PAPER

Design, synthesis and biological evaluation of hydrogen sulfide releasing derivatives of 3-*n*-butylphthalide as potential antiplatelet and antithrombotic agents†

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In the present study, a series of hydrogen sulfide (H₂S) releasing derivatives (**8a–g** and **9a–f**) of 3-*n*-butylphthalide (NBP) were designed, synthesized and biologically evaluated. The most promising compound **8e** significantly inhibited the adenosine diphosphate (ADP) and arachidonic acid (AA)-induced platelet aggregation *in vitro*, superior to NBP, ticlopidine hydrochloride and aspirin. Furthermore, **8e** could slowly produce moderate levels of H₂S *in vitro*, which could be beneficial for improving cardiovascular and cerebral circulation. Most importantly, **8e** protected against the collagen and adrenaline induced thrombosis in mice, and exhibited greater antithrombotic activity than NBP and aspirin in rats. Overall, **8e** could warrant further investigation for the treatment of thrombosis-related ischemic stroke.

1. Introduction

Ischemic stroke, a leading cause of death and long-term disability worldwide, is a major socioeconomic burden due to the lack of a suitable therapy.[1] Ischemic stroke is characterized by thrombosis initiated by abnormal platelet function, which results in insufficient cerebral blood flow, and further leads to ischemic brain damage.[2–5] Current drug therapies for prevention and treatment of ischemic stroke are primarily dependent on high affinity and single-target drugs. However, a drug directed against a single molecular target may not be effective in treatment of ischemic stroke because of its multi-factorial pathology. Therefore, there is a significantly unmet medical need for new drugs with multiple actions to combat ischemic stroke.

The racemic 3-*n*-butylphthalide (NBP) was approved by the State Food and Drug Administration (SFDA) of China in 2002 as a new anti-ischemic stroke drug, which has beneficial effects on stroke through multiple actions,[6–8] including inhibiting platelet aggregation and thrombosis, improving microcirculation, reducing the area of cerebral infarct and decreasing oxidative damage, *etc.* Despite these advantages, the clinical application of NBP has been limited due to its moderate potency.[9,10] To achieve the ideal anti-ischemic stroke drugs, a wide range of studies of structural modification and related research on NBP have been carried out. Previous studies have demonstrated that the potassium salt of 2-(1-hydroxypentyl)-benzoate (HPBA), a ring-opening derivative of NBP, has an improved aqueous solubility and comparable potency to NBP.[11] Recently, we also reported several ring-opening derivatives of NBP as potent anti-ischemic stroke agents.[12–14]

Hydrogen sulfide (H₂S) is considered as a new member of gasotransmitter family, following nitric oxide (NO) and carbon monoxide (CO). Recent studies have revealed that H₂S has a positive effect on cerebral ischemia reperfusion injury. Indeed, augmentation of H₂S production can induce vasodilatation, inhibit platelet aggregation, improve CBF, promote angiogenesis, and protect neurons against oxidative stress and free radicals, benefiting patients with ischemic stroke.[15–18] And a number of H₂S-releasing compounds have been developed and are already undergoing intensive investigation.[19–21]

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In this study, we hypothesized that H$_2$S-releasing derivatives of NBP could display synergistic effects exerted by both NBP and H$_2$S on platelet aggregation and thrombosis. Thereby, a series of hybrids from HPBA and H$_2$S were designed, synthesized and evaluated as potential antiplatelet and anti-thrombotic agents *in vitro* and *in vivo*.

