

Letters

Discovery of Potent Orally Active Thrombin Receptor (Protease Activated Receptor 1) Antagonists as Novel Antithrombotic Agents

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Abstract: Structurally novel thrombin receptor (protease activated receptor 1, PAR-1) antagonists based on the natural product himbacine are described. The prototypical PAR-1 antagonist **55** showed a K_i of 2.7 nM in the binding assay, making it the most potent PAR-1 antagonist reported. **55** was highly active in several functional assays, showed excellent oral bioavailability in rat and monkey models, and showed complete inhibition of agonist-induced ex vivo platelet aggregation in cynomolgus monkeys after oral administration.

Currently available antithrombotic therapy suffers from low efficacy and side effects mainly associated with bleeding.¹ Additionally, most of the marketed antithrombotic agents are not orally bioavailable.² Several efforts to achieve an orally active antithrombotic agent based on GpIIb/IIIa antagonism failed in clinical trials.³ Therefore, there exists an unmet clinical need for a potent, orally active antithrombotic agent with a better safety margin. Inhibition of thrombin mediated platelet activation represents a unique opportunity to discover novel antithrombotic agents with potent antiplatelet effects.

In addition to its fibrin generating properties, thrombin activates a number of cell types via proteolytic activation of specific G-protein-coupled cell surface receptors known as protease activated receptors (PARs).^{4–7} Four PARs are known, among which the prototypical PAR-1 receptor, also known as thrombin receptor, is widely distributed on human and monkey platelets, endothelial cells, and smooth muscle cells. Thrombin is the most potent activator of platelets, and thrombin mediated platelet activation plays a critical role in the pathophysiology of thrombosis.^{8–10} Activated platelets bind to fibrinogen, causing platelets to aggregate at the site of cardiovascular injury to form a thrombus that is further stabilized by a thrombin-generated fibrin network.^{11–13} Since PAR-1 antagonists are expected to be specific for the cellular activation of thrombin and do not inhibit fibrin generation, such an

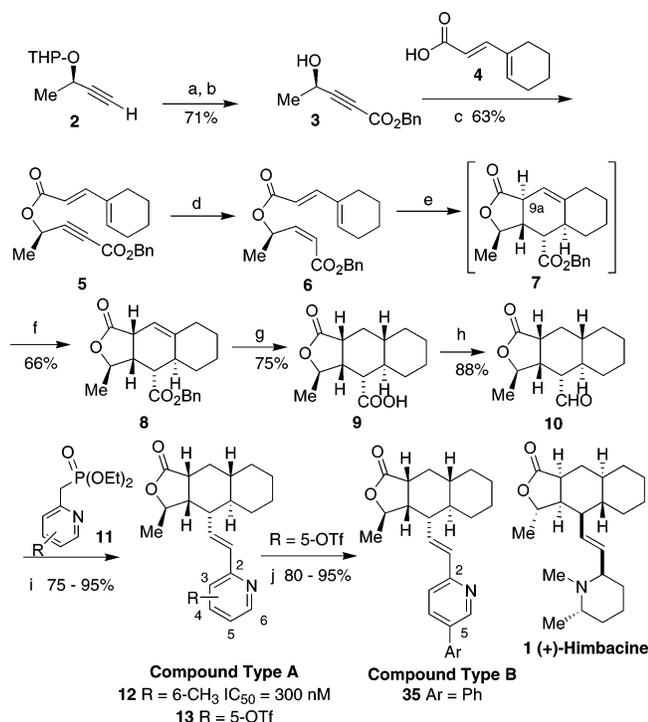
agent is likely to have less bleeding liability than the currently existing antithrombotic drugs. A PAR-1 antagonist may also inhibit thrombin mediated proinflammatory and proliferative processes in vascular endothelial cells and smooth muscle cells, offering additional cardiovascular utility in the treatment of atherosclerosis and restenosis.^{14,15}

The cellular activation of thrombin is mediated by a unique tethered ligand mechanism in which thrombin selectively cleaves the extracellular domain of the receptor at Arg₄₁-Ser₄₂. The newly unmasked amino terminus binds intramolecularly to the proximal receptor eliciting transmembrane signaling.^{16–19} There were several reports that strongly suggest the potential utility of a PAR-1 antagonist for the treatment of thrombotic disorders.^{20–26} These studies, while providing encouraging proof-of-concept for the antithrombotic effect of PAR-1 antagonism, employed modestly potent PAR-1 antagonists that required iv or subcutaneous administration of the drug.

