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Discovery and synthesis of a novel series of quinoline-based thrombin receptor (PAR-1) antagonists

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Abstract—The design, synthesis, and SAR studies of a structurally novel series of highly potent thrombin receptor (PAR-1) antagonists are described. Compound **30** is a highly potent thrombin receptor antagonist (IC_{50} = 6.3 nM), a related compound **36** showing efficacy in a monkey ex vivo study.

Thrombin is a multi-functional protease involved in hemostasis and wound healing.[1] It plays a key role in the coagulation cascade by converting fibrinogen to fibrin which is crosslinked to form a clot.[2] In addition, thrombin activates various cell types including platelets, leukocytes, endothelial cells, and vascular smooth muscle cells. The cellular activity of thrombin is mediated via proteolytic activation of specific cell surface receptors known as protease-activated receptors (PARs).[3–7] Four PAR subtypes are known: PAR-1, PAR-3, and PAR-4 are activated by thrombin, while PAR-2 is activated by trypsin and tryptase. PAR-1 is the most important of these receptors and the mechanism by which thrombin activates PARs such as PAR-1 is unique. Thrombin binds to the receptor through its exo-site anion binding region, and it cleaves the extracellular domain at Arg^{41}-Ser^{42} to reveal an amino terminus which binds intramolecularly to the receptor.[8–11] Thrombin receptor-activating peptides (TRAPs),[12] designed to mimic the amino terminus of the activated receptor, were shown to elicit a functional agonist response. Mutant receptors that do not contain cleavable functionality in the extracellular domain are unresponsive to thrombin but are activated by TRAPs.[10]

Inhibition of platelet activation by a PAR-1 antagonist is hypothesized to have significant utility in the treatment of thrombotic disorders such as platelet-driven arterial thrombosis. Additionally, a PAR-1 antagonist may be effective in treating vascular disorders such as atherosclerosis and restenosis by inhibiting proinflammatory and proliferative processes that are mediated by PAR-1 activation in endothelial and vascular smooth muscle cells. Because a thrombin receptor antagonist would only inhibit the cellular effects of thrombin and not its enzymatic role in fibrin generation, this mechanism of action would have a reduced bleeding liability relative to current antithrombotic agents.[13,14]

Recently, we have reported a structurally novel class of potent, orally active PAR-1 antagonists[15] (shown in Fig. 1). This study showed that an alkyl substitution was optimal at the 6-position, but not at the 5-position, whereas an aryl substitution was optimal at the 5-posi-

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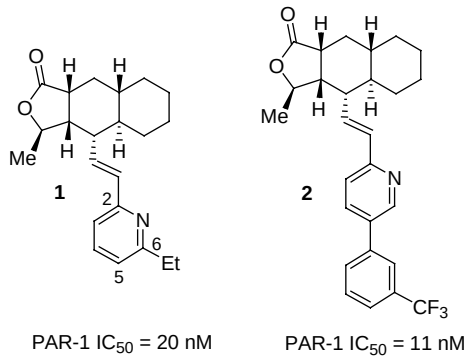
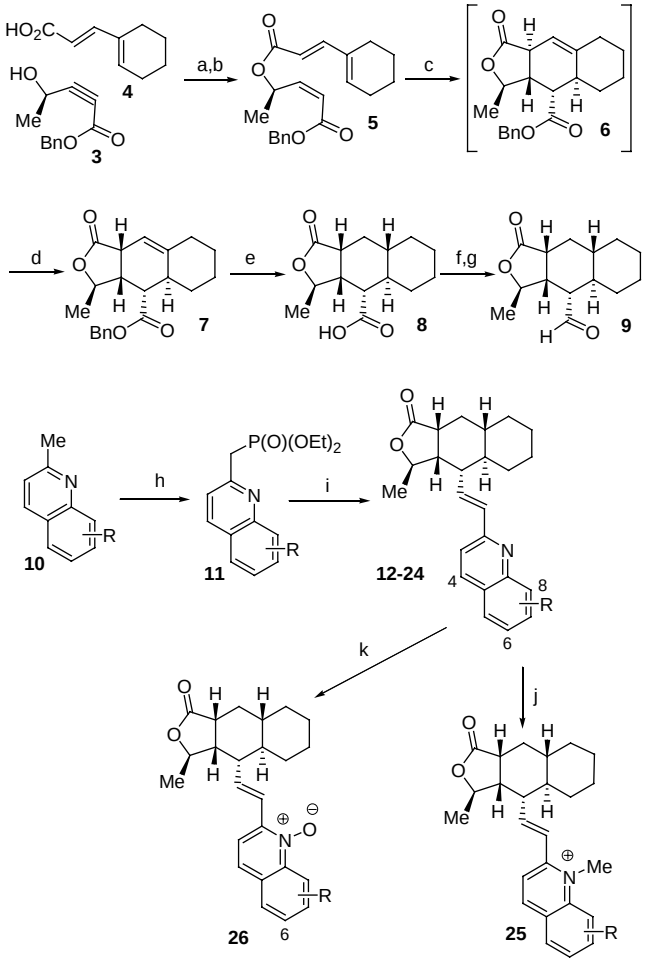


