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Lead Optimization of Ethyl 6-Aminonicotinate Acyl Sulfonamides as Antagonists of the P2Y$_{12}$ Receptor. Separation of the Antithrombotic Effect and Bleeding for Candidate Drug AZD1283

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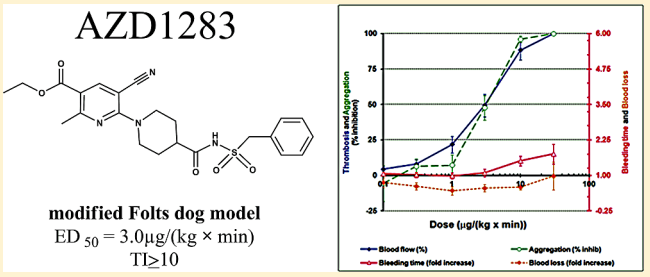
Supporting Information

ABSTRACT: Synthesis and structure–activity relationships of ethyl 6-aminonicotinate acyl sulfonamides, which are potent antagonists of the P2Y$_{12}$ receptor, are presented. Shifting from 5-chlorothienyl to benzyl sulfonamides significantly increased the potency in the residual platelet count assay. Evaluation of PK parameters in vivo in dog for six compounds showed a 10-fold higher clearance for the azetidines than for the matched-pair piperidines. In a modified Folts model in dog, both piperidine **3** and azetidine **13** dose-dependently induced increases in blood flow and inhibition of ADP-induced platelet aggregation with antithrombotic ED$_{50}$ values of 3.0 and 10 μg/kg/min, respectively. The doses that induced a larger than 3-fold increase in bleeding time were 33 and 100 μg/kg/min for **3** and **13**, respectively. Thus, the therapeutic index (TI) was ≥10 for both compounds. On the basis of these data, compound **3** was progressed into human clinical trials as candidate drug AZD1283.



INTRODUCTION

Acute arterial thrombosis is accountable for most cases of myocardial infarction and approximately 80% of strokes, which collectively are the most common causes of mortality and morbidity in the developed world.[1] By vascular damage, e.g., due to rupture of atherosclerotic plaque, platelets can get into contact with and adhere to subendothelial proteins such as collagen and von Willebrand factor.[2] This activates the platelets, which in turn release ADP from their dense granules. ADP activates the G$_i$-coupled P2Y$_{12}$ receptors,[3] which decrease the intracellular adenylyl cyclase activity and prolong intracellular calcium signaling, thereby stabilizing the formed platelet aggregates.[4]

The central role of the P2Y$_{12}$ receptor in platelet function makes it an attractive target for the development of novel antiplatelet therapies.[5] The "thienopyridines", including ticlopidine, clopidogrel, and prasugrel, are prodrugs, whose active metabolites bind irreversibly to the receptor, thus inhibiting the platelet for its entire life span.[6] However, preclinical data suggested that reversible binding could not only lead to a recovery of platelet function, but also increase the separation between antithrombotic effect and bleeding risk.[7,8]

Ticagrelor, which is the first reversibly binding, direct-acting P2Y$_{12}$ antagonist, was developed via a medicinal chemistry program from ATP, the natural antagonist of the P2Y$_{12}$ receptor, as the lead structure.[9] Other series of P2Y$_{12}$ antagonists include piperazinyl glutamate–pyridines/pyrimidines,[10] thienopyrimidines,[11] anthraquinones,[12] adenosine analogs,[13] dinucleoside polyphosphates and nucleotides,[14] and phenylpyrazole derivatives.[15]

We recently reported that a series of ethyl nicotinate derivatives are antagonists of the P2Y$_{12}$ receptor. These were featured by urea[16] (e.g., **1**, Figure 1) or sulfonylurea[17] (e.g., **2**) linkers. Further development of the urea series was hampered by low solubility and low microsomal stability. While the sulfonylurea series improved these issues, it remained to identify one compound that integrated high potency with desirable physicochemical and pharmacokinetic (PK) properties. In the present paper, we show how replacement of the sulfonylurea linker with an acyl sulfonamide linker followed by structure–property and in vivo dog PK and PD investigations of this new series led to the selection of compound **3** as a candidate drug (named AZD1283) for clinical development.

CHEMISTRY

The ethyl 6-chloronicotinates **4** and **5**[17] (Scheme 1) were treated with azetidine-3-carboxylic acid (3-aze) to give the azetidinylpyridines **6** and **7** and with piperidine-4-carboxylic acid (4-pip) to give the piperidinylpyridines **8** and **9**. The carboxylic acid functionalities of compounds **6**–**9** were activated with a coupling reagent such as 1-ethyl-3-(3-

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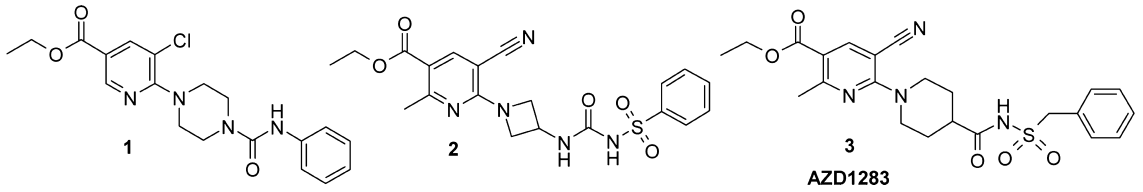
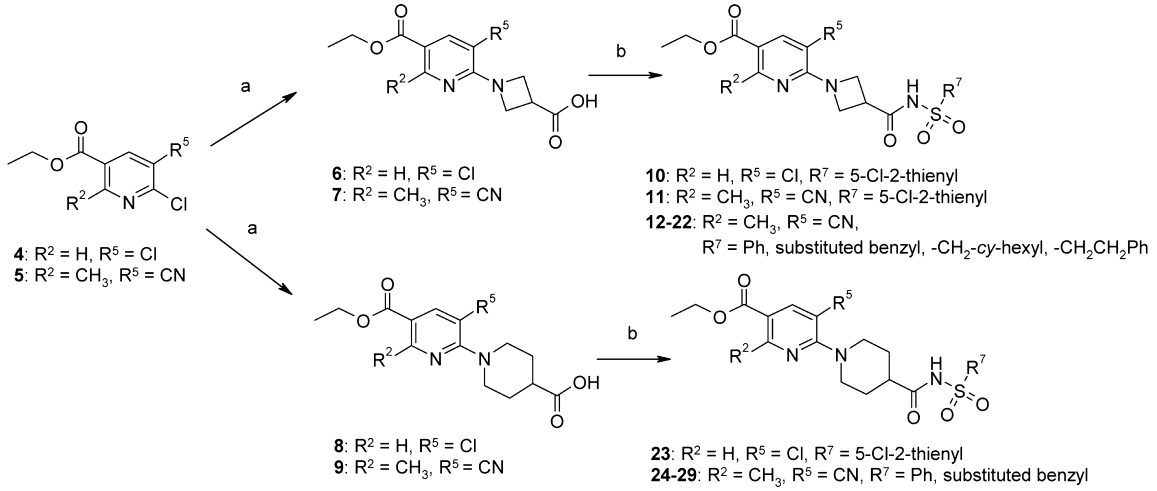


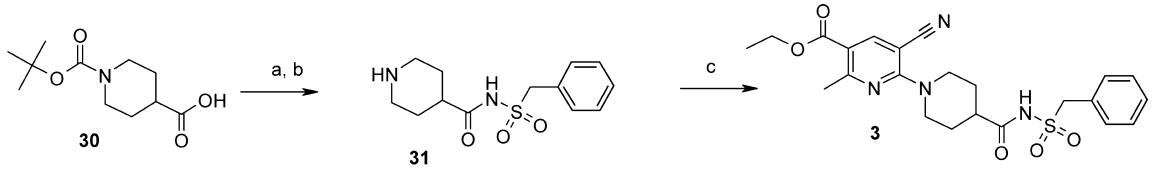
Figure 1. Examples of ethyl nicotinate derivatives, featured by urea (**1**), sulfonylurea (**2**), and acyl sulfonamide (**3**) linkers.