We report the discovery of high affinity, orally active, low molecular weight non-peptide PAR-1 antagonists based on the natural product himbacine (**1**).^{27,28} One of the synthetic analogues of himbacine, (\pm)-**12**, was identified in high-throughput screening as a lead for the PAR-1 antagonist program. The synthesis outlined in Scheme 1 employs a highly diastereoselective intramolecular Diels–Alder (IMDA) reaction to generate the tricyclic intermediate **9** in a protocol that was adapted from the total synthesis of himbacine.²⁷ The synthesis, which was originally carried out in the racemic form, commences with commercially available 3-butyn-2-ol, which was *O*-protected to give **2** and subsequently elaborated to the pentynoic acid benzyl ester **3** as described. Esterification of **3** with dienic acid **4** and subsequent Lindlar reduction of the triple bond gave the IMDA precursor **6**. Thermal cyclization of **6** gave 65% of the required tricyclic carboxylic ester **8** after a brief in situ treatment with DBU to epimerize the stereogenic center α to the lactone carbonyl group. Catalytic hydrogenation of **8** over platinum oxide effected diastereospecific reduction of the double bond as well as *O*-debenzylation to produce the tricyclic carboxylic acid **9**. The acid chloride generated from **9** was reduced to the aldehyde **10** by treatment with tributyltin hydride in the presence of palladium.²⁹ Horner–Wadsworth–Emmons reaction of aldehyde **10** with the appropriate phosphonate **11** gave the vinylpyridine derivatives in excellent yields. Halogen or *O*-triflate substituted pyridine (e.g., **13**) could be further elaborated via palladium catalyzed coupling reactions to alkyl, aryl, amino, and other substituted pyridine derivatives.

In vitro binding studies were carried out using purified human platelet membrane as PAR-1 source and tritiated high-affinity thrombin receptor activating peptide, alanine-*p*-fluorophenylalanine-arginine-cyclo-

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Scheme 1^a

^a Reagents and conditions: (a) *n*-BuLi/THF, BnOCOCl, -78 °C; (b) DOWEX/CH₃OH; (c) **4**, 4-pyrrolidinylpyridine, DCC/CH₂Cl₂; (d) H₂, Lindlar/THF; (e) *o*-xylene, 185 °C; (f) DBU; (g) H₂, PtO₂/CH₃OH; (h) (i) SOCl₂/toluene, 80 °C, (ii) Bu₃SnH, (Ph₃P)₄Pd; (i) **11**, *n*-BuLi(hex)/THF, 0 °C; (j) (i) PdCl₂(dppf), KOAc, diboron pinacol ester/dioxane 80 °C, (ii) Ar-Br, PdCl₂(dppf), K₃PO₄, 80 °C.

Table 1. SAR of Non-Aryl Substituted Pyridine Derivatives (Compound Type A)

