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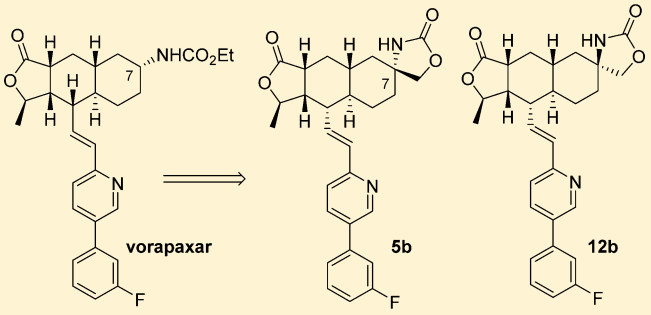
Himbacine-Derived Thrombin Receptor Antagonists: C_7-Spirocyclic Analogues of Vorapaxar

Mariappan V. Chelliah,* Keith Eagen, Zhuyan Guo, Samuel Chackalamannil,† Yan Xia,‡ Hsingan Tsai, William J. Greenlee,§ Ho-Sam Ahn, Stan Kurowski, George Boykow, Yunsheng Hsieh, and Madhu Chintala

Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033-1300, United States

Supporting Information

ABSTRACT: We have synthesized several C_7-spirocyclic analogues of vorapaxar and evaluated their in vitro activities against PAR-1 receptor. Some of these analogues showed activities and rat plasma levels comparable to vorapaxar. Compound **5c** from this series showed excellent PAR-1 activity (K_i = 5.1 nM). We also present a model of these spirocyclic compounds docked to the PAR-1 receptor based on the X-ray crystal structure of vorapaxar bound to PAR-1 receptor. This model explains some of the structure−activity relationships in this series.



KEYWORDS: *PAR-1 antagonist, vorapaxar, thrombin receptor, himbacine*

Platelets play a central role in balancing the interplay between blood clotting and bleeding. Any atherosclerotic plaque rupture in the artery will activate the aggregation of platelets, which is a major component of blood clot.[1] When a clot occludes a small vessel, it can prevent the blood flowing to the heart and cause heart attack, which is a major cause of death in developed countries. Drugs that inhibit platelet aggregation can be used to treat patients with prior cardiovascular events and prevent future heart attacks. Aspirin and thienopyridines are two commonly prescribed classes of antiplatelet agents. Aspirin works by blocking the activation of platelets by thromboxane, whereas thienopyridines work by irreversibly inhibiting the ADP receptor $P2Y_{12}$.[2,3] Despite the use of these drugs, heart attack is still a major cause of mortality. There is an unmet clinical need for novel drugs that work by an entirely different mechanism compared with aspirin and thienopyridines.

We have published on the activity of several series of antiplatelet agents, derived from the natural product himbacine, that work by a novel mechanism.[4−10] These agents are antagonists of the PAR-1 thrombin receptor (also called protease activated receptor, PAR), a G-protein coupled receptor, located on the platelet surface. Though two types of PAR receptors are present in human (PAR-1 and PAR-4), PAR-1 dominates the thrombin mediated platelet activation.[11−17] Thrombin cleaves the extra-cellular domain of these receptors exposing a tethered ligand, which activates these receptors leading to the aggregation of platelets, which ultimately lead to the formation of platelet rich thrombi. These thrombi can often occlude the coronary arteries leading to heart attack.

Optimization of our himbacine based lead led to the discovery of vorapaxar, a first-in-class antiplatelet agent that works by antagonism of the PAR-1 receptor.[8] In a recent Ph-III clinical trial, vorapaxar was evaluated against the secondary prevention of atherothrombotic events.[18,19] Vorapaxar showed a significant reduction of death from cardiovascular events. Thus, the proof of concept of treating platelet aggregation by the antagonism of PAR-1 receptor has been clinically validated.

Since the C_7-carbamate side-chain of vorapaxar is a site of metabolism,[20] as part of our structure−activity studies on vorapaxar analogues we were interested in analogues where the carbamate is cyclized in a spirocyclic fashion to give an analogue such as **5b** (Figure 1). We believed that this might address the carbamate metabolism observed in vorapaxar. In this letter we disclose the synthesis, PAR-1 activity, and a docking model of these novel spirocyclic analogues of vorapaxar.

