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# Himbacine derived thrombin receptor (PAR-1) antagonists: SAR of the pyridine ring

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**Abstract**—The structure–activity relationship (SAR) of the vinyl pyridine region of himbacine derived thrombin receptor (PAR-1) antagonists is described. A 2-vinylpyridyl ring substituted with an aryl or a heteroaryl group at the 5-position showed the best overall PAR-1 affinity and pharmacokinetic properties. One of the newly discovered analogs bearing a 5-(3-pyridyl) substituent showed excellent PAR-1 affinity ($K_i$ = 22 nM) and oral activity with reduced *C*log *P* and improved off-target selectivity compared to an earlier development candidate.


Cardiovascular disease (CVD) affects one third of US adult population and is the leading cause of death in the US.[1] The majority of CVD mortality and morbidity is associated with coronary artery diseases (CAD) caused by advancing atherosclerotic lesions.[2] Spontaneous rupture of an atherosclerotic plaque can result in the formation of an occlusive thrombus leading to myocardial infarction (MI) and unstable angina, which can often be fatal. Thrombin plays a key role in arterial thrombosis: it cleaves fibrin to fibrinogen and activates platelets, two key events in thrombus formation.[3] While thrombin is essential for its life-sustaining role in hemostasis and wound healing, thrombin's over-activity under pathological conditions often results in arterial or venous thrombosis which will lead to MI, unstable angina, or stroke. Various antithrombotic agents aim to attenuate either the endogenous production of thrombin or its proteolytic activity. However, their widespread use has been tempered by bleeding side effects and a lack of oral activity.[4]

Thrombin activates platelets and other cell types via proteolytic cleavage of specific cell surface G-protein coupled receptors known as protease activated receptors (PARs).[5] Four PARs are known (PAR1-4), among which PAR-1, also known as thrombin receptor, is the most abundant on human and monkey platelets. Among the various platelet activators, thrombin is the most potent. It has been proposed that thrombin activates platelet thrombin receptors via a 'tethered ligand' mechanism in which the newly unmasked amino terminus by thrombin of the extracellular loop intramolecularly ligates the receptor eliciting intracellular signal transduction which leads to platelet activation.[6] The cloning and characterization of PAR-1 in the early 1990s presented an opportunity for identifying small molecule PAR-1 antagonists as novel, orally active antithromotic agents.[6] Because thrombin is the most potent activator of platelet, and because a PAR-1 antagonist would selectively block thrombin-mediated platelet activation without inhibiting fibrin formation or platelet activation by other activators (e.g., ADP and thromboxane A2), a PAR-1 antagonist might achieve potent antiplatelet effects with less bleeding side effects than the currently available anticoagulants and antiplatelet agents.[7]

We have previously reported a series of PAR-1 antagonists **1**–**5** based on the natural product himbacine.[8,9] Structure–activity relationship (SAR) development at the pyridine 6 position led to the potent, albeit orally inactive, compound **2**. Further SAR development at the pyridine 5 position yielded the potent compound **4**

