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RESEARCH ARTICLE

Octahydrocyclopenta[*c*]pyridine and octahydrocyclopenta[*c*]pyran analogues as a protease activated receptor 1 (PAR1) antagonist

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Abstract Protease activated receptor 1 (PAR1) has been considered as a promising antiplatelet target to prevent thrombotic cardiovascular events in patients with prior myocardial infarction or peripheral arterial diseases. Previously, we found a series of octahydroindene analogues to have high potency on PAR1 and no significant cytotoxicity but poor metabolic stability in human and rat liver microsomes. We have designed and synthesized fused 6/5 heterobicycle analogues with octahydrocyclopenta[*c*]pyridine or octahydrocyclopenta[*c*]pyran core scaffold by the insertion of heteroatom at C5 of octahydroindene ring aiming to improvement of metabolic stability. Both heterobicycle analogues showed much more improved metabolic stability compared with octahydroindenes without remarkable decrease in activity. Compounds **22** (IC_{50} = 110 nM) and **33** (IC_{50} = 50 nM) from this series showed good activity on PAR1 with moderate metabolic stability.

Keywords Protease activated receptor 1 · 6/5 Fused heterobicycle · Octahydrocyclopenta[*c*]pyridine · Octahydrocyclopenta[*c*]pyran · Octahydroindene · Metabolic stability

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Introduction

The beneficial effects of antiplatelet therapy on cardiovascular diseases have been proven (David and Patrono 2007). Thrombin regulates platelet aggregation mainly through actions on protease activated receptor 1 (PAR1), platelet surface G-protein coupled receptor (Coughlin 2000, 2005; Hein et al. 1994; Ishii et al. 1993; Trejo et al. 1998; Vu et al. 1991; Zhang et al. 2012). PAR1 has been considered as a promising antiplatelet target to treat patients with prior cardiovascular events, leading to the reduction of the rate of cardiovascular death, myocardial infarction (MI), stroke, and urgent coronary revascularization (Chackalamannil et al. 2005; Chackalamannil and Xia 2006; Chelliah et al. 2012, 2014; Clasby et al. 2006; Dockendorff et al. 2012; Ramachandran et al. 2012). Vorapaxar, a first-in-class PAR 1 antagonist, has been approved by USFDA in 2014 as the indication for the reduction of thrombotic cardiovascular events in patients with a history of MI or with peripheral arterial disease (PAD), which indicates the clinical validation on this mechanism of action (Chackalamannil et al. 2008; Kosoglou et al. 2012; Morrow et al. 2012; Xia et al. 2010).

Previously, we identified fused 6/5 bicycle, octahydroindene as a novel scaffold for PAR1 antagonists (Lee 2011; Lee et al. 2013). Although these series of compounds showed high potency and no significant cytotoxicity, they were metabolically unstable in both human and rat live microsomes. We try to improve their metabolic stability by the insertion of heteroatom at C5 which might be the site of metabolism (Fig. 1). In this paper, we disclose the design, synthesis, PAR1 inhibitory activity, and metabolic stability of fused 6/5 heterobicycle compounds with octahydrocyclopenta[*c*]pyridine or octahydrocyclopenta[*c*]pyran core scaffold.

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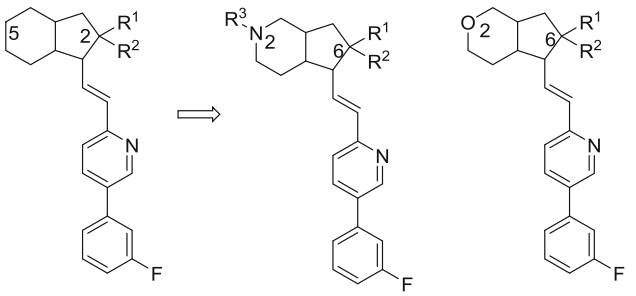


Fig. 1 Design of octahydrocyclopenta[*c*]pyrimidine and octahydrocyclopenta[*c*]pyran core

Materials and methods

Chemistry

Anhydrous solvents were dried by conventional methods. Reagents of commercial quality were used from freshly opened containers unless otherwise stated. For purification of products by column chromatography, Merck Silica gel 60 (230–400 mesh) was used. ^{1}H NMR spectra were recorded on a Varian Gemini 300 with TMS as an internal standard. Chemical shifts are reported in δ (ppm). Mass spectra were obtained with a Bruker instrument by using electron impact techniques.

*tert-Butyl(3-hydroxypropyl)carbamate (**2**)*

To the solution of 3-amino-1-propanol (14 mL, 66.6 mmol) in CH_2Cl_2 (150 mL) were added Et_3N (14 mL, 99.9 mmol) and di-*tert*-butyldicarbonate at 0°C. After stirring for 1 h at rt, the reaction mixture was concentrated under reduced pressure to remove all volatiles. The residue was diluted with water, and extracted with ethyl acetate. The organic layer was washed twice with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to give an off-white oil (1.1 g, yield 94 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.65 (m 2H), 3.28 (t, 2H, J = 5.9 Hz), 3.68 (t, 2H, J = 5.4 Hz), 4.86 (s, 1H), MS 175 (M^+).

*tert-Butyl(3-oxopropyl)carbamate (**3**)*

The solution of DMSO (9.74 mL, 137.1 mmol) and oxalyl chloride (5.8 mL, 68.6 mmol) in anhydrous CH_2Cl_2 (400 mL) was stirred for 15 min at −78 °C, and the compound **2** (8 g, 45.7 mmol) dissolved in anhydrous CH_2Cl_2 (30 mL) was added. The reaction mixture was continuously stirred for an additional 1 h at −78 °C. Subsequently, Et_3N (31.8 mL, 228.5 mmol) was added, and the reaction mixture was stirred for 1 h at rt. To the reaction NH_4Cl solution was added, and the layers were separated. An aqueous layer was extracted with CH_2Cl_2 twice. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was used for the next step without purification.

*Ethyl (E)-5-(t-butoxycarbonylamino)pent-2-enoate (**4**)*

To the mixture of NaH (1.5 g, 64 mmol, 60 % in an oil) in anhydrous THF (400 mL) was added ethyl 2-(diethoxyphosphoryl)acetate (13.6 mL, 68.6 mmol) at 0 °C, which was stirred for 10 min at 0 °C, and cooled to −78 °C. The compound **3** dissolved in anhydrous THF (30 mL) was added at −78 °C, and the mixture was stirred for 1 h at rt. After the solvent was removed, the residue was filtered through a silica gel pad using hexane/ethyl acetate (3:1) solution. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 9:1) to give a pale yellow oil (8 g, yield 74 % over 2steps).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, 3H, J = 7.1 Hz), 1.44 (s, 9H), 2.40 (q, 2H, J = 6.4 Hz), 3.26 (m, 2H), 4.18 (q, 2H, J = 7.1 Hz), 4.56 (s, 1H), 5.87 (m, 1H), 6.86 (m, 1H). MS 243 (M^+).

*tert-Butyl (E)-5-(hydroxypent-3-enyl)carbamate (**5**)*

To the solution of compound **4** (8 g, 32.9 mmol) in anhydrous CH_2Cl_2 (300 mL), diisobutylaluminium hydride (82.3 mL, 1 M in toluene) was added at −78°C, and the reaction mixture was stirred for 1 h ar rt. Methanol was added to the reaction and the mixture was filtered through a silica gel pad. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 2:1) to give a pale yellow oil (5.5 g, yield 83 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.21–2.27 (m, 2H), 3.15–3.22 (m, 2H), 4.09–4.13 (m, 2H), 4.56 (1H, s), 5.64–5.75 (m, 2H). MS 201 (M^+).

