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Research Article

Design, Synthesis and Pharmacological Evaluation of Novel Piperlongumine derivatives as Potential Antiplatelet Aggregation Candidate

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A series of novel piperlongumine derivatives (4a-i, 6a-i) were designed and synthesized. The inhibitory activities of platelet aggregation induced by ADP and AA in vitro have been evaluated by bron turbidimetry and liver microsomal incubated assay. The assay results show that compounds 4e and 6e exhibited remarkable potency to that of the positive control piplartine and aspirin.

Key words: antiplatelet aggregation, piperlongumine derivatives, thienopyridine

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Piper longum L. had been used as a crude drug for the treatment of the intestinal disorder, asthma and poorly peripheral blood circulation in Asia for decades. Piperlongumine (PL, Figure 1), also known as piperaceous amides or piplartine, is an alkaloid isolated from Piper longum L, which displays wide range of biological activities, such as anticancer (1,2), antidiabetic (3), antiplatelet aggregation (4), antidepression and antifungal activities (5).

Great effort had been paid to the structure modification of PL to make it more selective and potent to the disease. By replacing nitrogen atom of lactam in PL with carbon atom to increase anti-inflammatory activity, Zhou's group reach promising anti-inflammatory agent PL-0N (6). 10 amide alkaloid derivatives were synthesized by Lee's team. The antiproliferative activity against eight human tumour cell lines was evaluated. It was suggested that side chain length affects the ability to overcome the MDR cancer phenotype (7).

In particular, the antiplatelet aggregation activity of piperlongumine has attracted global attention. It was reported that piperlongumine inhibits platelet aggregation as a thromboxane A2 receptor antagonist (8). In addition, Park's team evaluated the anti-atherosclerotic potential of PL. It was assumed that PL significantly attenuated activation of NF-κB in response to PDGF-BB stimulation and inhibited PDGF-BB-induced PDGF receptor beta activation and suppressed downstream signalling molecules (4).

Thienopyridines, including ticlopidine, clopidogrel, prasugrel, (Figure 2) are wildly used in clinical for treating platelet aggregation by selectively blocking the binding of adenine diphosphate (ADP)-induced platelet aggregation (9). Currently, dual treatment with aspirin and clopidogrel is the ideal treatment of antiplatelet therapy for patients with acute coronary syndromes (ACS) and prevention of thrombotic events after percutaneous coronary intervention (PCI) with stenting (10). However, the bleeding risk, complications and individual risk still exist (11). Novel safer antiplatelet agents are urgently needed (12). The thienopyridine derivatives were designed and synthesized by Zhi's group and exhibit potent inhibition to ADP-induced aggregation than ticlopidine (13).

On careful review at piplartine and the thienopyridine derivatives, it was noticed that piplartine and 'grel' may share same skeleton. We also predicted that trimethoxyphenyl-acryloyl part and thienopyridine may be able to contribute the activity of antiplatelet aggregation. Therefore, in the present research, we report the design, synthesis and biological evaluation of the piplartine derivatives bearing thienopyridine structure (Figure 3; Table 1).

Chemistry

Target compounds **4a-i, 6a-i** were achieved via the synthetic route outlined in Scheme 1. Different substituted benzaldehyde was used as starting material. **4a-e** and **6a-e** were reached by Knoevenagel, amidation, condensation reaction and esterification. **6f-i** were reached by acylation

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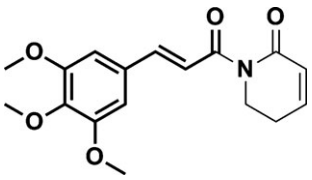


Figure 1: Piperlongumine.

of **7a-d**, which hydroxyl group was protected by acylation, using Et_3N as acid-binding agent, while **4f-i** were achieved using DCC as condensation agent, because of the hydroxyl group on the benzene ring.

Experimental Section

General

All of the target compounds were purified by column chromatography (CC) on silica gel 60 (200–300 mesh). Melting points were determined using a capillary apparatus (RDCSY-I) and are reported directly. NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer (Bruker, Fallanden, Switzerland), using TMS as an internal standard. IR spectra were measured with Shimadzu FTIR-8400S (Shimadzu,Tokyo, Japan). MS spectra were measured with a Hewlett-Packard 1100 LC/MSD spectrometer (Agilent, Waldbronn, Germany).

(*E*)-3-(3,4,5-trimethoxyphenyl)acrylic acid (2a)

A solution of 3,4,5-trimethoxybenzaldehyde (10.0 g, 51.0 mmol), malonic acid (6.41 g, 61.5 mmol) in benzene (150 mL) was treated with pyridine (35 mL), piperidine (2 mL). The reaction mixture was stirred at reflux for 6 h. The reaction mixture was cooled to room temperature and saturated $NaHCO_3$(liq.) (75 mL) was added. After 30 min, the aqueous phase was acidified with HCl (liq.) to pH = 4. Then, the separated solid was filtrated and filter was recrystallized with anhydrous ethanol, to provide the title compound as a white solid (7.92 g, 65.2%), m.p.124.3~125.7 °C.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (4a)

$SOCl_2$ (15 mL)was added to the solution of **1b** (3.00 g, 12.6 mmol) in toluene (45 mL). The reaction mixture was stirred at 80 °C for 5 h. The reaction mixture was evaporated to give the crude compound **3a**.