*Keywords*: Structure–activity relationship; SAR; Pyridine; Himbacine; Thrombin receptor antagonist; PAR-1 antagonist.
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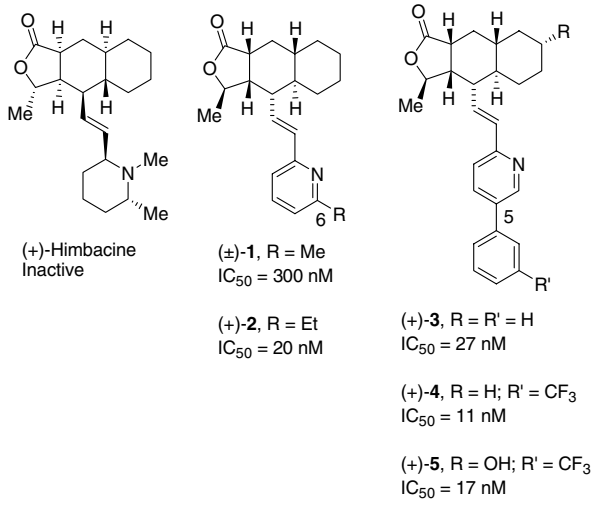
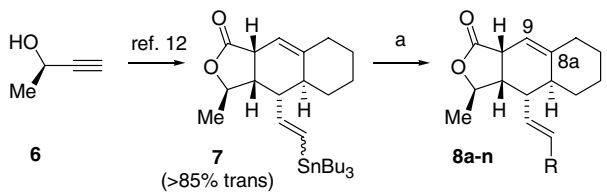
with excellent oral activity in the platelet aggregation assay in cynomolgus monkeys.[8] Compound **4**, however, showed induction of rat specific CYP 2B liver enzyme at higher doses with time-progressed lowering of the parent plasma levels. Such an apparent autoinduction pattern suggests potential lack of adequate drug exposure needed for the FDA-required long term rodent carcinogenicity studies. Compound **4** is highly hydrophobic ($C\log P = 6.0$) and showed slow metabolite clearance in our laboratory animal clearance studies. We suspect that the enzyme induction observed in rat could be attributed to the accumulation of metabolites. By studying the metabolic profiles of compound **4**, we identified compound **5** with better therapeutic window in the enzyme induction study in rat.[9] However, an off-target activity at the cannabinoid $CB_2$ receptor was observed for compound **5** in a GPCR counter-screen ($CB_2$ $K_i$ = 38 nM for **5**).[10] Due to the immunological implications of $CB_2$ mechanism,[11] this property was deemed inappropriate for an ideal development candidate. In order to further optimize the properties of this series, we undertook a comprehensive SAR study of the heteroaryl region addressed to replacing the pyridyl ring and the 5-phenyl substituent. Reported herein is the outcome of these studies which have resulted in the identification of potent, orally active thrombin receptor antagonists with reduced $C\log P$ and excellent $CB_2$ selectivity.




The synthesis of PAR-1 antagonists incorporating pyridine surrogates is shown in Scheme 1. Palladium catalyzed coupling of the previously reported tricyclic vinyl tin derivative **7**[12] with appropriate aryl or heteroaryl halides followed by chromatographic purification gave the *trans*-products **8a**–**n**. The aryl or heteroaryl halides are either commercially available or readily prepared from commercially available precursors using standard protocols. Our studies have shown that the presence of $C_{8a}$–$C_9$ double bond did not affect the PAR-1 $IC_{50}$ values considerably (e.g., compounds **8b** and **8d** had similar $IC_{50}$ as compounds **1** and **3**, respectively). Therefore, due to the ready availability of the tricylic intermediate **7** using established procedures, the unsaturated tricylic unit was initially explored for pyridine SAR studies.



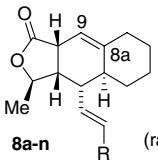

**Scheme 1.** Reagents and condition: (a) $Pd(PPh_3)_4$, RX (X = Cl, Br, or I), toluene, 100 °C.

Binding studies were carried out as previously reported using human platelet membranes as PAR-1 source and tritiated high affinity thrombin receptor activating peptide (haTRAP) as a radioligand.[13] PAR-1 $IC_{50}$ values or percentage of inhibition at 1 μM for compounds **8a**–**n** are shown in Table 1. The $IC_{50}$ values of **8a**–**d** are similar to those reported for the corresponding C8a–C9 saturated analogs, which establishes the fact that the presence of a C8a–C9 double bond does not affect PAR-1 binding, as discussed before. We did not identify any aryl or heteroaryl surrogates that are significantly better than the 2-pyridyl ring (or the 2-quinolyl ring). The phenyl replacement analogs **8e** and **8f** are essentially inactive. The 3-pyridyl compound **8g** is slightly less potent than the 2-pyridyl **8a** and the 3-quinolyl compound **8h** is ca. 10-fold less potent than the 2-quinolyl compound **8c**. Other heteroaryls such as the 2-thiozolyl (**8k**), the pyrazine (**8i**) or the pyrimidine (**8j**) also did not produce better potency than the 2-pyridyl (**8a**). The benzoxazole compound **8l** is less potent than the 2-quinolyl compound **8c**. These results may suggest that the basicity and orientation of the nitrogen lone pair of electrons in the 2-pyridyl or 2-quinolyl rings is important for the binding to the thrombin receptor. Finally, although the 6-Me substitution (**8b**) and 5-phenyl substitution (**8d**) gave ca. 10–100-fold improvement over 5,6-unsubstituted compound **8a**, the combination of 6-Me (or 6-Et) and 5-phenyl disubstituted compounds (**8m** and **8n**) did not give synergistic improvement in binding. The $IC_{50}$s are actually higher than the 5-phenyl substituted compound (**8d**) that has no substitution at the 6-position. Presumably, the alkyl substitutions at the 6-position in compounds **8m** and **8n** forced the 5-phenyl ring into an unfavorable binding conformation.

