Journal of Medicinal Chemistry

ECU Libraries

Subscriber access provided by ECU Libraries

Article

Optimization of P2Y12 Antagonist Ethyl 6-(4-((Benzylsulfonyl)carbamoyl)piperidin-1-yl)-5-cyano-2-methylnicotinate (AZD1283) Led to the Discovery of an Oral Antiplatelet Agent with Improved Drug-Like Properties

Deyu Kong, Tao Xue, bin guo, Jianjun Cheng, Shunying Liu, Jianhai Wei, Zhengyu Lu, Haoran Liu, Guoqing Gong, Tian Lan, Wenhao Hu, and Yushe Yang

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b01971 • Publication Date (Web): 07 Mar 2019

Downloaded from http://pubs.acs.org on March 8, 2019

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Optimization of $P2Y_{12}$ Antagonist Ethyl 6-(4-((Benzylsulfonyl)carbamoyl)piperidin-1-yl)-5-cyano-2-methylnicotinate (AZD1283) Led to the Discovery of an Oral Antiplatelet Agent with Improved Drug-Like Properties

Deyu Kong,[†,‡] Tao Xue,[‡] Bin Guo,[‡] Jianjun Cheng,[▽] Shunyin Liu,[†] Jianhai Wei,[†] Zhengyu Lu,[‡] Haoran Liu,[‡] Guoqing Gong,[∥] Tian Lan,[∥] Wenhao Hu,*[,†,§] and Yushe Yang*[,‡]

[†]Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai, 200062, PR China.

[‡]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China.

[▽]iHuman Institute, ShanghaiTech University, Shanghai 201210, China

[§]School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, China.

[ll]Department of Pharmacology, School of Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu Province 210009, China.

ABSTRACT: $P2Y_{12}$ antagonists are widely used as antiplatelet agents for the prevention and treatment of arterial thrombosis. Based on the scaffold of a known $P2Y_{12}$ antagonist AZD1283, a series of novel bicyclic pyridine derivatives were designed and synthesized. The cyclization of the ester substituent on the pyridine ring to the ortho methyl group led to lactone analogs of AZD1283 that showed significantly enhanced

metabolic stability in subsequent structure-pharmacokinetic relationship studies. The metabolic stability was further enhanced by adding a 4-methyl substituent to the piperidinyl moiety. Compound **58l** displayed potent inhibition of platelet aggregation in vitro as well as antithrombotic efficacy in a rat ferric chloride model. Moreover, **58l** showed a safety profile that was superior to what was observed for clopidogrel in a rat tail-bleeding model. These results support the further evaluation of compound **58l** as a promising drug candidate.

INTRODUCTION

Thromboembolic disorders, such as acute myocardial infarction and stroke, are still the most common causes of mortality and morbidity in the developed world.[1] Platelet activation plays a critical role in thrombotic complications.[2, 3] The activation process involves the production of several platelet activation agonists, including thrombin, thromboxane A_2 and adenosine diphosphate (ADP).[2] ADP is a key factor in platelet activation and aggregation,[4] and its mechanism involves the binding and activation of two G-protein coupled receptors (GPCRs), $P2Y_1$ and $P2Y_{12}$.[5] $P2Y_1$ is ubiquitously expressed and plays a role in initial activation, while the $P2Y_{12}$ receptor is primarily expressed in platelets and plays a central role in the amplification of platelet activation, aggregation and stable thrombus formation. The $P2Y_{12}$ receptor has been validated as an important drug target for the development of antithrombotic drugs.[6]

The therapeutic success of $P2Y_{12}$ receptor irreversible antagonist clopidogrel (**1**, **Figure 1**) in preventing atherothrombotic events in patients that have experienced acute coronary syndrome (ACS),[7] confirmed that $P2Y_{12}$ antagonism is a powerful therapeutic

strategy in the management and prevention of arterial thrombosis.[8, 9] However, clopidogrel is a thienopyridine prodrug that requires metabolic conversion by the cytochrome P450 system[10] to generate the active metabolite that irreversibly binds to the $P2Y_{12}$ receptor,[11] resulting in a slow onset and offset of its pharmacological action. Therefore, clopidogrel displayed obvious drawbacks, including delayed onset of action,[12] significant response variability,[13] and insufficient antiplatelet activity in some patients.[14] These drawbacks led to the development of more potent and reliable reversible $P2Y_{12}$ receptor antagonists, such as ticagrelor (**2**)[15] and cangrelor[16] (**3**). However, both of these drugs have their own contraindications and numerous issues that must be considered in clinical practice. For example, several adverse clinical effects, such as dyspnea, ventricular pauses and hyperuricemia were observed with ticagrelor, which could likely be attributed to the inhibition of equilibrative nucleoside transporter 1 by ATP analogs.[17] And cangrelor must be administered by intravenous injection.[15]

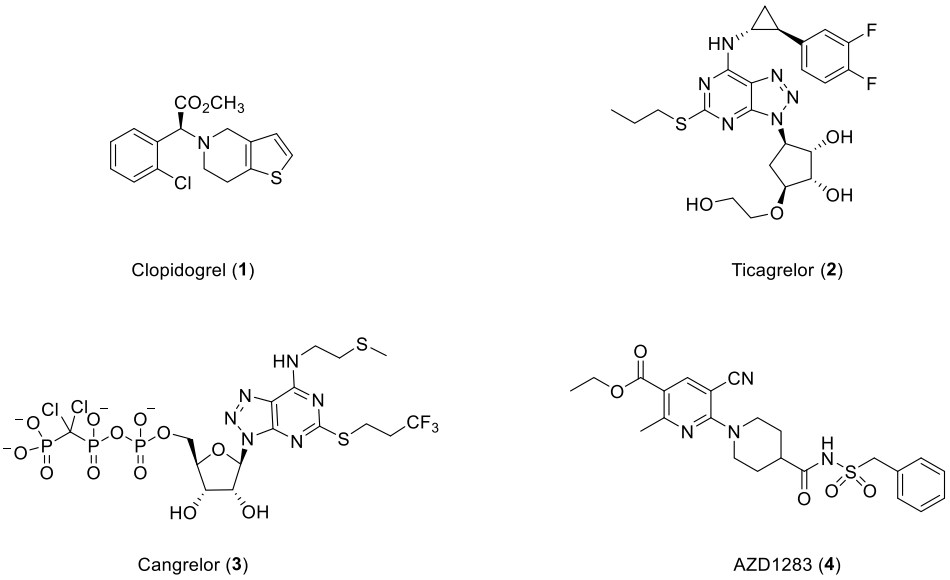


Figure 1. Structures of clopidogrel (**1**), ticagrelor (**2**), cangrelor (**3**) and AZD1283 (**4**).

Existing evidence suggests that reversible binding could not only lead to a rapid

recovery of platelet function but also increase the separation between the antithrombotic effect and bleeding risk.[18, 19] This hypothesis has inspired extensive efforts toward the discovery of novel, reversible and orally available $P2Y_{12}$ receptor antagonists with improved safety profiles.[20, 21] Among these antagonists, AZD1283[22] (**4**, **Figure 1**), an oral, reversible $P2Y_{12}$ receptor antagonist reported by AstraZeneca, showed potent antithrombotic efficacy and reduced bleeding effects in animal models and was advanced into human clinical trials. However, the development of this compound was terminated prior to phase II studies due to poor absorption and low metabolic stability of the ester.[23] Moreover, AZD1283 inhibited three CYP450-metabolizing enzymes, CYP2C9, CYP3A4 and CYP2C19.[22]

The X-ray crystal structure of AZD1283 bound to the human $P2Y_{12}$ receptor showed that a hydrogen bond between the ester carbonyl and Asn159 is crucial to its high affinity.[24, 25] Knowing that the ester group is susceptible to metabolic degradation, we envisioned that cyclization of this ester group to the ortho methyl would generate novel analogs of AZD1283 that retain its $P2Y_{12}$ activity while having much improved metabolic stability (**Figure 2**). Herein, we describe the structure-activity and structure-pharmacokinetic relationships of these bicyclic pyridine compounds and the identification of compound **58l** as a potential drug candidate for the development of novel antithrombotic agents.

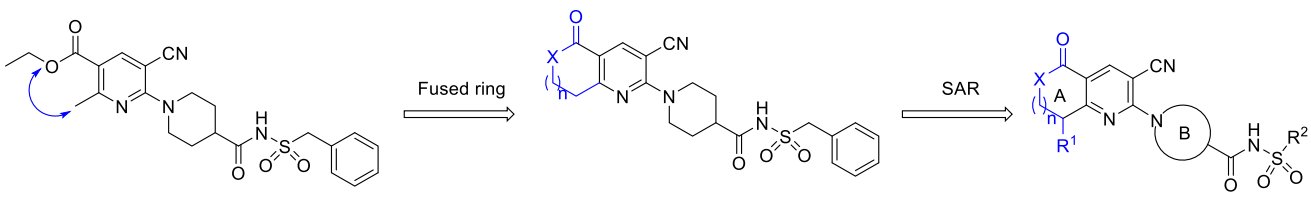


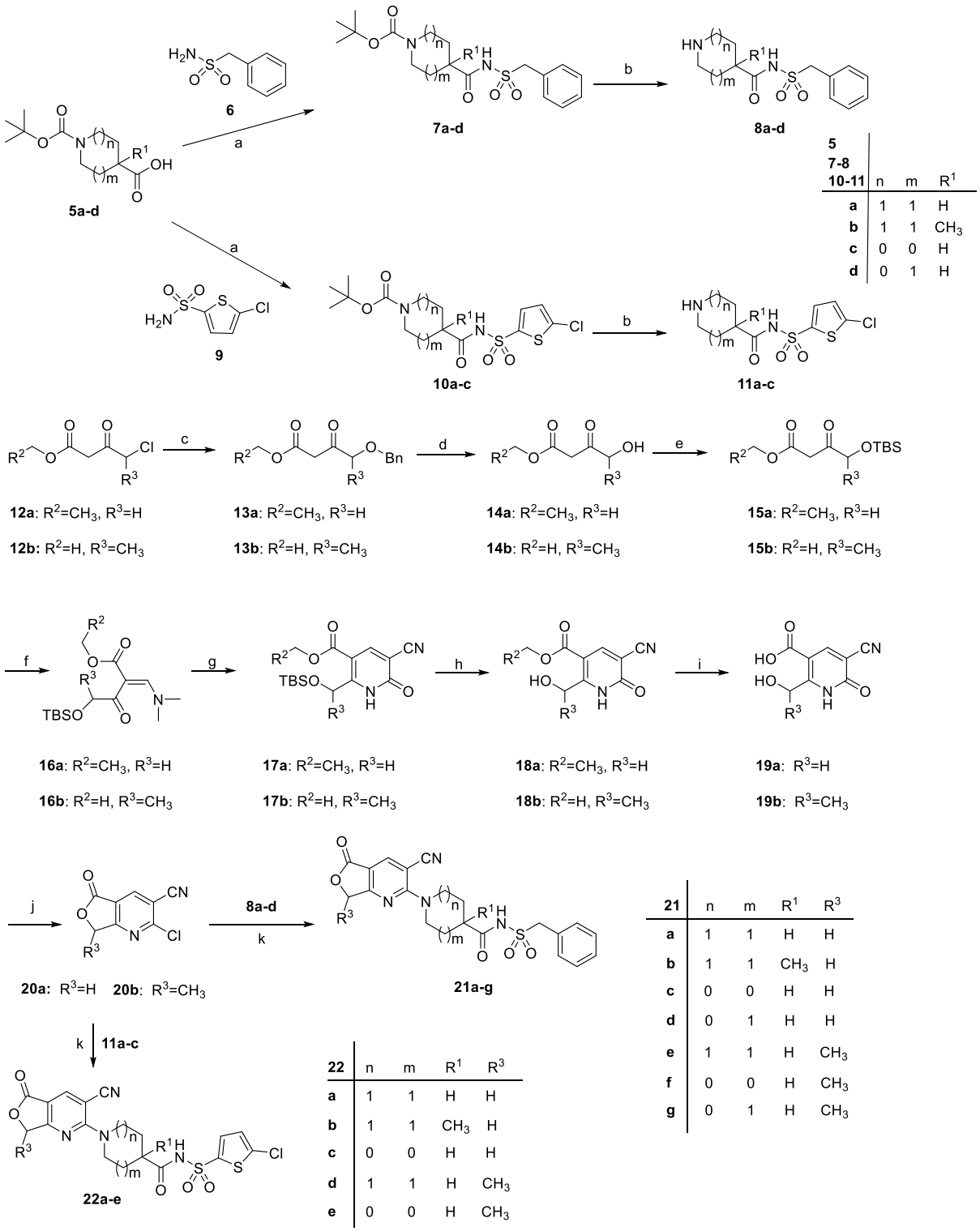
Figure 2. Design of novel, fused bicyclic pyridine antiplatelet agents.

RESULTS AND DISCUSSION

CHEMISTRY

The syntheses of five-membered lactone compounds **21a**–**g** and **22a**–**e** are shown in **Scheme 1**. For the synthesis of the right-hand amine moieties, carboxylic acids **5a**–**d** were used as starting materials, and they were activated with TBTU as a coupling reagent and then treated with sulfonamides **6** and **9** to produce acyl sulfonamides **7a**–**d**[22] and **10a**–**c**; Subsequent Boc deprotection provided amines **8a**–**d** and **11a**–**c**. To construct the left-hand fused pyridines, substituted acetoacetates **12a** and **12b** were used as starting materials, and they were treated with benzyl alcohol and then debenzylated to introduce the hydroxyl group (**14a**–**b**). The hydroxyl groups were subsequently protected as silyl ethers to afford **15a**–**b**, which were then subjected to an Aldol condensation to give intermediates **16a**-**b**. Cyclization of **16a**-**b** with cyanoacetamide gave pyridones **17a**–**b**. Cleavage of the silyl ether protecting groups and hydrolysis of the esters gave intermediates **19a**–**b**, which were refluxed with $POCl_3$ to give lactone-fused pyridines **20a**–**b**. Compounds **20a**–**b** were coupled with amines **8a**–**d** and **11a**–**c** to afford target compounds **21a**–**g** and **22a**–**e**.

Scheme 1. Synthesis of Compounds 21a–g and 22a–e[a]



5 7-8 10-11	n	m	R^1
a	1	1	H
b	1	1	CH_3
c	0	0	H
d	0	1	H

21	n	m	R^1	R^3
a	1	1	H	H
b	1	1	CH_3	H
c	0	0	H	H
d	0	1	H	H
e	1	1	H	CH_3
f	0	0	H	CH_3
g	0	1	H	CH_3

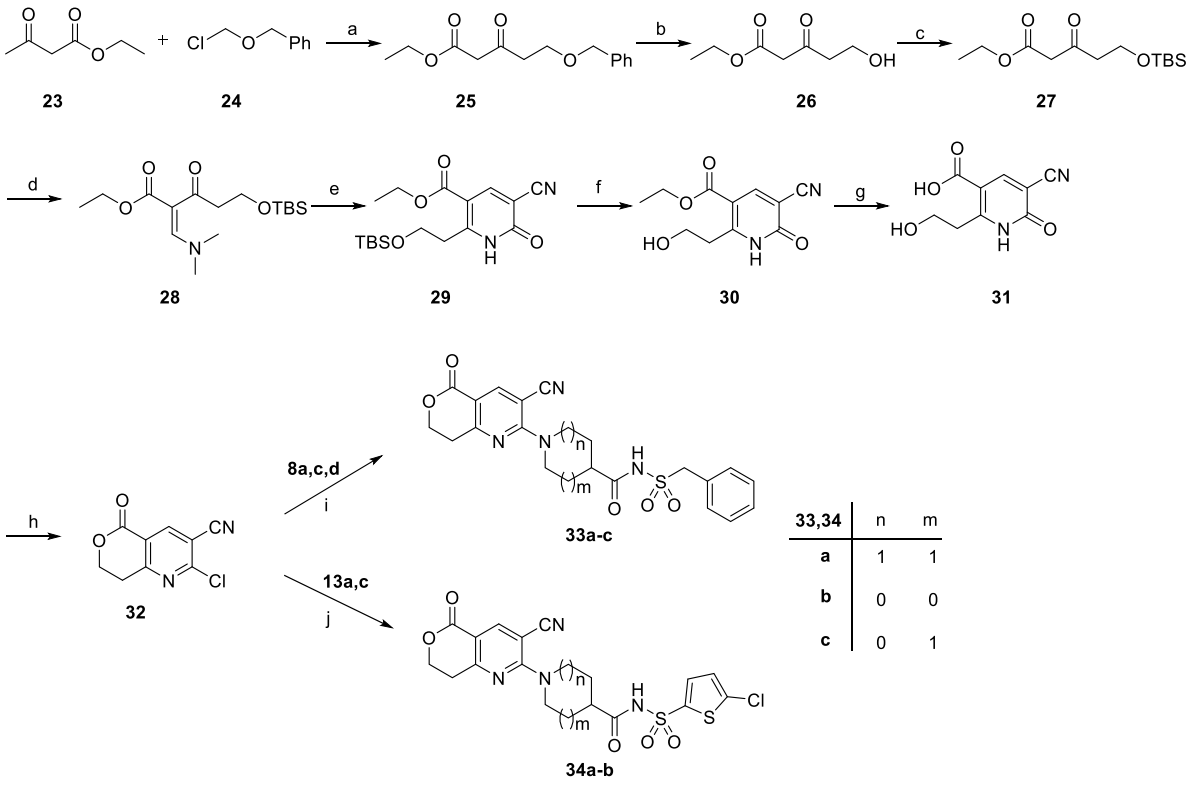
22	n	m	R^1	R^3
a	1	1	H	H
b	1	1	CH_3	H
c	0	0	H	H
d	1	1	H	CH_3
e	0	0	H	CH_3

[a]Reagents and conditions: (a) TBTU, triethylamine (TEA), THF, LiCl, rt, 18 h, 26–90%; (b) HCOOH, rt, 20 h, 80–96%; (c) BnOH, NaH, THF, 0 °C to rt, 91–92%; (d) H_2, Pd/C, EtOH, rt, 86–98%; (e) TBSCl, imidazole, DMAP, THF, rt, 49–70%; (f) DMF/DMA, 80 °C, 72–83%; (g) 2-Cyanoacetamide, NaOEt, EtOH, rt, 81–81%; (h) TBAF, THF, rt,

85–91%; (i) NaOH, MeOH, rt, 86–96%; (j) $POCl_3$, 100 °C, 56–58%;(k) TEA, EtOH, reflux, overnight, 69–85% for **21a**–**g**, 57–82% for **22a**–**e**

Six-membered lactone compounds **33a**–**c** and **34a**–**b** were prepared using a similar method, as shown in **Scheme 2**. Ethyl 5-hydroxy-3-oxopentanoate (**26**) was prepared and used in place of **14a** and **14b** in **Scheme 1** for the construction of pyridine-fused six-membered lactone **32**. Intermediate **32** were treated with amines **8a**, **8c**, **8d**, **13a** and **13c** to provide compounds **33a**–**c** and **34a**–**b** in good yields.