*tert-Butyl (E)-{5-[(tert-butyldimethylsilyl)oxy]pent-3-en-1-yl}carbamate (**6**)*

To the solution of compound **5** (11 g, 54.7 mmol) in THF (300 mL) were added imidazole (8.9 mg, 131.3 mmol) and *tert*-butyldimethylsilyl chloride (9.9 g, 65.6 mmol). After stirring for 1 h at rt, aqueous NH_4Cl solution was added to the reaction. The mixture was extracted with ethyl acetate. The organic layer was washed twice with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel

column chromatography (hexane:ethyl acetate = 10:1) to give a pale yellow (16 g, yield 93 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.89 (s, 9H), 1.45 (s, 9H), 2.20 (m, 2H), 3.15 (m, 2H), 4.11 (m, 2H), 4.52 (s, 1H), 5.56–5.62 (m, 2H). MS 315 (M^+).

*tert-Butyl (E)-{5-[(t-butyldimethylsily)loxy]pent-3-en-1-yl}(prop-2-yn-1-yl)carbamate (**7**)*

To anhydrous THF (15 mL) were added NaH (1.35 g, 57.03 mmol, 60 % in oil) and 15-crown-5 (11.2 mL, 57.03 mmol) at −30 °C. The compound **6** (6 g, 19.1 mmol) dissolved in anhydrous THF (180 mL) and propargyl bromide (10.5 mL, 95.08 mmol) were added at −30 °C and the mixture was stirred for 2 h at rt. Aqueous NH_4Cl solution was added to the reaction. The mixture was extracted with ethyl acetate. The organic layer was washed twice with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1) to give a pale yellow oil (4.0 g, yield 60 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.89 (s, 9H), 1.45 (s, 9H), 2.13 (m, 1H), 2.18–2.23 (m, 2H), 3.07–3.13 (m, 1H), 3.26–3.31 (m, 1H), 4.04–4.07 (m, 2H), 4.14 (m, 2H), 5.51–5.55 (m, 2H). MS 353 (M^+).

*tert-Butyl(±)-(4aS,5R)-5-{[(tert-butyldimethylsilyl)oxy]methyl}-6-oxo-1,3,4,4a,5,6-hexahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**8**)*

To the solution of compound **7** (4.0 g, 11.3 mmol) in anhydrous CH_2Cl_2 (150 mL) was added $Co_2(CO)_8$ (3.9 g, 11.3 mmol) at 0 °C, and the mixture was stirred at rt for 2 h to form a complex between the compound and Co. *N*-Methylmorpholine-*N*-oxide (1.37 g, 11.77 mmol) was added at 0 °C to the reaction mixture. After stirring for 2 h at rt, the mixture was filtered through a silica gel pad and the filtrate was concentrated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 7:1) to give a pale yellow oil (2.3 g, yield 53 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.08 (s, 9H), 1.58–1.64 (m, 1H), 2.09–2.18 (m, 2H), 2.83–2.88 (m, 1H), 3.56–3.64 (m, 1H), 3.80–3.89 (m, 2H), 4.02–4.13 (m, 1H), 4.15 (d, 1H, J = 13.4 Hz), 4.65 (d, 1H, J = 13.4 Hz), 5.88 (s, 1H). MS 381 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-5-{[(t-butyldimethylsilyl)oxy]methyl}-6-octahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**9**)*

To the solution of compound **8** (2.3 g, 6.03 mmol) in ethanol (3 mL) was added $Pd(OH)_2$ (0.23 g, 1.21 mmol). The mixture was stirred under hydrogen atmosphere (60 psi) for 10 h, and was filtered through a silica gel pad. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1) to give a pale yellow oil (1.7 g, yield 74 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.85 (s, 9H), 1.46 (s, 9H), 1.57–1.63 (m, 1H), 1.82–1.90 (m, 1H), 2.14–2.25 (m, 3H), 2.45–2.61 (m, 2H), 3.30–3.36 (m, 1H), 3.60–3.88 (m, 4H), 3.90–3.95 (m, 1H). MS 383 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-5-{[(t-butyldimethylsilyl)oxy]methyl}-6-hydroxy-6-methyloctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**10**)*

To the solution of ketone **9** (300 mg, 0.78 mmol) in anhydrous THF (10 mL) was added CH_3MgBr (1.0 M solution, 1.6 mL) at −78 °C, which was stirred for 1 h at rt, NH_4Cl was added to the reaction mixture. After removal of all volatiles under reduced pressure, the residue was diluted with CH_2Cl_2. The organic layer was washed twice with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated and purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to give a pale yellow oil (130 mg, yield 42 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.89 (s, 9H), 1.26 (s, 3H), 1.46 (s, 9H), 1.69–1.75 (m, 2H), 1.86–1.99 (m, 2H), 2.12–2.23 (m, 2H), 2.89–3.13 (m, 2H), 3.25–3.39 (m, 1H), 3.43–3.52 (m, 1H), 3.65 (m, 1H), 3.68–3.79 (m, 1H), 3.81–3.98 (m, 1H). MS 399 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-6-hydroxy-5-(hydroxymethyl)-6-methyloctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**11**)*

To the solution of compound **10** (130 mg, 0.325 mmol) in anhydrous THF (4 mL) was added tetrabutylammonium fluoride (1.0 M solution, 9 μL, 0.032 mmol) at 0 °C. The mixture was stirred for 1 h at rt, and was diluted with CH_2Cl_2. The organic layer was washed twice with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated and purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to give a pale yellow oil (85 mg, yield 92 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (s, 3H), 1.46 (s, 9H), 1.63–1.85 (m, 2H), 2.03 (m, 2H), 2.31–2.43 (m, 3H), 3.06 (m, 1H), 3.35–3.36 (m, 2H), 3.36–3.38 (m, 1H), 3.73–3.77 (m, 2H). MS 285 (M^+).

*tert-Butyl(±)-(4aR,5S,7aR)-5-formyl-6-hydroxy-6-methyloctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**12**)*

To the solution of alcohol **11** (85 mg, 0.3 mmol) in CH_2Cl_2 was added *o*-iodoxybenzoic acid (180 mg, 0.642 mmol), and the mixture was stirred for 2 h at rt. To the mixture was added saturated $NaHCO_3$ solution (1 mL). Layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, and dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give an aldehyde, which was used for the next step without further purification.

*tert-Butyl(±)-(4aR,5S,7aR)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-6-hydroxy-6-methyloctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**13**)*

In the solution of diethyl {[3′-fluoro(1,1′-biphenyl)4-yl]vinyl}Phosphonate (150 mg, 0.45 mmol) in anhydrous THF (2 mL) was slowly added n-BuLi (0.16 mL, 2.5 M toluene solution) at 0 °C. After stirring for 30 min, the aldehyde **12** dissolved in THF (1 mL) was slowly added. After stirring for 2 h at rt, the mixture was extracted with ethyl acetate. The organic layer was washed twice with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated and purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to give a pale yellow oil (68 mg, yield 50 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (s, 3H), 1.48 (s, 9H), 1.65–1.83 (m, 2H), 2.01–2.03 (m, 2H), 2.35–2.42 (m, 2H), 3.08 (q, 1H, J = 6.02 Hz), 3.35–3.36 (m, 2H), 3.38–3.41 (m, 1H), 3.53–3.55 (m, 1H), 6.64–6.73 (m, 1H), 6.78–6.83 (m, 1H), 7.21–7.27 (m, 2H), 7.34–7.46 (m, 2H), 7.79–7.82 (m, 1H), 8.75 (s, 1H). MS 452 (M^+).

