixture of *trans/cis* amide rotamers) δ 9.70 (s, 0.9H), 9.68 (s, 0.1H), 8.48 (d, *J* = 5.7 Hz, 1H), 7.82 (d, *J* = 8.3, 1.0 Hz, 0.1H), 7.74 (m, 1.9H), 7.62 - 7.57 (m, 0.1H), 7.60 - 7.48 (m, 2.8H), 7.37 (t, *J* = 4.6 Hz, 0.1H), 4.87 (dd, *J* = 7.8, 2.0 Hz, 0.1H), 4.77 - 4.68 (m, 0.9H), 4.57 (dd, *J* = 17.3, 5.5 Hz, 0.1H), 4.43 (dd, *J* = 17.6, 5.6 Hz, 0.9H), 4.26 (dd, *J* = 17.3, 3.9 Hz, 0.1H), 4.18 (dd, *J* = 17.6, 4.0 Hz, 0.9H), 3.73 - 3.59 (m, 1H), 3.56 - 3.42 (m, 1H), 2.44 - 2.02 (m, 4H); UPLC I (ESI) R_t 0.88 min, *m/z* 309.6 [M+H]⁺ (98%); LC-MS (A) R_t 6.7 min, *m/z* 309.0 [M+H]⁺ (97%); (134mg, 75%)

(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)quinoline-8-carboxamide (39)

^1H NMR (400 MHz, CDCl_3) (9/1 mixture of *trans/cis* amide rotamers) δ 12.07 - 11.97 (br s, 1H), 9.06 (dd, $J = 4.3$, 1.8 Hz, 1H), 8.84 (dd, $J = 7.4$, 1.6 Hz, 1H), 8.28 (dd, $J = 8.3$, 1.8 Hz, 1H), 7.99 (dd, $J = 8.1$, 1.5 Hz, 1H), 7.68 (t, $J = 8$ Hz, 1H), 7.51 (dd, $J = 8.3$, 4.3 Hz, 1H), 5.03 (d, $J = 7$ Hz, 0.1H), 4.86 (br d, $J = 6.2$ Hz, 0.9H), 4.72 (dd, $J = 18.0$, 4.2 Hz, 0.1H), 4.62 - 4.50 (dd, $J = 17.8$, 5.1 Hz, 0.9H), 4.36 (dd, $J = 17.8$, 4.2 Hz, 1H), 3.79 - 3.71 (m, 1H), 3.66 - 3.55 (m, 1H), 2.41 - 2.08 (m, 4H); UPLC I (ESI) R_t 1.31 min, m/z 309.5 $[\text{M}+\text{H}]^+$ (98%); LC-MS (A) R_t 12.2 min, m/z 309.0 $[\text{M}+\text{H}]^+$ (98%); (39mg, 48%)

Scheme S1: (S)-2-(5-cyanopyridin-2-ylamino)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)acetamide



Ethyl 2-(5-cyanopyridin-2-ylamino)acetate (S1)

KHCO_3 (1.908g, 19mmol) was added to a solution of ethyl 2-aminoacetate hydrochloride (1.330g, 9.53 mmol) and 6-chloronicotinonitrile (1.2g, 8.66mmol) in dry DMF. The mixture was stirred for 3h at 90 °C. After cooling to room temperature, the reaction was quenched with saturated sodium bicarbonate and extracted with ethyl acetate (3x), washed with brine, dried over sodium sulfate, filtered and evaporated. The product was purified using column chromatography with a hexane-ethyl acetate eluent.