Figure 1. Thrombin receptor antagonists.

tion and not tolerated at the 6-position. In order to explore the possibility of a synergistic effect between these positions, and to further refine this lead, we synthesized a series of quinoline derivatives (Scheme 1).

Propargylic alcohol **3** was readily available in both racemic and enantiopure forms from (±)-3-butyn-2-ol or (R)-3-butyn-2-ol, respectively, in three steps.[15] Propargylic alcohol **3** was coupled with acid **4**[16] and the product hydrogenated using Lindlar's catalyst to give triene **5**, which underwent an intramolecular Diels–Alder reaction at 185 °C to furnish **6**. After in situ treatment of **6** with DBU, tricyclic ester **7** was isolated in 66% yield for the 3 steps. The tricyclic ester **7** was converted to the acid **8** via hydrogenation which, in turn, was converted to the aldehyde **9** using tributyltin hydride-mediated reduction of its acid chloride derivative.[17] Phosphonates were synthesized via treatment of the lithium anion of a substituted 2-methylquinoline with diethylchlorophosphate. Finally, the targets **12**–**24** were synthesized via Horner–Wadsworth–Emmons reaction of the aldehyde **9** with the appropriate phosphonate. The *N*-methyl derivative **25** and the *N*-oxide **26** were synthesized by treatment of **12** and **14** with methyl iodide and *m*-CPBA, respectively.



Scheme 1. Synthesis of thrombin receptor antagonists. Reagents and conditions: (a) DCC, 4-pyrrolidinylpyridine, CH_2Cl_2, 63%; (b) Lindlar Catalyst, TEA, H_2, THF; (c) xylenes, 185 °C; (d) DBU, THF, 66% 3 steps; (e) PtO_2, H_2, MeOH, 75%; (f) $(COCl)_2$, DMF (cat), CH_2Cl_2; (g) Bu_3SnH, $Pd(Ph_3P)_4$, PhMe, 0 °C to rt, 88% 2 steps; (h) *t*-BuLi, $(EtO)_2POCl$, THF, −78 °C to rt, 68–96%; (i) *n*-BuLi (0 °C), then **9** 0 °C to rt, THF, 34–94%; (j) MeI, CH_2Cl_2, 99%; (k) mCPBA, CH_2Cl_2.

