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# TARGETING PAR1 TO IMPROVE CURRENT ANTIPLATELET STRATEGIES

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## SUMMARY

*Platelets are key mediators of thrombosis. Oral antiplatelet agents have an important role in the prevention of atherothrombotic complications. Current antiplatelet therapies combine the inhibition of cyclooxygenase-1 and ADP receptor antagonism, although the variability in the metabolic and functional responses together with the risk of bleeding in association with both strategies have prompted the investigation of new alternatives providing more rapid and consistent platelet inhibition. Thrombin plays a central role in platelet activation through its proteolytic action on protease-activated receptors (PARs). Among them, PAR1 is a high-affinity receptor for platelet activation at low thrombin concentrations. Inhibition of PAR1 represents a promising antiplatelet strategy, since there is evidence demonstrating that it prevents pathological thrombus growth due to excessive thrombin generation, without interfering with normal vascular healing or thrombin-mediated fibrin generation. In this review, some of the latest advances in PAR1 antagonists will be discussed.*

**Key words:** Antiplatelet agents – Protease-activated receptors – Thrombosis

## INTRODUCTION

Platelets play a key role in hemostasis, but they are also responsible for the pathological thrombus formation leading to clinical manifestations of acute atherothrombotic vascular disease (1-4). Atherothrombosis remains the leading cause of morbidity and mortality in Western societies. Therefore, there is great interest in developing pharmacological strategies to modulate platelet activation to prevent cardiovascular accidents. Platelet activation occurs via multiple pathways and current agents do not interfere with all of them.

Oral antiplatelet agents have an important role in the treatment of atherothrombotic complications, such as acute coronary syndrome, peripheral artery disease and cerebrovascular disease (5). While currently available oral antiplatelet agents such as aspirin and $P2Y_{12}$ receptor antagonists reduce the incidence of ischemic events, the residual risk for morbidity and mortality remains substantially elevated, since each of these agents inhibits only one of a number of platelet activation pathways (6, 7). Moreover, current antiplatelet agents are associated with an increased risk of bleeding (8-11) because they interfere with platelet activation pathways that are crucial for hemostasis. In an attempt to circumvent unwanted hemorrhagic risks associated with classic antiplatelet agents, new strategies are being developed focusing on those agonists that can be upregulated, such as thrombin, in the event of pathological hemostasis. Thrombin and thrombin receptors have therefore became major targets.

Thrombin is one of the strongest platelet-activating agents (12, 13), and its role in the physiology and pathology of hemostasis cannot be disregarded. In addition to cleaving fibrinogen and other soluble protein substrates, the coagulation protease thrombin triggers several responses in platelets, endothelial cells and other cells. Thrombin is thought to act at the sites of its production, interacting with specific receptors or coagulation factors. There are at least three different receptors for thrombin in platelets: the complex GPIb/V/IX (14, 15) and the protease-activated receptors PAR1 and PAR4 (16). Inhibition of the former would have a major impact on the adhesive properties of platelets, since the glycoprotein complex also acts as a receptor for von Willebrand factor (VWF). However, there is evidence demonstrating that inhibition of PAR1 prevents pathological thrombus growth without interfering with normal vascular healing or thrombin-mediated fibrin generation. Circulating levels of thrombin are normally low (17, 18), and increased levels should be expected locally in association with damaged vascular surfaces. Therefore, inhibition of PAR1 would have the advantage of not interfering with the main receptors involved in primary hemostasis, but instead inhibiting the possible activation of platelets related to excessive thrombin generation at sites of vascular damage.

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## THROMBIN FUNCTIONS

Thrombin is a multifunctional serine protease. Active thrombin is generated in the context of vascular injury when activation of the coagulation cascade triggers conversion of the circulating zymogen prothrombin to active protease. Among other functions, thrombin cleaves circulating protein substrates, leading to the conversion of fibrinogen to fibrin monomers or the activation of protein C. In addition, thrombin exhibits further effects acting on cells.

As mentioned earlier, thrombin is the strongest activator of platelets (12). Activation of platelets by thrombin leads to platelet shape change and secretion, synthesis and release of thromboxane A2 (TXA2) (19), expression of P-selectin (20) and CD40 ligand (21) at the platelet surface, and activation of the integrin $\alpha_{IIb}/\beta_3$ (22). The latter facilitates the binding of fibrinogen and VWF, potentiating platelet recruitment into aggregates. In addition, thrombin induces the expression of procoagulant activity on the platelet surface, promoting additional thrombin generation that further amplifies platelet activation.

Thrombin has additional effects on other cell types (reviewed in 13). In cultured endothelial cells, thrombin causes release of VWF, expression of P-selectin on the plasma membrane, production of chemokines and increased permeability of the cells. These events promote binding of platelets and leukocytes to the endothelial surface in vivo and local transudation of plasma proteins. Thrombin also acts as a mitogenic factor on fibroblast and vascular smooth muscle cells in culture, and triggers calcium signaling and other responses in T lymphocytes. These effects are due to the proteolytic activation of the specific cell-surface receptors PAR, PAR1 being the prototype, by thrombin.