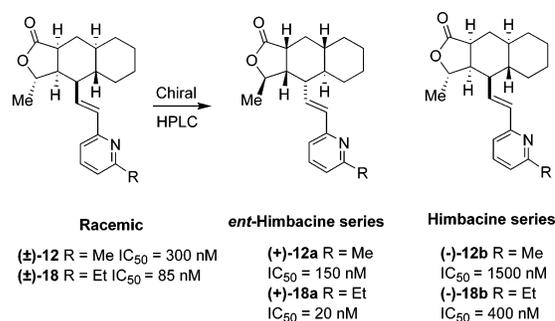
compd	R	IC ₅₀ (nM) ^a	compd	R	IC ₅₀ (nM) ^a
(±)- 12	6-CH ₃	300	(±)- 25	6- <i>cy</i> -Pr	210
(±)- 14	H	4000	(±)- 26	6-NHCH ₃	1250
(±)- 15	3-CH ₃	> 5000	(±)- 27	6-CH ₂ OH	1500
(±)- 16	4-CH ₃	2100	(±)- 28	6-CH ₂ OCH ₃	850
(±)- 17	5-CH ₃	1100	(±)- 29	6-CH ₂ Ph	900
(±)- 18	6-Et	85	(±)- 30	6-OCH ₃	inactive
(±)- 19	6-vinyl	150	(±)- 31	6-Ph	inactive
(±)- 20	6- <i>n</i> -Pr	250	(±)- 32	5-Bn	3681
(±)- 21	6- <i>n</i> -Bu	143	(±)- 33	5-OCH ₃	325
(±)- 22	6- <i>n</i> -hex	3500	(±)- 34	5-OBn	19
(±)- 23	6- <i>i</i> -Pr	725	(+)- 35	5-Ph	27
(±)- 24	6- <i>i</i> -Bu	550			

^a PAR-1 binding assay ligand: [³H]haTRAP, 10 nM (K_d = 15 nM).³⁰

hexylalanine-homoarginine-[³H]phenylalanine amide ([³H]haTRAP, K_d = 15 nM), as ligand as reported previously.³⁰ This assay, which was carried out in 96-well plates, tolerates long incubation time and up to 5% of DMSO concentration, which was important for solubilizing compounds of low aqueous solubility.

Initial SAR studies were directed at optimizing the substitution pattern on the pyridine ring. An examination of Table 1 indicates that the presence of an alkyl group at the C-6 position of the pyridine ring (**12**, **18**, **20–24**) is preferred over alkyl groups at other positions. The monosubstituted pyridine derivative **14** and the C-3, C-4, and C-5 substituted derivatives (**15–17**) were less active. An ethyl substituent at the 6-position (**18**) gave the best PAR-1 affinity. Other small hydrophobic

Scheme 2



groups at C-6 (**19–21**, **25**) also had comparable activity, whereas branched chain substituents (**23**, **24**) and polar substituents (**26**, **27**) were less active. Pyridine substituted at the 6-position with an *n*-hexyl group (**22**) was far less active, and the 6-phenyl derivative **31** was totally inactive.

In an effort to further characterize the PAR-1 antagonist profile of the himbacine series, **12** and **18** were resolved using chiral HPLC (Scheme 2). The (+)-enantiomer was found to be approximately 10 times more active than the (–)-enantiomer in each case. The absolute stereochemistry of the more active series was established using an enantiospecific synthesis starting from (*R*)-3-butyn-2-ol. Subsequent resolution of a number of active compounds confirmed that the *ent*-himbacine absolute stereochemistry is favored for PAR-1 antagonism.

In the radioligand binding assay, compound (+)-**18a** showed a K_i of 12 nM against PAR-1. In the haTRAP induced human platelet aggregation assay,³¹ this compound showed dose-dependent inhibition of aggregation with an IC₅₀ of 70 nM. This compound was further evaluated in an ex vivo²³ platelet aggregation assay in conscious cynomolgus monkeys after iv infusion (10 mg/kg, 30 min). Nearly complete inhibition of platelet aggregation induced by exogenously added peptide agonist (haTRAP) to the plasma drawn from the drug treated group was noted for 2 h. However, this compound showed poor oral bioavailability in rat (*f* < 3%) perhaps due to rapid metabolism (iv T_{1/2} < 1 h).

Having achieved excellent PAR-1 affinity and ex vivo efficacy, we further turned our attention to generating compounds with good oral bioavailability. Although C-5 alkyl substitution gave weaker PAR-1 affinity than the corresponding C-6 alkyl substitution (**17** vs **12**), the C-5 methoxy substituted derivative **33** showed modest activity and the corresponding *O*-benzyl analogue **34** showed excellent PAR-1 inhibition. However, **34** showed no oral bioavailability presumably because of rapid metabolism.