Scheme 2. Synthesis of Compounds 33a–c and 34a–b[a]

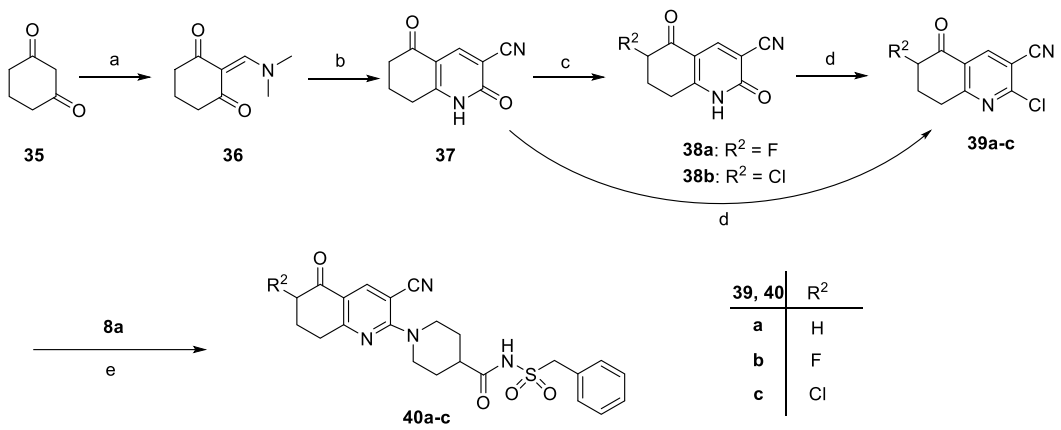


[a]Reagents and conditions: (a) NaH, *n*-BuLi, THF, rt, 43%; (b) H_2, Pd/C, EtOH, rt, 98%; (c) TBSCl, imidazole, DMAP, THF, rt, 81%; (d) DMF/DMA, 80 °C, 96%; (e) 2-cyanoacetamide, NaOEt, EtOH, rt, 81%; (f) TBAF, THF, rt, 98%; (g) NaOH, MeOH, rt, 94%; (h) $POCl_3$, 100 °C, 35%; (i) TEA, EtOH, reflux, overnight, 73–78% for **33a**–

c, 79–82% for **34a**–**b**

The synthesis of cyclic ketone compounds **40a**–**c** is depicted in **Scheme 3**. Commercially available dimedone **35** was first converted to enaminone **36** and then to pyridone **37** via a two-step Hantzsch-type reaction.[26] The α position of ketone **37** was substituted with a F to give **38a** and a Cl to give **38b**. These pyridones (**37**, **38a** and **38b**) were converted to the corresponding 2-chloropyridines (**39a**–**c**) in good yields under Vilsmeier conditions using $SOCl_2$/DMF. The coupling of **39a**–**c** with amine **8a** provided compounds **40a**–**c**.

Scheme 3. Synthesis of Compounds 40a–c[a]

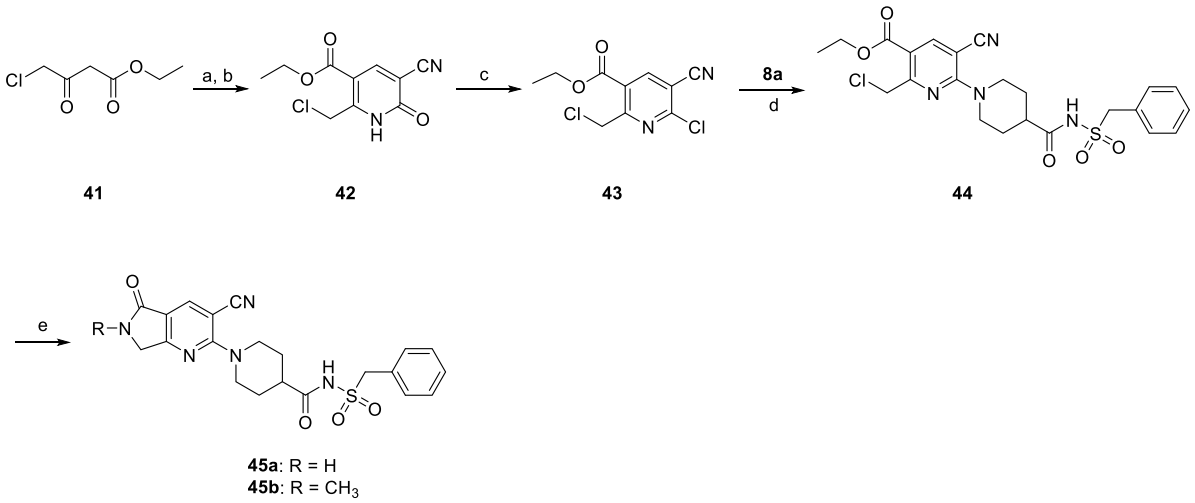


[a]Reagents and reaction conditions: (a) DMF/DMA, reflux, 99%; (b) malononitrile, NH_4OAc, CH_3CN, reflux, 53%; (c) Selectfluor, H_2SO_4, MeOH, 70% for **38b**, NCS, MeCN, PTSA, Ar, 80 °C, 65% for **38c**; (d) $SOCl_2$, DMF, DCM, 51–62%; (e) DIPEA, EtOH, reflux, 18 h, 61–78%

The preparations of compounds **45a**–**b** are outlined in **Scheme 4.** Ethyl 4-chloro-3-oxobutanoate (**41**) was used as the starting material, and it was converted to pyridine **42** via a Hantzsch-type reaction. Subsequent chlorination of **42** gave **43**, which was

treated with amine **8a** to afford intermediate **44**. **44** was reacted with ammonia and methylamine to afford compounds **45a** and **45b**, respectively.

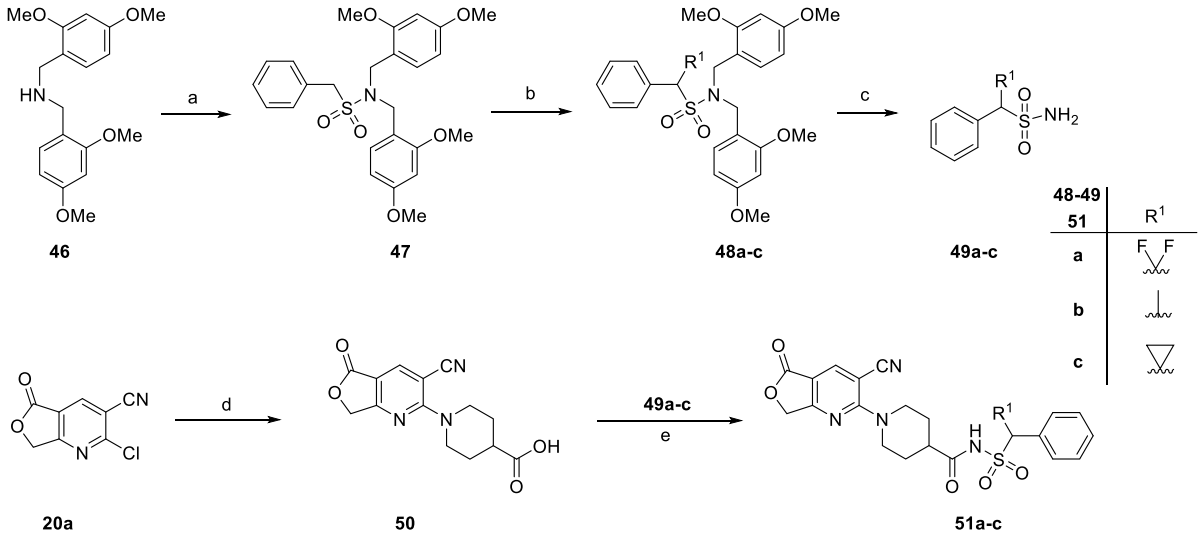
Scheme 4. Synthesis of Compounds 45a–b[a]



[a]Reagents and reaction conditions: (a) Ac_2O, triethoxymethane, 120 °C, 62%; (b) 2-cyanoacetamide, NaOEt, EtOH, rt, 12 h, 62% for two steps; (c) Oxalyl chloride, DMF, 70 °C, 54%; (d) TEA, EtOH, reflux, 84%; (e) amine, MeOH, rt, 76% for **45a**, methylamine, MeOH, rt, 69% for **45b**

Compounds **51a–c** were synthesized according to **Scheme 5**. Bis(2,4-dimethoxybenzyl)amine **46** was treated with benzylsulfonyl chloride to afford sulfonamide **47**. The α position of the sulfone was deprotonated with NaHMDS and then treated with *N*-fluorobenzenesulfonimide, methyl iodide[27] or 1,3,2-dioxathiolan-2,2-oxide[27] to give derivatives **48a–c**, respectively. Subsequent deprotection with TFA gave intermediates **49a–c**. For the left-hand fragment, chloropyridine **20a** was coupled to piperidine carboxylic acid to give intermediate **50**, which was then coupled to sulfonamides **49a–c** to produce compounds **51a–c** in good yields.

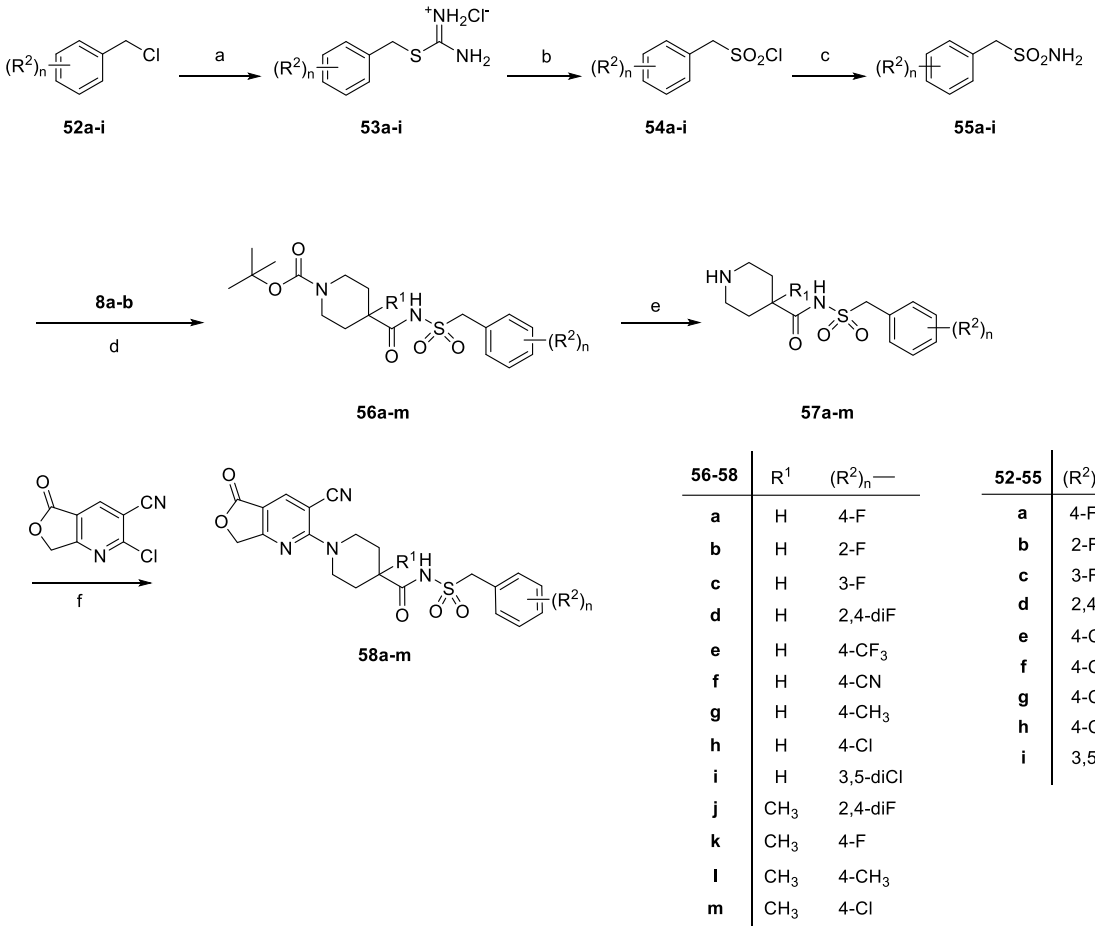
Scheme 5. Synthesis of Compounds 51a–c[a]



[a]Reagents and conditions: (a) Benzylsulfonyl chloride, DMAP, THF, rt, 60%; (b) NaHMDS, THF, –78 °C, Ar, *N*-fluorobenzenesulfonimide for **48a**, CH_3I for **48b**, 1,3,2-dioxathiolan-2,2-oxide (DTD) for **48c**, 66–78%; (c) TFA, DCM, rt, 70–82%; (d) DIPEA, EtOH, reflux, 70%; (e) EDCI, HOBt, DIPEA, 58–73%

Compounds **58a–m** were synthesized using an alternative route, as illustrated in **Scheme 6**. Derivatives of **55a**–**i** were prepared using various benzyl chlorides (**52a**–**i**) as starting materials. Intermediates **53a–i** were readily prepared by refluxing the benzyl chlorides with thiourea in ethanol, and these compounds were then subjected to NCS/HCl oxidative chlorination conditions to generate benzylsulfonyl chlorides **54a–i**.[28] The following four steps in the synthesis of target compounds **58a–m** were similar to those depicted in **Scheme 1**.

Scheme 6. Synthesis of Compounds 58a–m[a]



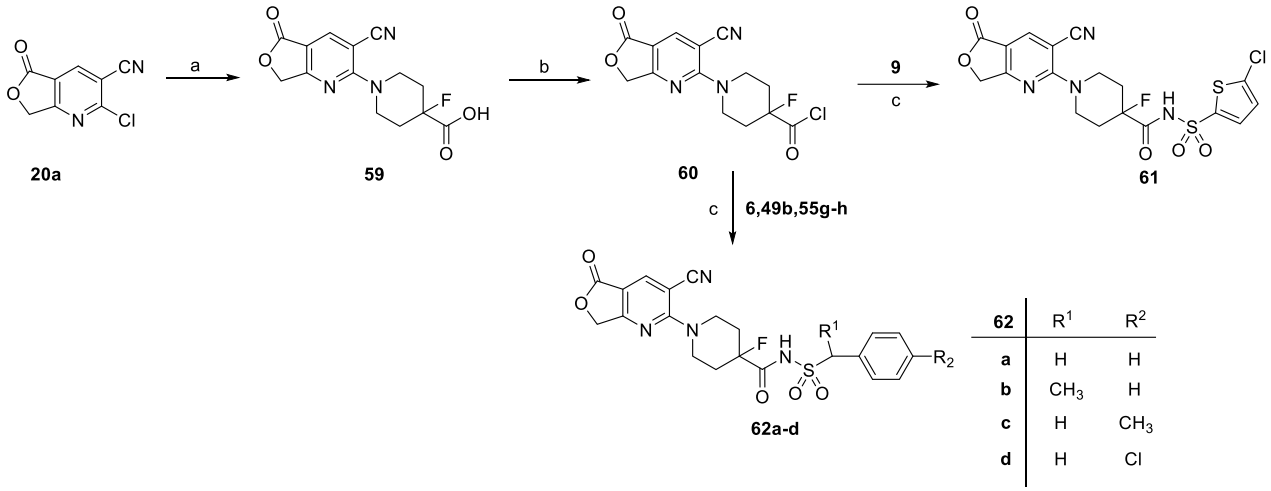
56-58	R^1	$(R^2)_n$—
a	H	4-F
b	H	2-F
c	H	3-F
d	H	2,4-diF
e	H	4-CF_3
f	H	4-CN
g	H	4-CH_3
h	H	4-Cl
i	H	3,5-diCl
j	CH_3	2,4-diF
k	CH_3	4-F
l	CH_3	4-CH_3
m	CH_3	4-Cl

52-55	$(R^2)_n$—
a	4-F
b	2-F
c	3-F
d	2,4-diF
e	4-CF_3
f	4-CN
g	4-CH_3
h	4-Cl
i	3,5-diCl

[a]Reagents and conditions: (a) EtOH, reflux, 2 h; (b) NCS, HCl, MeCN, rt; (c) NH_4OH, THF, rt, 25–68%; (d) TBTU, TEA, LiCl, rt, 48–84%; (e) HCOOH, rt, 74–91%; (f) DIPEA, EtOH, reflux, 12 h, 41–74%

Compounds **61** and **62a**–**d** were synthesized according to **Scheme 7**. Compound **20a** was treated with 4-fluoropiperidine-4-carboxylic acid to give acid **59**, which was converted to acid chloride **60** and then coupled to various sulfonamides to afford compounds **61** and **62a**–**d**.

