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Graphical abstract

$P2Y_{12}$ Binding IC_{50} (μM)	0.33	0.042	0.0065
Aq. solubility pH 6.5 (μM)	<0.1	90	83
CLint in HLM (μM/min/mg)	≥300	70	28

Synthesis, Structure-Property Relationships and Pharmacokinetic Evaluation of Ethyl 6-Aminonicotinate Sulfonylureas as Antagonists of the $P2Y_{12}$ Receptor

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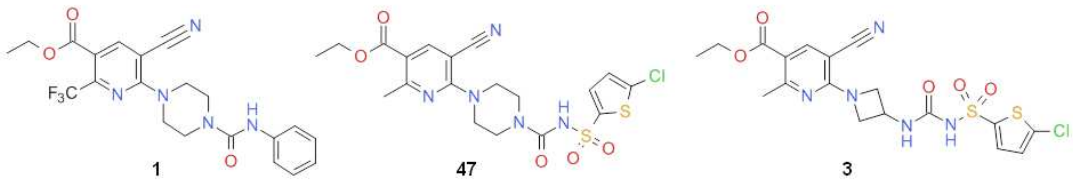
Graphical abstract



	1	47	3
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Abstract

The present paper describes the development of a new series of $P2Y_{12}$ receptor antagonists based on our previously reported piperazinyl urea series **1** (IC_{50} binding affinity = 0.33 μM, aq. solubility <0.1μM, microsomal CLint (HLM) ≥300 μM/min/mg). By replacement of the urea functionality with a sulfonylurea group we observed increased affinity along with improved stability and solubility as exemplified by **47** (IC_{50} binding affinity = 0.042 μM, aq solubility = 90 μM, microsomal CLint (HLM) = 70 μM/min/mg). Further improvements in affinity and metabolic stability was achieved by replacing the central piperazine ring with an 3-aminoazetidine as examplified by **3** (IC_{50} binding affinity = 0.0062 μM, aq. solubility = 83 μM, microsomal Clint (HLM) = 28 μM/min/mg). The improved affinity observerved in the in vitro binding assay also translated to the potency observed in the WPA aggregation assay (**47**: 19 nM and **3**: 9.5 nM) and the observed in vitro ADME properties translates to the in vivo PK properties observed in rat. In addition, we found that the chemical stability of the sulfonylureas during prolonged storage in solution was related to the sulfonyl urea linker and depended on the type of solvent and the substitution pattern of the sulfonyl urea functionality.

Keywords

$P2Y_{12}$ receptor, anti-platelet, sulfonylureas, solubility, stability.

1. Introduction

The platelet $P2Y_{12}$ receptor, also known as the ADP receptor, plays an important role in the amplification phase of platelet aggregation. $P2Y_{12}$ is a 7-transmembrane, G-protein-coupled receptor that is activated by adenosine diphosphate (ADP) [1]. When platelets are stimulated, they release ADP from their dense-granules. ADP interacts with the $P2Y_{12}$ receptor and causes a down-regulation of intracellular adenylyl-cyclase activity [2]. This results in decreased cyclic-AMP levels and prolonged calcium signalling which both stabilize the formed aggregates. The important role of the $P2Y_{12}$ receptor in platelet function makes it an attractive target for the development of novel anti-platelet therapies [3].

The first class of compounds to show benefit in clinical studies were thienopyridine pro-drugs like ticlopidine and clopidogrel, whose active metabolites bind irreversibly to the receptor [4]. However, preclinical data suggested that reversible binding would not only lead to a faster off-set of effect, but also improved separation between the anti-thrombotic effect and bleeding risk [5, 6]. The clinical benefits of ticagrelor, the first reversibly binding, direct-acting $P2Y_{12}$ antagonist, were demonstrated in the phase III PLATelet inhibition and patient Outcomes (PLATO) trial; in comparison with clopidogrel, ticagrelor significantly reduced the rate of the primary composite endpoint of myocardial infarction, stroke, and death from vascular causes [7]. Ticagrelor was developed via a medicinal chemistry program starting from adenosine triphosphate (ATP), the natural antagonist of the $P2Y_{12}$ receptor [8]. Other series of $P2Y_{12}$ antagonists are for example

piperazinyl-glutamate-pyridines [9], thienopyrimidines [10], anthroquinones [11], adenosine analogs [12], and dinucleoside polyphosphates and nucleotides [13].

The present paper describes the discovery of a novel series of ethyl 6-aminonicotinate sulfonylureas as potent antagonists of the $P2Y_{12}$ receptor. The sulfonylureas were developed from our recently reported series of piperazinyl-pyridine ureas [14], exemplified by compound **1** (Figure 1). Many compounds in the urea series had potencies in the submicromolar range, but the urea compounds generally suffered from low solubility and low metabolic stability in liver microsomes. Compounds to be tested in vivo by oral administration must be dissolved in order to be absorbed from the intestinal fluid, thus low solubility often results in low systemic exposure and poor in vivo activity [15]. Issues with low solubility can be adressed at different stages in the drug discovery process. In the early stage, one strategy is molecular design, in the later stage formulation can be an option, however neither of these have any guarantee of succes. Our strategy to use molecular design to increase the solubility ultimately led to the discovery of the series of sulfonyl ureas presented here. Likewise, our attempts to increase the microsomal stability of the novel series by replacement of the ethyl ester functionality in structures like **2** and **3** will be described.

Compounds in the sulfonylurea series are composed of a pyridine moiety (A), that is substituted in the 6-position with a cyclic amine (B ring), exemplified by a piperazine **2** (Figure 1) and an azetidine **3**, but also other mono- and bicyclic B-rings have been incorporated into the structure. A sulfonyl urea linker connects the A-B system to an aryl group (C). From a synthetic perspective, compounds like **2** and **3** were attractive target molecules since building blocks A, B and C could be cou pled by efficient parallel synthesis procedures.

[insert Figure 1]

In the present study, SAR (structure-activity relationships) arising from variations of the different parts of the molecules is presented. Firstly, the variations of the linker that led to the discovery of the sulfonyl urea linker will be described. To study the sulfonyl ureas further, three different A-rings (all ethyl nicotinates) with variations of the pyridine 2- and 5-substituents have been employed. Variations of the B-ring, based on shape matching to the azetidinyl and piperazinyl compounds, were made to test the effects of changing ring type, size, rigidity, and substitution pattern, while substituted phenyls, benzyls and 5-chloro-2-thienyl were introduced as C-rings. Then follows an investigation of the chemical stability in solution of the sulfonyl ureas. Finally, in vivo PK data in rat on six selected compounds are presented.

2. Chemistry

Four different 6-chloronicotinic acid or ester building blocks (A-rings) were used as starting materials (Figure 2). The 2-CF_3/5-CN building block (**4**) was described previously [14], the 2-H/5-Cl building blocks (**5** and **6**) were commercially available, and the 2-CH_3/5-CN building block (**7**) was made by a modified literature procedure [16, 17] via the corresponding pyridone [18]. The 6-chloro nicotinates **4**, **5**, and **7** were reacted with an amine (B-ring) to give 6-amino nicotinates (A-B). Subsequently, these were treated with (derivatives of) sulfonyl isocyanates to form sulfonyl ureas. Alternatively, a more convergent route was used, in which a B-ring and a C-ring were joined via a sulfonyl urea linker before being coupled with an A-ring.

[insert Figure 2]

A synthetically efficient method to introduce the 5-chloro-2-thienyl C-ring was needed. The 5-chlorothiophene-2-sulfonamide (**8**, Scheme 1) was commercially available, however its corresponding sulfonyl isocyanate was not. Consequently, chemistry procedures were developed to convert **8** into the corresponding sulfonyl isocyanate or synthetic equivalents thereof. Activation of **8** with *N*,*N'*-disuccinimide carbonate (DSC) [19], *N*,*N'*-carbonyldiimidazole (CDI), or *n*-butyl isocyanate/phosgene [20] generated intermediates that were reacted in situ. Alternatively, reaction of **8** with Troc-Cl (2,2,2-trichloroethyl chloroformate) [21] gave a stable intermediate **9** that could be isolated and stored. Compound **9** became the reagent-of-choice due to its easy preparation, handling, and the reasonably high yields obtained in reactions of **9** with A-B systems.

[Insert Scheme 1]

Ethyl 2-CF_3/5-CN/6-piperazinyl nicotinates were synthesized from the 6-chloro-nicotinate **4** as outlined in Scheme 2 [22]. Compound **4** was treated with two different piperazines to form piperazinyl-pyridines **10** and (±)-**11**, respectively. Treatment of **10** with the CDI-derivative of **8** gave **12**, while treatment with commercially available sulfonyl isocyanates produced sulfonyl ureas **13**-**18** in 18-84% yield. Treatment of (±)-**11** with the CDI-derivative of **8** gave (±)-**19** after *tert*-butyl deprotection.

[Insert Scheme 2]

Ethyl 2-H/5-Cl/6-piperazinyl nicotinates were synthesized as outlined in Scheme 3. Urea compound (±)-**22** was prepared in three steps, starting from commerically available (±)-**20**. Compounds **2**, **24**-**26**, and **28**-**32** were synthesized by treating the piperazinyl-pyridine **23** with

different iso(thio)cyanates. Compound **27** was made by reaction of **23** with the CDI-derivative of **8**. The nicotinic acid **33** was made by hydrolysis under basic conditions of ethyl nicotinate **2**.

[Insert Scheme 3]

Ethyl 6-azetidinyl and 6-piperidinyl nicotinates featured by two N-H functionalities in the sulfonylurea linker were synthesized as outlined in Scheme 4. Reaction of pyridines **5** and **7** with *tert*-butyl azetidin-3-ylcarbamate or *tert*-butyl piperidin-4-ylcarbamate provided intermediates **34**-**37**. Boc-deprotection of **34** and **36** followed by reaction with Troc-derivative **9** produced **3** and **42**, respectively, while Boc-deprotection of **34**-**37** followed by reaction with the appropriate sulfonylisocyanates produced **38**-**41** and **43**-**45**.