The solution of **1c** in DCM was added at 0 °C slowly to the mixture of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride (1.86 g, 13.4 mmol) and Et_3N (6 mL) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at room temperature for 2.5 h. The organic phase was washed with 1 mol/L Na_2CO_3(liq.), 1% hydrochloric acid and saturated brine, dried over sodium sulphate and evaporated in vacuo to give the crude product. The crude product was purified on silica using hexanes/ethyl acetate to provide the title compound as white solid (2.43 g, 53.3%), m.p.151.4~152.7 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.63 (d, J = 15.6 Hz, 1H, ArC**H**=), 7.16 (d, J = 5.1 Hz, 1H, Th**H**), 6.85–6.80 (m, 2H, -CH= and Th**H**), 6.76 (s, 2H, Ar**H**), 4.78 (s, 2H, Py-C$\mathbf{H}_2$), 3.98 (t, 2H, J = 4.5 Hz, Py-C$\mathbf{H}_2$), 3.91 (s, 6H, OC$\mathbf{H}_3$ × 2), 3.88 (s, 3H, OC$\mathbf{H}_3$), 2.96 (br s, 2H, Py-CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:166.0, 153.4, 143.0, 139.6, 132.4, 130.8, 125.2, 124.5, 123.6, 116.6, 105.1, 61.0, 56.2, 46.0, 43.3, 26.1; IR (KBr, cm^{-1}) υ: 2998.3, 2935.6, 2833.9, 1642.7, 1587.0, 1505.6, 1437.4, 1418.9, 1332.8, 1252.2, 1266.5, 1124.4, 1058.1, 1008.1, 977.9, 826.2, 717.9, 613.2; ESI-Mass for $C_{19}H_{21}NO_4S$: *m/z* (M^++H) 360.06.

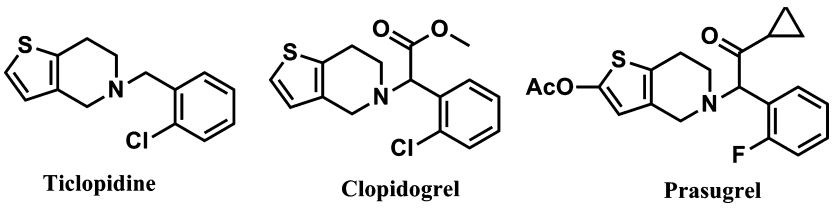


Figure 2: Structure of thienopyridines.

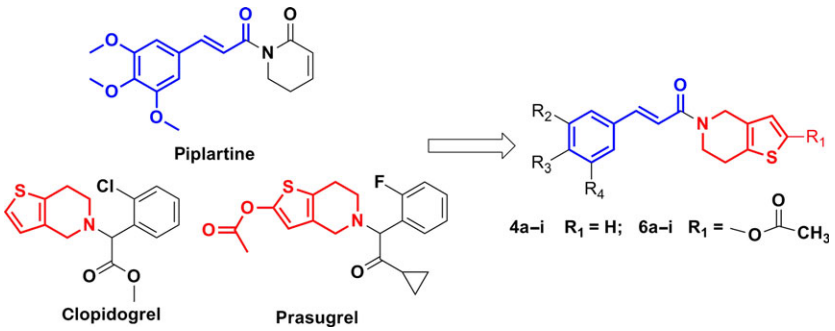
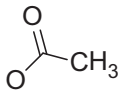
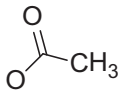
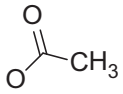
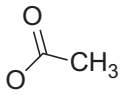
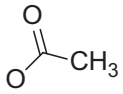
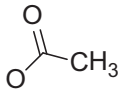
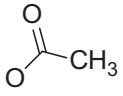
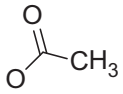
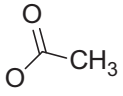
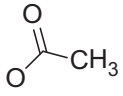
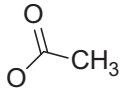
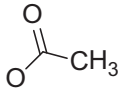
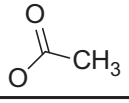


Figure 3: The design of piplartine derivatives.

Table 1: The structure of target compounds

Compd	R_1	R_2	R_3	R_4
4a	H	OCH_3	OCH_3	OCH_3
4b	H	H	OCH_3	H
4c	H	H	CH_3	H
4d	H	H	Cl	H
4e	H	H	NO_2	H
4f	H	H	OH	H
4g	H	OCH_3	OH	H
4h	H	OH	OCH_3	H
4i	H	OCH_3	OH	OCH_3
6a		OCH_3	OCH_3	OCH_3
6b		H	OCH_3	H
6c		H	CH_3	H
6d		H	Cl	H
6e		H	NO_2	H
6f		H		H
6g		H		H
6h		H	OCH_3	
6i		OCH_3		OCH_3