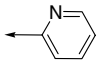
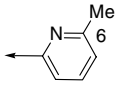
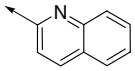
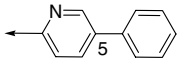
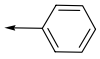
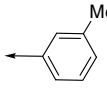
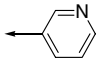
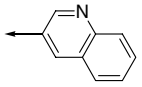
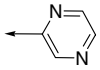
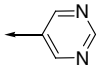
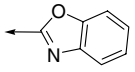
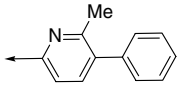
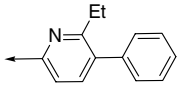
We next evaluated replacement of the 5-aryl ring on the pyridine with other groups, especially heteroaromatic rings, in an effort to lower the hydrophobicity. These compounds were synthesized conveniently from the pyridyl triflate intermediate **9** that we reported previously.[8] As shown in Scheme 2, the triflate **9** was converted to compounds **11a,b** through palladium catalyzed coupling reactions with appropriate organozinc reagents. The amino derivatives **11c**–**f** were prepared from **9** by the Buchwald amination protocol.[14] Compounds **11g**–**r** were prepared from **9** under Stille, Suzuki, or Miyaura[15] conditions.

The PAR-1 binding activities of 5-pyridyl derivatives **11a**–**r** are summarized in Table 2. The 5-alkyl substituted compounds **11a** and the cyclopentyl derivative **11b** are less potent than the phenyl derivative **3**. Interest-

**Table 1.**

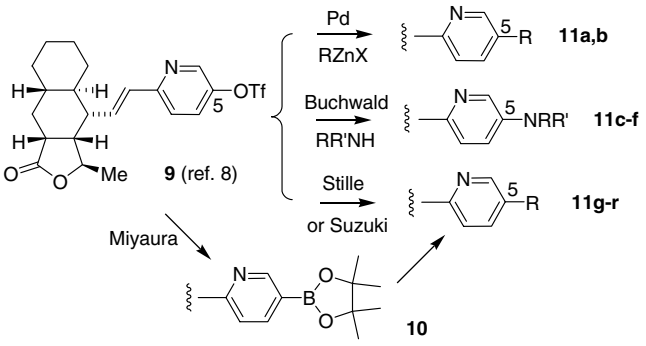


**8a-n** (racemic)

| Compound | R | Inhibition[a] @ 1 μM (%) | $IC_{50}$[a] (nM) |
|---|---|---|---|
| **8a** |  | 16 | 3600 |
| **8b** |  | | 350 |
| **8c** |  | | 150 |
| **8d** |  | | 43 |
| **8e** |  | 2 | |
| **8f** |  | −18 | |
| **8g** |  | 11 | |
| **8h** |  | 45 | |
| **8i** |  | 1 | |
| **8j** |  | 5 | |
| **8k** |  | 14 | |
| **8l** |  | 38 | |
| **8m** |  | | 130 |
| **8n** |  | | 285 |

[a] PAR-1 binding assay ligand: [$^3$H]haTRAP, 10 nM ($K_d$ = 15 nM).[13]

ingly, the 5-pyrrolidino compound **11c** with an $IC_{50}$ of 29 nM is as potent as the phenyl compound **3** and five times more potent than the cyclopentyl compound **11b**. Presumably, this is because the pyrrolidinyl ring can adopt a flatter conformation than the cyclopentyl