Scheme 7. Synthesis of Compounds 61 and 62a–d[a]



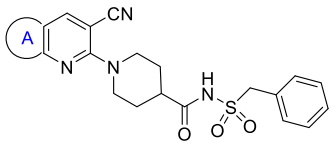
[a]Reagents and conditions: (a) DIPEA, EtOH, reflux, 80%; (b) $SOCl_2$, DMF, DCM; (c) TEA, DCM, 56–79%

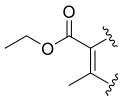
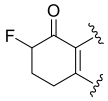
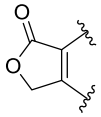
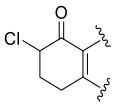
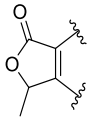
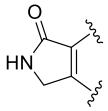
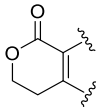
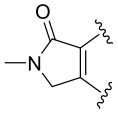
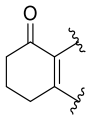
In Vitro Platelet Aggregation. All compounds were evaluated for their ability to inhibit ADP-induced human platelet-rich plasma (PRP) aggregation.[29-31] The platelet-rich plasma was prepared following a standard protocol, and the aggregation was measured by a double-channel aggregometer for platelet aggregation (see the Experimental Section for details).

As an initial exploration, five- or six-membered lactones were designed to replace the ethyl ester of compound AZD1283 because the lactone group could maintain the hydrogen bond with the receptor but also potentially improve the metabolic stability of the compound. As shown in **Table 1**, this hypothesis was confirmed with five-membered lactone compound **21a**, which exhibited slightly enhanced activity compared to AZD1283, but six-membered lactone analog **33a** showed no activity in the PRP aggregation assay. Additionally, when a methyl group was introduced to the α position of the five-membered lactone, a loss of activity was observed (**21e** versus **21a**). We then converted the lactone to a cyclic ketone or a lactam, both of which are lactone

bioisosteres, as in compounds **40a**–**c** and **45a**–**b**. Compared to the six-membered lactone, the simple six-membered cyclic ketone showed improved inhibitory activity (**40a** versus **33a**). Again, the introduction of α substituents decreased the activity to varying degrees (**40b**–**c** versus **40a**). Neither of the two lactam analogs **45a**–**b** inhibited platelet aggregation. These results indicate a very strict SAR at this moiety.

Table 1. In Vitro Antiplatelet Aggregation Potency of Various A-Ring Derivatives



compd	A-ring	IC_{50}[a] (μM)	compd	A-ring	IC_{50}[a] (μM)
AZD1283		3.60	**40b**		254.81
21a		2.35	**40c**		>300
21e		>300	**45a**		>300
33a		>300	**45b**		>300
40a		116.51			

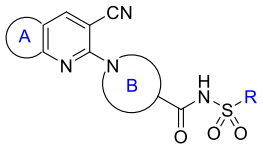
[a]ADP-induced platelet aggregation ([ADP] =10 μM, n = 2), human platelet-rich plasma (hPRP).

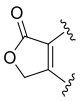
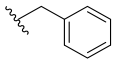
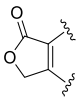
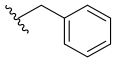
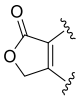
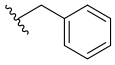
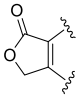
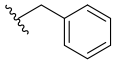
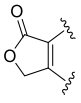
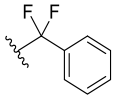
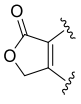
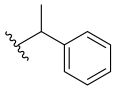
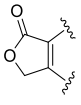
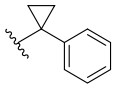
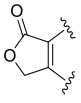
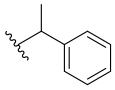
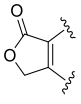
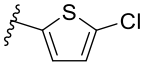
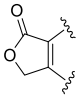
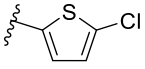
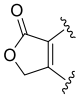
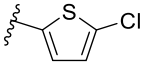
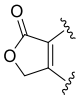
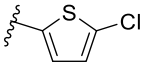
Based on the encouraging results of compound **21a**, a comprehensive SAR investigation was implemented around the B-ring and the aryl tail (R) (**Table 2**). Changing the piperidine to either an azetidine or a pyrrolidine led to a loss of potency (**21c–d** versus **21a**), so the piperidine ring was kept and substituents were introduced to

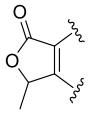
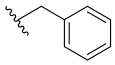
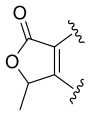
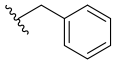
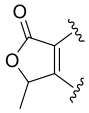
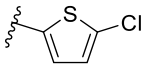
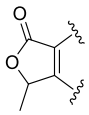
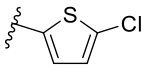
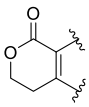
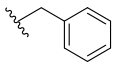
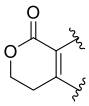
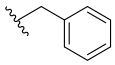
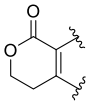
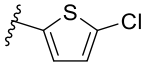
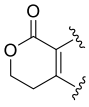
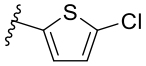
its 4 position, as this may block the metabolically labile α position of the carbonyl and therefore improve compound stability. As demonstrated by compound **21b**, the introduction of a methyl group led to a 2-fold decrease in potency compared to unsubstituted compound **21a**, while introducing a fluorine significantly increased the inhibitory activity (IC_{50} = 1.21 μM for **62a** versus 2.35 μM for **21a**). Another potential site for metabolic degradation is the benzyl position of the sulfonamide, so this position was structurally modified, and fluoride, cyclopropyl[32] or methyl groups were introduced at this position. However, we noticed that di-fluoro compound **51a** was chemically unstable upon storage under ambient conditions. Methyl- and cyclopropyl-substituted compounds **51b**, **51c** and **62b** exhibited 2- to 4-fold decreased potencies compared to compound **24a**. These findings showed that substituents at the benzyl position did not improve the inhibitory activity. Replacement of the benzyl substituent with a 5-Cl-thienyl moiety, which is a structural motif present in elinogrel,[31] slightly decreased the in vitro activity (**22a** vs **24a**). Interestingly, keeping this 5-Cl-thienyl moiety and adding a methyl substituent at the 4 position of the piperidinyl group increased the potency by a factor of 3 (**22b** vs **24a**), while fluoro-substituted compound **61** showed reduced potency. The structure-activity relationship was not consistent with that observed in the above benzyl series. Based on these results, the α-substituted five-membered lactone or six-membered lactone scaffolds were reevaluated. Unfortunately, neither changes in the B-ring nor the aryl tail group improved the inhibitory activities (**21f–g**, **22d**–**e**, **33b–c** and **34–b**) (IC_{50} >300 μM).

Table 2. In Vitro Antiplatelet Aggregation Potency of Various A-Ring, B-Ring and

Aryl Group (R) Analogs.



compd	A-ring	B-ring[a]	R	IC_{50}[b] (μM)
21c		3-aze		>300
21d		3-pyr		>300
21b		4-methyl-pip		6.68
62a		4-fluoro-pip		1.21
51a		4-pip		n.d.[c]
51b		4-pip		4.16
51c		4-pip		4.10
62b		4-fluoro-pip		9.95
22a		4-pip		4.47
22b		4-methyl-pip		1.64
61		4-fluoro-pip		37.06
22c		3-aze		193.12

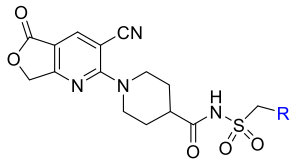
21f		3-aze		>300
21g		3-pyr		>300
22d		4-pip		>300
22e		3-aze		>300
33b		3-aze		>300
33c		3-pyr		>300
34a		4-pip		>300
34b		3-aze		>300

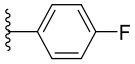
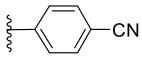
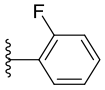
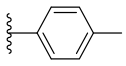
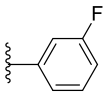
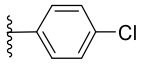
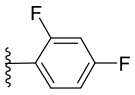
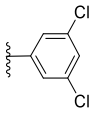
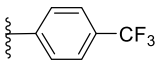
[a]3-aze=3-azetidinyl, 3-pyr=3-pyrrolyl, 4-pip=4-piperidinyl, 4-fluoro-pip=4- fluoro-piperidinyl, 4-methyl-pip=4-methyl-piperidinyl. [b]ADP-induced platelet aggregation ([ADP] =10 μM, n = 2), human platelet-rich plasma (hPRP). [c]n.d. = not determined.

Based on the reported cocrystal structure of AZD1283 bound to $P2Y_{12}$, the phenyl tail of AZD1283 occupies a hydrophobic pocket formed by the side chains of F252, R256, Y259, L276 and K2807.[24, 25] The reported SAR of the aromatic substituents at this position also showed that lipophilic substituents are favored on this phenyl ring.[33] Therefore, various small lipophilic substituents were introduced onto the phenyl group to improve the binding affinity of the compound. As shown in **Table 3**, the *para*-fluoro (**58a**) and *para*-chloro (**58h**) analogs displayed inhibitory activities comparable to that

of compound **24a**. However, shifting the fluoro substituent from the *para*- (**58a**) to the *ortho*- (**58b**) or *meta*- (**58c**) positions significantly reduced the activity. In addition, di-fluoro (**58d**) or di-chloro substitution (**58i**) also decreased the potency to varying degrees. Small alkyl groups, such as methyl (**58g**), provided comparable activity, but a trifluoromethyl (**58e**) or cyano (**58f**) substituent led to a 2- to 4-fold decrease in activity.

Table 3. In Vitro Antiplatelet Aggregation Potency of Various Phenyl Derivatives



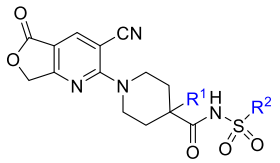
compd	R	IC_{50}[a] (μM)	compd	R	IC_{50}[a] (μM)
58a		3.50	**58f**		8.90
58b		23.72	**58g**		4.28
58c		70.49	**58h**		4.42
58d		5.20	**58i**		30.51
58e		15.90			

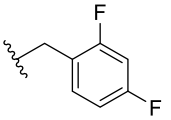
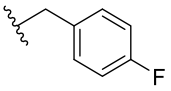
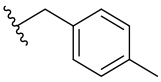
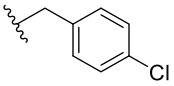
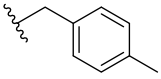
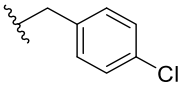
[a]ADP-induced platelet aggregation ([ADP] =10 μM, n = 2), human platelet-rich plasma (hPRP).

From the above SAR studies, the A-ring, B-ring and aryl tail groups were each optimized individually. Finally, we combined these fragments for the last round of modifications. As shown in **Table 4**, compounds **58l** and **58m** exhibited excellent activities in the hPRP assay, and they were both over 10-fold more potent than their corresponding 4-fluoro-substituted piperidinyl counterparts (**62c** and 6**2d**). However,

the 4-F/CH_3-substituted piperidinyl derivatives (**58j–k** and **62c–d**) did not display more potent antiplatelet activities than their unsubstituted piperidine counterparts.

Table 4. In Vitro Antiplatelet Aggregation Potency of B-Ring and Aryl Group (R) Derivatives.



compd	R^1	R^2	$IC_{50}{}^a$ (μM)
58j	—CH_3		8.76
58k	—CH_3		35.44
58l	—CH_3		2.94
58m	—CH_3		3.01
62c	—F		32.76
62d	—F		37.22

[a]ADP-induced platelet aggregation ([ADP] = 10 μM, n = 2), human platelet-rich plasma (hPRP).

On the basis of their in vitro parameters, a selection of compounds **21a**, **22a**, **22b** and **58l** were progressed for evaluation of metabolic stabilities in a liver microsome assay, and the data are listed in **Table 5**. The liver microsomal stability[34, 35] of AZD1283 in rat liver microsomes was very poor ($T_{1/2}$ = 6.08 min) but was better in dog microsomes ($T_{1/2}$ = 207 min) and human microsomes ($T_{1/2}$ = 65.0 min). The four new compounds

were much more stable in rat microsomes ($T_{1/2}$ = 76.0 – 152 min) and were comparable (81.3 and 79.1 min for **21a** and **22a**) or much more stable (203.9 and 208.3 min for **22b** and **58l**) in human microsomes. Compound **22b** showed more stable in dog microsomes than AZD1283. Compound **58l** showed the best stability in both rat and human liver microsomes with the lowest clearance values and longest half-life among these compounds. This further proved that replacing the ethyl ester of AZD1283 with the five-membered lactone could significantly improve the metabolic stability.

Table 5. Microsomal Stability of Selected Compounds[a]

compd	species	$T_{1/2}$[b] (min)	Cl_{int}[c] (mL/min/g protein)	MF%[d]
AZD 1283	rat	6.08	345	11.0
	dog	201	11.2	66.0
	human	65.0	32.3	38.0
21a	rat	145	14.5	75.0
	dog	116	18.2	54.0
	human	81.3	25.8	43.0
22a	rat	152	13.8	76.0
	dog	171	12.3	64.0
	human	79.1	26.6	42.0
22b	rat	76.0	27.6	61.7
	dog	200.3	10.5	67.2
	human	203.9	10.3	65.6
58l	rat	89.1	10.6	65.3
	dog	97.5	116.3	60.0
	human	208.3	10.1	66.0

[a]0.33 mg/mL microsomal protein, $NADP^+$-regenerating system, [inhibitor], 0.1 μM;

incubation at 37 °C; samples taken at 0, 7, 17, 30, and 60 min; determination of parent compound by MS. [b]$T_{1/2}$: elimination half-life. [c]Cl_{int}: intrinsic body clearance. [d]Calculated of metabolic bioavailability.

Pharmacokinetic Analysis. Based on their excellent in vitro activities in the hPRP assay, compounds **21a**, **22a**, **22b**, **58l**, **58m** and **62a** were selected for evaluation of their PK properties in Sprague−Dawley (SD) rats along, and AZD1283 was evaluated for comparison. All compounds were administered orally at a dose of 5 mg/kg. As summarized in **Tables 6**, AZD1283 showed poor PK properties, such as an extremely low plasma concentration (C_{max} = 25.9 ± 11 ng/mL) and overall drug exposure ($AUC_{0-\infty}$ = 34 ± 3.34 ng·h/mL). To our delight, all our new five-membered lactone compounds displayed much better PK properties, with AUCs 8- (**62a**) to 121-fold (**58l**) higher than that of AZD1283. Among these compounds, the overall PK profile of **58l** in rats was the most desirable, as it showed the highest maximal plasma concentration (C_{max} = 1661 ± 642 ng/mL), highest plasma exposure ($AUC_{0-\infty}$ = 4120 ± 2127 ng·h/mL), and longest elimination half-life ($T_{1/2}$ = 2.91 ± 1.09). 5-Chloro-thienyl analogs **22a** and **22b** displayed less favorable overall PK properties compared with that of **58l**, including moderate C_{max} values and lower plasma exposures. Notably, **22b** showed higher plasma exposure than **22a**, which supports the hypothesis that the introduction of a methyl group at the 4 position of the piperidine ring could improve the PK properties of the compound. However, the introduction of a fluoro substituent at the same position (compound **62a**) led to decreased plasma exposure and an overall worse PK profile compared to that of compound **21a**, which indicated that although it enhances in vitro

potency, fluoro substitution negatively impacted the PK properties. Although generally considered a metabolically liable group, a methyl substituent at the *para*-position of the benzene tail not only improved the in vitro antiplatelet activity of **58l** but also led to much better pharmacokinetic properties compared to those of chloro-substituted derivative **58m** (an interesting example of the "magic methyl" effect). The IV PK of **58l** in rats also was outstanding, with relatively low clearance (CL = 10.2 ±0.9 mL/min/kg), higher plasma exposure ($AUC_{0-\infty}$ = 8187 ± 673 ng·h/mL), and good oral bioavailability (F = 51%) at a dose of 5 mg/kg, which displayed much better than that of AZD1283 (data not shown). Taken together, compound **58l** has excellent pharmacokinetic parameters in rats.

Table 6. PK Properties of Selected Compounds in Male Rats after Oral Administration[a]

compd	dose (mg/kg)	C_{max} (ng/mL)	$T_{1/2}$ (h)	T_{max} (h)	$AUC_{0-\infty}$ (ng·h/mL)	MRT (h)
AZD	5	25.9 ± 11	1.68 ± 0.37	0.25	34.0 ± 3.34	2.67 ± 0.77
21a	5	271 ± 89	1.70 ± 0.85	0.25	384 ± 170	1.91 ± 0.41
22a	5	225 ± 144	2.40 ± 0.27	0.25	417 ± 217	2.31 ± 0.55
22b	5	479 ± 67	1.26 ± 0.45	0.25	1057 ± 632	1.98 ± 0.70
58l	5	1661 ± 642	2.91 ± 1.09	0.25	4120 ± 2127	3.64 ± 0.18
58m	5	877 ± 798	1.90 ± 1.29	0.25	1352 ± 754	2.58 ± 1.56
62a	5	149 ± 85	2.52 ± 3.0	0.25	291 ± 137	3.83 ± 4.38

[a]Sprague−Dawley rats (male). All values are represented as the mean ± SD. Abbreviations: C_{max}, peak plasma concentration of a drug after administration; T_{max},

time to reach C_{max}; $T_{1/2}$, elimination half-life; AUC, area under the concentration−time curve; MRT, mean residence time; AZD, AZD1283.