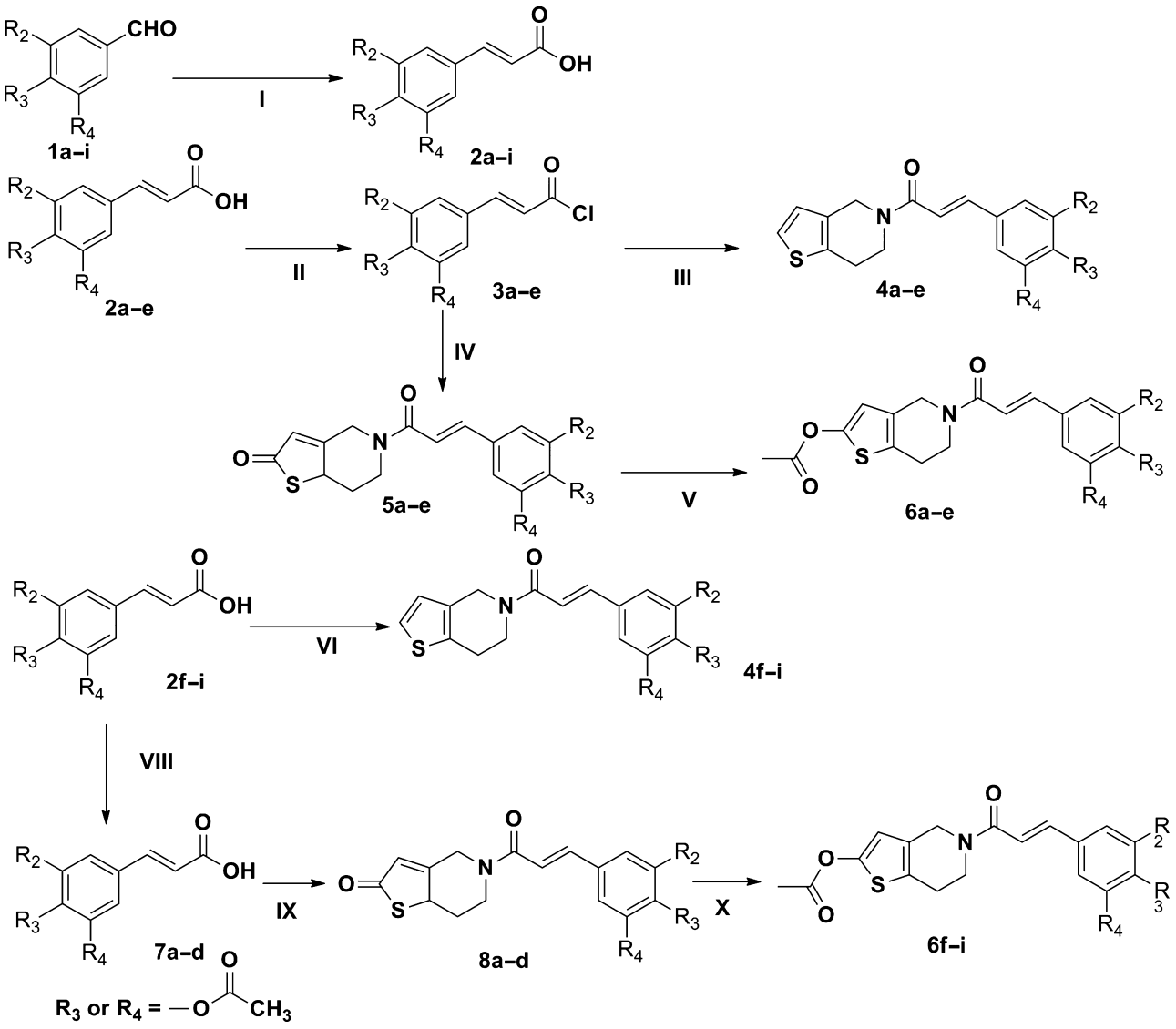
Compounds **4b-e** were synthesized following the same procedure described above for the preparation of **4a**.

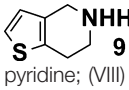
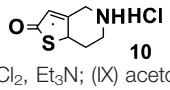
(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (4b)

Off-white solid,yield 61.2%,m.p.131.4~132.7 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (d, J = 15.2 Hz, 1H, ArC**H**=), 7.50 (d, J = 8.0 Hz, 2H, Ar**H**), 7.15 (d, J = 5.2 Hz, 1H, Th**H**), 6.90 (d, J = 8.4 Hz, 2H, Ar**H**), 6.83–6.79 (m, 2H, Th**H** and -C**H**=), 4.76 (s, 2H, Py-CH_2), 3.97 (br s, 2H, Py-CH_2), 3.84 (s, 3H, OCH_3), 2.94 (br s, 2H, Py-CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:166.3, 160.9, 142.6, 132.3, 129.4, 127.9, 125.2, 124.5, 123.5, 114.9, 114.2, 55.3, 45.9, 43.3, 26.0; IR (KBr, cm^{-1}) υ:3004.3, 2962.5, 2905.6, 1649.8, 1593.0, 1506.3, 1437.5, 1249.2, 1165.5, 1060.8, 1027.9, 980.1, 896.4, 824.6, 716.9; ESI-Mass for $C_{17}H_{17}NO_2S$: *m/z* (M^++H) 300.13.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-methylphenyl)prop-2-en-1-one (4c)

White solid,yield 63.7%,m.p.145.8~147.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.69 (d, J = 15.6 Hz, 1H, ArC**H**=), 7.45 (d, J = 7.6 Hz, 2H, Ar**H**), 7.19 (d, J = 8.0 Hz, 2H, Ar**H**), 7.15 (d, J = 5.2 Hz, 1H, Th**H**), 6.89 (d, J = 15.6 Hz,



Scheme 1: Reagents and conditions: (I) malonic acid, pyridine, benzene, 100 °C; (II) $SOCl_2$ toluene, 80 °C; (III) CH_2Cl_2, Et_3N;  (IV) CH_2Cl_2, Et_3N;  (V) acetic anhydride, acetonitrile, Et_3N; (VI) DCC, CH_2Cl_2; (VII) acetic anhydride, pyridine; (VIII) acetic anhydride, CH_2Cl_2, Et_3N; (IX) acetonitrile, Et_3N; (X) acetic anhydride, acetonitrile, Et_3N.

1H, -CH=), 6.83 (d, *J* = 4.8 Hz, 1H, Th**H**), 4.77 (s, 2H, Py-C**H**$_2$), 3.97 (br s, 2H, Py-C**H**$_2$), 2.95 (br s, 2H, Py-C**H**$_2$), 2.37 (s, 3H, C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:166.2, 142.9. 139.9, 132.4, 129.5, 127.6, 125.2, 124.5, 123.5, 116.5, 116.2, 45.9, 43.2, 25.9, 21.4; IR (KBr, cm^{-1}) υ: 3076.1, 2923.6, 2833.9, 1643.9, 1596.0, 1518.3, 1455.5, 1452.5, 1323.9, 1219.3, 1054.8, 1013.0, 968.1, 812.6, 737.9; ESI-Mass for $C_{17}H_{17}NOS$: *m/z* (M^++H) 284.08.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-chlorophenyl)prop-2-en-1-one (4d)

