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Discovery of a vorapaxar analog with increased aqueous solubility

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ABSTRACT

An analog of the thrombin receptor antagonist vorapaxar (SCH 530348) with increased aqueous solubility, compound **9c** (SCH 602539), was discovered through incorporation of polar substituents on the pyridine ring of the himbacine-derived lead series. This analog retained the excellent potency, pharmacokinetic and safety properties of vorapaxar while increasing the aqueous solubility by 20-fold. Also presented are in vivo evaluations of this compound in a cynomolgus monkey platelet aggregation assay and in a Folts model of thrombosis in anesthetized monkeys.

Atherothrombosis is triggered by the rupture of an atherosclerotic plaque in the coronary artery and it is the major cause of cardiovascular death.[1] Plaque rupture leads to a spectrum of clinical conditions, collectively known as acute coronary syndrome (ACS), that range from unstable angina to acute myocardial infarction.[2] In addition to surgical interventions such as stent implantation, the pharmacological treatment of ACS includes administration of antiplatelet agents such as aspirin (inhibitor of thromboxane A_2 biosynthesis) and clopidogrel (ADP antagonist). However, these agents suffer from several limitations such as modest efficacy and bleeding side effect, leaving room for a more potent oral antiplatelet agent with an improved safety margin. In our efforts to identify such an agent, we have reported the discovery of thrombin receptor antagonist vorapaxar (SCH 530348, **4**, Fig. 1), based on the molecular template of the natural product himbacine (**1**).[3] Vorapaxar is currently undergoing Phase-III clinical trials for ACS and secondary prevention of cardiovascular events.[4] In a Phase-II clinical trial, vorapaxar met the primary endpoint of absence of thrombolysis in myocardial infarction (TIMI) major plus minor bleeding and showed a numerical reduction in outcome end points such as myocardial infarction (MI) and major adverse cardiac events (MACE).[5,6]

The thrombin receptor, also known as protease activated receptor-1 (PAR-1), is the most potent cell surface inducer of platelet activation.[7] Mechanistically, thrombin activates PAR-1 by proteolytic cleavage of the extracellular loop of the G-protein coupled PAR.[8] The newly unveiled amino terminus acts as a tethered ligand that binds intramolecularly to the proximally located part of the receptor, causing intracellular signaling events. Since thrombin receptor activation represents the most potent platelet activation mechanism, a thrombin receptor antagonist was expected to produce potent antiplatelet effects. Additionally, since the procoagulant fibrin-generating activity of thrombin is unaffected and platelet activation needed for normal hemostasis by other platelet receptor agonists such as collagen are left intact, such an agent was expected to provide improved safety margin compared to currently available antiplatelet agents.[9]

Vorpaxar is a potent antagonist of PAR-1.[3] In a PAR-1 binding assay, vorapaxar showed a K_i of 8.5 nM and demonstrated a potent oral antiplatelet effect in a cynomolgus monkey model of ex vivo platelet aggregation (100% inhibition of platelet aggregation for 24 h after oral administration at 0.1 mg/kg).

Since vorapaxar has limited aqueous solubility (vide infra) making intravenous formulations challenging, we undertook an effort to identify analogs with greater aqueous solubility while retaining its excellent spectrum of activities. In this Letter we describe the successful outcome of these efforts that led to the discovery of compound **9c**, which is equipotent to vorapaxar at the PAR-1 receptor while being 20-fold more soluble in water. Also reported herein are in vivo studies carried out on **9c** including an oral platelet aggregation inhibition assay in a cynomolgus monkey model and an intravenous study in a Folts model of thrombosis in anesthetized monkeys.

In an effort to preserve the excellent potency and selectivity of the lead series, we evaluated polar heteroaryl groups at the C-5′ position of the pyridine moiety. Based on our previous SAR studies,[3,10] we knew that this part of the molecule was more tolerant to changes (Fig. 1). Scheme 1 illustrates the synthesis of target

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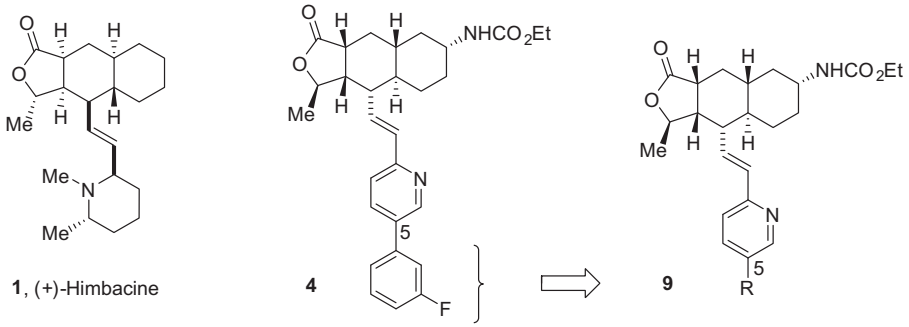


