Discovery of 1,3-Diaminobenzenes as Selective Inhibitors of Platelet Activation at the PAR1 Receptor

Citation

Dockendorff, Chris, Omozuanvbo Aisiku, Lynn VerPlank, James R. Dilks, Daniel A. Smith, Susanna F. Gunnink, Louisa Dowal, et al. 2012. Discovery of 1,3-diaminobenzenes as selective inhibitors of platelet activation at the PAR1 receptor. ACS Medicinal Chemistry Letters 3(3): 232-237.

Published Version

doi:10.1021/ml2002696

ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Discovery of 1,3-Diaminobenzenes as Selective Inhibitors of Platelet Activation at the PAR1 Receptor

Chris Dockendorff,*[†] Omozuanvbo Aisiku,[‡] Lynn VerPlank,[†] James R. Dilks,[‡] Daniel A. Smith,[‡] Susanna F. Gunnink,[‡] Louisa Dowal,[‡] Joseph Negri,[†] Michelle Palmer,[†] Lawrence MacPherson,[†] Stuart L. Schreiber,[†,§] and Robert Flaumenhaft*[‡]

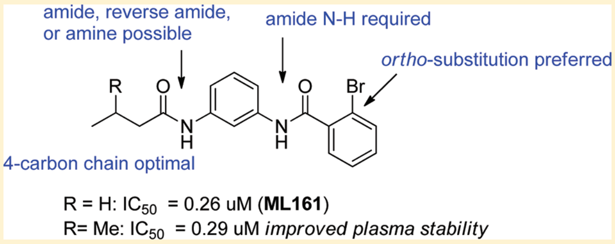
[†]Chemical Biology Platform and Probe Development Center, Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, Massachusetts 02142, United States

[‡]Division of Hemostasis and Thrombosis, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215, United States

[§]Howard Hughes Medical Institute, Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, Massachusetts 02142, United States

Supporting Information

ABSTRACT: A high-throughput screen of the NIH-MLSMR compound collection, along with a series of secondary assays to identify potential targets of hit compounds, previously identified a 1,3-diaminobenzene scaffold that targets protease-activated receptor 1 (PAR1). We now report additional structure–activity relationship (SAR) studies that delineate the requirements for activity at PAR1 and identify plasma-stable analogues with nanomolar inhibition of PAR1-mediated platelet activation. Compound **4** was declared as a probe (ML161) with the NIH Molecular Libraries Program. This compound inhibited platelet aggregation induced by a PAR1 peptide agonist or by thrombin but not by several other platelet agonists. Initial studies suggest that ML161 is an allosteric inhibitor of PAR1. These findings may be important for the discovery of antithrombotics with an improved safety profile.



KEYWORDS: *platelet activation, PAR1 inhibitor, allosteric inhibitor, 1,3-diaminobenzene, ML161, MLPCN MLSMR*

Antiplatelet agents are an important part of regimens for patients at risk for adverse cardiovascular events, decreasing the probability of such events by minimizing thrombus formation following rupture of atherosclerotic plaque.[1] However, contemporary antiplatelet drugs are only partially effective as evidenced by the substantial recurrence rate of arterial thrombosis despite current therapy.[2] Furthermore, the beneficial effect of antiplatelet agents is tempered by an increased risk of dangerous hemorrhagic complications.[3] Many developmental programs focused on new drug targets on platelets have been initiated over the past decade. Prominent among these programs have been those identifying and testing compounds that block protease-activated receptor 1 (PAR1)-mediated platelet activation.

Several inhibitors of PAR1 have been developed. The most advanced in clinical trials is vorapaxar (SCH530348), which was developed from a lead identified by a radioligand binding approach using a high affinity Thrombin Receptor Agonist Peptide.[4] Vorapaxar is a potent inhibitor of PAR1 but was associated with an increased risk of intracranial bleeding when used in combination with standard therapy in a phase III trial (TRA-CER).[5] Atopaxar (E5555) is a second PAR1 inhibitor in advanced clinical trials. Atopaxar therapy is associated with anti-PAR1 activity ex vivo; however, its use was associated with elevation of liver transaminases and QTc prolongation at higher doses in phase II trials.[6−8] Other small molecule PAR1 inhibitors have been described[9−13] but have not been tested in clinical trials. Safety and efficacy issues with current antiplatelet therapies, including investigational PAR1 antagonists, highlight the need for antiplatelet agents that may act via alternative mechanisms.