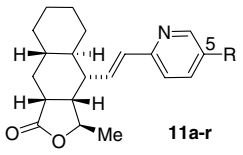

**Scheme 2.**

ring through conjugation of the nitrogen lone pair of electrons with the pyridyl ring and this flatter conformation more closely mimics that of the phenyl ring. Other cyclic amino analogs **11d–f** bearing polar or basic groups in the heterocyclic ring gave reduced binding affinity. A variety of heteroaryls (**11g–k** and **11n–o**) produced good PAR-1 affinity except the ones that are highly polar such as the 3-pyridine oxide (**11q**), and the imidazoles (**11l** and **11m**). The SAR of the 2-, 3-, and 4-pyridyl compounds (**11n, 11o**, and **11p**) parallels that of the *o*-, *m*-, and *p*-substituted 5-aryl groups. We have previously established that *ortho* and *meta* substituents on the phenyl ring are better than the *para* substitution.[8] The same SAR is emulated in the case of 2-, 3-, and 4-pyrdiyl groups where the 2-pyrdyl derivative (**11n**) and the 3-pyridyl derivative (**11o**) are highly active and the 4-pyridyl is two orders of magnitude less active (**11p**).

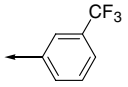
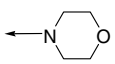
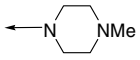
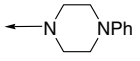
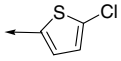
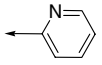
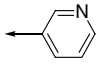
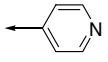
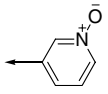
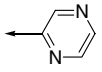
The pharmacokinetic profiles in a rapid rat model[16] for selected analogs were also studied (Table 2). Among the bipyridyl derivatives, the 3-pyridine derivative **11o** showed the highest plasma levels. The pyrrolidine derivative **11c** and the 3-pyridine compound **11o** were further evaluated in the ex vivo platelet aggregation assay in cynomolgus monkeys.[17] Compound **11c** showed only transient inhibition of platelet aggregation at 3 mpk. However, compound **11o** showed complete inhibition of platelet aggregation up to 4 h at 3 mpk and ca. 70% inhibition at 6 h. When compared to **4**, which gave complete inhibition up to 6 h, compound **11o** seems to have a shorter duration of action. Compounds **11c** and **11o** were also evaluated in the cannabinoid $CB_2$ receptor binding assay.[10] While compound **11c** showed a $K_i$ of 487 nM, compound **11o** is more selective with a $K_i$ of 13 μM.

In summary, the SAR of the pyridine ring for the himbacine derived PAR-1 antagonists was evaluated. The 2-vinyl pyridyl ring with suitable substitutions at the 5- or 6-position is important for PAR-1 binding. Both the basicity and the orientation of the lone pair electron on the pyridine nitrogen may be important. The C-5 position of the pyridine ring is amenable to considerable modification and the resulting compounds have produced good oral activity. Heteroaryls are generally well tolerated at the C-5 position of the pyridine ring with the

**Table 2.**




**11a-r**

| Compound | R | $IC_{50}$[a] (nM) | rat PK[b] $AUC_{(0-4\ h)}$, $C_{max}$ |
|---|---|---|---|
| (+)-**3**[8] | Ph | 27 | 451, 223 |
| (+)-**4**[8] |  | 11 | 3009, 1131 |
| (+)-**11a** | Et | 263 | |
| (+)-**11b** |  | 115 | |
| (+)-**11c** |  | 29 | |
| (+)-**11d** |  | 440 | |
| (+)-**11e** |  | 12% @1 μM | |
| (+)-**11f** |  | 3300 | |
| (±)-**11g** |  | 12 | |
| (±)-**11h** |  | 16 | 160, 109 |
| (±)-**11i** |  | 36 | 1062, 343 |
| (±)-**11j** |  | 11 | 434, 461 |
| (+)-**11k** |  | 47 | |
| (+)-**11l** |  | 1320 | |
| (+)-**11m** |  | 145 | |
| (+)-**11n** |  | 22 | 509, 251 |
| (+)-**11o** |  | 22 | 4228, 1823 |
| (+)-**11p** |  | 2144 | |
| (+)-**11q** |  | 207 | |
| (+)-**11r** |  | 88 | |

[a] PAR-1 binding assay ligand: [$^3$H]haTRAP, 10 nM ($K_d$ = 15 nM).[13]

[b] Compounds were dosed in 20% HPBCD. $AUC_{(0-4\ h)}$ measurements are given in ng h/ml and $C_{max}$ in ng/ml.[16]

3-pyridyl (**11o**) being the best with lower *C*log *P* (3.7 for **11o** vs 6.0 for compound **4**) and excellent $CB_2$ selectivity.

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