Synthesis of the spirocyclic oxazolidinone analogues **5a−d** (Scheme 1) starts with the known ketone **1**,[5] which was converted to the olefine **8** using standard Wittig olefination condition. Hydroboration of this alkene using 9-BBN gave compound **3** as the major isomer, which was converted to the carbamate **4** using trichloroacetyl isocyanate. Spirocyclization of carbamate **4** to give the oxazolidinone **5a** was achieved using rhodium mediated C−H insertion as described by Dubois.[21] The 3-fluorophenyl analogue **5b** was synthesized by ozonolysis of **5a** giving aldehyde **6**, which was coupled with the known

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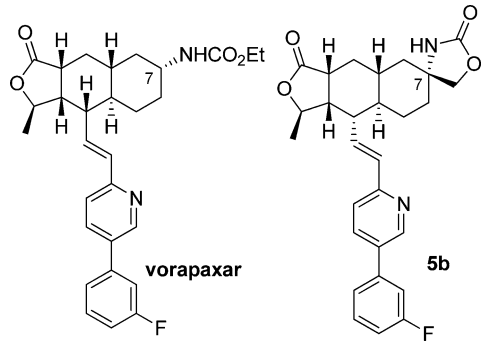
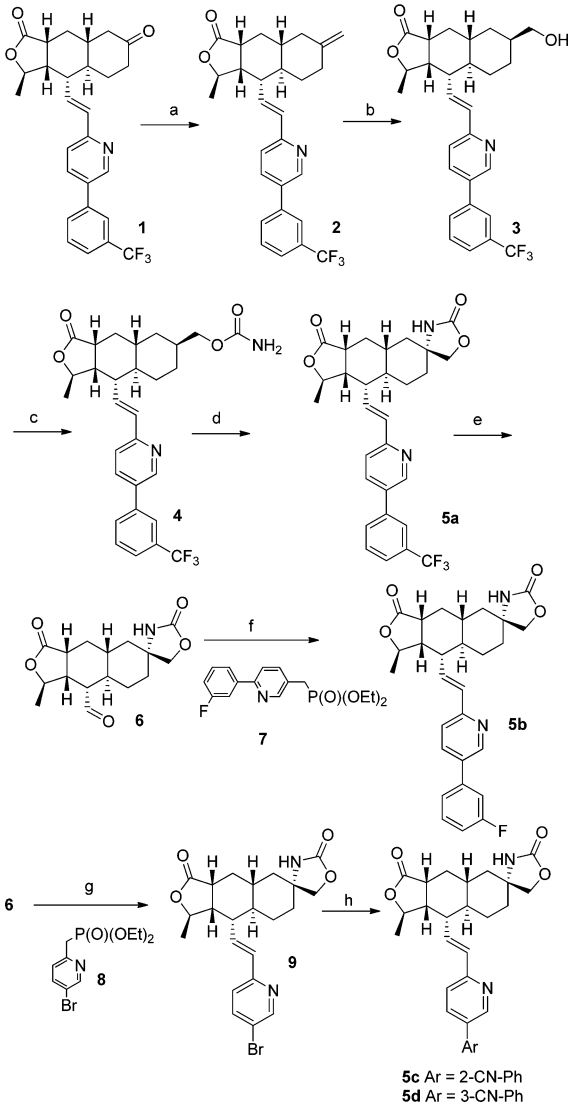


Figure 1. Spirocyclic analogue of vorapaxar.

Scheme 1. Synthesis of Spirocyclic Analogues 5a–d[a]