^1H NMR (400 MHz, CDCl_3) δ 8.38 (dd, $J = 2.2$, 0.5 Hz, 1H), 7.57 (dd, $J = 8.7$, 2.2 Hz, 1H), 6.48 (dd, $J = 8.8$, 0.7 Hz, 1H), 5.50 (s, 1H), 4.25 (q, $J = 7.1$ Hz, 2H), 4.18 (d, $J = 5.3$ Hz, 2H), 1.30 (t, $J = 7.1$ Hz, 3H); MS (ESI) m/z 228.0 $[\text{M} + \text{Na}]^+$; (1.05g, 59%)

2-(5-Cyanopyridin-2-ylamino)acetic acid (S2)

Ethyl 2-(5-cyanopyridin-2-ylamino)acetate was dissolved in a 1M solution of KOH in water-methanol 1-1. After 4h stirring, the methanol was evaporated and the water layer diluted, acidified to pH 4, extracted with EtOAc (4x). The EtOAc was washed with brine, dried over sodium sulfate, filtered and evaporated. The crude product was used without further purification.

¹H NMR (400 MHz, MeOD) δ 8.33 (dd, *J* = 2.3, 0.8 Hz, 1H), 7.64 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.65 (dd, *J* = 8.9, 0.7 Hz, 1H), 4.13 (s, 2H); (0.110g, 32%)

(S)-2-(5-Cyanopyridin-2-ylamino)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)acetamide (16)

N1-((ethylimino)methylene)-N3,N3-dimethylpropane-1,3-diamine hydrochloride (EDC) (0.119g, 0.62mmol) was added to a solution of 2-(5-cyanopyridin-2-ylamino)acetic acid (0.1g, 0.564mmol) in DMF. After 15 minutes, a solution of (S)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 2,2,2-trifluoroacetate (0.287g, 1.072mmol) and triethylamine (0.197mL, 1.41mmol) in DCM was added. After 24h, the mixture was extracted with 0.1N HCl, the aqueous layer was washed with DCM and neutralized to pH 10. The aqueous layer was extracted with DCM four times. The combined organic layers were washed with brine, dried over sodium sulfate, filtrated and evaporated. The crude product was purified with column chromatography using a ethyl acetate - methanol mixture to yield 0.035g (36%) pure product.

¹H NMR (400 MHz, CDCl₃) δ 8.39 (d, *J* = 1.8 Hz, 1H), 7.58 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.00 (br s, 1H), 6.53 (d, *J* = 8.8 Hz, 1H), 4.74 (dd, *J* = 8.0, 2.2 Hz, 1H), 5.72 (br s, 1H), 4.03 (dd, *J* = 17.6, 4.0 Hz, 1H), 4.09 - 4.18 (m, 3H), 3.68 - 3.57 (m, 1H), 3.50 - 3.37 (m, 1H), 2.37 - 2.08 (m, 4H); LC-MS(A) R_t 9.8 min, m/z 313.0 [M+H]⁺ (95%); (35mg, 19%)

(S)-1-(2-(1-Oxoisindolin-2-yl)acetyl)pyrrolidine-2-carbonitrile (18)

(S)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile² (0.079 g, 0.461 mmol) was added dropwise to the mixture of isoindolin-1-one (0.056 g, 0.419 mmol), tetrabutylammonium iodide (0.031 g, 0.084 mmol), 5ml of toluene and 5ml of aqueous NaOH 20% w/w solution. The mixture was stirred for 1 hour. The layers were separated, the aqueous layer was washed with toluene (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated in vacuum. The crude residue was purified on column using hexane-ethylacetate gradient to yield the target product. (0.04g, 36%)