We recently reported[14] a high-throughput screen of the National Institute of Health Molecular Libraries Small Molecule Repository (NIH-MLSMR) small-molecule library (~300000 compounds), undertaken to identify inhibitors of granule secretion and/or platelet activation.[15,16] The primary screen measured adenosine triphosphate (ATP) secreted from dense granules following SFLLRN-induced activation through PAR1[9−13] using a luciferin/luciferase detection system. Several chemically tractable scaffolds were identified that inhibited dense granule secretion. Target identification studies with hits from this screen showed that compounds with a 1,3-diaminobenzene core act at PAR1. This paper describes structure−activity relationship (SAR) studies of this class of

Received: November 10, 2011
Accepted: January 23, 2012
Published: January 30, 2012

PAR1 inhibitors as well as preliminary studies supporting an allosteric mode of action.

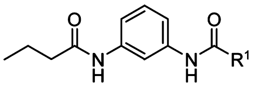
Several compounds were identified by high-throughput screening (HTS) with a 1,3-diamidobenzene core. Of these, compound **4** was selected as a starting point for SAR studies, as it showed acceptable potency in the primary assay measuring inhibition of dense granule release (IC_{50} = 1−10 μM).[17] Importantly, it was inactive in a luciferase counterscreen, and it showed little or no inhibition of platelet activation stimulated through other receptors (Figure S1 in the Supporting Information). Inhibition of PAR1 platelet activation by compound **4** was readily reversible. In addition, the compound did not inhibit phosphodiesterase 3A (PDE3A), which is present within platelets and promotes activation by suppressing cAMP levels.[18] Compound **4** also showed acceptable solubility in PBS (40 μM) and good inhibition in a standard assay with human platelets measuring SFLLRN-induced surface expression of P-selectin (Table 1),[19] a transmembrane cell adhesion molecule that is sequestered in platelet α-granules and expressed on the platelet surface following activation.[20]

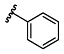
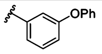
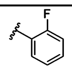
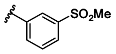
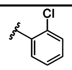
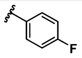
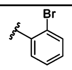
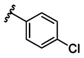
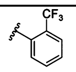
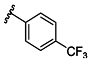
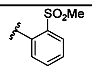
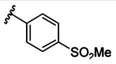
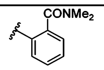
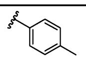
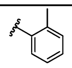
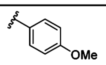
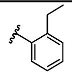
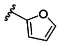
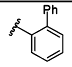
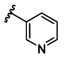
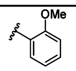
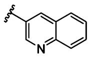
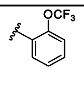
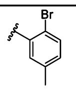
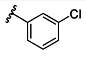
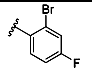
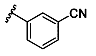
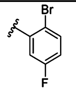
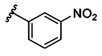
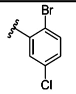
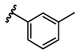
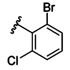
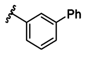
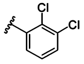
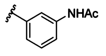
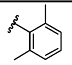
SARs of the 1,3-diamidobenzene scaffold are described in Tables 1−4. Compounds were prepared according to representative Schemes 1 or 2 (see the Supporting Information for details).

Keeping the west side of compound **4** fixed, the aryl substituent R^1 was investigated comprehensively (Table 1), and analogues were tested in the P-selectin assay. Maximal activities were observed with *ortho* substituents (compounds **2**−**12**); substituents at the *meta* and *para* positions had neutral or negative effects on potency (compounds **13**−**26**). The best results were observed with electron-withdrawing or neutral lipophilic *ortho* groups, with the bromide substituent of compound **4** proving optimal, giving an IC_{50} of 0.26 μM. Methyl (**8**) and ethyl (**9**) also showed good potencies, although a larger *ortho* substituent (phenyl, **10**) was not tolerated. A select number of heterocycles (furan, 3-pyridyl, and 3-quinolinyl, **27**−**29**) were poorly active or inactive. In an attempt to improve the potency, disubstituted analogues **30**−**36** were prepared, but all showed lower potencies than **4**.