2. Results

2.1. Strategy for the design of H$_2$S–NBP

It has been demonstrated that conjugation of an H$_2$S-donor moiety with a "native" molecule enhances therapeutic effect and/or reduces the adverse effect of the native molecule.[22–24] In view of the fact that ADTOH, 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione, a known H$_2$S-donor, is able to release H$_2$S *in vitro* and *in vivo*,[25] we designed a novel class of compounds through connecting ADTOH to HPBA with different linkers. In addition, amines, such as diethylamine or *N*-methyl piperazine, were introduced to the side chain of HPBA as a substituted acetate linkage, respectively. We expected that the ester bonds of H$_2$S–NBP would be cleaved *in vivo* by esterases to release HPBA and H$_2$S, the former would subsequently undergo ring closing to generate NBP; and that both NBP and

H$_2$S would synergistically inhibit platelet aggregation and thrombosis.

2.2. Chemistry

The synthesis of the target compounds **8a–g** and **9a–f** are illustrated in Scheme 1. The H$_2$S-donor ADTOH (compound **2**) was prepared as described previously[26] and converted to the corresponding bromo-substituted ethers **3a–g** using K$_2$CO$_3$ in acetone. NBP was subjected to saponification and sequential acidification to form the ring-opening acid **4**. Treatment of **4** with acetyl chloride or chloroacetyl chloride gave the acylates **5** or **6**. Compound **5** was condensed with **3a–g** to generate the target compounds **8a–g**. Treatment of **6** with diethylamine or *N*-methyl piperazine led to ester **7a** or **7b**, which was then reacted with **3c–d** to provide the target compounds **9a–f**. All of the new compounds were purified by column chromatography and characterized by ESI-MS, ^{1}H NMR, ^{13}C NMR, and HRMS. The individual compounds with a chemical purity of >95% (determined by HPLC analysis) were used for subsequent experiments.

2.2. Antiplatelet aggregation effects *in vitro*

The individual compounds were evaluated for inhibition of platelet aggregation in rabbit platelet rich plasma (PRP) in
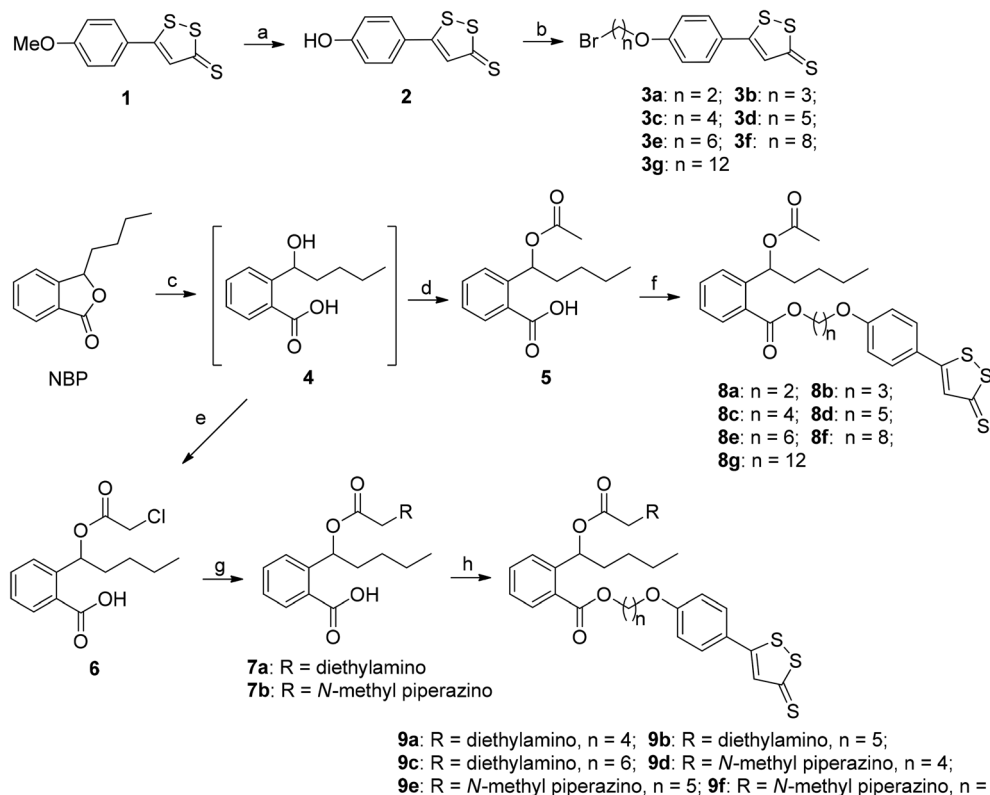
Scheme 1 Synthesis of **8a–g** and **9a–f**. *Reagents and conditions*: (a) pyridine hydrochloride, 215 °C, 0.5 h; (b) Br(CH$_2$)$_n$Br, K$_2$CO$_3$, acetone, reflux, 6–8 h; (c) (i) 2 M NaOH, CH$_3$OH–H$_2$O, 50 °C, 0.5 h; (ii) 5% HCl, −10 to 0 °C; (d) CH$_3$COCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, −10 °C, 5 h; (e) ClCH$_2$COCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, −10 °C, 5 h; (f) **3a–g**, K$_2$CO$_3$, acetone, reflux, 6–10 h; (g) diethylamine or *N*-methyl piperazine, K$_2$CO$_3$, acetone, reflux, 6–8 h; (h) **3c–e**, K$_2$CO$_3$, acetone, reflux, 4–8 h.

