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Letter

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Himbacine-Derived Thrombin Receptor Antagonists: C_7-Aminomethyl and C_{9a}-Hydroxy Analogues of Vorapaxar

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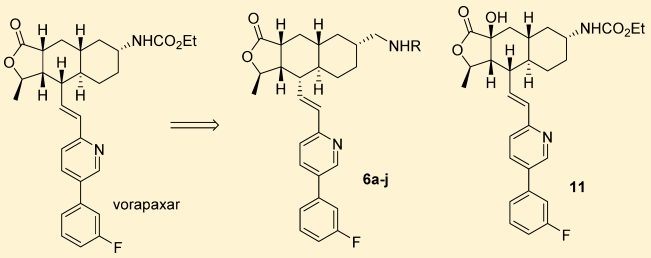
Supporting Information

ABSTRACT: We have synthesized several C_7-aminomethyl analogues of vorapaxar that are potent PAR-1 antagonists. Many of these analogues showed excellent in vitro binding affinity and pharmacokinetics profile in rats. Compound **6a** from this series showed excellent PAR-1 activity (K_i = 5 nM). We have also synthesized a C_{9a}-hydroxy analogue of vorapaxar, which showed very good PAR-1 affinity (K_i = 19.5 nM) along with excellent rat pharmacokinetic profile and ex vivo efficacy in the cynomolgus monkey.



KEYWORDS: *Himbacine, vorapaxar, PAR-1 antagonist, thrombin receptor, platelet aggregation*

Cardiovascular disease is a major cause of death in both developed and developing countries. A large number of these deaths is associated with platelet mediated atherothrombotic events.[1,2] When an atherosclerotic rupture occurs in the vascular endothelium, it leads to the adhesion of platelets to the site of injury, which subsequently form a platelet rich thrombus. Though the formation of thrombus plays a vital role in hemostasis, the formation of thrombi often occludes the coronary artery leading to acute coronary syndrome such as unstable angina and myocardial infarction. Thus, antiplatelet agents play a vital role in treating patients prone to atherothrombotic events. Aspirin and clopidogrel are two widely used antiplatelet agents for people afflicted by acute coronary syndrome. Aspirin works by the antagonism of thromboxane A2 (TXA2) mediated platelet activation, whereas clopidogrel works by the antagonism of the predominant ADP receptor $P2Y_{12}$. Though platelet activation is initiated by several factors, thrombin is the most potent activator of platelets. This activation is mediated by thrombin action on two GPCR receptors located on the surface of the platelets called thrombin receptors (also known as protease activated receptors; PAR-1 and PAR-4).[3−9] Among these two receptors, PAR-1 plays a major role in primate platelet activation. Agents that antagonize the activation of this PAR-1 receptor can be expected to be potent antiplatelet agents.

We have published a series of himbacine-derived antiplatelet agents that are potent antagonists of PAR-1 receptor.[10−16] Our systematic optimization of the himbacine based hit led to the discovery of vorapaxar (SCH530348), a potent PAR-1 antagonist.[14] Vorapaxar showed excellent ex vivo platelet aggregation inhibition in a preclinical monkey efficacy model.[17] In the phase-II clinical study, it met the primary end point of absence of TIMI major and minor bleeding.[18] In the phase-III clinical trial for the secondary prevention of atherothrombotic events, there was a significant reduction in overall ischemic events with vorapaxar when added to background antiplatelet therapy.[19,20] Thus, treatment of atherothrombotic events with antagonists of the PAR-1 receptor has been validated by the results of the phase-III clinical trial of vorapaxar.

In our lead optimization of the himbacine based thrombin receptor antagonists, we replaced the C_7 carbon of the tricyclic himbacine scaffold with nitrogen, which resulted in the discovery of **1** (Figure 1).[12] This heterohimbacine analogue **1** maintained the excellent antithrombotic activity while solving the critical enzyme induction and clearance issues encountered in the corresponding carbocyclic series.[12] When compound **1** was dosed in cynomolgus monkey, with 20% PEG-HPBCD as the dosing vehicle, it showed excellent ex vivo efficacy in the platelet aggregation inhibition assay. Unfortunately, this compound showed poor aqueous solubility (<2 μM) and the ex vivo efficacy was markedly reduced when the dosing vehicle was changed from 20% PEG-HPBCD to 0.4% methylcellulose. In the subsequent SAR study of this series, nitrogen atom of the C-ring was moved exocyclic to the ring to give the C_7-amino substituted tricyclic himbacine analogues, which led to the discovery of vorapaxar.[14] In this letter, we disclose the variation of this C-ring exocyclic amine to give the homologated analogues **6a−j** where a methylene linker is introduced between the amino group and the C-ring along with the carboxamide analogues **9a−h**. We also synthesized the C_{9a}-hydroxy analogue **11** of vorapaxar. This analogue was prepared in reflection of a recent disclosure of novel nor seco himbacine analogues as potent PAR-1 antagonists.[16] In the nor seco series,