Concurrent with our explorations at the east end of the scaffold, the alkyl chain at the west end was investigated (Table 2). Optimal potency was observed with a 3-carbon chain (**4**). Some potency was retained with the 2-carbon chain (**38**), but a 4-carbon chain (**39**) failed to demonstrate inhibition. Replacement of the alkyl chain with a phenyl ring (**40**) decreased activity at the target. We expected that branched alkyl chains could provide compounds with improved plasma stability (PS) over **4**, which showed moderate stability in human plasma (80% remaining after 5 h) but poor stability in mouse plasma (<2%). Mouse plasma stable compounds are required for study in a number of our in vivo disease models, so we attempted to address this liability of **4**. We hypothesized that branched alkyl chains could give compounds more resistant to proteases and esterases, and in fact, **42** and **43** showed some improvement. Compound **42** had only moderate potency but acceptable mouse PS (83% after 5 h). Cyclopentane **43** had acceptable potency (IC_{50} = 0.52 μM) but poor mouse PS (26%). The lack of activity observed with 1,4-diamide **44** indicates that the 1,3-substitution pattern about the central ring is important.

Table 1. SAR at the East End of the 1,3-Diamidobenzene Scaffold

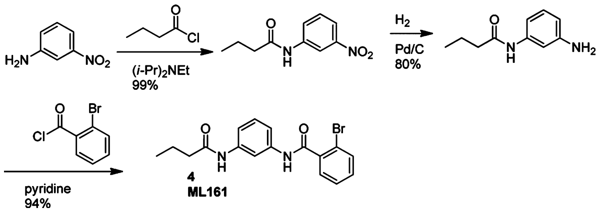


Cmpd	R^1	%inhib. IC_{50} (μM)[a]	Cmpd	R^1	%inhib. IC_{50} (μM)[a]
1		64%	**19**		65%[b]
2		99% 0.97	**20**		25%
3		>99% 1.10	**21**		85%
4		>99% 0.26	**22**		80%
5		99% 1.29	**23**		30%
6		21%	**24**		10%
7		48%	**25**		25%
8		98% 0.77	**26**		15%
9		>99% 0.52	**27**		30%
10		<1%	**28**		<1%
11		50%	**29**		10%
12		>99% 0.71	**30**		>99% 0.48
13		80%[b]	**31**		>99% 0.39
14		70%	**32**		>99% 0.60
15		55%	**33**		68% 0.38
16		88%	**34**		>99% 2.28
17		98% 2.20	**35**		99% 3.12
18		55%	**36**		98% 3.00

[a] % Inhibition of platelet P-selectin expression on human platelets induced by 5 μM SFLLRN. See the Supporting Information for details. The IC_{50} value was only determined for compounds inhibiting expression by at least 90% at 10 μM. The value is an average of three measurements. [b] Measured at 3 μM.

In a search for more potent, plasma-stable compounds, we continued our investigations by varying the central 1,3-diamidobenzene ring (Table 3). Several heterocyclic compounds were prepared, but neither pyridyl, benzimidizole, nor oxazole analogues (**45**−**50**) showed any significant activity in our assay. Substituents at the 2- and 6-positions of the central

Scheme 1. Synthesis of 4 (ML161)