White solid,yield 56.1%,169.5~170.9 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.65 (d, *J* = 15.2 Hz, 1H, ArC**H**=), 7.47 (d, *J* = 8.0 Hz, 2H, Ar**H**), 7.35 (d, *J* = 8.0 Hz, 2H, Ar**H**), 7.16 (d, *J* = 5.2 Hz, 1H, Th**H**), 6.92 (d, *J* = 15.6 Hz, 1H, -CH=), 6.83 (d, *J* = 4.8 Hz, 1H, Th**H**), 4.76 (s, 2H, Py-C**H**$_2$), 3.97 (br s, 2H, Py-C**H**$_2$), 2.95 (br s, 2H, Py-C**H**$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:165.7, 141.5, 135.4, 133.7, 132.3, 129.0, 128.9, 125.1, 124.5, 123.6, 118.0, 45.9, 43.3, 25.9; IR (KBr, cm^{-1}) υ:2926.1, 2869.3, 2833.4, 1607.3, 1646.2, 1487.7, 1439.9, 1401.0, 1326.2, 1215.6, 1182.7, 1081.0, 1048.1, 970.4, 805.9, 704.2; ESI-Mass for $C_{16}H_{14}ClNOS$: *m/z* (M^++H) 314.17.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-nitrophenyl)prop-2-en-1-one (4f)

White solid,yield 45.7%,191.3~192.4 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.24 (d, *J* = 8.7 Hz, 2H, Ar**H**), 7.75–7.67 (m,

3H, ArC**H= and** Ar**H**), 7.18 (d, *J* = 5.1 Hz, 1H, Th**H**), 7.09 (d, *J* = 15.6 Hz, 1H, -CH=), 6.84 (d, *J* = 3.6 Hz, 1H, Th**H**), 4.79 (s, 2H, Py-C$\mathbf{H}_2$), 3.94 (d, *J* = 30.3 Hz, 2H, Py-C$\mathbf{H}_2$), 2.99 (br s, 2H, Py-C$\mathbf{H}_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:165.0, 148.1, 141.5, 140.1, 128.4, 125.2, 124.4, 124.1, 123.8, 120.0, 121.8, 46.1, 44.0, 26.0; IR (KBr, cm^{-1}) υ:3106.0, 2929.6, 2851.8, 1649.8, 1605.0, 1512.3, 1449.5, 1344.9, 1216.3, 1183.4, 1108.6, 1051.8, 962.1, 839.5, 746.8, 708.0; ESI-Mass for $C_{16}H_{14}N_2O_3S$: *m/z* (M^++H) 315.06.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (5d)

NaOH (1.70 g, 42.5 mmol) was added to a solution of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride in dichloromethane (60.0 mL). The reaction was stirred at room temperature for 3.5 h. The organic phase was washed with water, dried over sodium sulphate and evaporated in vacuo to give the 4,5,6,7-tetrahydrothieno[3,2-c] pyridine.

To a solution of (*E*)-3-(4-hydroxyphenyl)acrylic acid (3.50 g, 21.3 mmol), 4,5,6,7-tetrahydrothieno[3,2-c]pyridine (3.00 g, 21.6 mmol) in dichloromethane (60.0 mL) was added DCC (5.30 g, 25.7 mmol). The reaction was stirred at 0 °C for 6 h. The organic phase was washed with water, dried over sodium sulphate and evaporated in vacuo to give the 4,5,6,7-tetrahydrothieno[3,2-c]pyridine to give the crude product. The crude product was purified on silica using DCM/MeOH to provide the title compound as a white solid (3.40 g, 54.1%). m.p.209.6~212.1 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.86 (s, 1H, O**H**), 7.57 (d, *J* = 8.4 Hz, 2H, Ar**H**), 7.44 (d, *J* = 15.0 Hz, 1H, ArC**H=**), 7.34 (d, *J* = 4.8 Hz, 1H, Th**H**), 7.13 (d, *J* = 15.6 Hz, 1H, -C**H=**), 6.90 (d, *J* = 3.0 Hz, 1H, Th**H**), 6.78 (d, *J* = 8.4 Hz, 2H, Ar**H**), 4.79 (s, 1H, Py-C$\mathbf{H}_2$), 4.62 (s, 1H, Py-C$\mathbf{H}_2$), 3.90 (d, *J* = 30.3 Hz, 2H, Py-C$\mathbf{H}_2$), 2.83(d, *J* = 23.1 Hz, 2H, Py-C$\mathbf{H}_2$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 165.5, 159.5, 142.2, 132.7, 129.8, 125.9, 125.4, 125.1, 123.6, 115.7, 114.2, 45.1, 43.1, 25.6; IR (KBr, cm^{-1}) υ: 3120.9, 3016.3, 2935.6, 2842.9, 1640.9, 1578.0, 1518.3, 1449.5, 1273.1, 1219.3, 1192.4, 1162.5, 1063.8, 983.1, 821.6, 764.8; ESI-Mass for $C_{16}H_{15}NO_2S$: *m/z* (M^++H) 286.15.

Compounds **4g-i** were synthesized following the same procedure described above for the preparation of **4f**.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (4g)

White solid,yield 56.4%,m.p.196.2~197.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.64 (d, *J* = 15.2 Hz, 1H, ArC**H=**), 7.16 (d, *J* = 5.2 Hz, 1H, Th**H**), 7.13 (d, *J* = 8.4 Hz, 1H, Ar**H**), 7.01 (s, 1H, Ar**H**), 6.93 (d, *J* = 8.4 Hz, 1H, Ar**H**), 6.83 (d, *J* = 4.8 Hz, 1H, Th**H**), 6.78 (d, *J* = 14.8 Hz, 1H, -C**H**=), 4.77 (s, 2H, Py-C$\mathbf{H}_2$), 4.06–3.99 (m, 1H, Py-C$\mathbf{H}_2$), 3.94 (s, 4H, Py-C$\mathbf{H}_2$ and OC$\mathbf{H}_3$), 2.97(br s, 2H, Py-C$\mathbf{H}_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:165.4, 148.5, 147.8, 142.5, 132.7, 126.6, 125.4, 125.1, 123.6, 122.5, 115.4, 114.6, 111.3; IR (KBr, cm^{-1}) υ:3100.0, 2929.6, 2842.9, 1766.5, 1640.9, 1581.1, 1524.2, 1452.5, 1362.8, 1297.0, 1243.2, 1162.5, 1120.6, 1057.8, 1030.9, 977.1, 896.4, 833.6, 797.7, 737.9; ESI-Mass for $C_{17}H_{17}NO_3S$: *m/z* (M^+−H) 314.17.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(3-hydroxy-3-methoxyphenyl)prop-2-en-1-one (4h)

