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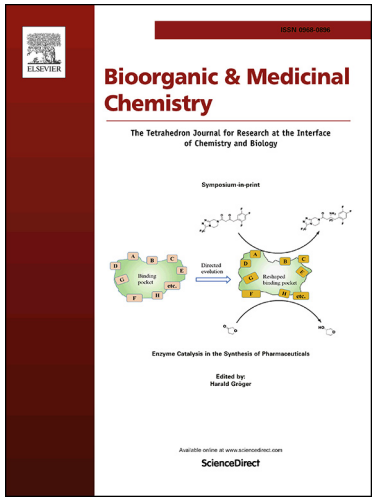

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Graphical Abstract

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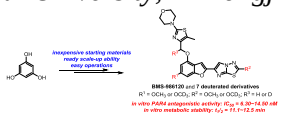

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ABSTRACT

BMS-986120 is a PAR4 antagonist that is being investigated as an antiplatelet agent in phase I clinical trial. An improved synthesis of **BMS-986120** has been developed. Based on the novel synthetic approach to **BMS-986120**, a series of deuterated derivatives of **BMS-986120** have been synthesized and biologically evaluated to search for more potent antiplatelet agents. The in vitro antiplatelet assay by turbidimetry demonstrated that **PC-2** and **PC-6** had IC_{50} values of 6.30 nM and 6.97 nM, respectively, versus **BMS-986120** with an IC_{50} of 7.80 nM. The result of in vitro metabolic stability study showed that all of the deuterated compounds had similar half-life ($T_{1/2}$) and intrinsic clearance (Clint) in comparison with **BMS-986120**. Further probing the metabolic profile of **BMS-986120** is worth being conducted.

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1. Introduction

Stroke and myocardial infarction are representative thromboembolic events that have significant morbidity and mortality all over the world.[1-6] Platelet plays a key role in the formation of thrombus. At the location of vascular damage, diverse agonists, such as ADP, collagen, epinephrine and thrombin can be released. These endogenous substances can activate platelet rapidly through the corresponding membrane receptors. Then, the secondary agonists such as ADP and thromboxane A2 released by the activated platelet can reinforce the platelet activation again. Finally, platelet activation and aggregation occur spontaneously through the common central pathway of the binding of GPIIb/IIIa to fibrinogen resulting in thrombi.[7,8] Thus, antiplatelet therapy prevailed in the clinical treatment and prevention of atherothrombotic events due to the role of platelet in the pathological mechanism of thrombus occurrence and progress.[9,10]

The drug combination of asprin and clopidogrel is widely used as the standard of care in clinical practice.[11] Nevertheless, "aspirin resistance" occurs in the patients with the probability of 5% - 60%, resulting in the increasing recurrence of thrombotic events in 5 years.[12] On the other hand, "clopidogrel resistance" happens in the CYP2C19 poor metabolizers who also experience high risk of failure in this fundamental "dual treatment".[13] Furthermore, this drug combination would increase the hazard of hemorrhage in the elderly patients, especially with cerebrovascular disease.[14] Therefore, agents with strong antithrombotic activity and low bleeding risk is urgently needed. Thrombin, a serine protease, is the terminal enzyme of the coagulation cascade through stimulation of the protease activated receptors (PARs) of platelets.[15] As members of the PAR family, PAR1 and PAR4 are both G protein-coupled receptors inserted in the cytomembrane of human platelets with a unique self-activation mechanism following cleavage by thrombin.[16] Unlike PAR1 that has the hirudin like thrombin binding domain, PAR4 is a low-affinity thrombin receptor that can only apperceive higher concentrations of thrombin while much lower concentrations of thrombin can be recognized by PAR1.[17] With different kinetics of signal transduction of aggregation in human platelets, PAR1 and PAR4 are believed to be involved in different phases of platelet activation. The activation of PAR1 can transmit the signal of hemostasis in the early stage of bleeding and the activation of PAR4 contributes to stabilize the formation of thrombus. This intriguing and valuable mechanism would make PAR4 to be a promising antiplatelet target with potent antithrombotic activity with low risk of bleeding.[18]

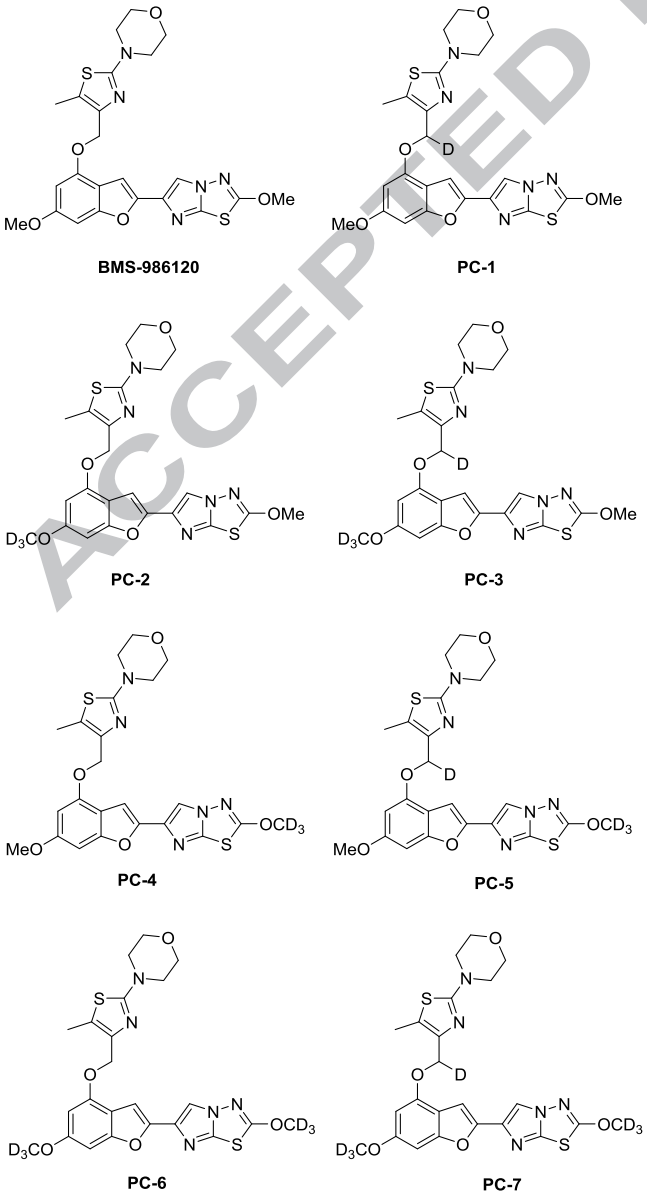


Figure 1. Structures of BMS-986120 and its deuterated derivatives.