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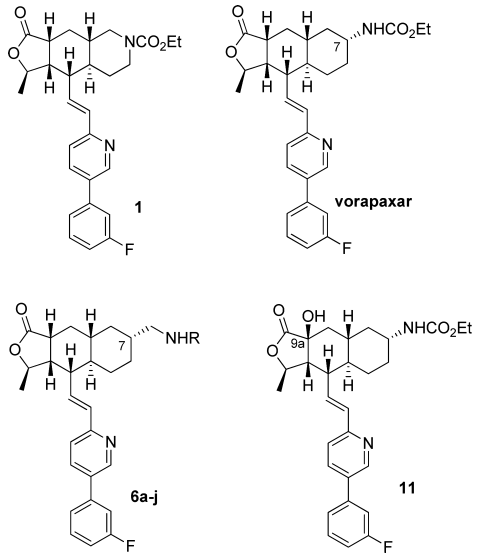


Figure 1. Himbacine-derived thrombin receptor antagonists.

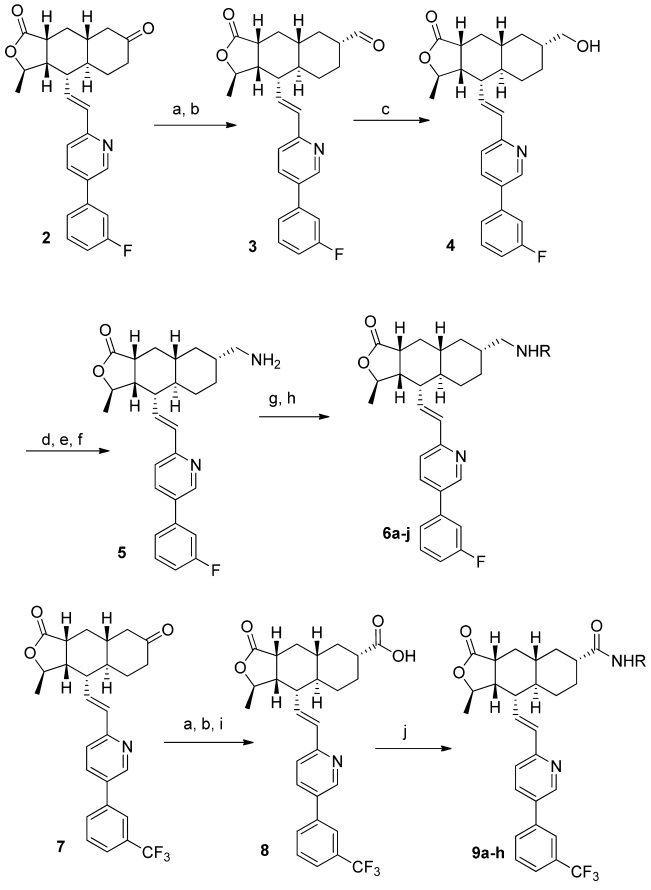
we observed a dramatic improvement in the rat plasma level by the introduction of a hydroxy functionality at the carbon alpha to the lactone carbonyl group. We wanted to see whether a similar improvement in pharmacokinetic profile will result for vorapaxar by the introduction of a similar C_{9a}-hydroxy group. Herein we describe the synthesis and the PAR-1 activity results of these analogues.

The synthesis of the C_7-aminomethyl analogues **6a−g** (Scheme 1) starts with the known ketone **2**,[14] which was subjected to a Wittig reaction followed by the hydrolysis of the resulting vinyl ether to give the aldehyde **3**. Reduction of this aldehyde gave the alcohol **4**, which was mesylated, and the mesylate was subsequently displaced with sodium azide to give the azide intermediate, which was reduced to the amine **5** using trimethyl phosphine. This amine was subsequently treated with chloroformates, acid chlorides, sulfonyl chlorides, and isocyanates to give the corresponding carbamates, sulfonamides, and ureas **6a−j**. The preparation carboxamide analogues **9a−h** started with the ketone **7**,[11] which was converted to the carboxylic acid **8**. Coupling of this acid with amines under standard condition gave the amide analogues **9a−h**.