[Insert Scheme 4]

Compounds **47**-**53** with an ethyl 2-CH_3/5-CN nicotinate A-ring were prepared as shown in Scheme 5. 6-Chloro nicotinic ester **7** was coupled with a B-ring, followed by treatment of the formed A-B system with the activated sulfonamide derivative of a C-ring. Reaction with the CDI-derivative of **8** produced **47**. Treatment with commercially available sulfonyl isocyanates gave **48**, **49**, and **53**, while reaction with the Troc-derivative **9** provided **50**, (±)-**51**, and **52**.

[Insert Scheme 5]

Nicotinic esters **59**-**63** (Scheme 6) were prepared from 5,6-dichloronicotinic acid **6**. Nucleophilic aromatic substitution with piperazine in the 6-position of compound **6** with subsequent acid catalyzed esterification gave 6-piperazinyl nicotinic ester intermediates **55**-**58**. Compounds **55**-**57** were reacted with the CDI-derivative of **8** to give **59**, **61**, and **63**, while **58** was reacted with the

isocyanate derivative of **8** to produce **60**. Given the low yields (7-57%) in the syntheses of **55-58**, an alternative esterification procedure was used in the synthesis of the *i*-propyl ester homolog **62**. This involved EDCI/HOBT activation of *N*-Boc-protected 6-piperazinyl nicotinic acid **64** followed by reaction with *i*-PrOH which gave the *i*-propyl ester **65** in high yield (92%), followed by Boc-deprotection and coupling with Troc-derivative **9** to provide **62**.

[Insert Scheme 6]

Ketone analogs **70** and **71** (Scheme 7) were synthesized via the Weinreb amide **67** by reaction with propyl and cyclopropyl Grignard reagents, respectively, followed by deprotection of the Boc-protected piperazine and reaction with **9**. 3-Propyl sulfonyl analog **74** was made from aminopyridine **72** while benzene analog **77** was made from the commercially available benzoic acid derivative **75**. In the latter case, nonaflate [23] served as a useful leaving group in a Buchwald-Hartwig reaction between **76** and *tert*-butyl piperazine-1-carboxylate.

[Insert Scheme 7]

3. Results and discussion

Affinity in a binding assay and potency in a GTPγS functional assay were determined in a first screen cycle [24]. The GTPγS functional assay is based on the principle that activation of a GPCR ($P2Y_{12}$) by an agonist (ADP) leads to conformational changes that favors binding of the receptor to a G-protein. Thereby, an agonist-receptor-G-protein ternary complex is formed which induces GDP bound on the G-protein α-subunit to exchange with GTPγ^{35}S. The effect of an added antagonist can thus be determined by the fraction of unbound GTPγ^{35}S. Most compounds with an

IC_{50}-value lower than 0.2 μM in the GTPγS assay were characterized further by additional in vitro screens. These included determination of the potency of the compounds in inhibiting fibrinogen-induced aggregation in a washed platelet assay (WPA) [5] permeability in Caco-2 monolayers in the apical to the basolateral direction (A to B) [25], and in vitro intrinsic clearance (metabolic stability, CLint) in rat liver microsomes (RLM) and human liver microsomes (HLM) [26]. A balancing of in vitro potency, permeability, microsomal stability, and structural diversity was applied when selecting compounds for determination of PK parameters in vivo in rat. The ultimate goal was that the selected set of compounds could be used for analyzing in vitro-in vivo correlations and thereby enable the prediction of in vivo PK parameters for other compounds in this series.

3.1 Improvement of solubility and study of the SAR of the linker

The sulfonylurea series was discovered from our efforts to improve the solubility of the ethyl 6-aminonicotinate urea compounds [14], exemplified by **1** (Figure 3, Table 1). Structural changes leading to reduced lipophilicity (**78**, **79**) or increased conformational flexibility (**80**, **81**) improved the solubility compared to **1**. Of these, only **80** gave a moderate increase (to 0.11 μM) in potency compared to **1**. Replacing the urea linker with thiourea (**24**), acylurea (**25**) or acylthiourea (**26**) did not lead to any improvement in potency compared to **78**. Comparison of thiourea **24** with urea **78** indicated that one strong hydrogen bond acceptor was needed for high binding affinity [27]. The introduction of an additional strong hydrogen bond acceptor as in **25** led to lower affinity compared to **82**.

Inspired by the fact that ADP, the natural agonist of $P2Y_{12}$, contains an acidic side chain, attempts were made to introduce acidic functionalities into structure **1**. Introduction of a carboxylate as exemplified by (±)-**22** increased the solubility and maintained the affinity of compound **1**.

Since sulfonamides had been reported as $P2Y_{12}$ inhibitors [28], it was attempted to lower the pKa of the urea linker of **1** by way of a sulfone. Thus, replacement of the urea linker of **1** with the acidic sulfonylurea functionality, a bioisostere of the carboxylic acid [29], produced sulfonylurea **2**.

Compound **2** showed a potency on par with urea **1** and provided a breakthrough in solving the issue with low solubility. The high solubility of sulfonylurea **2** was a consequence of the relatively low pKa of the NH proton in the sulfonylurea linker (pKa = 3.8, i.e. an acidity similar to formic acid) which made the compound appear as an anion at pH 6.8, where solubility is determined. Additional examples of an increase in solubility by replacement of the urea linker with a sulfonylurea linker include the matched-pairs **28**/**83** (increase from 10 to 210 μM) and **30**/**84** (increase from <0.09 to 490 μM).

Compound **33**, in which the ethyl ester of compound **2** had been replaced with the corresponding carboxylic acid, was found to be inactive [30]. This was analogous to what was observed in the urea series.

[Insert Figure 3]

Table 1. Binding affinity, potency, solubility, and lipophilicity of compounds leading to sulfonyl urea **2** [31]. See Figure 3 for structures.[a]

Compd No	Binding IC_{50} (μM)	GTPγS IC_{50} (μM)	Solubility at pH6.8 (μM)	clogP	logD pH7.4
1	0.33	0.68	<0.10	4.0	5.0
2	0.18	0.22	100	3.3	ND
(±)-**22**	0.68	2.2	96	3.3	ND
24	12	3.7	ND	4.1	ND
25	9.9	5.3	ND	3.0	ND
26	2.4	0.99	ND	4.2	ND
28	0.15	0.26	210[b]	0.7	ND
30	0.30	0.30	490[b]	0.9	ND
33	>33	ND	ND	2.8	<0.5
78	0.89	1.7	40	3.3	ND
79	0.92	2.6	90	2.0	3.7
80	0.47	0.11	2.2	3.7	5.0
81	1.3	0.51	15	3.5	5.0
82	2.4	1.7	ND	3.5	ND
83	1.4	2.6	10[b]	3.2	ND
84	0.60	1.1	<0.09[b]	3.7	ND

[a] ND = not determined. [b]Solubility at pH7.4.

3.2 SAR by structural variations of the A-, B- and C-rings

To understand the scope of the sulfonyl urea compounds, a library with structural variations of the A-, B- and C-rings of compound **2** was synthesized (Table 2). Variations of the pyridine A-

ring were made in the 2- and 5-positions and included the substitution patterns 2-CF_3/5-CN, 2-H/5-Cl, and 2-CH_3/5-CN.

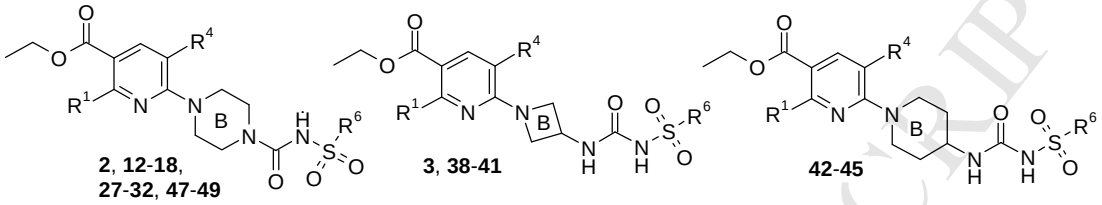
Pairwise comparison of the piperazinyls show that 2-CF_3/5-CN pyridines had similar or higher potency than the corresponding 2-H/5-Cl pyridines. In general, the 2-CF_3/5-CN pyridines had lower permeability in Caco-2 monolayers than the 2-H/5-Cl pyridines, by comparison of the pairs **16**/**30**, **17**/**31**, and **18**/**32**. In both piperazinyl sub-series **12-18** (2-CF_3/5-Cl pyridines) and **27-32** (2-H/5-Cl pyridines), monosubstitution on the phenyl C-ring in the 2- or 4-position with CH_3, Cl or F (4-position only) produced compounds with similar or lower potency than compound **2** in both binding and GTPγS assays.

In the 3-CH_3/5-CN pyridine sub-series **47**-**49**, a benzyl C-ring (**49**) provided binding affinity and potency in both the GTPγS and WPA assays similar to 5-chloro-2-thienyl (**47**), and **49** was the only piperazinyl that has low clearance in HLM. In the azetidinyl (**3** and **38**-**41**) and piperidinyl (**42**-**45**) sub-series the 5-Cl-2-thienyl remained the optimum C-ring to provide potency, as exemplified by compounds **3** and **42** having GTPγS and WPA potencies similar to **47**.

In general, compounds with a 5-Cl-thienyl C-ring had consistently high potency in WPA, but also had lower permeability in Caco-2 monolayers than compounds of similar logD (compare **27**/**30**, **47**/**49**, and **3**/**38**). Among the 2-H/5-Cl pyridines, azetidinyl **38** was similar in potency to the comparable piperazinyl **2** while piperidinyl **43** had a remarkably lower potency than **2**.

Comparison of pairs and triplets **3**/**42**/**47**, **40**/**44**, and **41**/**45**/**49**, showed the azetidinyls and piperazinyls to have similar logD, while the piperidinyls had logD 0.8-1.0 higher than the azetidinyls which is the likely reason for the piperidinyls having higher permeability in Caco-2 monolayers. Overall, the azetidinyls were the sub-series with highest stability in HLM.