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Table 1 The IC$_{50}$ values of **8a–g** and **9a–f** against platelet aggregation *in vitro*

Compd	IC$_{50}$ (mM)	
	ADP (10 μM)	AA (1.0 mM)
Control	—	—
Ticlid[a]	0.36 ± 0.04	—
Aspirin[b]	—	0.14 ± 0.02
NBP	0.74 ± 0.20	0.58 ± 0.06
8a	0.23 ± 0.09	0.15 ± 0.05
8b	0.20 ± 0.11	0.14 ± 0.06
8c	0.18 ± 0.10	0.14 ± 0.08
8d	0.17 ± 0.13	0.15 ± 0.07
8e	0.14 ± 0.06	0.09 ± 0.01
8f	0.19 ± 0.14	0.16 ± 0.05
8g	0.24 ± 0.12	0.20 ± 0.06
9a	0.23 ± 0.14	0.18 ± 0.03
9b	0.21 ± 0.13	0.17 ± 0.04
9c	0.19 ± 0.11	0.15 ± 0.06
9d	0.71 ± 0.19	0.37 ± 0.10
9e	0.52 ± 0.17	0.23 ± 0.09
9f	0.49 ± 0.14	0.23 ± 0.08

[a] Ticlid is an inhibitor of the ADP-induced platelet aggregation. [b] Aspirin is an inhibitor of the AA-induced platelet aggregation. IC$_{50}$ values (a dose achieved 50% inhibition of platelet aggregation) are expressed as mean ± SD ($n = 6$) and analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test.

response to the adenosine diphosphate (ADP) and arachidonic acid (AA) using the Born's turbidimetric method.[27] Ticlopidine hydrochloride (Ticlid) and aspirin, as inhibitors of the ADP- and AA-induced platelet aggregation,[28,29] respectively, were employed as positive controls, and NBP as a reference compound. The inhibitory activity of individual compounds at five different concentrations against the ADP (10 μM) and AA (1.0 mM) induced platelet aggregation. Data were calculated and expressed as the IC$_{50}$ values.

As shown in Table 1, all the target compounds presented better inhibitory effects than NBP both against the ADP- and AA-induced platelet aggregation. The compounds with 6-carbon alkanes (**8e**, **9c**) displayed the best inhibitory activity. Furthermore, the compounds containing a hydrogen atom (**8c–e**) exhibited stronger inhibitory activity than that bearing diethylamino and *N*-methyl piperazino moieties in the side chain (**9a–f**). Notably, **8e** (R = H, *n* = 6) was the most potent, whose IC$_{50}$ value (0.14 mM) on the ADP-induced platelet aggregation was significantly less than that of NBP (0.74 mM) and Ticlid (0.36 mM), and the IC$_{50}$ value (0.09 mM) on the AA-induced platelet aggregation was also less than that of NBP (0.58 mM) and aspirin (0.14 mM). These data indicated that **8e** was a potent inhibitor of both the ADP- and AA-induced platelet aggregation. Therefore, **8e** was chosen for the following investigations.