Synthesis of the C_{9a}-hydroxy analogue of vorapaxar **11** is described in Scheme 2. The amino functionality of the carbamate group was protected with di-*tert*-butyldicarbonate, and the resultant product was treated with lithium bis-(trimethylsilyl)amide to generate the C_{9a}-enolate. This enolate was stirred under an oxygen atmosphere to introduce the C_{9a}-hydroxy functionality. Deprotection of the *tert*-butyl carbamate group under acidic conditions gave the target **11**.

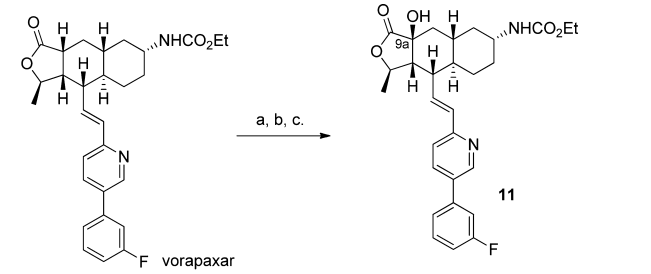
The above synthesized analogues were evaluated in the in vitro binding assay using PAR-1 receptors isolated from human platelets and [^{3}H]haTRAP as the radioligand.[21] Table 1 presents the PAR-1 binding affinity for the C_7-aminomethyl derivatives, and Table 2 presents the affinity for the carboxamide analogues. Binding affinity for compound **11** is presented in Scheme 2. Both the C_7-hydroxymethyl analogue **4** and the unsubstituted aminomethyl analogue **5** showed good binding affinity. The methyl carbamate **6a** (K_i = 5 nM) showed excellent binding affinity, while the ethylcarbamate **6b** (K_i = 14 nM) was 3-fold less active. Both the acetamide **6c** and the propionamide **6d** analogues showed very good affinity. Amides

Scheme 1. Synthesis of C_7-Aminomethyl Carbamates and Carboxamides[a]



[a]Reagents and conditions: (a) $Ph_3P^+CH_2OCH_3$ Cl^-, ^{t}BuOK, THF; (b) HCl in dioxane/water, rt; (c) sodium borohydride, THF−MeOH; (d) methanesulfonyl chloride, Et_3N; (e) sodium azide, DMSO, 65 °C; (f) Me_3P, H_2O; (g) ROCOCl, RCOCl, RSO_2Cl, RNCO; (h) TFA−DCM (for compounds **6e−g** only); (i) $NaClO_2$, H_2O_2, NaH_2PO_4; (j) RNH_2, HATU, Et_3N.

Scheme 2. Synthesis of C_{9a}-Hydroxy Analogue of Vorapaxar[a]



PAR-1 Ki = 8.5±3.2 nM
Rat $AUC_{0\text{-}6h}$ = 3064 ng.hrml (10mg/kg, po)

PAR-1 Ki = 19.5±5.5 nM
Rat $AUC_{0\text{-}6h}$ = 7431 ng.hrml (10 mg/kg, po)

[a]Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMAP, CH_3CN, 60 °C; (b) LHMDS, THF then oxygen; (c) TFA−DCM, 0 °C to rt.

6e−g containing amino groups indicate that polar groups are tolerated at this position. The amide derivative of piperidine-4-carboxylic acid **6g** showed very good affinity, though the corresponding analogues of alanine (**6e**) and proline (**6f**) analogues showed 2-fold less affinity. The methane sulfonamide

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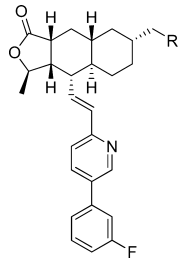
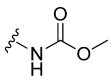
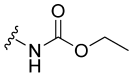
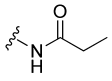
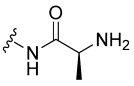
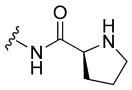
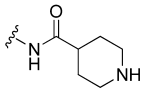
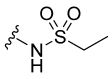
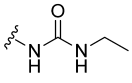
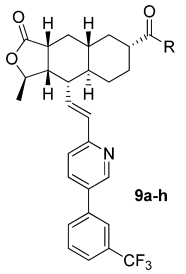
Table 1. Binding and PK Data for Compounds 4, 5, and 6a−j

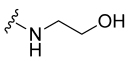
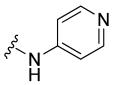
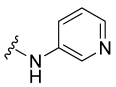
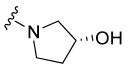
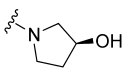


compd	R	Ki (nM) ± SEM[a]	Rat AUC[b]
4		8±0.1	2673
5		20±2	
6a		5±0.3	4863
6b		14±0	1213
6c		12±2	3813
6d		12.5±1.5	7728
6e		32±7	
6f		24±5	
6g		13.5±1.5	5
6h		15±0	7758
6i		14.5±3.5	
6j		12.9±5.1	567

[a]n = 2 or more. [a]AUC from 0 to 6 h in ng·h/mL and at 10 mg/kg oral dose (0.4% methylcellulose).