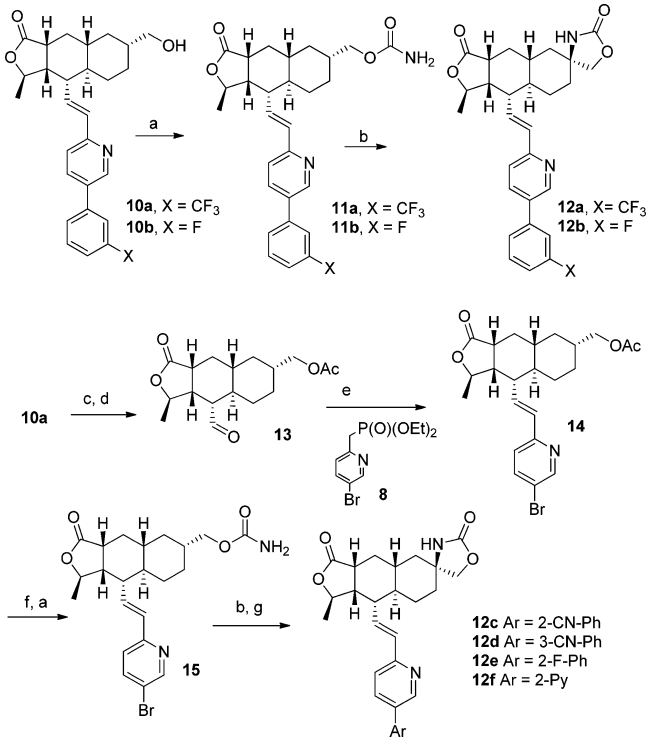


[a]Reagents and conditions: (a) $Ph_3P^+CH_3\ Br^-$, phenyl lithium, THF; (b) 9-BBN then $NaBO_3{\cdot}4H_2O$; (c) trichloroacetyl isocyanate then K_2CO_3; (d) $Rh_2(OAc)_4$, MgO, $PhI(OAc)_2$; (e) ozone, −78 °C then dimethyl sulfide, rt; (f) **7**, LHMDS, $Ti(O^iPr)_4$ then **6** ; (g) **8**, LHMDS, $Ti(OiPr)_4$ then **6**; (h) 2-cyano-phenyl boronic acid neopentylglycol ester or 3-cyano-phenyl boronic acid, $Pd(Ph_3P)_4$, K_2CO_3.

phosphonate **7**. To synthesize analogues where the phenyl substitution is varied further, we prepared bromo-substituted pyridyl intermediate **9** from aldehyde **6** and phosphonate **8**. This enabled direct Suzuki type cross-coupling reaction on **9** to afford the desired targets **5c–d**.

The synthesis of the isomeric oxazolidinone spirocyclic analogues, where the C_7-amino group takes the β-orientation, represented by structures **12a–f**, is presented in Scheme 2. The

Scheme 2. Synthesis of Spirocyclic Analogues 12a–f[a]



[a]Reagents and conditions: (a) trichloroacetyl isocyanate then K_2CO_3; (b) $Rh_2(OAc)_4$, MgO, $PhI(OAc)_2$; (c) acetyl chloride, triethyl amine, and DMAP; (d) ozone, −78 °C then dimethyl sulfide, rt; (e) **8**, LHMDS, $Ti(O^iPr)_4$ then **13**; (f) K_2CO_3, MeOH; (g) $ArB(OH)_2$, $Pd(Ph_3P)_4$, K_2CO_3 (for compounds **12c–e**), and 2-pyridylzinc chloride, $Pd(Ph_3P)_4$, THF (for compound **12f**).

alcoholic functionality of **10a** was converted to carboxamide **11a** using trichloroacetyl isocyanate, which gave the desired spirocyclic analogue **12a** upon rhodium facilitated C–H insertion.[21] Analogue **12b** was prepared in a similar fashion starting from the alcohol **10b**. To prepare analogues where the phenyl substitution is varied, intermediate **15** was prepared. Preparation of **15** starts with **10a**, where the alcoholic functionality was protected as the acetate and the double bond was cleaved by ozonolysis to give the aldehyde **13**. Coupling of this aldehyde with the phosphonate **8** gave **14**, which was converted to the carbamate **15**. Spirocyclization followed by cross-coupling of the aryl bromide with appropriately substituted aryl boronic acids or aryl zinc reagent gave the target analogues **12c–f**.

We also synthesized isomeric oxazolidinone analogues where the oxygen atom of the oxazolidinone ring is directly attached to the C_7-position of the carbocyclic ring as represented by the structures **20a–b** (Scheme 3). Reaction of **1** with dimethylsulfonium methylide gave, after chromatographic separation, epoxides **17a** and **17b**. Both epoxides were ring opened with sodium azide to give the azides **18a–b**, which on reduction