CYP450 Inhibition Assay. Another drawback of AZD-1283 is its high CYP450 inhibition, especially for the CYP2C19 subtype.[22] Therefore, we tested compounds **21a**, **22a**, **22b**, and **58l** along with AZD1283 for their abilities to inhibit five major CYP450 enzymes, and the results are summarized in **Table 7**. Indeed, as reported previously, AZD1283 showed strong inhibition of CYP2C19 (IC_{50} = 0.399 μM) and moderate inhibition of CYP2C9 and CYP3A4. The inhibitory activities of the four new compounds toward CYP2C19 and CYP2C9 were dramatically lower, and 5-chloro-thienyl substituted compounds **22a** and **22b** exhibited no CYP3A4 inhibition. Compounds **21a** and **58l** moderately inhibited CYP3A4 and were slightly more potent than AZD1283. However, in conjunction with its very weak inhibition of the other main subtypes, the overall profile of **58l** is comparable to that of AZD1283 in terms of drug-drug interaction potential. Additionally, by comparing the results of compounds **21a** and **58l** with **22a** and **22b**, it could be speculated that the benzyl group is associated with CYP3A4 inhibition, which suggested the benzyl tail as a possible site for further modifications.

Table 7. CYP450 Inhibition of Selected Compounds.

compd	IC_{50} (μM)[a]					
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4(M)[b]	CYP3A4(T)[c]
AZD	>25	6.62	0.399	>25	4.28	3.64
21a	>25	>25	>25	>25	8.01	3.53

22a	50	50	28.5	50	50	50
22b	50	50	7.75	50	50	50
58l	50	25	50	50	1.64	1.11

[a]IC_{50} refers to the concentration of compound that causes 50% inhibition of the enzyme activity. [b]Midazolam was used as the substrate. [c]Testosterone was used as the substrate. AZD, AZD1283.

hERG K^+ Channel Inhibition. To further evaluate the safely profiles of these compounds, we evaluated compounds **21a**, **22a**, **22b**, and **58l** in a human ether-a-go-go-related gene (hERG) assay, and none of the compounds inhibited hERG at concentrations as high as 40 μM. This excludes the QT interval prolongation-related cardiac toxicity of these compounds.

Solubility Assay. Good solubility is the key to druggability. Therefore, we tested compound **58l** and along with AZD1283 for their solubility in three different matrices **(Table 8)**. To our delight, compound **58l** displayed significantly better solubility, with maximal soluble concentration 6 to 18-fold higher than that of AZD1283.

Table 8. Solubility of AZD1283 and 58l in Different Matrices.

compd	maximal soluble concentration (μM)		
	intestinal juice	gastric juice	water
AZD1283	15.61±6.40	0.51±0.20	2.99±1.80
58l	90.90±2.69	4.00±2.53	54.2±15.31

In Vivo Antithrombotic Efficacy. Based on its in vitro potency in the hPRP assay and its excellent overall pharmacokinetic properties, the in vivo antithrombotic efficacy

of compounds **21a**, **22a**, **22b**, and **58l** were tested, and AZD1283 was evaluated for comparison. The ferric chloride model in Wistar rats was used to determine the efficacy of those antiplatelet agents. All tested compounds were administered orally at a dose of 10 mg/kg (**Figure 3**). Among these new five-membered lactone compounds, **21a**, **22b**, and **58l** resulted in a significant antithrombotic effect, whereas administration of AZD1283 caused no significant difference relative to the control group. Compound **58l** showed the best antithrombotic effect among these compounds, which was in accordance with its in vivo PK properties and the liver microsomal stability. To further determine the antithrombotic effect of compound **58l**, we tested it together with clopidogrel (the most commonly used $P2Y_{12}$ antagonist) in the same experimental model (**Figure 4**). Compound **58l** decreased thrombus weight in dose-dependent manner with an ED_{50} of 27 mg/kg, compared to that of 7 mg/kg for clopidogrel.

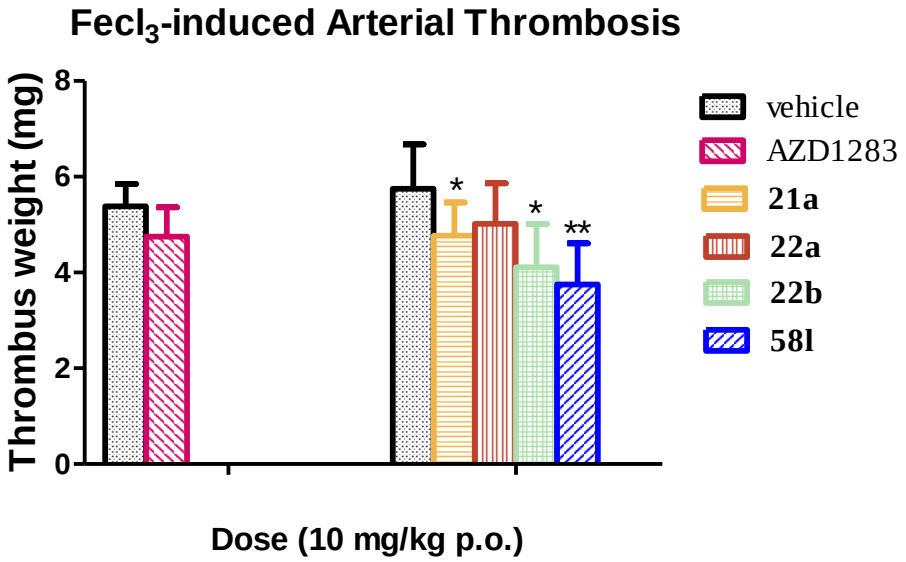


Figure 3. Dose-effects of AZD1283, **21a**, **22a**, **22b**, and **58l** on thrombus weight after $FeCl_3$-induced arterial injury in anesthetized Wistar rats at 1.5 h postdosing following oral administration at 10 mg/kg. Data are reported as the mean ± SEM (n = 10). ($^{*}p$ <

0.05, $^{**}p < 0.01$).

Bleeding Risk Assessment. Achieving efficient levels of platelet inhibition with $P2Y_{12}$ antagonists causes a concomitant increase in the risk of bleeding events. Therefore, we evaluated the effects of **58l** on both bleeding time and bleeding weight in rats after oral administration. The effects of **58l** were studied 1.5 h postdosing in a tail transection model. The effects of compound **58l** were compared to those of clopidogrel under the same conditions. The ED_{100} (ED_{100}: 2-fold prolongation of bleeding time of the control group) was used as the measure of bleeding time, and blood loss was defined as the amount of blood oozing out of the rat tail after placing a standardized surgical wound. As shown in **Figure 5**, **58l** and clopidogrel prolonged the bleeding time in a dose-dependent manner, but the calculated ED_{100} value of **58l** was 7.58 mg/kg, while that of clopidogrel was 2.36 mg/kg. In terms of bleeding weight, in the absence of any antithrombotic treatment (vehicle group), the surgical blood loss was quantified at 0.22 g of blood (**Table 8**). At different doses of clopidogrel (2.5−20 mg/kg), the surgical blood loss weight increased from 0.44 to 1.99 g of blood. At the tested doses of compound **58l** (2.5−40 mg/kg), the surgical blood loss weight increased from 0.24 to only 0.66 g.

Recent studies have suggested the application of a "therapeutic window" for the use of clopidogrel to prevent bleeding and thrombotic complications.[36, 37] For the new generation of $P2Y_{12}$ antagonists, such as prasugrel and ticagrelor, the concept of a therapeutic window is becoming more important because of their high potency and the associated increase in bleeding risk. In our studies in rats (**Table 9**), we observed that

at a dose of 40 mg/kg, compound **58l** inhibited thrombosis at a rate of 56%, which is equal to that of clopidogrel at 10 mg/kg (57% inhibition of thrombosis), and these rates are comparable to the high average rate of inhibition achieved with a daily dose of 75 mg of clopidogrel.[38] At these therapeutically equivalent doses, compound **58l** showed significantly lower bleeding weight and time compared to clopidogrel (**Table 9**), indicating it has a wider therapeutic window than clopidogrel and may therefore have more potential for use across a broader spectrum of ACS patients with fewer contraindications.

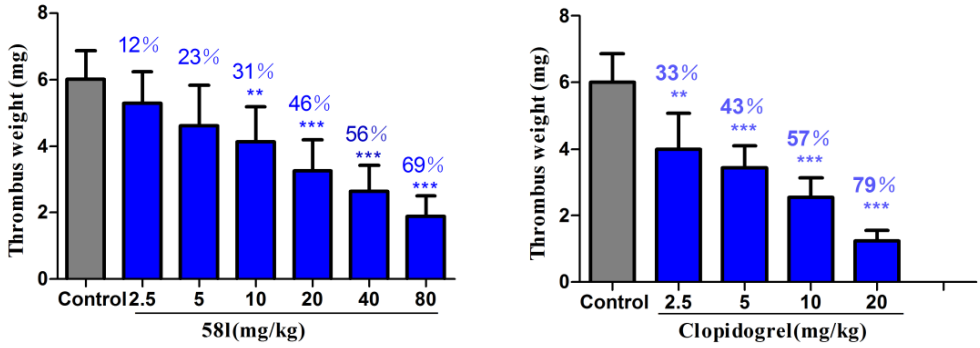


Figure 4. Dose-effects of clopidogrel and **58l** on thrombus weight after $FeCl_3$-induced arterial injury in anesthetized Wistar rats at 1.5 h postdosing following oral administration. **58l** (2.5−80 mg/kg), clopidogrel (2.5−20 mg/kg). Data are reported as the mean ± SEM (n = 10). ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

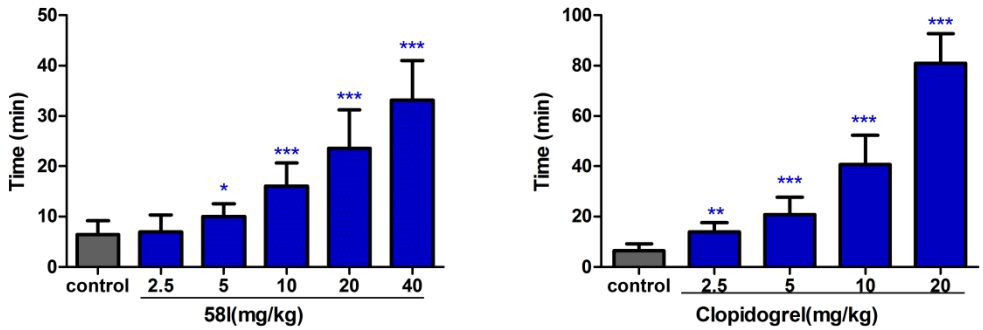


Figure 5. Dose-effects of **58l** and clopidogrel on tail bleeding time at 1.5 h postdosing following oral administration in rats. Data are reported as the mean ± SEM (n = 10) ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Table 8. Effects of 58l and Clopidogrel on Blood Loss in Rats

compd	dose (mg/kg)	bleeding weight (g)
Vehicle	-	0.22 ± 0.06
58l	2.5	0.24 ± 0.06
	5	0.31 ± 0.07
	10	0.39 ± 0.08
	20	0.43 ± 0.10
	40	0.66 ± 0.11
clopidogrel	2.5	0.44 ± 0.11
	5	0.70 ± 0.16
	10	1.20 ± 0.24
	20	1.99 ± 0.17

Dose-effects of **58l** and clopidogrel on tail blood loss at 1.5 h postdosing following oral

administration in rats. Data are reported as the mean ± SEM (n = 10).

Table 9. Effects of 58l and Clopidogrel on Bleeding Risk after Oral Administration in Rats.

compd	dose (mg/kg)	inhibition of thrombosis (%)	bleeding weight (g) [a]	bleeding time (min) [a]
58l	40	56	$0.66 \pm 0.11^{***}$	$33.09 \pm 7.88^{***}$
clopidogrel	10	57	$1.20 \pm 0.24^{***}$	$40.50 \pm 11.81^{***}$

[a]Data are reported as the mean ± SEM (n = 10) ($^{***}p < 0.001$).

CONCLUSION

In summary, to improve the metabolic stability of the reversible $P2Y_{12}$ antagonist AZD1283, a series of bicyclic pyridine analogs were designed and synthesized. Among these lactone, lactam and cyclic ketone derivatives, a pyridine-fused lactone was the most effective replacement for the open ester substituent of AZD1283. Further structural optimization focusing on the B-ring and aryl tail led to the discovery of several novel analogs, such as **21a**, **22a**, **22b** and **58l**, with high in vitro inhibitory activities in the platelet aggregation assay. Importantly, the pharmacokinetic properties of these compounds were much better than those of AZD1283 in rats, and they showed significantly enhanced metabolic stabilities in rat and human microsomes. Furthermore, among these new five-membered lactone compounds, **21a**, **22b**, and **58l** resulted in a significant antithrombotic effect in vivo, whereas administration of AZD1283 caused no significant difference relative to the control group. The best PK properties and antithrombotic effect in vivo were observed with compound **58l**, which was further

tested in an in vivo model of $FeCl_3$-induced carotid artery thrombosis injury to evaluate its antithrombotic efficacy as well as in a rat tail-bleeding model to assess its bleeding risk. Compound **58l** displayed potent and dose-dependent inhibition of platelet aggregation in vivo and resulted in decreased bleeding time and weight compared to clopidogrel.

Blood platelets are highly activated within the first one or two weeks in patients after experiencing ACS, and highly active platelet agents (clopidogrel or ticagrelor) may only be required for the initial treatment of ACS patients, which should potentially be switched a maintenance therapy using antiplatelet agents with lower activities but superior safety profiles.[38] Our results demonstrate that compound **58l** could be ideal for such maintenance treatment based on its reduced bleeding risk and wider therapeutic window. Further preclinical evaluation of compound **58l** is currently underway.

EXPERIMENTAL SECTION

Chemistry. All solvents and chemicals were used as purchased without further purification. Room temperature refers to 20−25 °C. Intermediates not described below were purchased from commercial vendors and were used as supplied unless stated otherwise. All reactions were monitored using thin-layer chromatography (TLC) on silica gel F-254 TLC plates. Column chromatography was carried out using silica gel (200−300 mesh). ^1H and ^{13}C NMR spectra were recorded on a Bruker 400, a Bruker 500 or a Bruker 600 NMR spectrometer using solvent residual as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are reported in Hertz (Hz). EI-MS spectra were obtained on a Finnigan MAT95

spectrometer, and ESI-MS spectra were obtained on a Krats MS 80 mass spectrometer. All final compounds were purified to >96% purity as determined by analytical HPLC (PLATISIL ODS 250 mm × 4.6 mm, particle size 5 μm) with acetonitrile/buffer (0.1% CF_3COOH and 0.1% NH_4OH in water, pH 3.5) as the mobile phase. Detailed synthetic procedures and spectral characterization data for all intermediates can be found in the Supporting Information.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)piperidine-4-carboxamide (21a).** A suspension of **20a** (30 mg, 0.16 mmol), **8a** (53 mg, 0.19 mmol) and TEA (47 mg, 0.46 mmol) in EtOH (10 mL) was stirred at 72 °C for 18 h. The reaction mixture was cooled to rt and a mixture of EtOAc (50 mL) and water (15 mL) was added. The organic phase was washed with water (3 × 15 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated to afford a crude product. The crude product was purified by chromatography on silica gel with dichloromethane/methanol (30 : 1) to afford **21a** as a white solid (58 mg, 85%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.62 (s, 1H), 8.57 (s, 1H), 7.40 (d, J = 6.5 Hz, 3H), 7.29 (d, J = 6.9 Hz, 2H), 5.26 (s, 2H), 4.70 (s, 2H), 4.51 (d, J = 13.5 Hz, 2H), 3.23 (t, J = 12.3 Hz, 2H), 2.61 (dd, J = 13.2, 9.2 Hz, 1H), 1.87 (d, J = 11.4 Hz, 2H), 1.67 (dt, J = 14.9, 7.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.10, 170.74, 167.66, 161.48, 143.28, 130.70, 129.62, 129.06, 128.64, 128.58, 117.39, 108.34, 93.08, 69.90, 57.44, 46.94, 41.24, 40.66, 27.44. MS(ESI) *m/z*: 441.1 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{21}H_{21}N_4O_5S$ ($[M + H]^+$) 441.1227, found: 441.1237.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-**

methylpiperidine-4-carboxamide (21b). Compound **21b** (92 mg, 78%) was prepared from **20a** (100 mg, 0.52 mmol) and **8b** (135 mg, 0.50 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.35 (s, 1H), 8.55 (s, 1H), 7.40–7.37 (m, 3H), 7.34–7.31 (m, 2H), 5.26–5.25 (s, 2H), 4.76 (s, 2H), 4.07–4.02 (m, 2H), 3.58–3.52 (m, 2H), 2.16–2.10 (m, 2H), 2.02–1.97 (m, 1H), 1.59–1.53 (m, 2H), 1.18 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 176.41, 170.71, 167.67, 161.37, 143.26, 130.72, 129.62, 128.66, 128.51, 117.45, 108.14, 92.90, 69.87, 57.86, 44.72, 42.19, 33.16, 23.14. MS (ESI) *m/z*: 453.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{21}N_4O_5S$ ($[M - H]^-$) 453.1238, found: 453.1239.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2yl)azetidine-3-carboxamide (21c).** Compound **21c** (75 mg, 70%) was prepared from **20a** (50 mg, 0.26 mmol) and **8c** (72 mg, 0.28 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.50 (s, 1H), 7.41–7.33 (m, 5H), 5.26 (s, 2H), 4.75 (s, 2H), 4.51–4.38 (m, 4H), 3.59 (ddd, J = 14.5, 8.9, 5.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.52, 171.29, 167.92, 160.19, 141.83, 130.73, 128.99, 128.72, 128.60, 116.58, 107.41, 90.11, 69.80, 57.66, 33.96, 28.96. MS (ESI) *m/z*: 413.2 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{19}H_{17}N_4O_5S$ ($[M + H]^+$) 413.0914, found 413.0922.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)pyrrolidine-3-carboxamide (21d).** Compound **21d** (90 mg, 71%) was prepared from **20a** (58 mg, 0.30 mmol) and **8d** (80 mg, 0.30 mmol) in the same manner as described for **21a.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.53 (s, 1H), 7.40–

7.35 (m, 3H), 7.30 (dd, *J* = 6.3, 3.0 Hz, 2H), 5.26 (s, 2H), 4.71 (s, 2H), 3.98–3.80 (m, 4H), 3.22–3.13 (m, 1H), 2.27–2.06 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.50, 171.16, 167.91, 157.83, 143.04, 130.62, 129.14, 128.62, 128.57, 128.52, 117.96, 106.89, 90.90, 69.83, 57.53, 51.16, 48.70, 28.13. MS (ESI) *m/z*: 425.1 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{20}H_{17}N_4O_5S$ ($[M - H]^-$) 425.0929, found: 425.0925.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-methylpiperidine-4-carboxamide (21e).** Compound **21e** (92 mg, 78%) was prepared from **20b** (100 mg, 0.52 mmol) and **8a** (135 mg, 0.50 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.56 (s, 1H), 7.44–7.36 (m, 3H), 7.33–7.26 (m, 2H), 5.51 (q, *J* = 6.8 Hz, 1H), 4.70 (s, 2H), 4.58–4.48 (m, 2H), 3.23 (t, *J* = 12.7 Hz, 2H), 2.68–2.57 (m, 1H), 1.92–1.82 (m, 2H), 1.76–1.60 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.11, 173.27, 166.72, 161.51, 143.46, 130.70, 129.07, 128.63, 128.56, 117.37, 107.95, 93.13, 77.39, 57.44, 46.89, 41.26, 27.43, 18.10. MS (ESI) *m/z*: 453.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{21}N_4O_5S$ ($[M - H]^-$) 453.1238, found: 453.1236.