## PROTEASE-ACTIVATED RECEPTOR PAR1

PARs are G protein-coupled receptors (GPCRs), or 7-transmembrane receptors. GPCRs comprise a large family of proteins that are involved in signaling pathways that control multiple physiological functions. Because of their functional ubiquity, GPCRs are potential drug targets in different pathological scenarios.

PARs utilize a unique mechanism to convert an extracellular proteolytic cleavage event into a transmembrane signal: these receptors carry their own ligands, which remain silent until activated by receptor cleavage. The proteolytic enzyme cleaves the extracellular loop of the GPCR, and the newly unmasked *N*-terminus acts as a tethered ligand that binds to the proximally located transmembrane loop of the GPCR (13) (Fig. 1).

Thrombin-mediated platelet activation in humans is known to occur through PAR1 and PAR4. In humans, PAR1 is widely distributed among cells and tissues, such as endothelial cells, smooth muscle cells, monocytes and fibroblasts. Human PAR4 mRNA has been shown to be expressed in a number of tissues, with high levels being present in lung, pancreas, thyroid, testis and small intestine. PAR1 is a high-affinity receptor for platelet activation at low thrombin concentrations, whereas PAR4 appears to play a supportive backup function for thrombin-induced platelet activation, mediating signaling only at more elevated thrombin concentrations.

Therefore, inhibition of PAR1 represents a rational approach to the development of novel antiplatelet agents for the treatment of patients with atherothrombotic complications. In addition, since thrombin has a mitogenic action on endothelial and smooth muscle cells (23, 24), PAR1 antagonism may also have additional therapeutic utility in the treatment of vascular disorders, such as atherosclerosis and restenosis following percutaneous revascularization.

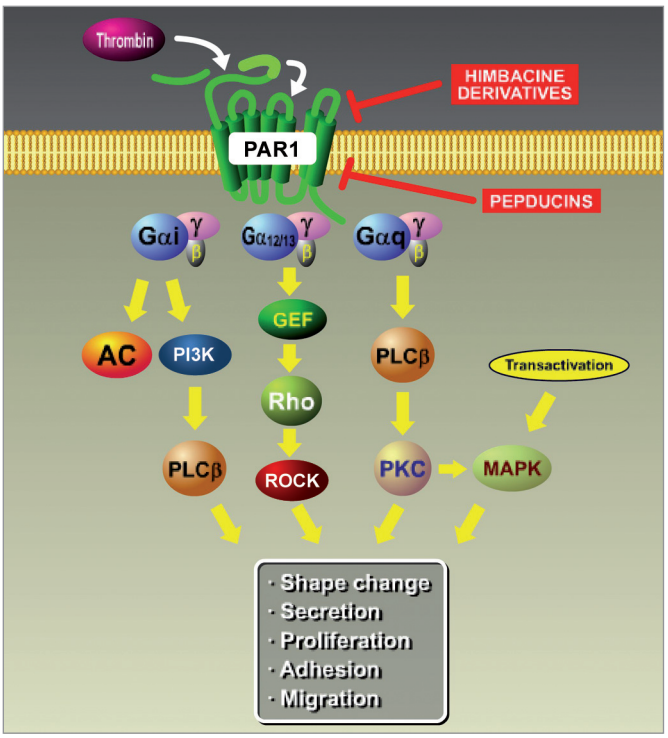



**Figure 1.** PAR1 signal transduction pathways. Thrombin can trigger signaling through PAR1 coupled to $G_i$, $G_{12/13}$ and $G_q$ proteins. After binding, an extracellular portion of the PAR molecule is cleaved. The newly exposed *N*-terminus of the PAR molecule binds to itself, inducing a conformational change that allows the binding of the G protein complex. Subunits $G_{\beta\gamma}$ are released. Activated $G_{\alpha i}$ inactivates adenylate cyclase (AC), which causes levels of cAMP to drop. Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) can be activated through $G_{\beta\gamma}$ subunits. Activated $G_{\alpha 12/13}$ activates guanine nucleotide exchange factor (GEF), which in turn activates Rho protein. Activated Rho binds and activates Rho-associated protein kinase (ROCK). Activated $G_{\alpha q}$ protein activates phospholipase C beta (PLCβ), which can activate protein kinase C (PKC) through phosphatidylinositol 4,5-bisphosphate ($PIP_2$) cleavage into inositol trisphosphate ($IP_3$) and diacylglycerol (DAG). Other pathways, such as the mitogen-activated protein kinase (MAPK) pathway, can be activated by transactivation of other receptors (i.e., the epidermal growth factor receptor [EGFR]).

## PAR1 ANTAGONISTS AS ANTIPLATELET AGENTS

PAR1 antagonists can be classified into three major chemical groups: peptide/peptidomimetic derivatives, non-peptide-based derivatives and small molecules targeting intracellular domains.

