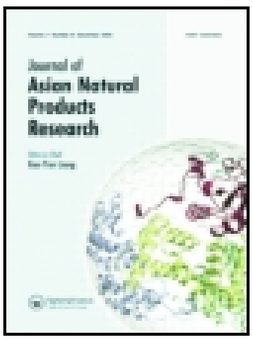

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Synthesis of phthalide derivatives and evaluation on their antiplatelet aggregation and antioxidant activities

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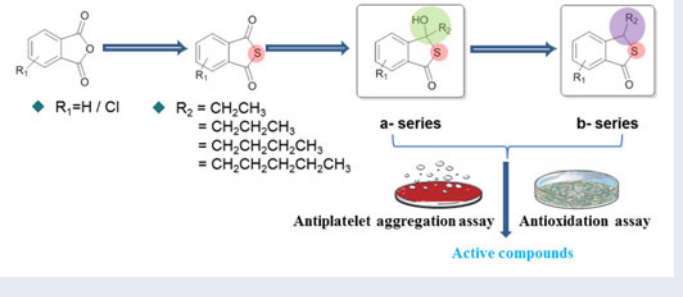
ABSTRACT

As part of our continuing efforts to discover structurally interesting bioactive phthalide derivatives, 23 of them with a structure incorporating thiophen or halogens were designed and synthesized, 17 of which are previously unreported. *In vitro* antiplatelet aggregation activity screening showed that **14b** could significantly inhibit platelet aggregation induced by arachidonic acid, compared with edaravone ($p < 0.01$). Meanwhile, oxidative damage models using SH-SY5Y and PC12 cells induced by H_2O_2 were built to evaluate the antioxidant activity of the phthalide derivatives. In SH-SY5Y cells, compared with aspirin, **1a** significantly increased the relative cell survival rate ($p < 0.05$). Compared with edaravone, **1a** ($p < 0.01$) and **15b** ($p < 0.05$) significantly increased the relative cell survival rate. In PC12 cells, **1a** ($p < 0.01$), **15b** ($p < 0.01$), and **12a** ($p < 0.05$) remarkably increased the cell survival rate compared with edaravone. The present study identified lead structures to develop potential anti-ischemic stroke agents.



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Phthalide derivative; antiplatelet aggregation; antioxidation

1. Introduction

Phthalides are widely distributed in Umbelliferae plants, such as *Ligusticum chuanxiong* and *Angelica sinensis*, and are usually modified by C-3 alkyl substitutions,

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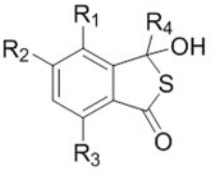
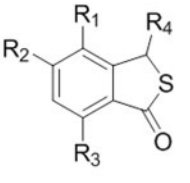
unsaturated benzene ring substitutions, and dimers. Phthalides are considered as a large class of characteristic compounds in these plants [1, 2]. In addition, a large number of phthalides are also found in the metabolites of fungi and molds, such as *Phomopsis archeri* [3] and *Anthocleista djalonensis* [4]. Phthalides possess various pharmacological activities, such as anticoagulation, antiplatelet aggregation, antioxidant, anti-inflammation, antimicrobial, and anti-tumor effects [5–11]. As a representative phthalide, *n*-butylphthalide (NBP) is used as a listed drug to treat ischemic stroke in China, and can reduce ischemic brain injury by improving blood fluidity, and through anti-thrombotic and anti-inflammatory effects [12]. In recent years, our research group has studied the effectiveness and mechanism of a phthalide derivative, senkynolide I. The results suggested that senkynolide I possesses an analgesic effect, could decrease plasma and intracerebral nitric oxide (NO) levels, and improved cerebral edema and cerebral infarction volume in rats with focal cerebral ischemia reperfusion injury. Unfortunately, none of these effects are strong enough for clinical use [13–15]. Meanwhile, 3-substituted benzo[*c*]thiophen-1(3*H*)-ones and phthalides with halogens had been reported previously which showed superior effects to NBP [16]. Therefore, as part of our continuing efforts to discover structurally interesting bioactive phthalide derivatives, 23 phthalide derivatives with structures incorporating thiophen or halogens were designed and synthesized (Figure 1).