Table 2. Binding Data for Compounds 9a−h



compd	R	Ki (nM) ± SEM[a]
9a		25±8
9b		35±11
9c		31±5
9d		98±22
9e		140±66
9f		239±7
9g		180±5.5
9h		384±5.5

[a]n = 2 or more.

(**6h**), ethane sulfonamide (**6i**), and *N*-ethyl urea (**6j**) analogues showed good affinity.

The carboxamide analogues **9a−c** containing *N*-methyl, *N*-ethyl, and *N*-isopropyl amides showed good affinity. However, introduction of polar group reduces the affinity as indicated by the amino ethanol amide analogue **9d**. Amides of 4-amino-pyridine and 3-aminopyridines **9e−f** showed considerable reduction in affinity. Both analogues of 3-hydroxy pyrrolidine (**9g−h**) showed reduced affinity indicating that polar groups at this position reduces the affinity. Also, the C_{9a}-hydroxy analogue of vorapaxar, **11**, showed good affinity (Scheme 2) indicating that the introduction of the hydroxyl group at the C_{9a} position is tolerated in this series

Selected analogues were evaluated in the rat pharmacokinetic assay at an oral dose of 10 mg/kg, and the plasma levels were analyzed up to 6 h. Several of these analogues showed good plasma levels. While the ethyl carbamate analogue **6b** showed good rat plasma level (AUC = 1213 ng·h/mL), the corresponding methyl carbamate analogues **6a** showed about 4-fold higher plasma level (AUC = 4863 ng·h/mL). Compared with the acetamide **6c**, the propionamide analogue **6d** showed 2-fold increase in plasma level (AUC = 7728 ng·h/mL). Analogue **6g** showed very poor plasma level (AUC = 5 ng·h/mL) indicating that the polar amino group severely affects the absorption. While methane sulfonamide **6h** showed excellent plasma level, the *N*-ethyl urea analogue **6j** showed a low level of plasma. Rat plasma level for the C_{9a}-hydroxy analogue **11** was excellent (AUC = 7431 ng·h/mL) as indicated in Scheme 2. Compared with vorapaxar (AUC = 3064 ng·h/mL), analogue **11** showed a 2-fold increase in rat plasma level. This observation is in parallel to the nor seco himbacine analogues where introduction of the hydroxy group at alpha to the carbonyl group showed increased plasma level.[16]

We evaluated compounds **11** and **6a** in the ex vivo platelet aggregation inhibition assay in cynomolgus monkey (Figure 2). The compound was given as an oral dose and blood was drawn

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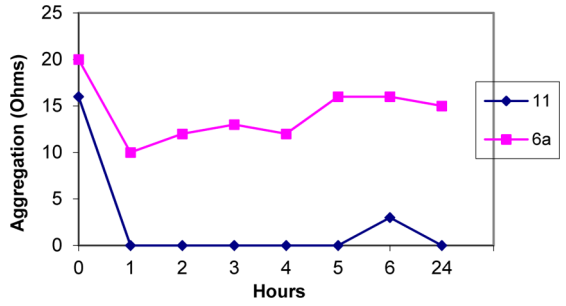

Figure 2. Ex vivo platelet aggregation inhibition in cynomolgus monkey following a single oral dose (1 mg/kg in 20% PEG-HPBCD) for **11** and **6a**.

at various points of time. The exogenous agonist peptide, haTRAP, was added as a 1 M solution to the blood sample, and the extent of aggregation induced by this agonist was quantified using an aggregometer as described before.[22] At a dose of 1 mg/kg, **11** completely inhibited platelet aggregation, and the inhibition was maintained at this level for up to 24 h. This clearly indicated that the introduction of the C_{9a}-hydroxy group, while improving the rat plasma level, also maintained excellent ex vivo efficacy profile. In marked contrast, compound **6a** showed only transient efficacy despite showing excellent binding affinity and rat plasma levels.