As the first-in-class PAR1 antagonist, **Vorapaxar** has undergone two phase III clinical trials. However, the intracranial bleeding risk hinders it's clinical usage in patients with a prior history of stroke or transient ischemic attack.[17] On the other hand, **BMS-986120** (**Figure 1**), an orally active, potent and selective PAR-4 antagonist has been proven to be safe and well tolerated by healthy subjects in phase I clinical trials.[19] As a part of our program aiming at the development of novel antiplatelet agents,[13] we wished to develop new type of PAR4 antagonists. When we tried to obtain **BMS-986120** as a positive control, we found that it was extremely difficult to acquire this compound in satisfactory yields following the literature method.[18] Here we report a practical synthesis of **BMS-986120**. Moreover, our assay result of the metabolic stability of **BMS-986120** in human liver microsomes showed that the half-life ($T_{1/2}$) of this compound was only about 11.1 min. In fact, the pharmacokinetic studies in a clinical phase I trial (NCT02439190) demonstrated that the half-life of **BMS-986120** was only 4 hours.[20] To overcome the metabolic instability of **BMS-986120**, we employed the deuteration technology[21-23] to design and synthesize a series of deuterated derivatives of **BMS-986120** (**Figure 1**). Biological evaluation of these deuterated derivatives has also been conducted.

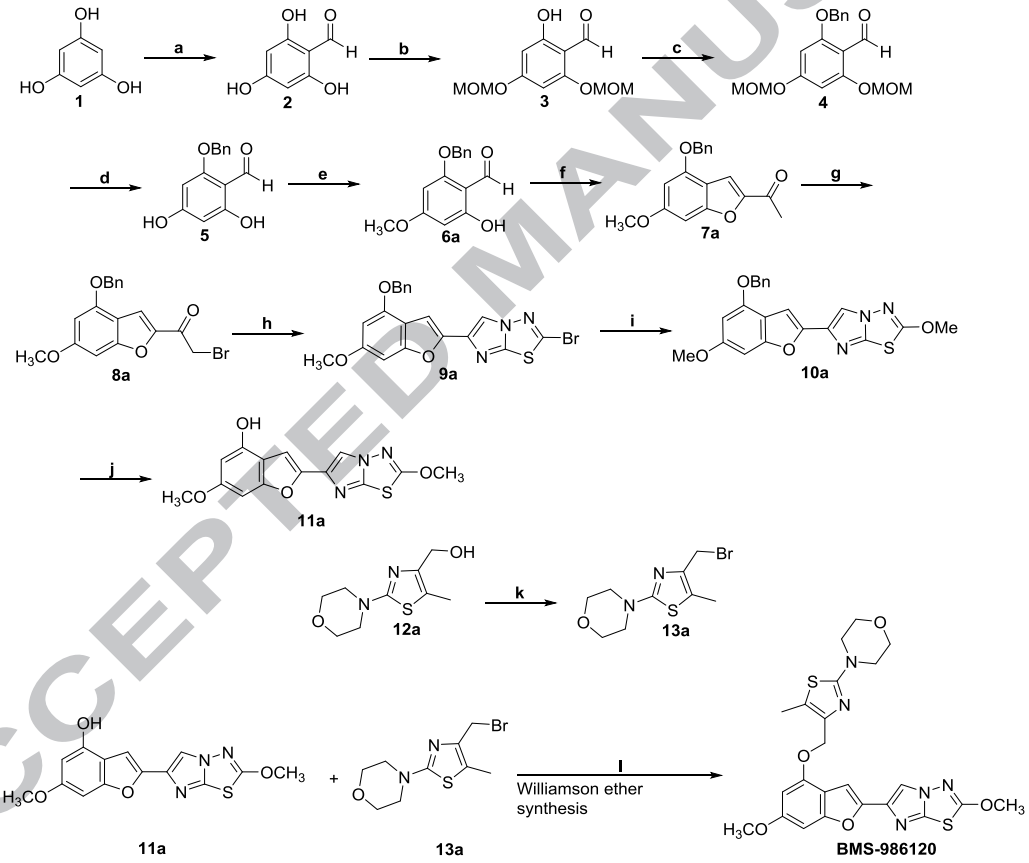
2. Result and Discussion

2.1. Chemistry

We found that it was not practical to synthesize **BMS-986120** following the literature methods.[18,24-26] Therefore, we have developed a new approach to preparation of **BMS-986120**. As shown in **Scheme 1**, formylation of phloroglucinol **1** via Vilsmeier-Haack reaction provided aldehyde **2** in 80% yield. Treatment of **2** with MOMCl/Et_3N in CH_3CN afforded the desired dually MOM-protected product **3** in 60% yield with high regioselectivity. Then benzylation of **3** gave compound **4** in 92% yield. Under reflux condition, the protective group MOM- of **4** could be easily removed by 2 N HCl (aq) to give dihydroxyl aldehyde **5**. Regioselective methylation of **5** was carried out via Mitsunobu reaction to give aldehyde **6a**. Reaction of **6a** with 1-chlorone acetone in acetone at reflux provided 2-acetyl benzofuran **7a**. Treatment of **7a** with $CuBr_2$ in ethyl acetate under refluxing condition provided bromide **8a** in 50% yield. Using this protocol for bromination reaction, low temperature and sophisticated multistep operations employed by the literature methods were avoided to obtain the desired product in reasonable yields. Reaction of **8a** with 5-bromo-1,3,4-thiadiazol-2-amine in isopropanol with heating furnished imidazothiadiazole **9a** that

could be used for further reactions without any purification. Treatment of **9a** with sodium methoxide under mild conditions delivered imidazothiadiazole **10a** smoothly. Debenzylation of **10a** with BCl_3 afforded the key intermediate **11a** in quantitative yield. Alcohol **12a** could be synthesized according to the procedure reported in the patent *WO 2013/163279 A1*. Initially, we tried to obtain **BMS-986120** through Mitsunobu reaction between **11a** and **12a** according to the literature method.[18] However, we found that this Mitsunobu reaction was extremely difficult probably due to the insolubility of **11a** in THF. We then tried to synthesize **BMS-986120** through Williamson ether synthesis. In this regard, bromination of alcohol **12a** with PBr_3 afforded bromide **13a** in 45% yield. A variety of bases (e.g. K_2CO_3, NaH, NaOH, *t*-BuONa) were used for this ether synthesis with varying yields (6-49%), indicating that a strong base was necessary for this reaction to occur. Finally, reaction of **11a** with **13a** in the presence of *t*-BuONa in DMF afforded **BMS-986120** in 49% yield.

Having established the optimized procedures, an array of deuterated derivatives of **BMS-986120** were synthesized to examine their in vitro antiplatelet aggregation activity and metabolic stability profile. As shown in **Scheme 2**, Mitsunobu reaction of **5** with deuterated methanol gave **6b**. Deuterated imidazothiadiazole **9b** could be obtained from **6b** following the procedure as described above (**scheme 1**). Reaction of **9b** with sodium methoxide/methanol or deuterated sodium methoxide/deuterated methanol under ambient temperature afforded **10b** or **10d**, respectively. In the similar pattern, reaction of **9a** with deuterated sodium methoxide/deuterated methanol gave **10c**. Debenzylation of **10b-d** with BCl_3 provided **11b-d** in 90-99% yields. Oxidation of alcohol **12a** by the Dess-Martin periodinane afforded aldehyde **14** in 65% yield. Reduction of **14** with sodium borodeuteride in deuterated methanol gave singly α-deuterated alcohol **12b** in 74% yield. Bromination of **12b** with PBr_3 afforded bromide **13b** in 44% yield. Finally, applying Williamson ether synthesis, reaction of **11a-d** with **13a-b** in the presence of *t*-BuONa in DMF provided all of the deuterated derivatives **PC-1** - **PC-7**, respectively.



Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF, rt, 80%; (b) MOMCl, Et_3N, CH_3CN, 0 °C, 60%; (c) BnBr, K_2CO_3, DMF, rt, 92%; (d) 2 N HCl (aq), MeOH, reflux, 60%; (e) PPh_3, DIAD, MeOH, THF, 0 °C - rt, 60%; (f) 1-chlorone acetone, K_2CO_3, acetone, reflux, 86%; (g) CuBr2, EtOAc, reflux, 50%; (h) 5-bromo-1,3,4-thiadiazol-2-amine, isopropanol, sealed tube, 80 °C - 130 °C; (i) MeONa in MeOH (4 M), $MeOH/CH_2Cl_2$ (v/v=3:1), rt, 32% (two steps); (j) BCl_3, pentamethylbenzene, dry CH_2Cl_2, -78 °C, 99%; (k) PBr_3, dry CH_2Cl_2, 0 °C - rt, 45%; (l) *t*-BuONa, dry DMF, rt, 49%.

2.2. Biological activity assessment

Antiplatelet aggregation assays

Platelet aggregation was measured with a PRECIL LBY-NJ4 4-channel aggregometer as previously described.[27] Briefly, 270 μL platelets were pipetted into aggregometer cuvettes and were incubated with 20 μL compounds for 5 min at 37 °C. Saline served as the vehicle. Platelets aggregation was stimulated with 10 μL PAR4-AP (AYPGKF-NH2) in the presence of magnetic beads, and the maximum platelet aggregation rate was determined by a lasting measurement of light transmittance for 5 min. For each compound tested, at least five concentrations were chosen and the curve of inhibition rate and concentration was derived, then the IC_{50} value of this compound was obtained by using Prism 6.0 (GraphPad, San Diego, CA). The results of antiplatelet aggregation assays are summarized in **Table 1**. Notably, single deuteration at the C-H bond connected to the phenyl oxygen led to a significant decrease in potency (**PC-1**, IC_{50} = 14.50 nM). Deuteration at 6- OCH_3 on the benzofuran ring of **BMS-986120** resulted in improved potency (**PC-2**, IC_{50} = 6.30 nM) in comparison to the parent compound (IC_{50} = 7.80 nM). **PC-3** with deuteration at both 6-OCH_3 and the C-H bond connected to the phenyl oxygen had a moderate potency (IC_{50} = 8.15 nM). Deuteration at 2'-OCH_3 on the imidazothiadiazole ring afforded **PC-4** with slightly decreased potency (IC_{50} = 8.74 nM). **PC-5** with deuteration at the imidazothiadiazole ring and the C-H bond connected to the phenyl oxygen was equally potent (IC_{50} = 7.69 nM) with **BMS-986120** (IC_{50} = 7.80 nM). Like **PC-2**, **PC-6** possessing both 6-OCD_3 and 2'-OCD_3 was more potent (IC_{50} = 6.97 nM) than **BMS-986120**, indicating that deuteration at 6-OCH_3 might be favourable for PAR4 binding and structural modifications at this position might be considered to improve potency. Further deuteration of **PC-6** at the C-H bond connected to the phenyl oxygen led to decreased potency (**PC-7**, IC_{50} = 9.45 nM), further implying that structural modifications at this position should be careful.

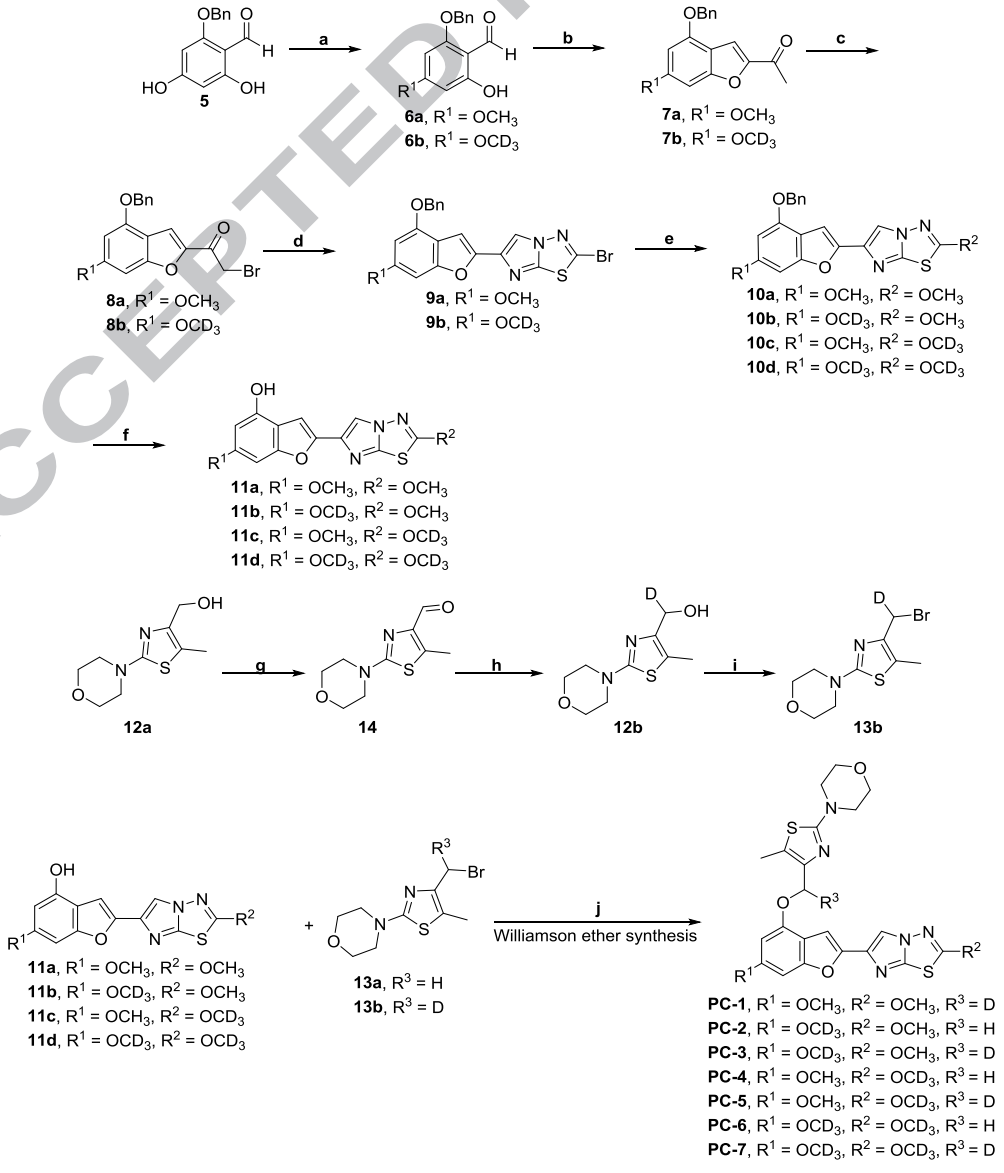
Table 1. In vitro antiplatelet activity of the deuterated derivatives of BMS-986120.