a- series

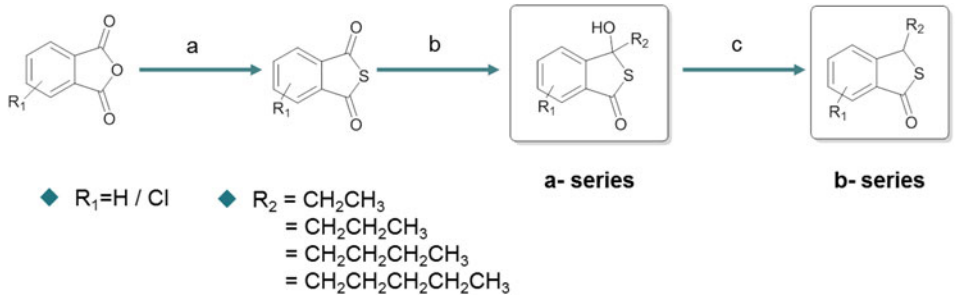


b- series

	a- series	b- series
1	R_1 = H, R_2 = H, R_3 = H, R_4 = butyl	R_1 = H, R_2 = H, R_3 = H, R_4 = butyl
2	R_1 = H, R_2 = H, R_3 = H, R_4 = isopropyl*	R_1 = H, R_2 = H, R_3 = H, R_4 = isopropyl*
3	R_1 = H, R_2 = Cl, R_3 = H, R_4 = butyl*	R_1 = H, R_2 = Cl, R_3 = H, R_4 = butyl*
4	R_1 = Cl, R_2 = H, R_3 = H, R_4 = butyl*	R_1 = Cl, R_2 = H, R_3 = H, R_4 = butyl*
5	-	R_1 = H, R_2 = H, R_3 = H, R_4 = ethyl
6	R_1 = H, R_2 = H, R_3 = H, R_4 = pentyl	R_1 = H, R_2 = H, R_3 = H, R_4 = pentyl
7	-	R_1 = H, R_2 = Cl, R_3 = H, R_4 = pentyl*
8	R_1 = H, R_2 = Cl, R_3 = H, R_4 = isopropyl*	R_1 = H, R_2 = Cl, R_3 = H, R_4 = isopropyl*
9	-	R_1 = H, R_2 = H, R_3 = Cl, R_4 = pentyl*
10	-	R_1 = H, R_2 = H, R_3 = Cl, R_4 = isopropyl*
11	-	R_1 = H, R_2 = H, R_3 = H, R_4 = propyl
12	R_1 = H, R_2 = Cl, R_3 = H, R_4 = propyl*	R_1 = H, R_2 = Cl, R_3 = H, R_4 = propyl*
13	R_1 = Cl, R_2 = H, R_3 = H, R_4 = propyl*	R_1 = Cl, R_2 = H, R_3 = H, R_4 = propyl*
14	-	R_1 = H, R_2 = Cl, R_3 = H, R_4 = ethyl*
15	-	R_1 = Cl, R_2 = H, R_3 = H, R_4 = ethyl*

"-" No corresponding compounds obtained; "*" Compounds not reported in literature.

Figure 1. The structures of phthalide derivatives.



Scheme 1. Rationale design of the title compounds. Reagents and conditions: (a) $Na_2S{\cdot}9H_2O$, THF, room temp, 4 h; (b) R_2MgX, THF, −5 °C, 0.5 h, and then room temp, 5 h; (c) AcOH, aqueous HI (57%), reflux, 0.5 h. THF, tetrahydrofuran.

2. Results and discussion

2.1. Chemistry

The synthesis of the a and b series of target compounds is outlined in Scheme 1. Starting from phthalic anhydride, 4-chloro phthalic anhydride, and 3-chloro phthalic anhydride, three intermediates of benzo[c]thiophene-1,3-dione **2** were prepared at 50–80% yield by reacting them with $Na_2S{\cdot}9H_2O$. Then, a series of target products were synthesized by treatment with corresponding Grignard reagents to introduce different alkyls and hydroxyls to the C-3 lactone ring. Finally, the b series of target products were obtained using a one-pot reaction comprising dehydration and reduction (Scheme 1).

2.2. Antiplatelet aggregation activity

Thromboembolism is associated with the pathophysiological process of cerebral ischemia, and previous studies have shown that platelets are an important factor affecting thrombosis [16–18]. Platelet status is an important parameter when evaluating the severity of cerebral ischemia [19]. Thus, all the compounds obtained were evaluated for their activity against platelet aggregation induced by arachidonic acid (AA) and adenosine diphosphate (ADP). The screening results showed that the phthalide derivatives selectively inhibited the platelet aggregation induced by ADP and AA (Supporting Information Table 3). The structure-activity analysis of the two inducers showed that the electronegativity, length, and space steric resistance of the C-3 substituents greatly influenced their activity; but no substitution of OH and smaller alkyl hindrance in C-3 benefited the antiplatelet aggregation activity. The results of ADP experiment showed that chloro-substituted phthalides had no marked influence on their activity, regardless of the substitution position. The results of AA experiment showed that, compared with the positive control drug Eda, **14b** could significantly inhibit platelet aggregation (Table 1). The electron-withdrawing group chlorine on the benzene ring might be beneficial to the antiplatelet aggregation activity; however, it was not influenced by the substitution site. Compared with the results in the literature [16], the bioactivities measured were not consistent well with each other, and the reason may be related to different measurement methods.

Table 1. Effect of phthalide derivatives on platelet aggregation and oxidative damage of SH-SY5Y/PC 12 cells induced by H_2O_2. ($\bar{x} \pm s$, $n = 3$).

	Aggregation inhibition rate (%)		Cell relative survival rate (%)	
NO.	ADP	AA	SH-SY5Y	PC 12
Asp	72.8 ± 1.1	56.0 ± 4.0	85.9 ± 4.9	88.2 ± 0.3
Eda	80.0 ± 4.1	50.2 ± 3.2	74.7 ± 6.7	56.8 ± 2.3
NBP	48.9 ± 7.8	23.3 ± 10.9	57.6 ± 4.5	46.5 ± 5.1
1a	33.4 ± 4.7	0.2 ± 3.9	97.1 ± 8.8$^{*,\#\#}$	74.5 ± 4.1$^{\#\#}$
12a	14.6 ± 7.8	15.6 ± 9.8	69.1 ± 7.3	67.4 ± 5.9$^{\#}$
14b	−8.0 ± 7.3	65.1 ± 5.5**	56.0 ± 7.5	47.8 ± 2.8
15b	54.5 ± 0.9	44.5 ± 7.1	92.7 ± 5.8$^{\#}$	93.8 ± 3.7$^{\#\#}$

$^{**}p < 0.01$ vs. Edaravone (Eda);
$^{*}p < 0.05$ vs. Asp,
$\#p < 0.05$,
$\#\#p < 0.01$ vs. Eda.
SPSS 21.0 software was used for data processing and one-way ANOVA was used for statistical analysis.

