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Discovery of Octahydroindenes as PAR1 Antagonists

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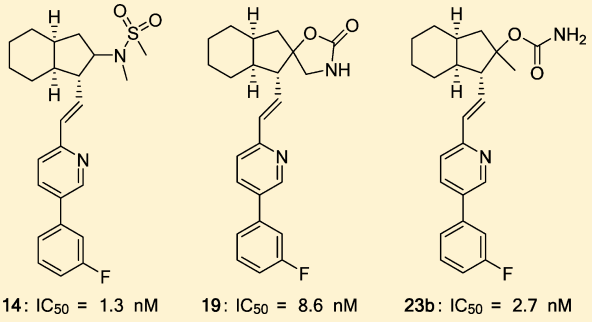
Supporting Information

ABSTRACT: Octahydroindene was identified as a novel scaffold for protease activated receptor 1 (PAR1) antagonists. Herein, the 2-position (C2) was explored for structure−activity relationship (SAR) studies. Compounds **14**, **19**, and **23b** showed IC_{50} values of 1.3, 8.6, and 2.7 nM in a PAR1 radioligand binding assay, respectively, and their inhibitory activities on platelet activation were comparable to that of vorapaxar in a platelet rich plasma (PRP) aggregation assay. This series of compounds showed high potency and no significant cytotoxicity; however, the compounds were metabolically unstable in both human and rat liver microsomes. Current research efforts are focused on optimizing the compounds to improve metabolic stability and physicochemical properties as well as potency.



KEYWORDS: *Octahydroindene, PAR1 antagonist, PRP aggregation, antiplatelet, bleeding*

The protease activated receptor 1 (PAR1) plays a critical role in thrombin mediated platelet aggregation but not in fibrin generation. It is thought to be a promising antithrombosis target with potentially less severe bleeding side effects.[1,2] PAR1 is activated through proteolytic cleavage of its extracellular loop by thrombin, and the resulting new amino acid terminus (SFLLRN) intramolecularly binds to the proximally located portion of the receptor, acting as a tethered ligand to cause transmembrane signaling.[3−6] Consequently, a strong interaction between PAR1 and its antagonist is required for the efficient blockage of the intramolecular binding of a tethered ligand with a very high local concentration.[7−13] Vorapaxar is a very potent and virtually irreversible PAR1 antagonist with very slow receptor association and dissociation rates.[14−17] Regardless the observation of an increased risk of bleeding in a phase III trial of vorapaxar on patients who had previously suffered a stroke,[18] there is still the potential to dissociate antiplatelet activity from bleeding risk.

We have designed the compounds described herein using the structure of vorapaxar as a starting point to identify new PAR1 antagonists. We kept its (3-fluorophenyl)pyridine-2-vinyl moiety fixed and attempted to modify the tricyclic ring of vorapaxar. We found that a 6/5 bicycle,[19] octahydroindene could serve as a core scaffold (Figure 1). The middle 6-membered ring of vorapaxar was replaced with a 5-membered ring, and its lactone ring was opened. The 2-position (C2) of the octahydroindene was further studied to determine structure−activity relationships (SARs). In this letter, we report the SAR of this series of compounds as PAR1 antagonists and

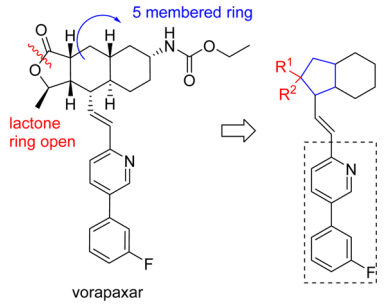


Figure 1. Design of octahydroindene core.

we further describe the biological and physicochemical profiles of some of the active compounds.

Primarily, ketal analogues at C2 were prepared to confirm that the substitution of an octahydroindene scaffold could retain the compound's ability to inhibit PAR1. The synthesis followed the conventional procedures outlined in Scheme 1 (see the Supporting Information for details). Reductive ring-opening of both *cis*- and *trans*-fused hexahydroisobenzofuran-1,3-diones using $LiAlH_4$ generated diols (**1a** and **1b**) and *O*-mesylation of alcohols were subsequently performed. An S_N2 reaction of mesylates (**2a** and **2b**) with cyanides (**3a** and **3b**) and a Dieckmann cyclization of dinitriles yielded three octahydroindenes (**4a**, **4b**, and **4c**).[20−22] The *trans*-fused

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Scheme 1. Syntheses of *cis*- and *trans*-Octahydroindenes