mixture of amide rotamers (8.5-1.5), major rotamer: ¹H NMR (400 MHz, CDCl₃) δ 7.86 (dd, *J* = 5.6, 2.6 Hz, 1H), 7.56 (td, *J* = 7.6, 1.2 Hz, 1H), 7.50 – 7.44 (m, 2H), 4.76 (dd, *J* = 7.3, 2.9 Hz, 1H), 4.70 (d, *J* = 17.7 Hz, 1H), 4.61 (d, *J* = 16.4 Hz, 1H), 4.51 (d, *J* = 16.7 Hz, 1H), 4.23 (d, *J* = 16.4 Hz, 1H), 3.77 – 3.70 (m, 1H), 3.69 – 3.62 (m, 1H), 2.35 – 2.09 (m, 4H); minor rotamer: ¹H NMR (400 MHz, CDCl₃) δ 8.08 (d, *J* = 1.4 Hz, 1H), 7.56 (td, *J* = 7.6, 1.2 Hz, 1H), 7.50 – 7.44 (m, 2H), 5.13 (dd, *J* = 7.7, 1.8 Hz, 1H), 4.83 (d, *J* = 5.9 Hz, 1H), 4.79 (d, *J* = 7.0 Hz, 1H), 4.46 (d, *J* = 17.4 Hz, 1H), 4.17 (d, *J* = 16.1 Hz, 1H), 3.77 – 3.70 (m, 1H), 3.55 – 3.46 (m, 1H), 2.35 – 2.09 (m, 4H); LC-MS(A) R_t 11.3 min, *m/z* 270.1 [M+H]⁺ (98%); (0.03g, 25%)

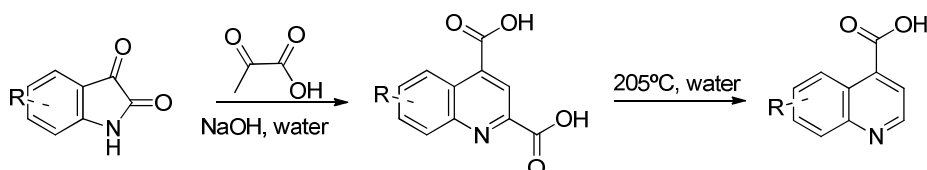
(S)-N-(2-(2-Cyanopyrrolidin-1-yl)-2-oxoethyl)-3-hydroxyquinoline-4-carboxamide (24)

To a 50mL round bottom flask, was added 3-hydroxyquinoline-4-carboxylic acid¹ (0.1 g, 0.529 mmol), acetonitrile (10 ml) and *N*-ethyl-*N*-isopropylpropan-2-amine (0.157 g, 1.216 mmol) at room temperature with stirring until a solution was observed. Then, di(-1*H* imidazol-1-yl)methanone (0.094 g, 0.581 mmol) was added in one portion and the mixture was held for 4h. The amine (S)-1-(2-aminoacetyl)pyrrolidine-2-carbonitrile 2,2,2-trifluoroacetate (0.198 g, 0.740 mmol) was added in one portion. The mixture was heated to 75 °C, held at 75°C for 5 h, cooled to room temperature and stirred at room temperature overnight. The volatiles were evaporated and the residue was redissolved in EtOAc, washed with 0.3N citric acid (2x), saturated sodium bicarbonate, brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified using column chromatography with ethyl acetate - methanol (95-5) mixture to yield the product

¹H NMR (400 MHz, CDCl₃) (8.5/1.5 mixture of *trans/cis* amide rotamers) δ 8.77 (d, *J* = 3.51 Hz, 1H), 8.24 - 8.17 (m, 1H), 8.10 (dd, *J* = 1.49, 7.97 Hz, 1H), 7.64 (ddd, *J* = 8.47, 7.06, 1.58 Hz, 1H), 7.59 (ddd, *J* = 8.28, 6.92,

1.48Hz, 1H), 7.41 (s, 1H), 4.82 (d, $J = 7.01$ Hz, 1H), 4.77 - 4.71 (m, 0.2H), 4.47 (dd, $J = 17.1, 4.8$ Hz, 0.15H), 4.47 (dd, $J = 17.76, 4.8$ Hz, 0.85H), 4.42 - 4.36 (m, 0.15H), 4.28 (dd, $J = 17.84, 3.76$ Hz, 0.85H), 3.78 - 3.69 (m, 1H), 3.57 (m, 1H), 2.43 - 2.20 (m, 4H); UPLC I (ESI) R_t 1.19 min, m/z 325.6 $[M+H]^+$ (98%); (0.076g, 44%)

Scheme S2. Synthesis of substituted quinoline carboxylic acids



General procedure 2: general procedure for the synthesis of quinoline-dicarboxylic acids (S3-S13)

NaOH (0.925g, 23.13mmol) was added to 7mL water with the corresponding isatin (0.7 g, 3.86 mmol), then sodium 2-oxopropanoate (0.509 g, 4.63 mmol) was added and the mixture refluxed for 2-4h. After cooling to room temperature, the pH was adjusted to 2, and the product was filtered off, washed with water, dried and used without further purification.