2.3. Antioxidation activity

As one of the important factors involved in neuronal injury, oxidative stress is associated with the pathophysiological processes of various neurological diseases, and is one of the main research strategies for anti-stroke treatment [20]. H_2O_2, a toxic byproduct of aerobic metabolism, can cross the cell membrane and participate in the Fenton reaction to generate the most active free radical, OH^-, result a series of chain reactions [21]. Oxidative damage models comprising SH-SY5Y and PC12 cells induced by H_2O_2 were used to evaluate the antioxidant activity of the compounds. The relative cell survival rate was determined using the cell counting kit-8 (CCK-8) method, with edaravone (Eda) and aspirin (Asp) as positive controls. The compounds exhibited some selective protection in both models (Supporting Information Table 3). The results of the SH-SY5Y cell experiment showed that compared with Asp, **1a** significantly increased the relative cell survival rate ($p < 0.05$). Compared with Eda, **1a** very significantly increased the relative cell survival rate ($p < 0.01$) and **15b** significantly increased the relative cell survival rate ($p < 0.05$) (Table 1). The length and steric hindrance of the C-3 side chain greatly influenced the oxidation activity, with ethyl and propyl groups showing better activity. In addition, the activity decreased with increasing alkyl side chain length. The results of the PC12 cell experiment showed that compared with Eda, **1a** and **15b** very significantly increased the relative cell survival rate ($p < 0.01$) and **12a** significantly increased the relative cell survival rate ($p < 0.05$) (Table 1). No explicit structure-activity relationships were observed.

In conclusion, 23 phthalide derivatives were designed and synthesized, among which seventeen were unreported. *In vitro* antiplatelet aggregation and antioxidant models were successfully constructed to evaluate the activity of the phthalide derivatives. The screening results indicated that the phthalide derivatives displayed selective inhibition of platelet aggregation induced by ADP and AA, and selective protection from oxidative damage induced by H_2O_2 in SH-SY5Y and PC12 cells. The two sets of experiments revealed the following structure-activity rules: Substituting ethyl and propyl for C-3 alkyl side chains was beneficial to the compound's activity; the substitution of C-3 hydroxyl reduced the original activity and

increased the structural instability. The introduction of electron-absorbing chlorine atoms on the benzene ring had no clear guiding significance for the change in activity, and was independent of the replacement position. The substitution of 2-sulfur atoms tended to increase the antioxidant activity, and most of the derivatives were superior to butylphthalide, which indicated a direction for further structural modification.

3. Experimental

3.1. General experimental procedures

Bruker Spectrospin AC-300P/AC 300 MHz spectrometers and Bruker AVANCE III 400 MHz spectrometers (Bruker, Reinstetten, Germany) recorded the corresponding nuclear magnetic resonance (NMR) spectra. Chemical shifts are indicated in δ-values (ppm) in relation to tetramethylsilane (TMS) used as an internal standard. The electrospray ionization mass spectrometry (ESIMS) was measured on an Aglilent LC/MS-6210 mass spectrometer (Aglilent Technologies Inc., California, USA). Semi-preparative high performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-6AD HPLC instrument (Shimadzu, Kyoto, Japan) with a Shim-pack Prep-octadecyl silica (ODS) column (Shimadzu, Kyoto, Japan, NO. 2025B41). Thin-layer chromatography (TLC) was performed on precoated silica gel plate (SGF_{254}, 0.2 mm, Yantai Chemical Industry Research Institute, Yantai, China). Flash column chromatography was performed using silica gel (200–300 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China). All solvents and reagents were of analytical pure grade, and no further purification was needed. All starting materials were commercially available.

3.2. Synthesis

3.2.1. Preparation of intermediates

I. Phthalic anhydride (2.96 g, 20 mmol) was dissolved in 4 ml tetrahydrofuran (THF); 4 ml $Na_2S{\cdot}9H_2O$ (2.40 g, 10 mmol) water solution was added one drop at a time. During the process, the reaction solution turned light yellow, and a small amount of white solids were precipitated. At room temperature, stirring was continued for 4 hours. 20 ml H_2O was added to the reaction solution to quench, and a large amount of white solids were precipitated out. 5 ml 2 mol/L HCl was adjusted to pH 6, and 1.27 g white crystals were extracted, with a yield of 85%.

II. Using 4-chloro-phthalic anhydride (3.65 g, 20 mmol) as raw material, 1.60 g of white powder was obtained with the yield of 87.6%, following the above method.

III. Using 3-chloro-phthalic anhydride (3.65 g, 20 mmol) as raw material, 1.40 g grey powder was obtained with a yield of 76.7%, following the above method.

