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Brief Article

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Development of a Series of (1-Benzyl-3-(6-methoxypyrimidin-3-yl)-5-(trifluoromethoxy)-1*H*-indol-2-yl)methanols as Selective Protease Activated Receptor 4 (PAR4) Antagonists with in Vivo Utility and Activity Against γ-Thrombin

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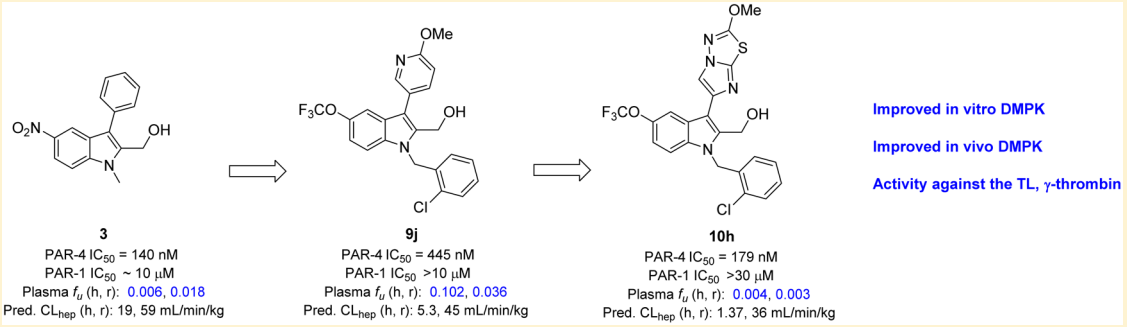
Supporting Information



ABSTRACT: Here, we describe the development of a series of highly selective PAR4 antagonists with nanomolar potency and selectivity versus PAR1, derived from the indole-based **3**. Of these, **9j** (PAR4 IC_{50} = 445 nM, PAR1 response IC_{50} > 30 μM) and **10h** (PAR4 IC_{50} = 179 nM, PAR1 response IC_{50} > 30 μM) maintained an overall favorable in vitro DMPK profile, encouraging rat/mouse in vivo pharmacokinetics (PK) and activity against γ-thrombin.

INTRODUCTION

Heart attack is the leading cause of death and morbidity in the western world,[1] with up to one-third of patients experiencing a second event within six months.[2] The role of platelet activation is reflected by the efficacy of antiplatelet reagents (aspirin + P2Y12 antagonist) in preventing the recurrent thrombosis that causes heart attack and stroke.[3,4] Despite this progress, 10−12% of patients on currently available antiplatelet therapeutics will face a second heart attack one year after being treated and a significant risk of bleeding.[5−7] Thus, there is an urgent need for additional antiplatelet therapeutics to prevent thrombosis without causing bleeding.

Thrombin is the terminal enzyme of the coagulation cascade responsible for platelet activation and the generation of fibrin, essential processes for both thrombosis and hemostasis. Human platelets respond to thrombin through two G protein-coupled receptors, PAR1 and PAR4.[8] PAR1 contains a hirudin like thrombin binding domain and therefore displays higher affinity for thrombin.[9] EC_{50}s for PAR1 (~0.2 nM) and PAR4 (~5 nM) differ by more than an order of magnitude, suggesting PAR4 is engaged after PAR1 as the concentration of thrombin increases at the local site of injury.

Thrombin receptor antagonists (TRAs) as a therapeutic alternative have been eagerly anticipated in cardiovascular medicine; vorapaxar is a PAR1 TRA that underwent two phase III clinical trials, TRA•CER,[10] and TRA 2°P.[11,12] After safety review, the TRA•CER trial was halted early and the TRA 2°P secondary prevention trial was partially discontinued due to an alarming increase in intracranial hemorrhage.[12] As a result, attention has turned to PAR4 as a potential target on the platelet that could strike the desired balance between safety and efficacy.