In summary, we have synthesized several C_7-aminomethyl and carboxamide analogues of vorapaxar. The aminomethyl analogues in general showed excellent binding affinity while maintaining excellent rat plasma levels for many analogues. Introduction of a hydroxy group at the C_{9a} position of vorapaxar improved rat plasma level while maintaining complete inhibition of platelet aggregation in cynomolgus monkey.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the preparation of compounds **6a**, **9a**, and **11** is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PAR-1, protease activated receptor-1; TXA2, thromboxane A2; PEG-HPBCD, poly(ethylene glycol)−hydroxypropyl-beta-cyclodextrin; haTRAP, high affinity thrombin receptor-activating peptide; ADP, adenosine diphosphate receptor; GPCR, G protein coupled receptor; PK, pharmacokinetics; DMAP, 4-dimethylaminopyridine; LHMDS, lithium bis(trimethylsilyl) amide; DCM, dichloromethane; HATU, 1-[bis-(dimethylamino) methylene]-1*H*-1,2,3-triazolo[4,5-b]-pyridinium 3-oxid hexafluorophosphate

■ REFERENCES

(1) Davi, G.; Patrono, C. Platelet Activation and Atherothrombosis. *N. Engl. J. Med.* **2007**, *357*, 2482−2494.

(2) Jackson, S. P.; Schoenwaelder, S. M. Antiplatelet Therapy: in Search of the 'Magic Bullet'. *Nat. Rev. Drug Discovery* **2003**, *2* (10), 775−789.

(3) Chackalamannil, S. *Burger's Medicinal Chemistry, Drug Discovery, and Development*, 7th ed.; Abraham, D. J., Rotella, D. P., Eds.; Wiley: New York, 2010; pp 409−476.

(4) 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.

(5) Coughlin, S. R. Protease-Activated Receptors in the Cardiovascular System. *Cold Spring Harbor Symp. Quant. Biol.* **2002**, *67*, 197−208.

(6) Coughlin, S. R. Protease-Activated Receptors in Vascular Biology. *J. Thromb. Haemostasis* **2001**, *86*, 298−307.

(7) Chackalamannil, S. Thrombin Receptor (Protease Activated Receptor-1) Antagonists as Potent Antithrombotic Agents with Strong Antiplatelet Effects. *J. Med. Chem.* **2006**, *49*, 5389−5403.

(8) Grand, R. J; Turnell, A.; Grabham, A. S.; Cellular, P. W. Consequences of Thrombin-Receptor Activation. *Biochem. J.* **1996**, *313*, 353−368.

(9) Coughlin, S. R. Protease-Activated Receptors in Hemostasis, Thrombosis and Vascular Biology. *J. Thromb. Haemostasis* **2005**, *3*, 1800−1814.

(10) Chackalamannil, S.; Xia, Y.; Greenlee, W. J.; Clasby, M.; Doller, D.; Tsai, H.; Asberom, T.; Czarniecki, M.; Ahn, H.-S.; Boykow, G.; Foster, C.; Agans-Fantuzzi, J.; Bryant, M.; Lau, J.; Chintala, M. Discovery of Potent Orally Active Thrombin Receptor (Protease Activated Receptor 1) Antagonists as Novel Antithrombotic Agents. *J. Med. Chem.* **2005**, *48*, 5884−5887.

(11) Clasby, M. C.; Chackalamannil, S.; Czarniecki, M.; Doller, D.; Eagen, K.; Greenlee, W.; Kao, G.; Lin, Y.; Tsai, H.; Xia, Y.; Ahn, H.-S.; Agans-Fantuzzi, J.; Boykow, G.; Chintala, M.; Foster, C.; Smith-Torhan, A.; Alton, K.; Bryant, M.; Hsieh, Y.; Lau, J.; Palamanda, J. Metabolism-Based Identification of a Potent Thrombin Receptor Antagonist. *J. Med. Chem.* **2007**, *50*, 129−138.

(12) Chelliah, M. V.; Chackalamannil, S.; Xia, Y.; Eagen, K.; Clasby, M. C.; Gao, X.; Greenlee, W. J.; Ahn, H.-S.; Agans-Fantuzzi, J.; Boykow, G.; Hsieh, Y.; Bryant, M.; Palamanda, J.; Chan, T.-M.; Hesk, D.; Chintala, M. Heterotricyclic Himbacine Analogs as Potent, Orally Active Thrombin Receptor (Protease Activated Receptor-1) Antagonists. *J. Med. Chem.* **2007**, *50* (21), 5147−5160.