*tert-Butyl (±)-(4aR,5S,7aR)-6-(carbamoyloxy)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-6-methyloctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**14**)*

To the solution of compound **13** (48 mg, 0.106 mmol) in anhydrous THF (2 mL) was added trichloroacetyl isocyanate (0.025 mL, 0.212 mmol). The mixture was stirred for 2 h at rt, and concentrated under reduced pressure to remove all volatiles. Methanol (2 mL), H_2O (0.1 mL) and K_2CO_3 (0.038 g, 0.245 mmol) were added to the residue. The mixture was stirred at rt until the starting material was disappeared in TLC. After completion of the reaction, the mixture was extracted with ethyl acetate The organic layer was washed twice with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated and purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to give a pale yellow solid (50 mg, yield 91 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (s, 3H), 1.50 (s, 9H), 1.85–1.87 (m, 2H), 2.01–2.02 (m, 2H), 2.41–2.44 (m, 1H), 2.47–2.51 (m, 1H), 2.90 (m, 2H), 3.36–3.45 (m, 2H), 3.53–3.55 (m, 1H), 4.52 (s, 2H), 6.60–6.64 (m, 1H), 6.73–6.82 (m, 1H), 7.08–7.11 (m, 1H), 7.25 (s, 1H), 7.38 (m, 2H), 7.43–7.54 (m, 1H), 7.83 (m, 1H), 8.81(d, 1H, J = 1.3 Hz). MS 495 (M^+).

*(±)-(4aR,5S,7aR)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-6-methyloctahydro-2H-cyclopenta[c]pyridine-6-yl carbamate (**15**)*

To the solution of compound **14** (50 mg, 0.101 mmol) in CH_2Cl_2 (2 mL) was added CF_3COOH (50 μL) at 0 °C. The reaction mixture was stirred for 1 h at rt, neutralized by 1 N NaOH, and extracted with CH_2Cl_2. The organic layer was washed twice with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 10:1) to give a pale yellow solid (36 mg, yield 90 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.21 (s, 3H), 2.01 (m, 1H), 2.41 (m, 1H), 2.48 (m, 1H), 2.53 (m, 1H), 2.71–2.86 (m, 3H), 2.96 (m, 1H), 4.58 (s, 2H), 6.57 (m, 1H), 6.77 (m, 1H), 7.11 (m, 1H), 7.28 (m, 1H), 7.32 (m, 2H), 7.43 (m, 1H), 7.83 (m, 1H), 8.77 (d, 1H, J = 0.9 Hz). MS 395 (M^+).

*(±)-(4aR,5S,7aR)-2-carbamoyl-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-6-methyloctahydro-2H-cyclopenta[c]pyridine-6-yl carbamate (**16**)*

To the solution of compound **15** (10 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) were added Et_3N (5 μL, 0.038 mmol) and triethylsilylisocyanate. The reaction mixture was stirred for 1 h at rt and diluted with CH_2Cl_2. The organic layer was washed twice with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:-MeOH = 15:1) to give an off white solid (10 mg, yield 90 %) .

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 3H), 1.94-2.02 (m, 2H), 2.21 (m, 1H), 2.27 (m, 1H), 2.42 (m, 1H), 2.98-3.12 (m, 2H), 3.32 (m, 1H), 3.44 (m, 1H), 3.78 (m, 1H), 4.35 (s, 1H), 4.51–4.53 (m, 2H), 6.58 (m, 1H), 6.80 (m, 1H), 7.10 (m, 1H), 7.28 (m, 1H), 7.33–7.37 (m, 2H), 7.43 (m, 1H), 7.84 (m, 1H), 8.77(d, J = 1.1 Hz, 1H). MS 438 (M^+).

*(±)-(4aR,5S,7aR)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-2-formyl-6-methyloctahydro-2H-cyclopenta[c]pyridine-6-yl carbamate (**17**)*

$(CH_3CO)_2O$ (3.6 μL, 0.038 mmol) and HCOOH (1.5 μL, 0.038 mmol) were stirred for 2 h at 50°C and added to the solution of compound **16** (10 mg, 0.025 mmol) in THF (1 mL). After stirring for 20 min at room temperature, the mixture was diluted with CH_2Cl_2 and washed twice brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 12:1) to give a white solid (10 mg, yield 90 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 3H), 2.00-2.09 (m, 2H), 2.27 (t, J = 1.4 Hz, 1H), 2.45–2.58 (m, 2H), 3.04–3.25 (m, 2H), 3.31–3.52 (m, 3H), 4.16 (m, 1H), 4.53–4.57 (m, 2H), 6.57 (m, 1H), 6.79 (m, 1H), 7.10 (m, 1H), 7.28 (m, 1H), 7.32 (d, J = 11.9 Hz, 1H), 7.45 (m, 1H), 7.84 (m, 1H), 8.09 (s, 1H), 8.79(s, 1H). MS 423 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-6-allyl-5-{[(tert-butyldimethylsilyl)oxy]methyl}-6-hydroxyoctahydro-2H-cyclopenta[c]pyidine-2-carboxylate (**18**)*

To the solution of ketone **17** (200 mg, 0.521 mmol) in THF (6 mL) was added allyl magnesium bromide (1.04 mL, 1.0 M in Et_2O) with stirring at −78 °C. After stirring for 10 min, the reaction mixture was warmed to 0 °C and stirred for 2 h at 0 °C. Saturated aqueous NH_4Cl solution (6 mL) was added to the mixture to stop the reaction. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to give a pale yellow oil (180 mg, yield 81 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.87 (s, 9H), 1.47 (s, 9H), 1.67–1.75 (m, 2H), 2.24–2.31 (m, 2H), 2.42–2.58 (m, 2H), 3.10–3.28 (m, 2H), 3.40–3.51 (m, 2H), 3.55–3.77 (m, 2H), 3.93 (m, 2H), 5.07 (d, 1H, J = 4.5 Hz), 5.85–5.94 (m, 2H). MS 425 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-5-{[(tert-butyldimethylsilyl)oxy]methyl}-6-hydroxy-9-(3-hydroxylpropyl)octahydro-2H-cyclopenta[c]pyidine-2-carboxylate (**19**)*

To the solution of compound **18** (180 mg, 0.423 mmol) in THF (5 mL) was slowly added borane-methylsulfide complex (0,63 mL, 2.0 M in THF, 1.27 mmol) with stirring at 0 °C. After stirring for 10 min, the reaction mixture was warmed to rt and was stirred for 2 h. EtOH (1 mL) was added to the reaction. To the mixture were added 3 N NaOH (1 mL) and 30 % H_2O_2 (1 mL), which was then heated at reflux with stirring for 2 h. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1) to give a pale yellow oil (85 mg, yield 45 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.90 (s, 9H), 1.47 (s, 9H), 1.50–1.70 (m, 4H), 1.74–1.76 (m, 3H), 2.03–2.12 (m, 4H), 3.03–3.08 (m, 1H), 3.22 (s, 2H), 3.39–3.42 (m, 1H), 3.62–3.76 (m, 6H). MS 443 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-5-{[(tert-butyldimethylsilyl)oxy]methyl}-5′-oxooctahydro-3′H-spiro(cyclopenta [c]pyidine-6,2′-furan)-2(1H)-carboxylate (**20**)*