Table 2. Effects on potency, solubility, logD, permeability in Caco-2 monolayers, and intrinsic clearance, CLint, in rat liver microsomes and human liver microsomes by variations of the pyridine substituents R^1 and R^4, the central ring (B), and the sulfonyl urea substituent R^6.[a]



Compd No	R^1	R^4	Central ring (B)	R^6	Binding IC_{50} (μM)	GTPγS IC_{50} (μM)	WPA IC_{50} (μM)	logD pH 7.4	Caco-2 A to B (10^{-6} cm/s)	CLint RLM L/M/H	CLint HLM L/M/H
12	CF_3	CN	paz	5-Cl-2-thienyl	0.079	0.039	0.038	1.1	L (0.076)	ND	ND
13	CF_3	CN	paz	Ph	0.17	0.22	0.12	ND	M (0.60)	H	H
14	CF_3	CN	paz	2-CH_3-Ph	0.43	0.38	ND	ND	ND	ND	ND
15	CF_3	CN	paz	2-Cl-Ph	0.69	0.47	ND	ND	ND	ND	ND
16	CF_3	CN	paz	4-CH_3-Ph	0.12	0.090	0.16	1.3	M (0.22)	H	H
17	CF_3	CN	paz	4-Cl-Ph	0.068	0.097	0.80	ND	L (0.13)	H	H
18	CF_3	CN	paz	4-F-Ph	0.23	0.21	ND	0.4	L (0.053)	H	M
2	H	Cl	paz	Ph	0.18	0.22	1.1	ND	M (0.28)	H	H
27	H	Cl	paz	5-Cl-2-thienyl	0.090	0.10	0.26	0.9	L (0.11)	H	H
28	H	Cl	paz	2-CH_3-Ph	0.15	0.26	ND	0.7	ND	H	H
29	H	Cl	paz	2-Cl-Ph	0.87	0.99	1.1	0.6	ND	ND	ND
30	H	Cl	paz	4-CH_3-Ph	0.30	0.30	0.19	0.9	H (2.5)	H	H
31	H	Cl	paz	4-Cl-Ph	0.52	0.38	ND	1.3	H (1.2)	H	H
32	H	Cl	paz	4-F-Ph	0.37	0.47	ND	0.6	M (0.54)	H	H
3	CH_3	CN	3-aze	5-Cl-2-thienyl	0.0063	0.025	0.0095	0.8	L (0.11)	H	M
38	H	Cl	3-aze	Ph	0.43	0.38	ND	0.8	H (3.9)	ND	ND
39	CH_3	CN	3-aze	Ph	0.062	0.19	0.19	0.4	M (0.41)	L	L

40	CH_3	CN	3-aze	4-Cl-Ph	0.086	0.078	0.094	1.1	H (1.2)	M	L
41	CH_3	CN	3-aze	Bn	0.079	0.082	0.44	1.1	H (1.2)	M	M
42	CH_3	CN	4-pip	5-Cl-2-thienyl	0.027	0.057	0.026	1.6	H (6.9)	H	H
43	H	Cl	4-pip	Ph	4.8	3.2	ND	1.5	ND	ND	ND
44	CH_3	CN	4-pip	4-Cl-Ph	0.19	0.26	0.21	2.1	H (19)	H	H
45	CH_3	CN	4-pip	Bn	0.088	0.15	ND	1.9	H (30)	H	M
47	CH_3	CN	paz	5-Cl-2-thienyl	0.042	0.034	0.019	1.0	L (0.058)	H	H
48	CH_3	CN	paz	Ph	0.14	0.13	ND	ND	M (0.30)	ND	ND
49	CH_3	CN	paz	Bn	0.033	0.054	0.020	0.9	M (0.37)	H	L

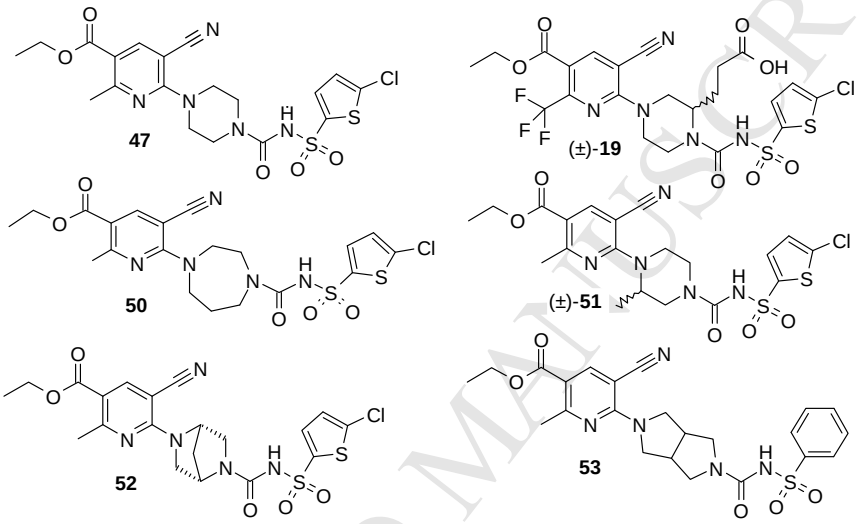
[a] paz = piperazinyl, 3-aze = 3-amino-azetidinyl, 4-pip = 4-amino-piperidinyl, WPA = washed platelet assay, in which the ability of the compounds to inhibit fibrinogen-induced aggregation is determined, Caco-2 = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon, CLint = intrinsic clearance, measured as metabolism in rat liver microsomes (RLM) and , human liver microsomes (HLM), L/M/H = low/ moderate/high. ND = not determined.

3.3 SAR of the central ring (B) and of the pyridine 3-substituent (R^2)

Next, the B-ring was varied exploratively (Table 3). We expected that changes in the conformation, rigidity, or size of the B-ring should modulate the binding mode, and thus potentially increase the binding affinity, of the molecules. Low-energy conformations of molecules with structurally different B-rings were shape-matched to compound **47** (Figure 4). As seen from the figure, the compounds with alternative B-rings, that were selected for synthesis, have functionalities that overlay the similar functionalities in **47**. Compound (±)-**19**, featured by a propanoic acid side chain on the B-ring, had potencies on level with **47** and was stable in HLM. Introduction of a 7-membered B-ring in the form of a 1,4-diazepane (**50**) gave a 7-fold (to 0.63 μM) reduction in the binding affinity and a 15-fold (to 0.24 μM) reduction in the GTPγS potency relative to **47**. Compound (±)-**51**, featured by a 2-methyl substituent on the B-ring, had a potency similar to **47**. The compounds **52** and **53** with bi-cyclic B-rings both had lower potency than **47**.

[Insert Figure 4]

Table 3. Effects on binding affinity, potency, Caco-2 permeability, and intrinsic clearance in rat liver microsomes and human liver microsomes by variations of the central ring.[a]



Compd No	Binding IC_{50} (μM)	GTPγS IC_{50} (μM)	WPA IC_{50} (μM)	logD pH 7.4	Caco-2 A to B (10^{-6} cm/s)	CLint RLM L/M/H	CLint HLM L/M/H
47	0.042	0.034	0.019	1.0	L (0.058)	H	H
(±)-**19**	0.095	0.11	0.031	ND	M (0.19)	H	L
50	0.63	0.24	ND	0.9	ND	ND	ND
(±)-**51**	0.064	0.037	0.031	1.6	M (0.31)	H	H
52	2.9	0.69	ND	ND	ND	ND	ND
53	0.24	0.58	ND	0.4	M (0.39)	L	L

[a], WPA = washed platelet assay, in which the ability of the compounds to inhibit fibrinogen-induced aggregation is determined, Caco-2 = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon, CLint = intrinsic clearance, measured as CYP450 catalyzed metabolism in rat liver microsomes (RLM) and human liver microsomes (HLM),), L/M/H = low/moderate/high. ND = not determined.

Since the increased structural complexity (e.g. chirality) introduced by replacement of the piperazine with other B-rings gave no significant increase in potency compared to compound **47**, the unsubstituted piperazine B-ring was retained in the subsequent variation of the pyridine 3-ethoxycarbonyl substituent (R^2) and replacement of the pyridine ring with a benzene ring (Table 4).

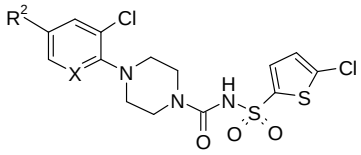
Minor structural variations of the pyridine 3-substituent were made, in part to study the SAR in the pyridine 3-position and in part to potentially identify substituents that could provide compounds with higher microsomal stability than the ethyl esters. Replacement of the 3-ethoxycarbonyl substituent with 3-carboxamides had given compounds of low potency in the urea series,[14] thus carboxamides were not included in the present study. Instead, we included a number of alkyl esters and ethyl ester isosteres in the form of two ketones and a sulfone.

Shortening of the ethyl ester in **27** to methyl ester **59** reduced the binding affinity 7-fold (to 0.65 μM) and the potency 32-fold (to 3.2 μM) in the GTPγS assay. Straight-chain elongations with *n*-propyl (**60**) and *n*-butyl (**61**) esters or with an alkyl chain having branching away from the ester functionality like in the iso-pentyl ester **63** gave potencies similar to the ethyl ester. Branching close to the ester carbonyl like in the iso-propyl ester **62** reduced the binding affinity and GTPγS potency 3-5 fold compared to the ethyl ester and clearance in RLM and HLM remained high. *n*-Propyl and cyclopropyl ketone analogs **70** and **71** had 9-13 fold lower GTPγS potencies than ethyl ester **27**, however the ketones were stable in both RLM and HLM. Sulfone analog **74** had more than a 100-fold lower GTPγS potency than **27**. Replacement of the pyridine ring of **27** with a benzene ring gave compound **77** that had a 7-fold lower binding affinity and a 18-fold lower potency in the GTPγS assay compared to **27**, which underlines the importance of the A-ring being a pyridine rather than a benzene.

In summary, replacement of the pyridine 3-ethoxycarbonyl substituent and of the pyridine with benzene did not improve the potency compared to compound **27**. Although ketones **70** and **71** had

a notably lower clearance in HLM, this was out-weighted by lower potencies, hence further development was made with the 3-ethoxycarbonyl pyridines.