In addition to delayed engagement, PAR4 displays a more robust and sustained responses in the platelet,[13] suggesting distinct roles for the receptors; contrary to prior convention, recent findings suggest PAR4 is more important for the thrombin-induced procoagulant effect on platelets.[13] Unfortunately, tool compounds to probe the role of PAR4 in hemostasis and thrombosis have been lacking and the field has relied almost exclusively on PAR4 antibodies.[14] Additionally, thrombin activates PARs through cleavage of the

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extracellular domain of the receptor, revealing an encrypted tethered ligand (TL) that binds intramolecularly to activate the receptor.[15] PARs can also be activated artificially with a synthetic soluble "activating peptide" (AP) corresponding in sequence to the naturally derived TL.[15,16] Becaue of its covalent nature, the TL is much more difficult to inhibit than AP, but the ability to target TL would be a critical attribute of an in vivo probe molecule.

PAR4 antagonist described to date in the literature (Figure 1), including YD-3 (**1**),[17] SEY-3 (**2**),[18] and ML354 (**3**),[19]

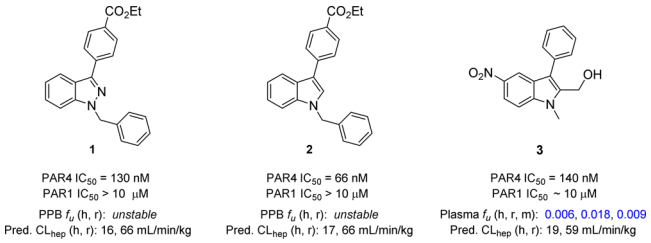


Figure 1. Structures and properties of reported PAR-4 antagonists.

proved to be selective versus PAR1 but suffer from both poor DMPK profiles as well as a lack of activity upon γ-thrombin activation. As we previously reported, the 2-methyl carbinol moiety within **3** plays an important role in maintaining potency while reducing lipophilicity, positively impacting free fraction and plasma stability.[19] A unique challenge remaining within **3** was the identification of an appropriate electron-withdrawing functionality to replace the aromatic nitro group, as limited success was found in our initial campaign (all analogues ~10 μM).[19] To further address this challenge, we now report the results from ongoing iterative lead optimization efforts within the indole chemotype, leading to compounds with comparable potency and selectivity while improving DMPK profiles and achieving activity against the TL.

RESULTS AND DISCUSSION

Chemistry. Our multidimensional optimization approach is summarized in Figure 2, and PAR4 antagonist activity was

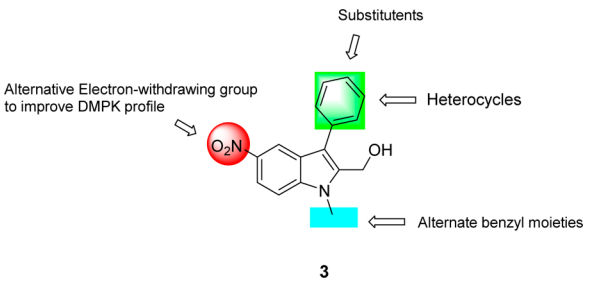
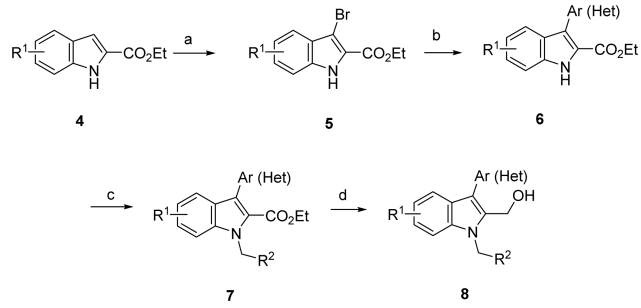


Figure 2. Library optimization strategy for **3** to improve PAR4 antagonist activity, selectivity, and the DMPK profile.