White solid,yield 42.1%,m.p.176.3~177.8 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.62 (d, *J* = 15.3 Hz, 1H, ArC**H=**), 7.20 (d, *J* = 2.1 Hz, 1H, Ar**H**), 7.15 (d, *J* = 5.4 Hz, 1H, Th**H**), 7.04 (dd, J_1=2.1 Hz, J_2=8.4 Hz, 1H, Ar**H**), 6.85–6.77 (m, 3H, **–CH=,** Th**H and ArH**), 4.76 (s, 2H, Py-C$\mathbf{H}_2$), 4.02 (m, 1H, Py-C$\mathbf{H}_2$), 3.92 (s, 4H, Py-C$\mathbf{H}_2$ and OC$\mathbf{H}_3$), 2.94(br s, 2H, Py-C$\mathbf{H}_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:166.4, 157.0, 148.3, 145.9, 143.0, 128.7, 125.2, 124.5, 123.5, 121.6, 115.2, 112.7, 110.6, 56.0, 45.9, 43.3, 24.9; IR (KBr, cm^{-1}) υ:3091.0, 2929.6, 2845.9, 1634.9, 1578.1, 1524.3, 1425.6, 1312.0, 1261.1, 1219.3, 1126.6, 1051.8, 1027.9, 977.1, 803.7, 737.9; ESI-Mass for $C_{17}H_{17}NO_3S$: *m/z* (M^+−H) 314.17.

(*E*)-1-(6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-en-1-one (4i)

Off-white solid,yield 47.8%,m.p.155.7~156.4 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.64 (d, *J* = 15.0 Hz, 1H, ArC**H=**), 7.16 (d, *J* = 5.1 Hz, 1H, Th**H**), 6.83 (d, *J* = 5.1 Hz, 1H, Th**H**), 6.81~6.76 (m, 3H, -C**H=** and Ar**H**), 4.77 (s, 2H, Py-C$\mathbf{H}_2$), 3.98 (t, *J* = 5.4 Hz, 2H, Py-C$\mathbf{H}_2$), 3.94 (s, 6H, OC$\mathbf{H}_3$ × 2), 2.96(t, *J* = 5.1 Hz, 2H, Py-C$\mathbf{H}_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:166.3, 157.0, 147.2, 143.5, 136.7, 132.3, 126.7, 125.2, 124.6, 123.6, 115.1, 104.9, 56.4, 46.1, 43.3, 25.0; IR (KBr, cm^{-1}) υ:3100.0, 2929.6, 2839.8, 1643.9, 1605.0, 1512.3, 1455.5, 1428.6, 1335.9, 1264.1, 1210.3, 1150.5, 1108.6, 1057.8, 974.1, 905.3, 824.6, 705.0; ESI-Mass for $C_{18}H_{19}NO_4S$: *m/z* (M^+−H) 344.59.

(*E*)-5-(3-(3,4,5-trimethoxyphenyl)acryloyl)-5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4*H*)-one (5a)

Compound **5a** was synthesized following the same procedure described above for the preparation of **4a**.

Yellow solid, 51.6%,m.p.87.4~89.6 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.52 (d, *J* = 15.3 Hz, 1H, ArC**H**=), 6.69–6.64 (m, 3H, Ar**H** and–C**H=**), 6.12 (s, 1H, Th**H**), 4.35–4.29 (m, 1H, Py-C$\mathbf{H}_2$), 4.20 (s, 1H, Py-C**H**), 3.82 (s, 9H, OC$\mathbf{H}_3$ × 3), 3.80(s, 2H, Py-C$\mathbf{H}_2$), 3.37 (br s, 1H, Py-C$\mathbf{H}_2$), 2.46 (br s, 2H, Py-C$\mathbf{H}_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:197.8, 165.9, 153.4, 144.4, 144.2, 139.9, 130.3, 127.3, 115.4, 105.2, 61.0, 56.3, 51.0, 44.5, 42.9, 22.6; IR (KBr, cm-1) υ:2938.5, 2839.4, 1682.5, 1644.3, 1583.7, 1505.5, 1455.2, 1418.6, 1333.4, 1270.1, 1246.1, 1188.6, 1125.5, 1002.7, 976.8, 825.9, 779.9, 646.9; ESI-Mass for $C_{19}H_{21}NO_5S$: *m/z* (M^++H) 376.10.