Table 4. Variations of the pyridine 3-ethoxycarbonyl substituent (R^2) and replacement of pyridine (X = N) with benzene (X = C).[a]



Compd No	R^2	X	Binding IC_{50} (μM)	GTPγS IC_{50} (μM)	WPA IC_{50} (μM)	logD pH7.4	Caco-2 A to B (10^{-6} cm/s)	CLint RLM L/M/H	CLint HLM L/M/H
27	-CO_2Et	N	0.090	0.10	0.26	ND	L (0.11)	H	H
59	-CO_2Me	N	0.65	3.2	ND	0.9	ND	ND	ND
60	-CO_2-*n*-Pr	N	0.21	0.24	ND	1.6	ND	ND	ND
61	-CO_2-*n*-Bu	N	0.095	0.15	ND	2.3	M (0.50)	ND	H
62	-CO_2-*i*-Pr	N	0.25	0.53	ND	1.5	ND	H	H
63	-CO_2-*i*-Pn	N	0.14	0.11	ND	2.7	M (0.44)	ND	H
70	-C(O)-*n*-Pr	N	0.56	0.89	ND	1.3	M (0.16)	H	M
71	-C(O)-*cy*-Pr	N	0.42	1.3	3.8	1.2	L (0.12)	L	L
74	$S(O)_2$-*n*-Pr	N	9.3	14	ND	ND	ND	ND	ND
77	-CO_2Et	C	0.66	1.8	ND	1.5	ND	H	ND

[a] WPA = washed platelet assay, in which the ability of the compounds to inhibit fibrinogen-induced aggregation is determined, Caco-2 = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon, CLint = intrinsic clearance, measured as metabolism in rat liver microsomes (RLM) and human liver microsomes (HLM), L/M/H = low/moderate/high. ND = not determined.

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3.4 Stability of the sulfonyl ureas during prolonged storage in solution

During the course of the project we noticed that some of the sulfonyl ureas having a piperazine B-ring were chemically instable during prolonged storage in acetonitrile (MeCN) or DMSO. For example, 10% remained of **12** after three months and 52% remained of **16** after four months at 5 °C in MeCN. Mass analysis of the fragments showed that this instability was solely due to cleavage of the sulfonyl urea linker, i.e. the ethyl ester functionality was stable under these conditions. No instability was observed during storage at room temperature of any of the sulfonyl ureas as solid materials. To investigate the stability during prolonged storage in solution of the piperazine sub-series compared to the azetidine and piperidine sub-series, the stability of solutions of two piperazines, **30** and **31**, one azetidine, **38**, and one piperidine, **43**, in MeCN and ethanol (EtOH), respectively, was studied by UV absorption in LC/MS during 21 days at room temperature [32]. The pair **30**/**31** was chosen in order to disclose any electronic influence of the C-ring (4-CH_3-phenyl vs 4-Cl-phenyl) on the stability of the sulfonyl urea linker. EtOH and MeCN were chosen as solvents to reveal any difference between stability in a nucleophilic (EtOH) and a less nucleophilic (MeCN) solvent.

As seen from the graphs (Figure 5), solutions of the piperazines **30** and **31** were the least stable, particularily in EtOH. During 21 days, the purity of **30** in EtOH decreased from 89% to 9% and in MeCN from 89% to 80%, while the purity of **31** in EtOH decreased from 95% to 32% and in MeCN from 95% to 74%. In marked contrast, the azetidine **38** was stable in both EtOH and MeCN while the piperidine **43** was stable in MeCN and slightly instable in EtOH (decrease from 97% to 89% purity). Compound **30** (4-CH_3, slightly electron rich phenyl) was less stable than **31** (4-Cl, electron poor phenyl) in EtOH, but more stable than **31** in MeCN. The latter observation is in concord with the above-mentioned stability in MeCN of **16** (4-CH_3, slightly electron rich phenyl) vs **12** (5-Cl-2-thienyl, electron poor C-ring). Detected degradation products by LC-

HRMS were the azetidinyl-, piperazinyl- or piperidinyl-pyridines, respectively, and, for solutions in EtOH, the ethyl sulfonyl carbamates. No degradation of the nicotinic ethyl esters was observed.

The observation that the stability of sulfonyl ureas in EtOH depended on their substitution pattern was also seen with tolbutamide derivatives [33]. In aqueous solution at neutral pH, where sulfonamides (usual pKa ≈ 4) exist as anions, the stability may be higher since the formed anion protects the linker against hydrolytic cleavage [34].

To conclude, long-time storage of solutions of piperidinyl and (most critically) piperazinyl pyridines in acetonitrile and ethanol (and probably in other solvents as well) should be avoided.

[Insert Figure 5]

3.5 Property profiling including in vivo PK

A general trend of the sulfonyl urea compounds was high intrinsic clearance in RLM. In HLM, the compounds showed more variation. This difference in intrinsic clearance reflected interspecies differences in expression of metabolizing enzymes [35]. The metabolism could be due to hydrolytic enzymes (esterases), CYP450, or other enzymes like oxidases. NADPH is needed for CYP450 to function, so to uncover if the metabolism in RLM and HLM was CYP450 dependent, the metabolism of an array of compounds **2**, **12**, **13**, **27**, **38**, **47**, and **48** was determined in assays from which NADPH had been excluded. No change in the degree of metabolism was observed for these compounds. It was thus concluded that the metabolism in microsomes was not CYP-dependent and was most likely due to the activity of esterases present in the liver microsomes. The major metabolites of the ethyl esters **19** and **30** in RLM and HLM was identified as the corresponding carboxylates. The interspecies difference in the activity of esterases was underlined when testing the metabolic stability of compounds (±)-**19**, **27**, **38**, **39**,

and **41** in rat plasma and human plasma, respectively. In human plasma, the compounds were stable [36] and showed a strong binding to human plasma protein with free fractions lower than 1.5% [37]. In rat plasma, the compounds were unstable and the corresponding carboxylate metabolites could be identified by HRMS.

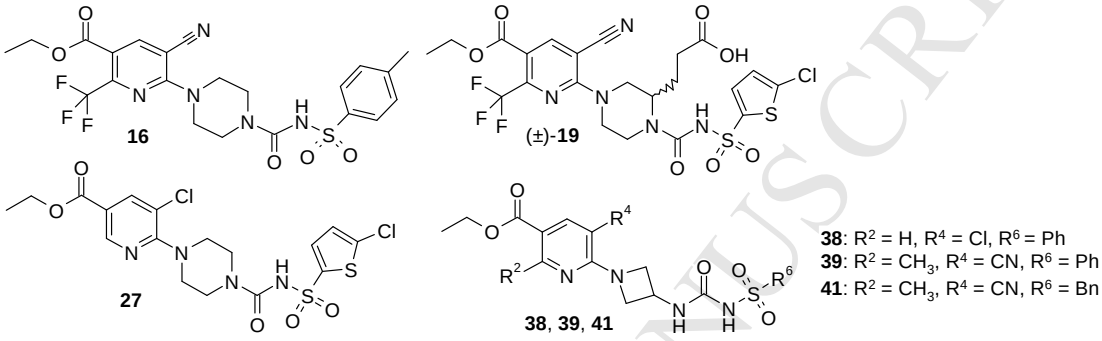
In vivo PK parameters in rat (Table 5) was determined for the six compounds **16**, (±)-**19**, **27**, **38**, **39**, and **41**. This was done to understand if the microsomal clearance and Caco-2 permeability in vitro could be correlated to the in vivo PK parameters of the compounds. Given our limited ressources for in vivo PK determination it was attempted to include compounds that were diverse with regards to structure and in vitro properties. For example was the moderately potent compound **38** included due to its comparatively high permeability in Caco-2 monolayers.

Compounds (±)-**19** and **41** had very low bioavailability (0.8-3%) while **16**, **27**, and **38** had moderate (15-20%), and **39** comparatively high (39%) bioavailability. Both **16** and (±)-**19** had a moderate Caco-2 permeability, but **16** had a remarkably higher bioavailability (20% vs 0.8%), despite being metabolically more labile than (±)-**19**. The high bioavailability of **39,** explained by a moderate Caco-2 permeability in combination with the lowest clearance (13 mL/min/kg), contributed to **39** having the longest half-lives (1.7 h (iv) and 9.0 h (po)) of the six compounds.

The triad of compounds **38**/**39**/**41** had the same B-ring and linker and were thus particularly interesting to compare. Compounds **38** and **39**, that were equipotent in GTPγS, differed only in the substitution pattern of the A-ring, and shifting from the 2-H, 5-Cl-pyridine (**38**) to the less lipophilic 2-CH_3, 5-CN-pyridine (**39**) led to reduced clearance (from 29 to 13 mL/min/kg) and increased bioavailability (from 20 to 39%) and oral half-life (from 2.1 to 9.0 h). Compounds **39** and **41**, that showed similar affinity and potency, differed only in the C-ring (phenyl vs benzyl) and replacing the phenyl of **39** with the more lipophilic benzyl of **41** significantly increased the clearance (from 13 to 31 mL/min/kg) and reduced both the bioavailability (from 39% to 3%) and the oral half-life (from 9.0 h to 1.3 h).

Overall was the observed clearance in vivo well-predicted by the intrinsic clearance in rat liver microsomes, as seen from Table 5. It was not possible to predict the bioavailability from an estimate using Caco-2 permeability and in vivo clearance data.

Table 5. Pharmacokinetic data in vivo (in rat) for six selected compounds.[a]



Compd No	N, iv	CL (mL/min/kg) ± SEM	$t_{1/2}$ iv (h)	N, po	*F* (%)[b] ± SEM	$t_{1/2}$ po (h)	WPA IC_{50} (µM)	logD pH 7.4	Caco-2 A to B (10^{-6} cm/s)	CLint RLM L/M/H
16	2	89 (±2)	1.5	2	20 (±10)	0.65	0.16	1.3	M (0.22)	H
(±)-**19**	2	46 (±4)	0.62	2	0.83 (±0.53)	4.0	0.031	ND	M (0.19)	H
27	1	36	0.92	2	15 (±0)	2.4	0.26	ND	L (0.11)	H
38	2	29 (±2)	0.17	3	20 (±2)	2.1	ND	0.8	H (3.9)	ND
39	1	13	1.7	3	39 (±13)	9.0	0.19	0.4	M (0.41)	L
41	1	31	0.55	2	3.0 (±1.1)	1.3	0.44	1.1	H (1.2)	M

[a]In vitro data are included for reference. CL = clearance, *F* = bioavailability, WPA = washed platelet assay, in which the ability of the compounds to inhibit fibrinogen-induced aggregation is determined, Caco-2 = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon, CLint = intrinsic clearance, measured as metabolism in rat liver microsomes (RLM), L/M/H = low/moderate/high. ND = not determined. [b]The compounds were administered as solutions in TEG/DMA/H_2O 1:1:1 (**39** and **41**) or TEG/EtOH/H_2O 50:5:45.