***N*-(Benzylsulfonyl)-1-(3-cyano-7-methyl-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)azetidine-3-carboxamide (21f).** Compound **21f (**123 mg, 76%) was prepared from **20b** (80 mg, 0.38 mmol) and **8c** (98 mg, 0.38 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.86 (s, 1H), 8.50 (s, 1H), 7.41–7.32 (m, 5H), 5.50 (q, *J* = 6.8 Hz, 1H), 4.76 (s, 2H), 4.62–4.28 (m, 4H), 3.64–3.53 (m, 1H), 1.51 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.09, 171.28, 166.98, 160.26, 142.00, 130.74, 129.00, 128.70, 128.59, 116.56, 107.04, 90.25, 77.25, 57.67,

33.95, 18.22. MS(ESI) *m/z*: 425.1 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{20}H_{17}N_4O_5S$ ([M − H]$^-$) 425.0925, found: 425.0927.

***N*-(Benzylsulfonyl)-1-(3-cyano-7-methyl-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)pyrrolidine-3-carboxamide (21g).** Compound **21g** (91 mg, 69%) was prepared from **20b** (62 mg, 0.30 mmol) and **8d** (80 mg, 0.30 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.51 (d, J = 1.8 Hz, 1H), 7.42–7.33 (m, 3H), 7.33–7.26 (m, 2H), 5.50 (qd, J = 6.8, 2.6 Hz, 1H), 4.72 (s, 2H), 4.00–3.79 (m, 4H), 3.22–3.12 (m, 1H), 2.27–2.06 (m, 2H), 1.52 (dd, J = 6.8, 2.4 Hz, 3H). ^{13}C NMR(126 MHz, DMSO-d_6) δ 173.72, 172.44, 166.97, 157.87, 157.81, 143.23, 130.63, 129.13, 128.62, 128.56, 117.95, 106.52, 91.03, 90.99, 77.28, 57.52, 51.13, 48.70, 18.25, 18.22.MS (ESI) *m/z*: 439.1 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{19}N_4O_5S$ ([M − H]$^-$) 439.1082, found: 439.1080.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)piperidine-4-carboxamide (22a).** Compound **22a** (75 mg, 67%) was prepared from **20a** (50 mg, 0.24 mmol) and **11a** (89 mg, 0.29 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 8.57 (s, 1H), 7.68 (d, J = 4.1 Hz, 1H), 7.29 (d, J = 4.1 Hz, 1H), 5.26 (s, 2H), 4.47 (d, J = 13.6 Hz, 2H), 3.25 (t, J = 11.4 Hz, 2H), 2.68 (s, 1H), 1.90 (d, J = 9.1 Hz, 2H), 1.59 (dd, J = 20.9, 11.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.35, 170.71, 167.65, 161.51, 143.22, 137.77, 136.60, 133.88, 127.72, 117.34, 108.35, 93.14, 69.88, 46.91, 41.24, 27.23. MS (ESI) *m/z*: 465.1 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{18}H_{14}ClN_4O_5S_2$ [M − H]$^-$ 465.0100, found: 465.0110.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-methylpiperidine-4-carboxamide (22b).** Compound **22b** (83 mg, 68%) was prepared from **20a** (100 mg, 0.48 mmol) and **11b** (154 mg, 0.48 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.31 (s, 1H), 8.53 (s, 1H), 7.68 (d, J = 3.9 Hz, 1H), 7.27 (d, J = 4.1 Hz, 1H), 5.24 (s, 2H), 4.04 (dd, J = 9.8, 5.6 Hz, 2H), 3.52–3.46 (m, 2H), 2.13–2.08 (m, 2H), 1.60–1.53 (m, 2H), 1.20 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 175.83, 170.69, 167.65, 161.34, 143.22, 133.85, 129.62, 127.63, 117.41, 108.16, 92.93, 69.86, 44.74, 42.22, 33.14, 23.04.MS (ESI) *m/z*: 479.1 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{19}H_{16}ClN_4O_5S_2$ ($[M - H]^-$) 479.0252, found: 479.0246.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)azetidine-3-carboxamide (22c).** Compound **22c** (56 mg, 62%) was prepared from **20a** (40 mg, 0.21 mmol) and **11c** (70 mg, 0.25 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (s, 1H), 7.70 (d, J = 4.1 Hz, 1H), 7.29 (d, J = 4.1 Hz, 1H), 5.22 (s, 2H), 4.56–4.44 (m, 2H), 4.39–4.28 (m, 2H), 3.64 (ddd, J = 11.3, 9.0, 5.8 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.46, 170.81, 167.90, 160.22, 141.77, 137.74, 136.65, 133.98, 127.75, 116.55, 107.40, 90.15, 69.77, 34.10. MS (ESI) *m/z*:436.9 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{16}H_{11}N_4O_5ClS_2$ ($[M - H]^-$) 436.9776, found: 436.9783.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-7-methyl-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)piperidine-4-carboxamide (22d).** Compound **22d** (140 mg, 62%) was prepared from **20b** (100 mg, 0.48 mmol) and **11a** (148 mg, 0.48

mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 8.54 (s, 1H), 7.68 (d, J = 4.1 Hz, 1H), 7.29 (d, J = 4.1 Hz, 1H), 5.49 (q, J = 6.8 Hz, 1H), 4.54–4.44 (m, 2H), 3.28–3.19 (m, 2H), 2.71–2.63 (m, 1H), 1.91 (dd, J = 13.1, 2.8 Hz, 2H), 1.64–1.53 (m, 2H), 1.50 (d, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.73, 167.21, 162.02, 143.91, 138.23, 137.12, 134.41, 128.23, 117.83, 108.45, 93.66, 77.88, 47.33, 41.74, 27.71, 18.58. MS (ESI) *m/z*: 479.0 $[M-H]^-$. HRMS (ESI) *m/z* calcd for $C_{19}H_{16}ClN_4O_5S_2$ ($[M-H]^-$) 479.0256, found: 479.0259.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-7-methyl-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)azetidine-3-carboxamide (22e).** Compound **22e** (82 mg, 65%) was prepared from **20b** (60 mg, 0.28 mmol) and **11c** (81 mg, 0.32 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.18 (d, J = 4.0 Hz, 1H), 5.47 (q, J = 6.7 Hz, 1H), 4.64–4.18 (m, 4H), 3.56–3.46 (m, 1H), 1.48 (d, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.11, 167.00, 160.22, 141.96, 126.99, 116.60, 106.79, 90.08, 77.22, 45.76, 34.87,18.21. MS(ESI) *m/z*:451.0 ($[M-H]^-$). HRMS (ESI) *m/z* calcd for $C_{17}H_{12}ClN_4O_5S_2[M-H]^-$ 450.9943, found: 450.9945.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)piperidine-4-carboxamide (33a).** Compound **33a** (30 mg, 74%) was prepared from **32** (78 mg, 0.37 mmol) and **8a** (29 mg, 0.11 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.35 (s, 1H), 7.44–7.37 (m, 3H), 7.32–7.27 (m, 2H), 4.70 (s, 2H), 4.58–4.53 (m, 2H), 4.52 (t, J = 6.1 Hz, 2H), 3.20 (dd, J = 18.5, 6.7 Hz, 2H), 3.04 (t, J = 6.1 Hz, 2H), 2.61 (ddd, J = 11.1, 9.4, 3.7 Hz, 1H),

1.86 (dd, *J* = 12.8, 2.5 Hz, 2H), 1.71–1.61 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.11, 163.08, 162.95, 159.05, 146.39, 130.70, 129.06, 128.64, 128.57, 117.36, 110.54, 90.93, 65.73, 57.44, 46.40, 41.35, 30.61, 27.47. MS (ESI) *m/z*: 453.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for Ion Formula $C_{23}H_{23}N_4O_4S$ ($[M - H]^-$) 453.1238, found: 453.1243.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)azetidine-3-carboxamide (33b).** Compound **33b** (123 mg, 76%) was prepared from **32** (50 mg, 0.24 mmol) and **8b** (67 mg, 0.26 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.33 (s, 1H), 7.42–7.37 (m, 3H), 7.36–7.31 (m, 2H), 4.75 (s, 2H), 4.50 (t, *J* = 6.0 Hz, 2H), 4.48–4.28 (m, 4H), 3.62–3.52 (m, 1H), 3.03 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.37, 163.90, 163.14, 158.44, 144.91, 130.72, 129.01, 128.71, 128.59, 116.45, 109.91, 88.50, 65.59, 57.66, 33.99, 30.54. MS (ESI) *m/z*: 425.1 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{20}H_{17}N_4O_5S$ ($[M - H]^-$) 425.0925, found: 425.0927.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)pyrrolidine-3-carboxamide (33c).** Compound **33c** (118 mg, 73%) was prepared from **32** (78 mg, 0.37 mmol) and **8c** (100 mg, 0.73 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (s, 1H), 8.32 (s, 1H), 7.42–7.35 (m, 3H), 7.30 (dd, *J* = 6.5, 2.8 Hz, 2H), 4.73 (s, 2H), 4.51 (t, *J* = 6.0 Hz, 2H), 3.98–3.78 (m, 4H), 3.03 (t, *J* = 6.0 Hz, 2H), 2.25–2.05 (m, 2H), 2.03–1.95 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.91, 163.92, 163.67, 156.41, 146.62, 131.13, 129.51, 129.17, 129.09, 118.37, 109.85, 89.67, 66.15, 58.03, 51.32, 48.77, 31.15.MS (ESI) *m/z*: 439.1 $[M - H]^-$. HRMS (ESI) *m/z* calcd for Ion Formula $C_{21}H_{19}N_4O_5S$ ($[M - H]^-$)

439.1082, found: 439.1086.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-5-oxo-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)piperidine-4-carboxamide (34a).** Compound **37a** (91 mg, 79%) was prepared from **32** (50 mg, 0.24 mmol) and **11a** (89 mg, 0.29 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 8.34 (s, 1H), 7.67 (d, J = 4.1 Hz, 1H), 7.29 (d, J = 4.1 Hz, 1H), 4.54–4.48 (m, 4H), 3.24–3.15 (m, 2H), 3.02 (t, J = 6.1 Hz, 2H), 2.66 (tt, J = 11.4, 4.0 Hz, 1H), 1.89 (dd, J = 13.2, 2.6 Hz, 2H), 1.61–1.49 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.36, 163.05, 162.93, 159.06, 146.35, 133.87, 127.72, 117.32, 110.54, 90.97, 65.71, 46.35, 41.35, 30.58, 27.26.MS (ESI) *m/z*: 479.0 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{19}H_{16}ClN_4O_5S_2$ ($[M - H]^-$) 479.0258, found: 479.0256.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-5-oxo-7,8-dihydro-5H-pyrano[4,3-b]pyridin-2-yl)azetidine-3-carboxamide (34b).** Compound **34b** (140 mg, 82%) was prepared from **32** (80 mg, 0.38 mmol) and **11c** (108 mg, 0.38 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.59 (d, J = 4.0 Hz, 1H), 7.22 (d, J = 4.1 Hz, 1H), 4.47 (t, J = 6.0 Hz, 2H), 4.45–4.22 (m, 4H), 3.10 (td, J = 11.9, 7.1 Hz, 1H), 2.99 (t, J = 6.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.21, 163.87, 163.13, 158.43, 144.86, 139.86, 135.19, 132.51, 127.24, 116.47, 109.74, 88.39, 65.56, 45.74, 34.64, 30.52. MS (ESI) *m/z*: 451.0 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{17}H_{12}ClN_4O_5S_2$ ($[M - H]^-$) 450.9943, found: 450.9948.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,6,7,8-tetrahydroquinolin-2-yl)piperidine-4-carboxamide (40a).** A suspension of **39a** (30 mg, 0.15 mmol), **8a** (49

mg 0.17 mmol), and DIPEA (19 mg, 0.44 mmol) in EtOH (10 mL) was stirred at 72 °C for 18 h. The reaction mixture was cooled to rt and poured into a mixture of EtOAc (50 mL) and water (15 mL). The organics were washed with water (3 × 15 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated to afford the crude material. The crude was purified by chromatography on silica gel with dichloromethane/methanol (30:1) to afford **40a** as a white solid (60 mg, 74%) . ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.25 (s, 1H), 7.42–7.37 (m, 3H), 7.29 (dd, J = 7.4, 1.8 Hz, 2H), 4.69 (s, 2H), 4.54 (d, J = 13.4 Hz, 2H), 3.19–3.13 (m, 2H), 2.90 (t, J = 6.1 Hz, 2H), 2.62–2.58 (m, 1H), 2.57–2.52 (m, 2H), 2.04–2.00 (m, 2H), 1.85 (dd, J = 13.0, 2.6 Hz, 2H), 1.64 (ddd, J = 15.5, 12.7, 3.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 194.26, 174.18, 167.33, 158.64, 143.75, 130.70, 129.09, 128.63, 128.56, 118.86, 117.68, 90.64, 57.44, 46.36, 41.45, 37.40, 32.46, 27.48, 20.88. MS (ESI) *m/z*: 451.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{23}H_{23}N_4O_4S$ ($[M - H]^-$) 451.1445, found 451.1446.

***N*-(Benzylsulfonyl)-1-(3-cyano-6-fluoro-5-oxo-5,6,7,8-tetrahydroquinolin-2-yl)piperidine-4-carboxamide (40b).** Compound **40b** (51 mg, 61.4%) was prepared from **39b** (40 mg, 0.18 mmol) and **8a** (60 mg, 0.27 mmol) in the same manner as described for **40a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.60 (s, 1H), 8.31 (s, 1H), 7.42–7.35 (m, 3H), 7.32–7.25 (m, 2H), 5.32 (dd, J = 12.4, 5.3 Hz, 1H), 4.68 (s, 2H), 4.59–4.50 (m, 2H), 3.18 (t, J = 12.5 Hz, 2H), 3.14–3.08 (m, 1H), 2.95 (ddd, J = 17.7, 7.9, 4.1 Hz, 1H), 2.63–2.55 (m, 1H), 2.27–2.15 (m, 2H), 2.02–1.94 (m, 1H), 1.85 (dd, J = 13.0, 2.4 Hz, 2H), 1.63 (ddd, J = 15.2, 13.1, 3.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 189.64, 189.51, 174.11, 166.00, 158.58, 144.58, 130.70, 129.07, 128.64, 128.57, 117.46,

117.16, 90.91, 89.44, 57.45, 46.36, 46.32, 41.36, 30.21, 30.12, 27.49. MS (ESI) *m/z*: 469.1[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{23}H_{22}FN_4O_4S$ [M − H]$^-$ 469.1351, found 469.1355.