Scheme 2. Synthesis of 68

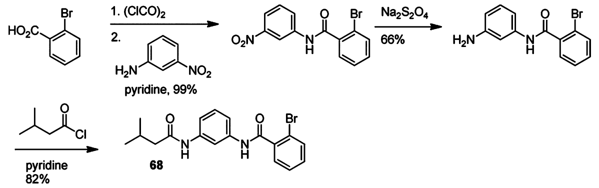
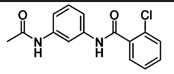
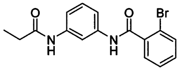
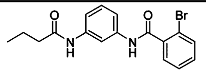
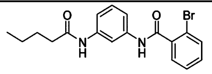
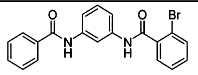
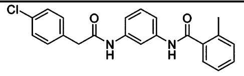
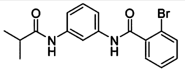
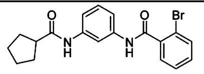
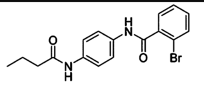
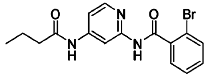
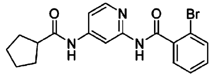
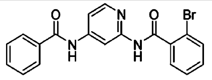
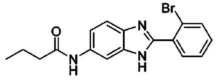
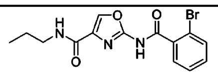
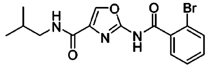
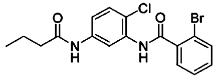
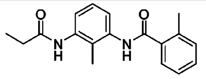
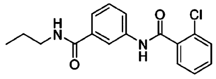
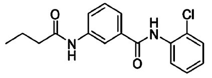


Table 2. SAR at the West End of the 1,3-Diamidobenzene Scaffold[a]

Cmpd	Structure	%inhib. IC$_{50}$ (μM)[a]
37		35%
38		95%[b] 1.68
4		>99% 0.26
39		<1%
40		67%
41		40%
42		88% 1.41
43		91% 0.52
44		1%

[a]See the Table 1 footnotes.

Table 3. SAR at the Central Ring[a]

Cmpd	Structure	%inhib. IC$_{50}$ (μM)[a]
45		<1%
46		<1%
47		11%
48		39%
49		<1%
50		<1%
51		35%
52		5%
53		99% 1.80
54		67%

[a]See the Table 1 footnotes.

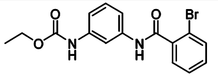
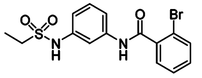
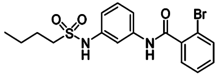
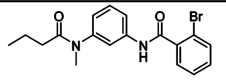
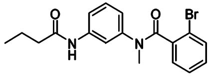
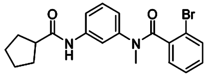
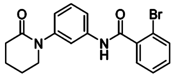
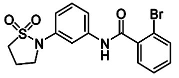
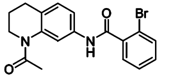
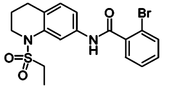
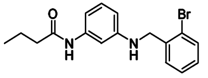
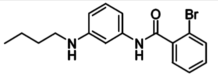
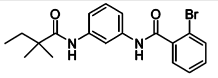
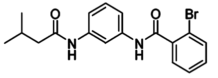
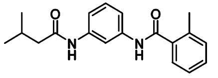
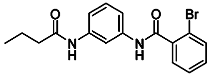
ring were not tolerated (**51** and **52**), although alternative groups could be worth investigating. Two compounds with reverse amides were screened (**53** and **54**), and the compound with the reverse amide at the western position (**54**) maintained much of the activity of the parent compound (**3**).

To explore SARs further and to search for compounds with improved potencies and physicochemical and biological properties (especially PS), additional compounds were prepared and screened. These results are listed in Table 4, which includes plasma protein binding (PPB) and PS. Replacement of the western propylamide of **4** with a carbamate (**55**) or sulfonamides (**56** and **57**) gave compounds with decreased potencies. *N*-Methyl amides **58**−**60** were inactive, which suggests that both amides act as hydrogen bond donors with the target. The constrained analogues **61**−**64** were similarly inactive. Removal of the eastern carbonyl group of **4** was tolerated somewhat (**65**), but interestingly, much of the activity was retained with removal of the western carbonyl group in aniline **66**.