4. Conclusions

In conclusion, we have discovered a series of ethyl 6-aminonicotinate sulfonylureas that are antagonists of the $P2Y_{12}$ receptor. The series was developed from our previously reported urea linker series [14]. A molecular design strategy in which a urea linker was replaced with a sulfonyl urea linker led to compounds with improved solubility and microsomal stability. The structural features of the compounds facilitated the use of parallel synthesis methodology. SAR studies showed that the 2-H/5-Cl-pyridines had similar or lower potency in the GTPγS assay than the corresponding 2-CF_3/5-CN pyridines. Replacement of the central piperazine with rings having different size or rigidity than the piperazine gave no improvement in potency. The substituent on the sulfonyl of the sulfonylurea linker was varied and presented 5-Cl-2-thienyl, 4-CH_3-phenyl, 4-Cl-phenyl and benzyl as the most promising substituents to provide potency in the GTPγS and WPA assays. Replacement of the ethyl ester functionality with *n*-propyl and *n*-butyl esters gave unchanged GTPγS potency, while ketone and sulfone analogs gave lower potency. Replacement of the pyridine ring with a benzene ring gave significantly lowered potency.

The chemical stability of the sulfonyl urea linker by prolonged storage in solutions of acetonitrile or ethanol depended on the type of solvent and the substitution pattern of the nitrogens in the linker; thus 3-azetidinyl and 4-piperidinyl-pyridines were more stable than 4-piperazinyl-pyridines.

In vivo PK parameters in rat were determined for six selected compounds. Of these, (±)-**19** had the highest potency (0.031 μM) in the WPA assay, but had low bioavailability (0.8%). Compared to (±)-**19**, compound **39** had a much higher bioavailability (39%), but a 6-fold lower potency in the WPA assay. In conclusion, although one single molecule that integrated all the desired properties was not identified, the present study provided a solid foundation for continued research to

optimize this type of compounds. Our further progress along this line will be reported in due course.

5. Experimental Section

5.1 General methods. Commercially available chemicals were used as provided by the commercial supplier. In general, reactions were performed under a nitrogen atmosphere. Reactions in microwave reactors were performed by single node heating in a Smith Creator, Smith Synthesizer, or an Emrys Optimizer using microwave vials from Personal Chemistry (now Biotage). Concentration of solutions was done in vacuo on a rotary evaporator at temperatures below 50 °C.

Thin-layer chromatography was made using TLC silica gel 60 F_{254} from Merck KGaA, Darmstadt. Flash chromatography was performed with either standard glass- or plastic-columns using Merck silica-gel grade 9385, 60Å (0.063-0.200 mm) from Sigma-Aldrich or on a Biotage Horizon High Performance Flash Chromatography (HPFC) system using Biotage silica gel. Preperative HPLC was performed on a Waters YMC-ODS AQS-3 120 Angstrom 3 x 500 mm or on a Waters Delta Prep System using Kromasil C8, 10 µm columns. Gradient: 95% 0.1M ammonium acetate buffer: 5% $CH_3CN \rightarrow$ 100% CH_3CN.

^{1}H NMR measurements were performed on a Varian Mercury VX 400 spectrometer, operating at a ^{1}H frequency of 400 MHz or on Varian UNITY plus 400, 500 and 600 spectrometers, operating at ^{1}H frequencies of 400, 500 and 600 MHz, respectively. Chemical shifts are given in ppm with the solvent as internal standard. Coupling constants are given in Hz. *Note that with CD_3OD as NMR solvent the signals from exchangable protons are not observed, and thus not reported.*

The purity of screening compounds was determined by analytic HPLC. Low resolution mass spectra (electrospray ionization) were acquired on a Waters ZQ quadrupole spectrometer coupled to an Agilent Technologies 1100-series HPLC. The HPLC retention time were recorded through standard gradient 5% to 95% acetonitrile (10 mM ammonium acetate, pH4) over 4 minutes using a Synergy MAX-RP C12 (4 μm; 50 mm x 3 mm ID) column with a flow rate of 2 mL/min. All screening compounds had purity ≥ 95%.

5.2. Chemistry

5.2.1. Ethyl 5-chloro-6-(4-{[(phenylsulfonyl)amino]carbonyl}piperazin-1-yl)nicotinate (2).

23 (0.054 g, 0.20 mmol) was dissolved in THF (1.0 mL). TEA (0.030 g, 0.30 mmol) was added at 0 °C, followed by benzenesulfonyl isocyanate (0.048 g, 0.26 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at rt for 17 h. The reaction mixture was then stirred gently with PS-TRIS (0.050 g, loading 4.35 mmol/g) and PS-NCO (0.100 g, loading 1.53 mmol/g) at rt for 1 h. The resins were filtered off and the solvents evaporated. The crude product was purified by flash chromatography (EtOAc/pentane 1:1, then gradient to 100% EtOAc). Yield: 0.022 g (24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (3H, t, *J* = 7.2 Hz), 3.49 (1H, s), 3.54-3.57 (8H, m), 4.38 (2H, q, *J* = 7.2 Hz), 7.56-7.59 (2H, m), 7. 62-7.67 (2H, m), 8.06-8.12 (1H, m), 8.15 (1H, d, *J* = 2.0 Hz), 8.75 (1H, d, *J* = 2.0 Hz). MS $^m/_z$: 453 (M+1).

5.2.2. Ethyl 6-(3-(3-(5-chlorothiophen-2-ylsulfonyl)ureido)azetidin-1-yl)-5-cyano-2-methylnicotinate (3). 34

(1.00 g, 2.77 mmol) was dissolved in DCM (10 mL). HCl (4 M in 1,4-dioxane, 13.9 mL, 55.5 mmol) was added slowly and the reaction mixture was stirred at rt for 16 h. The mixture was concentrated to afford a solid, which was used crude assuming 100% conversion.

Ethyl 6-(3-aminoazetidin-1-yl)-5-cyano-2-methylnicotinate dihydrochloride (0.200 g, 0.60 mmol) and **9** (0.336 g, 0.90 mmol) were dissolved in DMA (2 mL). DIPEA (1.05 mL, 6.0 mmol) was added and the reaction mixture was heated to 100 °C for 1 h. The reaction mixture was cooled to rt and concentrated. The crude was dissolved in EtOAc (40 mL) and washed with NH_4Cl (2 x 40 mL, saturated, aq solution). The organic phase were dried ($MgSO_4$) and concentrated. The crude was purified by flash chromatography (30-50% EtOAc in hexanes, then 50% EtOAc in hexanes with 0.5% AcOH). Yield: 0.034 g (11%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, t, *J* = 7.1 Hz), 2.61 (3H, s), 4.11-4.19 (2H, m), 4.23 (2H, q, *J* = 7.1 Hz), 4.46-4.58 (3H, m), 4.85 (1H, br s), 7.26 (1H, d, *J* = 4.1 Hz), 7.40-7.49 (1H, m), 7.63 (1H, d, *J* = 4.1 Hz), 8.27 (1H, br s). HRMS: Calc. for $C_{19}H_{21}N_5O_5S_2Cl$ $(M+1)^+$, 498.0673; found: 498.0671.

5.2.3. Ethyl 6-chloro-5-cyano-2-methylnicotinate (7). Ethyl 5-cyano-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (2.00 g, 9.70 mmol) [18(b)] was suspended in DCM (40 mL). Oxalyl chloride (6.16 g, 4.25 mL, 48.5 mmol) was added. DMF (0.071 g, 0.080 mL, 0.97 mmol) was added slowly and the reaction mixture was heated to reflux for 18 h. The mixture was cooled to rt, diluted with DCM (250 mL) and poured onto ice-water (250 mL). The organic phase was separated, washed with brine (250 mL), $NaHCO_3$ (2 x 250 mL, saturated, aq solution), and brine (250 mL), dried (Na_2SO_4) and concentrated. Yield: 2.18 g (98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (3H, t, *J* = 7.1 Hz), 4.40 (2H, q, *J* = 7.1 Hz), 2.91 (3H, s), 8.49 (1H, s). MS $^m/_z$: 223 (M-1).

5.2.4. 2,2,2-Trichloroethyl [(5-chloro-2-thienyl)sulfonyl]carbamate (9). 8 (15.00 g, 75.9 mmol) was suspended in a bi-phasic mixture of NaOH (9.11 g, 55.4 mmol) in water (100 mL) and DCM (250 mL). The reaction mixture was stirred rapidly at 0 °C and 2,2,2-trichloroethyl chloroformate (30.1 mL, 133 mmol) was added drop-wise. The reaction mixture was slowly allowed to reach rt and stirred for 18 h. HCl (concentrated) was added drop-wise till pH < 1,

followed by dilution with DCM (500 mL). The organic phase was separated, dried ($MgSO_4$) and concentrated. The material was purified by flash chromatography (EtOAc/hexanes 1:3 to 1:1). Yield: 20.67 g (73%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.76 (2H, s), 6.99 (1H, d, *J* = 4.2 Hz), 7.71 (1H, d, *J* = 4.2 Hz), 7.74 (1H, br s). MS $^m/_z$: 372 (M-1).

5.2.5. Ethyl 5-cyano-6-piperazin-1-yl-2-(trifluoromethyl)nicotinate (10). 4 (1.00 g, 3.41 mmol) and piperazine (0.928 g, 10.8 mmol) were mixed in EtOH (3 mL) and TEA (0.727 g, 7.18 mmol) was added. The reaction mixture was heated in a microwave oven at 170 ^{0}C for 20 minutes. The mixture was diluted with DCM (200 mL), and the organic phase was washed with $NaHCO_3$ (saturated, aq solution), and brine, dried (Na_2SO_4) and concentrated. The crude was purified by flash chromatography (DCM/MeOH 100:1 to 30:1). Yield: 0.751 g (67%). ^{1}H NMR (400, CD_3OD): δ 1.36 (3H, t, *J* = 7.14 Hz), 2.93-2-99 (4H, m), 3.92-3.98 (4H, m), 4.34 (2H, q, *J* = 7.22 Hz), 8.42 (1H, s). MS ^{m}/z: 329 (M+1).