(*E*)-5-(3-(3,4,5-trimethoxyphenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6a)

To the solution of **5a** (2.00 g, 5.33 mmol), acetic anhydride (1.5 mL) in acetonitrile (40 mL), Et_3N (1.5 mL) was added. The reaction mixture was stirred at room temperature for 5 h, then evaporated in vacuo. The residue was solved in DCM and washed with water. The organic phase was dried over sodium sulphate and evaporated in vacuo to give the crude product. The crude product was purified on silica using hexanes/ethyl acetate 20–33% to provide the title compound as an off- white solid (1.36 g, 61.3%), m.p.121.8~123.7 °C; 1H NMR (CDCl3, 400 MHz) δ: 7.61 (d, J = 15.2 Hz, 1H, ArCH=), 6.81–6.72 (m, 3H, ArH and -CH=), 6.20 (s, 1H, ThH), 4.66 (s, 2H, Py-CH2), 4.00 (br s, 2H, Py-CH2), 3.91 (s, 6H, OCH_3 × 2), 3.88 (s, 3H, OCH3), 2.85 (br s, 2H, Py-CH2), 2.30 (s, 3H, CH3); 13C NMR (CDCl3, 100 MHz) δ:167.8, 166.0, 153.4, 150.1, 143.2, 139.6, 130.8, 116.8, 116.4, 111.7, 111.4, 105.1, 61.0, 56.2, 45.7, 43.1, 25.4, 20.7; IR (KBr, cm-1) υ:2938.5, 2836.9, 1763.5, 1643.9, 1587.0, 1500.3, 1422.6, 1335.9, 1273.1, 1186.4, 1126.6, 1057.8, 1004.0, 884.4, 827.6, 722.9; ESI-Mass for C21H23NO6S: *m/z* (M^++H) 418.01.

Compounds **6b-e** were synthesized following the same procedure described above for the preparation of **6a**.

(*E*)-5-(3-(4-methoxyphenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6b)

Off- white solid,yield 63.6%,m.p.126.3~127.4 °C;^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.68 (d, J = 15.3 Hz, 1H, ArC**H**=), 7.50 (d, J = 8.7 Hz, 2H, Ar**H**), 6.90 (d, J = 8.7 Hz, 2H, Ar**H**), 6.79 (d, J = 15.3 Hz, 1H, -C**H**=), 6.41 (s, 1H, Th**H**), 4.65 (s, 2H, Py-C$\mathbf{H}_2$), 3.96 (t, J = 5.4 Hz, 2H, Py-C$\mathbf{H}_2$), 3.83 (s, 3H, OC$\mathbf{H}_3$), 2.84(t, J = 5.4 Hz, 2H, Py-C$\mathbf{H}_2$), 2.29 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:167.7, 166.3, 160.9, 150.0, 142.8, 129.4, 127.9, 115.0, 114.6, 114.2, 111.8, 111.3, 55.4, 45.6, 43.0, 25.4, 20.7; IR (KBr, cm^{-1}) υ:2938.5, 2839.9, 1757.5, 1643.9, 1599.0, 1503.3, 1440.5, 1371.8, 1303.0, 1252.2, 1207.3, 1144.5, 1030.9, 977.1, 926.3, 890.4, 818.6, 728.9; ESI-Mass for $C_{19}H_{19}NO_4S$: *m/z* (M^++H) 357.98.

(*E*)-5-(3-(4-methylphenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6c)

Off- white solid,yield 57.2%,m.p.117.2~118.6 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68 (d, J = 15.2 Hz, 1H, ArC**H**=), 7.44 (d, J = 8.0 Hz, 2H, Ar**H**), 7.19 (d, J = 7.6 Hz, 2H, Ar**H**), 6.87 (d, J = 15.6 Hz, 1H, -C**H**=), 6.41 (s, 1H, Th**H**), 4.66 (s, 2H, Py-C$\mathbf{H}_2$), 3.96 (br s, 2H, Py-C$\mathbf{H}_2$), 2.84(br s, 2H, Py-C$\mathbf{H}_2$), 2.37 (s, 3H, C$\mathbf{H}_3$), 2.29 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:167.7, 166.2, 150.1, 143.1, 140.0, 132.4, 129.5, 127.8, 116.4, 116.1, 111.7, 111.3, 45.6, 43.0, 25.4, 21.4, 20.7; IR (KBr, cm^{-1}) υ:2935.6, 2896.7, 2848.8, 1757.5, 1646.8, 1596.0, 1494.4, 1434.6, 1365.8, 1219.3, 1144.5, 1042.9, 983.1, 893.4, 812.6; ESI-Mass for $C_{19}H_{19}NO_3S$: *m/z* (M^++H) 342.07.

(*E*)-5-(3-(4-chlorophenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6d)

Off- white solid,yield 62.5%,m.p.115.4~116.2 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.65 (d, J = 15.6 Hz, 1H, ArC**H**=), 7.47 (d, J = 8.7 Hz, 2H, Ar**H**), 7.35 (d, J = 8.7 Hz, 2H, Ar**H**), 6.89 (d, J = 15.3 Hz, 1H, -C**H**=), 6.41 (s, 1H, Th**H**), 4.65 (s, 2H, Py-C$\mathbf{H}_2$), 3.96 (br s, 2H, Py-C$\mathbf{H}_2$), 2.84(br s, 2H, Py-C$\mathbf{H}_2$), 2.29 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:167.7, 165.7, 150.1, 141.7, 135.5, 133.7, 129.1, 124.8, 118.1, 117.8, 111.7, 111.3, 45.7, 43.0, 25.4, 20.7; IR (KBr, cm^{-1}) υ:2920.6, 2866.8, 1748.5, 1643.9, 1604.9, 1584.1, 1503.3, 1428.6, 1362.8, 1332.9, 1225.3, 1204.3, 1150.5, 1087.7, 1042.8, 971.1, 893.4, 812.6; ESI-Mass for $C_{18}H_{16}ClNO_3S$: *m/z* (M^++H) 361.96.

(*E*)-5-(3-(4-nitrophenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6e)

Off- white solid,yield 32.1%,m.p.127.6~128.3 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.24 (d, J = 8.7 Hz, 2H, Ar**H**), 7.74–7.67 (m, 3H, ArC**H**= and Ar**H**),7.10–7.05 (m, 1H, -C**H**=), 6.42 (s, 1H, Th**H**), 4.67 (s, 2H, Py-C$\mathbf{H}_2$), 4.00 (t, J = 12.0 Hz, 2H, Py-C$\mathbf{H}_2$), 2.87 (br s, 2H, Py-C$\mathbf{H}_2$), 2.30 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:167.7, 165.0, 150.3, 148.1, 141.4, 140.2, 128.4, 126,8, 124.1, 122.0, 121.6, 111.7, 45.8, 43.1, 25.4, 20.7; IR (KBr, cm^{-1}) υ: 2930.3, 2895.2, 2840.6, 1754.8, 1645.9, 1607.5, 1508.2, 1440.9, 1203.9, 1104.6, 1059.8, 960.5, 838.8, 755.5; ESI-Mass for $C_{18}H_{16}N_2O_5S$: *m/z* (M^++H) 373.00.