***N*-(Benzylsulfonyl)-1-(6-chloro-3-cyano-5-oxo-5,6,7,8-tetrahydroquinolin-2-yl)piperidine-4-carboxamide (40c).** Compound **40c** (61 mg, 62%) was prepared from **39b** (50 mg，0.21 mmol) and **8a** (65 mg，0.23 mmol) in the same manner as described for **40a**. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 11.62 (s, 1H), 8.35 (s, 1H), 7.43–7.37 (m, 3H), 7.31–7.27 (m, 2H), 4.97 (dd, *J* = 8.7, 4.1 Hz, 1H), 4.69 (s, 2H), 4.61–4.55 (m, 2H), 3.24–3.16 (m, 2H), 3.03 (t, *J* = 6.1 Hz, 2H), 2.65–2.60 (m, 1H), 2.37–2.29 (m, 1H), 1.99 (dt, *J* = 12.6, 7.1 Hz, 1H), 1.86 (dd, *J* = 12.9, 2.7 Hz, 2H), 1.65 (qd, *J* = 12.5, 3.7 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 187.34, 174.15, 166.00, 158.49, 145.18, 130.72, 128.66, 128.59, 117.45, 116.65, 91.11, 59.60, 57.43, 46.37, 46.31, 41.39, 30.27, 29.97, 27.52. MS(ESI) *m/z*: 485.25 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{23}H_{22}ClN_4O_4S$ ([M − H]$^-$) 485.1056, found: 485.1054.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyridin-2-yl)piperidine-4-carboxamide (45a).** To a solution of **44** (44 mg, 0.09 mmol) in EtOH (10 mL) was add ammonium hydroxide (0.3 mL) dropwise at 0 ℃. The resulting solution was stirred for 5 h at room temperature then evaporated. The residue was purified by silica gel chromatography with dichloromethane/methanol (20:1) to yield **45a** (29 mg, 75.7%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.63 (s, 1H), 8.56 (s, 1H), 8.30 (s, 1H), 7.40 (dd, *J* = 4.9, 2.2 Hz, 3H), 7.29 (dd, *J* = 7.2, 2.2 Hz, 2H), 4.68 (s, 2H), 4.38 (d, *J* = 13.6 Hz, 2H), 4.33 (s, 2H), 3.13 (t, *J* = 11.6 Hz, 2H), 2.55 (dd,

J = 19.1, 7.6 Hz, 1H), 1.84 (d, *J* = 10.4 Hz, 2H), 1.68 (dd, *J* = 21.2, 11.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.75, 167.63, 162.10, 140.56, 131.17, 128.96, 128.91, 118.34, 116.93, 93.26, 57.87, 47.82, 47.32, 42.30, 28.10. MS (ESI) *m/z*: 438.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{20}N_5O_4S$ ($[M - H]^-$) 438.1241, found: 438.1247.

***N*-(Benzylsulfonyl)-1-(3-cyano-6-methyl-5-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyridin-2-yl)piperidine-4-carboxamide (45b).** Compound **45b** (31 mg, 68.9%) was prepared from **44** (50 mg, 0.10 mmol) in the same manner as described for **45a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.28 (s, 1H), 7.43–7.38 (m, 3H), 7.30 (dd, *J* = 7.3, 1.9 Hz, 2H), 4.69 (s, 2H), 4.41 (s, 2H), 4.38 (d, *J* = 13.4 Hz, 2H), 3.17–3.10 (m, 2H), 3.03 (s, 3H), 2.61–2.55 (m, 1H), 1.85 (dd, *J* = 12.9, 2.6 Hz, 2H), 1.73–1.64 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.78, 166.54, 165.28, 161.97, 140.08, 131.19, 129.60, 129.11, 129.06, 118.28, 116.93, 93.22, 57.94, 53.39, 47.76, 41.95, 29.23, 27.95. MS (ESI) *m/z*: 452.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for Ion Formula $C_{22}H_{22}N_5O_4S$ ($[M - H]^-$) 452.1398, found: 452.1392.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((difluoro(phenyl)methyl)sulfonyl)piperidine-4-carboxamide (51a) A mixture of **50** (100 mg, 0.35 mmol), EDCI (87 mg, 0.46 mmol, and HOBT (62 mg, 0.46 mmol) in DCM (5 mL) wasstirred at rt for 30 min. **49a** (72 mg, 0.35 mmol) and DIPEA (225 mg, 1.75 mmol) were added, and the reaction mixture was stirred at rt for 16 h. The mixture was diluted with DCM (60 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 purified by silica gel chromatography with dichloromethane/methanol (20:1) to yield

51a (96 mg, 58%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.61 (d, J = 7.2 Hz, 3H), 7.52 (d, J = 6.8 Hz, 2H), 5.25 (s, 2H), 4.45–4.38 (m, 2H), 3.18–3.10 (m, 2H), 2.01 (ddd, 1H), 1.88–1.83 (m, 2H), 1.62–1.55 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.78, 167.71, 161.39, 143.27, 131.54, 129.63, 128.27, 127.04, 126.99, 126.95, 117.44, 108.09, 92.86, 69.89, 53.57, 47.23, 41.81, 28.11. MS (ESI) *m/z*: 475.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{17}F_2N_4O_5S$ ($[M - H]^-$) 475.0893, found: 475.0898.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-*N*-((1-phenylethyl)sulfonyl)piperidine-4-carboxamide (51b). Compound **51b** (81 mg, 73%) was prepared from **50** (70.5 mg, 0.25 mmol) and **49c** (50 mg, 0.27 mmol) in the same manner as described for **51a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.57 (s, 1H), 8.56 (s, 1H), 7.41–7.38 (m, 3H), 7.38–7.34 (m, 2H), 5.26 (s, 2H), 4.80 (q, J = 7.2 Hz, 1H), 4.51–4.45 (m, 2H), 3.25–3.18 (m, 2H), 2.58 (ddd, J = 11.2, 7.0, 4.2 Hz, 1H), 1.93–1.85 (m, 2H), 1.78–1.70 (m, 2H), 1.67 (d, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.94, 170.74, 167.66, 161.47, 143.27, 134.23, 129.10, 128.78, 128.50, 117.37, 108.33, 93.07, 69.89, 61.66, 46.94, 46.90, 41.21, 27.58, 27.22, 14.68. MS (ESI) *m/z*: 453.3 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{22}N_4O_5S$ ($[M - H]^-$) 453.1235, found: 453.1238.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-*N*-((1-phenylcyclopropyl)sulfonyl)piperidine-4-carboxamide (51c). Compound **51c** (106 mg, 63%) was prepared from **50** (100 mg, 0.35 mmol) and **49c** (70 mg, 0.35 mmol) in the same manner as described for **51a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21–8.17 (m,

1H), 7.49 (d, *J* = 4.2 Hz, 2H), 7.41–7.34 (m, 3H), 5.11 (s, 2H), 4.67–4.56 (m, 2H), 3.29–3.19 (m, 2H), 2.45 (m, 1H), 2.10–2.00 (m, 4H), 1.92–1.79 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.13, 170.29, 167.78, 161.82, 143.15, 133.86, 131.89, 129.59, 128.73, 117.15, 109.11, 93.89, 70.00, 47.26, 45.59, 42.03, 27.88, 13.64. MS (ESI) *m/z*: 465.3 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{23}H_{21}N_4OS$ ($[M - H]^-$) 465.1238, found: 465.1240.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((2-fluorobenzyl)sulfonyl)piperidine-4-carboxamide (58a). Compound **58a** (50 mg, 57%) was prepared from **20a** (40 mg, 0.20 mmol) and **57a** (60 mg, 0.20 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.74 (s, 1H), 8.55 (s, 1H), 7.48–7.36 (m, 2H), 7.29–7.22 (m, 2H), 5.25 (s, 2H), 4.74 (s, 2H), 4.51 (d, *J* = 13.4 Hz, 2H), 3.27–3.20 (m, 2H), 2.64 (ddd, *J* = 10.7, 9.2, 3.4 Hz, 1H), 1.93–1.86 (m, 2H), 1.73–1.64 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.47, 171.25, 169.50, 168.16, 161.98, 143.77, 133.85, 131.67, 125.15, 117.88, 116.90, 116.17, 108.84, 93.58, 70.39, 51.61, 47.45, 41.83, 27.87. MS (ESI) *m/z*: 457.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{18}FN_4O_5S$ ($[M - H]^-$) 457.0987, found: 457.0986.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((3-fluorobenzyl)sulfonyl)piperidine-4-carboxamide (58b). Compound **58b** (60 mg, 66%) was prepared from **20a** (40 mg, 0.20 mmol) and **57b** (60 mg, 0.20 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.55 (s, 1H), 7.48–7.42 (m, 1H), 7.26–7.21 (m, 1H), 7.15–7.09 (m, 2H), 5.25 (s, 2H), 4.74 (s, 2H), 4.49 (d, *J* = 13.5 Hz, 2H), 3.26–3.19 (m, 2H), 2.64–2.57 (m, 1H), 1.89–

1.81 (m, 2H), 1.71–1.61 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.57, 171.23, 168.15, 161.98, 143.77, 131.12, 131.05, 127.37, 117.97, 117.87, 116.17, 116.01, 108.86, 93.59, 70.39, 57.42, 47.41, 41.73, 27.89. MS (ESI) *m/z*: 457.3[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{18}FN_4O_5S$ ([M − H]$^-$) 457.0987, found: 457.0987.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((4-fluorobenzyl)sulfonyl)piperidine-4-carboxamide (58c). Compound **58c** (52 mg, 74%) was prepared from **20a** (30 mg, 0.16 mmol) and **57c** (50 mg, 0.17 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H), 8.59 (s, 1H), 7.36 – 7.30 (m, 2H), 7.25 (t, *J* = 8.7 Hz, 2H), 5.27 (s, 2H), 4.71 (s, 2H), 4.52 (d, *J* = 11.6 Hz, 2H), 3.23 (t, *J* = 12.3 Hz, 2H), 2.03 – 1.96 (m, 1H), 1.91 – 1.84 (m, 2H), 1.73 – 1.62 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.63, 171.23, 168.16, 163.78, 161.97, 143.76, 133.33, 133.26, 125.92, 117.88, 116.08, 115.91, 108.82, 93.57, 70.39, 57.07, 47.44, 41.76, 27.94. MS (ESI) *m/z*: 457.2 [M − H]$^-$. HR MS (ESI) *m/z* calcd for $C_{21}H_{19}N_4O_5S$ ([M − H]$^-$) 457.0982, found: 457.0987.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((2,4-difluorobenzyl)sulfonyl)piperidine-4-carboxamide (58d). Compound **58d** (83 mg, 68%) was prepared from **20a** (50 mg, 0.26 mmol) and 57**d** (100 mg, 0.31 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.77 (s, 1H), 8.57 (s, 1H), 7.47 (dd, *J* = 15.2, 8.5 Hz, 1H), 7.37–7.32 (m, 1H), 7.20–7.15 (m, 1H), 5.27 (s, 2H), 4.74 (s, 2H), 4.58–4.45 (m, 2H), 3.29–3.20 (m, 2H), 2.65 (ddd, *J* = 10.4, 9.0, 3.8 Hz, 1H), 1.96–1.86 (m, 2H), 1.75–1.64 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 188.64, 173.99, 170.74, 167.66, 161.49, 143.28, 134.52, 129.62, 127.87, 117.38,

112.92, 112.04, 108.35, 105.51, 104.14, 93.09, 69.89, 50.69, 46.95, 41.35, 27.37. MS (ESI) *m/z*: 475.2[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{17}F_2N_4O_5S$ ([M − H]$^-$) 475.0893, found: 475.0889.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((4-(trifluoromethyl)benzyl)sulfonyl)piperidine-4-carboxamide (58e). Compound **58e** (87 mg, 75%) was prepared from **20a** (44 mg, 0.23 mmol) and **57e** (80 mg, 0.23 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.69 (s, 1H), 8.57 (s, 1H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 5.26 (s, 2H), 4.85 (s, 2H), 4.52 (d, *J* = 13.5 Hz, 2H), 3.27–3.21 (m, 2H), 2.67–2.60 (m, 1H), 1.91–1.86 (m, 2H), 1.69 (ddd, *J* = 15.0, 12.8, 3.5 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.16, 170.74, 167.66, 161.50, 143.27, 133.85, 131.62, 125.48, 125.45, 117.38, 108.35, 93.10, 69.89, 56.93, 46.94, 41.26, 27.42. MS (ESI) *m/z*: 507.1 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{18}F_3N_4O_5S$ ([M − H]$^-$) 507.0945, found: 507.0943.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((4-cyanobenzyl)sulfonyl)piperidine-4-carboxamide (58f). Compound **58f** (50 mg, 44%) was prepared from **20a** (50 mg, 0.26 mmol) and **57f** (95 mg, 0.31 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.72 (s, 1H), 8.57 (s, 1H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.50 (d, *J* = 8.3 Hz, 2H), 5.27 (s, 2H), 4.51 (d, *J* = 13.5 Hz, 2H), 3.24 (t, *J* = 11.5 Hz, 2H), 2.62 (tt, *J* = 11.1, 4.0 Hz, 1H), 1.88 (dd, *J* = 13.0, 2.7 Hz, 2H), 1.68 (ddd, *J* = 15.4, 13.0, 3.8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.13, 170.74, 167.66, 161.50, 143.27, 134.64, 132.46, 131.71, 118.46, 117.38, 111.45, 108.35, 93.11, 69.89, 57.16, 46.94, 41.28, 27.42. MS (ESI) *m/z*: 464.1 [M − H]$^-$. HRMS

(ESI) *m/z* calcd for $C_{22}H_{18}N_5O_5S$ ([M − H]$^-$) 464.102, found 464.1027.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((4-methylbenzyl)sulfonyl)piperidine-4-carboxamide (58g). Compound **58g** (87 mg, 78%) was prepared from **20a** (50 mg, 0.25 mmol) and **57g** (73 mg, 0.25 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 11.57 (s, 1H), 8.57 (s, 1H), 7.21 (d, J = 7.9 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 5.27 (s, 2H), 4.64 (s, 2H), 4.54–4.49 (m, 2H), 3.27–3.21 (m, 2H), 2.62 (ddd, J = 11.1, 9.5, 3.9 Hz, 1H), 2.31 (s, 3H), 1.91–1.86 (m, 2H), 1.72–1.66 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 174.09, 170.74, 167.67, 161.48, 143.28, 138.11, 130.58, 129.13, 125.98, 117.39, 108.34, 93.08, 69.90, 57.14, 46.95, 41.22, 39.52, 27.45, 20.73. MS (ESI) *m/z*: 453.3 [M − H]$^-$. HR MS (ESI) *m/z* calcd for $C_{22}H_{22}N_4O_5S$ ([M − H]$^-$) 453.1238, found 453.1231.

***N*-((4-Chlorobenzyl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)piperidine-4-carboxamide (58h).** Compound **58h** (53 mg, 64%) was prepared from **20a** (33 mg, 0.17 mmol) and **57h** (60 mg, 0.19 mmol) in the same manner as described for **21a**. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.71 (s, 1H), 8.59 (s, 1H), 7.50 – 7.42 (m, 2H), 7.35 (d, J = 1.6 Hz, 1H), 7.27–7.23 (m, 1H), 5.27 (s, 2H), 4.75 (s, 2H), 4.51 (d, J = 13.1 Hz, 2H), 3.27–3.19 (m, 2H), 2.03–1.95 (m, 1H), 1.89–1.82 (m, 2H), 1.73–1.62 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 174.09, 170.74, 167.65, 161.48, 143.27, 133.00, 131.49, 130.48, 130.38, 129.39, 128.64, 117.36, 108.37, 93.11, 69.90, 56.76, 46.91, 41.25, 27.44. MS (ESI) *m/z*: 473.1 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{18}FN_4O_5S$ ([M − H]$^-$) 473.0681, found 473.0683.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((3,5-

dichlorobenzyl)sulfonyl)piperidine-4-carboxamide (58i). Compound **58i** (50 mg, 42%) was prepared from **20a** (45 mg, 0.23 mmol) and **57i** (70 mg, 0.24 mmol) in the same manner as described for **21a**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.76 (s, 1H), 8.56 (s, 1H), 7.69 (s, 1H), 7.33 (s, 2H), 5.26 (s, 2H), 4.78 (s, 2H), 4.51 (d, J = 10.5 Hz, 2H), 3.27–3.22 (m, 2H), 2.68–2.56 (m, 1H), 1.92–1.82 (m, 2H), 1.71– 64 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 174.14, 170.80, 167.73, 161.55, 143.33, 134.07, 133.24, 129.41, 128.40, 117.39, 108.47, 93.20, 69.96, 56.29, 46.95, 41.34, 27.50. MS (ESI) *m/z*: 507.2[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{17}Cl_2N_4O_5S$ ([M − H]$^-$) 507.0302, found 507.0302.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((2,4-difluorobenzyl)sulfonyl)-4-methylpiperidine-4-carboxamide (58j). Compound **58j** (63 mg, 55%) was prepared from **20a (**50 mg, 0.26 mmol) and **57k** (75 mg, 0.24 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.50 (s, 1H), 7.48 (dd, J = 15.2, 8.4 Hz, 1H), 7.34–7.27 (m, 1H), 7.19–7.14 (m, 1H), 5.24 (s, 2H), 4.79 (s, 2H), 4.08–4.02 (m, 2H), 3.64–3.57 (m, 2H), 2.16–2.10 (m, 2H), 1.59–1.54 (m, 2H), 1.21 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.57, 170.72, 167.66, 161.38, 161.34, 143.29, 143.25, 117.45, 108.05, 92.77, 69.86, 45.32, 42.25, 34.07, 24.96. MS (ESI) *m/z*: 489.1 [M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{19}F_2N_4O_5S$ ([M − H]$^-$) 489.1040, found: 489.1039.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-N-((4-fluorobenzyl)sulfonyl)-4-methylpiperidine-4-carboxamide (58k). Compound **58k** (40 mg, 43%) was prepared from **20a (**43 mg, 0.22 mmol) and **57k** (60 mg, 0.2 mmol)

in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.55 (s, 1H), 7.39–7.34 (m, 2H), 7.23 (t, J = 8.8 Hz, 2H), 5.25 (s, 2H), 4.77 (s, 2H), 4.04 (ddd, J = 13.4, 6.0, 3.4 Hz, 2H), 3.60–3.53 (m, 2H), 2.16–2.10 (m, 2H), 1.60–1.53 (m, 2H), 1.19 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 176.46, 170.70, 167.66, 163.31, 161.39, 143.24, 132.88, 132.81, 125.39, 117.45, 115.53, 115.36, 108.15, 92.93, 69.87, 56.99, 44.71, 42.20, 33.15, 23.09. MS (ESI) *m/z*: 471.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{20}FN_4O_5S$ $([M - H]^-)$ 471.1133, found: 471.1137.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-methyl-N-((4-methylbenzyl)sulfonyl)piperidine-4-carboxamide (58l). Compound **58l** (260 mg, 73%) was prepared from **20a** (160 mg, 0.81 mmol) and **57l** (250 mg, 0.81 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.55 (s, 1H), 7.22–7.17 (m, 4H), 5.26 (s, 2H), 4.71 (s, 2H), 4.08–4.00 (m, 2H), 3.56–3.48 (m, 2H), 2.28 (s, 3H), 2.16–2.11 (m, 2H), 1.59–1.52 (m, 2H), 1.19 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 176.37, 170.71, 167.66, 161.38, 143.25, 138.12, 130.61, 129.05, 125.95, 117.44, 108.14, 92.91, 69.87, 57.60, 44.71, 42.19, 33.16, 23.22, 20.70. MS (ESI) *m/z*: 467.2$[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{23}H_{23}N_4O_5S$ $([M - H]^-)$ 467.1394, found: 467.1395.