evaluated through $\alpha_{IIb}\beta 3$ activation upon PAR4-AP stimulation using a PAC-1 binding assay to drive SAR.[20] Scheme 1 highlights the four-step route to access analogues **8**, surveying diversity at three positions.[20] Here, bromination of commercial 5- or 6-substituted indoles **4** occurred smoothly, affording 3-bromo congeners **5** in 90–99% yield. Suzuki couplings with aryl or heteroaryl boronic acids afforded analogues **6** in good yields, followed by alkylation to deliver derivatives **7**. Finally, LAH reduction of the esters provided putative PAR4

Scheme 1. Synthesis of PAR4 Antagonist Analogues 8[a]

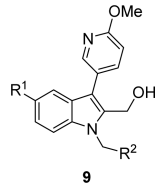


[a]Reagents and conditions: (a) THF, NBS (1.0 equiv), 0 °C, 1 h, 90–99%; (b) aryl/heteroarylboronic acid (1.5 equiv), $Pd(PPh_3)_4$ (0.1 equiv), Na_2CO_3 (2.0 equiv), DMF–H_2O (4:1), μw, 120 °C, 15 min, 76–95%; (c) K_2CO_3 (2 equiv), benzyl or alkyl bromide (2 equiv, DMF, 45 °C, 16 h, 60–90%; (d) LAH, THF, 0 °C, 1 h, 50–85%.

antagonists **8** in yields ranging from 50 to 85%. Initially, we held the 3-positon constant with a 2-methoxypyridine moiety, providing analogues **9**, as this 3-position substituent showed modest activity (~10 μM) in analogues lacking the 5-NO_2 group. If a competent NO_2 replacement could be identified in the context of the 2-methoxypyridine moiety, we hoped to see an increase in PAR4 activity.

Our screening strategy relied on an initial single-point screen at 10 μM and assessing the maximum % of PAC-1 binding, followed by full concentration–response curves (CRCs) to determine PAR4 IC_{50}s. As shown in Table 1, initial SAR for

Table 1. Initial SAR of Analogues 9



compd	R^1	R^2	PAR4-AP % max PAC-1[a]	PAR4-AP PAC-1 IC_{50} (μM), pIC_{50} ± SEM[b]
9a	CF_3	H	106.5	>10
9b	OCF_3	Ph	53.4	>10
9c	OCF_3	2-FPh	89.0	>10
9d	CF_3	3-OMePh	108.5	>10
9e	OCF_3	3-FPh	80.6	>10
9f	OCF_3	2,4-diFPh	19.7	5.11, 5.29 ± 0.33
9g	OCF_3	3-CF_3Ph	19.7	4.82, 5.32 ± 0.04
9h	OCF_3	3-ClPh	95.5	>10
9i	OCF_3	3-BrPh	27.1	2.19, 5.66 ± 0.33
9j	OCF_3	2-ClPh	4.95	0.45, 6.31 ± 0.05

[a]Initial single point screens were conducted using 10 μM analogue, and values indicate the percentage of max PAC-1 binding after PAR4-AP stimulation of human platelets. [b]Average of three independent determinations.

series **9** was steep, with NO_2 replacements such as CF_3 (**9a** and **9d**) and, in many cases, OCF_3 (**9b**, **9c**, **9e**, and **9h**) being devoid of PAR4 activity. Moreover, the nature of the benzyl moiety was critically important for PAR4 inhibitory activity. For example, **9f**, with a 2,4-diF benzyl group, displayed submicromolar activity (IC_{50} = 820 nM), highlighting that the undesirable 5-NO_2 moiety could be replaced while maintaining PAR4 activity. However, the most potent analogue

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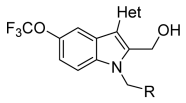
J. Med. Chem. XXXX, XXX, XXX–XXX

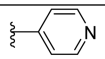
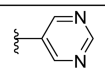
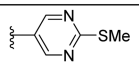
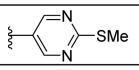
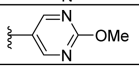
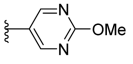
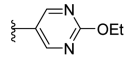
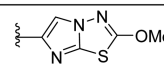
in this initial series **9** was **9j**, a 2-Cl benzyl derivative, with an IC_{50} of 445 nM.