C-5 aryl substituted derivatives were examined, and their data are presented in Table 2. The C-5 phenyl substituted derivative **35**, which had an IC₅₀ of 27 nM, was the first compound in this series to show a promising pharmacokinetics profile. In preliminary rat pharmacokinetics studies, which measured plasma levels of the parent compound for 4 h after oral administration of the drug in 20% hydroxypropyl-β-cyclodextrin (HP-BCD), modest plasma levels were detected (AUC_{0–4h} = 917 nM·h; C_{max} = 453 nM). The limited plasma levels were presumed to be due to the facile metabolism of the

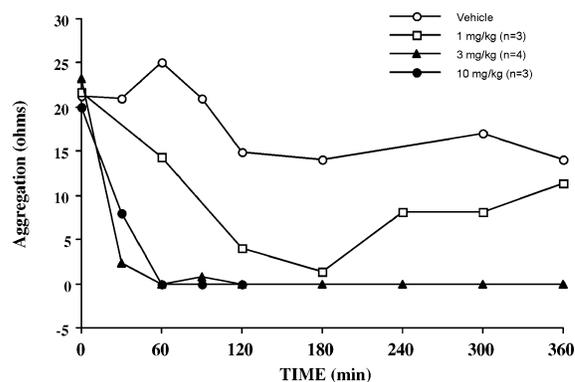
Table 2. SAR of 5-Aryl Substituted Pyridine Derivatives (Compound Type B)

compd	Ar	IC ₅₀ (nM) ^a	rat PK ^b AUC, C _{max}
(+)- 35	Ph	27	917, 453
(±)- 36	(<i>p</i> -CH ₃)-phenyl	325	ND
(±)- 37	(<i>p</i> -OCH ₃)-phenyl	204	ND
(±)- 38	(<i>p</i> -F)-phenyl	467	ND
(±)- 39	(<i>p</i> -Cl)-phenyl	1000	ND
(±)- 40	(<i>p</i> -CF ₃)-phenyl	>300	ND
(+)- 41	(<i>o</i> -CH ₃)-phenyl	14	2805, 1222
(+)- 42	(<i>o</i> -CF ₃)-phenyl	46	2350, 1004
(+)- 43	(<i>o</i> -CO ₂ Et)-phenyl	44	ND
(+)- 44	(<i>o</i> -OCH ₃)-phenyl	11	413, 419
(+)- 45	(<i>o</i> -F)-phenyl	22	2467, 1077
(+)- 46	(<i>o</i> -Cl)-phenyl	26	4285, 1785
(+)- 47	(<i>m</i> -F)-phenyl	35	3553, 1516
(+)- 48	(<i>m</i> -Cl)-phenyl	10	4112, 1457
(+)- 49	(<i>m</i> -Br)-phenyl	25	ND
(+)- 50	(<i>m</i> -CN)-phenyl	25	ND
(+)- 51	(<i>m</i> -CH ₃)-phenyl	13	331, 380
(+)- 52	(<i>m</i> - <i>i</i> Pr)-phenyl	19	258, 289
(+)- 53	(<i>m</i> -OCH ₃)-phenyl	28	12, 20
(+)- 54	(<i>m</i> -SO ₂ NH ₂)-phenyl	100	ND
(+)- 55	(<i>m</i> -CF ₃)-phenyl	11	6116, 2300

^a PAR-1 binding assay ligand: [³H]haTRAP, 10 nM ($K_d = 15$ nM).³⁰ ^b Compounds were dosed in 20% HPBCD. AUC measurements are given in nM·h and C_{max} in nM.

unsubstituted phenyl ring. In an effort to further optimize the binding and pharmacokinetics properties of **35**, substitutions on the phenyl ring were examined. As the data in Table 2 indicate, para substitution of the phenyl ring of **35** resulted in weaker PAR-1 binding (**36–40**). However, several ortho and meta substituted phenyl compounds showed excellent PAR-1 affinity. More importantly, several of these compounds showed improved pharmacokinetics parameters in the rat pharmacokinetics screening model. Compounds with halogens at the ortho and meta positions in general showed excellent PAR-1 binding and good plasma levels in rats after oral dosing (e.g., **45–48**). The *m*-(trifluoromethyl)-phenyl derivative **55** had the best overall profile in this group with an IC₅₀ of 11 nM against PAR-1, an AUC of 6116 nM·h, and C_{max} of 2300 nM in the rat pharmacokinetics model.