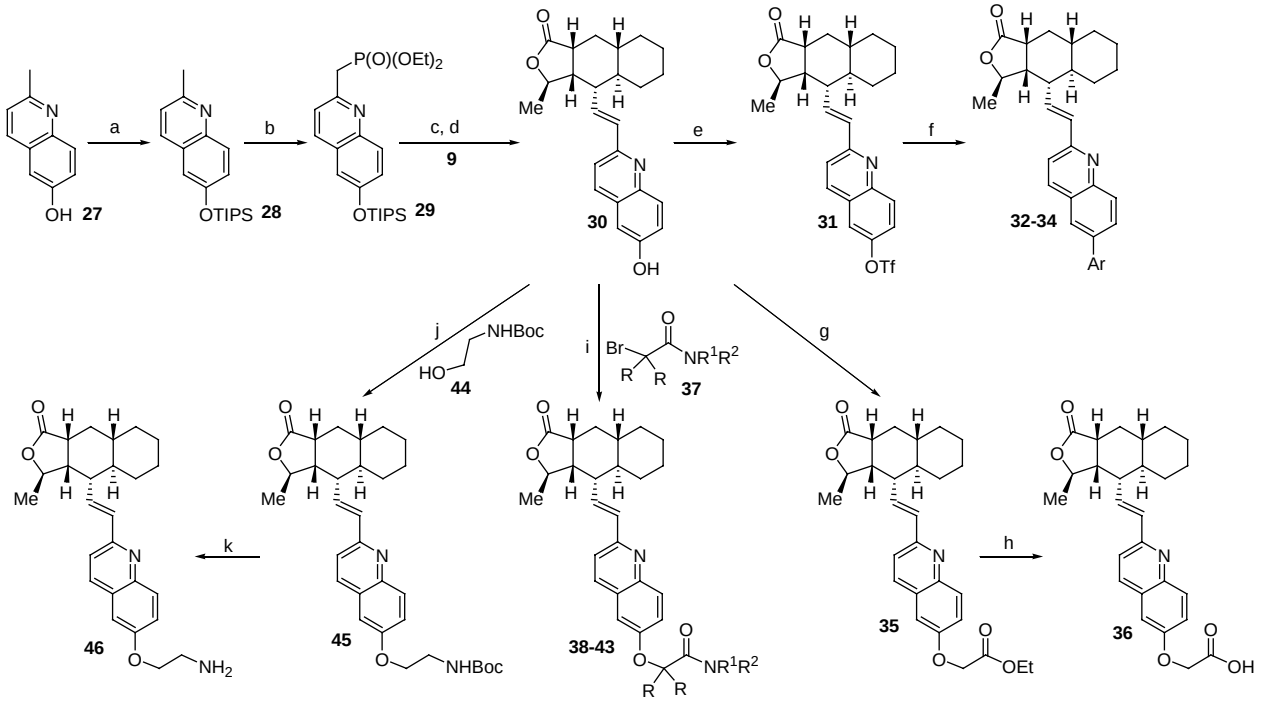
Compounds **12**–**26** were screened in the in vitro binding assay using purified human platelet membranes as a PAR-1 source and tritiated high affinity thrombin receptor activating peptide, alanine-*p*-fluorophenylalanine-arganine-cyclohexylalanine-homoarganine-[^{3}H]phenylalanine amide, ([^{3}H]haTRAP) as the ligand as previously reported.[15,18] Representative SAR is shown in Table 1. These initial results showed that substitution at the 6-position of the quinoline ring with an electron-donating group gave the most potent compounds. The 6-methoxy-substituted compound **14** gave comparable binding data (IC_{50} = 15 nM) to lead structures **1** and **2** thus supporting our initial hypothesis. Substitution, however, at other positions or quaternization of the pyridyl nitrogen led to substantially less active compounds. Although not shown in the table, removal of the olefin linker via hydrogenation led to at least a 10-fold loss in activity.

Encouraged by these results, we then set about further exploring the SAR of the 6-position of the quinoline moiety. A representative synthesis of various analog types is outlined in Schemes 2 and 3. Phenol **27** was protected as its TIPS ether and converted to the phosphonate **29**