### Peptide or peptidomimetic PAR1 antagonists

These inhibitory strategies are based on the sequence of the small peptides that selectively activate the thrombin receptor: SFLLRN or

SFLLR, referred as thrombin receptor-activating peptide (TRAP). Structure–activity relationship (SAR) studies have provided both a basic understanding of the peptide ligand side-chain structural requirements and on the relative tolerance with regard to their interactions with the human thrombin receptor. A three-point model basic structure constituted by the ammonium group, the center of the benzene ring and the central carbon of the guanidine group has been used in conjunction with indole, indazole and benzimidazolone to design candidate peptidomimetic structures. The most interesting compounds have developed from an indole-based series, some of them being able to inhibit human platelet aggregation induced by TRAP (reviewed in 25 and 26).

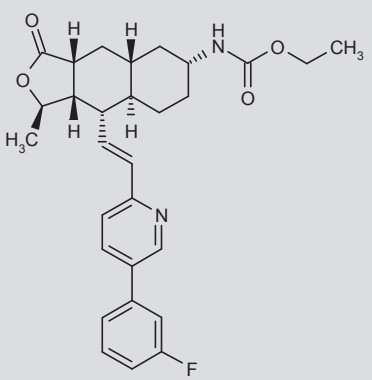
### Non-peptide-based derivatives

From the examination of SARs performed on peptidomimetic antagonists or from high-throughput screening, it has been determined that it is possible to develop simpler structures of lower molecular mass retaining the antagonist activity (reviewed in 25 and 26). The pyrroloquinazoline analogues were the first reported to have good PAR1 affinity and promising activity in functional assays, although PAR1-independent effects were also observed (27) in animal models. PAR1 antagonists based on cyclic guanidine and amidine templates have also been patented. A bicyclic amidine derivative (E-5555) has been tested in phase II trials, as will be discussed below. In addition, several patents related to the PAR1 antagonists are based on the core structure of the natural product himbacine. Some of these compounds are included in this review.

The text below lists a series of different anti-PAR1 agents ordered according to their level of clinical development as antiplatelet agents.

#### *SCH-530348 (vorapaxar)*

The novel antiplatelet agent SCH-530348 (vorapaxar) is a himbacine analogue that acts as a potent, orally active PAR1 antagonist, inhibiting thrombin-mediated platelet activation without interfering with thrombin-mediated cleavage of fibrinogen (28, 29). It has demonstrated rapid absorption and distribution, with peak plasma levels reached within 60-90 minutes following the oral administration of single or multiple doses. SCH-530348 is metabolized and eliminated primarily by the biliary and gastrointestinal routes.




SCH-530348

Preclinical studies

Incubation of human platelet-rich plasma with SCH-530348 resulted in inhibition of thrombin-induced and TRAP-induced platelet aggregation, with $IC_{50}$ values of 47 and 25 nM, respectively, without affecting the aggregation induced by other platelet agonists such as ADP, the TXA2 mimetic U-46619 or collagen (28, 30). SCH-530348 did not affect prothrombin time or activated partial thromboplastin time, suggesting that the potential for bleeding events may not be increased. In studies performed in cynomolgus monkeys, SCH-530348 demonstrated a bioavailability of 86% (28). Oral administration of SCH-530348 at doses of 0.1 mg/kg or above completely inhibited platelet aggregation for 24 hours (30). SCH-530348, either alone or in combination with aspirin plus clopidogrel, did not increase surgical blood loss or bleeding times versus placebo and aspirin plus clopidogrel, respectively (28).

Clinical studies

The route of administration is oral and the route of elimination is through the gastrointestinal and biliary tracts. SCH-530348 shows a high bioavailability and a half-life of 126-269 hours (29, 31). SCH-530348 given to healthy volunteers as a single dose (5-40 mg) caused a mean inhibition of > 90% of TRAP-induced platelet aggregation for more than 72 hours (29, 31, 32). A 40-mg loading dose followed by a 2.5-mg once-daily dose of SCH-530348 effectively inhibited TRAP-induced platelet aggregation throughout 28 days (28).

In the phase II TRA-PCI study, SCH-530348 was administered to 1,030 patients who were scheduled for angiography and possible stenting (29). Patients received 10, 20 or 40 mg of SCH-530348 or placebo. Those patients who underwent primary percutaneous coronary intervention (PCI) received the standard therapy of clopidogrel (300 or 600 mg) and heparin/bivalirudin and continued taking an oral dose of SCH-530348 (0.5, 1.0 or 2.5 mg) for 60 days. Those patients who underwent coronary artery bypass graft or medical management, but not PCI, continued in a separate cohort. Primary endpoints were the incidence of clinically significant minor or major bleedings and secondary endpoints were death or major adverse coronary events. SCH-530348 was not associated with an increase in major and minor bleedings versus placebo: the primary endpoint was reached in 2%, 3% and 4% of patients, respectively, taking 10, 20 or 40 mg of SCH-530348 versus 3% of patients in the placebo group. During the maintenance dose regimen, 2%, 4% and 3% of patients, respectively, receiving 0.5, 1.0 or 2.5 mg SCH-530348 reached a primary endpoint versus 2% of patients receiving placebo. The combined endpoint of death/major adverse cardiovascular events/stroke occurred less frequently in the SCH-530348 group compared with placebo: 6% versus 9%.