In the radioligand binding assay, **55** showed a K_i of 2.7 nM against PAR-1, making it the most potent PAR-1 antagonist reported to date. Scatchard plots of saturation binding in the presence and absence of **55** were consistent with a competitive binding profile to the PAR-1. The effect of **55** on aggregation responses to thrombin (1 U/mL), haTRAP (1 μM), ADP (20 μM), and collagen (5 μg/mL) in human platelet rich plasma (PRP)²¹ was evaluated in a comparative experiment. Compound **55** showed robust inhibition of thrombin-induced platelet aggregation with an IC₅₀ of 44 nM and haTRAP induced platelet aggregation with an IC₅₀ of 24 nM, whereas it showed no inhibition of ADP and collagen induced aggregation of platelets. In the thrombin induced calcium transient assay³² in human coronary artery smooth muscle cells, this compound showed a K_d of 2.6 nM. The compound inhibited thrombin-induced thymidine incorporation³² in human coronary artery smooth muscle cells with a K_i of 13.0 nM. Detailed pharmacokinetics parameters of **55** were determined in rat and cynomolgus monkey models. In rat, **55** showed an oral bioavailability of 30% at a dose of 10 mg/kg. The C_{max} following

**Figure 1.** Inhibition of ex vivo haTRAP-induced platelet aggregation by **55** in cynomolgus monkeys ($n = 3$) after oral administration at 1, 3, and 10 mg/kg.

oral dose was 1.16 μM, and the half-life was 3.2 h following intravenous administration. The oral bioavailability of **55** in cynomolgus monkey was 50%, and the half-life was 12.4 h after intravenous administration.

The effect of **55** on haTRAP induced ex vivo platelet aggregation in whole blood in conscious, fasted cynomolgus monkeys was determined (Figure 1). After oral administration of the drug, blood samples were collected at various intervals and aggregation response to 1 μM haTRAP was performed in a whole blood aggregometer. The compound showed dose-dependent inhibition of platelet aggregation up to the time point measured. While the aggregation response to 1 mg/kg showed some recovery at the 6 h time point, aggregation inhibition observed for the 3 and 10 mg/kg groups was sustained throughout the experiment.

Compound **55** did not affect clotting parameters (PT, prothrombin time; APTT, activated partial thromboplastin time), confirming that its mechanism of action is not by active site inhibition of thrombin or other coagulation proteases. The compound was selective against a number of GPCRs, ion channels, and receptors that it was tested against at the CEREP laboratories and was inactive in the PAR-2 and PAR-4 functional assays.

In summary, we have reported here the first instance of orally active PAR-1 antagonists that are also highly potent. The prototypical PAR-1 antagonist **55** (SCH 205831) with a K_i of 2.7 nM was more potent than any PAR-1 antagonists reported to date in receptor binding and several functional assays. The unique properties of this series are further underscored by the excellent oral bioavailability of **55** in rat and monkey models, excellent potency against thrombin and haTRAP induced human platelet aggregation, and the sustained and complete ex vivo platelet aggregation inhibition in cynomolgus monkey model after oral administration. We believe that the high potency of **55** as an antiplatelet agent is due to its tight binding to the thrombin receptor where it effectively competes against the tethered ligand that enjoys considerable entropic advantage over an incoming antagonist. Further mechanistic and in vivo studies of these compounds will be published.