The following compounds we synthesized using General procedure 2:

Unless noted in the NMR, the carboxylic acid protons were not observed in the ^1H NMR.

5-Chloroquinoline-2,4-dicarboxylic acid (S3)

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.22 - 8.16 (dd, $J = 7.3, 2.4$ Hz, 1H), 7.98 - 7.90 (s, 1H), 7.88 - 7.81 (m, 2H); UPLC I (ESI) R_t 0.60 min, m/z 252.4 $[M+H]^+$ (95%)

5-Bromoquinoline-2,4-dicarboxylic acid (S4)

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.27 (dd, $J = 8.5, 1.1$ Hz, 1H), 8.16 (dd, $J = 7.6, 1.1$ Hz, 1H), 8.07 (s, 1H), 7.83 (dd, $J = 8.4, 7.7$ Hz, 1H); UPLC I (ESI) R_t 1.02 min, m/z 296.5, 298.5 $[M+H]^+$ (95%)

6-Chloroquinoline-2,4-dicarboxylic acid (S5)

^1H NMR (400 MHz, DMSO- d_6) δ 9.01 (d, J = 2.3 Hz, 1H), 8.37 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.82 (dd, J = 9.0, 2.4 Hz, 1H); MS (ESI) m/z 252.6 $[\text{M}+\text{H}]^+$

6-(Trifluoromethoxy)quinoline-2,4-dicarboxylic acid (S6)

^1H NMR (400 MHz, DMSO- d_6) δ 8.96 (s, 1H), 8.58 (s, 1H), 8.41 (d, J = 9.23 Hz, 1H), 7.95 (d, J = 9.38 Hz, 1H); MS (ESI) m/z 323.9 $[\text{M}+\text{Na}]^+$; (0.190g, 73%)

6-Fluoroquinoline-2,4-dicarboxylic acid (S7)

^1H NMR (400 MHz, DMSO- d_6) δ 8.60 (dd, J = 11.05, 2.86 Hz, 1H), 8.57 (s, 1H), 8.35 (dd, J = 9.33, 5.81 Hz, 1H), 7.89 (ddd, J = 9.27, 8.15, 2.92 Hz, 1H); MS (ESI) m/z 236.1 $[\text{M}+\text{H}]^+$

6-Methoxyquinoline-2,4-dicarboxylic acid (S8)

^1H NMR (400 MHz, DMSO- d_6) δ 8.54 - 8.47 (s, 1H), 8.29 - 8.24 (d, J = 2.8 Hz, 1H), 8.19 - 8.13 (d, J = 9.2 Hz, 1H), 7.63 - 7.57 (dd, J = 9.3, 2.8 Hz, 1H), 3.95 (s, 3H); UPLC I (ESI) R_t 1.01 min, m/z 248.5 $[\text{M}+\text{H}]^+$ (99%)

7-Chloroquinoline-2,4-dicarboxylic acid (S9)

^1H NMR (400 MHz, DMSO- d_6) δ 8.89 - 8.82 (d, J = 9.2 Hz, 1H), 8.53 - 8.45 (s, 1H), 8.33 - 8.28 (d, J = 2.3 Hz, 1H), 7.92 - 7.85 (dd, J = 9.1, 2.3 Hz, 1H); UPLC I (ESI) R_t 1.24 min, m/z 252.3 $[\text{M}+\text{H}]^+$ (96%)