3.2.2. Preparation of a-series derivatives

Because the synthesis steps were very similar, **2a** was taken as an example. Dry phthalic anhydride (4.50 g, 27.44 mmol) was dissolved in 40 ml THF, slowly drop with isopropyl magnesium chloride (anhydrous THF solution, 28 ml, 60.36 mmol) below $-5\,^{\circ}C$, until the reaction fluid changed from yellow to orange. The reaction

was continued at low temperature for 0.5 h, and moved to room temperature for 5 h. The reaction was quenched with a saturated ammonium chloride solution below 0 °C. The reaction mixture was acidified to pH 2 with 10% HCl, and extracted with EtOAc, then dried with anhydrous Na_2SO_4. Yellow oily products were obtained by chromatographic separation with a yield of 30%. The yield of this reaction step is generally 20–30%.

3.2.2.1. 3-Butyl-3-hydroxybenzo[c]thiophen-1(3H)-one (compound 1a). $C_{12}H_{14}O_2S$, yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.50–7.62 (3H, m, ArH), 7.39 (1H, t, $J = 7.2$ Hz, ArH), 4.62 (1H, s, OH), 2.30 (1H, td, $J = 4.5$, 13.8 Hz, CH_2), 2.03 (1H, td, $J = 4.2$, 12.3 Hz, CH_2), 1.54–1.58 (1H, m, CH_2), 1.22–1.32 (2H, m, CH_2), 1.04–1.15 (1H, m, CH_2), 0.83 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 195.8, 153.0, 134.8, 134.3, 129.8, 123.8, 123.3, 95.9, 42.5, 27.8, 22.6, 13.9. ESI-MS *m/z*: 221.8 $[M\text{-}H]^-$. The spectroscopic data of **1a** were in agreement with those of known compound reported in literatures [16].

3.2.2.2. 3-Hydroxy-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 2a). $C_{11}H_{12}O_2S$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.60 (3H, m, ArH), 7.43 (1H, t, H-4, ArH), 3.98 (1H, s, OH), 2.47–2.56 (1H, m, CH), 1.23 (3H, d, $J = 6.3$ Hz, CH_3), 0.63 (3H, d, $J = 6.6$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 196.4 (C-1), 152.9 (C-3a), 135.2 (C-1), 134.4 (C-5), 129.9 (C-4), 123.6 (C-7a), 123.2 (C-6), 100.7 (C-3), 77.6 (C-1), 77.2 (C-1), 76.7 (C-1), 38.8 (C-8), 19.2 (C-10), 17.8 (C-9). ESI-MS *m/z*: 209 $[M + H]^+$, 231 $[M + Na]^+$.

3.2.2.3. 3-Butyl-5-chloro-3-hydroxybenzo[c]thiophen-1(3H)-one (compound 3a). $C_{12}H_{13}ClO_2S$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.58 (2H, d, $J = 8.1$ Hz, ArH), 7.45 (1H, d, $J = 8.1$ Hz, ArH), 3.76 (1H, s, OH), 2.30–2.39 (1H, m, CH_2), 2.02–2.11 (1H, m, CH_2), 1.10–1.44 (4H, m, 2 × CH_2), 0.89 (3H, t, CH_3, $J = 6.9$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 194.2, 154.6, 141.3, 133.2, 130.4, 124.4, 124.3, 95.1, 42.5, 27.9, 22.7, 14.0. ESI-MS *m/z*: $[M + H]^+$ 257:259 = 3:1.

3.2.2.4. 3-Butyl-7-chloro-3-hydroxybenzo[c]thiophen-1(3H)-one (compound 4a). $C_{12}H_{13}ClO_2S$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.67 (1H, d, $J = 7.5$ Hz, ArH), 7.61 (1H, d, $J = 7.8$ Hz, ArH), 7.44 (1H, t, $J = 7.5$ Hz, ArH), 3.39 (1H, s, OH), 2.50–2.64 (2H, m, CH_2), 1.55–1.67 (2H, m, CH_2), 1.25–1.39 (2H, m, CH_2), 0.82 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 193.4, 146.8, 138.1, 136.1, 131.4, 131.2, 122.3, 96.6, 39.3, 27.8, 22.6, 14.0. ESI-MS *m/z*: $[M + H]^+$ 257:259 = 3:1.

3.2.2.5. 3-Hydroxy-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 5a). $C_{10}H_{10}O_2S$, light yellow oil. ESI-MS *m/z*: 195 $[M + H]^+$, 217 $[M + Na]^+$.

3.2.2.6. 3-Hydroxy-3-pentylbenzo[c]thiophen-1(3H)-one (compound 6a). $C_{13}H_{16}O_2S$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.61–7.71 (m, 3H, ArH), 7.49 (1H, t, $J = 7.5$ Hz, ArH), 2.33–2.43 (1H, m, CH_2), 2.02–2.15 (1H, m, CH_2), 1.17–1.30

(6H, m, 3 × CH_2), 0.86 (3H, t, $J = 6.3$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 195.0, 153.0, 134.9, 134.3, 130.0, 123.8, 123.5, 95.8, 42.8, 31.7, 25.5, 22.5, 14.1. ESI-MS m/z: 237 $[M + H]^+$, 261 $[M + Na]^+$. The spectroscopic data of **6a** were in agreement with those of known compound reported in literatures [16].