In order to examine whether each moiety (**2**, **3e** and **5**) of **8e** contributes to the inhibitory activity of this compound, we further investigated their corresponding effects on the ADP-induced platelet aggregation *in vitro*. As shown in Fig. 1, compounds **2**, **3e** and **5** inhibited the ADP-induced platelet aggregation by 28.04%, 30.05% and 26.80%, respectively, but each
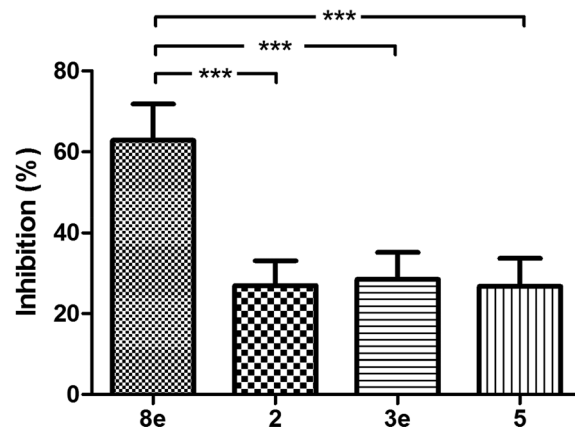

Fig. 1 Inhibition of **8e**, **2**, **3e** and **5** on the ADP-induced platelet aggregation *in vitro*. Rabbit platelet suspensions were preincubated with the testing compound (200 μM) at 37 °C for 5 min and exposed to 10 μM of ADP, followed by continually monitoring. Rabbit platelet suspensions that had been treated with the vehicle and exposed to ADP were used as positive controls. Data are expressed as mean ± SD of each group (*n* = 6) for two separate experiments. ***$P < 0.001$ by one-way ANOVA followed by *post hoc* Tukey's test.
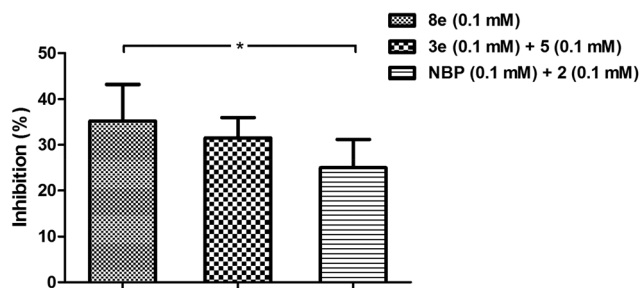

Fig. 2 Inhibition of **8e** and combinations of its moieties on the ADP-induced platelet aggregation *in vitro*. Data are expressed as mean ± SD of each group (*n* = 6) for two separate experiments. *$P < 0.05$ by one-way ANOVA followed by *post hoc* Tukey's test.

of them was substantially less effective than **8e** (62.90%), suggesting that these moieties may have synergistic effects on the inhibition of **8e** against the ADP-induced platelet aggregation.

To confirm the synergistic effects between these moieties, we then tested the moieties of **8e** in combination. Obviously **8e** had a better antiplatelet activity than NBP together with **2** (*$P < 0.05$, Fig. 2) which supports our hypothesis that **8e** affect the platelet aggregation greater than the combination of its mother compound-NBP and H$_2$S releasing moiety-ADTOH (compound **2**). Although the combination of **3e** and **5** showed weaker inhibition on platelet aggregation than **8e**, no statistical significance was found between them.