5.2.6. (±)-Ethyl 6-[3-(3-*tert*-butoxy-3-oxopropyl)piperazin-1-yl]-5-cyano-2-(trifluoromethyl)nicotinate ((±)-11). 4 (0.250 g, 0.90 mmol) and *tert*-butyl 3-piperazin-2-ylpropanoate (0.192 g, 0.90 mmol) were dissolved in EtOH (2 mL). TEA (0.15 mL, 1.1 mmol) was added. The solution was heated in a microwave oven at 150 °C for 20 minutes. The mixture was concentrated, diluted with DCM (50 mL), washed with water (50 mL), dried ($MgSO_4$) and concentrated. The crude was purified by flash chromatography (DCM/MeOH 50:1). Yield: 0.162 g (40%).^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (3H, t, *J* = 7.2 Hz), 1.44 (9H, s), 1.58-1.84 (3H, m), 2.35 (2H, t, *J* = 7.7 Hz), 2.75-2.83 (1H, m), 2.85-2.93 (2H, m), 3.10-3.16 (1H, m), 3.18-3.28 (1H, m), 4.35 (2H, q, *J* = 7.2 Hz), 4.59-4.67 (2H, m), 8.34 (1H, s). MS ^{m}/z: 457 (M+H).

5.2.7. Ethyl 6-[4-({[(5-chloro-2-thienyl)sulfonyl]amino}carbonyl)piperazin-1-yl]-5-cyano-2-(trifluoromethyl)nicotinate (12). 9 (0.181 g, 0.91 mmol) and CDI (0.148 g, 0.91 mmol) were dissolved in DCM (5 mL) and DIPEA (1.59 mL, 9.14 mmol) was added. The reaction mixture was stirred at rt for 4 h. **10** (0.300 g, 0.91 mmol) was added and the mixture was heated at reflux for 16 h. The mixture was cooled to rt and concentrated. Flash chromatography (3:7 EtOAc/hexanes to 99:1 EtOAc/AcOH) gave the product as a solid. Yield: 0.060 g (12%). ^{13}C NMR (151 MHz, d_6-DMSO) δ 162.83, 157.10, 151.62, 148.75, 145.91 (q, $J_{C\text{-}C\text{-}F}$ = 35.1 Hz), 139.22, 135.85, 133.08, 127.10, 120.20 (q, $J_{C\text{-}F}$ = 275.5 Hz), 116.48, 114.83, 93.67, 61.76, 46.10, 40.03, 13.72. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.29 (3H, t, *J* = 7.1 Hz), 3.53-3.60 (4H, m), 3.86-3.93 (4H, m), 4.29 (2H, q, *J* = 7.1 Hz), 7.24 (1H, d, *J* = 4.1 Hz), 7.62 (1H, d, *J* = 4.1 Hz), 8.57 (1H, s), 11.43 (1H, br s). HRMS: Calc. for $C_{19}H_{17}N_5O_5F_3S_2Cl$ $(M+1)^+$, 552.0390; found: 552.0411.

5.2.8. Ethyl 5-cyano-6-(4-(phenylsulfonylcarbamoyl)piperazin-1-yl)-2-(trifluoromethyl)nicotinate (13). 10 (0.210 g, 0.640 mmol) was dissolved in DCM (10 mL). Benzenesulfonylisocyante (0.352
g, 1.92 mmol) and TEA (0.10 mL, 1.0 mmol) were added and the reaction mixture was stirred over night at rt. The mixture was concentrated and purified by preparative HPLC. Yield: 0.093 g (27%). ^{1}H NMR (400 MHz,$CDCl_3$): δ 1.37 (3H, t, *J* = 7.2 Hz), 3.59-3.64 (4H, m), 3.67 (1H, s), 3.93-4.01 (4H, m), 4.37 (2H, q, *J* = 7.2 Hz), 7.52-7.60 (2H, m), 7.61-7.68 (1H, m), 8.05-8.07 (1H, m), 8.07-8.10 (1H, m), 8.36 (1H, s). MS ^{m}/z: 510 (M-1).

5.2.9. Ethyl 5-cyano-6-[4-({[(2-methylphenyl)sulfonyl]amino}carbonyl)piperazin-1-yl]-2-(trifluoromethyl)nicotinate (14). 10 (0.066 g, 0.20 mmol) and 2-methylbenzenesulfonyl isocyanate (0.047 g, 0.24 mmol) were dissolved in DCM (1.0 mL) and TEA (0.080 mL, 0.60

mmol) was added. The reaction mixture was stirred at rt for 14 h and then evaporated. The crude material was purified by preparative HPLC. Yield: 0.066 g (62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 (3H, t, *J* = 7.1 Hz), 2.66 (1H, s), 2.69 (3H, s), 3.61-3.66 (4H, m), 3.93-3.98 (4H, m), 4.37 (2H, q, *J* = 7.1 Hz), 7.28-7.38 (2H, m), 7.44-7.51 (1H, m), 8.08-8.12 (1H, m), 8.36 (1H, s). MS $^m/_z$: 526 (M+1).

5.2.10. (±)-3-{1-({[(5-Chloro-2-thienyl)sulfonyl]amino}carbonyl)-4-[3-cyano-5-[ethoxy(hydroxy)methyl]-6-(trifluoromethyl)pyridin-2-yl]piperazin-2-yl}propanoic acid ((±**)-19). 8** (0.027 g, 0.14 mmol), CDI (0.034 g, 0.21 mmol), and DIPEA (0.10 mL, 0.58 mmol) were dissolved in DCM (1 mL) and the mixture was stirred at rt for 3.5 h. A solution of (±)-**11** (0.063 g, 0.14 mmol) and DIPEA (0.14 mL, 0.81 mmol) in DCM (1 mL) was added. The reaction mixture was stirred at rt for 24 h and then in a sealed vial at 40 °C (oil bath heating) for 16 h. The mixture was concentrated and the residue was purified by preparative HPLC. Yield: 0.056 g (60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.2 Hz), 1.52 (9H, s), 1.69-1.81 (1H, m), 1.83-1.95 (1H, m), 2.34-2.42 (2H, m), 3.09 (dt, 1H, *J* = 3.2 and 12.5 Hz), 3.36-3.50 (2H, m), 4.02-4.10 (1H, m), 4.29 (1H, d, *J* = 13.9 Hz), 4.37 (2H, q, *J* = 7.2 Hz), 4.51-4.66 (2H, m), 6.91 (1H, d, *J* = 4.0 Hz), 7.65 (1H, d, *J* = 4.0 Hz), 8.37 (1H, s). MS ^{m}/z: 680 (M+H).

(±)-Ethyl 6-[3-(3-*tert*-butoxy-3-oxopropyl)-4-({[(5-chloro-2-thienyl)sulfonyl]amino)carbonyl)piperazin-1-yl]-5-cyano-2-(trifluoromethyl)nicotinate (0.056 g, 0.082 mmol) was dissolved in DCM (4 mL) and TFA (1 mL) was added. The reaction mixture was stirred at rt for 1 h and then concentrated. The crude was purified by preparative HPLC. Yield: 0.046 g (90%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.27 (3H, t, *J* = 7.1 Hz), 1.53-1.75 (2H, m), 2.02-2.14 (1H, m), 2.16-2.28 (1H, m), 3.14-3.40 (3H, m), 4.06-4.16 (1H, m), 4.26 (2H, q, *J* = 7.1 Hz), 4.30-4.45 (3H, m), 6.90 (1H, d, *J* = 3.8 Hz), 7.14 (1H, d, *J* = 3.8 Hz), 7.64 (1H, br s), 8.49 (1H, s). MS ^{m}/z: 624 (M+H).

5.2.11. Ethyl 5-chloro-6-piperazin-1-ylnicotinate (23). A mixture of **5** (2.20 g, 10.0 mol), piperazine (1.03 g, 12.0 mol), TEA (1.21 g, 12.0 mol), and EtOH (99%, 20.0 mL) was stirred until a clear solution appeared. This solution was split into 10 microwave vials. The vials were heated in a microwave oven at 120 °C for 10 minutes. The reaction mixtures were combined, K_2CO_3 (80 mL,10% aq solution) was added and the aq phase was extracted with EtOAc (3 x 80 mL).The combined organic extract was concentrated and the crude material was purified by flash chromatography (DCM/MeOH/TEA 9:1:0.1). Yield: 1.60 g (61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, *J* = 7.2 Hz), 1.77 (1H, br s), 3.01-3.05 (4H, m), 3.51-3.55 (4H, m), 4.36 (2H, t, *J* = 7.2 Hz), 8.12 (1H, d, *J* = 2.0 Hz), 8.75 (1H, d, *J* = 2.0 Hz). MS $^m/_z$: 270 (M+1).

5.2.12. Ethyl 5-chloro-6-[4-({[(5-chloro-2-thienyl)sulfonyl]amino}carbonyl)piperazin-1-yl]nicotinate (27). 8 (0.073 g, 0.37 mmol) and CDI (0.078 g, 0.48 mmol) were mixed in DCM (1.50 mL) and the reaction mixture was stirred for 1.5 h at rt. A solution of **23** (0.100 g, 0.37 mmol) and DIPEA (0.35 mL, 3.44 mmol) in DCM (1.50 mL) was added and the reaction mixture was stirred at 40 °C for 16 h. The mixture was concentrated and the crude was purified by preparative HPLC. Yield: 0.110 g (60%).

^{1}H NMR (400 MHz,$CDCl_3$): δ 1.38 (3H, t, *J* = 7.0 Hz), 3.54-3.64 (8H, m), 4.37 (2H, q, *J* = 7.0 Hz), 6.93 (1H, d, *J* = 4.1 Hz), 7.65 (1H, d, *J* = 4.1 Hz), 8.15 (1H, d, *J* = 1.9 Hz), 8.74 (1H, d, *J* = 1.9 Hz). MS $^m/_z$: 494 (M+1).