3.2.2.7. 5-Chloro-3-hydroxy-3-pentylbenzo[c]thiophen-1(3H)-one (compound 7a). $C_{13}H_{15}ClO_2S$, light yellow oil. ESI-MS m/z: $[M + H]^+$ 271:273 = 3:1, $[M + Na]^+$ 293:295 = 3:1.

3.2.2.8. 5-Chloro-3-hydroxy-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 8a). $C_{11}H_{11}ClO_2S$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.56–7.59 (2H, m, ArH), 7.45 (1H, dd, $J = 1.6$, 8.4 Hz, ArH), 2.48–2.55 (1H, m, CH), 1.26 (3H, d, $J = 6.4$ Hz, CH_3), 0.70 (3H, d, $J = 6.4$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 194.3, 154.5, 141.4, 133.7, 130.5, 124.3, 124.1, 99.8, 38.8, 19.1, 17.9. ESI-MS m/z: $[M + H]^+$ 243:245 = 3:1.

3.2.2.9. 4-Chloro-3-hydroxy-3-pentylbenzo[c]thiophen-1(3H)-one (compound 9a). $C_{13}H_{15}ClO_2S$, light yellow oil. ESI-MS m/z: $[M + H]^+$ 271:273 = 3:1.

3.2.2.10. 7-Chloro-3-hydroxy-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 10a). $C_{11}H_{11}ClO_2S$, light yellow oil. ESI-MS m/z: $[M + H]^+$ 243:245 = 3:1.

3.2.2.11. 3-Hydroxy-3-propylbenzo[c]thiophen-1(3H)-one (compound 11a). $C_{11}H_{12}O_2S$, light yellow oil. ESI-MS m/z: 209 $[M + H]^+$.

3.2.2.12. 5-Chloro-3-hydroxy-3-propylbenzo[c]thiophen-1(3H)-one (compound 12a). $C_{11}H_{11}ClO_2S$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.57–7.59 (2H, m, ArH), 7.45 (1H, dd, $J = 1.2$, 8.4 Hz, ArH), 3.63 (1H, s, OH), 2.29–2.37 (1H, m, CH_2), 2.05 (1H, td, $J = 4.4$, 12.0 Hz, CH_2), 1.66–1.74 (1H, m, CH_2), 1.23–1.26 (1H, m, CH_2), 0.95 (3H, t, $J = 3.2$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 194.0, 154.6, 141.3, 133.2, 130.5, 124.4, 124.3, 95.0, 44.9, 19.2, 14.0. ESI-MS m/z: $[M + H]^+$ 243:245 = 3:1.

3.2.2.13. 4-Chloro-3-hydroxy-3-propylbenzo[c]thiophen-1(3H)-one (compound 13a). $C_{11}H_{11}ClO_2S$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.67 (1H, d, $J = 7.6$ Hz, ArH), 7.61 (1H, d, $J = 8.0$ Hz, ArH), 7.45 (1H, d, $J = 8.0$ Hz, ArH), 3.17 (1H, br s, OH), 2.60–2.68 (1H, m, CH_2), 2.43–2.51 (1H, m, CH_2), 1.62–1.65 (1H, m, CH_2), 1.18–1.21 (1H, m, CH_2), 0.96 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 193.2, 146.8, 138.1, 136.1, 131.4, 131.2, 122.3, 96.5, 41.7, 19.2, 14.0. ESI-MS m/z: $[M + H]^+$ 243:245 = 3:1.

3.2.2.14. 5-Chloro-3-ethyl-3-hydroxybenzo[c]thiophen-1(3H)-one (compound 14a). $C_{10}H_9ClOS$, light yellow oil. ESI-MS m/z: $[M + H]^+$ 213:215 = 3:1.

3.2.2.15. 4-Chloro-3-ethyl-3-hydroxybenzo[c]thiophen-1(3H)-one (compound 15a). $C_{10}H_9ClO_2S$, light yellow oil. ESI-MS m/z: $[M + H]^+$ 229:231 = 3:1.

3.2.3. Preparation of b-series derivatives

Because of the very similar synthesis steps, **2b** was taken as an example. **2a** was dissolved in 2 ml glacial acetic acid, added 57% HI after reflux, and continued reflux reaction for 0.5 h. Then the mixture was washed by a saturated solution of $NaHSO_3$ until a negative potassium iodine-starch test was given. The solution was evaporated in a vacuum to remove acetic acid, then ethyl acetate was added to the residues. Anhydrous Na_2SO_4 was added to the resulted solution to remove the water. After removing Na_2SO_4 by filtration, the filtrate was subject to column chromatography to give the title compound. The yield is generally 70–80%.

3.2.3.1. 3-Butylbenzo[c]thiophen-1(3H)-one (compound 1b). $C_{12}H_{14}OS$, yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.78 (1H, d, $J = 7.5$ Hz, ArH), 7.62 (1H, t, $J = 7.5$ Hz, ArH), 7.53 (1H, d, $J = 7.5$ Hz, ArH), 7.45 (1H, t, $J = 7.5$ Hz, ArH), 4.83 (1H, d, $J = 9.6$ Hz, CH), 2.30 (1H, dd, $J = 9.9$, 18.9 Hz, CH_2), 1.82 (1H, dd, $J = 9.9$, 20.4 Hz, CH_2), 1.38–1.64 (4H, m, $2 \times CH_2$), 0.91 (3H, t, $J = 6.0$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 197.6, 151.6, 136.1, 133.4, 128.3, 125.2, 123.8, 51.7, 36.3, 30.3, 22.6, 14.0. ESI-MS *m/z*: 207 $[M + H]^+$, 229 $[M + Na]^+$. The spectroscopic data of **1b** were in agreement with those of known compound reported in literatures [16].