Compound No.	IC_{50} (nM)[a]
BMS986120	7.80±0.16
PC-1	14.50±1.18
PC-2	6.30±0.20
PC-3	8.15±0.36
PC-4	8.74±0.07
PC-5	7.69±0.47
PC-6	6.97±0.24
PC-7	9.45±0.09

[a]In vitro inhibition of PAR4-AP (75 μmol)-induced mice platelet aggregation; values are means from at least three independent dose-response curves.

Metabolic stability in human liver microsomes

In vitro metabolic study using liver microsomes is the most common approach to early estimation and prediction of in vivo metabolic stability of a compound.[28] To evaluate the metabolic stability of the deuterated derivatives of **BMS-986120**, human liver microsomes were employed. Half-life ($T_{1/2}$) and intrinsic clearance (Clint) of the test compounds are summarized in **Table 2**. The results show that there is no significant improvement of in vitro metabolic stability for all the deuterated compounds ($T_{1/2}$ = 11.2 ~ 12.5 min) in comparison with **BMS-**



Scheme 2. (a) PPh_3, DIAD, MeOH (for **6a**) or CD_3OD (for **6b**), THF, 0 °C - rt, 50% - 60%; (b) 1-chlorone acetone, K_2CO_3, acetone, reflux, 80% - 86%; (c) $CuBr_2$, EtOAc, reflux, 43% - 55%; (d) 5-bromo-1,3,4-thiadiazol-2-amine, isopropanol, sealed tube, 80 °C - 130 °C; (e) MeONa in MeOH (4 M), MeOH/CH_2Cl_2 (v/v=3:1), rt, 32% (two steps) for **10a**; or CD_3ONa in CD_3OD (4 M), CD_3OD/CH_2Cl_2 (v/v=3:1), rt, 32% - 40% (two steps) for **10b-d**; (f) BCl_3, pentamethylbenzene, dry CH_2Cl_2, -78 °C, 90% - 99%; (g) Dess-Martin periodinane, dry CH_2Cl_2, rt, 65%; (h) $NaBD_4$, CD_3OD, 0 °C - rt, 74%; (i) PBr_3, dry CH_2Cl_2, 0 °C - rt,, 44%; (j) *t*-BuONa, dry DMF, rt, 22% - 45%.

986120 ($T_{1/2}$ = 11.1 min). This indicates that deuteration at the three sites (6-OCH_3, 2'- OCH_3 and the C-H bond connected to the phenyl oxygen) might not be suitable for improving metabolic stability.

Table 2. The metabolic stability of the deuterated derivatives of BMS-986120 in human liver microsomes.

Compound No.	$T_{1/2}$ (min)[a,c]	Clint (μL/min/mg protein)[b]
BMS-986120	11.1±0.33	125±3.74
PC-1	11.2±0.22	124±2.42
PC-2	11.6±0.18	120±1.81
PC-3	11.3±0.18	123±1.91
PC-4	11.8±0.61	118±6.02
PC-5	11.2±0.14	124±1.50
PC-6	11.4±0.18	121±1.92
PC-7	12.5±0.27	111±2.42

[a]$T_{1/2}$ = ln2/k = 0.693/k. [b]Clint (intrinsic clearance) (μL/min/mg protein) = Ln(2)*1000/$T_{1/2}$/Protein Concentration. [c]The absolute value k of the slope is measured by the natural logarithm of the percentage of the remaining quantity of the test compound as a function against time.

3. Conclusions

In summary, a practical synthesis of PAR4 antagonist **BMS-986120** has been developed, featuring by inexpensive starting materials, ready scale-up ability, and easy operations. Based on this novel synthetic approach, a series of deuterated derivatives of **BMS-986120** have been synthesized and biologically evaluated. The results of antiplatelet aggregation assays showed that compounds **PC-2** and **PC-6** with deuteration at 6-OCH_3 were more potent than the parent compound, indicating that structural modifications at this position might be favourable for PAR4 binding. In vitro metabolic stability studies using human liver microsomes showed that structural modifications at the three sites (6-OCH_3, 2'- OCH_3 and the C-H bond connected to the phenyl oxygen) might not be suitable for improving metabolic stability. Further investigation on the metabolic profile and structural modifications of **BMS-986120** are ongoing in our laboratory.

4. Experimental section

All commercially available solvents and reagents were used without further purification. ^1H and ^{13}C NMR spectra were recorded on an ACF* 300Q Bruker, ACF* 400Q Bruker or ACF* 500Q Bruker spectrometer in $CDCl_3$, with Me_4Si as the internal reference, or in DMSO-d_6. Data for ^1H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet or unresolved, br s = broad singlet, coupling constant (s) in Hz, integration). Low- and high resolution mass spectra (LRMS and HRMS) were recorded in electron impact mode. Reactions were monitored by TLC on silica gel HSGF254 plates (Yantai Jiangyou Company, China). Column chromatography was carried out on silica gel (300−400 mesh, Qingdao Ocean Chemical Company, China). The purity of all final compounds was determined to be ≥95% by analytical HPLC (equipment: SHIMADZU LC-20AT system with a SPD-20A UV detector; column, Phecda C18, 4.6 mm × 250 mm, eluting with 80% MeOH + 20% H_2O + 0.1% (for **PC-1**-**PC-5** and **PC-7**) or 0.5% (for **BMS-986120** and **PC-6**) TFA, flow rate 1 mL/min, oven temperature 25 °C, detection UV 254 nm).

2,4,6-trihydroxybenzaldehyde (2)[29]

A 100 mL round bottom flask was charged with phloroglucin (3 g, 24 mmol) and DMF (30 mL). After the flask was flushed with Ar and covered with the rubber plug, $POCl_3$ (2.2 mL, 24 mmol) was added dropwise under the condition of ice bath. After completion of adding, the mixture was stirred at room temperature for 4 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with ice cold water (50 mL), the slurry was extracted with CH_2Cl_2 (20 mL × 3). The combined organic extracts were washed with 20% LiCl aqueous solution (10 mL × 2), brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 2:1) to provide 2,4,6-trihydroxybenzaldehyde (2.9 g, 80%) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 11.46 (s, 2H), 10.65 (s, 1H), 9.93 (s,

1H), 5.79 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.9, 167.2, 164.1, 104.6, 94.2. MS (ESI): *m/z* 153.0 [M-H]$^-$.

