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Himbacine derived thrombin receptor (PAR-1) antagonists: Structure–activity relationship of the lactone ring

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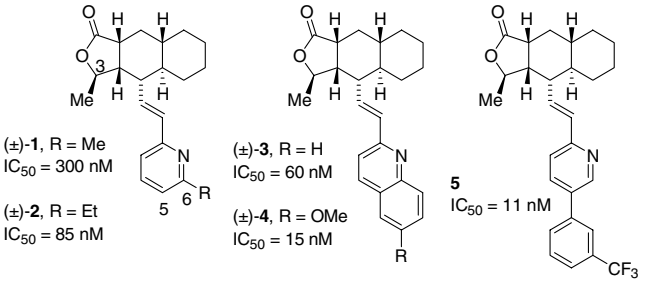
Abstract—The structure–activity relationship (SAR) of the lactone ring of himbacine derived thrombin receptor (PAR-1) antagoinsts (e.g., **2**–**5**) is described. The effect of the lactone carbonyl group on binding to PAR-1 is dependent on the substitution pattern of the pyridine ring. A stereoselective intramolecular Michael addition reaction to the vinyl pyridine group was observed for these pyridine analogs of himbacine in basic conditions at elevated temperature.

Thrombin plays essential roles in hemostasis and wound healing.[1] While it stops bleeding through clot formation in the coagulation cascade, overactivity under pathological conditions often results in arterial or venous thrombosis which will lead to cardiovascular events such as myocardial infarction or stroke. Various agents that attenuate the activity of thrombin have been used as antithrombotic drugs, including indirect or direct thrombin inhibitors (e.g., heparin or hirudin). However, their wider use has been tempered by a lack of oral activity and bleeding side effects.[2] Thrombin plays two roles in thrombus formation: the first is the enzymatic cleavage of soluble fibrinogen to form fibrin which cross links to form the matrix of the clot. Another is the activation of platelet aggregation through thrombin receptors on the platelets.[3] Fibrin and aggregated platelets together comprise the insoluble thrombus. Thrombin is the most potent activator of platelet aggregation, although other activators such as ADP and thromboxane A2 also contribute. The cloning and characterization of the thrombin receptor or protease-activated receptor-1 (PAR-1) in the early 1990s presented an attractive approach of using small molecule PAR-1 antagonist as a novel orally active antithrombotic agent.[4] Because thrombin is the most potent activator of platelet aggregation, and because a PAR-1 antagonist would selectively block the action of thrombin on platelet aggregation without affecting formation of fibrin or platelet aggregation by other activators, a PAR-1 antagonist might achieve good antithrombotic effects with less bleeding side effects.[5–7]



We recently described a novel series of small molecule PAR-1 antagonists derived from the natural product himbacine (**2**–**5**).[8,9] These compounds have a vinyl pyridine appendage attached to the central ring of the tricyclic lactone core instead of the vinyl piperidine structure as in himbacine. We have discovered that the pyridine ring of the lead compound **1** is an important structural motif for PAR-1 binding. Optimization of structure–activity relationships (SARs) at the 5- or 6- positions