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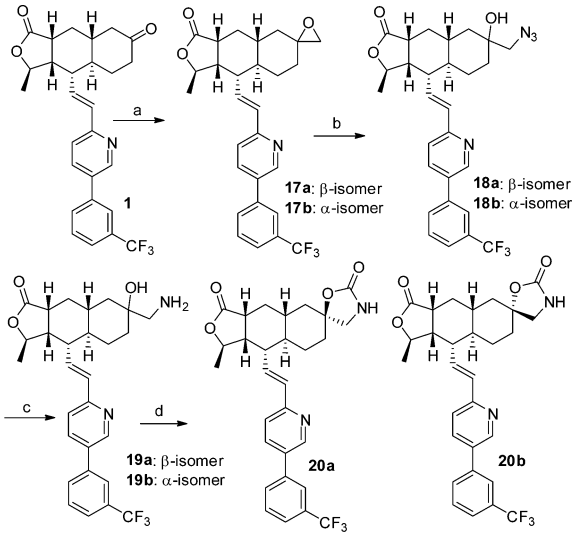
Scheme 3. Synthesis of Spirocyclic Analogues 20a−b[a]

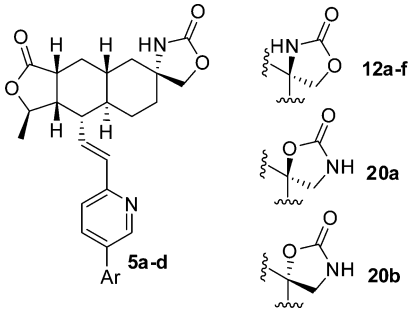


[a]Reagents and conditions: (a) NaH, trimethylsulfonium iodide, DMSO; (b) NaN_3, NH_4Cl, DMF, 60 °C; (c) Me_3P, ethyl acetate−H_2O; (d) triphosgene, DIPEA.

with trimethyl phosphine followed by treatment with triphosgene gave the oxazolidinone analogues **20a−b**.

In vitro activities for the target compounds were determined using [^{3}H]haTRAP as the radioligand as described before.[22] PAR-1 receptors isolated from human platelets were used for this study. The inhibition constants (K_i) for the spirocyclic analogues are presented in Table 1. Among the spirocyclic analogues **5a−d**, the 2-cyanophenyl analogue **5c** (K_i = 5.1 nM) shows the best activity. This trend continues in the isomeric oxazolidinone analogues **12a−f** where the 2-cyanophenyl analogue **12c** (K_i = 8.9 nM) shows excellent activity. Unlike the vorapaxar analogues, the spirocyclic series appears to be sensitive to the effect of phenyl substitution as represented by the marked reduction in potency for the 3-cyanophenyl analogue **5d** (K_i = 267 nM) and pyridyl analogue **12f** (K_i = 232 nM). Among the two spirocyclic analogues **20a** and **20b** where the oxygen of the oxazolidinone ring is directly attached to the C_7-carbon, the β-isomer **20a** showed better activity than the corresponding α-isomer **20b**. Representative analogues were also evaluated in rat pharmacokinetic studies. Analogues **5a**, **5b**, and **20a** were dosed orally at 10 mg/kg, and they showed good plasma levels as indicated by their AUCs up to the 6 h time point that was evaluated.

Table 1. Inhibition Constant and Pharmacokinetic Data for Compounds 5a−d, 12a−f, and 20a−b



compd	Ar	K_i (nM) ± SEM[a]	rat AUC[b]
5a	3-CF_3−Ph	21 ± 9	4920
5b	3-F−Ph	15.5 ± 1.5	3245
5c	2-CN−Ph	5.1 ± 0.5	
5d	3-CN−Ph	267 ± 19.5	
12a	3-CF_3−Ph	26.5 ± 11.5	
12b	3-F−Ph	28 ± 13	
12c	2-CN−Ph	8.9 ± 3.1	
12d	3-CN−Ph	43 ± 10	
12e	2-F−Ph	25 ± 1	
12f	2-pyridyl	232 ± 21	
20a	3-CF_3−Ph	31.5 ± 4.5	3202
20b	3-CF_3−Ph	392 ± 78	