***N*-((4-Chlorobenzyl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-methylpiperidine-4-carboxamide (58m).** Compound **58m** (64 mg, 63%) was prepared from **20a (**68 mg, 0.21 mmol) and **57m** (40 mg, 0.21 mmol) in the same manner as described for **21a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.39 (s, 1H), 8.56 (s, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 5.26 (s, 2H), 4.79 (s, 2H), 4.08

– 4.00 (m, 2H), 3.60–3.52 (m, 2H), 2.17–2.09 (m, 2H), 1.60–1.54 (m, 2H), 1.19 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.21, 168.16, 161.91, 143.74, 133.05, 129.04, 117.95, 108.66, 93.45, 70.37, 57.58, 45.21, 42.72, 33.64, 23.59. MS (ESI) *m/z*: 467.2[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{20}ClN_4O_5S$ ([M − H]$^-$) 487.0848, found: 487.0842.

***N*-((5-Chlorothiophen-2-yl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-fluoropiperidine-4-carboxamide (61).** Compound **6** (60 mg, 0.30 mmol) was added to a solution of **65** (60 mg, 0.19 mmol) and TEA (38 mg, 0.37 mmol) in DCM (5 mL) at 0 °C, and the reaction mixture was stirred at rt for 2 h. The mixture was diluted with DCM (30 mL), washed with $NaHCO_3$(2 × 10 mL, saturated, aq solution) and brine (40 mL), dried ($MgSO_4$) and concentrated. The crude material was purified purified by silica gel chromatography with dichloromethane/methanol (20:1) to yield **61** (50 mg, 56%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.25 (d, J = 3.9 Hz, 1H), 7.00 (d, J = 3.9 Hz, 1H), 5.28 (s, 2H), 4.44–4.37 (m, 2H), 3.46–3.40 (m, 2H), 2.17–2.08 (m, 2H), 1.90–1.84 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.46, 170.86, 167.72, 161.46, 146.07, 143.24, 130.88, 128.21, 125.79, 117.41, 108.38, 94.00, 93.10, 92.56, 69.95, 44.11, 32.86, 32.68. MS (ESI) *m/z*: 483.1[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{18}H_{13}ClFN_4O_5S_2$ ([M − H]$^-$) 483.0005, found: 482.9996.

***N*-(Benzylsulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-fluoropiperidine-4-carboxamide (62a).** Compound **62a** (92 mg, 78%) was prepared from **60 (**80 mg, 0.26 mmol) and **9** (45 mg, 0.26 mmol) in the same manner as described

for **61**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.63 (s, 1H), 7.44–7.36 (m, 3H), 7.34–7.26 (m, 2H), 5.29 (s, 2H), 4.76 (s, 2H), 4.51 (d, J = 13.6 Hz, 2H), 3.47–3.39 (m, 2H), 2.27–2.12 (m, 2H), 2.04–1.97 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.72, 167.61, 161.44, 143.27, 130.73, 128.70, 128.59, 117.32, 108.71, 94.08, 93.35, 92.58, 69.93, 57.70, 43.23, 31.37, 31.20. MS (ESI) *m/z*: 457.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{18}FN_4O_5S$ $([M - H]^-)$ 457.0987, found: 457.0984.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-fluoro-N-((1-phenylethyl)sulfonyl)piperidine-4-carboxamide (62b). Compound **62b** (58 mg, 56%) was prepared from **60 (**105 mg, 0.33 mmol) and **49b** (61 mg, 0.33 mmol) in the same manner as described for **61**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.93 (s, 1H), 8.61 (s, 1H), 7.41–7.36 (m, 5H), 5.28 (s, 2H), 4.85 (q, J = 7.2 Hz, 1H), 4.52–4.45 (m, 2H), 3.45–3.37 (m, 2H), 2.22–2.07 (m, 2H), 2.06–1.96 (m, 2H), 1.69 (d, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.20, 168.10, 161.93, 143.75, 134.35, 129.63, 129.40, 129.04, 117.80, 109.23, 94.56, 93.86, 93.06, 70.42, 62.62, 43.68, 15.22. MS (ESI) *m/z*: 471.2 $[M - H]^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{27}FN_3O_3Cl_2S$ $([M - H]^-)$ 471.1145, found: 471.1137.

1-(3-Cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-fluoro-N-((4-methylbenzyl)sulfonyl)piperidine-4-carboxamide (62c). Compound **62c** (60 mg, 69%) was prepared from **60 (**60 mg, 0.19 mmol) and **55g** (57 mg, 0.28 mmol) in the same manner as described for **61**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.97 (s, 1H), 8.60 (s, 1H), 7.22–7.13 (m, 4H), 5.28 (s, 2H), 4.60 (s, 2H), 4.54–4.43 (m, 2H), 3.48–3.39 (m, 3H), 2.30 (s, 3H), 2.24–2.09 (m, 2H), 2.04–1.94 (m, 2H). ^{13}C NMR (126 MHz,

DMSO-d_6) δ 171.44, 170.74, 167.63, 161.42, 143.26, 137.75, 130.56, 128.99, 128.81, 117.34, 108.63, 94.09, 93.28, 92.60, 69.93, 57.23, 45.73, 43.42, 31.70, 31.53, 20.73. MS (ESI) *m/z*: 471.2[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{22}H_{20}FN_4O_5S$ ([M − H]$^-$) 471.1144, found: 471.1135.

***N*-((4-Chlorobenzyl)sulfonyl)-1-(3-cyano-5-oxo-5,7-dihydrofuro[3,4-b]pyridin-2-yl)-4-fluoropiperidine-4-carboxamide (62d).** Compound **62d** (52 mg, 57%) was prepared from **60 (**60 mg, 0.19 mmol) and **55h** (57 mg, 0.28 mmol) in the same manner as described for **61**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (s, 1H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H), 5.28 (s, 2H), 4.75 (s, 2H), 4.51 (d, *J* = 13.6 Hz, 2H), 3.47–3.40 (m, 2H), 2.25–2.09 (m, 2H), 2.05–1.98 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.75, 170.01, 167.66, 161.50, 143.28, 133.60, 132.58, 128.64, 128.06, 117.35, 108.75, 94.16, 93.41, 92.66, 69.96, 56.91, 43.28, 31.45, 31.27. MS (ESI) *m/z*: 491.2[M − H]$^-$. HRMS (ESI) *m/z* calcd for $C_{21}H_{17}ClFN_4O_5S$ ([M − H]$^-$) 491.0598, found: 491.0589.

ADP-Induced Human PRP Aggregation Assay.[29-31, 39] Healthy volunteers that had not taken aspirin or other nonsteroidal anti-inflammatory drugs for at least two weeks were recruited, and written informed consent was obtained before blood collection. Blood samples were collected using 36 mL syringe tubes containing 6 mL of ACD solution (85 mM sodium citrate, 71.38 mM citric acid and 27.78 mM glucose). The platelet-rich plasma (PRP) was obtained by centrifugation of the blood at 300 g for 10 min at room temperature. The supernatant PRP was transferred to a fresh tube, and the remaining portion was centrifuged at 2000 g for 10 min to obtain platelet-poor plasma

(PPP), which was transferred into another tube for use in the assay. The platelet aggregation was analyzed using a double-channel aggregometer (Model 400VS, Chrono-Log, Haverston, PA, USA) with stirring (900 rpm) at 37 ℃. The assayed dose-response concentrations of the test compounds and AZD1283 were 3, 10, 30, 100 and 300 μM in 0.5% DMSO. The aggregometer system was calibrated with PRP (0% aggregation control = baseline) and PPP (100% aggregation control). Fresh PRP (400 μL) was added to a cuvette and incubated at 37 ℃ for 1 min with stirring before being transferred to the aggregometer. The baseline was set using PPP as a blank. The solution of the test compound (20 μL) was added to the fresh PRP, and then the mixture was warmed at 37 ℃ for 2 min. Platelets were stimulated with adenosine 5-diphosphate (20 μL, 10 μM final concentration; ADP-sodium salt, Aladdin Chemical Co, China). Platelet aggregation was monitored for 5 min by recording the variations of optical density according to the method described by G. V. Born.[39] The aggregation inhibition rate was calculated as follows: inhibition rate (%) = [(control tube maximal aggregation response − test tube maximum aggregation response) / control tube maximum aggregation response] × 100%. The IC_{50} values were calculated by nonlinear regression using XLfit software. All experiments using human subjects were performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Fudan University.

Metabolic Stability Assay. The assay was performed using liver microsomes from male Sprague–Dawley rats (BD Gentest, USA), dog and humans. The test compounds (final concentration of 0.1 μM in 0.01% DMSO with 0.005% bovine serum albumin)

were incubated with live microsomes (0.33 mg/mL in 0.1 M tris(hydroxymethyl) aminomethane/hydrochloric acid buffer (pH 7.4), cofactor $MgCl_2$ (5 mM) and NADPH (1 mM)) at 37 °C for 60 min. Aliquots were taken at 0, 7, 17, 30 and 60 min, and the enzymatic reaction was stopped by protein precipitation with methanol. After centrifugation, the samples were analyzed by LC/MS/MS. The metabolic stability of the compounds is presented as the in vitro half-life ($T_{1/2}$), clearance (Cl_{int}) and metabolic bioavailability (MF%) in rat and human liver microsomes as previously described.[34, 35]

Rat Pharmacokinetic Studies. The Pharmacokinetic parameters of compounds AZD-1283, **21a**, **22a**, **22b**, **58l**, **58m** and **62a** were subjected to PK studies on male Sprague−Dawley rats weighing between (200 and 220 g) with three animals in each group. The tested compounds (5% DMSO +95% HPMC) were administered orally at a dose of 5 mg/kg. Serial specimens (0.3 mL) were collected via the retrobulbar vein 0.25 h, 0.5 h, 1.0 h, 2.0 h, 4.0 h, 8.0 h, and 24h after administration and quantified by liquid chromatography(LC)/tandem mass spectrometry (MS/MS). Pharmacokinetic parameters were calculated from the mean plasma concentration by noncompartmental analysis. The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China).

CYP450 Inhibition Cocktail Assay. Experiments were performed in 96-well plates with final incubation volume of 100 μL per well. Each well contained human liver microsomes (20 μL, final concentration 0.3 mg/mL), test compound (50 μL) or positive control inhibitor, and the probe substrates (20 μL, in 0.1 M Tris (pH 7.4)). After a pre-

incubation at 37 °C for 10 min, the reaction started with the addition of NADPH (10 μL) to make a solution of 1 mM final concentration. The plates were incubated at 37 °C for 15 min before being quenched by the addition of acetonitrile (100 μL) with a mixture of internal standard (propranolol, nadolol) (50 nM). After the quenching, plates were centrifuged and supernatants were analyzed by LC/MS/MS. The inhibition rate was determined at five different concentrations of the test compound. The substrates used in the assays included phenacetin (CYP1A2), tolbutamide (CYP2C9), mephenytoin (CYP2C19), dextromethorphan CYP2D6), midazolam (CYP3A4(M)) and testosterone (CYP3A4 (T)).

hERG K^+ Channel Inhibition Assay. Whole-cell recordings were performed using automated QPatch (Sophion). The cells were voltage clamped at a holding potential of −80 mV. The hERG current was activated by depolarizing at +20 mV for 5 s, after which the current was brought back to −50 mV and kept for 5 s to remove the inactivation and to observe the deactivating tail current. The maximum amount of tail current was used to determine hERG current amplitude. Compound stock solutions (10 or 30 mM in DMSO) were prepared before the experiments. The stock solutions were diluted to test concentrations upon use. After achieving break-in (whole-cell) configuration, the cells were recorded for 120 s to assess current stability. The voltage protocol described above was then applied to the cells every 20 s throughout the whole procedure. Only stable cells with recording parameters above threshold were allowed for subsequent drug additions. External solution containing 0.1% DMSO (vehicle) was applied to the cells to establish the baseline. The current was allowed to stabilize for 3

min before test compound was added. The cells were kept in the test solution until the compound's effect reached a steady state for a minimum of 3 min. Drug washout was performed with external solution until the recovery of the current reached a steady state. Cisapride was used as the positive control. Data were analyzed using Assay Software provided by Sophion, XLFit, or Graphpad Prism 6.0.

Solubility Assay. NaCl (20 mg) and 12 M HCl aqueous solution (70 μL) were mixed and the mixture was diluted to 10 mL with water to prepare gastric juice (pH = 1.2). KH_2PO_4 (68 mg) was dissolved in 2500 μL of water followed by the addition of 770 μL 0.2 M NaOH aqueous solution and 5 mL water, 0.2 M NaOH aqueous solution or 0.2 M HCl aqueous solution was used to adjust the pH of mixture to 6.8 ± 0.1, and the mixture was diluted to 10 mL with water to prepare intestinal juice. Tested compound (3 mg) was added to different matrix (500 μL), followed by shaking the mixtures at 37 °C for 24 h. Then, the mixtures were centrifuged for 30 min, supernatants were centrifuged again for 30 min. Finally, supernatants were diluted 1:1 by water and were centrifuged for 5 min. Analysis carried out by LC/MS/MS.

Rat $FeCl_3$ Thrombosis Model. The $FeCl_3$ model was performed based on previous reports with slight modifications.[40-42] Male rats (250−300 g, n = 10 per dose group) were acclimatized in the laboratory (room temperature of 22 ± 2 °C and humidity 60%) at least 7 days, and no abnormal behavior was observed during the adaptation period. Solutions of compound **21a**, **21b**, **58l**, clopidogrel or vehicle suspended in 0.5% carboxymethylcellulose sodium (CMC-Na) were administered as single oral doses by oral gavage at 10 mL/kg. The rats were anesthetized by an intraperitoneal injection of

2% pentobarbital sodium salt hydrate at a dose of 50 mg/kg and then placed on a heating pad (to maintain body temperature). A 1.5-cm-long portion of the left carotid artery was isolated through a neck skin incision, and a 1.0 × 1.5 cm plastic strip catheter was inserted to protect the nearby tissue. At 1.5 h after drug administration, a strip of filter paper (0.8 × 1.0 cm) saturated with 20% $FeCl_3$ in water was placed on the anterior of the carotid artery for 10 min to induce thrombus formation. Thirty minutes after application of the filter paper, the artery was dissected free. The thrombus was removed, cleaned in saline, blotted dry, and weighed. All experiments were conducted in accordance with and approved by the Animal Care and Use Committee of China Pharmaceutical University (Nanjing, China).

Rat Tail-Bleeding Model. The rat tail-bleeding model as described previously with minor modifications.[38] Male rats (250−300 g, n = 10 per dose group) were acclimatized in the laboratory (room temperature of 22 ± 2 °C and humidity 60%) at least 7 days, and no abnormal behavior was observed during the adaptation period. The rats were administered **21a**, **22a**, **22b**, **58l**, clopidogrel or vehicle by oral gavage. After 1.5 h, they were anesthetized by intraperitoneal injection of 2% pentobarbital sodium salt hydrate at a dose of 50 mg/kg for transection. The animals were then placed on a heating pad (to maintain their body temperature) with their tails straightened. Rat tails were transected 4-mm from the tip with a scalpel blade and immediately immersed into a 20 mL graduated cylinder filled with 10 mL of saline held at 37 °C. The observation period was stopped when no additional bloodstains were observed in a period of thirty seconds. All experiments were conducted in accordance with and approved by the Animal Care

and Use Committee of China Pharmaceutical University (Nanjing, China).

■ASSOCIATED CONTENT

Supporting Information.

Preparation procedures and characterization data of all intermediates, and molecular formula strings (CSV) of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■AUTHOR INFORMATION

Corresponding Authors

* Wenhao Hu: Tel: 86-20-39943117; Fax: 86-022-39943000; E-mail: huwh9@mail.sysu.edu.cn.

* Yushe Yang: Tel: 86-21-50806786; Fax: 86-21-50806786; E-mail: ysyang@simm.ac.cn.

Notes

The authors declare no competing financial interest.

■ACKNOWLEDGMENTS

The work was supported by the National Natural Science Foundation of China (21332003), the Program for Guangdong Introducing Innovative and Entrepreneurial Teams (No. 2016ZT06Y337) and the Youth Innovation Promotion Association of Chinese Academy of Sciences (2016262).