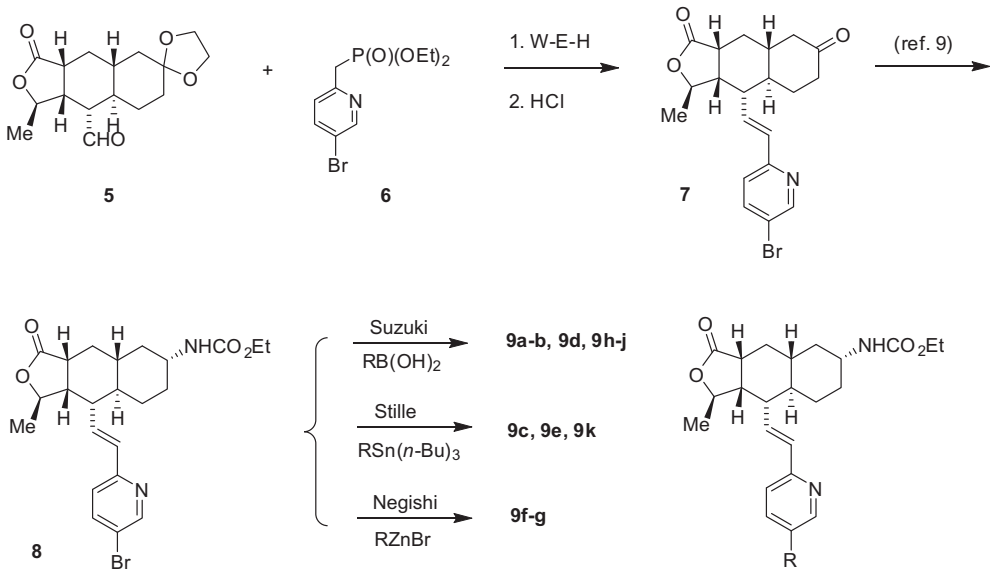
Figure 1.

compounds **9a**–**k**. Utilizing our previously reported procedures,[11] a Wadsworth–Emmons–Horner reaction between the aldehyde **5**[11] and the phosphonate **6**[12] followed by hydrolysis provided the ketone **7**. Ketone **7** was then converted to the intermediate **8** in a similar sequence as we previously described (reductive amination, isolation of the major α isomer, and carbamate formation).[3] The intermediate **8** was then converted to target compounds **9a**–**k** through palladium-catalyzed coupling reactions of the bromopyridine **8** with appropriate boronic acids, organotin reagents, or organozinc reagents under the Suzuki, Stille, or Negishi protocols as shown in Scheme 1.

Thrombin receptor (PAR1) binding studies on compounds **9a**–**k** were carried out as previously reported using human platelet membranes as the PAR-1 source and tritiated high affinity thrombin receptor activating peptide (haTRAP) as the radioligand.[13] PAR-1 K_i values for compounds **9a**–**k** are shown in Table 1. The 2-cyanophenyl (**9a**) and the 3-methoxyphenyl (**9b**) substitutions are well tolerated giving similar K_i's as compound **4**. Of the pyridyl substituted compounds (**9c**–**e**), only the 2-pyridyl (**9c**) is potent. The 3- and 4-pyridyl derivatives are roughly 10-fold less potent (see **9d** and **9e**). The 5-methyl-2-pyridyl group (**9f**) was tolerated but the 4-methyl-2-pyridyl group (**9g**) was not. A 4-methoxy substitution on the 3-pyridyl improved the potency three fold (**9i** vs **9d**). Other heteroaryl groups such as pyrimidine (**9h**), furan (**9j**), and thiazole (**9k**) gave moderate to poor potency.

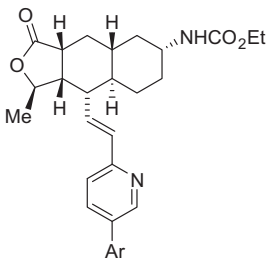
Table 2 summarizes the solubility and pharmacokinetic properties of the more potent compounds in this series. The solubility assay was done using a high-throughput kinetic solubility test.[15] The pharmacokinetic assay was performed in rat in a high-throughput format as reported.[16] As shown in Table 2, polar substitutions on the phenyl ring (**9a** and **9b**) increased the solubility only slightly compared to compound **4**. Changing the phenyl group to heteroaryl groups had a more pronounced effect on solubility with the 2-pyridyl (**9c**) having the highest solubility. Compound **9c** also gave high plasma levels in the rat pharmacokinetics model as shown by the high AUC and C_{max} values. Compound **9c** was further evaluated in a 24 h equilibrium solubility assay and it was found to be 20 times more soluble than compound **4** (67 μM vs 2.8 μM, Table 2).