2-hydroxy-4,6-bis(methoxymethoxy)benzaldehyde (3)[30]

In argon atmosphere, Et_3N (0.57 mL, 4.07 mmol) and MOMCl (4.07 mmol in 5 mL anhydrous acetonitrile) were added dropwise successively to a solution of 2,4,6-trihydroxybenzaldehyde (0.30 g, 1.95 mmol) in anhydrous acetonitrile (6 mL) at 0 °C. The mixture was stirred at 0 ^{o}C for 80 min. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with ice cold water (10 mL), the slurry was extracted with ethyl acetate (5 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10:1) to provide 2-hydroxy-4,6-bis (methoxymethoxy)benzaldehyde (0.28 g, 60%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 10.10 (s, 1H), 6.29 (d, *J* = 1.8 Hz, 1H), 6.20 (d, *J* = 1.8 Hz, 1H), 5.31 (s, 2H), 5.26 (s, 2H), 3.44 (s, 3H), 3.39 (s, 3H). ^{13}C NMR (75 MHz $CDCl_3$) 192.3, 165.8, 161.4, 119.7, 107.1, 96.8, 94.8, 94.3, 94.3, 56.8, 56.6. MS (ESI): *m/z* 241.1 [M-H]$^-$.

2-(benzyloxy)-4,6-bis(methoxymethoxy)benzaldehyde (4)[31]

2-Hydroxy-4,6-bis(methoxymethoxy)benzaldehyde (0.12 g, 0.50 mmol), benzyl bromide (0.07 mL, 0.55 mmol) and potassium carbonate (0.14 g, 1.0 mmol) were added to DMF (3 mL) and then stirred at room temperature for 4 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with ice cold water (5 mL), the slurry was extracted with ethyl acetate (5 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1) to provide 2-(benzyloxy)-4,6-bis(methoxymethoxy)benzaldehyde (0.15 g, 92%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.48-7.31 (m, 5H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.38 (d, *J* = 2.0 Hz, 1H), 5.24 (s, 2H), 5.17 (s, 2H), 5.15 (s, 2H), 3.51 (s, 3H), 3.47 (s, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.7, 163.6, 163.0, 161.3, 136.2, 128.7, 128.1, 127.2, 110.9, 96.2, 95.1, 94.9, 94.4, 70.8, 56.7, 56.5. MS (ESI): *m/z* 355.1 [M+Na]$^+$.

2-(benzyloxy)-4,6-dihydroxybenzaldehyde (5)[31]

2 N HCl aqueous solution (0.25 mL) was added to a solution of 2-(benzyloxy)-4,6-bis(methoxymethoxy)benzaldehyde (0.18 g, 0.53 mmol) in MeOH (3 mL). The mixture was stirred under reflux for 3 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with saturated sodium bicarbonate solution (5 mL), the slurry was extracted with ethyl acetate (3 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1) to provide 2-(benzyloxy)-4,6-dihydroxybenzaldehyde (0.077 g, 60%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.92 (s, 1H), 10.02 (s, 1H), 7.53-7.30 (m, 6H), 6.08 (d, *J* = 1.8 Hz, 1H), 5.88 (d, *J* = 1.6 Hz, 1H), 5.18 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.7, 167.3, 165.2, 162.9, 136.2, 128.5, 128.0, 127.4, 104.9, 95.2, 92.6, 69.8. MS (ESI): *m/z* 245.0 [M+H]$^+$.

2-(benzyloxy)-6-hydroxy-4-methoxybenzaldehyde (6a)[18]

In argon atmosphere, 2-(benzyloxy)-4,6-dihydroxybenzaldehyde (0.50 g, 2.0 mmol), triphenylphosphine (0.52 g, 2.0 mmol) and absolute methanol (0.073 mL, 1.80 mmol) were added to anhydrous THF (10 mL). Diisopropyl azodicarboxylate (0.40 mL, 2.10 mmol) was added dropwise to the aforementioned system at 0 ^{o}C . The mixture was stirred under at room temperature for 4 h. TLC analysis indicated that the reaction was completed. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 40:1) to provide 2-(benzyloxy)-6-hydroxy-4-methoxybenzaldehyde (0.31 g, 60%) as a white solid.^{1}H NMR (300 MHz, $CDCl_3$) δ 12.50 (s, 1H), 10.17 (s, 1H), 7.42-7.35 (m, 5H), 6.03 (d, *J* = 2.0 Hz, 1H), 5.99 (d, *J* = 2.0 Hz, 1H), 5.09 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 191.5, 167.9, 165.1, 162.3, 136.1, 128.5, 128.0, 127.5, 105.6, 93.4, 92.1, 70.0, 55.9. MS (ESI): *m/z* 281.1 [M+Na]$^+$.

2-(benzyloxy)-6-hydroxy-4-(methoxy-d_3)benzaldehyde (6b)

Following a procedure similar to that described for the preparation of **6a** except that methanol was replaced by methanol-d_4, the title compound was obtained as a white solid in 50% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.50 (s, 1H), 10.17 (s, 1H), 7.42-7.35 (m, 5H), 6.03 (d, *J* = 2.0 Hz, 1H), 5.99 (d, *J* = 2.0 Hz, 1H), 5.09 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.8, 168.1, 166.4, 162.6, 135.8, 128.7, 128.4, 127.4, 106.2, 93.3, 91.8, 70.5, 54.9 (hept, *J* = 21.8 Hz). MS (ESI): *m/z* 284.1 [M+Na]$^+$.

1-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)ethan-1-one (7a)[18]

Chlorone acetone (1.88 mL, 23.75 mmol) and anhydrous potassium carbonate (3.5 g, 25.73 mmol) was added to a solution of 2-(benzyloxy)-6-hydroxy-4-methoxybenzaldehyde (5.11 g, 19.79 mmol) in acetone (50 mL). The mixture was stirred under reflux condition for 7 h and then filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ dichloromethane = 1:2) to provide 1-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)ethan-1-one (5.05 g, 86%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, *J* = 0.78 Hz, 1H), 7.48-7.36 (m, 5H), 6.65 (dd, *J* = 1.8, 0.78 Hz, 1H), 6.40 (d, *J* = 1.8 Hz, 1H), 5.15 (s, 2H), 3.84 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.4, 162.1, 157.3, 153.8, 150.6, 136.4, 128.4, 127.9, 127.7, 112.8, 111.4, 96.6, 88.5, 69.8, 55.9, 25.9. MS (ESI): *m/z* 295.2 [M-H]$^-$.

1-(4-(benzyloxy)-6-(methoxy-d_3)benzofuran-2-yl)ethan-1-one (7b)

Following a procedure similar to that described for the preparation of **7a**, the title compound was obtained as a white solid in 80% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.48-7.36 (m, 5H), 6.65 (s, 1H), 6.40 (d, *J* = 1.8 Hz, 1H), 5.15 (s, 2H), 2.53 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.1, 162.5, 158.0, 154.3, 151.1, 136.2, 128.7, 128.2, 127.5, 112.3, 112.1, 96.4, 88.2, 70.3, 55.0 (hept, *J* = 21.8 Hz). 26.0. MS (ESI): *m/z* 322.1 [M+Na]$^+$.