To the solution of compound **19** (85 mg, 0.191 mmol) in CH_2Cl_2 (2 mL) was added pyridium chlorochromate (124 mg, 0.575 mmol) with stirring at rt, The resulting dark brown suspension was stirred for 6 h at rt. A Celite 545 (100 mg) was added to the mixture, which was stirred for 30 min. The reaction mixture was filtered through a Celite 545 pad using CH_2Cl_2 (30 mL). The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1) to give a pale yellow oil (62 mg, yield 74 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.88 (s, 9H), 1.46 (s, 9H), 1.63–1.68 (m, 2H), 1.76–1.83 (m, 2H), 1.89–2.03 (m, 2H), 2.11–2.28 (m, 2H), 2.51–2.59 (m, 3H), 3.14 (m, 1H), 3.33 (m, 2H), 3.66–3.75 (m, 4H). MS 439 (M^+).

*tert-Butyl(±)-(4aR,5R,7aR)-5-(hydroxymethyl)-5′-oxooctahydro-3′H-spiro(cyclopenta [c]pyidine-6,2′-carboxylate (**21**)*

Using the compound **20** (62 mg, 0.141 mmol) as a starting material, the same reaction to prepare compound **11** was proceeded to give a pale yellow oil (36 mg, yield 78 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 1.60 (m, 1H), 1.83 (m, 1H), 1.97–2.05 (m, 4H), 2.29 (m, 1H), 2.38–2.45 (m, 1H), 2.55–2.64 (m, 2H), 3.15(m, 1H), 3.23 (m, 1H), 3.41 (m, 1H), 3.56 (m, 1H), 3.79 (m, 1H), 4.25–4.31(m, 2H). MS 325 (M^+).

*tert-Butyl(±)-(4aR,5S,7aR)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-5′-oxooctahydro-3′H-spiro(cyclopenta [c]pyidine-6,2′-furan)-2(1H)-carboxylate (**22**)*

Using the compound **21** (35 mg, 0.101 mmol) as a starting material, the same reactions to prepare compound **12** and **13** were proceeded sequentially to give a pale yellow oil (17 mg, yield 35 % over 2 steps).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.47(s, 9H), 1.59–1.62(m, 1H), 1.82–1.83(m, 1H), 1.99–2.07(m, 4H), 2.17–2.25(m, 1H), 2.31–2.48(m, 1H), 2.50–2.54(m, 2H), 2.93–2.99(m, 1H), 3.11–3.19(t, $J = 8.2$ Hz, 1H), 3.31–3.33(m, 1H), 3.42–3.60(m, 1H), 3.73–3.81(m, 1H), 4.15(m, 1H), 6.65–6.74(m, 2H), 7.10(t, $J = 1.5$ Hz, 1H), 7.35–7.37(m, 2H), 7.44–7.46(m, 1H), 7.83(d, $J = 4.5$ Hz, 1H), 8.77(s, 1H). MS 492 (M^+).

*(±)-(4aR,5S,7aR)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}decahydro-5′H- spiro(cyclopenta [c]pyidine-6,2′-furan)-5′-one (**23**)*

Using the compound **22** (16 mg, 0.032 mmol) as a starting material, the same reaction to prepare compound **15** was proceeded to give a pale yellow oil (10 mg, yield 78 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.72 (s, 4H), 2.01–2.07 (m, 2H), 2.19–2.22 (m, 2H), 2.45–2.53 (m, 2H), 2.75 (m, 2H), 2.88 (m, 1H), 2.94 (m, 1H), 3.16 (m, 1H), 6.62(m, 1H), 6.71 (m, 1H), 7.09 (m, 1H), 7.29 ~ 7.37 (m, 2H), 7.43 (m, 1H), 7.82 (m, 1H), 8.77 (d, 1H, $J = 1.1$ Hz). MS 392 (M^+).

*(±)-(4aR,5S,7aR)-5-(E)-2-{5-(3-fluorophenyl)pyridine-2-yl]vinyl}-5′-oxooctahydro-3′H-spiro(cyclopenta [c]pyidine-6,2′-furan)-2(1H)-carbaldhyde (**24**)*

Using the compound **22** (10 mg, 0.025 mmol) as a starting material, the same reaction to prepare compound **17** was proceeded to give a pale yellow solid (9 mg, yield 84 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.97–2.11 (m, 4H), 2.23–2.38 (m, 2H), 2.43–2.52 (m, 2H), 2.96–3.11 (m, 2H), 3.20(m, 1H), 3.34 (m, 1H), 3.54 (m, 1H), 3.80 (m, 1H), 4.08 (m, 1H), 6.2 (m, 1H), 6.75 (m, 1H), 7.06 (m, 1H), 7.35 (m2H), 7.47(m, 1H), 7.83 (m, 1H), 8.08 (s, 1H), 8.77 (s, 1H). MS 420 (M^+).

*3-(Prop-2-yn-1-yloxy)propan-1-ol (**25**)*

To the solution of propargyl bromide (20 g, 168.12 mmol) and 1,3-propandiol (25.5 g, 336.24 mmol) was added the pulverized NaOH (8 g, 201.74 mmol) with stirring at 0°C. After stirring for 24 h at rt, the mixture was diluted with water, and extracted with $CHCl_3$. The organic layer was washed twice with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 3:1) to give an of white oil (14 g, yield 73 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.82 (m, 2H), 2.47 (s, 1H), 3.69 (t, 2H, $J = 5.7$ Hz), 3.74 (t, 2H, $J = 5.4$ Hz), 4.16 (s, 2H). MS 114 (M^+).

*3-(Prop-2-yn-1-yloxy)propanal (**26**)*

To the solution of compound **25** (14 g, 122.65 mmol) in anhydrous CH_2Cl_2 (400 mL) were added molecular sieve, sodium acetate (50.3 g, 613.25 mmol) and pyridinium chlorochromate (65.8 g, 306.66 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C, and filtered through a Celite pad, and extracted with ether. The filtrate was concentrated and purified by silica gel column chromatography (hexane:ethyl acetate = 3:1) to afford an off white oil (6 g, yield 44 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.46 (s, 1H), 2.68 (t, 2H, $J = 6.06$ Hz), 3.88 (t, 2H, $J = 6.06$ Hz), 4.16 (s, 2H), 9.79 (s, 1H). MS 112 (M^+).

*Ethyl (E)-5-(Prop-2-yn-1-yloxy)pent-2-enoatel (**27**)*

Using the compound **26** (90 mg, 0.80 mmol) as a starting material, the same reaction to prepare compound **4** was proceeded to give a pale yellow oil (80 mg, yield 59 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.48 (s, 1H), 2.51(q, 2H, $J = 6.39$ Hz, $J = 13.0$ Hz), 3.65 (t, 2H, $J = 6.39$ Hz), 3.73 (s, 3H), 4.15 (s, 2H), 5.94–5.88 (m, 1H), 6.99–6.94 (m, 1H). MS 182 (M^+).