7-Bromoquinoline-2,4-dicarboxylic acid (S10)

^1H NMR (400 MHz, DMSO- d_6) δ 8.79 - 8.74 (d, J = 9.2 Hz, 1H), 8.53 - 8.40 (m, 2H), 8.04 - 7.90 (dd, J = 9.1, 2.1 Hz, 1H); UPLC I (ESI) R_t 1.27 min, m/z 296.8, 298.8 $[\text{M}+\text{H}]^+$ (99%)

8-Chloroquinoline-2,4-dicarboxylic acid (S11)

^1H NMR (400 MHz, DMSO- d_6) δ 8.74 (d, J = 2.63 Hz, 1H), 8.70 (ddd, J = 8.04, 2.63, 1.8 Hz, 1H), 8.11 (dd, J = 8.04, 1.8 Hz, 1H), 7.70 (dd, J = 8.4, 8.04 Hz, 1H); MS (ESI) m/z 273.9 $[\text{M}+\text{Na}]^+$;

8-Bromoquinoline-2,4-dicarboxylic acid (S12)

^1H NMR (400 MHz, DMSO- d_6) δ 14.02 (s, 2H), 8.79 - 8.81 (dd, J = 8.6, 1.2 Hz, 1H), 8.29 - 8.31 (m, 1H), 8.50 (s, 1H), 7.70 - 7.74 (m, 1H); MS (ESI) m/z 296.3, 298.4 $[\text{M}+\text{H}]^+$;

2-(4-Methoxyphenyl)quinoline-4-carboxylic acid (S13)

Isatin (0.5g, 3.4mmol) was added to a 2M NaOH 20mL solution. After stirring, 1-(4-methoxyphenyl)ethanone (0.510g, 3.4mmol) was added and the mixture was refluxed for 4h. After cooling to room temperature, the pH was adjusted to 2, and the product was filtered off, dried and used without further purification. (0.68g, 71,6%)

^1H NMR (400 MHz, DMSO- d_6) : δ 8.61 (dd, J = 8.5, 0.8 Hz, 1H), 8.41 (s, 1H), 8.30 – 8.24 (m, 2H), 8.12 (dd, J = 8.4, 0.6 Hz, 1H), 7.82 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.66 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.14 – 7.09 (m, 2H), 3.85 (s, J = 6.9 Hz, 3H); MS (ESI) m/z 280.0 $[\text{M} + \text{H}]^+$

General procedure 3 : General procedure for the synthesis of quinoline-carboxylic acids (S14 - S23)

The corresponding quinoline dicarboxylic acid (**S3** - **S12**)(0.103g, 0.35mmol) was transferred in a 15mL pressure tube, 6 mL water was added. The closed tube was heated to 205°C for 2h unless otherwise noted. (See products) After slow cooling of the tube, the resulting precipitate was filtered and washed with water to yield white crystals.

The following compounds we synthesized using General procedure 3:

Unless noted in the NMR, the carboxylic acid protons were not observed in the ^1H NMR.

5-Chloroquinoline-4-carboxylic acid (S14)

^1H NMR (400 MHz, DMSO- d_6) δ 8.83 - 8.76 (d, J = 4.3 Hz, 1H), 7.98 - 7.92 (dd, J = 7.4, 2.4 Hz, 1H), 7.70 - 7.63 (m, 2H), 7.25 - 7.19 (d, J = 4.4 Hz, 1H); UPLC I (ESI) R_t 1.13 min, m/z 208.5 $[\text{M}+\text{H}]^+$ (98%)

5-Bromoquinoline-4-carboxylic acid (S15)

The starting material was reacted in a pressured tube for 50 minutes at 205°C with stirring.