3.2.3.2. 3-Isopropylbenzo[c]thiophen-1(3H)-one (compound 2b). $C_{11}H_{12}O_2S$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70 (1H, d, $J = 7.8$ Hz, ArH), 7.61 (1H, t, $J = 7.5$ Hz, ArH), 7.41–7.51 (2H, m, ArH), 4.94 (1H, d, $J = 2.7$ Hz, CH), 2.58–2.68 (1H, m, CH), 1.18 (3H, d, $J = 6.6$ Hz, CH_3), 0.62 (3H, d, $J = 6.6$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 197.8, 150.7, 136.7, 133.4, 128.2, 125.1, 123.6, 58.7, 32.6, 22.9, 15.0. ESI-MS *m/z*: 193 $[M + H]^+$.

3.2.3.3. 3-Butyl-5-chlorobenzo[c]thiophen-1(3H)-one (compound 3b). $C_{12}H_{13}ClOS$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.71 (1H, d, $J = 8.4$ Hz, ArH), 7.51 (1H, br s, ArH), 7.43 (1H, d, $J = 8.1$ Hz, ArH), 4.78–4.81 (1H, m, CH), 2.23–2.27 (1H, m, CH_2), 1.73–1.81 (1H, m, CH_2), 1.32–1.45 (4H, m, $2 \times CH_2$), 0.93 (3H, t, $J = 6.0$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 196.0, 153.0, 140.3, 133.3, 129.0, 125.5, 124.7, 51.1, 36.2, 30.2, 22.6, 14.0. ESI-MS *m/z*: $[M + H]^+$ 241:243 = 3:1.

3.2.3.4. 3-Butyl-7-chlorobenzo[c]thiophen-1(3H)-one (compound 4b). $C_{12}H_{13}ClOS$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70 (1H, d, $J = 7.8$ Hz, ArH), 7.60 (1H, d, $J = 7.8$ Hz, ArH), 7.42 (1H, t, $J = 7.5$ Hz, ArH), 4.88 (1H, dd, $J = 2.1$, 9.6 Hz, CH_2), 2.56–2.60 (1H, m, CH_2), 1.70–1.79 (2H, m, CH_2), 1.25–1.52 (4H, m, $2 \times CH_2$), 0.84 (3H, t, $J = 6.9$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 196.3, 147.9, 138.3, 134.2, 132.0, 129.8, 122.4, 51.0, 34.1, 30.1, 22.4, 14.0. ESI-MS *m/z*: $[M + H]^+$ 241:243 = 3:1.

3.2.3.5. 3-Ethylbenzo[c]thiophen-1(3H)-one (compound 5b). $C_{10}H_{10}OS$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.79 (1H, d, $J = 7.5$ Hz, ArH), 7.62 (1H, t, $J = 7.2$ Hz, ArH), 7.54 (1H, d, $J = 7.8$ Hz, ArH), 7.45 (1H, t, $J = 7.2$ Hz, ArH), 4.83 (1H,

dd, $J = 3.3$, 8.7 Hz, CH), 2.32–2.38 (1H, m, CH_2), 1.84–1.94 (1H, m, CH_2), 1.04 (3H, t, $J = 7.5$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 197.6, 151.2, 136.3, 133.4, 128.3, 125.2, 123.8, 53.0, 29.3, 11.8. ESI-MS *m/z*: 179 $[M + H]^+$. The spectroscopic data of **5b** were in agreement with those of known compound reported in literatures [16].

3.2.3.6. 3-Pentylbenzo[c]thiophen-1(3H)-one (compound 6b). $C_{13}H_{16}OS$, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.79 (1H, d, $J = 7.8$ Hz, ArH), 7.62 (1H, t, $J = 7.5$ Hz, ArH), 7.53 (1H, d, $J = 7.8$ Hz, ArH), 7.45 (1H, t, $J = 7.2$ Hz, ArH), 4.83 (1H, dd, $J = 3.6$, 9.6 Hz, CH), 2.24–2.32 (1H, m, CH_2), 1.77–1.85 (1H, m, CH_2), 1.50–1.59 (1H, m, CH_2), 1.25–1.45 (3H, m, 2 × CH_2), 0.89 (3H, t, $J = 6.6$ Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 197.5, 151.6, 136.1, 133.3, 128.3, 125.2, 123.8, 51.7, 36.6, 31.6, 27.8, 22.5, 14.1. ESI-MS *m/z*: 221 $[M + H]^+$. The spectroscopic data of **6b** were in agreement with those of known compound reported in literatures [16].

3.2.3.7. 5-Chloro-3-pentylbenzo[c]thiophen-1(3H)-one (compound 7b). $C_{13}H_{15}ClOS$, light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70 (1H, d, $J = 8.4$ Hz, ArH), 7.51 (1H, br s, ArH), 7.44 (1H, d, $J = 8.4$ Hz, ArH), 4.78–4.82 (1H, m, CH), 2.24–2.27 (1H, m, CH_2), 1.77–1.80 (1H, m, CH_2), 1.25–1.44 (6H, m, 3 × CH_2), 0.88–0.90 (3H, m, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 195.9, 153.0, 140.3, 133.3, 128.9, 125.5, 124.7, 51.1, 36.5, 31.6, 27.8, 22.5, 14.1. ESI-MS *m/z*: $[M + H]^+$ 255:256 = 3:1.