*(E)-5-(Prop-2-yn-1-yloxy)pent-2-en-1-ol (**28**)*

Using the compound **27** (150 mg, 0.89 mmol) as a starting material, the same reaction to prepare compound **5** was proceeded to give a pale yellow oil (100 mg, yield quantitative).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.37 (q, 2H, $J = 6.54$, 12.7 Hz), 2.44 (t, 2H, $J = 4.03$ Hz), 3.58 (t, 2H, $J = 6.63$ Hz), 4.14 (s, 2H), 5.74–5.70 (m, 2H). MS 140 (M^+).

*(E)-tert-Butyldimethyl{[5-(Prop-2-yn-1-yloxy)pent-2-en-1-yl]oxy}silane (**29**)*

Using the compound **28** (150 mg, 0.89 mmol) as a starting material, the same reaction to prepare compound **6** was proceeded to give a pale yellow oil (42 mg, yield 45 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.84 (s, 9H), 2.29–2.34 (m, 2H), 2.35 (s, 1H), 3.49 (t, 2H,

J = 6.87 Hz), 4.06–4.06 (m, 4H), 5.56–5.58 (m, 2H). MS 254 (M^+).

*(±)-(4aR,5S)-5-(tert-butyldimethylsilyloxymethyl)-3,4,4a,5-tetrahydrocyclopenta[c]pyran-6(1H)-one (**30**)*

Using the compound **29** (500 mg, 1.96 mmol) as a starting material, the same reaction to prepare compound **8** was proceeded to give a pale yellow oil (250 mg, yield 45 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.08 (s, 9H), 1.58 ~ 1.64 (m, 1H), 2.09–2.18 (m, 2H), 2.83–2.88 (m, 1H), 3.56–3.64 (m, 1H), 3.80–3.89 (m, 2H), 4.02–4.13 (m, 1H), 4.15 (d, 1H, J = 13.4 Hz), 4.65 (d, 1H, J = 13.4 Hz), 5.88 (s, 1H). MS 282 (M^+).

*(±)-(4aR,5R,7aS)-5-{[(tert-butyldimethylsilyl)oxy]methyl}hexahydrocyclopenta[c]pyran-6(1H)-one (**31**)*

Using the compound **30** (250 mg, 0.88 mmol) as a starting material, the same reaction to prepare compound **9** was proceeded to give a pale yellow oil (110 mg, yield 44 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.85 (s, 9H), 1.57–1.63 (m, 1H), 1.82–1.90 (m, 1H), 2.14–2.25 (m, 3H), 2.45–2.61 (m, 2H), 3.30–3.36 (m, 1H), 3.60–3.88 (m, 4H), 3.90–3.95 (m, 1H). MS 284 (M^+).

*(±)(4aR,5S,7aR)-5-{(E)-2-[5-(3-Fluorophenyl)pyridin-2-yl]vinyl}-6-methyloctahydrocyclopenta[c]pyran-6-ol (**32**)*

Using the compound **31** as a starting material, the same reactions to prepare compound **10**, **11**, **12**, and **13** were sequentially proceeded to give a pale yellow oil (yield 18, 82, 59, and 14 %, each).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 3H), 1.89–2.21 (m, 3H), 2.45–2.50 (m, 3H), 3.14 (t, 1H, J = 15 Hz), 3.48–3.80 (m, 4H), 6.65–6.81 (m, 2H), 7.08–7.84 (m, 6H), 8.77 (s, 1H). MS 353 (M^+).

*(±)(4aR,5S,7aR)-5-{(E)-2-[5-(3-Fluorophenyl)pyridin-2-yl]vinyl}-6-methyloctahydrocyclopenta[c]pyran-6-yl carbamate (**33**)*

Using the compound **32** (3 mg, 0.0084 mmol) as a starting material, the same reaction to prepare compound **14** was proceeded to give a pale yellow oil (1.2 mg, yield 36 %).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.61 (s, 3H), 2.0 (brs, 2H), 2.38 (m, 3H), 2.77 (m, 1H), 3.20 (t, J = 5.0 Hz, 1H), 3.50 (t, J = 5.0 Hz, 1H), 3.73 (brs, 2H), 4.60 (brs, 2H), 6.61 (d, J = 17.6, 1H), 6.79 (m, 1H), 7.07 (t, J = 7.5 Hz, 1H), 7.23–7.47 (m, 4H), 7.83 (d, J = 2.5 Hz, 1H), 8.78 (s, 1H). MS 396 (M^+).

Evaluation of inhibitory effect on PAR 1 using radioligand binding assay

Preparation of platelet membrane

20 unit of platelet concentrate (Red Cross Blood Services, Daejeon) was centrifuged for 20 min at 100 g to remove red blood cells. The supernatant was collected and centrifuged for 15 min (3000 g). The resulting precipitate was mixed well with Buffer A (200 mL) (10 mM Tris Cl, pH 7.5, 5 mM EDTA, 150 mM NaCl) and centrifuged for 10 min at 4400 g. The resulting precipitate was mixed well with Buffer A (200 mL) and centrifuged again for 10 min at 4400 g. The resulting precipitate was mixed well with Buffer B (30 mL) (10 mM Tris Cl, pH 7.5, 5 mM EDTA). Then, the mixture was homogenized twenty times using a Dounce homogenizer and centrifuged for 20 min at 41,000×g. The resulting precipitate was mixed well with Buffer C (40 mL) (20 mM Tris Cl, pH 7.5, 1 mM EDTA, (0.1 mM DTT)). This mixture was divided in 5 mL portions, quickly cooled by liquid nitrogen and stored at −80 °C. The portions stored at −80 °C were dissolved, homogenized five times using a Dounce homogenizer and centrifuged for 20 min at 41,000×g. The resulting precipitate was mixed well with Buffer D (20 mL) (10 mM Tris ethanolamine HCl, pH 7.4, 5 mM EDTA) and centrifuged for 20 min at 41,000×g. Then, the resulting precipitate was mixed well with Buffer E (20 mL) (50 mM Tris Cl, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, (1 % DMSO)). This mixture was divided in 250 μL portions, quickly cooled by liquid nitrogen and stored at −80 °C. Blood within 48 h after the blood collection must be used and the blood should be kept at 4 °C after it is washed with Buffer A.

Evaluation of inhibitory effect on PAR1

First, the buffer for binding reaction (39 μL) was distributed into the reaction plate (Nunc 96 well plate # 269620). The human platelet membrane was diluted with the buffer for binding reaction (50 mM Tris HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.1 % BSA) to prepare 2× concentration (the final 1× concentration: 0.15 mg/mL), and 50 μL each was added to the reaction plate. The compound was diluted with DMSO to prepare 10× solution, and 10 μL each was added to the reaction plate and mixed by pipetting. To the positive control was added DMSO (10 μL) and to the nonselective binding control was added 10× unlabeled haTRAP (10 μL) (hexaamino acid thrombin receptor antagonistic peptide; final concentration 100 μM). The radio-labeled ligand ([^{3}H]-haTRAP, alanine-*p*-fluorophenylalanine-arginine-cyclohexylalanine-homoarginine-[^{3}H]tyrosine-NH_2) was diluted with DMSO to

prepare 100× concentration, and 1 µL each was added to the reaction plate and mixed by pipetting (the final concentration of the radio-labeled ligand was decided as the concentration showing the binding of 60 % Bmax in the saturation experiment). Then, reaction plate was treated in a plate stirrer (Heidolph, Titramax 1000) for 15 s at 900 rpm to mix and incubated for 60 min at 30 °C. During the incubation, the unifilter GF/C plate (Perkin Elmer, # 6005174) was wetted with 0.1 % polyethyleneimine (100 µL). After completion of the reaction, the unifilter GF/C plate was located in a Milipore Vacuum Manifold. The reaction mixture was transferred and combined using a pipette and washed six times with cooled washing buffer (100 µL) (50 mM Tris HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA). The unifilter GF/C plate was dried at rt. Microscint-20 scintillation cocktail solution (Perkin Elmer, # 6013621) (40 µL) was added to each well and its radioactivity was measured by Packard TOPCOUNT scintillation counter to calculate the IC_{50} value.