Two additional trials in patients with acute coronary syndrome (ACS) or ischemic stroke have confirmed the favorable safety profile of SCH-530348 observed in the TRA-PCI study (33, 34).

Two phase III clinical trials have been conducted: the TRA-CER (Thrombin Receptor Antagonist for Clinical Event Reduction in ACS) and the TRA 2P-TIMI 50 (Thrombin Receptor Antagonist in Secondary Prevention of atherothrombotic ischemic events) trials.

The TRA-CER study was designed to determine whether SCH-530348, when added to the existing standard of care (aspirin, clopidogrel) for preventing heart attack and stroke in patients with ACS, will yield additional benefit over the existing standard of care in preventing heart attack and stroke. The primary efficacy endpoint of the study was the first occurrence of any component of the composite of cardiovascular death, myocardial infarction, stroke, recurrent ischemia with rehospitalization and urgent coronary revascularization. The study was also designed to assess the risk of bleeding with SCH-530348 added to the standard of care versus the standard of care alone. The TRA-CER study evaluated a 40-mg loading dose and 2.5-mg maintenance dose of SCH-530348 in 12,944 patients with ACS without ST segment elevation. After a median follow-up of 502 days (interquartile range: 349-667), the primary endpoint occurred in 1,031 of 6,473 patients receiving vorapaxar versus 1,102 of 6,471 patients receiving placebo (Kaplan-Meier 2-year rate: 18.5% vs. 19.9%; hazard ratio [HR]: 0.92; 95% confidence interval [CI]: 0.85-1.01; $P = 0.07$). A composite of death from cardiovascular causes, myocardial infarction or stroke occurred in 822 patients in the vorapaxar group versus 910 in the placebo group (14.7% and 16.4%, respectively; HR: 0.89; 95% 95% CI: 0.81-0.98; $P = 0.02$). Rates of moderate and severe bleeding were 7.2% in the vorapaxar group and 5.2% in the placebo group (HR: 1.35; 95% CI: 1.16-1.58; $P < 0.001$). Intracranial hemorrhage rates were 1.1% and 0.2%, respectively (HR: 3.39; 95% CI: 1.78-6.45; $P < 0.001$). Rates of nonhemorrhagic adverse events were similar in the two groups. It was concluded that in patients with ACS, the addition of vorapaxar to standard therapy did not significantly reduce the primary composite endpoint but significantly increased the risk of major bleeding, including intracranial hemorrhage (35).

The TRA 2P-TIMI 50 trial was designed to determine whether SCH-530348 when added to the existing standard of care for preventing heart attack and stroke in patients with a known history of atherosclerosis would yield additional benefit over the existing standard of care without SCH-530348 in preventing heart attack and stroke. It was planned to enroll 19,000 patients with a history of cardiovascular disease receiving a maintenance dose of 2.5 mg of SCH-530348 or placebo. The primary efficacy endpoint of the study is the first occurrence of any component of the composite of cardiovascular death, myocardial infarction, stroke and urgent coronary revascularization. The TRA 2P-TIMI 50 study is ongoing but not recruiting participants. In contrast with TRA-CER, fewer patients in TRA 2P-TIMI 50 were taking both aspirin and a $P2Y_{12}$ inhibitor. Therefore, the results from the second vorapaxar study should give greater insight into the effect of blocking PAR1 in different groups of patients, including many patients who were not taking the combination of a $P2Y_{12}$ inhibitor and aspirin.

A recent study performed in Japanese and Caucasian subjects revealed that there were no differences in the pharmacodynamics and pharmacokinetics of the thrombin receptor antagonist depending on ethnic aspects (31).

### *E-5555 (atopoxar)*

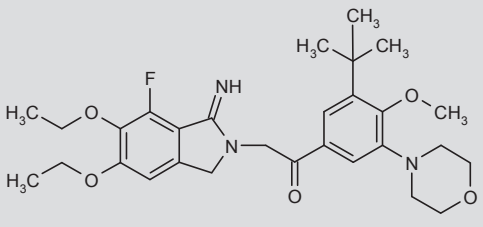
E-5555 is a reversible PAR1 antagonist that interferes with platelet signaling. The drug has been tested in phase II trials in patients with coronary artery disease (CAD) and shows antithrombotic and antiinflammatory effects (36).

#### Preclinical studies

The in vitro effects of escalating concentrations of E-5555 (20, 50 and 100 ng/mL) on platelet function were tested in healthy volunteers and CAD patients treated with aspirin (ASA) with or without clopidogrel. Platelet inhibition was usually moderate, present already at 20 ng/mL, and was not dose-dependent without TRAP stimulation. E-5555 caused 10-15% inhibition of ADP- and collagen-induced platelet aggregation in plasma, but not in whole blood. TRAP-induced aggregation was inhibited almost completely. Platelet endothelial cell adhesion molecule (PECAM-1), expression and activity of the integrin $\alpha_{IIb}/\beta_3$, platelet glycoprotein Ib (GPIb), thrombospondin, vitronectin receptor expression and formation of platelet–monocyte aggregates were also significantly reduced by E-5555. The authors suggested that the antiplatelet potency of ASA alone and the combination of ASA and clopidogrel may be enhanced by E-5555, providing a rationale for their synergistic use (38).