2.3. Antithrombotic activity of 8e in mice

We then evaluated the antithrombotic activity of **8e** on a mouse model of thromboembolism, that is, an acute vascular occlusion initiated by intravascular platelet induced aggrega-
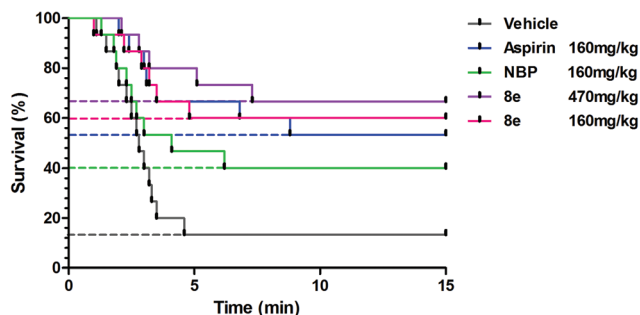
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Fig. 3 The survival of mice after injection of 0.21 mg kg^{-1} collagen and 44.5 μg kg^{-1} adrenaline and subsequent oral administration of **8e**, NBP and aspirin, respectively, at the indicated doses and time points, and the results were expressed as the percentage of mice alive (n = 15) as a function of time.

tion through infusion of a mixture of collagen and adrenaline into the jugular vein.[30] Male ICR mice were randomized and treated orally with vehicle, NBP (160 mg kg^{-1}), aspirin (160 mg kg^{-1}), or **8e** (160 mg kg^{-1} and 470 mg kg^{-1}, which are equal mass to and equal molar to that of NBP, respectively) for seven days. Two hours after the last administration, individual mice were injected intravenously with a mixture of collagen (0.21 mg kg^{-1}) and adrenaline (44.5 μg kg^{-1}) to induce acute systemic vascular thromboembolism. As shown in Fig. 3, treatment with **8e** (470 mg kg^{-1}) significantly reduced the onset of hemiplegia and death in mice, and the percentage of survivors (66.7%) was greater than that of aspirin (60.0%) and NBP (53.3%), while the percentage (60.0%) by treatment with **8e** (160 mg kg^{-1}) was slightly greater than that of NBP and comparable to that of aspirin in this model at the same dosage. These results clearly demonstrated that **8e** efficiently inhibited acute systemic intravascular thrombosis in mice leading to protective effects.

2.4. Antithrombotic activity of 8e in rats

Parallelly, we investigated the antithrombotic activity of **8e** in a rat arterio-venous (A-V) shunt model mimicking arterial thrombosis.[31] SD rats were randomized and treated intragastrically (i.g.) with normal saline (sham group), NBP (80 mg kg^{-1}), aspirin (80 mg kg^{-1}), and **8e** (80 and 235 mg kg^{-1}). After a 7-day oral administration of individual compounds, the wet and dry thrombus weights were measured (Fig. 4). All four treatment groups showed significant difference from the sham group on wet weight of thrombus. Treatment with **8e** at a dose of 80 or 235 mg kg^{-1}, which is equal mass to or equal molar to that of NBP, reduced wet weight by 27.87% and 37.16%, respectively, more effective than treatment with NBP (15.02%), and the potency of the higher dose group was similar to that of aspirin (36.74%). Similarly, four treatment groups shared significant difference from the sham group on dry weight of thrombus. **8e** at 235 mg kg^{-1} reduced the dry weight of thrombus by 32.58%, which was more potent than both NBP (18.72%) and aspirin (30.60%). Treatment with **8e** at 80 mg kg^{-1} also effectively reduced the thrombus dry weight by 26.34%, which was higher than NBP but lower than aspirin. These results were consistent with our previous research, and clearly indicated that **8e** was a potent thrombosis inhibitor *in vivo*.