(*E*)-3-(4-acetoxyphenyl)acrylic acid (7a)

To the solution of (E)-3-(4-hydroxyphenyl)acrylic acid (6.00 g, 36.6 mmol) in Ac_2O (12 mL),pyridine (2 mL) was added. The reaction mixture was stirred at room temperature for 3.5 h. Water was added and filtered. The filter was solved in water. Then saturated Na_2CO_3 (liq.) was added to pH = 10. The mixture was filtered. The filtrate was acidified with con.HCl to pH = 3, then solid appears. The mixture was filtered, and the crude product was obtained. White solid, 6.17 g,yield 81.9%,m.p.216.4~218.7.

(*E*)-4-(3-(2-acetoxy-6,7-dihydrothieno[3,2-c]pyridin-5(4*H*)-yl)-3-oxoprop-1-en-1-yl)phenyl acetate (7b)

Off-white solid, yield 47.6%,m.p.103.6~106.2 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.68 (d, J = 15.6 Hz, 1H, ArC**H**=), 7.55 (d, J = 8.7 Hz, 2H, Ar**H**), 7.11 (d, J = 8.7 Hz, 2H, Ar**H**), 6.87 (d, J = 15.3 Hz, 1H, -C**H**=), 6.41 (s, 1H, Th**H**), 4.65 (s, 2H, Py-C$\mathbf{H}_2$), 3.95 (br s, 2H, Py-C$\mathbf{H}_2$), 2.84(br s, 2H, Py-C$\mathbf{H}_2$), 2.31 (s, 3H, C$\mathbf{H}_3$), 2.29 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:169.2, 167.7, 165.9, 151.6, 150.1, 142.0, 133.0, 128.9, 122.0, 117.8, 117.4, 111.8, 111.3, 45.7, 43.0, 25.4, 21.2, 20.7; IR (KBr, cm^{-1})

υ:3068.0, 2927.1, 2839.9, 1751.6, 1645.9, 1613.9, 1585.1, 1424.9, 1370.5, 1047.0, 909.2, 896.4, 848.4; ESI-Mass for $C_{20}H_{19}NO_5S$: *m/z* (M^++H) 386.00.

Compounds **6g-i** were synthesized following the same procedure described above for the preparation of **6f**.

(*E*)-5-(3-(4-acetoxy-3-methoxyphenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6g)

Off-white solid, yield 51.2%,m.p.130.1~130.9 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.65 (d, *J* = 15.6 Hz, 1H, ArC**H**=), 7.17 (dd, J_1 = 1.5 Hz, J_2 = 8.1 Hz, 1H, Ar**H**), 7.09–7.03 (m, 2H, Ar**H**), 6.84 (d, *J* = 15.3 Hz, 1H, -C**H**=), 6.42 (s, 1H, Th**H**), 4.65 (s, 2H, Py-C$\mathbf{H}_2$), 3.96 (br s, 2H, Py-C$\mathbf{H}_2$), 3.88 (s, 3H, OC$\mathbf{H}_3$), 2.85(br s, 2H, Py-C$\mathbf{H}_2$), 2.33 (s, 3H, C$\mathbf{H}_3$), 2.29 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:168.8, 167.7, 165.8, 151.3, 150.1, 142.3, 140.9, 134.2, 128.4, 124.8, 123.1, 120.4, 117.9, 117.6, 111.6, 55.9, 45.7, 43.0, 25.4, 20.7, 20.6; IR (KBr, cm^{-1}) υ:3068.0, 3010.3, 2930.3, 2869.4, 1767.6, 1649.1, 1613.9, 1591.5, 1511.4, 1456.9, 1399.3, 1300.0, 1255.2, 1123.8, 1047.0, 973.3, 890.0, 822.8, 745.9; ESI-Mass for $C_{21}H_{21}NO_6S$: *m/z* (M^++H) 416.00.

(*E*)-5-(3-(3-acetoxy-4-methoxyphenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6h)

Off-white solid, yield 37.1%,m.p.140.6~142.2 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.62 (d, *J* = 15.3 Hz, 1H, ArC**H**=), 7.38 (dd, J_1 = 2.1 Hz, J_2 = 8.4 Hz, 1H, Ar**H**), 7.27 (s, 1H, Ar**H**), 7.96 (d, *J* = 8.4 Hz, 1H, Ar**H**), 6.77 (d, *J* = 14.4 Hz, 1H, -C**H**=), 6.41 (s, 1H, Th**H**), 4.65 (s, 2H, Py-C$\mathbf{H}_2$), 3.99 (br s, 2H, Py-C$\mathbf{H}_2$), 3.86 (s, 3H, OC$\mathbf{H}_3$), 2.84 (br s, 2H, Py-C$\mathbf{H}_2$), 2.34 (s, 3H, C$\mathbf{H}_3$), 2.29 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ:168.9, 167.7, 165.9, 151.3, 150.1, 142.4, 140.9, 134.2, 128.4, 123.2, 120.4, 117.9, 117.5, 111.6, 56.0, 45.7, 43.0, 25.4, 20.7; IR (KBr, cm^{-1}) υ: 3093.6, 3055.2, 2923.8, 2843.8, 1767.6, 1649.1, 1604.3, 1511.4, 1434.5, 1364.1, 1300.0, 1268.0, 1127.1, 1053.4, 1027.8, 886.9, 822.8; ESI-Mass for $C_{21}H_{21}NO_6S$: *m/z* (M^++H) 416.00.