^1H NMR (400 MHz, DMSO- d_6) δ 9.01 - 8.91 (d, J = 4.3 Hz, 1H), 8.16 - 8.07 (dd, J = 8.4, 1.2 Hz, 1H), 8.05 - 7.98 (dd,

$J = 7.6, 1.2 \text{ Hz, 1H}$), $7.75 - 7.69 \text{ (dd, } J = 8.4, 7.5 \text{ Hz, 1H)}$, $7.60 - 7.54 \text{ (d, } J = 4.4 \text{ Hz, 1H)}$; UPLC I (ESI) R_t 1.36 min, m/z 252.4, 254.4 $[M+H]^+$ (98%).

6-Chloroquinoline-4-carboxylic acid (S16)

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 8.96 (d, $J = 2.47 \text{ Hz, 1H}$), 8.80 (d, $J = 4.33 \text{ Hz, 1H}$), 7.95 (d, $J = 8.99 \text{ Hz, 1H}$), 7.66 (dd, $J = 8.96, 2.53 \text{ Hz, 1H}$), 7.57 (d, $J = 4.32 \text{ Hz, 1H}$); MS (ESI) m/z 205.8 $[M-H]^+$

6-(Trifluoromethoxy)quinoline-4- carboxylic acid (S17)

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 9.03 (d, $J = 2.8 \text{ Hz, 1H}$), 8.74 (d, $J = 4.5 \text{ Hz, 1H}$), 8.27 (d, $J = 8.8 \text{ Hz, 1H}$), 8.01 (dd, $J = 9.0, 2.80 \text{ Hz, 1H}$), 7.64 (d, $J = 5.3 \text{ Hz, 1H}$); MS (ESI) m/z 258.0 $[M+H]^+$, 255.7 $[M-H]^+$;

6-Fluoroquinoline-4- carboxylic acid (S18)

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 9.05 (d, $J = 4.38 \text{ Hz, 1H}$), 8.52 (dd, $J = 11.19, 2.90 \text{ Hz, 1H}$), 8.21 (dd, $J = 9.27, 5.86 \text{ Hz, 1H}$), 8.03 (d, $J = 4.31 \text{ Hz, 1H}$), 7.79 (ddd, $J = 9.24, 8.20, 2.94 \text{ Hz, 1H}$); MS (ESI) m/z 192.1 $[M+H]^+$, 189.9 $[M-H]^+$

6-Methoxyquinoline-4-carboxylic acid (S19)

The starting material was reacted in a pressured tube for **1.5** hour at 200°C with stirring.

$^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 8.92 - 8.83 (d, $J = 4.5 \text{ Hz, 1H}$), 8.23 - 8.13 (d, $J = 3.0 \text{ Hz, 1H}$), 8.08 - 7.99 (d, $J = 9.2 \text{ Hz, 1H}$), 7.99 - 7.88 (d, $J = 4.5 \text{ Hz, 1H}$), 7.55 - 7.45 (dd, $J = 9.0, 3.0 \text{ Hz, 1H}$), 3.96 - 3.85 (s, 3H); UPLC I (ESI) R_t 0.44 min, m/z 204.5 $[M+H]^+$ (99%)

7-Chloroquinoline-4-carboxylic acid (S20)

The starting material was reacted in a pressured tube for **1.5** hour at 200°C with stirring. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 14.27 - 13.34 (br s, 1H), 9.15 - 9.06 (d, $J = 4.4 \text{ Hz, 1H}$), 8.83 - 8.71 (d, $J = 9.1 \text{ Hz, 1H}$), 8.24 - 8.15 (d, $J = 2.3 \text{ Hz, 1H}$), 8.02 - 7.93 (d, $J = 4.4 \text{ Hz, 1H}$), 7.83 - 7.74 (dd, $J = 9.2, 2.3 \text{ Hz, 1H}$); UPLC I (ESI) R_t 1.15 min, m/z 208.4 $[M+H]^+$ (98%)

7-Bromo-4-quinoline-carboxylic acid (S21)

The starting material was reacted in a pressured tube for **50 min** at 200°C with stirring.