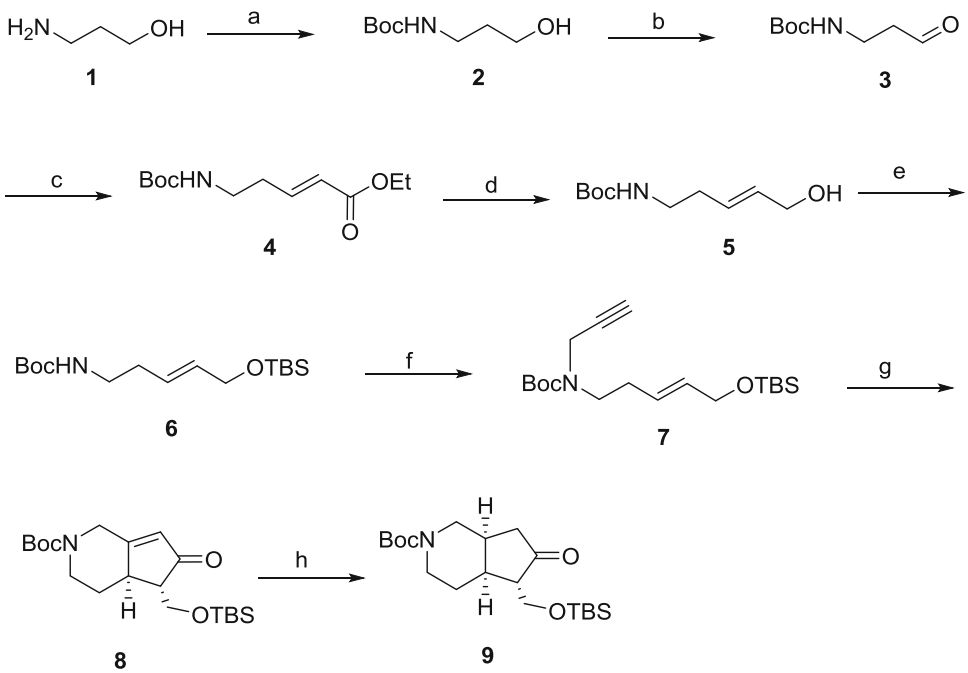
Results and discussion

Chemistry

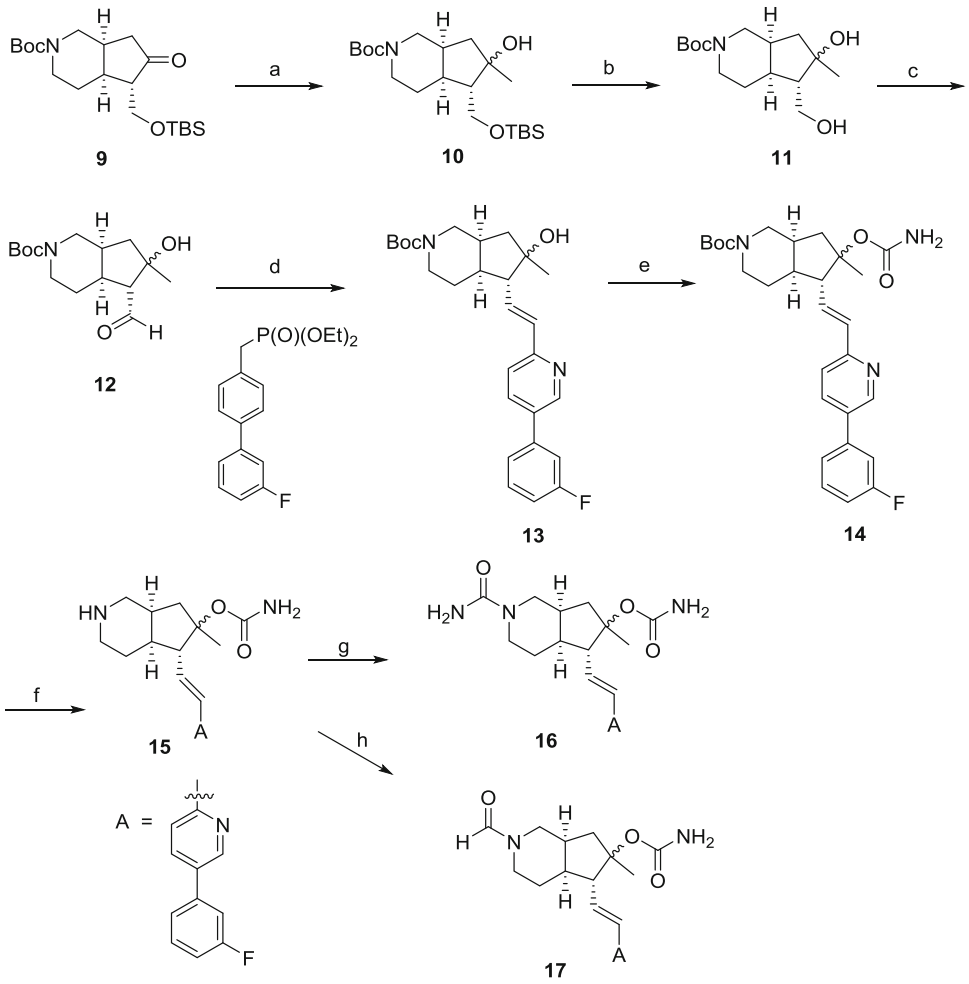
We have designed octahydrocyclopenta[*c*]pyridine and octahydrocyclopenta[*c*]pyran analogues by the insertion of heteroatom into C5 of octahydroindene ring, with the expectation on improvement of metabolic stability (Fig. 1).

Both cyclopentapyridine and cyclopentapyran rings were manipulated using an intramolecular Pauson–Khand reaction (PKR) (Jeong 1993; Kavanagh et al. 2009). The acyclic enyne PKR precursor **7** was prepared in straightforward method (Scheme 1). The primary amine **1** was protected with *t*-butylcarbamoyl group (Boc), and the primary alcohol group of **2** was oxidized to yield the aldehyde **3** by Swern reaction (Mancuso and Swern 1981), which was converted to the allylic ester **4** using Hornor-Wadsworth Emmons reaction (HWE) (Boutagy and Thomas 1974). The reduction of allylic ester to alcohol **5** was performed using diisobutylaluminium hydride (DIBAL-H) in CH_2Cl_2. Subsequent protection of primary alcohol moiety with *tert*-butyldimethylsilyl (TBS), and then alkylation of carbamate **6** using propargyl bromide gave PKR precursor **7**. The standard PKR conditions gave cyclopentenone **8**, and following a stereoselective hydrogenation from convex face afforded cis-fused bicyclic ring **9** (Kavanagh et al. 2009). Racemic mixtures were used for the next steps without separation. We decide to prepare the racemic mixture for biological evaluation until we found a compound with desirable activity and property.