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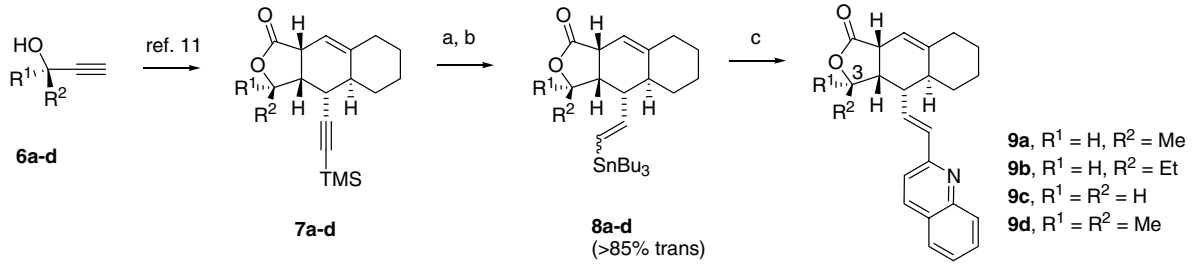
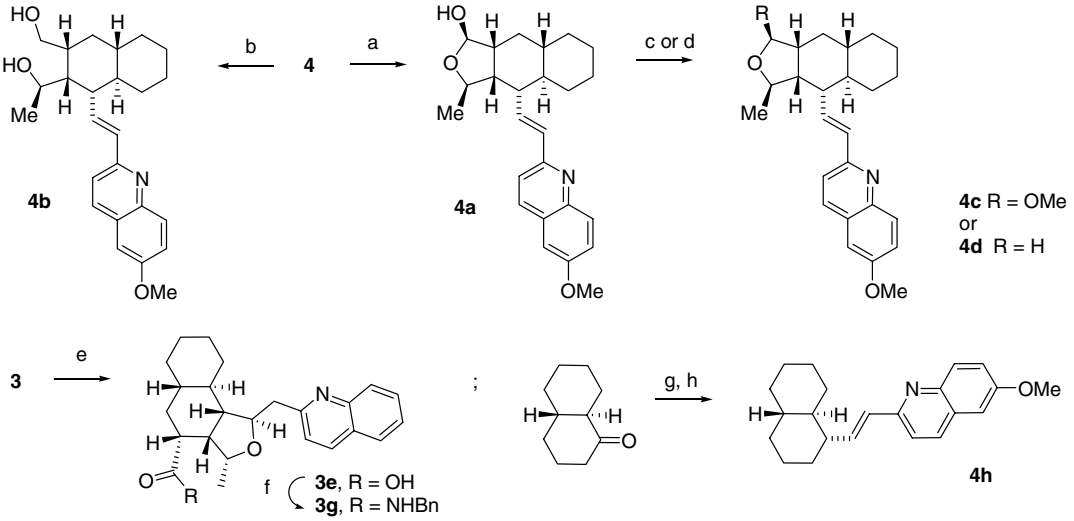
of the pyridine ring led to potent PAR-1 antagonists such as compounds **4** and **5**. Additionally, the 5-phenyl substituted pyridine compounds such as **5** displayed good oral activity.[8] In addition to the pyridine ring, the lactone ring also caught our attention as a potential structural element that can affect PAR-1 binding.[10] We were interested in the effects of substitutions at the C-3 position of the lactone ring and the importance of the lactone functional group. Here we describe the results of our studies which help to define the pharmacophore for PAR-1 binding for the himbacine derived PAR-1 antagonists. During the SAR studies, we discovered an interesting stereospecific intramolecular Michael addition reaction for these pyridine analogs of himbacine.

The synthesis of compounds with different substitutions on the lactone C-3 carbon is shown in Scheme 1. The tricyclic alkyne intermediates **7a–d** were synthesized from commercially available alkynyl alcohols **6a–d** as described previously.[11] The trimethylsilyl group of **7a–d** was cleaved and the resulting alkyne was heated with tributyltin hydride and AIBN to give the vinyltin intermediates **8a–d** which contain >85% of the trans isomer. Palladium catalyzed coupling reactions of **8a–d** with 2-chloroquinoline followed by separation gave the trans products **9a–d**.

The synthetic transformations of the lactone ring of the lead compounds **2–5**[8,9] are exemplified in Scheme 2. Diisobutylaluminum hydride reduction of compound **4** gave the lactol **4a**[12] which was either converted to the 2-methoxytetrahydrofuran derivative **4c**[13] with boron trifluoride etherate and methanol or to the tetrahydrofuran derivative **4d** with boron trifluoride etherate and triethyl silane. Alternatively, the lactone ring of **4** was reduced with lithium aluminum hydride to give diol **4b**. Other lactone compounds such as **2**, **3**, and **5** underwent similar transformations to give the derivatives shown in Table 2. We then attempted to open up the lactone ring to obtain a hydroxy acid derivative. The lactone ring is quite stable to acidic conditions.[14] In basic conditions (10% KOH in dioxane) at room temperature, the lactone compounds were converted to a highly polar intermediate product first but upon neutralization and work up were recovered unchanged. In more vigorous basic conditions (10% KOH in dioxane at reflux), however, they were converted in good yield to products which are consistent by spectroscopic analysis with a



Scheme 1. Reagents and conditions: (a) K_2CO_3, MeOH, rt, 80%; (b) Bu_3SnH, AIBN, toluene, 120 °C, 80%; (c) $Pd(PPh_3)_4$, 2-chloroquinoline, toluene, 100 °C, 80%.