In a final attempt to address the mouse PS liability of **4**, we made additional branched alkyl analogues, building upon the moderate results of **42** and **43**. The more highly branched α,α-dimethylamide **67** was very plasma stable but poorly active, but moving the branching point further from the amide carbonyl (**68**) gave a compound nearly equipotent to **4** (IC$_{50}$ = 0.29 μM) with good human PS (90% after 5 h) and mouse PS sufficient for in vivo work (65% after 5 h), as well as adequate solubility for a probe compound (20 μM in PBS). The preparation of **4** is described in Scheme 1,[21] and the synthesis of the mouse plasma-stable analogue **68** is depicted in Scheme 2. Prior to these more recent studies, compound **4** was formally nominated as a molecular probe (ML161) for the study of platelet activation.[14]

The selectivity at PAR1 is supported by studies involving various platelet activators in the presence of ML161. Washed human platelets were separately treated with the peptide AYPGKF (a PAR4 agonist), PMA (a protein kinase C activator), U46619 (a thromboxane receptor agonist), or collagen (an agonist of collagen receptors). ML161 did not inhibit these activators to any significant degree (Figure S1 in the Supporting Information). ML161 displayed dose-depend-

Table 4. Analogues Designed To Improve PS[a]

Cmpd	Structure	%inhib. IC$_{50}$ (μM)[a]	PPB[b] human/ mouse	PS[c] human/ mouse
55		98% 2.64	95% 87%	85% 56%
56		27%	93% 69%	99% >99%
57		31%	ND ND	97% >99%
58		8%	98% 85%	90% 85%
59		<1%	89% ND	19% <1%
60		<1%	95% 90%	64% 40%
61		9%	82% 72%	94% 82%
62		8%	91% 76%	>99% 98%
63		10%	99% 89%	92% 71%
64		26%	98% 96%	>99% 97%
65		60%	99% ND%	73% 1%
66		>99% 0.34	97% 96%	99% 97%
67		21%	95% 91%	>99% >99%
68		>99% 0.29	93% 87%	90% 65%
69		>99% 1.12	96% 83%	>99% 69%
4		>99% 0.26	97% ND	80% 2%

[a]See the Table 1 footnotes. [b]PPB (% bound). [c]PS (% remaining after 5 h). ND, not determined.

ent inhibition of thrombin-induced platelet activation, as measured by P-selectin expression (Figure S2 in the Supporting Information). Inhibition of PAR2, a widely distributed protease-activated receptor, was not evaluated since this receptor is not present on platelets. ML161 displayed selective inhibition of SFLLRN and thrombin-induced platelet aggregation, both of which operate via PAR1, and had no effect on AYPGKF, thromboxane, or ADP-induced platelet aggregation, which are all agonists at alternative platelet GPCRs (Figure S3 in the Supporting Information).

Continued work on the 1,3-diaminobenzene scaffold was inspired by our discovery that its mode of action at PAR1 may differ from reported PAR1 orthosteric inhibitors.[22,23] Activation of platelets via PAR1 cleaved by thrombin[24] leads to multiple downstream effects and observable phenotypic changes, including granule release and shape change characterized by extended pseudopodia. Blockade of PAR1 by orthosteric antagonists, such as those currently under clinical investigation,[4,25] would be expected to inhibit all phenotypic changes. In contrast, **4** inhibits granule secretion, but not platelet shape change, as monitored in a SFLLRN-induced platelet aggregation assay.[26] We hypothesize that it may be acting in an allosteric manner to inhibit select G-protein-coupled pathways mediated by PAR1. Our preliminary studies suggest that it inhibits Gαq, which is required for granule release, but not Gα12/13 signaling, which affects shape change. Additional evidence for a nonorthosteric inhibition mechanism was obtained by evaluating dose−response curves of SFLLRN-induced P-selectin expression in the presence of varying concentrations of **4** (Figure 1). Instead of a rightward shift of the dose−response curve, as expected of an orthosteric inhibitor, **4** demonstrated insurmountable antagonism at higher doses, consistent with a noncompetitive inhibitory mechanism. A quinolone derivative, termed JF5, was previously discovered in our laboratories as an inhibitor of platelet activation and has been recently characterized as a PAR1 inhibitor that requires helix 8 on the intracellular face of PAR1 for its inhibitory activity.[27] Additional investigations are underway to determine if compounds such as **4** share a similar binding site.