Having identified optimal benzyl moieties in analogues **9**, our next SAR exploration focused on the identification of alternate heterocyclces for the 2-methoxypyridine in analogues **10** (Table 2). Here, a more basic, unsubstituted 4-pyridyl analogue, **10a**, was inactive as was an unsubstituted pyrimidine **10b**. Interestingly, introduction of either a alkoxy group or a thiomethyl moiety to the 2-position of the pyrimidine core, as in **10c–g**, restored PAR4 inhibition; however, PAR4 IC_{50}s varied from 490 nM (**10d**) to >10 μM (**10f**). Introduction of either the 2-methoxy or a 2-thiomethoxy group had significant impact. Comparison of **10b** (IC_{50} > 10 μM) to **10c** (IC_{50} = 1.49 μM) and **10b** (IC_{50} > 10 μM) to **10e** (IC_{50} = 1.50 μM) highlights how this small polar moiety enhances PAR4 inhibition. As we were concerned that 2-substituted pyrimidines with OMe or SMe functionalities may be electrophilic in vivo and be displaced by biological nucleophiles, we also surveyed a variety of pyrimidine replacements and found that only a 2-methoxy-6-methylimidazo[2,1-*b*][1,3,4]thiadiazole bicyclic ring system, as in **10h**, was active. Moreover, it was the most potent analogue made in the series (PAR4 IC_{50} = 179 nM) and on par with **3**. Recently, Bristol-Myers Squibb disclosed a novel benzofuran-based series of PAR4 antagonists (Figure 3), exemplified by **12** (BMS986120), that also possesses the 2-methoxy-6-methylimidazo[2,1-*b*][1,3,4]thiadiazole bicyclic ring system.[21]

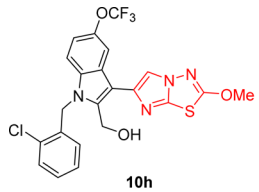
Table 2. Initial SAR of Analogues 10



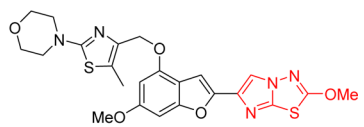
10

Cmpd.	Het	R	PAR4-AP % Max PAC-1[a]	PAR4-AP PAC-1 IC_{50} (μM), pIC_{50}±SEM[b]
10a		2-ClPh	101.2	>10
10b		2,4-diFPh	96.1	>10
10c		2,4-diFPh	15.6	1.35, 5.87±0.25
10d		2-ClPh	4.39	0.49, 6.31±0.09
10e		2-ClPh	7.95	1.50, 5.82±0.25
10f		2,4-diFPh	53.6	>10
10g		2-ClPh	35.4	4.91, 5.31±0.27±0.28
10h		2-ClPh	3.2	0.18, 6.75±0.07

[a]Initial single point screens were conducted using 10 μM analogue and values indicate the percentage of Max PAC-1 binding after PAR4-AP stimulation of human platelets. [b]Average of three independent determinations.



10h

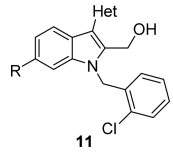


12, BMS986120

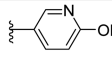
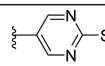
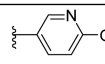
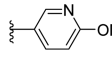
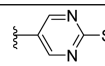
Figure 3. Structures of **10h** and **12**, PAR4 anatgonists containing the –methoxy-6-methylimidazo[2,1-*b*][1,3,4]thiadiazole bicyclic ring system.

Finally, we elected to explore the impact of 6- versus 5-substiuents on the indole core in analogues **11** (Table 3), as this had never been assessed. Interestingly, simple alkoxy moieties (**11a–c**) did afford modest PAR4 inhibition, but once again, the 6-OCF_3 group, in the context of 2-OMe and 2-SMe pyrimidines, **11d** (IC_{50} = 930 nM) and **11e** (IC_{50} = 210 nM), respectively, showed submicromlar potency. Thus, multiple new PAR4 antagonists resulted that proved worthy of further profiling.