Scheme 1. Synthesis of Ethyl 6-Azetidinylnicotinate and 6-Piperidinylnicotinate Derivatives[a]



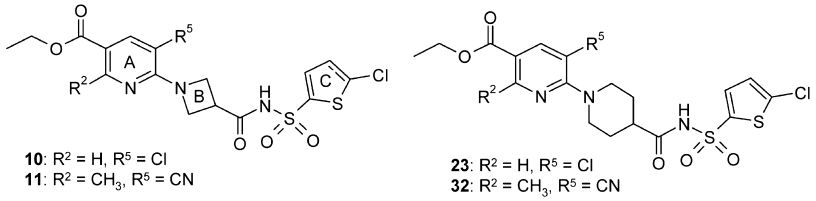
[a]Reagents and typical reaction conditions: (a) (e.g., for **6**) **4**, azetidine-3-carboxylic acid, *N*,*N*-diisopropylethylamine (DIPEA), DMA, 120 °C, 18 h (73%); (b) (e.g., for **10**) **6**, EDC, HOBT, DCM, rt, 30 min, then addition of 5-chlorothiophene-2-sulfonamide, DIPEA, rt, 18 h (56%).

Scheme 2. Synthesis of Ethyl 6-Piperidinylnicotinate Derivative 3 by the Reverse Method[a]



[a]Reagents and reaction conditions: (a) $PhCH_2SO_2NH_2$, TBTU, triethylamine (TEA), THF, LiCl, rt, 22 h (72%); (b) HCOOH, rt, 26 h (76%); (c) **5**, DIPEA, DMA, 60 °C,18 h (83%).

Table 1. SAR of Ethyl Nicotinate Derivatives with 5-Choro-2-thienyl Acyl Sulfonamides[a]



compd no.	R^2	R^5	central ring	binding IC_{50} (μM)	GTPγS IC_{50} (μM)	WPA IC_{50} (μM)	RPC IC_{50} (μM)	log *D*, pH 6.8[22]	Caco-2 A to B (10^{-6} cm/s)	CLint DLMs, L/M/H
10	H	Cl	3-aze	0.50	0.31	0.48		2.3	M (0.5)	L
11	CH_3	CN	3-aze	0.059	0.099	0.068	>22	2.6	M (0.2)	L
23	H	Cl	4-pip	0.20	0.13	0.74		2.8	H (10)	H
32	CH_3	CN	4-pip	0.039	0.080	0.039	>22	3.1	H (6)	M

[a]3-aze = 3-azetidinyl, 4-pip = 4-piperidinyl. In the washed platelet assay (WPA) the ability of the compounds to inhibit fibrinogen-induced aggregation is determined. The residual platelet count (RPC) assay determines the number of single platelets that remain after induction of platelet aggregation in whole blood using ADP as an agonist. Thus, the effect of an antagonist is determined by an increase in the residual platelet count. Lipophilicity, given by log *D* (*D* = distribution coefficient), was determined by a chromatographic method. Caco-2 A to B = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon. CLint DLMs = intrinsic clearance, as a measure for metabolic stability, in dog liver microsomes. L/M/H = low/moderate/high.

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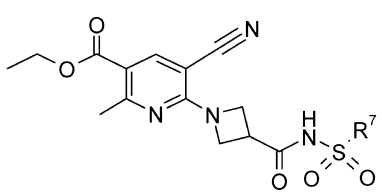
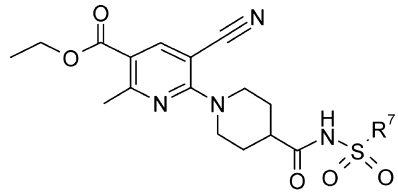
Table 2. SAR of Ethyl Nicotinate Derivatives with Phenyl, Benzyl, Cyclohexylmethyl, and Phenethyl Acyl Sulfonamides[a]





compd no.	central ring	R^7	binding IC_{50} (μM)	GTPγS IC_{50} (μM)	RPC IC_{50} (μM)	log D, pH 6.8	solubility, pH 6.8 (μM)	Caco-2 A to B (10^{-6} cm/s)	CLint DLMs (μL/min/mg)	CLint[25] HLMs, L/M/H	CYP 2C9 IC_{50} (μM)	CYP 3A4 IC_{50} (μM)
12	3-aze	Ph	0.20	0.84		1.7		0.2 (M)	<12 (L)	M		
13	3-aze	Bn	0.017	0.033	2.6	2.0	120	0.3 (M)	<12 (L)	L	>50	>50
14	3-aze	$-CH_2CH_2Ph$	0.22			2.2		0.3 (M)				
15	3-aze	$-CH_2$-c-hexyl	0.089	0.18		2.6		3 (H)				
16	3-aze	2-CH_3Bn	0.067	0.18		2.2						
17	3-aze	3-CF_3Bn	0.11	0.29						M		
18	3-aze	3-CH_3Bn	0.032	0.065	19	2.2						
19	3-aze	4-ClBn	0.010	0.023	2.8	2.7	134	0.2 (M)	26 (M)	M	7.1	3.3
20	3-aze	4-FBn	0.012	0.020	1.2	2.3	297	0.1 (L)	29 (M)	L	>20	>20
21	3-aze	4-CH_3Bn	0.006	0.019	1.8	2.2	69	0.6 (M)	<12 (L)	H	20	20
22	3-aze	4-CH_2OHBn	0.23	0.73		1.1				L		
24	4-pip	Ph	0.12	0.71		2.3				L		
3	4-pip	Bn	0.011	0.025	3.2	2.8	104	20 (H)	<12 (L)	L	3.3	20
25	4-pip	4-FBn	0.015	0.038	2.9	2.8	63	8 (H)	17 (M)	M	5.9	4.5
26	4-pip	4-OCH_3Bn	0.03	0.058	9.0	2.8	17	40 (H)		M	15	>17
27	4-pip	4-CH_3Bn	0.007	0.023	2.9	3.2	0.20	40 (H)	<12 (L)	H	>18	>17
28	4-pip	4-*i*-PrBn	0.16	0.25		4.2	2.0	40 (H)		H	2.4	>2
29	4-pip	2,4-di-FBn	0.011	0.016	2.7	2.9		5 (H)	20 (M)	H	4.1	1.4

[a]3-aze = 3-azetidinyl, 4-pip = 4-piperidinyl, and RPC = residual platelet count assay. Lipophilicity, given by log D (D = distribution coefficient), was determined by a chromatographic method. Caco-2 A to B = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon. CLint = intrinsic clearance in DLMs (dog liver microsomes) or HLMs (human liver microsomes). L/M/H = low/moderate/high.

(dimethylamino)propyl)carbodiimide hydrochloride (EDC)/1-hydroxybenzotriazole (HOBT),[18] *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluromium hexafluorophosphate (HATU), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), or bromotrispyrrolidinophosphonium hexafluorophosphate (PyBrop) and then treated with different sulfonamides to produce the acyl sulfonamides **10**–**29**. Compound **3** (Scheme 2) was prepared by a reverse method in which coupling of **30** with benzyl sulfonamide, followed by boc deprotection with formic acid, provided intermediate **31**, which was treated with the ethyl 6-chloronicotinate derivative **5** to provide the candidate drug **3**. By this sequence it was possible to precipitate **3** from the reaction mixture, thus enabling larger scale synthesis. Especially the coupling of **30** with benzyl sulfonamide was problematic due to the poor nucleophilicity of benzyl sulfonamide, but it was found that in THF, in the presence of lithium chloride (LiCl), a TBTU coupling readily afforded **31**. Compound **32** (Table 1) was prepared by similar procedures.

RESULTS AND DISCUSSION

Compounds were tested in vitro for binding affinity for $P2Y_{12}$ receptors in chinese hamster ovarian (CHO) cell membranes.[7] The potency of the compounds was determined in a functional guanosine 5′-*O*-[γ-thio]triphosphate (GTPγS)[7,19] assay using a similar membrane preparation. The GTPγS assay is based on the principle that activation (by ADP) of $P2Y_{12}$ favors binding of the receptor to a G-protein. The formed ADP–$P2Y_{12}$–G-protein ternary complex induces the G-protein-bound GDP to exchange with GTPγ^{35}S, which is not hydrolyzed by GTPases; thus, the effect of an added antagonist can be determined by the fraction of unbound GTPγ^{35}S. Potency in a residual platelet count (RPC) assay[20] was determined for most compounds. This assay determines the number of single platelets that remain after induction of platelet aggregation in whole blood using ADP as an agonist. Thus, the effect of an antagonist can be determined by an increase in the residual platelet count. A few compounds from the early-phase project were tested for potency in a functional human washed platelet aggregation assay (WPA) that determines the ability of the compounds to inhibit fibrinogen-induced aggregation.[7]

Ethyl 5-cyano-2-methylnicotinate derivatives (Table 1) showed a 5–8-fold higher binding affinity and a 7–17-fold higher potency in the WPA assay than the matched-pair ethyl 5-chloronicotinate derivatives (pairs **10**/**11** and **23**/**32**). On the basis of these differences in activity, the 2-methyl/5-cyanopyridine substitution pattern was retained in further structure–activity relationship (SAR) investigations. A similar difference in activity as a function of the pyridine substitution pattern was observed in the previously reported sulfonylurea series.[17] Another similarity with the sulfonylurea series was that the carboxylic acid of **13**, formed by hydrolysis of the ethyl ester, was inactive in both the binding and GTPγS assays.