2.5. Assay of hydrogen sulfide release *in vitro*

The target compounds were designed to be NBP derivatives, which could release H$_2$S. In order to show that this was indeed the case, the levels of H$_2$S produced by **8e** were measured in phosphate buffer and rat liver homogenate,[32] respectively. As shown in Fig. 5, little but still detectable H$_2$S release was observed when **8e** was incubated with phosphate buffer. However, in liver homogenate, **8e** could produce moderate levels of H$_2$S peaking at 60 min which remained slightly elevated for a further 30 min, suggesting that **8e** could slowly release H$_2$S in liver homogenate, which would be expected to increase the duration of intracellular H$_2$S exposure leading to the enhanced potency of **8e** relative to NBP. Based on the chemical structure of **8e**, we speculated that it could be firstly metabolized by esterases to release HPBA, which would subsequently undergo ring closure to generate NBP, and H$_2$S donor ADTOH. Incubation of **8e** in buffer released much
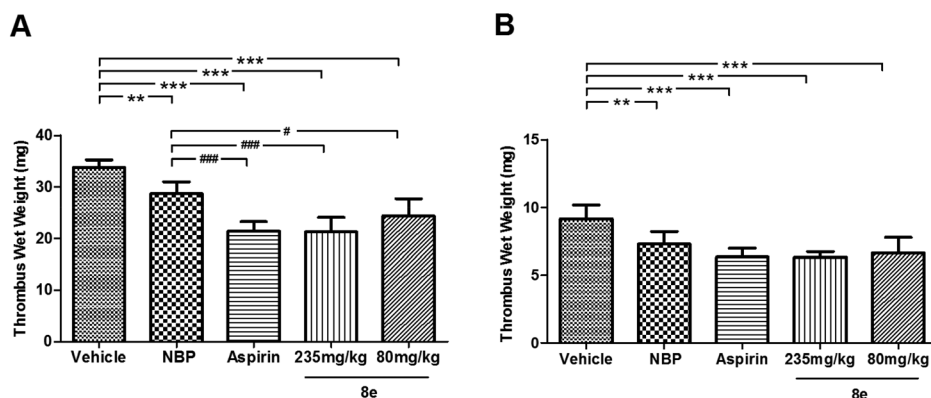

Fig. 4 Effects of **8e**, NBP and aspirin on thrombus wet weights (A) and dry weights (B) in rats. SD rats were treated with the indicated doses of each drug by gavage daily for seven days. Two hours after the last treatment, the rats were subjected to the A-V shunt using a silk thread for 15 min. The wet and dry weights of the threads were measured. Data are expressed as mean ± SD of each group (n = 12) and analyzed by ANOVA and *post hoc* Tukey test. ***P < 0.001, **P < 0.01 *vs.* the control group, ###P < 0.001, #P < 0.05 *vs.* the NBP group.
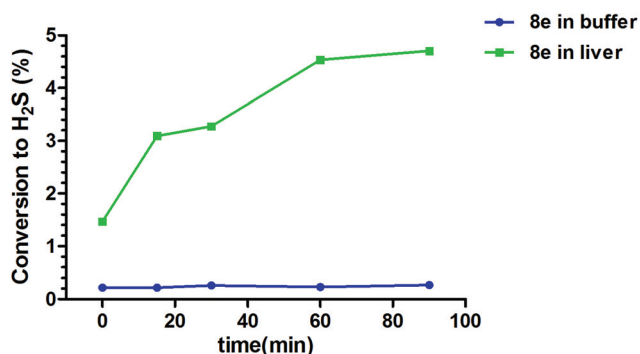
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Fig. 5 Release of H$_2$S from **8e** *in vitro*. **8e** (100 μM) was incubated (37 °C) for 90 min with fresh rat liver homogenate or buffer *in vitro*. H$_2$S concentration was determined. Data are expressed as accumulate % conversion of **8e** to H$_2$S at each time point, $n = 6$.

smaller quantities of H$_2$S which also implied that the gas released from the parent molecule is mainly as a result of metabolic events.