The antithrombotic activity of E-5555 in vivo was evaluated in a photochemically induced thrombosis model using guinea pigs (37). Oral administration of E-5555 at 30 and 100 mg/kg prolonged the time to occlusion by 1.8- and 2.4-fold, respectively, compared with controls. Furthermore, E-5555 did not prolong bleeding time in guinea pigs at the highest tested dose of 1000 mg/kg. Intravenous administration of 1 mg/kg tissue-type plasminogen activator (t-PA) significantly prolonged bleeding time, and its effects were not altered by the oral coadministration of 300 mg/kg E-5555.

In a rat model of intimal thickening after balloon injury in vivo (38), E-5555 selectively inhibited rat aortic smooth muscle cell (SMC) proliferation induced by thrombin and TRAP with $IC_{50}$ values of 0.16 and 0.038 μM, respectively. E-5555 did not inhibit rat SMC proliferation induced by basic fibroblast growth factor and platelet-derived growth factor at concentrations up to 1 μM. Repeated oral administration of 30 mg/kg E-5555 once daily for 16 days significantly




E-5555

reduced neointimal formation. It was concluded that E-5555 could have therapeutic potential for restenosis and chronic atherothrombotic disease.

Clinical studies

The phase II LANCELOT ACS (Lessons from ANtagonizing the Cellular Effects of Thrombin-Acute Coronary Syndromes) study tested the safety and tolerability of atopaxar in 603 unstable angina or non-ST elevated myocardial infarction patients randomized to placebo or to a 400-mg loading dose of atopaxar followed by a daily dose of 50, 100 or 200 mg for 12 weeks (39). In addition, nearly all patients were treated with aspirin and > 75% were taking aspirin in combination with clopidogrel or ticlopidine. The primary objective of this study was to investigate the safety and tolerability of atopaxar at three dose levels in patients with ACS. The secondary objectives were to determine the effect of atopaxar on major adverse coronary events and transient ischemia, and explore the pharmacokinetics of atopaxar and its effects on inflammatory markers. The incidence of Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) major or minor bleeding did not differ significantly between the combined atopaxar and placebo groups (3.08% vs. 2.17%; $P = 0.63$), and there was no dose-related trend ($P = 0.80$). The incidence of CURE major bleeding was numerically higher in the atopaxar group compared with the placebo group (1.8% vs. 0%; $P = 0.12$). The incidence of cardiovascular death, myocardial infarction, stroke, or recurrent ischemia was similar between the atopaxar and placebo arms (8.03% vs. 7.75%; $P = 0.93$). The incidence of cardiovascular death, myocardial infarction or stroke was 5.63% in the placebo group and 3.25% in the combined atopaxar group ($P = 0.20$). Dose-dependent trends for efficacy were not seen. Atopaxar significantly reduced ischemia on continuous ECG monitoring at 48 hours compared with placebo (relative risk: 0.67; $P = 0.02$). Transient dose-dependent transaminase elevation and relative QTc prolongation were observed with the highest doses of atopaxar. From this study it was concluded that in patients with ACS, atopaxar significantly reduced early ischemia on Holter monitoring without a significant increase in major or minor bleeding.

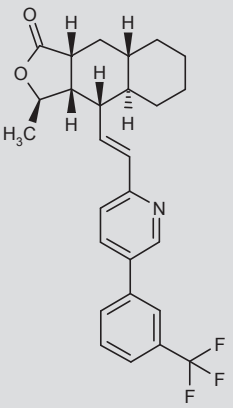
Two multicenter, randomized, double-blind, placebo-controlled phase II studies assessed the safety and efficacy of the oral PAR1 antagonist E-5555 in addition to standard therapy in Japanese patients with ACS or high-risk CAD, with promising results (40).

### *SCH-205831*

SCH-205831 is a potent, selective and orally active PAR1 antagonist derived from the natural product himbacine (41).

Preclinical studies

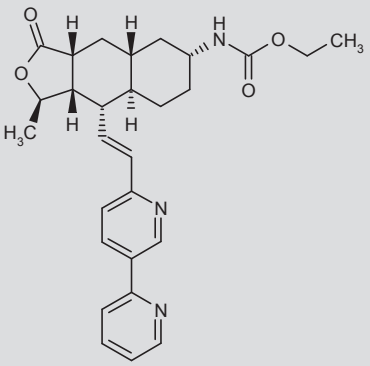
SCH-205831 competes with the thrombin receptor-activating peptide for PAR1 with a $K_i$ of 2.7 nM. SCH-205831 inhibits TRAP-induced platelet aggregation in washed human platelets with an $IC_{50}$ of 65 nM. Oral administration of SCH-205831 to cynomolgus monkeys caused a dose-dependent and complete inhibition of TRAP-induced platelet aggregation for 6 hours post-dosing. SCH-205831 was also evaluated in two models of thrombosis in baboons. SCH-205831 inhibited platelet deposition by 50-70% onto uncoated stents in an arteriovenous shunt model of thrombosis in these animals. SCH-205831 at 10 mg/kg prevented platelet deposition by 90% in an endarterectomy model of arteriovenous shunt thrombosis in baboons. Platelet aggregation responses to TRAP but not to ADP were inhibited by SCH-205831. Template bleeding times and coagulation parameters were unchanged (41).