The higher lipophilicity (Δ log D = 0.5) of piperidinylpyridines in comparison to the matched-pair azetidinylpyridines (pairs **23**/**10** and **32**/**11**) was reflected in the piperidinylpyridines having higher permeability in Caco-2 monolayers[21] and higher intrinsic clearance (CLint) in dog liver microsomes (DLMs).

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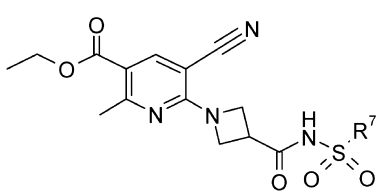
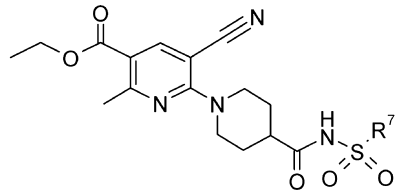
Table 3. In Vivo PK in Dog for a Selection of Potent Acyl Sulfonamides[a]

compd no.	central ring	R^7	RPC IC_{50} (μM)	Caco-2 A to B (10^{-6} cm/s)	CLint DLMs (μL/min/mg)	log *D*, pH 6.8	dog PPB (% free)	*N*, iv	dog CL (mL/min/kg)[b]	*N*, po	dog *F* (%)[b]	dog $t_{1/2}$, po (h)[b]
13	3-aze	Bn	2.4	M (0.3)	<12 (L)	2.0	1.9	2	9.8 ± 1.3	2	100 ± 29	3.1 ± 0
20	3-aze	4-FBn	1.1	L (0.1)	29 (M)	2.3	1.3	2	9.4 ± 0.1	2	27 ± 3	5.9 ± 0.4
21	3-aze	4-CH_3Bn	1.8	M (0.6)	<12 (L)	2.2	0.82	2	4.5 ± 0.5	1	69	7.5
3	4-pip	Bn	3.1	H (20)	<12 (L)	2.8	0.35	3	0.75 ± 0.25	2	100 ± 11	8.0 ± 0.5
27	4-pip	4-CH_3Bn	2.8	H (40)		3.2	0.59	1	0.50	1	92	13
29	4-pip	2,4-di-FBn	2.7	H (5)	20 (M)	2.9	0.14	2	1.1 ± 0.7	2	53 ± 23	6.6 ± 1.2

[a]In vitro data have been included for reference. 3-aze = 3-azetidinyl, 4-pip = 4-piperidinyl, RPC = residual platelet count assay, Caco-2 A to B = permeability in the apical (A) to basolateral (B) direction in adenocarcinoma cells from human colon, CLint = intrinsic clearance, and DLMs = dog liver microsomes. Lipophilicity is given by log *D* (*D* = distribution coefficient). PPB = plasma protein binding, CL = clearance, *F* = bioavailability, and L/M/H = low/moderate/high. [b]The compounds were administered as solutions in TEG/DMA/H_2O (1:1:1) or TEG/EtOH/H_2O (50:5:45).

Surprisingly, the potency in the RPC assay was low for the 5-chlorothienyl compounds **11** and **32** despite a high potency in WPA. This was observed only for 5-chlorothienyl-containing compounds. Further exploration of thienyl groups carrying methyl and/or chloro substituents did not increase the potency in RPC.

Further variations (Table 2) including phenyl (**12**), benzyl (**13**), phenylethyl (**14**), and cyclohexyl (**15**) derivatives showed superior binding affinity for the benzylic compound in the azetidine subseries; this was also observed for the phenyl/benzyl matched pair **24**/**3** in the piperidine subseries. Notably, the benzylic compounds **3** and **13** were potent in the higher order residual platelet count assay (RPC)[20] in which the two tested 5-chlorothienyls **11** and **32** showed low (IC_{50} >22 μM) potency. Consequently, the effect of introducing substituted benzyls in the azetidinyl- and piperidinylpyridines was investigated.

In the azetidine subseries, methyl substitution led to a 6-fold lower potency in the GTPγS assay when in the 2-position (**16**) and unchanged potency when in the 3- or 4-position (**18**, **21**), compared to the unsubstituted benzyl compound **13**. Also 4-chloro (**19**, lipophilic and electron-withdrawing) and 4-fluoro (**20**, electron-withdrawing) substituents led to unchanged GTPγS potency. Substituents that led to a lower potency in GTPγS are exemplified by the 3-trifluoromethyl substituent (**17**, bulky, electron-withdrawing), which lowered the potency 6-fold compared to the 3-methyl (**18**), and 4-hydroxymethyl (**22**, hydrophilic, electronically neutral),[23] which lowered the potency 38-fold compared to 4-methyl substitution (**21**).

In the piperidine subseries, the introduction of substituents such as fluoro (**25**), methoxy (**26**), or methyl (**27**) in the benzyl 4-position had no effect on the GTPγS potency compared to an unsubstituted benzyl substituent (**3**). Introduction of the sterically more demanding 4-isopropyl (**28**) group led to a 10-fold decrease in potency compared to 4-methyl (**27**). In both the azetidine and piperidine subseries, the introduction of further substituents on the aromatic part of the benzyl substituent, exemplified by the 2,4-difluorobenzyl derivative **29**, in the best cases led to no or very minor increases in potency compared to mono- and unsubstituted benzyl compounds.

The generally high aqueous solubility[24] at pH 6.8 was likely due to the acidity of the acyl sulfonamide linker (for example, pK_a(**3**) = 4.6, pK_a(**13**) = 3.6, pK_a(**21**) = 3.4, and pK_a(**27**) = 4.6).[24]

The in vitro pharmacokinetic (PK) profile indicated in general better permeability for 4-piperidines than for 3-azetidines, as examplified by the matched pair **3**/**13**. The intrinsic clearance was low or moderate for all compounds in DLMs and for most compounds in HLMs; however, no correlation was observed between the clearance values in the two different types of microsomes. The most striking example is compounds **21** and **27**, which showed low clearance in DLMs but high clearance in HLMs. Metabolite identification in HLMs indicated oxidation of the 4-methyl substituents as the main metabolic pathway in addition to ester hydrolysis.

In Vivo PK Parameters in Dog. On the basis of their in vitro parameters, a selection of compounds from Table 2 were progressed for evaluation of PK parameters in dog. For some compounds in Table 2, CYP450 inhibition was a possible liability. This was considered when selecting compounds for further evaluation. In total, six compounds (Table 3) were selected. Two of these (**3** and **13**) were selected in spite of their inhibition of two of the CYP450-metabolizing enzymes, CYP2C9 and CYP3A4. Whether or not this was a risk factor in later development (potential for drug−drug interactions) would depend on the dose of the selected drug candidate and should be weighted against the other properties of the compounds.

The azetidines showed higher clearance in vivo than the matched-pair piperidines, by comparison of the pairs **3**/**13** (13-fold) and **21**/**27** (9-fold). Although the protein binding was lower for the azetidines, this seems unlikely to explain their higher clearance.[26] No correlation was observed between the intrinsic clearance in DLMs and the clearance in vivo. For example, the two azetidines **13** (low intrinsic clearance in DLMs) and **20** (moderate intrinsic clearance in DLMs) both showed similar low to moderate clearance in vivo. This could point to the possibility of **13** having additional metabolic pathways in vivo.