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Supporting Information Available: Experimental procedures for platelet aggregation studies, PAR-1 binding assay, and synthesis and characterization of intermediates and final products. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Bisacchi. Anticoagulants, Antithrombotics, and Hemostatics. In *Burger's Medicinal Chemistry and Drug Discovery*, 6th ed.; Abraham, D. J., Ed.; John Wiley and Sons: Hoboken, NJ, 2003; Vol. 3, pp 283–338.
- (2) Gould, W. R.; Leadley, R. J. Recent Advances in the Discovery and Development of Direct Coagulation Factor Xa Inhibitors. *Curr. Pharm. Des.* **2003**, *9*, 2337–2347.
- (3) Kereiakes, D. J. Oral Blockade of the Platelet Glycoprotein IIb/IIIa Receptor: Fact or Fancy? *Am. Heart J.* **1999**, *138* (1, Part 2), S39–S46.
- (4) Coughlin, S. R. How the Protease Thrombin Talks to Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11023–11027.
- (5) Coughlin, S. R. Protease-Activated Receptors. In *Handbook of Cell Signaling*; Bradshaw, R. A., Dennis, E. A., Eds.; Elsevier: San Diego, CA, 2004; Vol. 1, pp 167–171.
- (6) Coughlin, S. R. Protease-Activated Receptors in the Cardiovascular System. *Cold Spring Harbor Symp. Quant. Biol.* **2002**, *67*, 197–208.
- (7) Coughlin, S. R. Protease-Activated Receptors in Vascular Biology. *Thromb. Haemostasis* **2001**, *86*, 298–307.
- (8) Harker, L. A. Pathogenesis of Thrombosis. In *Hematology*; Williams, W. J., Beutler, E., Erslev, A. J., Lictman, M. A., Eds.; McGraw-Hill: New York, 1990; pp 1559–1568.
- (9) Colter, B. S. The Role of Platelets in Arterial Thrombosis and the Rationale for Blockade of Platelet GpIIb/IIIa Receptors as Antithrombotic Therapy. *Eur. Heart J.* **1995**, *16* (Suppl. L), 11–15.
- (10) Sambrano, G. R.; Weiss, E. J.; Zheng, Y.-W.; Huang, W.; Coughlin, S. R. Role of Thrombin Signaling in Platelets in Hemostasis and Thrombosis. *Nature* **2001**, *413*, 74–78.
- (11) Jarvis, G. E.; Atkinson, B. T.; Frampton, J.; Watson, S. P. Thrombin-Induced Conversion of Fibrinogen to Fibrin Results in Rapid Platelet Trapping Which Is Not Dependent on Platelet Activation or GPIIb. *Br. J. Pharmacol.* **2003**, *138*, 574–583.
- (12) Ruggeri, Z. M.; Dent, J. A.; Saldivar, E. Contribution of Distinct Adhesive Interactions to Platelet Aggregation in Flowing Blood. *Blood* **1999**, *94*, 172–178.
- (13) Herman, A. G. Rationale for the Combination of Anti-Aggregating Drugs. *Thromb. Res.* **1998**, *92* (1, Suppl. 1), S17–S21.
- (14) Chackalamannil, S. Thrombin Receptor Antagonists as Novel Therapeutic Targets. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 417–427.
- (15) Seiler, S. M.; Bernatowicz, M. S. Peptide-Derived Protease-Activated Receptor-1 (PAR-1) Antagonists. *Curr. Med. Chem.: Cardiovasc. Hematol. Agents* **2003**, *1*, 1.
- (16) 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.
- (17) Coughlin, S. R. Thrombin Signaling and Protease-Activated Receptors. *Nature* **2000**, *407*, 258–264.
- (18) Hung, D. T.; Vu, T.-H.; Nelken, N. A.; Coughlin, S. R. Thrombin-Induced Events in Non-Platelet Cells Are Mediated by the Unique Proteolytic Mechanism Established for the Cloned Platelet Thrombin Receptor. *J. Cell Biol.* **1992**, *116*, 827–832.
- (19) Vu, T.-K. H.; Wheaton, V. I.; Hung, D. T.; Charo, I.; Coughlin, S. R. Domains Specifying Thrombin-Receptor Activation. *Nature* **1991**, *353*, 674–677.