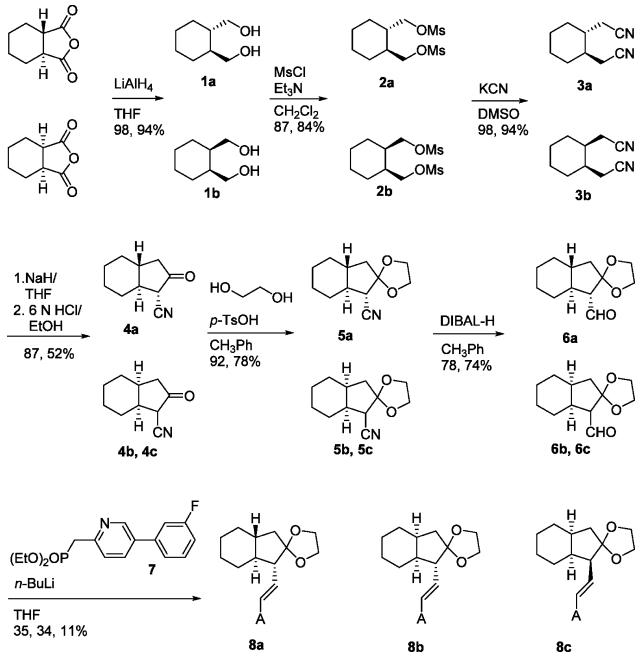
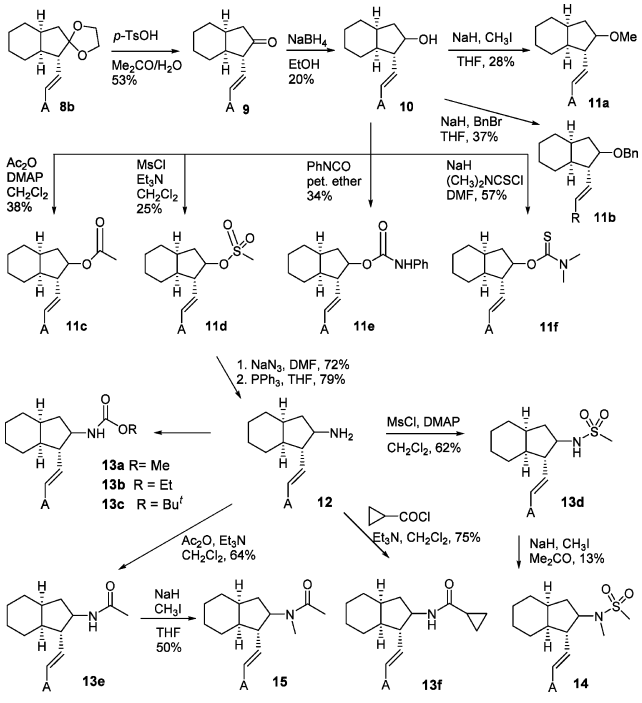


Table 1. Binding Data of *cis*- and *trans*-6/5 Bicyclic Dioxolanes

cmpd	vorapaxar[b]	8a	8b	8c
IC_{50} (μM)[a]	0.0011	0.47	0.026	2.1

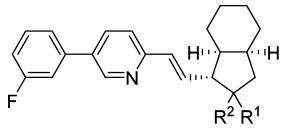
[a]PAR1 binding assay ligand [^{3}H]haTRAP, 10 nM. The value is an average of three measurements. [b]Vorapaxar was synthesized and evaluated by our laboratory as a reference standard.

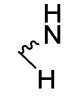
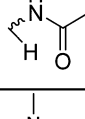
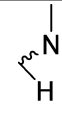
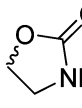
Scheme 2. Syntheses of 2-Oxy and 2-Amine Derivatives



compound **3a** yielded only one epimer (**4a**), which might be thermodynamically stable; however, the *cis*-fused compound **3b** gave a mixture of epimers of **4b** and **4c** as a 3:1 ratio. After ketal formation, reduction of nitriles to aldehydes (**6a**, **6b**, and **6c**) using DIBAL-H and a Horner−Wadsworth−Emmons (HWE) reaction with phosphonate **7** were performed to produce the corresponding dioxolanes (**8a**, **8b**, and **8c**).[10] Racemic mixtures were used for the biological assay without separation. A PAR1 binding assay was carried out to evaluate the activity of compounds to inhibit PAR1 using human platelet membrane as the receptor source and [^{3}H]-labeled high affinity thrombin receptor activation peptide ([^{3}H]−haTRAP, alanine-*p*-fluoro-

Table 2. SAR at C2 of Octahydroindene Derivatives[a]



Cmpd	R^1, R^2	IC_{50} (μM)	Cmpd	R^1, R^2	IC_{50} (μM)
8b		0.026	**9**		1.1
10		0.22	**11a**		0.067
11b		0.28	**11c**		0.067
11d		0.0064	**11e**		0.36
11f		0.12	**12**		0.23
13a		0.98	**13b**		2.0
13c		7.8	**13d**		0.050
13e		0.053	**13f**		0.23
14		0.0013	**15**		0.0054
19		0.0086	**22**		0.033
23a		0.043	**23b**		0.0027

[a]See Table 1 footnotes.