On the basis of their favorable pharmacokinetic profiles and high stabilities in HLMs, **3** and **13** were selected for further

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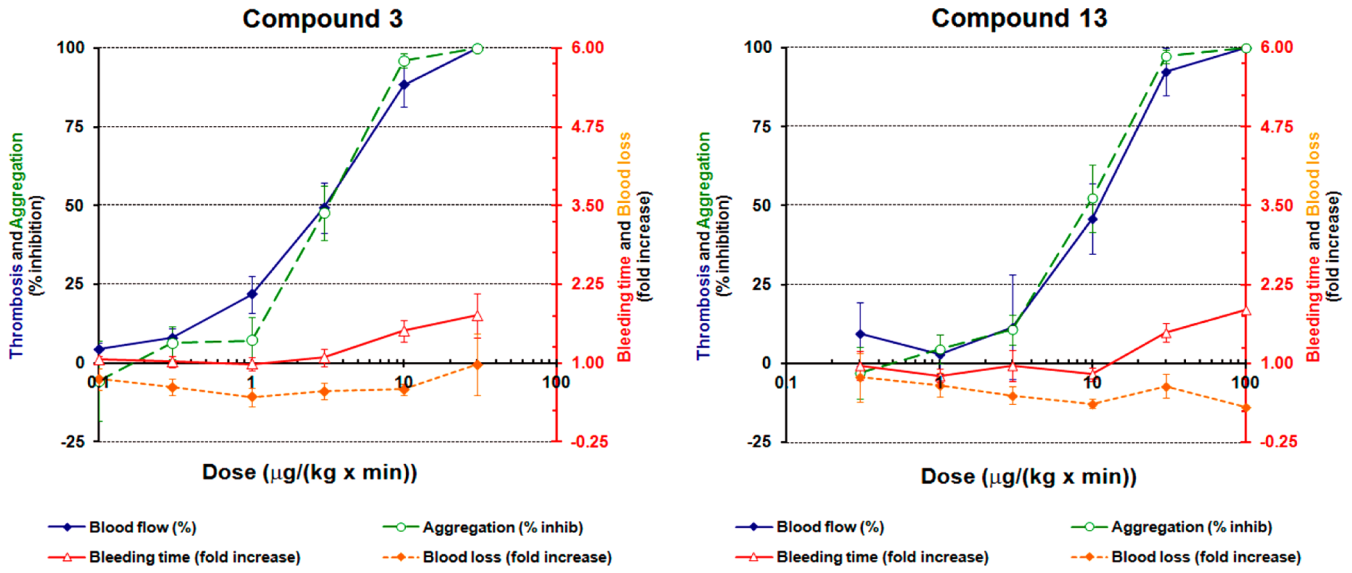

Figure 2. Effects of compounds **3** ($N = 6$) and **13** ($N = 5$) on blood flow, aggregation, bleeding time, and blood loss vs dose. In each graph, the left *y*-axis describes the inhibition (%) of thrombosis (blood flow) and aggregation time, while the right *y*-axis describes the fold increase of bleeding time and blood loss as a function of the dose given at the *x*-axis.

evaluation of their in vivo antithrombotic potential and associated bleeding risk in a modified Folts dog model.

Evaluation of the in Vivo and ex Vivo Antithrombotic Effect and Bleeding Time in a Modified Folts Dog Model. In a modified Folts dog model[8] the platelet/vessel wall interactions were evaluated by measurement of femoral artery blood flow after mechanical damage of the endothelium followed by approximately 80% stenosis. A blood flow pattern comprising thrombus growth followed by mechanical restoration of artery is known as cyclic flow reductions (CFRs), and an in vivo antithrombotic effect is seen as an abolition of the CFRs. In vivo (blood flow) and ex vivo (platelet aggregation measured with impedance aggregometry) antithrombotic effects, bleeding time, and blood loss (incision in the tongue) were measured during a 30 min control period followed by five consecutive 30 min periods with increasing doses of the test compounds. The results are summarized in Figure 2.

Both **3** and **13** induced dose-dependent increases in blood flow and inhibition of ADP-induced platelet aggregation, showing an antithrombotic ED_{50} for **3** and **13** of 3.0 and 10 μg/(kg × min), respectively. The two compounds displayed a similar bleeding time pattern; the doses that induced a larger than 3.5-fold increase in bleeding time ($ED_{BT>3.5}$) were greater than 33 and 100 μg/(kg × min) for **3** and **13**, respectively. Thus, the therapeutic index (TI), defined as antithrombotic $ED_{50}/ED_{BT>3.5}$, was ≥10 for both compounds. This is at least similar to the increase in TI previously reported for the reversibly binding antagonist ticagrelor (ED_{50} = 1.02 μg/(kg × min); TI > 5.2) and the irreversibly binding antagonist clopidogrel (ED_{50} = 15 mg/kg; TI = 2.3).

CONCLUSIONS

In conclusion, a novel series of ethyl 6-aminonicotinate acyl sulfonamides were discovered to be antagonists of the $P2Y_{12}$ receptor. An advantage of this structural class was that screening compounds could be prepared in a few synthetic steps. SAR investigations showed an increase in potency in the residual platelet count assay by replacement of a 5-chlorothienyl with a benzyl substitutent. Introduction of substitutents on the benzyl group gave no further improvement in potency and in many cases led to higher microsomal clearance. PK parameters in vivo in dog for six compounds showed higher clearance for the azetidines than for the matched-pair piperidines.

One azetidine (**13**) and one piperidine (**3**) were evaluated for an antithrombotic effect in vivo and possible associated bleeding risk in a modified Folts dog model. Both compounds induced dose-dependent increases in blood flow and inhibition of ADP-induced platelet aggregation, displaying a therapeutic index ≥10 in the separation of efficacy and bleeding time. The piperidine **3** showed the strongest antithrombotic effect and was therefore progressed to human clinical trials as candidate drug AZD1283.

EXPERIMENTAL SECTION

General Description. Commercially available chemicals were used as provided by the commercial supplier. Noncommercial or expensive sulfonamides were made by stirring the corresponding commercially available sulfonyl chloride (0.75 mmol) at rt in a saturated solution of ammonia in MeOH (5 mL) until completion of the reaction by LC/MS detection. After evaporation of ammonia and MeOH, the crude was dissolved in MeOH (5 mL). Optionally, DMF (2 mL) was added to dissolve the crude. The solution were then filtered through an ISOLUTE SCX-2 ion-exchange resin (while rinsing with MeOH) and then concentrated to give a material which was used without further purification. As a special case, the sulfonamide used in the synthesis of **19** was prepared from 4-(chloromethyl)benzyl alcohol in analogy to a literature procedure.[27]

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

Thin-layer chromatography was performed on silica gel 60 F_{254} (Merck KGaA, Darmstadt). Flash chromatography was performed with either standard glass or plastic columns using Merck silica gel grade 9385, 60 Å (0.063−0.200 mm) from Sigma-Aldrich or on a Biotage Horizon high-performance flash chromatography (HPFC) system using Biotage silica gel. Preparative HPLC was performed on a Waters YMC-ODS AQS-3 120 Å 3 × 500 mm. Gradient: 95% 0.1 M

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aq NH_4OAc buffer/5% CH_3CN → 100% CH_3CN. Alternatively, preparative HPLC was performed on a Waters Fraction Lynx purification system with a Kromasil C8 column (5 μm; 100 mm × 20 mm i.d.) with gradients of CH_3CN in 0.1 M aq NH_4OAc buffer as the mobile phase. MS-triggered fraction collection was used. Mass spectra were recorded on either a Micromass ZQ single quadrupole or a Micromass Quattro micro instrument, both equipped with a pneumatically assisted electrospray interface. HRMS of isolated compounds was performed on a Waters XEVO qToF instrument operated in positive electrospray mode using a Waters Acquity iClass UPLC system, with a 2.1 × 50 mm 1.7 μm CSH C18 column and a linear gradient of 5−95% acetonitrile (1 mM ammonium formate, 10 mM formic acid, pH 3) in 2.5 min (system controlled by Waters MassLynx 4.1 software). ^1H NMR measurements were performed on a Varian Mercury VX 400 spectrometer, operating at a ^1H frequency of 400 MHz, or on Varian UNITY plus 400, 500, and 600 spectrometers, operating at ^1H frequencies of 400, 500, and 600 MHz, respectively. Chemical shifts are given in parts per million with the solvent as the internal standard. Coupling constants are given in hertz.

For some samples with DMSO as the NMR solvent, a so-called wet DMSO NMR experiment was used. This was conducted in the following manner: A sample of a concentrated solution of the compound in $(CH_3)_2SO$ was diluted with $(CD_3)_2SO$. Since a substantial amount of $(CH_3)_2SO$ was present in the sample, a prescan was run and analyzed to automatically suppress the $(CH_3)_2SO$ and H_2O peaks at 2.54 and 3.30 ppm, respectively. In the wet DMSO NMR experiment, the ^1H NMR signal from the methine proton of the piperidinyl group coincided with the suppressed $(CH_3)_2SO$ signal at 2.54 ppm; however, the chemical shift for the methine proton could be determined by H,H COSY, and this signal is reported for each piperidinyl compound.