SCH-205831

### *SCH-602539*

SCH-602539 is an analogue of the thrombin receptor antagonist SCH-530348 (vorapaxar) with increased aqueous solubility due to the incorporation of polar substituents on the pyridine ring of the himbacine-derived lead series (42).



SCH-602539

Preclinical studies

SCH-602539 inhibited thrombosis in a dose-dependent manner in the Folts model of thrombosis in anesthetized cynomolgus monkeys. When administered together, SCH-602539 and cangrelor exhibited additive and synergistic antithrombotic effects (43).

### Small molecules/peptides targeting intracellular domains of PAR1

A more recent approach to receptor inhibition has arisen from the observation that *N*-palmitoylated peptides, termed pepducins, based on the third intracellular loop of certain GPCRs can cause activation and/or inhibition of G protein signaling only in the presence of the parent GPCR. These lipopeptides can penetrate the cell membrane and have demonstrated efficacy to control platelet-dependent hemostasis and thrombosis, tumor growth, invasion and angiogenesis, as well as to improve sepsis outcomes in mice (44). Pepducins have been successfully designed against a wide variety of GPCRs, including PAR1. Attachment of a palmitate lipid to peptides based on the *N*-terminal portion of the i3 loop of PAR1 yielded P1pal-12 pepducin, whose sequence is pal-RCLSSSAVANRS-$NH_2$. This peptide lacked agonist activity but was a full antagonist of PAR1-dependent inositol phosphate production and $Ca^{2+}$ signaling in platelets and recombinant systems (45).

In recent studies in which screening a small-molecule library for inhibitors of platelet activation was performed, a family of compounds that modified PAR1-mediated granule secretion was identified (46). The most potent inhibitory compound was termed JF5. In aggregation studies, inhibition by JF5 was overcome in a PAR1 mutant in which the eighth helix (H8) was deleted, confirming a role for H8 in JF5 activity. Evaluation of downstream signaling showed that JF5 was selective with regard to G protein coupling, blocking signaling mediated by $G_{\alpha q}$ but not $G_{\alpha 12}$. The compound inhibited thrombus formation in vivo following vascular injury with an $IC_{50}$ of ~1 mg/kg. These results indicate a role for H8 in conferring sensitivity to small molecules, and show that this sensitivity can be exploited to control platelet activation during thrombus formation.

## FINAL CONSIDERATIONS

Research on PAR1 antagonists can be approached through the outer surface (non-peptide derivatives), but also from the inside by entering the cell (pepducins, among other compounds) (Fig. 1). The search for effective PAR1 antagonists as antiplatelet agents has finally led to good clinical candidates such as SCH-530348 and E-5555. In this setting, in view of the results from the clinical trials, addition of anti-PAR1 strategies to standard antiplatelet therapies (to block cyclooxygenase-1 and/or the $P2Y_{12}$ receptor) should be directed to the right patient population in order to avoid increasing the risk of bleeding.

Moreover, considering the ubiquity of PAR receptors, research on antagonists most likely will lead to the development of antiplatelet drugs, as well as drugs useful for the treatment of inflammatory, proliferative and neurodegenerative diseases.

## DISCLOSURES

The authors state no conflicts of interest.