Table 3. Initial SAR of Analogues 11



11

Cmpd.	Het	R	PAR4-AP % Max PAC-1[a]	PAR4-AP PAC-1 IC_{50} (μM), pIC_{50}±SEM[b]
11a		OMe	4.31	1.39, 5.86±0.10
11b		OMe	21.3	4.15, 5.38±0.03
11c		OEt	19.7	3.76, 5.43±0.09
11d		OCF_3	12.3	0.82, 6.09±0.15
11e		OCF_3	5.57	0.21, 6.69±0.8

[a]Initial single point screens were conducted using 10 μM analogue, and values indicate the percentage of max PAC-1 binding after PAR4-AP stimulation of human platelets. [b]Average of three independent determinations.

Molecular Pharmacology. Of the six submicromolar PAR4 antagonists identified (**9f**, **9j**, **10d**, **10h**, **11d**, and **11e**), all were selective versus the key antitarget PAR1 (IC_{50}s > 30 μM). On the basis of physiochemical and DMPK data (vide infra), only **9j** and **10h** were subjected to more detailed molecular pharmacology profiling. As shown in Figure 4A, **9j** was an equipotent PAR4 antagonist at both assay readouts (both PAC-1, IC_{50} = 445 nM, and P-Selectin, IC_{50} = 435 nM).

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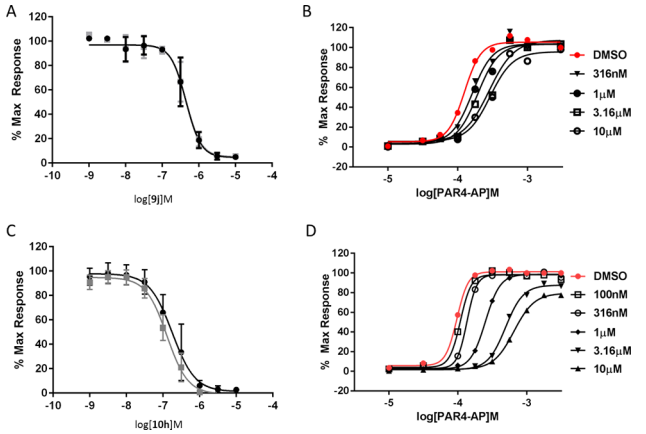

Figure 4. Molecular pharmacology profile of **9j** and **10h**. (A) PAR4-AP concentration−response curves (n = 3) with equivalent inhibition of both PAC-1 (IC_{50} = 445 nM) and P-Selectin (IC_{50} = 435 nM). (B) Progressive fold-shift inhibition assay with **9j**, showing complex mode of PAR4 inhibition. (C) PAR4-AP concentration−response curves (n = 3) with comparable inhibition of both PAC-1 (IC_{50} = 179 nM) and P-Selectin (IC_{50} = 132 nM). (D) Progressive fold-shift inhibition assay with **10h**, also showing a complex mode of PAR4 inhibition. Note: compound solubility was not an issue at the concentrations evaluated, i.e., no precipitate was observed.

A progressive fold-shift inhibition assay/Schild analysis with **9j** (Figure 4B) displayed an unexpected mixed competitive/noncompetitive mode of PAR4 inhibition with neither a clean, parallel rightward shift in the concentration−response curve (competitive) nor a defined decrease in AP-max (non-competitive). Similarly, **10h** displayed comparable PAR4 antagonism (Figure 4C) at both assay readouts (both PAC-1, IC_{50} = 179 nM, and P-Selectin, IC_{50} = 132 nM). However, a progressive fold-shift inhibition assay/Schild analysis with **10h** (Figure 4D) displayed a similar, complex mode of inhibition yet clearly more potent. The mixed mechanism of PAR4 inhibition is intriguing and suggests with further SAR exploration that it might be possible to develop both competitive and non-competitive inhibitors of PAR4 and then validate each in vivo. In addition, both **9j** and **10h** were devoid of activity at PAR1 (IC_{50}s > 30 μM).