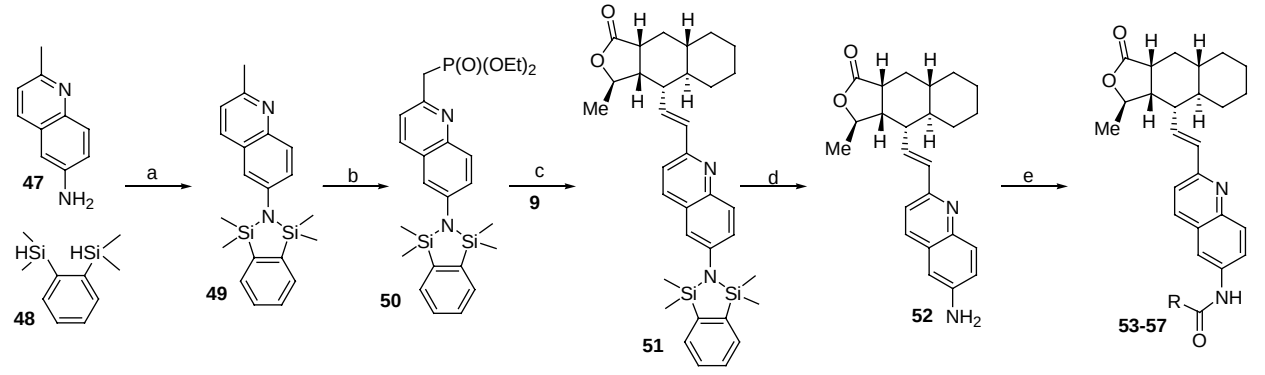
Table 1. SAR of quinoline derivatives of **1**

Compound	R group	PAR-1 IC_{50} (nM)[a]
(±)**12**	—	60
(±)**13**	6-Me	100
(±)**14**	6-OMe	15
(±)**15**	6-Cl	155
(±)**16**	6-F	730
(±)**17**	7-Me	150
(±)**18**	8-OMe	700
(±)**19**	8-Me	390
(±)**20**	4-OMe	Inactive[b]
(±)**21**	4-OBn	Inactive[b]
(±)**22**	6-OMe, 5-Cl	800
(+)**23**	6-OCF_3	Inactive[b]
(+)**24**	5,6-Dimethoxy	50
(±)**25**	—	Inactive[b]
(±)**26**	6-OMe	620

[a] PAR-1 binding assay ligand: [^{3}H]haTRAP, 10 nM (K_d = 15 nM).[15,18]
[b] Inactive at a concentration of 1000 μM.



Scheme 2. Reagents and conditions: (a) TIPSCl, imidazole, DMF, 88%; (b) LDA, $(EtO)_2POCl$, THF, −78 °C to rt, 82%; (c) *n*-BuLi (0 °C), then **9** 0 °C to rt, THF; (d) TBAF, THF, 0 °C, 65% 2 steps; (e) Tf_2O, TEA, CH_2Cl_2, 99%; (f) $Pd(Ph_3P)_4$ (5%), K_2CO_3, $ArB(OH)_2$, PhMe/EtOH/H_2O (4:2:1); (g) ethyl bromoacetate, NaH, DMF; (h) LiOH, MeOH/H_2O (3:1); (i) R = H, NaH, DMF; R = Me, NaH, 1,4-dioxane, 100 °C; (j) Ph_3P, DEAD, THF; (k) TFA, CH_2Cl_2.



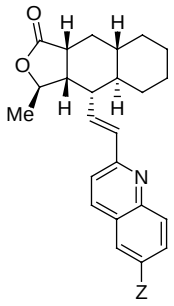
Scheme 3. Reagents and conditions: (a) $(Ph_3P)_3RhCl$, 120 °C, 41%; (b) LDA, $(EtO)_2POCl$, THF, −78 °C to rt, 44%; (c) *n*-BuLi (0 °C), then **9** 0 °C to rt, THF, 77%; (d) 1 N HCl, THF/MeOH (1:1), 80%; (e) RCOCl, TEA, CH_2Cl_2, 0 °C.

in the usual manner. Horner–Wadsworth–Emmons reaction of **29** with the aldehyde **9** followed by fluoride-mediated deprotection gave the key intermediate **30**. The 6-aryl-substituted analogs **32**–**34** were synthesized via Suzuki reaction of the triflate **31**. The O-linked analogs **36**, **38**–**43**, **45**, and **46** were synthesized via alkylation or Mitsunobu chemistry followed by derivatization when appropriate. The N-linked derivatives **53**–**57** provided more of a challenge. Finding an appropriate protecting group for the aminoquinoline **47** proved to be a problem since the group would have to survive strongly basic reaction conditions. Additionally, protecting group removal via catalytic hydrogenation was not feasible because we required an olefin linker in the final targets for activity.