Starting from **9**, we prepared 6-methy-6-hydroxy **13**, and 6-methyl-6-carbamate **14** compounds by published procedures (Lee et al. 2013), which were found to be active on PAR1 in our previous work (Scheme 2). *N*-Boc was further derivatized to *N*H **15**, *N*-carbamoyl **16** and *N*-formyl compound **17** in a straightforward method. *N*-Boc was deprotected using TFA to give secondary amine **15**, which was carbamoylated using $(CH_3)_3SiNCO$ (Mizuhara et al.



Scheme 1 Reagents and conditions **a** Boc_2O, Et_3N, CH_2Cl_2, rt, 94 %; **b** DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C; **c** $P(O)(OEt)_2CH_2COOEt$, NaH, −78 °C to rt, THF, 74 % (2 steps from **2** to **4**); **d** DIBAL-H, CH_2Cl_2, −78 °C, 83 %; **e** imidazole, TBDMSCl, THF, rt, 93 %; **f** NaH, 15-crown-5, propargyl bromide, −30 °C to rt, 60 %; **g** $Co_2(CO)_8$, NMMO, CH_2Cl_2, rt, 53 %; **h** $Pd(OH)_2$, EtOH, rt, 74 %



Scheme 2 Reagents and conditions **a** CH_3MgBr, THF, −78 °C to rt, 2 h, 42 %; **b** TBAF, THF, 0 °C to rt, 1 h, 92 %; **c** IBX, CH_2Cl_2, rt, 2 h; **d** *n*-BuLi, THF, −78 °C to rt, 1 h, 50 % (2 steps from **11** to **13**); **e** (i) CCl_3CONCO, THF, (ii) K_2CO_3, MeOH, rt, 91 %; **f** TFA, CH_2Cl_2, 0 °C to rt, 1 h, 90 %; **g** $(CH_3)_3SiNCO$, Et_3N, CH_2Cl_2, rt, 1 h, 90 %; **h** Ac_2O, HCOOH, THF, 50 °C to rt, 1 h, 90 %

2012). The formylation of **15** was performed using acetic anhydride and formic acid to give **17** (Scott et al. 1983).

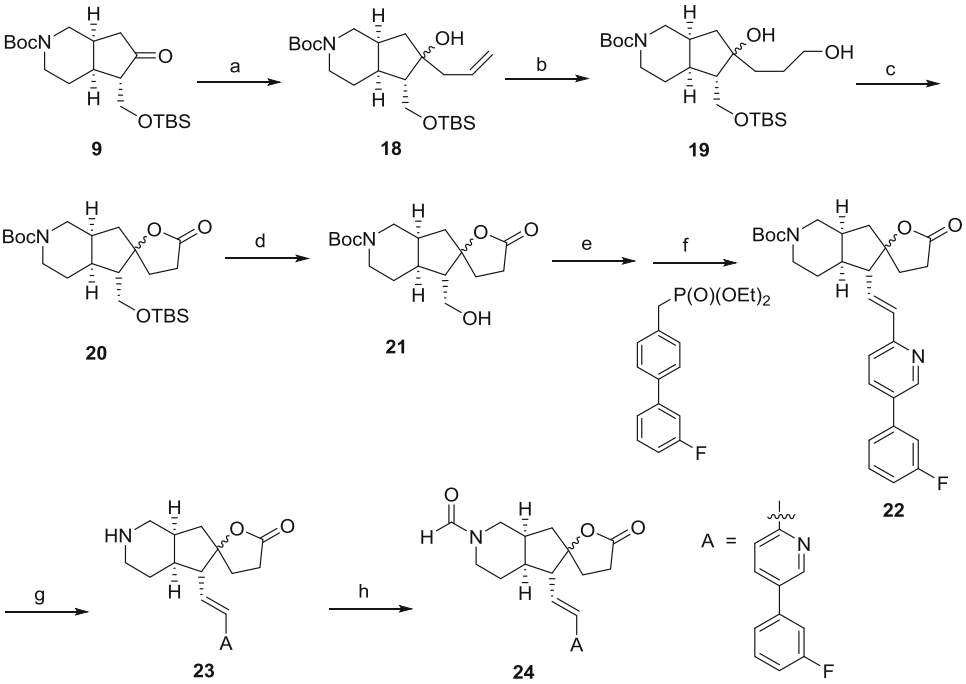
We additionally prepared spironolactone compound **22**–**24**. 2-Ketone **9** was allylated with Grignard regent to provide **18**, following hydroboration and oxidation using borane-methylsulfide (BMS) and H_2O_2 provided 6-hydroxy-6-propanol **19** outlined in Scheme 3 (Makino et al. 2002). Oxidation of **19** using pyridium chlorochromate (PCC) afforded spironolactone compound **20**. The TBS of **20** was deprotected, following oxidation of alcohol to aldehyde using 2-iodoxybenzoic acid (IBX), finally HWE reaction gave the compound **22**. *N*-Boc of **22** was further derivatized to NH (**23**) and *N*-formyl (**24**) moiety.

Outlined in Scheme 4, we prepared octahydrocyclopenta[*c*]pyran compounds according to the similar method to prepare octahydrocyclopenta[*c*]pyridines. The monoalkylation of 1,3-propanediol with propargyl bromide using NaOH gave **25**, of which alcohol was oxidized to aldehyde **26** using PCC. Hornor-Wadsworth Emmons reaction with **26**, following reduction of allylic ester **27** to alcohol **28** using DIBAL-H, and protection of primary alcohol functionality with TBS provided PKR precursor **29**. *Cis*-fused 6/5 bicyclic compound **31** was prepared by standard PKR, and subsequent hydrogenation from convex face (Kavanagh et al. 2009). 6-Methyl-6-hydroxy **32** and 6-methyl-6-carbamate **33** compounds of this octahydrocyclopenta[*c*]pyrans were prepared using previous methods.

Evaluation of biological activity

We evaluated PAR1 inhibitory activity of octahydrocyclopenta[*c*]pyridine and octahydrocyclopenta[*c*]pyran compounds using [^{3}H]-labeled high affinity thrombin receptor activation peptide ([^{3}H]-haTRAP) as a radioligand as described in our previous work (Ahn et al. 2000; Andrade-Gordon et al. 1999). Additionally, we determined metabolic stability of compounds in human and rat liver microsomes, which was represented as R_{50}, time when 50 % of the compound remains upon incubation with human and rat liver microsomes.