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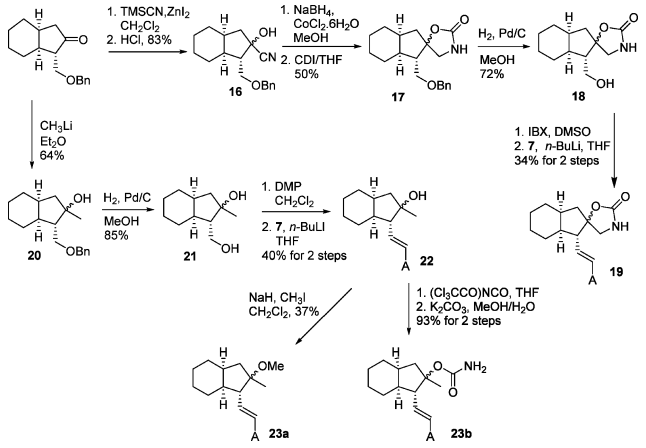
Scheme 3. Syntheses of 2-Quaternary and Spiro Derivatives



phenylalanine-arginine-cyclohexylalanine-homoarginine-[^{3}H]-tyrosine-NH$_2$) as the ligand (Table 1).[23,24] In this assay, vorapaxar, which was synthesized in our laboratory for use as a reference standard, showed an IC$_{50}$ value of 0.0011 μM. One of the *cis*-fused dioxolanes (**8b**, IC$_{50}$ = 0.026 μM) exhibited a much higher activity level than the *trans* compound (**8a**, IC$_{50}$ = 0.47 μM) and the other *cis* compound (**8c**, IC$_{50}$ = 2.1 μM).

We also synthesized the 2-oxy and 2-amine derivatives shown in Scheme 2 (see the Supporting Information for details) and evaluated their PAR1 inhibitory activity to study the SAR at C2 of *cis*-octahydroindene (Table 2). The ketal **8b** was hydrolyzed to ketone **9**, and then reduced to alcohol **10**, which resulted in the formation of two diastereoisomers that were separated and evaluated. However, the diastereoisomers showed no marked differences in activity. We then decided to prepare the mixture for further manipulation until we found a good activity profile for the compound. The yield of the ketone to alcohol reduction reaction was unusually low (20%) because a compound in which the double bond had migrated was substantially formed along with several unidentified side products. Alkylation and acylation of alcohol **10** were performed under standard conditions. The ketone **9** exhibited significantly less activity (IC$_{50}$ = 1.1 μM), while alcohol **10**, benzyloxy derivative **11b**, phenylcarbamate **11e**, and dimethylcarbamothioate **11f** were moderately active (IC$_{50}$ = 0.12−0.36 μM). The methyl ether **11a** and acetate **11c** showed a slightly improved potency over alcohol **10** but were weaker than ketal **8b**. The mesylate **11d** showed the highest increase in activity (IC$_{50}$ = 0.0064 μM). The addition of another H-bond acceptor or a bulky substituent at C2 might be beneficial for activity.

Amine **12** was prepared by nucleophilic substitution of mesylate **11d** to an azide and a subsequent reduction using PPh$_3$. The compound **12** was further derivatized to carbamate, amide, and methanesulfonamide compounds. The potencies of amine **12** and cyclopropylamide **13f** were similar to that of alcohol **10**, but carbamates **13a−13c** showed a marked reduction in affinity, especially when the size was increased. The incorporation of a large substituent at C2 was not tolerated. Methanesulfonamide **13d** and acetamide **13e** showed IC$_{50}$ values of 0.050 and 0.053 μM, respectively, and these values were similar to those of methoxy compound **11a** and acetate **11c**. *N*-Methylation of **13d** and **13e** led to a 10−25-fold improvement in activity with IC$_{50}$ values of 1.3 and 5.4 nM, respectively. The potency of *N*-methylmethanesulfonamide (**14**) was comparable to that of vorapaxar. The bulky substituent, which is close to C2, seemed to be favorable for PAR1 binding, but a large substituent far from C2 was deleterious, as observed with carbamates **13a−13c**. For vorapaxar, it has been reported previously that the methyl at the lactone ring gives optimal activity.[11]