■ ABBREVIATIONS USED

ADP, adenosine diphosphate; ACS, acute coronary syndrome; aze, azetidinyl; CYP450, cytochrome P450; CbzCl, benzyl chloroformate; CLint, intrinsic clearance; DMF/DMA, *N*,*N*-dimethylformamide dimethyl acetal; DMAP, 4-(dimethylamino)-

pyridine; DIPEA, *N*,*N*-diisopropylethylamine; DTD, 1,3,2-dioxathiolan-2,2-oxide; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; GPCRs, G-protein coupled receptors；HOBt, 1-hydroxybenzotria-zole; hPRP, human platelet rich plasma ; hERG, human *ether-à-go-go*-related gene; HLMs, human liver microsomes; NCS, *N*-chlorosuccinimide; pip, piperidinyl; pyr, pyrrolyl; SAR, structure-activity relationship; SPR, structure-pharmacokinetic relationship; SD, Sprague−Dawley; TBSCl, *tert*-butyldimethylsilyl chloride; TBTU, O-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate; TEA, triethylamine; TFA, trifluoroacetate.

REFERENCES

(1) Michelson, A. D. Antiplatelet therapies for the treatment of cardiovascular disease. *Nat. Rev. Drug Discovery* **2010**, 9, 154−169.

(2) Davì, G.; Patrono, C. Platelet activation and atherothrombosis. *N. Engl. J. Med.* **2007**, 357, 2482−2494.

(3) Jennings, L. K. Mechanisms of platelet activation: need for new strategies to protect against platelet-mediated atherothrombosis. *Thromb. Haemostasis* **2009**, 102, 248−257.

(4) Hollopeter, G.; Jantzen, H. M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V.; Yang, R. B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P. B. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **2001**, 409, 202−207.

(5) Abbracchio, M. P.; Burnstock, G.; Boeynaems, J. M.; Barnard, E. A.; Boyer, J. L.; Kennedy, C.; Knight, G. E.; Fumagalli, M.; Gachet, C.; Jacobson, K. A.; Weisman, G. A. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy.

Pharmacol. Rev. **2006**, 58, 281−341.

(6) Dorsam, R. T.; Kunapuli, S. P. Central role of the $P2Y_{12}$ receptor in platelet activation. *J. Clin. Invest.* **2004,** 113, 340−345.

(7) Fox, K. A.; Mehta, S. R.; Peters, R.; Zhao, F.; Lakkis, N.; Gersh, B. J.; Yusuf, S.; Benefits and risks of the combination of clopidogrel and aspirin in patients undergoing surgical revascularization for non-ST-elevation acute coronary syndrome: the clopidogrel in unstable angina to prevent recurrent ischemic events (CURE) trial. *Circulation* **2004**, 110, 1202−1208.

(8) Steg, P. G.; James, S. K.; Atar, D.; Badano, L. P.; Blomstrom Lundqvist, C.; Borger, M. A.; Di Mario, C.; Dickstein, K.; Ducrocq, G.; Fernandez-Aviles, F.; Gershlick, A. H.; Giannuzzi, P.; Halvorsen, S.; Huber, K.; Juni, P.; Kastrati, A.; Knuuti, J.; Lenzen, M. J.; Mahaffey, K. W.; Valgimigli, M.; van't Hof, A.; Widimsky, P.; Zahger, D.; Bax, J. J.; Baumgartner, H.; Ceconi, C.; Dean, V.; Deaton, C.; Fagard, R.; FunckBrentano, C.; Hasdai, D.; Hoes, A.; Kirchhof, P.; Knuuti, J.; Kolh, P.; McDonagh, T.; Moulin, C.; Popescu, B. A.; Reiner, Ž.; Sechtem, U.; Sirnes, P. A.; Tendera, M.; Torbicki, A.; Vahanian, A.; Windecker, S.; Hasdai, D.; Astin, F.; Åström-Olsson, K.; Budaj, A.; Clemmensen, P.; Collet, J.-P.; Fox, K. A.; Fuat, A.; Gustiene, O.; Hamm, C. W.; Kala, P.; Lancellotti, P.; Maggioni, A. P.; Merkely, B.; Neumann, F.-J.; Piepoli, M. F.; Van de Werf, F.; Verheugt, F.; Wallentin, L. ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur. Heart J.* **2012**, 33, 2569−2619.

(9) O'Gara, P. T.; Kushner, F. G.; Ascheim, D. D.; Casey, D. E., Jr.; Chung, M. K.; de

Lemos, J. A.; Ettinger, S. M.; Fang, J. C.; Fesmire, F. M.; Franklin, B. A.; Granger, C. B.; Krumholz, H. M.; Linderbaum, J. A.; Morrow, D. A.; Newby, L. K.; Ornato, J. P.; Ou, N.; Radford, M. J.; Tamis-Holland, J. E.; Tommaso, C. L.; Tracy, C. M.; Woo, Y. J.; Zhao, D. X.; Anderson, J. L.; Jacobs, A. K.; Halperin, J. L.; Albert, N. M.; Brindis, R. G.; Creager, M. A.; DeMets, D.; Guyton, R. A.; Hochman, J. S.; Kovacs, R. J.; Kushner, F. G.; Ohman, E. M.; Stevenson, W. G.; Yancy, C. W. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *J. Am. Coll. Cardiol.* **2013**, 61, e78−e140.

(10) Mega, J. L.; Close, S. L.; Wiviott, S. D.; Shen, L.; Hockett, R. D.; Brandt, J. T.; Walker, J. R.; Antman, E. M.; Macias, W.; Braunwald, E.; Sabatine, M. S. Cytochrome P-450 polymorphisms and response to clopidogrel. *N. Engl. J. Med.* **2009**, 360, 354−362.

(11) Hagihara, K.; Kazui, M.; Ikenaga, H.; Nanba, T.; Fusegawa, K.; Takahashi, M.; Kurihara, A.; Okazaki, O.; Farid, N. A.; Ikeda, T. Comparison of formation of thiolactones and active metabolites of prasugrel and clopidogrel in rats and dogs. *Xenobiotica* **2009**, 39, 218−226.

(12) Müller, I.; Seyfarth, M.; Rüdiger, S.; Wolf, B.; Pogatsa-Murray, G.; Schömig, A.; Gawaz, M. Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement. *Heart* **2001**, 85, 92−93.

(13) Gurbel, P. A.; Bliden, K. P.; Hiatt, B. L.; O'Connor, C. M. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet

reactivity. *Circulation* **2003**, 107, 2908−2913.

(14) Sibbing, D.; Braun, S.; Morath, T.; Mehilli, J.; Vogt, W.; Schomig, A.; Kastrati, A.; von Beckerath, N. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J. Am. Coll. Cardiol.* **2009**, 53, 849−856.

(15) Kalra, K.; Franzese, C. J.; Gesheff, M. G.; Lev, E. I.; Pandya, S.; Bliden, K. P.; Tantry, U. S.; Gurbel, P. A. Pharmacology of antiplatelet agents. *Curr. Atheroscler. Rep.* **2013**, 15, 371.

(16) Baker, D. E.; Ingram, K. T. Cangrelor. *Hosp. Pharm.* **2015**, 50, 922−929.

(17) Armstrong, D.; Summers, C.; Ewart, L.; Nylander, S.; Sidaway, J. E.; van Giezen, J. J. Characterization of the adenosine pharmacology of ticagrelor reveals therapeutically relevant inhibition of equilibrative nucleoside transporter 1. *J. Cardiovasc. Pharmacol. Ther.* **2014**, 19, 209−219.

(18) VAN Giezen, J. J.; Nilsson, L.; Berntsson, P.; Wissing, B. M.; Giordanetto, F.; Tomlinson, W.; Greasley, P. J. Ticagrelor binds to human $P2Y_{12}$ independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. *J. Thromb. Haemostasis* **2010**, 7, 1556−1565.

(19) Van Giezen, J. J.; Berntsson, P.; Zachrisson, H.; Björkman, J. A. Comparison of ticagrelor and thienopyridine $P2Y_{12}$ binding characteristics and antithrombotic and bleeding effects in rat and dog models of thrombosis/hemostasis. *Thromb. Res.* **2009**, 124, 565−571.

(20) Mcfadyen, J. D.; Schaff, M.; Peter, K. Current and future antiplatelet therapies:

emphasis on preserving haemostasis. *Nat. Rev. Cardiol.* **2018**, 15, 181−191.

(21) Cattaneo, M. Platelet P2 receptors: old and new targets for antithrombotic drugs. *Expert Rev. Cardiovasc. Ther.* **2007**, 5, 45−55.

(22) Bach, P.; Antonsson, T.; Bylund, R.; Bjorkman, J. A.; Osterlund, K.; Giordanetto, F.; van Giezen, J. J.; Andersen, S. M.; Zachrisson, H.; Zetterberg, F. 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. *J. Med. Chem.* **2013**, 56, 7015−7024.

(23) Zetterberg, F.; Svensson, P. State of affairs: Design and structure-activity relationships of reversible $P2Y_{12}$ receptor antagonists. *Bioorg. Med. Chem. Lett.* **2016**, 26, 2739−2754.

(24) Zhang, K.; Zhang, J.; Gao, Z. G.; Zhang, D.; Zhu, L.; Han, G. W.; Moss, S. M.; Paoletta, S.; Kiselev, E.; Lu, W.; Fenalti, G.; Zhang, W.; Muller, C. E.; Yang, H.; Jiang, H.; Cherezov, V.; Katritch, V.; Jacobson, K. A.; Stevens, R. C.; Wu, B.; Zhao, Q. Structure of the human $P2Y_{12}$ receptor in complex with an antithrombotic drug. *Nature* **2014**, 509, 115−118.

(25) Paoletta, S.; Sabbadin, D.; von Kugelgen, I.; Hinz, S.; Katritch, V.; Hoffmann, K.; Abdelrahman, A.; Strassburger, J.; Baqi, Y.; Zhao, Q.; Stevens, R. C.; Moro, S.; Muller, C. E.; Jacobson, K. A. Modeling ligand recognition at the $P2Y_{12}$ receptor in light of X-ray structural information. *J. Comput. Aided Mol. Des.* **2015**, 29, 737−756.

(26) Al-Mousawi, S.; Abdelkhalik, M. M.; John, E.; Elnagdi, M. H. Enaminones as building blocks in heterocyclic syntheses: a new approach to polyfunctionally

substituted cyclohexenoazines. *ChemInform* **2010**, 40, 689−695.

(27) Gillman, K. V.; Starrett, J. E.; Parker, M. F.; Xie, K.; Bronson, J. J.; Marcin, L. R.; McElhone, K. E.; Bergstrom, C. P.; Mate, R. A.; Williams, R.; Meredith, J. E.; Burton, C. R.; Barten, D. M.; Toyn, J. H.; Roberts, S. B.; Lentz, K. A.; Houston, J. G.; Zaczek, R.; Albright, C. F.; Decicco, C. P.; Macor, J. E.; Olson, R. E. Discovery and evaluation of BMS-708163, a potent, selective and orally bioavailable γ-secretase inhibitor. *Med. Chem. Lett.* 2010, 1, 120−124.

(28) Yang, Z.; Xu, J. ChemInform abstract: convenient and environment-friendly synthesis of sulfonyl chlorides from S-alkylisothiourea salts via N-chlorosuccinimide chlorosulfonation. *ChemInform* **2013**, 45, 1675−1682.

(29) Zhang, S.; Zhang, S.; Hu, L.; Zhai, L.; Xue, R.; Ye, J.; Chen, L.; Cheng, G.; Mruk, J.; Kunapuli, S. P. Nucleotide-binding oligomerization domain 2 receptor is expressed in platelets and enhances platelet activation and thrombosis. *Circulation* **2015**, 131, 1160−1170.

(30) Levade, M.; David, E.; Garcia, C.; Laurent, P. A.; Cadot, S.; Michallet, A. S.; Bordet, J. C.; Tam, C.; Sié, P.; Ysebaert, L. Ibrutinib treatment affects collagen and von Willebrand factor-dependent platelet functions. *Blood* **2014**, 124, 3991−3995.

(31) Boldron, C.; Besse, A.; Bordes, M. F.; Tissandie, S.; Yvon, X.; Gau, B.; Badorc, A.; Rousseaux, T.; Barre, G.; Meneyrol, J.; Zech, G.; Nazare, M.; Fossey, V.; Pflieger, A. M.; Bonnet-Lignon, S.; Millet, L.; Briot, C.; Dol, F.; Herault, J. P.; Savi, P.; Lassalle, G.; Delesque, N.; Herbert, J. M.; Bono, F. N-[6-(4-Butanoyl-5-methyl-1H-pyrazol-1-yl)pyridazin-3-yl]-5-chloro-1-[2-(4-methyl piperazin-1-yl)-2-oxoethyl]-1H-indole-3-

carboxamide (SAR216471), a novel intravenous and oral, reversible, and directly acting $P2Y_{12}$ antagonist. *J. Med. Chem.* **2014**, 57, 7293−7316.

(32) Talele, T. T. The "cyclopropyl fragment" is a versatile player that frequently appears in preclinical/clinical drug molecules. *J. Med. Chem.* **2016,** 59, 8712−8756.

(33) Giordanetto, F.; Bach, P.; Zetterberg, F.; Antonsson, T.; Bylund, R.; Johansson, J.; Sellen, M.; Brown, D.; Hidestahl, L.; Berntsson, P.; Hovdal, D.; Zachrisson, H.; Bjorkman, J. A.; van Giezen, J. J. Optimization of ketone-based $P2Y_{12}$ receptor antagonists as antithrombotic agents: pharmacodynamics and receptor kinetics considerations. *Bioorg. Med. Chem. Lett.* **2014**, 24, 2963−2968.

(34) RS, O. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab. Dispos.* **1999**, 27, 1350−1359.

(35) Lau, Y. Y.; Krishna, G.; Yumibe, N. P.; Grotz, D. E.; Sapidou, E.; Norton, L.; Chu, I.; Chen, C.; Soares, A. D.; Lin, C. C. The use of in vitro metabolic stability for rapid selection of compounds in early discovery based on their expected hepatic extraction ratios. *Pharmaceutical Research* **2002**, 19, 1606−1610.

(36) Sibbing, D.; Schulz, S.; Braun, S.; Morath, T.; Stegherr, J.; Mehilli, J.; Schömig, A.; Von, B. N.; Kastrati, A. Antiplatelet effects of clopidogrel and bleeding in patients undergoing coronary stent placement. *J. Thromb. Haemostasis* **2010**, 8, 250−256.

(37) Lipiecki, J.; Dupuy, P.; Slama, M.; Teiger, E.; Wittenberg, O.; Hanssen, M. 032 Differences in clinical presentation and 6-month outcomes in different age classes of elderly patients (75-80, 81-85, and >85 years). Data from prospective, nationwide,

French PAPI registry. *Arch. Cardiovasc. Dis. Suppl.* **2010**, 2, 11−11.

(38) Delesque-Touchard, N.; Pflieger, A. M.; Bonnet-Lignon, S.; Millet, L.; Salel, V.; Boldron, C.; Lassalle, G.; Herbert, J. M.; Savi, P.; Bono, F. SAR216471, an alternative to the use of currently available $P2Y_{12}$ receptor inhibitors? *Thromb. Res.* **2014**, 134, 693−703.

(39) Born, P. G. V. R. Aggregation of blood platelets by adenosine diphosphate (ADP) and its reversal. *Nature* **1962**, 194, 927−929.

(40) Kurz, K. D.; Main, B. W.; Sandusky, G. E. Rat model of arterial thrombosis induced by ferric chloride. *Thromb. Res.* **1990**, 60, 269−280.

(41) Liu, A. C.; Zhao, L. X.; Shu-Wen, Y. U.; Lou, H. X. Pre-treatment with puerarin affects pharmacokinetics of warfarin, but not clopidogrel, in experimental rats. *Chin. J. Nat. Med.* **2015**, 13, 257−263.

(42) Caroff, E.; Hubler, F.; Meyer, E.; Renneberg, D.; Gnerre, C.; Treiber, A.; Rey, M.; Hess, P.; Steiner, B.; Hilpert, K.; Riederer, M. A. 4-((R)-2-{[6-((S)-3-Methoxypyrrolidin-1-yl)-2-phenylpyrimidine-4-carbonyl]amino}-3-phosphonopropionyl)piperazine-1-carboxylic acid butyl ester (ACT-246475) and its prodrug (ACT-281959), a novel $P2Y_{12}$ receptor antagonist with a wider therapeutic window in the rat than clopidogrel. *J. Med. Chem.* **2015**, 58, 9133−9153.

Table of Contents Graphic

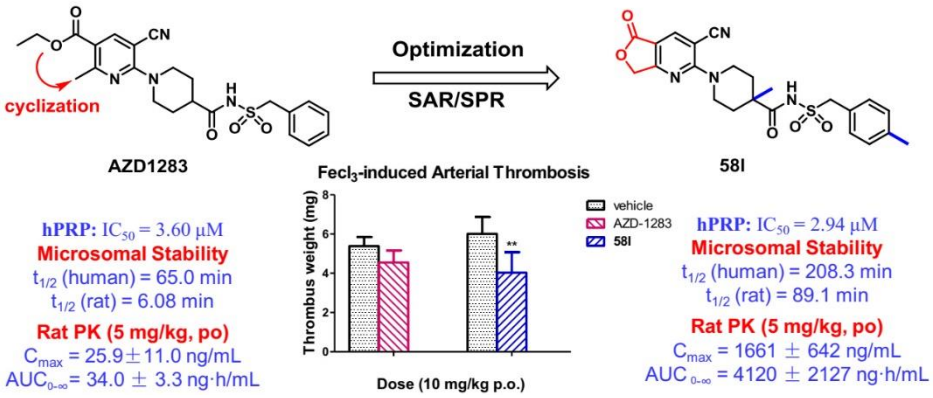


Table of Contents graphic