(*E*)-5-(3-(4-acetoxy-3,5-dimethoxyphenyl)acryloyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (6i)

White solid, yield 41.7%,m.p.136.8~137.7 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.61 (d, *J* = 15.3 Hz, 1H, ArC**H**=), 6.82 (d, *J* = 15.3 Hz, 1H, -C**H**=), 6.76 (s, 2H, Ar**H**), 6.43 (s, 1H, Th**H**), 4.66 (s, 2H, Py-C$\mathbf{H}_2$), 3.97 (br s, 2H, Py-C$\mathbf{H}_2$), 3.86 (s, 6H, OC$\mathbf{H}_3$×2), 2.85(br s, 2H, Py-C$\mathbf{H}_2$), 2.35 (s, 3H, C$\mathbf{H}_3$), 2.30 (s, 3H, C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 168.6, 167.7, 165.8, 152.3, 150.1, 142.9, 133.6, 129.9, 118.0, 117.5, 111.7, 111.3, 104.5, 56.2, 45.7, 43.0, 24.2, 20.7, 20.5; IR (KBr, cm^{-1}) υ: 3074.4, 3007.1, 2978.3, 2943.1, 2847.0, 1761.2, 1642.7, 1604.3, 1505.0, 1460.1, 1421.7, 1370.5, 1338.4, 1194.3, 1130.3, 1063.0, 1011.7, 883.6, 832.4, 794.7; ESI-Mass for $C_{22}H_{23}NO_7S$: *m/z* (M^++H) 446.00.

Pharmacology

Each chemical compound was dissolved in 20% dimethyl sulfoxide (DMSO) in water. The artery blood sample collected from Male New Zealand rabbit was mixed with just enough sodium citrate with a concentration 3.8% to prevent clotting, and centrifuged at room temperature for 5 min at 800 rpm to give platelet-rich plasma (PRP). The remaining blood was further centrifuged for 15 min at 599.4 *g* to give platelet-poor plasma (PPP). Aspirin and Piplartine were used as reference. The tested target compounds were given at 0.8, 4, 20, 100, 500 μg/mL separately. A 20 μL of blank solvent or test compounds was added to 170 μL of PRP, and after 5 min, the aggregation was initiated by adding 10 μL of ADP (4 μmol/L), in each well of 96-well plates, respectively. Optical density (OD) at 630 nm was measured with Microplate Reader (BioTek ELX800) after 1, 2, 4, 6 min of the addition. All the experiments were performed in triplicate. The platelet aggregation rate was expressed as PAR (%) and was calculated as follows:

$$\text{PAR}\,(\%) = (1 - \text{OD1/OD0}) \times 100\%.$$

Here, OD0 and OD1 indicated the optical density before the ADP was added and after the ADP was added, respectively. The following equation was used to calculate the inhibition rate of *platelet* aggregation (IR).

$$\text{IR}\,(\%) = (1 - \text{PAR test/PAR blank}) \times 100\%.$$

Here, PAR test and PAR blank represented the PAR of the tested compounds and the blank solvent, respectively.

Table 2: The inhibitory activities of **4a-4i** and **6a-6i** against platelet aggregation induced by AA or ADP (*n* = 4) *in vitro*

	IC_{50}/mM	
Compd	AA	ADP
4a	20.751 ± 1.450	6.710 ± 0.697
4b	7.794 ± 0.390	13.085 ± 1.943
4c	259.381 ± 16.148	41.483 ± 2.610
4d	13.234 ± 1.130	8.428 ± 0.733
4e	**0.130 ± 0.023**	**0.403 ± 0.080**
4f	21.520 ± 1.323	17.602 ± 1.443
4g	2.138 ± 0.243	0.310 ± 0.070
4h	0.403 ± 0.066	7.012 ± 0.374
4i	0.332 ± 0.076	2.806 ± 0.778
6a	21.556 ± 1.313	4.484 ± 0.567
6b	21.962 ± 1.192	14.366 ± 1.316
6c	10.127 ± 0.703	0.241 ± 0.077
6d	4.671 ± 0.323	0.466 ± 0.071
6e	**0.108 ± 0.014**	**3.145 ± 0.720**
6f	16.005 ± 1.110	3.185 ± 0.453
6g	26.092 ± 1.832	2.309 ± 0.730
6h	85.418 ± 3.718	1.710 ± 0.479
6i	13.246 ± 1.184	0.077 ± 0.013
Piperlongumine	5.489 ± 0.348	11.121 ± 0.790
Aspirin	4.431 ± 0.319	12.588 ± 1.313

Bold indicates compounds 4e and 6e exhibit best platelet aggregation inhibit rate.

The ability of the tested compounds to inhibit platelet aggregation was evaluated by $IC_{50,}$ calculated from the inhibition ratio linearity regression equation, which was fitted based on the inhibition ratio.

Results and Discussion

The synthesized piperlongumine derivatives were evaluated for their inhibitory effect on AA/ADP-induced platelet aggregation by Born's Method (14) and liver microsomal incubated assay *in vitro* (15), while aspirin and piperlongumine were used as a positive control. The assay results are summarized in Table 2. The results demonstrate that compared with positive control, all the target compounds had moderate IC_{50} value. Compound **6a**-**e** showed better inhibition effect on platelet aggregation induced by AA. It was speculated that these may be due to the elctro-withdraw effect of the substituent group. Especially, compounds **4e**, **6e** display obviously superior inhibition effect on platelet aggregation induced by AA/ADP.

Conclusion

In summary, 18 piperlongumine derivatives were synthesized and evaluated for their inhibitory effect on AA/ADP-induced platelet aggregation. The assay results indicated that **4e**, **6e** exhibited comparable potency with that of aspirin and piperlongumine.

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