1-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoethan-1-one (8a)[18]

To a suspension of $CuBr_2$ (2.95g,13.24mmol) in ethyl acetate (50 mL) was added a solution of 1-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)ethan-1-one (3.3 g, 11 mmol) in ethyl acetate (10 mL) dropwise under reflux condition through a dropping funnel. The mixture was stirred under reflux condition for 6 h and then filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/dichloromethane = 1:1) to provide 1-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoethan-1-one (2.1 g, 50%) as a yellow sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ

7.72 (s, 1H), 7.47-7.36 (m, 5H), 6.66 (s, 1H), 6.41 (s, 1H), 5.16 (s, 2H), 4.35 (s, 2H), 3.86 (s, 3H). MS (ESI): *m/z* 397.0 $[M+Na]^+$.

1-(4-(benzyloxy)-6-(methoxy-d_3)benzofuran-2-yl)-2-bromoethan-1-one (8b)

Following a procedure similar to that described for the preparation of **8a**, the title compound was obtained as a white solid in 43% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.47-7.34 (m, 5H), 6.65 (s, 1H), 6.40 (s, 1H), 5.16 (s, 2H), 4.35 (s, 2H). MS (ESI): *m/z* 400.0 $[M+Na]^+$.

6-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoimidazo[2,1-b][1,3,4]thiadiazole (9a)[18]

1-(4-(Benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoethan-1-one (1.17 g, 3.12 mmol), 5-bromo-1,3,4-thiadiazol-2-amine (0.67 g, 3.74 mmol) and 10 mL isopropanol were added to a pressure flask. After the flask was flushed with Ar and capped, the mixture was heated under 80 °C for 6 h and then 130 °C for 3 h, TLC analysis indicated that the reaction was completed. The solvent was evaporated in vacuo. The residue was used for next step reaction without further purification.

6-(4-(benzyloxy)-6-(methoxy-d3)benzofuran-2-yl)-2-bromoimidazo[2,1-b][1,3,4]thiadiazole (9b)

Following a procedure similar to that described for the preparation of **9a**, the crude title compound was obtained.

6-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (10a)[18]

The crude product 6-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-bromoimidazo [2,1-b][1,3,4]thiadiazole (1.25 g) obtained from the previous step was added to a mixture solvents of CH_2Cl_2 (90 mL) and MeOH (30 mL). Sodium methoxide-methanol solution (2.3 mL, 25 wt.%) was then added to the aforementioned mixture. The mixture was stirred at room temperature for 1 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with saturated ammonium chloride solution (20 mL), the slurry was extracted with ethyl acetate (30 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel （petroleum ether/ethyl acetate = 8:1） and recrystallization in petroleum ether/ethyl acetate/dichloromethane to provide 6-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (0.63 g, two step yield: 32%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.49-7.33 (m, 5H), 7.09 (s, 1H), 6.70 (s, 1H), 6.40 (s, 1H), 5.19 (s, 2H), 4.20 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 159.1, 156.4, 152.6, 149.4, 142.3, 137.1, 135.9, 128.7, 128.0, 127.3, 113.6, 110.3, 98.8, 96.0, 88.9, 70.4, 59.8(hept, J = 21.7 Hz), 55.9.

6-(4-(benzyloxy)-6-(methoxy-d_3)benzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (10b)

Following a procedure similar to that described for the preparation of **10a**, the title compound was obtained as a white solid in 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.49-7.32 (m, 5H), 7.09 (s, 1H), 6.69 (s, 1H), 6.40 (s, 1H), 5.19 (s, 2H), 4.20 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 159.0, 156.2, 152.5, 149.3, 142.2, 137.0, 135.7, 128.6, 127.9, 127.2, 113.4, 110.1, 98.7, 95.9, 88.8, 70.3, 59.7(hept, J = 21.7 Hz), 55.0. MS (ESI): *m/z* 433.1 $[M+Na]^+$.

6-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-(methoxy-d3)imidazo[2,1-b][1,3,4]thiadiazole (10c)

Following a procedure similar to that described for the preparation of **10a**, the title compound was obtained as a white solid in 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.49-7.33 (m, 5H), 7.09 (s, 1H), 6.69 (s, 1H), 6.40 (s, 1H), 5.19 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 159.0, 156.3, 152.5, 149.3, 142.2, 137.0, 135.8, 128.6, 127.9, 127.2, 113.4, 110.3, 98.7, 95.9, 88.8, 70.3, 58.9 (hept, J = 22.5 Hz), 55.8. MS (ESI): *m/z* 411.1 $[M+H]^+$.

6-(4-(benzyloxy)-6-(methoxy-d3)benzofuran-2-yl)-2-(methoxy-d3)imidazo[2,1-b][1,3,4]thiadiazole (10d)

Following a procedure similar to that described for the preparation of **10a**, the title compound was obtained as a white solid in 40% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.49-7.32 (m, 5H), 7.09 (s, 1H), 6.69 (s, 1H), 6.40 (s, 1H), 5.19 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 158.9, 156.2, 152.5, 149.3, 142.1, 137.0, 135.7, 128.5, 127.9, 127.2, 113.4, 110.1, 98.6, 95.8, 88.8, 70.2, 58.8 (hept, J = 22.8 Hz), 54.9 (hept, J = 22.0 Hz). MS (ESI): *m/z* 436.1 $[M+Na]^+$.

6-methoxy-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-ol (11a)[18]

In argon atmosphere, to a solution of 6-(4-(benzyloxy)-6-methoxybenzofuran-2-yl)-2-methoxyimidazo[2,1-b][1,3,4]thiadiazole (0.34 g, 0.83 mmol) and pentamethylbenzene (0.86 g, 5.81 mmol) in CH_2Cl_2 (20 mL) was added 1 M BCl_3 in CH_2Cl_2 (2.18 mL, 2.16 mmol) dropwise at -78 ^{o}C. The mixture was stirred at -78 ^{o}C for 1 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with 5% sodium bicarbonate solution (10 mL), the slurry was filtered and washed with toluene (5 mL × 3). The filter cake was dried in vacuum desiccator to provide 6-methoxy-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran- 4-ol (0.25 g, 99%) as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.32 (s, 1H), 6.99 (s, 1H), 6.65 (s, 1H), 6.26 (s, 1H), 4.20 (s, 3H), 3.75 (s, 3H).

4-(4-(bromomethyl)-5-methylthiazol-2-yl)morpholine (13a)

To a solution of (5-methyl-2-morpholinothiazol-4-yl)methanol (250 mg, 1.16 mmol) in CH_2Cl_2 (5 mL) was added PBr_3 (60 μL, 0.58 mmol in 5 mL CH_2Cl_2) dropwise at 0 °C. The mixture was stirred at room temperature for 4 h. After quenching the reaction mixture with saturated sodium bicarbonate solution (5 mL), the slurry was extracted with ethyl acetate (5 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 8:1) to provide 4-(4-(bromomethyl)-5-methylthiazol-2-yl)morpholine (145 mg, 45%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.39 (s, 2H), 3.80-3.77 (m, 4H), 3.41-3.38 (m, 4H), 2.29 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.3, 143.7, 121.0, 66.3, 48.5, 26.7, 11.3. MS (ESI): *m/z* 197.1 $[M-Br]^+$.