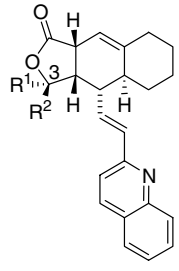
Scheme 2. Reagents and conditions: (a) DIBAL, toluene, −78 °C, 1.5 h, 95%; (b) LAH, THF, rt, 0.5 h, 96%; (c) $BF_3{\cdot}OEt_2$, MeOH, 0 °C to rt, 3 h, 60%; (d) $BF_3{\cdot}OEt_2$, Et_3SiH, −78 °C to rt, 3 h, 75%; (e) 10% KOH–dioxane (1:1), reflux, 20 h, 85%; (f) $BnNH_2$, EDCI, HOBt, DMF, 16 h, 46%; (g) i—diethyl (isocyanomethyl)phosphonate, *n*-BuLi, ether, −78 to 0 °C; ii—HCl (concd); (h) diethyl (6-methoxy-quinolin-2-ylmethyl)phosphonate (Ref. 9), *n*-BuLi, THF, 0 °C to rt.

tetrahydrofuran structure such as **3e** (Scheme 2). Apparently the lactone was hydrolyzed under the basic conditions and the intermediate hydroxyl acid underwent an intramolecular Michael addition of the secondary hydroxyl group to the vinyl pyridine to form the tetrahydrofuran ring. The acid **3e** was further derivatized with benzylamine to give the benzyl amide **3g**. An X-ray analysis of **3g** established the stereochemistry around the tetrahydrofuran ring.[15] The cyclization appears to be stereoselective since only one diastereoisomer was observed for the product. Attempts to selectively protect the primary hydroxyl group of the diols such as **4b** also resulted in formation of tetrahydrofuran products from intramolecular Michael addition of the secondary hydroxyl group to the vinyl pyridine group. Finally, an analog **4h** of compound **4** where the lactone ring was removed was prepared by a two-step sequence from the commercially available *trans*-1-decalone: a one carbon homologation by the procedure of Moskal and Van Leusen[16] and Wadsworth–Emmons–Horner olefination of the resulting aldehyde using diethyl (6-methoxyquinolin-2-ylmethyl)phosphonate.[9]

The PAR-1 binding activities of the compounds were measured by the previously reported in vitro assay using purified human platelet membrane.[17] The IC_{50} values for the compounds that have different substitutions at the lactone C-3 position are shown in Table 1.[18] Changing the methyl group to an ethyl group (**9a** and **9b**) decreased the binding affinity by 10-fold. Removing the C-3 methyl group (**9c**) or having an additional methyl group (**9d**) at the C-3 carbon did not affect the binding (**9c**, **9d** $\approx$ **9a**). Since no apparent improvement in binding was observed we decided to use the C-3 monomethyl group in our subsequent SAR studies due to the ease in stereoselective synthesis from chiral starting materials (both enantiomers of **6a** are commercially available).

The PAR-1 binding activities of the lactone carbonyl modified compounds are listed in Table 2. The lactone moiety is the most potent group for PAR-1 binding. Conversions from the lactones (**2**–**5**) to the lactols (**2a**–**4a**), diols (**2b**, **4b**, and **5b**), or lactol ethers (2-methoxytetrahydrofurans **3c** and **4c**), or removal of the lactone carbonyl group to give the tetrahydrofuran derivatives (**3d**–**5d**) gave weaker binding at the receptor. However, the degree of reduction in binding affinity upon conversion of the lactones to other functional groups is dependent on whether the lactones have the optimal pyridine moiety for PAR-1 binding. With less than optimal pyridine moiety this reduction is more pronounced than compounds with optimal pyridine moiety. For example, in the less than optimal 6-ethylpyridine series, the lactol **2a** and the diol **2b** are about 40-fold less potent than the lactone **2**, whereas in the 6-methoxyquinoline series where the lactone **4** has a higher affinity for the PAR-1 receptor, the lactol **4a** and the diol **4b** are only about 4- to 5-fold less potent than the lactone **4**. In the 5-(3-trifluoromethyl-phenyl)-pyridine series where we have discovered some of our most potent PAR-1 antagonists, the diol **5b** is almost as potent as the lactone **5**. Compounds that retain a H-bond acceptor property of the lactone carbonyl oxygen such as the lactols (**2a**–**4a**), diols (**2b**, **4b**, and **5b**), and lactol ethers (2-methoxytetrahydrofurans **3c** and **4c**) gave better binding. Compounds that lack the H-bond acceptor property such as the tetrahydrofuran derivatives **3d**–**5d** gave much weaker binding (ca. 40-fold or more) even in the 5-(3-trifluoromethyl-phenyl)-pyridine series. The