As mentioned earlier, no small molecule PAR4 antagonist reported to date has demonstrated PAR4 inhibition against the tethered ligand (TL). As an ideal tool compound that would possess activity against the TL, we evaluated the ability of both **9j** and **10h** to inhibit PAR4 when activated by 100 nM γ-thrombin (Figure 5). While the results with **9j** were variable and weak (IC_{50} > 10 μM with P-Selectin and IC_{50} = 9.4 μM, 48.7% max inhibition with PAC-1), efficacy was noted against the TL. However, **10h** proved far more effective against the TL,

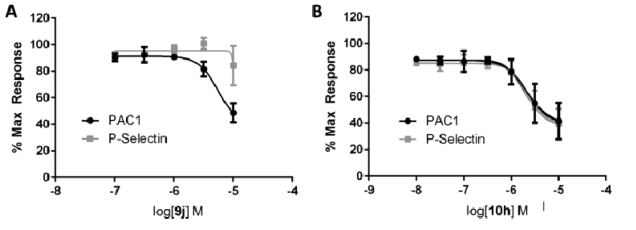

Figure 5. Molecular pharmacology profile of (A) **9j** and (B) **10h** against activation of PAR4 with the tethered ligand, 100 nM γ-thrombin.

affording comparable inhibition (IC_{50}s of 4.35 μM and 39.4% maximum inhibition). Thus, while weak partial antagonists against the TL, **9j** and **10h** represent the first reported small molecule PAR4 antagonists to have activity.

DMPK Disposition Attributes. Of the six submicromolar PAR4 antagonists identified (**9f**, **9j**, **10d**, **10h**, **11d**, and **11e**), **9f**, **11d**, and **11e** quickly fell out of contention as in vivo probes due to either high plasma protein binding (rat and human f_u < 0.001) or high predicted in vitro clearance in microsomes that was equal to or greater than hepatic blood flow (rat CL_{hep} > 60 mL/min/kg and human CL_{hep} > 20 mL/min/kg).[20] Table 4

Table 4. DMPK Characterization of Select PAR4 Antagonists

	9j	10d	10h
MW	462.8	479.9	508.9
TPSA	54.2	57.3	69.8
cLogP	5.2	5.3	5.1
CYP (1A2, 2C9, 2D6, 3A4) IC_{50} (μM)	4.2,6.8, 0.12, 14	2.9, 6.1, <0.1, 17.6	>30, 11.2, 1.2, >30
In Vitro PK			
rat CL_{HEP} (mL/min/kg)	45.1	58	36.1
human CL_{HEP} (mL/min/kg)	5.3	18.5	1.1
rat PPB (f_u)	0.04	0.001	0.003
human PPB (f_u)	0.10	0.002	0.004
in Vivo Rat PK (IV Cassette, 0.2 mg/kg, 0−6 h)			
$t_{1/2}$ (h)	2.83	0.25	6.1
CL_p (mL/min/kg)	118	380	45
V_{ss} (L/kg)	21.4	5.82	21.1