5.2.13. Ethyl 5-chloro-6-[4-({[(2-methylphenyl)sulfonyl]amino}carbonyl)piperazin-1-yl]nicotinate (28). 23 (0.067 g, 0.25 mmol) was dissolved in DCM (1.5 mL) and 2-methylbenzenesulfonyl isocyanate (0.074 g, 0.38 mmol) was added. The reaction mixture was stirred at rt for 14 h and then concentrated. The crude material was purified by preparative HPLC.

Yield: 0.038 g (33%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.41 (3H, t, *J* = 7.1 Hz), 2.71 (3H, s), 3.38-3.42 (4H, m), 3.44 (1H, s), 3.48-3.53 (4H, m), 4.38 (2H, q, *J* = 7.1 Hz), 7.30-7.36 (2H, m), 7.43-7.48 (1H, m), 8.04-8.08 (1H, m), 8.14-8.16 (1H, m), 8.70-8.72 (1H, m). MS $^m/_Z$: 467 (M+1).

5.2.14. Ethyl 6-(3-(tert-butoxycarbonylamino)azetidin-1-yl)-5-cyano-2-methylnicotinate (34). 7 (6.20 g, 29.4 mmol), *tert*-butyl azetidin-3-ylcarbamate (5.07 g, 29.4 mmol), and DIPEA (5.13 mL, 29.4 mmol) were dissolved in DCE (40 mL) and the reaction mixture was stirred at rt for 1 h. The mixture was concentrated and then diluted with EtOAc (40 mL). The organic phase was washed with $NaHCO_3$ (2 x 30 mL, saturated, aq solution), dried ($MgSO_4$) and concentrated. The crude was purified by flash chromatography (EtOAc/hexanes 1:6). Yield: 7.00 g (66%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.2 Hz), 1.46 (9H, s), 2.70 (3H, s), 4.18-4.22 (2H, m), 4.30 (2H, q, *J* = 7.2 Hz), 4.59 (1H, m), 4.67-4.72 (2H, m), 5.00 (1H, m), 8.26 (1H, s). MS $^m/_Z$: 361 (M+1).

5.2.15. Ethyl 6-(3-(tert-butoxycarbonylamino)azetidin-1-yl)-5-chloronicotinate (35). 5 (1.00 g, 4.50 mmol) and *tert*-butyl azetidin-3-ylcarbamate (0.765 g, 4.44 mmol) were dissolved in DMA (10 mL). DIPEA (1.66 g, 9.50 mmol) was added and the reaction mixture was heated at reflux for 16 h. The reaction mixture was cooled to rt and concentrated. The crude was dissolved in EtOAc (100 mL) and extracted with saturated aq $NaHCO_3$/ brine 1:1 (80 mL). The organic phase were dried ($MgSO_4$) and concentrated. The crude was purified by flash chromatography (4:1 hexanes/EtOAc). Yield: 1.02 g (50%). MS $^m/_Z$: 355 (M+1).

5.2.16. Ethyl 6-(4-aminopiperidin-1-yl)-5-cyano-2-methylnicotinate dihydrochloride (36). 7 (2.00 g, 8.90 mmol) and *tert*-butyl piperidin-4-ylcarbamate (1.78 g, 8.90 mmol) were dissolved in EtOH (50 mL). DIPEA (4.65 mL, 26.7 mmol) was added and the reaction mixture was heated at

94 °C for 4 h. The mixture was cooled to rt and concentrated. The material was dissolved in EtOAc (50 mL) and washed with NH_4Cl (2 x 30 mL, saturated, aq solution). The organic phase was washed with brine (30 mL), dried ($MgSO_4$) and concentrated. Yield: 3.30 g (95%). MS $^{m}/_{z}$: 389 (M+1).

5.2.17. Ethyl 5-chloro-6-(3-(3-(phenylsulfonyl)ureido)azetidin-1-yl)nicotinate (38). 35 (1.00 g, 2.80 mmol) was dissolved in DCM (4 mL). HCl (4M in 1,4-dioxane, 1.80 mL, 14.0 mmol) was added and the reaction mixture was stirred for 16 h at rt. The solvent was concentrated and then co-evaporated with hexanes and toluene, respectively, and used in the next step without further purification. Yield: 0.480 g (102%).

Ethyl 6-(3-aminoazetidin-1-yl)-5-chloronicotinate, dihydrochloride (0.150 g, 0.41 mmol) was suspended in DCM (5 mL) and TEA (0.21 mL, 1.52 mmol) was added. Benzenesulfonyl isocyanate (0.045 mL, 0.335 mmol) was added and the reaction mixture was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (20 mL) and washed with NH_4Cl (3 x 20 mL saturated, aq solution). The organic phase was dried ($MgSO_4$) and concentrated. The crude was purified by flash chromatography (1:1 EtOAc/hexanes to 1:1 EtOAc/hexanes with 1% AcOH). Yield: 0.042 g (31%). ^{13}C NMR (151 MHz, d_6-DMSO): δ 163.97, 156.89, 151.13, 148.11, 140.26, 137.76, 133.13, 128.97, 127.19, 116.52, 116.11, 113.58, 73.96, 60.53, 59.30, 40.89, 14.14. ^{1}H NMR (d_6-DMSO): δ 1.29 (3H, t, J = 7.2 Hz), 4.08-4.13 (2H, m), 4.27 (2H, q, J = 7.2 Hz), 4.37-4.45 (1H, m), 4.46-4.51 (2H, m), 7.24-7.30 (1H, m), 7.59-7.64 (2H, m), 7.67-7.72 (1H, m), 7.90-7.97 (3H, m), 8.56 (1H, m), 10.96 (1H, s). HRMS: Calc. for $C_{18}H_{19}N_4O_5SCl$ $(M+1)^+$, 439.0843; found: 439.0845.

5.2.18. *N*-(1-(3-cyano-5-(ethoxy(methoxy)methyl)-6-methylpyridin-2-yl)azetidin-3-ylcarbamoyl)benzenesulfonamide (39). Prepared by the procedure of **38** from **34** (1.00 g, 2.77

mmol) and benzenesulfonyl isocyanate (0.098 mL, 0.73 mmol), replacing TEA with DIPEA. Yield: 0.224 g (63%) of a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, *J* = 7.1 Hz), 1.58 (1H, br s), 2.72 (3H, s), 4.15-4.27 (2H, m), 4.32 (2H, q, *J* = 7.1 Hz), 4.61-4.77 (3H, m), 7.11 (1H, s), 7.53-7.62 (2H, m), 7.64-7.73 (1H, m), 7.84-7.93 (2H, m), 8.29 (1H, s). MS $^{m}/_{z}$: 444 (M+1).

5.2.19. 4-Chloro-*N*-*(*1-(3-cyano-5-(ethoxy(methoxy)methyl)-6-methylpyridin-2-yl)azetidin-3-ylcarbamoyl)benzenesulfonamide (40). Prepared by the procedure of **38** from **34** (1.00 g, 2.77 mmol) and 4-chlorobenzenesulfonyl isocyanate (0.103 mL, 0.69 mmol), replacing TEA with DIPEA (0.502 mL, 2.88 mmol). The reaction was complete after 2 h. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.29 (3H, t, *J* = 7.1 Hz), 2.61 (3H, s), 4.07-4.16 (2H, m), 4.23 (2H, q, *J* = 7.1 Hz), 4.39-4.55 (3H, m), 7.34-7.40 (1H, m), 7.70 (2H, d, *J* = 8.6 Hz), 7.91 (2H, d, *J* = 8.6 Hz), 8.28 (1H, m), 11.10 (1H, s). MS $^{m}/_{z}$: 478 (M+1).

5.2.20. *N*-(1-(3-cyano-5-(ethoxy(methoxy)methyl)-6-methylpyridin-2-yl)azetidin-3-ylcarbamoyl)-1-phenylmethanesulfonamide (41). 34 (1.00 g, 2.77 mmol) was dissolved in DCM (10 mL). TFA (6.40 mL, 83.2 mmol) was added slowly. The reaction mixture was stirred at rt for 30 minutes. The mixture was concentrated to afford ethyl 6-(3-aminoazetidin-1-yl)-5-cyano-2-methylnicotinate bis(trifluoroacetate) as a solid, which was used crude assuming a 100% conversion. CDI (0.054 g, 0.33 mmol) and $BnSO_2NH_2$ (0.057 g, 0.33 mmol) were dissolved in DCE (2 mL) and stirred at rt for 16 h. 6-(3-Aminoazetidin-1-yl)-5-cyano-2-methylnicotinate bis(trifluoroacetate) (0.210 g, 0.333 mmol) in DCE (2 mL) and DIPEA (0.580 mL, 3.33 mmol) were added and the reaction mixture was stirred at rt for 2 h. The reaction mixture was concentrated. EtOAc (40 mL) was added and the organic phase was washed with saturated $NaHCO_3$ (2 x 30 mL). The organic phase was dried ($MgSO_4$) and concentrated. The crude was purified by trituration (EtOAc/hexanes 1:1). Yield: 0.073 g (48%, 2 steps) as a solid. ^{13}C NMR

(151 MHz, d_6-DMSO) δ 164.31, 164.10, 157.72, 152.12, 145.81, 130.74, 129.53, 128.48, 128.38, 116.90, 113.32, 86.12, 60.53, 58.39, 57.96, 40.39, 25.29, 14.04. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.30 (3H, t, J = 7.1 Hz), 2.63 (3H, s), 4.18-4.19 (2H, m), 4.23 (2H, q, J = 7.1 Hz), 4.55 (3H, m), 4.69 (2H, m), 7.09 (1H, s), 7.32-7.42 (5H, m), 8.30 (1H, s), 10.47 (1H, s). HRMS: Calc. for $C_{21}H_{23}N_5O_5S$ $(M+1)^+$, 458.1498; found: 458.1512.