The purity of the 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 was recorded through a standard gradient from 5% to 95% CH_3CN in 10 mM aq NH_4OAc over 4 min using a Synergy MAX-RP C12 (4 μm; 50 mm × 3 mm i.d.) column with a flow rate of 2 mL/min. All screening compounds had purity ≥95%.

Ethyl 6-(4-[(Benzylsulfonyl)carbamoyl]piperidin-1-yl)-5-cyano-2-isopropylnicotinate (3). A suspension of **30** (7.73 g, 1.96 mmol) and TBTU (16.18 g, 50.4 mmol) in THF (80 mL) and TEA (10.89 g, 15.0 mL, 107.7 mmol) was stirred at rt for 45 min. 1-Phenylmethanesulfonamide (7.35 g, 42.92 mmol) and LiCl (0.46 g, 10.85 mmol) were added, and the reaction mixture was stirred at rt for 22 h. The mixture was concentrated, diluted with EtOAc (100 mL), washed with diluted HCl, dried ($MgSO_4$), and concentrated. Addition of *tert*-butyl methyl ether (TBME) (100 + 50 mL) facilitated precipitation. The mixture was stirred at rt for 1 h and filtered, and the solids were washed with TBME (50 mL). Yield: 9.34 g (72%).

A suspension of *tert*-butyl 4-[(benzylsulfonyl)carbamoyl]piperidine-1-carboxylate (6.45 g, 16.86 mmol) in formic acid (61.0 g, 50.0 mL, 46.0 mmol) was stirred at rt for 26 h, concentrated, and coconcentrated with water (25 mL). Water (35 mL) was added, and the pH was adjusted to 6 with NH_4OH (saturated, aq solution). The mixture was stirred at rt for 18 h and filtered, and the solids were washed with cold water (35 mL) and dried in vacuo. Yield: 3.61 g (76%) of **31** as a white powder. ^1H NMR (400 MHz, d_6-DMSO): δ 1.57−1.72 (2H, m), 1.72−1.84 (2H, m), 2.08−2.19 (1H, m), 2.72−2.85 (2H, m), 3.07−3.17 (2H, m), 4.24 (2H, s), 7.19−7.29 (5H, m), 8.12 (1H, br s). MS: *m/z* 283 (M + 1).

A suspension of **5** (0.671 g, 2.99 mmol), **31** (1.00 g, 3.14 mmol), and DIPEA (1.56 mL, 8.96 mmol) in DMA (20 mL) was stirred at 60 °C for 18 h. The reaction mixture was cooled to rt and poured into a mixture of EtOAc (200 mL) and NH_4Cl (100 mL, saturated, aq solution). The organics were washed with water (3 × 100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated to afford the crude material. The crude was purified by flash chromatography (EtOAc/hexanes (3:7) with 0.5% AcOH). Yield: 1.17 g (83%) as a solid. ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, *J* = 7.0 Hz), 1.77−1.91 (4H, m), 2.37−2.44 (1H, m), 2.73 (3H, s), 3.10−3.17 (2H, m), 4.33 (2H, q, *J* = 7.0 Hz), 4.64−4.68 (4H, m), 7.36−7.41 (5H, m), 8.36 (1H, s). MS: *m/z* 471 (M + 1). HRMS: *m/z* calcd for $C_{23}H_{26}N_4O_5S$ (M + 1)$^+$, 471.1702, found 471.1683.

1-[3-Chloro-5-(ethoxycarbonyl)pyridin-2-yl]azetidine-3-carboxylic Acid (6). **4** (3.68 g, 16.5 mmol) and azetidine-3-carboxylic acid (2.50 g, 24.8 mmol) were suspended in DMA (50 mL). DIPEA (6.38 g, 8.60 mL, 49.5 mmol) was added, and the reaction mixture was heated at 120 °C for 18 h. The mixture was cooled to rt and concentrated. The crude material was dissolved in DCM (300 mL), and the solution was washed with 1 N HCl (150 mL), dried ($MgSO_4$), and concentrated. The crude was purified by flash chromatography (1:3 EtOAc/hexanes to 1:3 EtOAc/hexanes, 0.5% AcOH). Yield: 3.44 g (73%) as a solid. ^1H NMR (400 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.1 Hz), 3.54−3.61 (1H, m), 4.34 (2H, q, *J* = 7.1 Hz), 4.53−4.63 (4H, m), 8.00 (1H, s), 8.67 (1H, s). MS: *m/z* 283 (M − 1).

1-[3-Cyano-5-(ethoxycarbonyl)-6-methylpyridine-2-yl]-azetidine-3-carboxylic Acid (7). A suspension of **5** (51.00 g, 227 mmol), azetidine-3-carboxylic acid (24.09 g, 238 mmol), and DIPEA (88.22 g, 118.9 mL, 681 mmol) in EtOH (250 mL) was heated at reflux for 1 h. The reaction mixture was cooled to rt and added dropwise to a solution of $KHSO_4$ (154.5 g, 1135 mmol) in water (3000 mL). The formed solids were collected by filtration and dried under vacuum. Yield: 65.33 g (100%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.1 Hz), 2.72 (3H, s), 3.59−3.68 (1H, m), 4.31 (2H, q, *J* = 7.1 Hz), 4.55−4.68 (4H, m), 8.28 (1H, s). MS: *m/z* 290 (M + 1).

1-[3-Chloro-5-(ethoxycarbonyl)pyridin-2-yl]piperidine-4-carboxylic Acid (8). **4** (5.00 g, 22.7 mmol) and piperidine-4-carboxylic acid (4.40 g, 34.1 mmol) were suspended in DMA (50 mL). DIPEA (8.83 g, 11.9 mL, 68.2 mmol) was added, and the reaction mixture was heated at 120 °C for 2 h and then cooled to rt and concentrated. The crude was diluted with DCM (300 mL), washed with HCl (1 M, 150 mL), dried ($MgSO_4$), concentrated, and purified by flash chromatography (1:4 EtOAc/hexanes to 1:3 EtOAc/hexanes, 0.5% AcOH). Yield: 6.36 g (90%) as a solid. ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, *J* = 7.1 Hz), 1.88−1.97 (2H, m), 2.03−1.12 (2H, m), 2.57−2.66 (1H, m), 2.99−3.09 (2H, m), 4.02−4.11 (2H, m), 4.36 (2H, q, *J* = 7.1 Hz), 8.12 (1H, s), 8.74 (1H, s). MS: *m/z* 311 (M − 1).

1-[3-Cyano-5-(ethoxycarbonyl)-6-methylpyridin-2-yl]-piperidine-4-carboxylic Acid (9). A reaction mixture of **5** (3.00 g, 13.4 mmol), piperidine-4-carboxylic acid (1.90 g, 14.7 mmol), and TEA (2.70 g, 2.0 mL, 26.7 mmol) was refluxed for 10 min. The mixture was concentrated, water (50 mL) and EtOAc (50 mL) were added, and the water phase was acidified to pH 3 with HCl. The EtOAc phase was separated, and the water phase was extracted with additional EtOAc (40 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The crude material was purified by preparative HPLC. Yield: 1.90 g (45%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, *J* = 7.1 Hz), 1.82−1.94 (2H, m), 2.05−2.13 (2H, m), 2.66−2.73 (1H, m), 2.74 (3H, s), 3.26−3.38 (2H, m), 4.33 (2H, q, *J* = 7.1 Hz), 4.54−4.65 (2H, m), 8.36 (1H, s). MS: *m/z* 318 (M + 1).