highlights representative physiochemical and DMPK profiles of **9j**, **10d**, and **10h**. We were concerned about the lability of the 2-SMe pyrimidine moiety in **10d**, and this concern was born out, as **10d** showed little fraction unbound in rat or human plasma and predicted hepatic clearance near blood flow in each species and superhepatic clearance in vivo. In contrast, the pyrimidine bioisostere **10h** displayed low predicted hepatic clearance in human microsomes (CL_{hep} = 1.1 mL/min/kg) and low to moderate in rat (CL_{hep} = 36.1 mL/min/kg). In vivo rat PK showed a 6.1 h half-life with moderate clearance (CL_p = 45 mL/min/kg); however, **10h** was highly protein bound in both species. PAR4 antagonist **9j** represented middle ground between the **10d** and **10h**. Analogue **9j** displayed low predicted hepatic clearance in human microsomes (CL_{hep} = 5.3 mL/min/kg) and moderate in rat (CL_{hep} = 45.1 mL/min/kg), and the best free drug levels to date (human f_u = 0.10, rat f_u = 0.04). In vivo rat PK showed a 2.8 h half-life with high total plasma clearance (CL_p = 118 mL/min/kg) and a high volume of distribution at steady state (V_{ss} = 21.4 L/kg). As mouse is the species in which the thrombosis models are performed, and in which PAR4 is expressed on platelets, we performed an IV PK study (0.2 mg/kg, PEG:saline (1:1) for **9j** in five male c57bl/6 mice to mimic the route of administration in these models. Plasma levels of **9j** were collected at a single 40 min time point and were incredibly consistent (plasma 450 ± 20 ng/mL, 1000 ± 50 nM) and above the in vitro IC_{50} of **9j**. When corrected for free drug levels, the unbound plasma concentration of **9j** was reduced to ~38 nM; however, a question to be addressed in later studies and with more refined PAR4 inhibitors is if efficacy will be driven by total or free drug levels in plasma.

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J. Med. Chem. XXXX, XXX, XXX−XXX

CONCLUSION

In summary, we report significant advances in the field of small molecule PAR4 antagonists relative to the known art **1**−**3**, for which we have addressed the plasma instability of **1** and **2** and found suitable replacements for the aromatic nitro group of **3**. New PAR4 antagonists described here have also lowered plasma protein binding (increased fraction unbound, e.g., free drug principle) as well as both improved in vitro and in vivo DMPK such that **9j** may serve as a first-generation PAR4 in vivo tool. Importantly, both **9j** and **10h** are the first reported small molecules to show PAR4 inhibitory activity against the native ligand, γ-thrombin, and were inactive against PAR1 (IC_{50}s > 30 μM). Finally, the mode of inhibition for **9j** and **10h** is complex, showing a mixed competitive/noncompetitive mechanism of action, suggesting it might be possible to develop both competitive and noncompetitive PAR4 antagonists. Additional refinements and in vivo studies are in progress and will be reported in due course.

EXPERIMENTAL SECTION

Chemistry. The general chemistry, experimental information, and syntheses of all other compounds, including **10h**, are supplied in the Supporting Information, as well as pharmacology and DMPK methods.

(1-(2-Chlorobenzyl)-3-(6-methoxypyridin-3-yl)-5-(trifluoromethoxy)-1*H*-indol-2-yl)methanol (9j): Ethyl 3-bromo-5-(trifluoromethoxy)-1*H*-indole-2-carboxylate. To a THF solution (3 mL) of commercially available ethyl 5-(trifluoromethoxy)-1*H*-indole-2-carboxylate (300 mg, 1.098 mmol, 1 equiv) was added dropwise the THF (5 mL) solution of NBS (214.7 mg, 1.206 mmol, 1.1 equiv). The mixture was stirred at room temperature for 2 h. Then THF was removed under reduced pressure and the crude product was purified via ISCO flash chromatography (0−10% ethyl acetate in hexane) (382 mg, 99%). LCMS: R_T = 1.276 min, MS (ESI^+) m/z = 351.8 $[M + H]^+$. ^1H NMR (400.1 MHz, $CDCl_3$): 1.46 (t, J = 7.1, 3H), 4.44−4.50 (q, J = 7.1, 2H), 7.23 (d, J = 1.4, 1H), 7.38−7.42 (dd, J = 4.7, 8.9, 1H), 7.53 (d, J = 16.1, 1H), 9.12 (s, 1H).