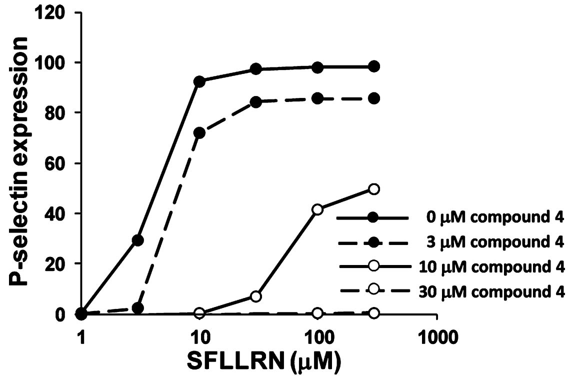


Figure 1. Dose−response curves of SFLLRN-induced P-selectin expression in the presence of varying concentrations of the PAR1 inhibitor **4**.

In summary, the 1,3-diaminobenzene scaffold was identified as an inhibitor of platelet activation via a high-throughput screen performed with platelet-rich plasma. Medicinal chemistry studies delineated the requirements for optimal potency of this scaffold at PAR1. These include (1) a secondary benzamide at the eastern side of the molecule, with a small (ethyl or smaller) electron-neutral or electron-withdrawing *ortho* substituent; (2) a 1,3-substitution pattern about the central benzene ring; and (3) a secondary amine or amide on the western side of the molecule, with a linear or branched aliphatic chain fewer than four carbons long. Potency was maintained and PS improved with the addition of a methyl group β to the western amide (**68**). We have demonstrated that compounds such as **4** (ML161) may inhibit PAR1 in an allosteric fashion, which could enable the selective modulation of platelet activation pathways. Allosteric inhibition of PAR1 could provide saturable receptor binding and selective modulation of downstream G-protein signaling pathways. These pharmacological attributes may decrease the risk of life-threatening hemorrhage in the setting of anti-PAR1 therapy.[28] Analogues of the 1,3-diaminobenzene scaffold will be important probes for evaluating this hypothesis.

ASSOCIATED CONTENT

Supporting Information

Additional assay results (Figures S1 to S3), assay protocols, and synthetic procedures and characterization data for **4**, **66**, and **68**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 1-617-714-7460. E-mail: cjdocken@broadinstitute.org (C.D.). Tel: 1-617-735-4005. E-mail: rflaumen@bidmc.harvard.edu. (R.F.).

Funding

This work was funded by the NIH-MLPCN program (1 U54 HG005032-1 awarded to S.L.S.) and P01-HL-87203 (R.F.), an Established Investigator Award from the American Heart Association (R.F.), and R03 MH-84076-01 (R.F.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Stephen Johnston, Chris Johnson, and Mike Lewandowski of the Broad Institute for analytical chemistry support, Benito Munoz for reviewing the manuscript, and Robert Gould for helpful discussions.

ABBREVIATIONS

ATP, adenosine triphosphate; HTS, high-throughput screening; IC_{50}, concentration of inhibitor giving half-maximal activity; NIH, National Institute of Health; MLPCN, Molecular Libraries Probe Center Network; NIH-MLSMR, National Institute of Health Molecular Libraries Small Molecule Repository; PAR1, protease-activated receptor 1; PPB, plasma protein binding; PBS, phosphate-buffered saline; PS, plasma stability; SAR, structure−activity relationship

REFERENCES

(1) For a review, see, for example: Michelson, A. D. Antiplatelet therapies for the treatment of cardiovascular disease. *Nature Rev. Drug Discovery* **2010**, *9*, 154−169.

(2) See, for example: Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Br. Med. J.* **2002**, *324*, 71−86.

(3) See, for example: Wiviott, S. D.; et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* **2007**, *357*, 2001−2015.

(4) Discovery of SCH 530348: Chackalamannil, S.; Wang, Y.; Greenlee, W. J.; Hu, Z.; Xia, Y.; Ahn, H.-S.; Boykow, G.; Hsieh, Y.; Palamanda, J.; Agans-Fantuzzi, J.; Kurowski, S.; Graziano, M.; Chintala, M. Discovery of a novel, orally active himbacine-based thrombin receptor antagonist (SCH 530348) with potent antiplatelet activity. *J. Med. Chem.* **2008**, *51*, 3061−3064.