Ethyl 5-Chloro-6-[3-({[(5-chloro-2-thienyl)sulfonyl]amino}-carbonyl)azetidin-1-yl]nicotinate (10). A mixture of **6** (0.150 g, 0.53 mmol), EDC (0.131 g, 0.68 mmol), and HOBT (0.093 g, 0.68 mmol) in DCM (5 mL) was stirred at rt for 30 min. 5-Chlorothiophene-2-sulfonamide (0.208 g, 1.05 mmol) and DIPEA (0.207 g, 0.280 mL, 1.58 mmol) were added, and the reaction mixture was stirred at rt for 18 h. The mixture was diluted with DCM (70 mL), washed with NH_4Cl (2 × 40 mL, saturated, aq solution) and brine (40 mL), dried ($MgSO_4$), and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes (3:7), 0.5% AcOH, to EtOAc/hexanes (7:3), 0.5% AcOH). Yield: 0.137 g (56%) as a solid. ^1H NMR (400 MHz, $CDCl_3$): δ 1.37 (3H, t, *J* = 7.1 Hz), 3.40−3.47 (1H, m), 4.34 (2H, q, *J* = 7.1 Hz), 4.46−4.53 (4H, m), 6.99 (1H, d, *J* = 4.6 Hz), 7.72 (1H, d, *J* = 4.6 Hz), 8.01 (1H, s), 8.66 (1H, s). MS: *m/z* 464 (M + 1). HRMS: *m/z* calcd for $C_{23}H_{26}N_4O_5S$ (M + 1)$^+$, 463.9908, found 463.9916.

Ethyl 6-[3-({[(5-Chloro-2-thienyl)sulfonyl]amino}carbonyl)-azetidin-1-yl]-5-cyano-2-methylnicotinate (11). **11** was prepared by the procedure of **10** from **7** (0.258 g, 0.89 mmol) and 5-chlorothiophene-2-sulfonamide (0.353 g, 1.78 mmol). The crude product was purified by flash chromatography (0−100% EtOAc in heptane followed by 0−40% MeOH in DCM). Yield: 0.061 g (15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, t, J = 7.0 Hz), 2.60 (3H, s), 3.55−3.68 (1H, m), 4.22 (2H, q, J = 7.0 Hz), 4.26−4.28 (2H, m), 4.37−4.46 (2H, m), 7.22 (1H, d, J = 4.2 Hz), 7.68 (1H, d, J = 4.2 Hz), 8.25 (1H, s). MS: m/z 469 (M + 1). HRMS: m/z calcd for $C_{18}H_{17}ClN_4O_5S_2$ (M + 1)$^+$, 469.0407, found 469.0401.

Ethyl 5-Cyano-2-methyl-6-(3-{[(phenylsulfonyl)amino]-carbonyl}azetidin-1-yl)nicotinate (12). **7** (0.072 g, 0.25 mmol) and benzenesulfonamide (0.060 g, 0.38 mmol) were dissolved in DMF (2 mL). DIPEA (0.162 g, 0.218 mL, 1.25 mmol) was added, followed by a solution of HATU (0.100 g, 0.26 mmol) in DMF (2 mL). The reaction mixture was stirred at rt for 16 h and then concentrated. The crude material was purified by preparative HPLC. Yield: 0.075 g (70%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.24 (3H, t, J = 7.2 Hz), 2.55 (3H, s), 3.47−3.57 (1H, m), 4.11−4.22 (2H, m), 4.18 (2H, q, J = 7.2 Hz), 4.30−4.40 (2H, m), 7.56−7.62 (2H, m), 7.64−7.69 (1H, m), 7.87−7.92 (2H, m), 8.23 (1H, s), 12.40 (1H, s). MS: m/z 429 (M + 1).

Ethyl 6-(3-[(Benzylsulfonyl)carbamoyl]azetidin-1-yl)-5-cyano-2-methylnicotinate (13). Phenylmethanesulfonamide (0.068 g, 0.38 mmol) was added to a solution of **7** (0.073 g, 0.25 mmol) and DIPEA (0.164 g, 0.222 mL, 1.27 mmol) in DMF (2 mL). A solution of HATU (0.079 g, 0.28 mmol) dissolved in DMF (1 mL) was added, and the reaction mixture was stirred at rt for 16 h. The mixture was concentrated, and the residue was purified by preparative HPLC. Yield: 0.060 g (54%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.23 (3H, t, J = 7.2 Hz), 2.57 (3H, s), 3.43 (1H, m), 4.17 (2H, q, J = 7.1 Hz), 4.23 (2H, t, J = 7.1 Hz), 4.34 (2H, t, J = 8.9 Hz), 4.68 (2H, s), 7.29 (5H, m), 8.33 (1H, s), 11.75 (1H, s). MS: m/z 443 (M + 1).

Ethyl 5-Cyano-2-methyl-6-(3-[(phenethylsulfonyl)-carbamoyl]azetidin-1-yl)nicotinate (14). **14** was prepared by the procedure of **13** from 2-phenylethanesulfonamide (0.078 g, 0.37 mmol) and **7** ((0.073 g, 0.25 mmol). Yield: 0.044 g (39%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.23 (3H, t, J = 7.2 Hz), 2.48 (2H, m, overlap with DMSO signal), 2.55 (3H, s), 2.95 (2H, t, J = 7.7 Hz), 3.45 (1H, m), 4.17 (4H, m), 4.34 (2H, m), 7.25−7.12 (5H, m), 8.23 (1H, s). MS: m/z 457 (M + 1).

Ethyl 5-Cyano-6-(3-{[(cyclohexylmethyl)sulfonyl]-carbamoyl}azetidin-1-yl)-2-methylnicotinate (15). **15** was prepared by the procedure of **13** from 1-cyclohexylmethanesulfonamide (0.065 g, 0.37 mmol) and **7** (0.073 g, 0.25 mmol). Yield: 0.012 g (11%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 0.98−1.25 (8H, m), 1.60−1.50 (3H, m), 1.74 (3H, m), 2.55 (3H, s), 3.26 (2H, d, J = 6.0 Hz), 3.58 (1H, m), 4.17 (2H, q, J = 7.1 Hz), 4.28 (2H, m), 4.41 (2H, m), 8.23 (1H, s). MS: m/z 449 (M + 1).

Ethyl 6-(3-{[(2-Methylbenzyl)sulfonyl]carbamoyl}azetidin-1-yl)-5-cyano-2-methylnicotinate (16). **16** was prepared by the procedure of **13** from 1-(2-methylphenyl)methanesulfonamide (0.010 g, 0.050 mmol) and **7** (0.073 g, 0.25 mmol). Yield: 0.002 g (2%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.24 (3H, t, J = 7.2 Hz), 2.32 (3H, s), 2.57 (3H, s), 3.40 (1H, m; overlapped by signal from water), 4.18 (2H, m), 4.30 (2H, m), 4.39 (2H, m), 4.63 (2H, s), 7.15 (4H, m), 8.24 (1H, s). MS: m/z 457 (M + 1).

Ethyl 5-Cyano-2-methyl-6-(3-{[[3-(trifluoromethyl)benzyl]-sulfonyl]carbamoyl}azetidin-1-yl)nicotinate (17). **17** was prepared by the procedure of **13** from (3-(trifluoromethyl)phenyl)-methanesulfonamide (0.10 g, 0.38 mmol) and **7** (0.073 g, 0.25 mmol). Yield: 0.050 g (39%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.24 (3H, t, J = 7.1 Hz), 2.57 (3H, s), 3.48 (1H, m), 4.18 (4H, m), 4.35 (2H, t, J = 8.8 Hz), 4.78 (2H, s), 7.57 (3H, m), 7.69 (1H, d, J = 6.6 Hz), 8.24 (1H, s). MS: m/z 511 (M + 1).

Ethyl 5-Cyano-2-methyl-6-(3-{[(3-methylbenzyl)sulfonyl]-carbamoyl}azetidin-1-yl)nicotinate (18). **18** was prepared by the procedure of **13** from (3-methylphenyl)methanesulfonamide (0.037 g, 0.20 mmol) and **7** (0.073 g, 0.25 mmol). Yield: 0.019 g (15%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.32 (3H, t, J = 7.1 Hz), 2.33 (3H, s), 2.65 (3H, s), 3.53−3.62 (1H, m), 4.21−4.34 (4H, m), 4.39−4.47 (2H, m), 4.72 (2H, s), 7.13−7.32 (4H, m), 8.33 (1H, s), 11.81 (1H, br s). MS: m/z 457 (M + 1).

Ethyl 6-(3-{[(4-Chlorobenzyl)sulfonyl]carbamoyl}azetidin-1-yl)-5-cyano-2-methylnicotinate (19). **19** was prepared by the procedure of **13** from (4-chlorophenyl)methanesulfonamide (0.105 g, 0.38 mmol) and **7** (0.073 g, 0.25 mmol). Yield: 0.057 g (48%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.24 (3H, t, J = 7.1 Hz), 2.58 (3H, s), 3.45 (1H, m), 4.18 (2H, q, J = 7.1 Hz), 4.23 (2H, m), 4.36 (2H, m), 4.65 (2H, s), 7.29 (2H, d, J = 8.5 Hz), 7.37 (2H, d, J = 8.5 Hz), 8.25 (1H, s). MS: m/z 477 (M + 1).