## REFERENCES


1. Ross, R., Glomset, J.A. *The pathogenesis of atherosclerosis.* N Engl J Med 1976, 295(7): 369-77.
2. Fuster, V., Badimon, L., Badimon, J.J., Chesebro, J.H. *The pathogenesis of coronary artery disease and the acute coronary syndromes.* N Engl J Med 1992, 326(5): 310-8.
3. Fuster, V., Badimon, L., Badimon, J.J., Chesebro, J.H. *The pathogenesis of coronary artery disease and the acute coronary syndromes.* N Engl J Med 1992, 326(4): 242-50.
4. Jackson, S.P. *Arterial thrombosis – Insidious, unpredictable and deadly.* Nat Med 2011, 17(11): 1423-36.
5. Tran, H., Anand, S.S. *Oral antiplatelet therapy in cerebrovascular disease, coronary artery disease, and peripheral arterial disease.* JAMA 2004, 292(15): 1867-74.
6. Roth, G.J., Stanford, N., Majerus, P.W. *Acetylation of prostaglandin synthase by aspirin.* Proc Natl Acad Sci U S A 1975, 72(8): 3073-6.
7. Foster, C.J., Prosser, D.M., Agans, J.M. et al. *Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs.* J Clin Invest 2001, 107(12): 1591-8.
8. Yusuf, S., Zhao, F., Mehta, S.R., Chrolavicius, S., Tognoni, G., Fox, K.K. *Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation.* N Engl J Med 2001, 345(7): 494-502.
9. Wiviott, S.D., Braunwald, E., McCabe, C.H. et al. *Prasugrel versus clopidogrel in patients with acute coronary syndromes.* N Engl J Med 2007, 357(20): 2001-15.
10. Garcia-Rodriguez, L.A., Hernandez-Diaz, S., de Abajo, F.J. *Association between aspirin and upper gastrointestinal complications: Systematic review of epidemiologic studies.* Br J Clin Pharmacol 2001, 52(5): 563-71.
11. Peters, R.J., Mehta, S.R., Fox, K.A. et al. *Effects of aspirin dose when used alone or in combination with clopidogrel in patients with acute coronary syndromes: observations from the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) study.* Circulation 2003, 108(14): 1682-7.
12. Davey, M.G., Luscher, E.F. *Actions of thrombin and other coagulant and proteolytic enzymes on blood platelets.* Nature 1967, 216(5118): 857-8.
13. Coughlin, S.R. *Protease-activated receptors in hemostasis, thrombosis and vascular biology.* J Thromb Haemost 2005, 3(8): 1800-14.
14. Harmon, J.T., Jamieson, G.A. *The glycocalicin portion of platelet glycoprotein Ib expresses both high and moderate affinity receptor sites for thrombin. A soluble radioreceptor assay for the interaction of thrombin with platelets.* J Biol Chem 1986, 261(28): 13224-9.
15. Ramakrishnan, V., DeGuzman, F., Bao, M., Hall, S.W., Leung, L.L., Phillips, D.R. *A thrombin receptor function for platelet glycoprotein Ib-IX unmasked by cleavage of glycoprotein V.* Proc Natl Acad Sci U S A 2001, 98(4): 1823-8.
16. Coughlin, S.R. *How the protease thrombin talks to cells.* Proc Natl Acad Sci U S A 1999, 96(20): 11023-7.
17. Hursting, M.J., Stead, A.G., Crout, F.V., Horvath, B.Z., Moore, B.M. *Effects of age, race, sex, and smoking on prothrombin fragment 1.2 in a healthy population*. Clin Chem 1993, 39(4): 683-6.
18. Colman, R.W., Marder, V.J., Salzman, E.W., Hirsh, J. *Overview of hemostasis*. In: Hemostasis and Thrombosis, 3rd Ed. Colman, R.W., Marder, V.J., Salzman, E.W., Hirsh, J. (Eds.). Lippincott: Philadelphia 1994, 3-18.
19. Hamberg, M., Svensson, J., Samuelsson, B. *Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides.* Proc Natl Acad Sci U S A 1975, 72(8): 2994-8.