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

To gain insight into protein ligand interactions and understand the SAR, models of compounds complexed with PAR1 were built by docking using the crystal structure of vorapaxar bound to PAR-1.[23] As in vorapaxar, the tricyclic region of the compounds discussed here binds to the extracellular loop region, with the spirocycle pointing toward an opening to the solvent, while the biaryl region binds to a hydrophobic pocket toward the trans-membrane region. As a representative example, we have provided a model of compound **5c** bound to the PAR-1 receptor (Figure 2). On the basis of the model, we attempt to rationalize the observed SAR. In general, the SAR in the **5a−d** and **12a−f** series follows a similar trend. For example, within each series, substitutions at the 2-position with a nitrile have the most potency enhancement (**5c** and **12c**). This can be explained by the binding of the biaryl region of these compounds to a subpocket involving residues Pro236, Leu237, Leu262, Leu263, and Lys240 near the bottom of the inhibitor binding site. Substituent 2-CN−Ph in **5c** and **12c** would fit nicely to the pocket. However, 2-F−Ph

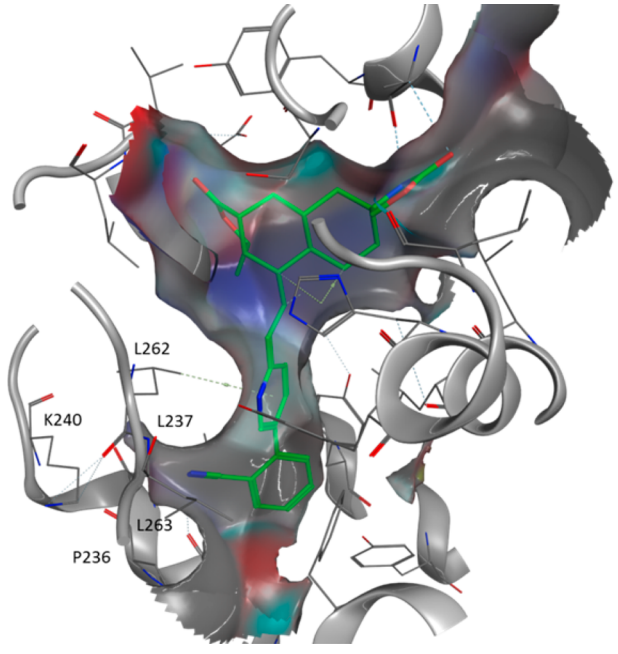


Figure 2. Model of compound **5c** bound to the PAR-1 receptor. The inhibitor is represented as stick (green carbon) and the protein as cartoon. The binding site pocket and select key residues are also shown.

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(**12e**) is slightly short and does not interact efficiently with the protein. The increase in K_i of 2-pyridyl (**12f**) may be partly attributed to the desolvation penalty of burying a polar atom in the hydrophobic environment.

Although the substituent 3-CF_3 is tolerated in **20a**, the isomeric compound **20b** shows ∼10-fold increase in K_i. We attribute this K_i shift to the difference in the stereo chemical orientation between these two isomers. From modeling, the NH in spirocycle **5a** makes a hydrogen bond with the backbone C═O of the protein; in spirocycle **12a**−**f** and **20a**, this NH is replaced by a CH_2 group, which is still tolerated (the NH in spirocycle **12a**−**f** and O in **20a** is close to His336). However, for **20b**, the corresponding atom is an oxygen, which would cause electrostatic conflict with the C═O of the protein.

In summary, we have synthesized several C_7-spirocyclic analogues of vorapaxar, evaluated their PAR-1 activities, and used the vorapaxar/PAR-1 crystal structure to build a docking model for these analogues. Compared with the vorapaxar series, the spirocyclic analogues appear to be more sensitive to phenyl substitution. The 2-cyanophenyl analogues **5c** and **12c** showed excellent in vitro activities, which could be explained by the excellent fit into a subpocket in the trans-membrane domain of the protein.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the preparation of all the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(M.V.C.) E-mail: mchelliah@aol.com.

Present Addresses

†(S.C.) Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854−8020, United States.

‡(Y.X.) School of Environmental and Municipal Engineering, Qingdao Technological University, 11 Fushun Road, Qingdao, Shandong 266033, China.

§(W.J.G.) MedChem Discovery Consulting, LLC, 115 Herrick Avenue, Teaneck, New Jersey 07666, United States.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PAR-1, protease activated receptor-1; haTRAP, high affinity thrombin receptor-activating peptide; ADP, adenosine diphosphate receptor; 9-BBN, 9-borabicyclo[3.3.1]nonane; DMAP, dimethylaminopyridine; LHMDS, lithium bis(trimethylsilyl) amide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DIPEA, *N*,*N*-diisopropylethylamine; PK, pharmacokinetics; AUC, area under the curve; SAR, structure−activity relationship

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