3.2.3.8. 5-Chloro-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 8b). $C_{11}H_{11}ClOS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (1H, d, $J = 8.0$ Hz, ArH), 7.49 (1H, d, $J = 1.2$ Hz, ArH), 7.44 (1H, dd, $J = 1.2$, 8.4 Hz, ArH), 4.90 (1H, d, $J = 3.2$ Hz, CH), 2.57–2.64 (1H, m, CH), 1.19 (3H, d, $J = 6.8$ Hz, CH_3), 0.68 (3H, d, $J = 6.8$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 196.2, 152.2, 140.4, 135.3, 129.0, 125.4, 124.7, 58.1, 32.7, 22.8, 15.2. ESI-MS *m/z*: $[M + H]^+$ 227:229 = 3:1.

3.2.3.9. 4-Chloro-3-pentylbenzo[c]thiophen-1(3H)-one (compound 9b). $C_{13}H_{15}ClOS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.50 (1H, t, $J = 7.6$ Hz, ArH), 7.42 (1H, d, $J = 7.6$ Hz, ArH), 7.40 (1H, t, $J = 8.0$ Hz, ArH), 4.74 (1H, dd, $J = 3.6$, 10.0 Hz, CH), 2.18–2.28 (1H, m, CH_2), 1.76–1.79 (1H, m, CH_2), 1.33–1.37 (1H, m, CH_2), 1.25–1.32 (5H, m, CH_2), 0.88 (3H, t, $J = 6.8$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 194.1, 154.7, 133.4, 131.7, 131.4, 130.5, 123.9, 49.5, 36.8, 31.6, 27.6, 22.5, 14.1. ESI-MS *m/z*: $[M + H]^+$ 255:257 = 3:1.

3.2.3.10. 7-Chloro-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 10b). $C_{11}H_{11}ClOS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.50 (1H, t, $J = 8.0$ Hz, ArH), 7.42 (1H, d, $J = 8.0$ Hz, ArH), 7.40 (1H, d, $J = 7.6$ Hz, ArH), 4.85 (1H, d, $J = 3.6$ Hz, CH), 2.58–2.63 (1H, m, CH), 1.18 (3H, d, $J = 6.8$ Hz, CH_3), 0.64 (3H, d, $J = 6.8$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 194.3, 154.0, 133.5, 132.0, 131.7, 130.6, 123.8, 56.4, 32.9, 22.9, 14.9. ESI-MS *m/z*: $[M + H]^+$ 227:229 = 3:1.

3.2.3.11. 3-Propylbenzo[c]thiophen-1(3H)-one (compound 11b). $C_{11}H_{12}OS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (1H, d, $J = 7.6$ Hz, ArH),

7.55–7.59 (1H, m, ArH), 7.49 (1H, t, $J = 7.6$ Hz, ArH), 7.39 (1H, t, $J = 7.2$ Hz, ArH), 4.79 (1H, dd, $J = 3.6$, 8.4 Hz, CH), 2.19–2.23 (1H, m, CH_2), 1.70–1.74 (1H, m, CH_2), 1.50–1.53 (1H, m, CH_2), 1.39–1.41 (1H, m, CH_2), 0.93 (1H, d, $J = 7.6$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 197.2, 151.4, 135.7, 133.2, 128.0, 125.1, 123.5, 51.3, 38.5, 21.3, 13.7. ESI-MS *m/z*: 193 $[M + H]^+$. The spectroscopic data of **11b** were in agreement with those of known compound reported in literatures [16].

3.2.3.12. 7-Chloro-3-isopropylbenzo[c]thiophen-1(3H)-one (compound 12b). $C_{11}H_{11}ClOS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (1H, d, $J = 8.4$ Hz, ArH), 7.51 (1H, br s, ArH), 7.43 (1H, dd, $J = 1.2$, 8.4 Hz, ArH), 4.80 (1H, dd, $J = 3.6$, 9.6 Hz, CH), 2.22–2.25 (1H, m, CH_2), 1.77–1.82 (1H, m, CH_2), 1.54–1.58 (1H, m, CH_2), 1.41–1.49 (1H, m, CH_2), 1.00 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 195.9, 153.0, 140.3, 134.6, 129.0, 125.5, 124.8, 50.9, 38.6, 21.5, 13.9. ESI-MS *m/z*: $[M + H]^+$ 227:229 = 3:1.

3.2.3.13. 4-Chloro-3-propylbenzo[c]thiophen-1(3H)-one (compound 13b). $C_{11}H_{11}ClOS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (1H, d, $J = 7.6$ Hz, ArH), 7.59 (1H, d, $J = 7.2$ Hz, ArH), 7.41 (1H, t, $J = 7.6$ Hz, ArH), 4.88 (1H, dd, $J = 2.8$, 9.6 Hz, OH), 2.52–2.54 (1H, m, CH_2), 1.71–1.76 (1H, m, CH_2), 1.51–1.53 (1H, m, CH_2), 1.39–1.42 (1H, m, CH_2), 0.97 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 196.2, 147.9, 138.2, 134.2, 131.9, 129.8, 122.4, 50.8, 36.5, 21.4, 13.7. ESI-MS *m/z*: $[M + H]^+$ 227:229 = 3:1.