(5) Tricoci, P.; et al. Thrombin-receptor antagonist vorapaxar in acute coronary syndromes. *N. Engl. J Med.* **2012**, *366*, 20−33.

(6) Goto, S.; Ogawa, H.; Takeuchi, M.; Flather, M. D.; Bhatt, D. L. Double-blind, placebo-controlled Phase II studies of the protease-activated receptor 1 antagonist E5555 (atopaxar) in Japanese patients with acute coronary syndrome or high-risk coronary artery disease. *Eur. Heart J.* **2010**, *31*, 2601−2613.

(7) O'Donoghue, M. L.; et al. Safety and tolerability of atopaxar in the treatment of patients with acute coronary syndromes: the lessons from antagonizing the cellular effects of Thrombin-Acute Coronary Syndromes Trial. *Circulation* **2011**, *123*, 1843−1853.

(8) Wiviott, S. D.; Flather, M. D.; O'Donoghue, M. L.; Goto, S.; Fitzgerald, D. J.; Cura, F.; Aylward, P.; Guetta, V.; Dudek, D.; Contant, C. F.; Angiolillo, D. J.; Bhatt, D. L. Randomized trial of atopaxar in the treatment of patients with coronary artery disease: The lessons from antagonizing the cellular effect of Thrombin-Coronary Artery Disease Trial. *Circulation* **2011**, *123*, 1854−1863.

(9) Kato, Y.; Kita, Y.; Nishio, M.; Hirasawa, Y.; Ito, K.; Yamanaka, T.; Motoyama, Y.; Seki, J. In vitro antiplatelet profile of FR171113, a novel non-peptide thrombin receptor antagonist. *Eur. J. Pharmacol.* **1999**, *384*, 197−202.

(10) Andrade-Gordon, P.; Maryanoff, B. E.; Derian, C. K.; Zhang, H. C.; Addo, M. F.; Darrow, A. L.; Eckardt, A. J.; Hoekstra, W. J.; McComsey, D. F.; Oksenberg, D.; Reynolds, E. E.; Santulli, R. J.; Scarborough, R. M.; Smith, C. E.; White, K. B. Design, synthesis, and biological characterization of a peptide-mimetic antagonist for a tethered-ligand receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12257−12262.

(11) Ahn, H.-S.; Boykow, G.; Burnett, D. A.; Caplen, M. A.; Czarniecki, M.; Domalski, M. S.; Foster, C.; Manna, M.; Stamford, A. W.; Wu, Y. Structure-activity relationships of pyrroloquinazolines as thrombin receptor antagonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2073−2078.

(12) Nantermet, P. G.; Barrow, J. C.; Lundell, G. F.; Pellicore, J. M.; Rittle, K. E.; Young, M.; Freidinger, R. M.; Connolly, T. M.; Condra, C.; Karczewski, J.; Bednar, R. A.; Gaul, S. L.; Gould, R. J.; Prendergast, K.; Selnick, H. G. Discovery of a nonpeptidic small molecule antagonist of the human platelet thrombin receptor (PAR-1). *Bioorg. Med. Chem. Lett.* **2002**, *12*, 319−323.

(13) Perez, M.; et al. Discovery of novel protease activated receptors 1 antagonists with potent antithrombotic activity in vivo. *J. Med. Chem.* **2009**, *52*, 5826−5836.

(14) VerPlank, L.; Dockendorff, C.; Negri, J.; Perez, J. R.; Dilks, J.; MacPherson, L.; Palmer, M.; Flaumenhaft, R.; Schreiber, S. L. Probe reports from the NIH Molecular Libraries Program. *Chemical Genetic Analysis of Platelet Granule Secretion—Probe 3*, **2010**; https://mli.nih.gov/mli/?dl_id=1254.