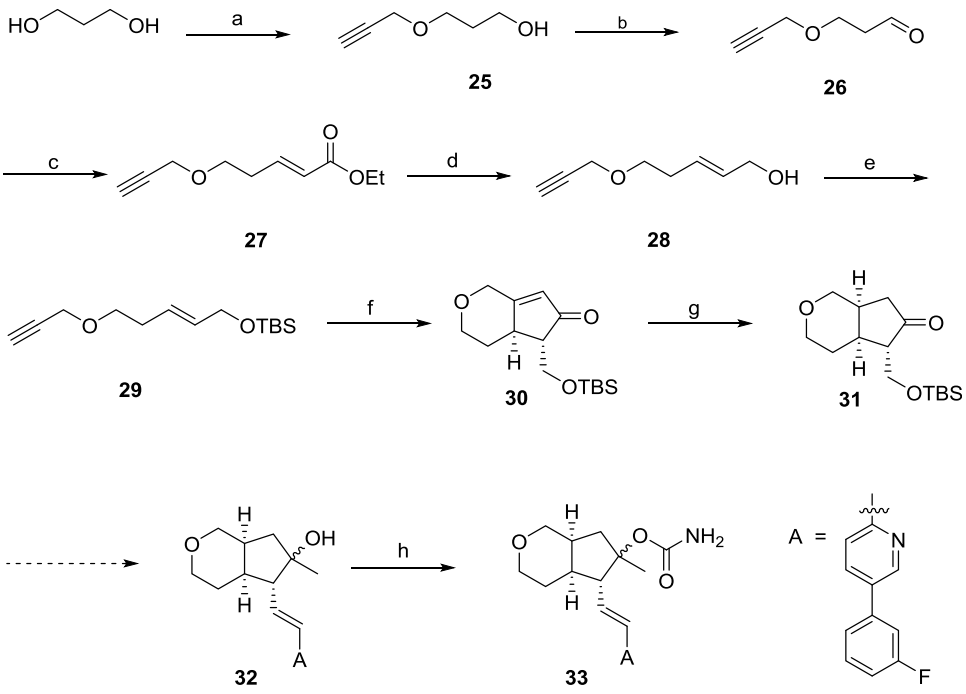
Even though their activities were decreased compared with those of octahydroindenes, both cyclopentapyridine



Scheme 3 Reagents and conditions: **a** allylmagnesium bromide, THF, −78 °C to rt, 2 h, 81 %; **b** (i) $BH_3{\cdot}SMe_2$, THF, (ii) 3 N NaOH, H_2O_2, 0 °C to rt, 45 %; **c** PCC, CH_2Cl_2, rt, 6 h, 74 %; **d** TBAF, THF, 0 °C to rt, 1 h, 78 %; **e** IBX, DMSO, rt, 1 h; **f** *n*-BuLi, THF, −78 °C to rt, 1 h, 35 % (2 step yields); **g** TFA, CH_2Cl_2, 0 °C to rt, 1 h, 78 %; **h** Ac_2O, HCOOH, THF, 50 °C to rt, 1 h, 84 %

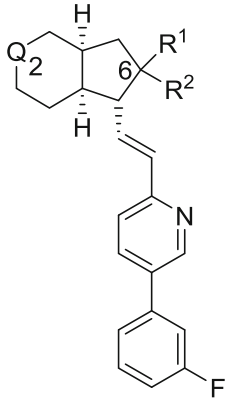
and cyclopentapyran derivatives still represented the significant inhibitory activity on PAR1 (Table 1).

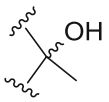
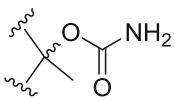
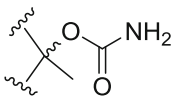
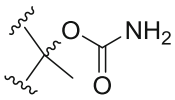
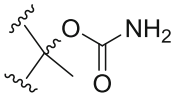
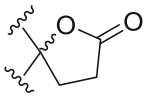
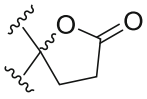
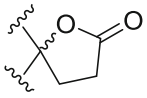
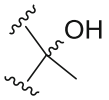
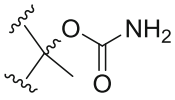
The 6-methyl-6-carbamate compound (**14**, $IC_{50} = 0.36$ μM) showed better activity than 6-methyl-6-hydroxy compound (**13**, $IC_{50} = 2.8$ μM) of *N*-Boc cyclopentapyridine, which is the same trend with octahydroindene's. Among 6-methyl-6-carbamate compounds of cyclopentapyridine (**14–17**), *N*-formyl compound (**17**, $IC_{50} = 0.065$ μM) showed the best activity, following *N*-Boc (**14,** $IC_{50} = 0.36$ μM), NH (**15**, $IC_{50} = 0.62$ μM), and *N*-carbamoyl (**16**, $IC_{50} = 1.2$ μM). As our expectation, the metabolic stability of cyclopentapyridines was generally much more improved than that of octahydroindenes. Especially, NH (**15**) and *N*-Boc (**16**) compounds showed an excellent metabolically stability both in human and rat liver microsomes. The most active compound **17** showed marginal metabolic stability ($R_{50} = 20.2$ min in human, 16.2 min in rat, each) but much better than



Scheme 4 Reagents and conditions **a** propargyl bromide, NaOH, 0 °C to rt, 24 h, 73 %; **b** PCC, CH_2Cl_2, 0 °C to rt, 44 %; **c** $PO(OEt)_2CH_2COOEt$, NaH, THF, 1 h, 59 %; **d** DIBAL-H, CH_2Cl_2, −78°, 1 h, 80 %; **e** TBSCl, imidazole, DMF, rt, 30 min, 45 %; **f** $Co_2(CO)_8$, NMO, 0 °C to rt, 2 h, 45 %; **g** H_2, $Pd(OH)_2$, EtOAc, rt, 60 psi, 44 %; **h** (i) CCl_3CONCO, THF, (ii) K_2CO_3, MeOH, rt, 36 %

Table 1 Inhibitory activity on PAR1 and metabolic stability data for compounds



Compound	C2 (Q)	C6 (R^1, R^2)	IC_{50} (μM)[a]	Metabolic stability (R_{50}, min)[c]	
				Human	Rat
Vorapaxar[b]			0.015	83.2	32.4
13	NBoc		2.8		
14	NBoc		0.36	57.5	82.0
15	NH		0.62	122.0	138.6
16	$NC(O)NH_2$		1.2	79.6	207.6
17	NCOH		0.065	20.2	16.2
22	NBoc		0.11	69.4	26.0
23	NH		2.5	38.1	18.2
24	NCOH		0.61	17.2	11.2
32	O		1.0	36.1	19.6
33	O		0.05	26.0	37.0

[a] PAR1 binding assay ligand [^{3}H]haTRAP, 10 nM; The value is an average of three measurements

[b] Vorapaxar was synthesized and evaluated by our laboratory as a reference standard

[c] Time when 50 % of the compound remains upon incubation with human and rat liver microsomes

octahydroinden's. 6-Spironolactone compounds (**22**–**24**) were also active on PAR1 but a little weaker than 6-methyl-6-carbamates in general. In 6-spironolactone series, *N*-Boc compound (**22**, $IC_{50} = 0.11$ μM) showed the best activity, which is more potent than 6-methyl-6-carbamate with *N*-Boc (**14**, $IC_{50} = 0.36$ μM). The compound **22** showed reasonable metabolic stability ($R_{50} = 69.4$ min in human, 36.0 min in rat, each), especially good in human liver microsome.

Cyclopetapyran compound with 6-Methyl-6-carbamare **33** showed a good activity ($IC_{50} = 50$ nM) with moderate metabolic stability ($R_{50} = 26.0$ min in human, 37.0 min in rat, each).

Conclusions

From this studies, we found that the modification of octahydroindene core to octahydrocyclopenta[*c*]pyridine and octahydrocyclopenta[*c*]pyran generally improved metabolic stability, while maintaining the significant activity on PAR1. Compound **22** ($IC_{50} = 110$ nM) and **33** ($IC_{50} = 50$ nM) showed good activity on PAR1 with moderate metabolic stability. We will keep trying to optimize this series of compounds to improve both the activity and druggable property.

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