The BSB (benzostabase) group[19] was eventually chosen because it possessed the desired stability and ease of removal under mildly acidic conditions. The key intermediate **52** was converted to amides **53**–**57** via treatment with various acid chlorides in the presence of triethylamine.

The PAR-1 binding data for this series of compounds are shown in Table 2. The most potent compound was the phenol **30** with a PAR-1 IC_{50} of 6.3 nM. Other compounds of note were **36**, **40**, **52**, and amide **55** all with IC_{50} in the 30–50 nM range. We observed that increasing steric bulk within a subseries generally led to a loss in activity; for example, **40** and **41** in the hydroxyacetamide subseries and **55**, **57**, and **56** in the quinolinylalkylamide subseries. For proof-of-principle studies we used an ex vivo model of platelet aggregation in cynomolgus monkeys. After administration of the drug, blood samples were collected at various intervals and aggregation

Table 2. SAR of quinoline 6-position



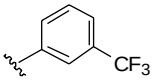
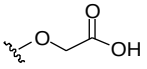
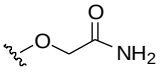
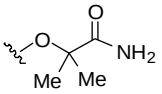
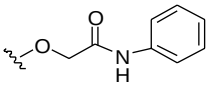
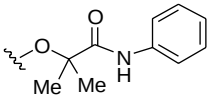
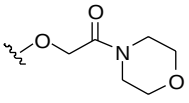
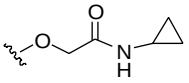
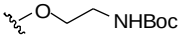
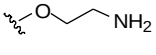
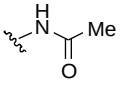
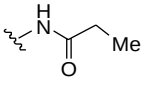
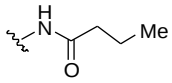
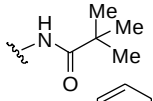
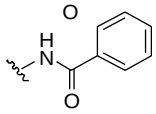
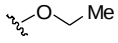
Compound	Z	PAR-1 IC_{50} (nM)[a]
(+)**30**	OH	6.3
(+)**32**		300
(+)**33**		50
(+)**34**		300
(+)**36**		29
(+)**38**		1000
(+)**39**		800
(+)**40**		25
(+)**41**		500
(+)**42**		500
(+)**43**		290
(±)**45**		275
(±)**46**		Inactive[b]
(+)**52**	NH_2	30
(+)**53**		100
(+)**54**		68

Table 2 (*continued*)

Compound	Z	PAR-1 IC_{50} (nM)[a]
(+)**55**		28
(+)**56**		2151
(+)**57**		219
(±)**58**		125
(+)**59**		1250

[a] PAR-1 binding assay ligand: [^{3}H]haTRAP, 10 nM (K_d = 15 nM).[15,18]
[b] Inactive at a concentration of 1000 μM.

response to 1 μM of haTRAP was measured in a whole blood aggregometer. When tested in our ex vivo model of platelet aggregation, compound **36** showed almost complete inhibition 2 h after IV dosing in cynomolgus monkeys. It was not active when dosed orally due to poor oral bioavailability.[20] Surprisingly, compounds **14** and **30** did not show substantial activity in the ex vivo model of platelet aggregation, which is presumably due to rapid conjugation and clearance.

In conclusion, we have discovered a series of potent PAR-1 receptor antagonists. The most potent compound up to this point is **30** with an IC_{50} of 6.3 nM. A closely related compound, **36**, was shown to have activity in an ex vivo platelet aggregation model. Efforts toward producing orally active antagonist in this series will be reported in due course.

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20. Compound **36** showed an $AUC_{(0-4h)}$ of 17 ng h/ml after dosing at 10 mg/kg when dosed in 20% HPBCD. For comparison compound **30** had an $AUC_{(0-4h)}$ of 826 ng h/ml when dosed under similar conditions but did not show substantial activity in the ex vivo model of platelet aggregation.