(15) Flaumenhaft, R.; Sim, D. S. The platelet as a model for chemical genetics. *Chem. Biol.* **2003**, *10*, 481−486.

(16) Flaumenhaft, R.; Dilks, J. R. Discovery-based strategies for studying platelet function. *Mini Rev. Med Chem.* **2008**, *8*, 350−357.

(17) Briefly, compounds were incubated in platelet-rich plasma in 384-well plates at a concentration of 7.5 μM (for the primary screen) or at a dose response (for confirmation assays) for 30 min. Next, a mixture of the CellTiter-Glo (Promega) reagent (for ATP detection with a luciferase/luciferin system) and SFLLRN (EC_{50} for platelet activation ~5 μM) was used. Each plate was incubated for 15 min at 22 °C, and then, luminescence resulting from ATP-driven oxyluciferin production was measured by a plate reader. See ref 13 for full details.

(18) Sim, D. S.; Merrill-Skoloff, G.; Furie, B. C.; Furie, B.; Flaumenhaft, R. Initial accumulation of platelets during arterial thrombus formation in vivo is inhibited by elevation of basal cAMP levels. *Blood* **2004**, *103*, 2127−2134.

(19) For analysis of P-selectin expression, 20 μL of gel-filtered platelets (0.5-1 × 108/mL) were incubated with the indicated antagonists and subsequently stimulated with 5 μM SFLLRN (EC_{50} for platelet activation ~5 μM) for 10 min. Following stimulation, 10 μL of reaction mixture was transferred to 5 μL of PE-conjugated AC1.2 anti-P-selectin antibody. Phosphate-buffered saline (PBS; 500

μL) was added after a 20 min incubation at 37 °C, and the platelets were analyzed immediately by flow cytometry. See the Supporting Information for further details.

(20) Review: Furie, B.; Furie, B. C.; Flaumenhaft, R. A journey with platelet P-selectin: The molecular basis of granule secretion, signalling and cell adhesion. *Thromb. Haemostasis* **2001**, *86*, 214−221.

(21) At the present time, compound **4** (ML161) is also commercially available from ChemBridge Corp.

(22) Review: Chen, C.; Maryanoff, B. E.; Andrade-Gordon, P. Thrombin receptor modulators: Medicinal chemistry, biological evaluation, and clinical application. In *Thrombin: Physiology and Disease*; Maragoudakis, M., Tsopanoglou, N., Eds.; Springer: New York, NY, 2009; pp 205−236.

(23) Review: Chackalamannil, S. Thrombin receptor (Protease Activated Receptor-1) antagonists as potent antithrombotic agents with strong antiplatelet effects. *J. Med. Chem.* **2006**, *49*, 5389−5403.

(24) The serine protease thrombin cleaves the N terminus of PAR1, which then acts as a tethered ligand to activate signaling through intramolecular ligation of the ortherosteric binding site. SFLLRN peptide is derived from this tethered ligand and is an agonist of PAR1 when applied externally. For the initial discovery, see Vu, T.-K. H.; Hung, D. T.; Wheaton, V. I.; Coughlin, S. R. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **1991**, *64*, 1057−1068.

(25) Serebruany, V. L.; Kogushi, M.; Dastros-Pitei, D.; Flather, M.; Bhatt, D. L. The in-vitro effects of E5555, a protease-activated receptor (PAR)-1 antagonist, on platelet biomarkers in healthy volunteers and patients with coronary artery disease. *Thromb. Haemostasis* **2009**, *102*, 111−119.

(26) Details will be disclosed in a full paper currently in preparation.

(27) Dowal, L.; Sim, D. S.; Dilks, J. R.; Blair, P.; Beaudry, S.; Denker, B. M.; Koukos, G.; Kuliopulos, A.; Flaumenhaft, R. Identification of an antithrombotic allosteric modulator that acts through helix 8 of PAR1. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 2951−2956.

(28) For a discussion,see Dowal, L.; Flaumenhaft, R. Targeting platelet G-Protein Coupled Receptors (GPCRs): Looking beyond conventional GPCR antagonism. *Curr. Vasc. Pharmacol.* **2010**, *8*, 140−154.