Ethyl 6-(3-{[(4-Fluorobenzyl)sulfonyl]carbamoyl}azetidin-1-yl)-5-cyano-2-methylnicotinate (20). **20** was prepared by the procedure of **13** from (4-fluorophenyl)methanesulfonamide (0.088 g, 0.38 mmol) and **7** (0.073 g, 0.25 mmol). Yield: 0.050 g (43%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.24 (3H, t, J = 7.1 Hz), 2.57 (3H, s), 3.49 (1H, m), 4.20 (4H, m), 4.36 (2H, m), 4.65 (2H, s), 7.13 (2H, t, J = 8.8 Hz), 7.32 (2H, m), 8.25 (1H, s). MS: m/z 461 (M + 1).

Ethyl 6-(3-{[(4-Methylbenzyl)sulfonyl]carbamoyl}azetidin-1-yl)-5-cyano-2-methylnicotinate (21). Thionyl chloride (0.119 g, 1.0 mmol) was added to a solution of **7** (0.058 g, 0.20 mmol) in DCM (1.0 mL) at 0 °C, and the reaction mixture was stirred at rt for 30 min. The mixture was concentrated and coconcentrated with DCM. The residue was dissolved in pyridine (1 mL) at 0 °C, and 1-(4-methylphenyl)methanesulfonamide (0.044 g, 0.24 mmol) was added. After the mixture was stirred at rt for 2 h, 4-(dimethylamino)pyridine (DMAP) (a few crystals) was added, and stirring at rt was continued for 19 h. 2-(*tert*-Butylimino)-2-(diethylamino)-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) (0.055 g, 0.20 mmol) was added, and stirring at rt was continued for 22 h. LC/MS showed no conversion. HATU (0.152 g, 0.40 mmol) and DIPEA (0.259 g, 2.0 mmol) were added, and the mixture was stirred at rt for 20 h. The mixture was concentrated, and the crude was purified by preparative HPLC. Yield: 0.019 g (15%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.32 (3H, t, J = 7.0 Hz), 2.32 (3H, s), 2.66 (3H, s), 3.51−3.60 (1H, m), 4.20−4.37 (4H, m), 4.38−4.47 (2H, m), 4.70 (2H, s), 7.17−7.28 (4H, m), 8.34 (1H, s), 11.77 (1H, br s). MS: m/z 457 (M + 1).

Ethyl 5-Cyano-6-(3-{[[4-(hydroxymethyl)benzyl]sulfonyl]-carbamoyl}azetidin-1-yl)-2-methylnicotinate (22). 4-Chloromethyl benzyl alcohol (1.35 g, 8.60 mmol) and imidazole (0.763 g, 11.2 mmol) were dissolved in DCM (8.6 mL), and *tert*-butyldimethylsilyl chloride (1.43 g, 9.50 mmol) was added in portions at 0 °C. The reaction mixture was stirred at rt for 1 h and then quenched with $KHSO_4$ (0.5 M aq solution, 10 mL). The organic phase was separated and concentrated to give a crude that was used in the next step without further purification assuming a 100% conversion.

Sodium 3-methoxy-3-oxopropane-1-sulfinate[27] (1.76 g, 10.1 mmol) was dissolved in DMSO (20 mL). A solution of *tert*-butyl{[4-(chloromethyl)benzyl]oxy}dimethylsilane (2.40 g, 8.4 mmol) in DMSO (5 mL) was added, and the reaction mixture was stirred at rt for 16 h. Water (30 mL) was added, and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give a product that was used in the next step without further purification. Yield: 3.10 g (95%). MS: m/z 404 (ammonia adduct).

Methyl 3-{[4-[[(*tert*-butyldimethylsilyl)oxy]methyl]benzyl]-sulfonyl}propanoate (3.10 g, 8.00 mmol) was dissolved in THF (20 mL), and sodium methoxide (3.2 M in methanol) was added until all starting material had been consumed according to LC/MS. A solution of hydroxylamine *O*-sulfonic acid (2.27 g, 20.0 mmol) and sodium acetate (2.50 g, 30.0 mmol) in water (30 mL) was added, and the reaction mixture was stirred at rt for 16 h. The mixture was extracted with EtOAc (2 × 30 mL), dried (Na_2SO_4), and concentrated. Yield: 2.50 g (99%). MS: m/z 314 (M + 1).

A mixture of **7** (0.100 g, 0.34 mmol), {4-[[(*tert*-butyldimethylsilyl)-oxy]methyl]phenyl}methanesulfonamide (0.130 g, 0.41 mmol), PyBrop (0.241 g, 0.52 mmol), and DIPEA (0.445 g, 0.60 mL, 3.45 mmol) in DCM (4.5 mL) was stirred at rt for 1 h. Water (4.5 mL) was added, and the organic phase was separated and concentrated. Yield: 0.119 g (57%).

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The crude was dissolved in TFA, and the reaction mixture was stirred at rt for 15 min. Yield: 0.018 g (19%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.30 (3H, t, J = 7.1 Hz), 2.63 (3H, s), 3.34−3.45 (1H, m), 4.24 (2H, q, J = 7.1 Hz), 4.27−4.34 (2H, m), 4.34−4.43 (2H, m), 4.47 (2H, d, J = 5.4 Hz), 4.48−4.56 (2H, m), 5.16 (1H, t, J = 5.6 Hz), 7.24 (4H, s), 8.29 (1H, s), 11.69−11.91 (1H, m). MS: m/z 473 (M + 1).

Ethyl 5-Chloro-6-[4-({[(5-chloro-2-thienyl)sulfonyl]amino}carbonyl)piperidin-1-yl]nicotinate (23). 23 was prepared by the procedure of **10** from **8** (0.250 g, 0.80 mmol) and 5-chlorothiophene-2-sulfonamide (0.316 g, 1.60 mmol). Yield: 0.095 g (24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, t, J = 7.1 Hz), 1.83−1.98 (4H, m), 2.42−2.50 (1H, m), 2.92−2.98 (2H, m), 4.09−4.13 (2H, m), 4.36 (2H, q, J = 7.1 Hz), 6.97 (1H, d, J = 4.1 Hz), 7.71 (1H, d, J = 4.1 Hz), 8.12 (1H, d, J = 1.7 Hz), 8.73 (1H, d, J = 1.7 Hz). MS: m/z 492 (M + 1).

Ethyl 5-Cyano-2-methyl-6-[4-[(phenylsulfonyl)carbamoyl]-piperidin-1-yl]nicotinate (24). A mixture of **9** (0.067 g, 0.21 mmol), TBTU (0.080 g, 0.25 mmol), and DIPEA (0.136 g, 183 mL, 1.05 mmol) in DCM (2 mL) was stirred at rt for 10 min. Benzenesulfonamide (0.039 g, 0.25 mmol) was added, and the reaction mixture was stirred at rt for 16 h and then washed with $KHSO_4$ (2 mL, 0.1 M aq solution), separated, and concentrated. The crude was purified by preparative HPLC. Yield: 0.047 g (49%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.29 (3H, t, J = 7.1 Hz), 1.38−1.54 (2H, m), 1.78−1.87 (2H, m), 2.47−2.54 (1H, m), 2.61 (3H, s), 3.07−3.19 (2H, m), 4.24 (2H, q, J = 7.1 Hz), 4.40−4.52 (2H, m), 7.54−7.76 (3H, m), 7.90 (2H, d, J = 7.5 Hz), 8.31 (1H, s), 12.13−12.25 (1H, m). MS: m/z 457 (M + 1).

Ethyl 6-[4-({[(4-Fluorobenzyl)sulfonyl]amino}carbonyl)-piperidin-1-yl]-5-cyano-2-methylnicotinate (25). A mixture of **9** (0.350 g, 1.10 mmol), EDC (0.274 g, 1.43 mmol), HOBt (0.194 g, 1.43 mmol), and DIPEA (0.713 g, 5.51 mmol) in DCM (8 mL) was stirred at rt for 30 min, and 1-(4-fluorophenyl)methanesulfonamide (0.271 g, 1.43 mmol) was added. Stirring at rt was continued for 16 h. $KHSO_4$ (0.5 M aq solution, 2 mL) was added, and the aq phase was extracted with DCM. Combined organic phases were concentrated to give a crude which was purified by preparative HPLC. Yield: 0.429 g (80%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.30 (3H, t, J = 7.2 Hz), 1.56−1.69 (2H, m), 1.80−1.88 (2H, m), 2.57 (1H, m), 2.64 (3H, s), 3.13 (2H, m), 4.24 (2H, q, J = 7.2 Hz), 4.53 (2H, m), 4.68 (2H, s), 7.20−7.27 (2H, m), 7.30−7.35 (2H, m), 8.33 (1H, s), 11.60 (1H, br s).