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.01 - 8.91 (d, *J* = 4.3 Hz, 1H), 8.16 - 8.07 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.05 - 7.98 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.75 - 7.69 (dd, *J* = 8.4, 7.5 Hz, 1H), 7.60 - 7.54 (d, *J* = 4.4 Hz, 1H); UPLC I (ESI) R_t 1.19 min, *m/z* 252.4, 254.4 [M+H]⁺ (99%)

8-Chloroquinoline-4-carboxylic acid (S22)

The starting material was reacted in a pressured tube for **90 min** at 200°C with stirring.

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.16 (d, *J* = 4.34 Hz, 1H), 8.67 (dd, *J* = 8.62, 1.22 Hz, 1H), 8.06 (dd, *J* = 7.53, 1.21 Hz, 1H), 8.03 (d, *J* = 4.34 Hz, 1H), 7.71 (dd, *J* = 8.58, 7.57 Hz, 1H); MS (ESI) *m/z* 208.1 [M+H]⁺;

8-Bromoquinoline-4-carboxylic acid (S23)

¹H NMR (400 MHz, CDCl₃) δ 9.20 (d, *J* = 4.39 Hz, 1H), 8.70 (s, 1H), 8.87 (d, *J* = 8.61 Hz, 1H), 8.15 (d, *J* = 7.52 Hz, 1H), 8.04 (s, 1H); MS (ESI) *m/z* 252.4, 254.4 [M+H]⁺;

Solubility measurements:

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

Log D protocol

20 μL of a 10 mM DMSO stock solution of the compound was added to 990 μL saturated PBS (pH 7.4). To this mixture was 990 μL of octanol added. The mixture was shaken for 2 hours at 37°C, then at room temperature for 10 minutes. 4 μL of octanol was added to 996 μL methanol and analyzed in triplicate. The PBS is injected

directly without any further dilution and analyzed in triplicate. The experiment was done in duplicate. A standard curve of six points was made to fit the measured concentrations.

Plasma stability protocol

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

Metabolic stability protocol

Liver microsomes (20 mg protein/ml), NADPH regenerating system solutions A & B and 5 mM stock compound solution (100% DMSO) are prepared. The reaction mixture finally contains 713 µL purified water, 200 µL 0.5 M potassium phosphate pH 7.4, 50 µL NADPH regenerating system solution A (BD Biosciences Cat. No. 451220), 10 µL NADPH regenerating system solution B (BD Biosciences Cat. No. 451200) and 2 µL of the compound stock solution (10 µM final concentration). The reaction mixture is warmed to 37°C for 5 minutes in a water bath and the reaction is initiated by addition of 25 µL of liver microsomes (0.5 mg protein/ml final concentration). At different time points (0 min – 15 min – 30 min – 60 min – 120 min – 240 min – 360 min – 24h), 20 µL is withdrawn and 80 µL cold acetonitrile is added on ice for 10 minutes. Then the mixtures are centrifuged at 13 000 rpm for 5 min at 4°C. The supernatant is further diluted in 90% water:MeCN (1/25) to fall within the range of the LC/MS² analysis. At each time point, the compound is analyzed using LC/MS/MS with the same UPLC system as described above. A standard curve of six points was made to fit the measured concentrations. Verapamil was used as a positive control.

Inhibition constant (K_i) measurement.

The inhibition constant of compounds **7** and **26** for the FAP active site (K_i) was determined using the same assay conditions as for the IC_{50} determination. Three different substrate concentrations (0.5, 1 and 2 mM Ala-Pro-pNA) and 10 different inhibitor concentrations (0 – 1 μ M) were used. Assuming competitive inhibition, the data were fitted to eq (1) using GraFit software to calculate the K_i .

$$v = \frac{V_{\max} [S]}{[S] + K_M \left(1 + \frac{[I]}{K_i} \right)} \quad \text{eq (1)}$$

Compound **7**: K_i 3,0 +/-0,4 nM

Compound **26**: K_i 4,1 +/- 0,6 nM

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