Ethyl 3-(6-Methoxypyridin-3-yl)-5-(trifluoromethoxy)-1*H*-indole-2-carboxylate. To a microwave vial was added ethyl 3-bromo-5-(trifluoromethoxy)-1*H*-indole-2-carboxylate (300 mg, 0.852 mmol, 1 equiv), 6-methoxy-3-pyridinyl-boronic acid (169.5 mg, 1.107 mmol, 1.3 equiv), $Pd(PPh_3)_4$ (98.5 mg, 0.085 mmol, 0.1 equiv), Na_2CO_3 (180.6 mg, 1.704 mmol, 2 equiv), DMF (4 mL), and water (1 mL). The vial was sealed and put in the microwave reactor and then heated for 15 min at 120 °C. On completion, the solvents were passed through a Celite pad. The solvents were removed under reduced pressure and pure 3-(6-methoxypyridin-3-yl)-5-(trifluoromethoxyl)-1*H*-indole-2-carboxylate was afforded; ISCO flash chromatography (0−20% ethyl acetate in hexane) (253.2 mg, 78%). LCMS: R_T = 1.233 min. MS (ESI^+) m/z = 380.8 $[M + H]^+$. ^1H NMR (400.1 MHz, $CDCl_3$): 1.27 (t, J = 7.1, 3H), 4.05 (s, 3H), 4.29−4.34 (q, J = 7.1, 2H), 6.90 (d, J = 8.5, 1H), 7.26−7.27 (m, 1H), 7.43 (s, 1H), 7.45 (d, J = 9.0, 1H), 7.81−7.84 (dd, J = 2.4, 8.5, 1H), 8.37 (d, J = 2.1, 1H), 9.1(s, 1H).

(1-(2-Chlorobenzyl)-3-(6-methoxypyridin-3-yl)-5-(trifluoromethoxy)-1*H*-indol-2-yl)methanol (9j). To a dry vial was added 3-(6-methoxypyridin-3-yl)-5-(trifluoromethoxyl)-1*H*-indole-2-carboxylate (10 mg, 0.039 mmol, 1 equiv), a series of 2-chlorobenzyl bromide (1.5 equiv), K_2CO_3 (5.5 mg, 0.078 mmol, 2 equiv), and DMF. The mixture was stirred at room temperature for 18 h. The desired indole esters were purified by the Gilson reverse-phase preparative LC (CH_3CN/H_2O/TFA). To the solution of the indole esters in THF was added LAH (1 M in THF) (2 equiv) at 0 °C, and the solution was allowed to stir for 1 h. Then, 2 N HCl aqueous (10 μL) was added to consume the remaining LAH, followed by addition of 10 μL of saturated $NaHCO_3$ aqueous. The solvents of reaction were removed on a heated air-blowing block, and then the title compound was purified by the Gilson reverse-phase preparative LC, with >95% purity by UV215 nm, UV254 nm, and ELSD analysis on LCMS. LCMS: R_T = 1.229 min. MS (ESI^+) m/z = 463.0 $[M + H]^+$. ^1H NMR (400.1 MHz, DMSO-d_6): 3.84 (s, 1H), 3.93 (s, 3H), 4.51 (s, 2H), 5.71 (s, 2H), 6.33−6.35 (m, 1H), 6.98−7.01 (dd, J = 0.5, 8.5, 1H), 7.16−7.20 (m, 2H), 7.28−7.31 (m, 1H), 7.45−7.48 (m, 2H), 7.54−7.56 (m, 1H), 7.91−7.94 (dd, J = 2.5, 8.5, 1H), 8.36 (d, J = 1.8, 1H). ^{13}C NMR (DMSO-d_6): 162.4, 146.7, 142.7, 140.1, 138.6, 135.0, 134.7, 131.0, 129.3, 128.8, 127.6, 127.5 (q, J = 259.6), 127.3, 126.7, 122.7, 116.2, 112.1, 111.5, 111.2, 110.6, 53.2, 53.0, 48.7. HRMS (TOF, ES+) $C_{23}H_{19}ClF_3N_2O_3$ $[M + H]^+$ calcd mass 463.1031, found 463.1035.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00928.

Experimental procedures and spectroscopic data for selected compounds, detailed pharmacology and DMPK methods (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

PAR4, selective protease activated receptor 4; CRC, concentration−response-curve; PPB, plasma protein binding

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