3.   Discussion and conclusions

As far as structure–activity relationships are concerned, in general, extension of the carbon chain (CH$_2$)$_n$ connecting to the H$_2$S releasing moiety (n = 2–6), the platelet aggregation inhibitory activity of the compounds was enhanced, and the activity was declined while n = 8. Furthermore, the compounds containing a hydrogen atom linking to the acetyl group in the side-chain exhibited stronger inhibitory activity than that bearing diethylamino and *N*-methylpiperazino moieties in the side chain. As a matter of fact, **8e** exhibited the strongest inhibitory activity among all compounds. Interestingly, all of the structural moieties of **8e** substantially inhibited the ADP-induced platelet aggregation but much less than **8e**, suggesting that they may act synergistically. Moreover, administration of **8e** alone had better antiplatelet aggregation activity than combinations from its corresponding moieties. However, the precise SAR of the target compounds remains to be further investigated.

In summary, we designed and synthesized a series of novel H$_2$S releasing derivatives of NBP, and found that compound **8e** was the most potent in inhibiting the ADP- and AA-induced platelet aggregation *in vitro*, superior to NBP, Ticlid and aspirin. Furthermore, **8e** could produce moderate levels of H$_2$S *in vitro*, which could be beneficial for improving cardiovascular and cerebral circulation. Most importantly, **8e** protected against the collagen and adrenaline induced thrombosis in mice, and exhibited greater antithrombotic activity in rats. Therefore, our findings may aid in the design of new therapeutic reagents for the treatment of thrombosis-related ischemic stroke and other vascular diseases.

4.   Experimental section

4.1.   Chemistry

General. Melting points are uncorrected and were measured in open capillary tubes using a Gallenkamp melting point

apparatus. ^{1}H and ^{13}C NMR spectral data were obtained from a Bruker Avance 300 MHz spectrometer at 300 K using TMS as an internal standard. MS spectra were recorded on a Mariner Mass Spectrum (ESI). Analytical and preparative TLC were performed on silica gel GF/UV 254, and the chromatograms were conducted on silica gel (200–300 mesh) and visualized under UV light at 254 and 365 nm. The purities of the compounds were characterized by HPLC analysis (LC-10A HPLC system consisting of LC-10ATvp pumps and a SPD-10Avp UV detector) and HRMS (Agilent technologies LC/MSD TOF). The target compounds **8a–g** and **9a–f** with a chemical purity of >95% (see the ESI†).

All experiments were approved and performed in accordance with National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985), and all animal experimental protocols were approved by the Animal Research Protection Committee of China Pharmaceutical University, Nanjing, China.

5-(4-Hydroxyphenyl)-3H-1,2-dithiole-3-thione   *(2)*. Pyridine hydrochloride (68.0 g, 590 mmol) was added to **1** (25.0 g, 100 mmol) in a dry flask, mixed and then heated to melt at 215 °C under argon protection for 0.5 h. After being cooled to 100 °C, warm water (200 mL) was added and hot-filtered. The cake was placed in a beaker, and 10% NaOH (300 mL) was added. This was stirred for 4 h, filtered, the cake was dissolved in water (3 L), and then adjusted to pH 2 with concentrated hydrochloric acid. The red precipitate was filtered and washed to neutral pH using water, and then dried in a vacuum desiccator to yield a yellow solid (18.0 g, 76.5%), mp 172–174 °C (found: C, 47.78; H, 2.67; O, 7.08; S, 42.53. Calcd for C$_9$H$_6$OS$_3$: C, 47.76; H, 2.67; O, 7.07; S, 42.50%); ^{1}H NMR (CDCl$_3$, 300 Hz, δ): 10.47 (brs, 1H, OH), 7.84 (d, 2H, J = 8.8 Hz, ArH), 7.70 (s, 1H, =CH), 6.88 (d, 2H, J = 8.8 Hz, ArH).