5-methyl-2-morpholinothiazole-4-carbaldehyde (14)

Pyridine (45 μL, 0.56 mmol) and Dess-Martin Periodinane (119 mg, 0.28 mmol) were added successively to a solution of (5-methyl-2-morpholinothiazol-4-yl) methanol (50 mg, 0.23 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 1.5 h. TLC analysis indicated that the reaction was completed. The suspension was filtered. The filter cake was washed with CH_2Cl_2 (5 mL × 2). The combined filtrate was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1) to provide 5-methyl-2-morpholinothiazole-4-carbaldehyde

(32 mg, 65%) as a pale blue solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.91 (s, 1H), 3.82-3.77 (m, 4H), 3.48-3.44 (m, 4H), 2.65 (s, 3H).

(5-methyl-2-morpholinothiazol-4-yl)methan-d-ol (12b)

Sodium borodeuteride (37 mg, 0.88 mmol) was added to a solution of 5-methyl-2-morpholinothiazole-4-carbaldehyde (187 mg, 0.88 mmol) in deuterated methanol (8 mL) at 0 ^{o}C. Then the mixture was stirred at room temperature for 5 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with ice cold water (5 mL), the slurry was extracted with ethyl acetate (5 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 1:3) to provide (5-methyl-2-morpholinothiazol-4-yl)methan-d-ol (140 mg, 74%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.45 (s, 1H), 3.81-3.78 (m, 4H), 3.41-3.38 (m, 4H), 2.27 (s, 3H).

4-(4-(bromomethyl-d)-5-methylthiazol-2-yl)morpholine (13b)

To a solution of (5-methyl-2-morpholinothiazol-4-yl)methan-d-ol (140 mg, 0.65 mmol) in CH_2Cl_2 (5 mL) was added PBr_3 (61 μL, 0.65 mmol in 5 mL CH_2Cl_2) dropwise at 0 °C. The mixture was stirred at room temperature for 4 h. After quenching the reaction mixture with saturated sodium bicarbonate solution (5 mL), the slurry was extracted with ethyl acetate (5 mL × 3). The combined organic extracts were washed with brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 4:1) to provide 4-(4-(bromomethyl)-5-methylthiazol-2-yl) morpholine (80 mg, 44%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.38 (s, 1H), 3.80-3.77 (m, 4H), 3.42-3.38 (m, 4H), 2.29 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2, 143.4, 120.9, 66.2, 48.5, 26.3 (t, J = 23.3 Hz), 11.2. MS (ESI): *m/z* 198.1 $[M-Br]^+$.

4-(4-(((6-methoxy-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)-5-methylthiazol-2-yl)morpholine (BMS-986120)[18]

A 25 mL round bottom flask was charged with 6-methoxy-2-(2-methoxyimidazo [2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-ol (50 mg, 0.158 mmol), ^{t}BuONa (22 mg 0.236 mmol) and DMF (5 mL). After the flask was flushed with Ar and covered with the rubber plug, a solution of 4-(4-(bromomethyl)-5-methylthiazol-2-yl)morpholine (65 mg, 0.236 mmol)in DMF (3 mL) was added dropwise to the above mixture. After completion of adding, the mixture was stirred at room temperature for 3 h. TLC analysis indicated that the reaction was completed. After quenching the reaction mixture with ice cold water (5 mL), the slurry was diluted with ethyl acetate (15 mL). The separated organic extract was washed with ice cold water (10 mL), brine and then dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (petroleum ether/dichloromethane/ethyl acetate/triethylamine = 8:3:0.5:0.2) to provide 4-(4-(((6-methoxy-2-(2-methoxyimidazo[2,1-b] [1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)-5-methylthiazol-2-yl)morpholine (38 mg, 47%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.05 (s, 1H), 6.69 (d, J = 1.4 Hz, 1H), 6.50 (d, J = 1.4 Hz, 1H), 5.05 (s, 2H), 4.20 (s, 3H), 3.84 (s, 3H), 3.82-3.79 (m, 4H), 3.44-3.40 (m, 4H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 168.4, 158.9, 156.3, 152.7, 149.1, 142.9, 142.2, 135.9, 121.2, 113.5, 110.2, 98.9, 96.0, 89.0, 66.3, 65.1, 59.8, 55.9, 48.6, 11.2.MS (ESI): *m/z* 514.1 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{23}N_5O_5S_2Na^+$ $[M+Na]^+$ 536.1033, found: 536.1035. HPLC purity: 98.6%, retention time = 6.71 min.

4-(4-(((6-methoxy-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl-d)-5-methylthiazol-2-yl)morpholine (PC-1)

Following a procedure similar to that described for the preparation of **1** except that 4-(4-(bromomethyl)-5-methylthiazol-2-yl)morpholine was replaced by 4-(4-(bromomethyl-d)-5-methylthiazol-2-yl, the title compound was obtained as a white solid in 45% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 1H), 4.20 (s, 3H), 3.85 (s, 3H), 3.84-3.78 (m, 4H), 3.47-3.38 (m, 4H), 2.37 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.5, 168.4, 158.9, 156.2, 152.6, 149.0, 142.9, 142.2, 135.8, 121.2, 113.4, 110.2, 98.9, 96.0, 88.9, 66.3, 64.8 (t, J = 21.7 Hz), 59.8, 55.9, 48.6, 11.2. MS (ESI): *m/z* 515.1 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{23}DN_5O_5S_2^+$ $[M+H]^+$ 515.1276, found: 515.1282. HPLC purity: 95.1%, retention time = 7.51 min.

4-(4-(((6-(methoxy-d3)-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)-5-methylthiazol-2-yl)morpholine (PC-2)

Following a procedure similar to that described for the preparation of **1** and **8** except that methanol was replaced by deuterated methanol, the title compound was obtained as a white solid in 38% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 2H), 4.20 (s, 3H), 3.84-3.78 (m, 4H), 3.47-3.38 (m, 4H), 2.37 (s, 3H). MS (ESI): *m/z* 517.0 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{21}D_3N_5O_5S_2^+$ $[M+H]^+$ 517.1402, found: 517.1401. HPLC purity: 95.3%, retention time = 7.60 min.

4-(4-(((6-(methoxy-d3)-2-(2-methoxyimidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl-d)-5-methylthiazol-2-yl)morpholine (PC-3)

Following a procedure similar to that described for the preparation of **PC-1** and **PC-2**, the title compound was obtained as a white solid in 31% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 1H), 4.20 (s, 3H), 3.84-3.78 (m, 4H), 3.47-3.38 (m, 4H), 2.37 (s, 3H). MS (ESI): *m/z* 518.0 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{20}D_4N_5O_5S_2^+$ $[M+H]^+$ 518.1464, found: 518.1469. HPLC purity: 95.4%, retention time = 7.55 min.

4-(4-(((6-methoxy-2-(2-(methoxy-d3)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)-5-methylthiazol-2-yl)morpholine (PC-4)

Following a procedure similar to that described for the preparation of **1** and **16** except that methanol was replaced by deuterated methanol and sodium methoxide was replaced by deuterated sodium methoxidethe, the title compound was obtained as a white solid in 33% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 2H), 3.85 (s, 3H), 3.84-3.78 (m, 4H), 3.47- 3.38 (m, 4H), 2.37 (s, 3H). MS (ESI): *m/z* 517.1 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{20}D_3N_5O_5S_2Na^+$ $[M+Na]^+$ 539.1221, found: 539.1186. HPLC purity: 98.8%, retention time = 7.58 min.