Because the compounds with a short and bulky substituent at C2 exhibited improved PAR1 inhibition, quaternary and spiro compounds were additionally synthesized (Scheme 3). Initially, we attempted to form the spiro ring after introducing a pyridine-2-vinyl moiety to improve the synthetic throughput. Contrary to our expectations, the reaction was very restricted, presumably due to the presence of the pyridine-2-vinyl group, which can act as a Michael acceptor. In a subsequent synthesis attempt, the spiro oxazolidinone ring was incorporated before performing the HWE reaction according to the established procedures.[25,26] Oxidation of alcohol **18** to aldehyde using 2-iodobenzoic acid (IBX), followed by an immediate HWE reaction produced compound **19**.[27] 2-Methyl-2-alcohol **20** was prepared by the same procedure as **19** and was subsequently derivatized to methyl ether **23a** and carbamate **23b**.[28] As anticipated, the introduction of an additional 2-methyl group and spiro ring made the compounds 5 to 10 times more potent. Spiro oxazolidinone **19** and its open structure **23b** were highly active with IC$_{50}$ values of 8.6 and 2.7 nM, respectively.

We also examined the inhibitory effects of several of the active compounds on platelet activation via a human platelet aggregation assay induced with haTRAP (Table 3).[29,30] While the inhibitory activity on washed platelet aggregation (WPA) was well correlated with PAR1 binding affinity, only 3

Table 3. Functional Assays and Properties

			metabolic stability (R$_{50}$, min)[b]		
cmpd	WPA IC$_{50}$[a] (μM)	PRP IC$_{50}$[a] (μM)	human	rat	cytotox. at 30 μM (% ATP)[c]
vorapaxar[d]	0.0015	0.12	83.2	32.4	102%
8b	0.41	4.1	4.5	3.4	97%
11d	0.006	N.A.	3.5	6.5	94%
13d	0.14	3.9	273	105	N.A
14	0.0022	0.097	2.5	2.2	95%
15	0.21	8.9	5.6	5.2	98%
19	0.0072	0.21	4.4	6.7	101%
23b	0.0029	0.10	4.3	5.8	99%

[a]The value is an average of three measurements. Aggregation inducer, haTRAP; preincubation 10 min. [b]Time when 50% of the compound remains upon incubation with human and rat liver microsomes. [c]In HepG2 cell line, ATP contents were determined by a % compared to those of vehicle-treated control at 24 h after addition of compound (30 μM). [d]See the Table 1 footnotes.

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compounds (**14**, **19**, and **23b**) represented significant efficacy (IC_{50} = 0.097, 0.21, and 0.10 μM, each) in the human platelet rich plasma (PRP) aggregation assay. These results are comparable to that obtained for vorapaxar (IC_{50} = 0.12 μM). In addition to the affinity of the compound for PAR1, the PRP assay reflects the compound's physicochemical properties, including plasma stability. None of the compounds exhibited cytotoxicity as determined by ATP contents in the HepG2 cell line; however, the compounds were not stable when incubated in human and rat liver microsomes. The compounds must be optimized to improve their metabolic stability.

In conclusion, we have identified a novel 6/5 bicycle core, octahydroindene scaffold that functions as a PAR1 antagonist. From the SAR studies at the C2 position, it was found that short and bulky substitution at C2 led to an improvement in activity. The compounds **14**, **19**, and **23b** were comparably potent to vorapaxar in PAR1 binding and PRP aggregation assays. While this series of compounds was generally not cytotoxic, the compounds were not metabolically stable in human and mouse liver microsomes. We are currently trying to optimize the compounds described herein to improve metabolic stability as well as potency.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures and characterization data; assay protocols. 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.

■ ACKNOWLEDGMENTS

[^{3}H]−haTRAP (Ala-Phe(p-F)-Arg-ChA-Har-[^{3}H]Tyr-NH_2): Cold peptides were prepared by Peptron (Korea, www.peptron.co.kr), and the radiolabeling of ligands was done by Moravek, USA.

■ ABBREVIATIONS

PAR1, protease-activated receptor 1; SAR, structure−activity relationship; HWE, Horner−Wadsworth−Emmons; DIBAL-H, diisobutylaluminum hydride; haTRAP, high affinity thrombin receptor activation peptide; IBX, 2-iodobenzoic acid; WPA, washed platelet aggregation; PRP, platelet rich plasma

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