5.2.21. Ethyl 6-(4-(3-(5-chlorothiophen-2-ylsulfonyl)ureido)piperidin-1-yl)-5-cyano-2-methylnicotinate (42). 36 (3.30 g, 8.5 mmol) was dissolved in HCl (4 M in 1,4-dioxane, 31.9 mL, 127 mmol). The reaction mixture was stirred at rt for 48 h and concentrated to afford ethyl 6-(4-aminopiperidin-1-yl)-5-cyano-2-methylnicotinate dihydrochloride a solid, that was used crude assuming 100% conversion. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.31 (3H, t, J = 7.1 Hz), 1.53-1.68 (2H, m), 2.02-2.12 (2H, m), 2.65 (3H, s), 3.14-3.27 (2H, m), 3.30-3.43 (1H, m), 4.25 (2H, q, J = 7.1 Hz), 4.50-4.60 (2H, m), 8.17-8.29 (2H, m), 8.37 (1H, s). MS $^m/_z$: 362 (M+1). Ethyl 6-(4-aminopiperidin-1-yl)-5-cyano-2-methylnicotinate dihydrochloride (0.250 g, 0.692 mmol) and **9** (0.387 g, 1.04 mmol) were dissolved in DMA (2 mL). DIPEA (1.21 mL, 6.92 mmol) was added and the reaction mixture was heated to 100 °C for 1 h. The mixture was cooled to rt and concentrated. The crude was dissolved in EtOAc (40 mL) and washed with saturated aq NH_4Cl (2 x 40 mL). The organic phase was dried ($MgSO_4$) and concentrated. The crude was purified by flash chromatography (3:7 to 1:1 EtOAc/hexanes then 1:1 EtOAc/hexanes with 0.5% AcOH). Yield: 0.011 g (3%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.30 (3H, t, J = 7.1 Hz), 1.40-1.53 (2H, m), 1.80-1.90 (2H, m), 2.63 (3H, s), 3.17-3.27 (2H, m), 3.66-3.78 (1H, m), 4.24 (2H, q, J = 7.1 Hz), 4.39-4.50 (2H, m), 6.67-6.76 (1H, m), 7.26 (1H, d, J = 4.1 Hz), 7.62 (1H, d, J = 4.1 Hz), 8.33 (1H, s), 10.9-11.0 (1H, s). MS $^m/_z$: 512 (M+1).

5.2.22. Ethyl 6-(4-(3-(4-chlorophenylsulfonyl)ureido)piperidin-1-yl)-5-cyano-2-methylnicotinate (44). Prepared by the procedure of **38** from **36** (3.30 g, 8.50 mmol) and 4-chlorobenzenesulfonyl isocyanate (0.084 mL, 0.56 mmol), replacing TEA with DIPEA (0.482 mL, 2.77 mmol). Yield: 0.182 g (65%) as a solid. ^{13}C NMR (151 MHz, d_6-DMSO) δ 164.07, 163.17, 157.82, 150.66, 147.24, 139.10, 138.01, 129.26, 129.11, 117.68, 114.06, 88.42, 60.58, 46.49, 45.67, 31.29, 25.29, 14.04. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.29 (3H, t, *J* = 7.1 Hz), 1.37-1.49 (2H, m), 1.77-1.86 (2H, m), 2.62 (3H, s), 3.14-3.25 (2H, m), 3.60-3.70 (1H, m), 4.24 (2H, q, *J* = 7.1 Hz), 4.38-4.47 (2H, m), 6.63 (1H, m), 7.69 (2H, d, *J* = 8.6 Hz), 7.91 (2H, d, *J* = 8.6 Hz), 8.31 (1H, s), 10.64 (1H, s). HRMS: Calc. for $C_{22}H_{24}N_5O_5S$ $(M+1)^+$, 506.1265; found: 506.1277.

5.2.23. Ethyl 6-(4-(3-(benzylsulfonyl)ureido)piperidin-1-yl)-5-cyano-2-methylnicotinate (45). Prepared by the procedure of **41** from **36** (3.30 g, 8.50 mmol) and $BnSO_2NH_2$ (0.200 g, 1.17 mmol). The crude was purified by trituration (hexanes/Et_2O/DCM 2:2:1). Yield: 0.185 g (69%) as a solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.31 (3H, t, *J* = 7.1 Hz), 1.39-1.56 (2H, m), 1.88-2.00 (2H, m), 2.64 (3H, s), 3.77-3.89 (1H, m), 4.25 (2H, q, *J* = 7.1 Hz), 4.39-4.49 (2H, m), 4.69 (2H, s), 6.32-6.41 (1H, m), 7.29-7.45 (5H, m), 8.34 (1H, s), 9.90 (1H, s). HRMS: Calc. for $C_{23}H_{27}N_5O_5S$ $(M+1)^+$, 486.1811; found: 486.1830.

5.2.24. Ethyl 5-cyano-2-methyl-6-(piperazin-1-yl)nicotinate (46). **7** (2.00 g, 8.90 mmol) and piperazine (2.30 g, 26.7 mmol) were suspended in EtOH (30 mL). TEA (1.35 g, 13.4 mmol) was added and the reaction mixture was heated in a microwave oven at 160 ^{0}C for 25 minutes. The mixture was concentrated, diluted with DCM (30 mL), washed with $NaHCO_3$ (30 mL, saturated, aq solution) and brine (30 mL), dried (Na_2SO_4) and concentrated. Yield: 2.44 g (100%). ^{1}H NMR

($CDCl_3$): δ 1.37 (3H, t, *J* = 7.2 Hz), 2.71 (3H, s), 2.96-3.02 (4H, m), 3.88-3.95 (4H, m), 4.10 (1H, s), 4.31 (2H, q, *J* = 7.2 Hz), 8.28 (1H, s). MS ^{m}/z: 275 (M+1).

5.2.25. Ethyl 6-[4-({[(5-chloro-2-thienyl)sulfonyl]amino}carbonyl)piperazin-1-yl]-5-cyano-2-methylnicotinate (47). A solution of CDI (0.443 g, 2.70 mmol), **8** (0.407 g, 2.00 mmol) and DIPEA (1.5 mL) in DCM (15 mL) was stirred at rt for 4 h. A solution of **46** (0.407 g, 1.50 mmol) and DIPEA (1 mL) in DCM (10 mL) was added and the reaction mixture was stirred at 40 °C for 18 h. The organic phase was washed with water (25 mL) and concentrated. The crude was purified by preparative HPLC. Yield: 0.527 g (55%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.29 (3H, t, *J* = 7.1 Hz), 2.63 (3H, s), 3.48-3.55 (4H, m), 3.80-3.87 (4H, m), 4.23 (2H, q, *J* = 7.1 Hz), 7.21 (1H, d, *J* = 4.0 Hz), 7.60 (1H, *J* = 4.0 Hz), 8.34 (1H, s). The signal from the N-H proton likely coincided with either the DMSO signal (2.50 ppm) or the water signal (3.32 ppm). MS $^{m}/_{z}$: 498 (M+1).

5.2.26. Ethyl 5-cyano-2-methyl-6-(4-{[(phenylsulfonyl)amino]carbonyl}piperazin-1-yl)nicotinate (48). **46** (0.274 g, 1.00 mmol) was dissolved in DCM (5 mL) and benzenesulfonylisocyanate (0.263 g, 1.44 mmol) was added. The reaction mixture was stirred at rt for 18 h. Water (2 drops) was added and the mixture was concentrated. The crude material was purified by flash chromatography (DCM/EtOAc 4:1 to 0:1). Yield 0.061 g (13%). ^{1}H NMR (500 MHz, d_6-DMSO): δ 1.31 (3H, t, *J* = 7.3 Hz), 2.64 (3H, s), 3.30 (1H, s), 3.45-3.53 (4H, m), 3.75-3.80 (4H, m), 4.26 (2H, q, *J* = 7.3 Hz), 7.39-7.45 (3H, m), 7.77-7.81 (2H, m), 8.35 (1H, s). MS ^{m}/z: 456 (M-1).

5.2.27. Ethyl 6-(4-(benzylsulfonylcarbamoyl)piperazin-1-yl)-5-cyano-2-methylnicotinate (49). CDI (0.152 g, 0.934 mmol) and $BnSO_2NH_2$ (0.200 g, 1.17 mmol) were dissolved in DCE (2

mL) and the reaction mixture was stirred at rt for 16 h. A solution of **46** x 2 HCl (0.200 g, 0.576 mmol) in DCE (2 mL) and DIPEA (0.502 mL, 2.88 mmol) was added and the reaction mixture was stirred at rt for 16 h. The mixture was diluted with EtOAc (40 mL), washed with 2 M HCl (2 x 30 mL), NH_4Cl (2 x 30 mL, saturated, aq solution), and H_2O (2 x 30 mL), dried ($MgSO_4$) and concentrated. The crude solid product was purified by trituration (hexanes/Et_2O/DCM 2:2:1). Yield: 0.156 g (58%) as a solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.31 (3H, t, J = 7.1 Hz), 2.66 (3H, s), 3.52-3.62 (4H, m), 3.80-3.90 (4H, m), 4.26 (2H, q, J = 7.1 Hz), 4.76 (2H, s), 7.33-7.42 (5H, m), 8.35 (1H, s), 10.5-10.6 (1H, br s). MS $^m/_z$: 472 (M+1).

Supporting Information

Supporting information available: Experimental procedures for compounds **15-18**, **21**, (±)-**22**, **24-26, 29-33**, **37**, **43**, **50**, (±)-**51**, **52-54**, **59-63**, **67**, **70**, **71**, **74**, **77** and description of the screening assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Conflict of Interest Disclosure

The authors declare no competing financial interst.

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Abbreviations

3-aze, 3-amino-azetidinyl; CDI, *N*,*N*'-carbonyl diimidazole; CLint, intrinsic clearance; *cy*-Pr, cyclopropyl; Caco-2, adenocarcinoma cells from human colon; DIPEA, *N*,*N*-diisopropylethylamine; DMA, dimethylacetamide; DMAP, 4-dimethylamino-pyridine; DSC, EDCI, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; *N*,*N*'-disuccinimide carbonate; GTPγS, guanosine 5'-O-[g-thio]triphosphate; HLM, human liver microsomes; HOBT, 1-hydroxybenzotriazole; LC, liquid chromatography; MES, 2-morpholinoethanesulphonic acid monohydrate; MW, single node heating in a microwave oven; paz, piperazinyl; 4-pip, 4-amino-piperidinyl; PS, polymer-supported; RLM, rat liver microsomes; rt, room temperature; TEA, triethylamine; TEER, transepithelial electrical resistance; TEG, triethyleneglycol; Troc-Cl, 2,2,2-trichloroethyl chloroformate; WPA, washed platelet assay.

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