- (20) Zhang, H.-C.; White, K. B.; McComsey, D. F.; Addo, M. F.; Andrade-Gordon, P.; Derian, C. K.; Oksenberg, D.; Maryanoff, B. E. High Affinity Thrombin Receptor (PAR-1) Ligands: A New Generation of Indole-Based Peptide Mimetic Antagonists with a Basic Amine at the C-terminus. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2199–2203.
- (21) Derian, C. K.; Damiano, B. P.; Addo, M. F.; Darrow, A. L.; D'andrea, M. R.; Nedelman, M.; Zhang, H.-C.; Maryanoff, B. E.; Andrade-Gordon, P. Blockade of the Thrombin Receptor Protease-Activated Receptor-1 with a Small-Molecule Antagonist Prevents Thrombus Formation and Vascular Occlusion in Nonhuman Primates. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 855–861.
- (22) 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.
- (23) Zhang, H.-C.; Derian, C. K.; Andrade-Gordon, P.; Hoekstra, W. J.; McComsey, D. F.; White, K. B.; Poulter, B. L.; Addo, M. F.; Cheung, W.-M.; Damiano, B. P.; Oksenberg, D.; Reynolds, E. E.; Pandey, A.; Scarborough, R. M.; Maryanoff, B. E. Discovery and Optimization of a Novel Series of Thrombin Receptor (PAR-1) Antagonists: Potent, Selective Peptide Mimetics Based on Indole and Indazole Templates. *J. Med. Chem.* **2001**, *44*, 1021–1024.
- (24) Cook, J. J.; Sitko, G. R.; Bednar, B.; Condra, C.; Mellott, M. J.; Feng, D.-M.; Nutt, R. F.; Shafer, J. A.; Gould, R. J.; Connolly, T. M. An Antibody against the Exo-Site of the Cloned Thrombin Receptor Inhibits Experimental Arterial Thrombosis in the African Green Monkey. *Circulation* **1995**, *91*, 2961–2971.
- (25) Chackalamannil, S.; Ahn, H.-S.; Xia, Y.; Doller, D.; Foster, C. Potent Non-Peptide Thrombin Receptor Antagonists. *Curr. Med. Chem.: Cardiovasc. Hematol. Agents* **2003**, *1*, 37.
- (26) Selnick, H. G.; Barrow, J. C.; Nantermet, P. G.; Connolly, T. M. Non-Peptide Small Molecule Antagonists of the Human Platelet Thrombin Receptor (PAR-1). *Curr. Med. Chem.: Cardiovasc. Hematol. Agents* **2003**, *1*, 47.
- (27) Chackalamannil, S.; Davies, R. J.; Asberom, T.; Doller, D.; Leone, D. A Highly Efficient Total Synthesis of (+)-Himbacine. *J. Am. Chem. Soc.* **1996**, *118*, 9812–9813.
- (28) Doller, D.; Chackalamannil, S.; Czarniecki, M.; McQuade, R. M.; Ruperto, V. Design, Synthesis and Structure–Activity Relationship Studies of Himbacine Derived Muscarinic Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 901–906.
- (29) Four, P.; Guibe, F. Palladium-Catalyzed Reaction of Tributyltin Hydride with Acyl Chlorides. A Mild, Selective and General Route to Aldehydes. *J. Org. Chem.* **1981**, *46*, 4439–4445.
- (30) Ahn, H.-S.; Foster, C.; Boykow, G.; Arik, L.; Smith-Torhan, A.; Hesk, D.; Chatterjee, M. Binding of a Thrombin Receptor Tethered Ligand Analogue to Human Platelet Thrombin Receptor. *Mol. Pharmacol.* **1997**, *51*, 350–356.
- (31) Bednar, B.; Condra, C.; Gould, R. J.; Connolly, T. M. Platelet Aggregation Monitored in a 96 Well Microplate Reader Is Useful for Evaluation of Platelet Agonists and Antagonists. *Thromb. Res.* **1995**, *77*, 453–463.
- (32) Ahn, H.-S.; Foster, C.; Boykow, G.; Stamford, A.; Manna, M.; Graziano, M. Inhibition of Cellular Action of Thrombin by *N*-3-Cyclopropyl-[4-91-methylethyl]phenylmethyl]-7*H*-pyrrolo[3,2-*f*]quinoxaline-1,3-diamine (SCH 79797), a Nonpeptide Thrombin Receptor Antagonist. *Biochem. Pharmacol.* **2000**, *60*, 1425–1434.

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