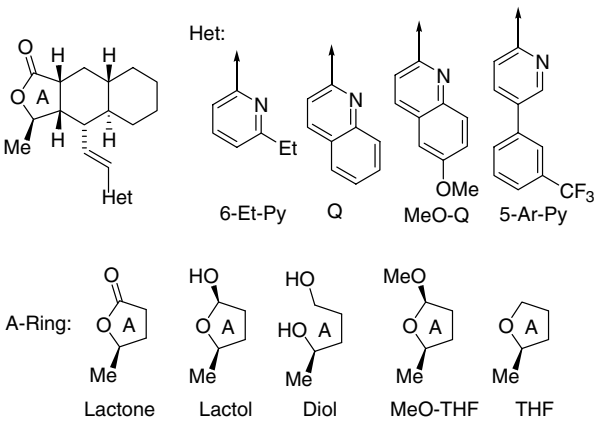
Table 1.



9a, R^1 = H, R^2 = Me
9b, R^1 = H, R^2 = Et
9c, R^1 = R^2 = H
9d, R^1 = R^2 = Me

Compound	R^1	R^2	IC_{50} (nM)
(±)-**3**			60
(±)-**9a**	H	Me	150
(±)-**9b**	H	Et	1500
(±)-**9c**	H	H	200
(±)-**9d**	Me	Me	150

Table 2.



Compound	A-ring	Het	IC_{50} (nM)
(±)-**2**	Lactone	6-Et-Py	85
(±)-**3**	Lactone	Q	60
(±)-**4**	Lactone	MeO-Q	15
5	Lactone	5-Ar-Py	11
(±)-**2a**	Lactol	6-Et-Py	3500
(±)-**3a**	Lactol	Q	2000
(±)-**4a**	Lactol	MeO-Q	62
(±)-**2b**	Diol	6-Et-Py	3750
(±)-**4b**	Diol	MeO-Q	74
5b	Diol	5-Ar-Py	18
(±)-**3c**	MeO–THF	Q	7000
(±)-**4c**	MeO–THF	MeO-Q	217
(±)-**3d**	THF	Q	8000
(±)-**4d**	THF	MeO-Q	623
5d	THF	5-Ar-Py	426
(±)-**4h**			7000
3e			5000
3g			Inactive

compound **4h** that lacks the lactone ring altogether decreased the binding by two orders of magnitude comparing with compound **4**. The intramolecular Michael addition products (**3e** and **3g**) are significantly less active as shown in Table 2.

In summary, the SAR of the lactone ring for PAR-1 binding for the himbacine derived PAR-1 antagonists was evaluated. The C-3 monomethyl group is the preferred group for stereoselective synthesis of these himbacine derived PAR-1 antagonists. The lactone ring is found to be an important structural motif for PAR-1 binding. Changes to the lactone functional group tend to give significant loss of binding affinity. This is likely due to the hydrogen-bond acceptor property of the lactone carbonyl group. However, the contribution toward binding affinity of the lactone group is less than that of the pyridine group. Changes to the lactone are more tolerated for the optimal pyridine groups such as the 6-methoxyquinoline or the 5-(3-trifluoromethylphenyl)-pyridine group. This SAR information is useful in designing new PAR-1 antagonists derived from himbacine. A stereoselective intramolecular Michael addition reaction was observed for these pyridine analogs of himbacine in basic conditions at elevated temperatures. The resulting tricyclic rings have highly defined conformation and may serve as template for new lead discoveries. Additional SAR results for the himbacine derived PAR-1 antagonists will be published in due course.

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12. Compound **4a** was isolated as a single diastereomer. The stereochemistry of the hydroxyl group was assigned based on the small coupling constant for the anomeric proton (J <2 Hz). This result is in agreement with the result observed by Prof. Kozikowski and co-workers.[10]
13. Compound **4c** was isolated as a single diastereomer. The stereochemistry of the methoxy group was assigned based on the small coupling constant for the anomeric proton (J <2 Hz).
14. Conditions examined: reflux with 6 N HCl in dioxane; reflux in 30% H_2SO_4; reflux with 33% HBr in AcOH; reflux with BBr_3 in 1,2-dichloroethane.
15. CCDC 608102 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK. Fax: +44 1223 336033.
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18. Compound **3** was included for reference. From our unpublished results the SAR in the unsaturated tricyclic lactone series generally parallels that in the saturated series with ca. 2-fold decreases in binding potency.