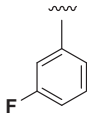
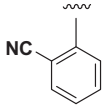
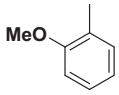
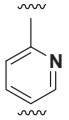
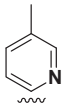
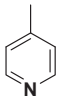
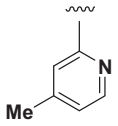
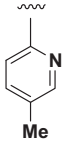
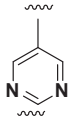
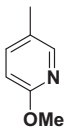
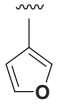
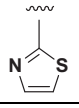
Compound **9c** was selected for further pharmacological evaluations as shown in Table 3. It inhibited TRAP-induced platelet aggregation in washed human platelets with an IC_{50} of 0.18 μM. In the ex vivo platelet aggregation assay in cynomolgus monkeys after oral administration,[17] compound **9c** showed complete inhibition of platelet aggregation at 0.3 mpk for 24 h. Even at 0.1 mg/kg this compound completely inhibited agonist-induced platelet aggregation up to 6 h with ca. 70% inhibition occurring at 24 h (Fig. 2). It is worth noting that, being a pyridine derivative, compound **9c** did not show untoward activities in our enzyme (p450) inhibition or enzyme induction (PXR)[18] assays. It has good permeability in the



Scheme 1.

Table 1



Compound number	Ar	K_i (nM) ± SEM
4		8 ± 1
9a		15 ± 7
9b		12 ± 5
9c		19 ± 1
9d		240 ± 47
9e		250 ± 19
9f		42 ± 4
9g		144 ± 70
9h		337 ± 55
9i		49 ± 18
9j		61 ± 22
9k		75 ± 28

Caco-2 assay and good oral exposures in monkey pharmacokinetic assays.

Table 2

Compound number	Kinetic solubility (μM)	Rat AUC, C_{max} (ng h/mL, ng/mL)
4	<5[a]	3064, 974
9a	12	5218, 1571
9b	<5	319, 368
9c	75[a]	10, 096, 3069
9f	12	NA
9i	12	NA
9j	12	NA

[a] Equilibrium solubility: **4**, 2.8 μM; **9c**, 67 μM; NA, not available.

Table 3

Assays	**9c**
Platelet aggregation in washed human platelets	IC_{50} = 0.18 μM
p450 Inhibition	CYP3A4, CYP2D6, CYP2C9 & CYP2C19 IC_{50}: >30 μM (co-incubation & pre-incubation)
PXR	0.13 @ 10 μM
Caco-2 permeability	283 (nm/s)
Monkey pharmacokinetics	1 mpk, po, methylcellulose: AUC_{0-24h} = 2520 ng h/mL, C_{max} = 236 ng/mL, T_{max} = 0.5 h 0.1 mpk, po, methylcellulose: AUC_{0-24h} = 50 ng h/mL; C_{max} = 16 ng/mL; T_{max} = 1 h

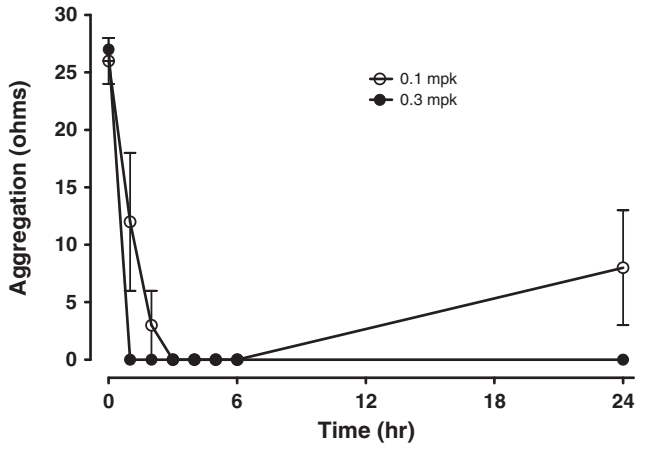


Figure 2. Inhibition of ex vivo haTRAP-induced platelet aggregation by compound **9c** in cynomolgus monkeys (n = 3) after oral administration at 0.3 and 0.1 mg/kg.

Compound **9c** was evaluated alone and in combination with the ADP antagonist cangrelor in a Folts model of thrombosis in anesthetized monkeys.[19] Increasing doses of **9c**, cangrelor or both were administered intravenously to the same animal in a sequential manner and the ability to inhibit cyclic flow reductions (CFR's) was monitored. Compound **9c** inhibited thrombosis in the Folts model in monkeys in a dose-dependent manner. The efficacy of **9c** was additive to that of cangrelor.

In summary, we have discovered a potent thrombin receptor antagonist **9c** with 20-fold increased aqueous solubility compared to vorapaxar through incorporation of polar substituents on the pyridine ring of the lead series. Compound **9c** showed PAR-1 affinity and antiplatelet effect in vivo in a cynomolgus monkey model comparable to those of vorapaxar. It also showed excellent pharmacokinetic properties and no untoward activities such as enzyme inhibition or enzyme induction. The increased aqueous

solubility of compound **9c** facilitated its evaluation in a Folts model of thrombosis via intravenous administration. In this study compound **9c** showed dose-dependent antithrombotic efficacy and this efficacy was additive when co-administered with the ADP antagonist cangrelor.

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