Ethyl 5-Cyano-6-[4-({[(4-methoxybenzyl)sulfonyl]amino}carbonyl)piperidin-1-yl]-2-methylnicotinate (26). 26 was prepared by the procedure of **25** from **9** (0.040 g, 0.13 mmol) and 1-(4-methoxyphenyl)methanesulfonamide (0.032 g, 0.16 mmol). Yield: 0.034 g (45%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.32 (3H, d, J = 7.1 Hz), 1.55−1.68 (2H, m), 1.77−1.86 (2H, m), 2.27−2.36 (1H, m), 2.66 (3H, s), 3.16−3.25 (2H, m), 3.74 (3H, s), 4.22−4.30 (3H, m), 4.42−4.51 (2H, m), 6.85 (2H, br d, J = 8.5 Hz), 7.16 (2H, br d, J = 8.5 Hz), 8.33 (1H, s). MS: m/z 523 (M + 1).

Ethyl 6-[4-({[(4-Methylbenzyl)sulfonyl]amino}carbonyl)-piperidin-1-yl]-5-cyano-2-methylnicotinate (27). 27 was prepared by the procedure of **25** from **9** (1.00 g, 3.15 mmol) and 1-(4-methylphenyl)methanesulfonamide (0.67 g, 3.62 mmol). Yield: 0.69 g (45%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.30 (3H, t, J = 7.1 Hz), 1.56−1.68 (2H, m), 1.79−1.87 (2H, m), 2.29 (3H, s), 2.41−2.60 (1H, m), 2.64 (3H, s), 3.09−3.18 (2H, m), 3.29 (1H, s), 4.24 (2H, q, J = 7.1 Hz), 4.48−4.56 (2H, m), 4.59 (2H, s), 7.13−7.21 (4H, m), 8.33 (1H, s). MS: m/z 485 (M + 1).

Ethyl 6-[4-({[(4-Isopropylbenzyl)sulfonyl]amino}carbonyl)-piperidin-1-yl]-5-cyano-2-methylnicotinate (28). 28 was prepared by the procedure of **25** from **9** (0.159 g, 0.50 mmol) and 1-(4-isopropylphenyl)methanesulfonamide (0.128 g, 0.60 mmol). Yield: 0.144 g (56%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.18 (6H, d, J = 6.8 Hz), 1.32 (3H, t, J = 7.1 Hz), 1.54−1.67 (2H, m), 1.75−1.85 (2H, m), 2.20−2.30 (1H, m), 2.65 (3H, s), 2.80−2.89 (1H, m), 3.18−3.28 (2H, m), 4.19 (2H, s), 4.26 (2H, q, J = 7.1 Hz), 4.38−4.47 (2H, m), 7.09−7.18 (4H, m), 8.32 (1H, s). MS: m/z 513 (M + 1).

Ethyl 6-[4-({[(2,4-Difluorobenzyl)sulfonyl]amino}carbonyl)-piperidin-1-yl]-5-cyano-2-methylnicotinate (29). 29 was prepared by the procedure of **25** from **9** (0.032 g, 0.10 mmol) and 1-(2,4-difluorphenyl)methanesulfonamide (0.023 g, 0.11 mmol). Yield: 0.018 g (34%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.31 (3H, t, J = 7.1 Hz), 1.59−1.69 (2H, m), 1.84−1.92 (2H, m), 2.65 (4H, m), 3.10−3.25 (2H, m), 4.25 (2H, q, J = 7.1 Hz), 4.49−4.60 (2H, m), 4.73 (2H, s), 7.13−7.23 (1H, m), 7.29−7.39 (1H, m), 7.40−7.50 (1H, m), 8.34 (1H, s), 11.75 (1H, s). MS: m/z 507 (M + 1).

Ethyl 6-[4-({[(5-Chloro-2-thienyl)sulfonyl]amino}carbonyl)-piperidin-1-yl]-5-cyano-2-methylnicotinate (32). A mixture of **30** (7.00 g, 30.5 mmol), EDC (7.02 g, 36.6 mmol), and HOBT (4.95 g, 36.6 mmol) in DCM (200 mL) was stirred at rt for 30 min. 5-Chlorothiophene-2-sulfonamide (7.54 g, 38.2 mmol) and DIPEA (16.0 mL, 91.6 mmol) were added, and the reaction mixture was stirred at rt for 20 h. The mixture was diluted with DCM (500 mL), washed with NH_4Cl (3 × 200 mL, saturated, aq solution), dried ($MgSO_4$), and concentrated. The crude was purified by flash chromatography (EtOAc/hexanes (1:3), 1% AcOH). Yield: 11.3 g (90%) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (9H, s), 1.55−1.65 (2H, m), 1.79−1.82 (2H, m), 2.92−2.37 (1H, m), 2.73−2.80 (2H, m), 4.06−4.11 (2H, m), 6.96 (1H, d, J = 4.1 Hz), 7.69 (1H, d, J = 4.1 Hz), 8.11 (1H, br s).

A suspension of *tert*-butyl 4-{[(5-chlorothiophene-2-yl)sulfonyl]-carbamoyl}piperidine-1-carboxylate (11.3 g, 27.6 mmol) in THF (500 mL) was treated with HCl (4 M in 1,4-dioxane, 138 mL, 552 mmol), and the reaction mixture was stirred at rt for 20 h and then concentrated. Yield: 9.52 g (100%) as a solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.58−1.68 (2H, m), 1.87−1.90 (2H, m), 2.52−2.59 (1H, m), 2.80−2.88 (2H, m), 3.22−3.25 (2H, m), 7.29 (1H, d, J = 4.1 Hz), 7.67 (1H, d, J = 4.1 Hz), 8.51 (1H, br s), 8.82 (1H, br s). MS: m/z 309 (M + 1).

Compound **5** (0.20 g, 0.89 mmol), *N*-(5-chlorothiophene-2-ylsulfonyl)piperidine-4-carboxamide, hydrochloride (0.40 g, 1.3 mmol), and DIPEA (0.46 g, 0.62 mL, 3.6 mmol) were dissolved in DMA (2 mL). The reaction mixture was heated at 160 °C for 30 min. The mixture was cooled, diluted with EtOAc (75 mL), washed with NH_4Cl (2 × 40 mL, saturated, aq solution) and brine (40 mL), dried ($MgSO_4$), and concentrated. Yield: 0.198 g (45%) as a white solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.30 (3H, t, J = 7.1 Hz), 1.50−1.59 (4H, m), 1.80 (2H, d, J = 11.0 Hz), 2.42−2.56 (1H, m), 2.63 (3H, s), 3.15 (2H, d, J = 11.9 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.49 (2H, d, J = 13.5 Hz), 7.28 (1H, d, J = 4.1 Hz), 7.67 (1H, d, J = 4.1 Hz), 8.32 (1H, s). MS: m/z 497 (M + 1).

■ ASSOCIATED CONTENT

Supporting Information

Description of or references for the screening assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

aze, azetidinyl; br, broad; BEMP, 2-(*tert*-butylimino)-2-(diethylamino)-1,3-dimethylperhydro-1,3,2-diazaphosphorine; CDI, *N*,*N*′-carbonyldiimidazole; CFR , cyclic flow reduction; CHO, chinese hamster ovarian; CLint, intrinsic clearance; DIPEA, *N*,*N*-diisopropylethylamine; DLMs, dog liver microsomes; DMAP, 4-(dimethylamino)pyridine; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; GTPγS, guanosine 5′-*O*-[γ-thio]triphosphate; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluromium hexafluorophosphate; HLMs, human liver microsomes; HOBT, 1-hydroxybenzotriazole; log *D*, logarithm of the distribution coefficient; MW, single-node heating in a microwave oven; pip, piperidinyl; PyBroP, bromotrispyrrolidinophosphonium hexafluorophosphate; pyrr, pyrrolidinyl; RLMs, rat liver microsomes; RPC, residual platelet count; TBME, *tert*-butyl methyl ether; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TEA, triethylamine; WPA, washed platelet assay

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