3.2.3.14. 5-Chloro-3-ethylbenzo[c]thiophen-1(3H)-one (compound 14b). $C_{10}H_9ClOS$, light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (1H, d, $J = 8.4$ Hz, ArH), 7.51 (1H, br s, ArH), 7.45 (1H, d, $J = 8.4$ Hz, ArH), 4.80 (1H, dd, $J = 3.6$, 8.8 Hz, CH), 2.29–2.35 (1H, m, CH_2), 1.88–1.93 (1H, m, CH_2), 1.05 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 195.9, 152.6, 140.4, 137.1, 134.9, 129.0, 125.5, 124.8, 52.4, 29.2, 11.7. ESI-MS *m/z*: $[M + H]^+$ 213:215 = 3:1.

3.2.3.15. 4-Chloro-3-ethylbenzo[c]thiophen-1(3H)-one (compound 15b). $C_{10}H_9ClOS$, light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (1H, dd, $J = 1.2$, 7.6 Hz, ArH), 7.59 (1H, dd, $J = 1.2$, 7.6 Hz, ArH), 7.41 (1H, t, $J = 7.6$ Hz, ArH), 4.88 (1H, dd, $J = 2.8$, 8.8 Hz, CH), 2.52–2.58 (1H, m, CH_2), 1.88–1.95 (1H, m, CH_2), 0.98 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 196.1, 147.5, 138.5, 134.1, 132.0, 129.9, 122.4, 52.3, 27.3, 11.6. ESI-MS *m/z*: $[M + H]^+$ 213:215 = 3:1, $[M + Na]^+$ 235:237 = 3:1.

3.3. In vitro antiplatelet aggregation assay

Blood was drawn from rabbit aorta abdominalis and mixed with 3.8% sodium citrate (9:1, v/v). After centrifugation at 900 rpm for 5 min at room temperature to obtain platelet-rich plasma (PRP), the remaining blood was further centrifuged at 3000 rpm for another 15 min to obtain platelet-poor plasma (PPP). A microtiter plate method for detection of platelet aggregation was used according to previous literatures [22, 23].

Microscopically, PRP count was adjusted to 5×10^8/ml using PPP. Briefly, PRP (90 μl) was preincubated in 96-well plates in duplicate for 10 min at 37 °C with vehicle, the individual compounds, or reference drugs (ASP and Eda) at the same concentrations (100 μM). After the samples were shaken and mixed, the absorbance value was measured at 570 nm with microplate reader (Synergy 4 MLFPTAD microplate reader, BioTek Instruments, Inc.). The absorbance value was measured continuously for two times until the datum was stable. The average absorbance of two times per well was recorded as A_{PRP}. A_{PPP} was measured in the same way as A_{PRP}, except 90 μl PPP was added into 5 μl blank solvent. The samples were mixed with 5 μl inducer ADP or AA, and vibrated for 5 min. The mean absorbance of the parallel wells was denoted as A_{sample}. Results indicated by absorbance value $\bar{x} \pm s$ at 570 nm, aggregation rate (*AR*) and aggregation inhibition rate (*AIR*) of each group were calculated according to the following formula:

$$AR = \frac{A_{PRP} - A_{sample}}{A_{PRP} - A_{PPP}} \times 100\%$$

$$AIR = \frac{AR_{control} - AR_{experiment}}{AR_{control}} \times 100\%$$

$AR_{control}$ represented the platelet aggregation rate of the blank control group; $AR_{experiment}$ represented the platelet aggregation rate of the drug group.

3.4. In vitro antioxidant assay

SH-SY5Y cells were cultured in F12K Dulbecco's modified Eagle's medium (DMEM) culture medium containing 10% fetal bovine serum, under the condition of 37 °C and 5% CO_2. After 2–3 days, the mixture of 0.25% trypsin and 0.02% ethylene diamine tetraacetic acid (EDTA) (1:1) was used to cells passage once at 1:5, and the logarithmic growth stage cells were taken for the next experiment. The treatment of PC 12 cell culture was the same. The toxicity of the synthetic compounds was detected by CCK-8 method first. Based on the CCK-8 experimental results, compounds with cytotoxic $IC_{50} > 30$ μM were selected for further experiments. Cells (1×10^5 cells/ml, 100 μl) were inoculated in 96-well plates, to join the H_2O_2 solution to 300 μM, compounds to 30 μM. The samples were incubated at 37 °C, 5% CO_2 for 24 h, and the cell survival rate was determined by CCK-8 method. The medium was changed to the medium containing 10% CCK-8 reagent. After incubating for 4 h, the absorbance of 450 nm was measured with an enzyme marker (H1M microplate reader, BioTek Instruments, Inc.), that is, the OD values [24, 25]. The cell relative survival rate of the tested individual compounds on the antioxidant activity was calculated as the following formula: Cell relative survival rate (CRSR) = $(OD_{experiment} - OD_{model})/(OD_{control} - OD_{model}) \times 100\%$. Control group (control), dimethylsulfoxide (DMSO) was added, but H_2O_2 and compounds were not added. Model group (model), H_2O_2 and DMSO were added, but no compounds treatment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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