(13) Clasby, M. C.; Chackalamannil, S.; Czarniecki, M.; Doller, D.; Eagen, K.; Greenlee, W. J.; Lin, Y.; Tagat, J. R.; Tsai, H.; Xia, Y.; Ahn, H.; Agans-Fantuzzi, J.; Boykow, G.; Chintala, M.; Hsieh, Y.; McPhail, A. T. Himbacine Derived Thrombin Receptor Antagonists: Discovery of a New Tricyclic Core. *Bioorg. Med. Chem. Lett.* **2007**, *17* (13), 3647−3651.

(14) Chackalamannil, S.; Wang, Y.; Greenlee, W. J.; Hu, Z.; Xia, Y.; Ahn, H.; 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* (11), 3061−3064.

(15) Xia, Y.; Chackalamannil, S.; Greenlee, W. J.; Wang, Y.; Hu, Z.; Root, Y.; Wong, J.; Kong, J.; Ahn, H.; Boykow, G.; Hsieh, Y.; Kurowski, S.; Chintala, M. Discovery of a Vorapaxar analogue with Increased Aqueous Solubility. *Bioorg. Med. Chem. Lett.* **2010**, *20* (22), 6676−6679.
(16) Chelliah, M. V.; Chackalamannil, S.; Xia, Y.; Eagen, K.; Greenlee, W. J.; Ahn, H.; Agans-Fantuzzi, J.; Boykow, G.; Hsieh, Y.; Bryant, M.; Chan, T.; Chintala, M. Discovery of Nor-Seco Himbacine Analogs as Thrombin Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2012**, *22* (7), 2544−2549.
(17) Chintala, M.; Shimizu, K.; Ogawa, M.; Yamaguchi, H.; Doi, M.; Jensen, P. Basic and Translational Research on Proteinase-Activated Receptors: Antagonism of the Proteinase-Activated Receptor 1 for Thrombin, a Novel Approach to Antiplatelet Therapy for Atherothrombotic Disease. *J. Pharmacol. Sci.* **2008**, *108*, 433−438.
(18) Richard, C. B.; David, J. M.; Lisa, K. J.; Karen, S. P.; Jinglan, P.; Alan, N.; Khaled, M. Z.; Gail, B.; John, S.; Diane, J.; Kenneth, W. M.; Frans, V. W.; Enrico, V.; Robert, A H. Safety and Tolerability of SCH 530348 in Patients Undergoing Non-Urgent Percutaneous Coronary Intervention: a Randomised, Double-Blind, Placebo-Controlled Phase II Study. *Lancet* **2009**, *373*, 919−928.
(19) Tricoci, P.; Huang, Z.; Held, C.; Moliterno, D. J.; Armstrong, P. W.; Van de Werf, F.; White, H. D.; Aylward, P. E.; Wallentin, L.; Chen, E.; Lokhnygina, Y.; Pei, J.; Leonardi, S.; Rorick, T. L.; Kilian, A. M.; Jennings, L. H. K.; Ambrosio, G.; Bode, C.; Cequier, A.; Cornel, J. H.; Diaz, R.; Erkan, A.; Huber, K.; Hudson, M. P.; Jiang, L.; Jukema, J. W.; Lewis, B. S.; Lincoff, A. M.; Montalescot, G.; Nicolau, J. C.; Ogawa, H.; Pfisterer, M.; Prieto, J. C.; Ruzyllo, W.; Sinnaeve, P. R.; Storey, R. F.; Valgimigli, M.; Whellan, D. J.; Widimsky, P.; Strony, J.; Harrington, R. A.; Mahaffey, K. W. Thrombin-Receptor Antagonist Vorapaxar in Acute Coronary Syndromes. *N. Engl. J. Med.* **2012**, *366* (15), 20−33.
(20) Morrow, D. A.; Braunwald, E.; Bonaca, M. P.; Ameriso, S. F.; Dalby, A. J.; Fish, M. P.; Fox, K. A.; Lipka, L. J.; Liu, X.; Nicolau, J. C.; Ophuis, A. J.; Paolasso, E.; Scirica, B. M.; Spinar, J.; Theroux, P.; Wiviott, S. D.; Strony, J.; Murphy, S. A. Vorapaxar in the Secondary Prevention of Atherothrombotic Events. *N. Engl. J. Med.* **2012**, *366* (15), 1404−1413.
(21) 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.
(22) 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* (13), 2199−2203.