20. Stenberg, P.E., McEver, R.P., Shuman, M.A., Jacques, Y.V., Bainton, D.F. *A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation.* J Cell Biol 1985, 101(3): 880-6.
21. Henn, V., Slupsky, J.R., Grafe, M., Anagnostopoulos, I., Forster, R., Muller-Berghaus, G., Kroczek, R.A. *CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells.* Nature 1998, 391(6667): 591-4.
22. Hughes, P.E., Pfaff, M. *Integrin affinity modulation.* Trends Cell Biol 1998, 8(9): 359-64.
23. Kataoka, H., Hamilton, J.R., McKemy, D.D. et al. *Protease-activated receptors 1 and 4 mediate thrombin signaling in endothelial cells.* Blood 2003, 102(9): 3224-31.
24. McNamara, C.A., Sarembock, I.J., Gimple, L.W., Fenton, J.W. Jr., Coughlin, S.R., Owens, G.K. *Thrombin stimulates proliferation of cultured rat aortic smooth muscle cells by a proteolytically activated receptor.* J Clin Invest 1993, 91(1): 94-8.
25. Chackalamannil, S. *Thrombin receptor (protease activated receptor-1) antagonists as potent antithrombotic agents with strong antiplatelet effects.* J Med Chem 2006, 49(18): 5389-403.
26. Cirino, G., Severino, B. *Thrombin receptors and their antagonists: An update on the patent literature.* Expert Opin Ther Patents 2010, 20(7): 875-84.
27. Di Serio, C., Pellerito, S., Duarte, M. et al. P*rotease-activated receptor 1-selective antagonist SCH79797 inhibits cell proliferation and induces apoptosis by a protease-activated receptor 1-independent mechanism.* Basic Clin Pharmacol Toxicol 2007, 101(1): 63-9.
28. Chintala, M., Shimizu, K., Ogawa, M., Yamaguchi, H., Doi, M., Jensen, P. *Basic and translational research on proteinase-activated receptors: Antagonism of the proteinase-activated receptor 1 for thrombin, a novel approach to antiplatelet therapy for atherothrombotic disease.* J Pharmacol Sci 2008, 108(4): 433-8.
29. Becker, R.C., Moliterno, D.J., Jennings, L.K. et al. *Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: A randomised, double-blind, placebo-controlled phase II study.* Lancet 2009, 373(9667): 919-28.
30. Chackalamannil, S., Wang, Y., Greenlee, W.J. et al. *Discovery of a novel, orally active himbacine-based thrombin receptor antagonist (SCH 530348) with potent antiplatelet activity.* J Med Chem 2008, 51(11): 3061-4.
31. Kosoglou, T., Reyderman, L., Tiessen, R.G et al. *Pharmacodynamics and pharmacokinetics of the novel PAR-1 antagonist vorapaxar (formerly SCH 530348) in healthy subjects.* Eur J Clin Pharmacol 2011.
32. Angiolillo, D.J., Bhatt, D.L., Gurbel, P.A., Jennings, L.K. *Advances in antiplatelet therapy: agents in clinical development.* Am J Cardiol 2009, 103(3, Suppl.): 40A-51A.
33. Goto, S., Yamaguchi, T., Ikeda, Y. et al. *Safety and exploratory efficacy of the novel thrombin receptor (PAR-1) antagonist SCH530348 for non-ST-segment elevation acute coronary syndrome.* J Atheroscler Thromb 2010, 17(2): 156-64.
34. Shinohara, Y., Goto, S., Doi, M., Jensen, P. S*afety of the novel protease-activated receptor-1 antagonist vorapaxar in Japanese patients with a history of ischemic stroke*. J Stroke Cererovasc Dis 2010.
35. Tricoci, P., Huang, Z., Held, C. et al.; the TRACER Investigators. *Thrombin-receptor antagonist vorapaxar in acute coronary syndromes.* N Engl J Med 2012, 366(1): 20-33.
36. Serebruany, V.L., Kogushi, M., Dastros-Pitei, D., Flather, M., Bhatt, D.L. *The in-vitro effects of E5555, a protease-activated receptor (PAR)-1 antagonist, on platelet biomarkers in healthy volunteers and patients with coronary artery disease.* Thromb Haemost 2009, 102(1): 111-9.
37. Kogushi, M., Matsuoka, T., Kawata, T. et al. *The novel and orally active thrombin receptor antagonist E5555 (atopaxar) inhibits arterial thrombosis without affecting bleeding time in guinea pigs.* Eur J Pharmacol 2011, 657(1-3): 131-7.
38. Kogushi, M., Matsuoka, T., Kuramochi, H. et al. *Oral administration of the thrombin receptor antagonist E5555 (atopaxar) attenuates intimal thickening following balloon injury in rats.* Eur J Pharmacol 2011, 666(1-3): 158-64.
39. O'Donoghue, M.L., Bhatt, D.L., Wiviott, S.D. et al.; LANCELOT-ACS Investigators. *Safety and tolerability of atopaxar in the treatment of patients with acute coronary syndromes: The lessons from antagonizing the cellular effects of Thrombin–Acute Coronary Syndromes Trial.* Circulation 2011, 123(17): 1843-53.
40. Goto, S., Ogawa, H., Takeuchi, M., Flather, M.D., Bhatt, D.L.; J-LANCELOT (Japanese-Lesson from Antagonizing the Cellular Effect of Thrombin) Investigators. *Double-blind, placebo-controlled phase II studies of the protease-activated receptor 1 antagonist E5555 (atopaxar) in Japanese patients with acute coronary syndrome or high-risk coronary artery disease.* Eur Heart J 2010, 31(21): 2601-13.
41. Chintala, M., Ahn, H., Foster, C., Xia, Y., Chackalamannil, S. *Antithrombotic effects of SCH 205831: A potent, selective and orally active antagonist of the PAR-1 thrombin receptor.* J Thromb Haemost [20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005] 2005, 3: Abst OR286.
42. Xia, Y., Chackalamannil, S., Greenlee, W.J. et al. *Discovery of a vorapaxar analog with increased aqueous solubility.* Bioorg Med Chem Lett 2010, 20(22): 6676-9.
43. Chintala, M., Strony, J., Yang, B., Kurowski, S., Li, Q. *SCH 602539, a protease-activated receptor-1 antagonist, inhibits thrombosis alone and in combination with cangrelor in a Folts model of arterial thrombosis in cynomolgus monkeys.* Arterioscler Thromb Vasc Biol 2010, 30(11): 2143-9.
44. Dimond, P., Carlson, K., Bouvier, M. et al. *G protein-coupled receptor modulation with pepducins: Moving closer to the clinic.* Ann NY Acad Sci 2011, 1226: 34-49.
45. Covic, L., Misra, M., Badar, J. et al. *Pepducin-based intervention of thrombin-receptor signalling and systemic platelet activation.* Nat Med 2002, 8(10): 1161-5.
46. Dowal, L., Sim, D.S., Dilks, J.R. et al. *Identification of an antithrombotic allosteric modulator that acts through helix 8 of PAR1.* Proc Natl Acad Sci U S A 2011, 108(7): 2951-6.