4-(4-(((6-methoxy-2-(2-(methoxy-d3)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl-d)-5-methylthiazol-2-yl)morpholine (PC-5)

Following a procedure similar to that described for the preparation of **PC-1** and **PC-4**, the title compound was obtained as a white solid in 25% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 1H), 3.85

(s, 3H), 3.84-3.78 (m, 4H), 3.47-3.38 (m, 4H), 2.37 (s, 3H). MS (ESI): *m/z* 518.1 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{19}D_4N_5O_5S_2Na^+$ $[M+Na]^+$ 540.1284, found: 540.1284. HPLC purity: 96.3%, retention time = 7.52 min.

4-(4-(((6-(methoxy-d3)-2-(2-(methoxy-d3)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl)-5-methylthiazol-2-yl)morpholine (PC-6)

Following a procedure similar to that described for the preparation of **PC-2** and **PC-5**, the title compound was obtained as a white solid in 34% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 2H), 3.84-3.78 (m, 4H), 3.47-3.38 (m, 4H), 2.37 (s, 3H). MS (ESI): *m/z* 520.1 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{18}D_6N_5O_5S_2^+$ $[M+H]^+$ 520.1590, found: 520.1591. HPLC purity: 97.5%, retention time = 6.46 min.

4-(4-(((6-(methoxy-d3)-2-(2-(methoxy-d3)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzofuran-4-yl)oxy)methyl-d)-5-methylthiazol-2-yl)morpholine (PC-7)

Following a procedure similar to that described for the preparation of **PC-1** and **PC-6**, the title compound was obtained as a gray solid in 22% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.06 (s, 1H), 6.69 (s, 1H), 6.51 (s, 1H), 5.06 (s, 1H), 3.84-3.78 (m, 4H), 3.47-3.38 (m, 4H), 2.37 (s, 3H). MS (ESI): *m/z* 521.1 $[M+H]^+$. ESI-HRMS: calcd. for $C_{23}H_{16}D_7N_5O_5S_2Na^+$ $[M+Na]^+$ 543.1472, found: 543.1470. HPLC purity: 97.1%, retention time = 7.23 min.

Assessment of antiplatelet activity

Animal: Institute of Cancer Research (ICR) mice (male, 18-22 g) were purchased from Nanjing Qinglongshan Animal Center (Nanjing, Jiangsu, China). The animals were acclimatized for at least 1 day prior to use.

Preparation of mice Gel-filtered platelets: Gel-filtered platelets were prepared as described previously.[32]nticoagulated blood was taken out from the Abdominal aorta of chloral hydrate-anesthetized mice by using 2.5 mL syringe, and centrifugated at 200×g for 10 min at room temperature to get platelet-rich plasma (PRP). Filtered the PRP by using the column packed with Sepharose 2B beads and eluted with modified Tyrode`s buffer(5.56 mM glucose, 137 Mm NaCl, 2.7 mM KCl, 2.56 mM $NaH_2PO_4 \cdot 2H_2O$, 20 mM HEPES, 137 mM $MgCl_2 \cdot 6H_2O$, 0.1% BSA, pH = 7.4） 1.5 mL tubes. Collected the high concentration Gel-filtered platelets, and adjusted to 3×10^8 platelets/mL using modified Tyrode's buffer.

Metabolic stability of the compounds in human liver microsomes

Materials: Midazolam maleate (Art. No. 171250, Lot No. 200401), as a reference compound, was purchased from National Institute for the Control of Pharmaceutical and Biological Products. Human liver microsomes (150-donor with mixed gender) were from IVT (Lot No. IQF; 20 mg/mL).

Solution preparation: 1. Preparation of stock solutions and working fluids of the test substances and the reference compound: The test substance was dissolved in DMSO and prepared into 10 mM stock solution. Appropriate amount of the reference compound was used to prepare 10 mM stock solution in DMSO (-20 °C fridge and the validity period was 3 months.). The stock solution was diluted with MeOH to 100 μM as a working fluid. 2. Preparation of stop solution containing internal standard: Terfenadine and Tolbutamide were respectively weighed for about 1 mg to prepare 1 mg/mL stock solution in DMSO (-20 °C in fridge and the validity period was 3 months.). The Terfenadine stock solution and Tolbutamide stock solution were diluted with acetonitrile to 25 ng/mL (Ter) and 50 ng/mL (Tol) as the stop solution containing mixed internal standard. 3. Phosphoric acid buffer: 1.83 g $K_2HPO_4 \cdot 3H_2O$ and 0.28 g KH_2PO_4 were dissolved in 200 mL deionized water, diluted to 50 mM, pH = 7.4 and reserved in fridge at 4°C. 4. Preparation of liver microsomal working solution: The 20 mg/mL liver microsome solution were freshly diluted to 0.63 mg/mL by phosphate buffer solution as a working solution just before use. 5. Preparation of NADPH solution: The appropriate amount of NADPH was freshly dissolved in phosphate buffer to 5 mM just before use.

Experimental procedure: Microsomal incubation system was prepared by mixing liver microsome working fluid (0.63 mg/mL, 197.5 μL) and the test compound or reference compound working fluid (100 μM, 2.5 μL). The mixture was preincubated in triplicate at 37 °C in the shaking water bath for 1 h. Then, NADPH (5 mM, 50 μL) solution was added. The final concentration of the test compound and liver microsomes in the mixture was 1 μM and 0.5 mg/mL, respectively. At time 0, 5, 15, 30, and 60 min, stop solution was added to terminate the incubation. After quenching, the mixture was centrifuged and filtered. The supernatant was then diluted and 5 μL was injected into liquid chromatography–mass spectrometer/mass spectrometer to detect the remaining amount of the test compound. Percentage of the test compound remaining was calculated by dividing the peak area of the test compound remaining at each time point by the peak area of the test compound at time 0 min and multiplying by 100%. Natural logarithm of percentage of the test compound remaining against time could be plotted. Half-life ($T_{1/2}$) and intrinsic clearance (Clint) of the test compound could be calculated based on the absolute value of slope of the above plot and the following formula: $T_{1/2}$ = ln2/k = 0.693/k; Clint (intrinsic clearance) (μL/min/mg protein) = Ln(2)*1000/$T_{1/2}$/protein concentration.

LC-MS/MS analysis method: Chromatographic column: Waters HSS C18 column 2.5 μ (2.1 mm * 50 mm); Mobile phase: A phase: H_2O with 0.1% TFA, B phase: ACN with 0.1% TFA, Flow rate: 0.6 mL/min; Column temperature: 30 °C; Injector temperature: 4 °C; Injected sample volume: 5 μL; Running time: 2 min.

Conflicts of interest

There are no conflicts to declare.

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Supplementary Material

Supplementary data associated with this article can be found, in the on line version, at

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