General procedure for the preparation of compounds 3a–g. To a mixture of **2** (0.226 g, 1.0 mmol), appropriate amounts of dibromoalkanes (3.0 mmol), K$_2$CO$_3$ (0.690 g, 5.0 mmol), and acetone (20 mL) were added under constant stirring and argon protection. It was then heated to 65 °C and refluxed for 6–8 h. After completion of the reaction (monitored by TLC), the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash chromatography (PE–acetone 10 : 1 v/v) to give the title compounds (70–83%).

5-(4-(2-Bromoethoxy)phenyl)-3H-1,2-dithiole-3-thione (3a). The title compound was obtained as a reddish brown solid (80%), mp 134–136 °C. IR (cm^{-1}, KBr): ν_{max} 595, 819, 1184, 1400, 1486, 1599. ^{1}H NMR (300 Hz, CDCl$_3$): δ 3.68 (t, 2H, J = 6.0 Hz, CH$_2$Br), 4.36 (t, 2H, J = 6.0 Hz, ArOCH$_2$), 7.00 (d, 2H, J = 8.8 Hz, ArH), 7.40 (s, 1H, =CH), 7.63 (d, 2H, J = 8.8 Hz, ArH).

5-(4-(3-Bromopropoxy)phenyl)-3H-1,2-dithiole-3-thione   *(3b)*. The title compound was obtained as a reddish brown solid (78%), mp 131–133 °C. IR (cm^{-1}, KBr): ν_{max} 591, 815, 1185, 1400, 1485, 1600. ^{1}H NMR (300 Hz, CDCl$_3$): δ 2.32–2.40 (m, 2H, CH$_2$CH$_2$Br), 3.62 (t, 2H, J = 6.3 Hz, CH$_2$Br), 4.12 (t, 2H, J = 5.8 Hz, ArOCH$_2$), 7.00 (d, 2H, J = 8.7 Hz, ArH), 7.40 (s, 1H, =CH), 7.63 (d, 2H, J = 8.7 Hz, ArH).

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5-(4-(4-Bromobutoxy)phenyl)-3H-1,2-dithiole-3-thione (3c). The title compound was obtained as a reddish brown solid (81%), mp 129–131 °C. IR (cm^{-1}, KBr): ν_{max} 590, 823, 1189, 1400, 1489, 1600. ^{1}H NMR (300 Hz, CDCl$_3$): δ 1.95–2.43 (m, 4H, CH$_2$CH$_2$CH$_2$Br), 3.60 (t, 2H, J = 6.4 Hz, CH$_2$Br), 4.07 (t, 2H, J = 6.0 Hz, ArOCH$_2$), 6.97 (d, 2H, J = 8.8 Hz, ArH), 7.40 (s, 1H, =CH), 7.62 (d, 2H, J = 8.8 Hz, ArH).

5-(4-((5-Bromopentyl)oxy)phenyl)-3H-1,2-dithiole-3-thione (3d). The title compound was obtained as a reddish brown solid (75%), mp 125–127 °C. IR (cm^{-1}, KBr): ν_{max} 592, 826, 1184, 1400, 1482, 1600. ^{1}H NMR (300 Hz, CDCl$_3$): δ 1.60–1.70 (m, 2H, CH$_2$CH$_2$CH$_2$Br), 1.81–2.45 (m, 4H, CH$_2$CH$_2$CH$_2$CH$_2$Br), 3.46 (t, 2H, J = 6.6 Hz, CH$_2$Br), 4.04 (t, 2H, J = 6.2 Hz, ArOCH$_2$), 6.96 (d, 2H, J = 8.8 Hz, ArH), 7.40 (s, 1H, =CH), 7.61 (d, 2H, J = 8.8 Hz, ArH).

5-(4-((6-Bromohexyl)oxy)phenyl)-3H-1,2-dithiole-3-thione (3e). The title compound was obtained as a reddish brown solid (83%), mp 122–124 °C. IR (cm^{-1}, KBr): ν_{max} 596, 827, 1189, 1400, 1482, 1599. ^{1}H NMR (300 Hz, CDCl$_3$): δ 1.25–1.33 (m, 2H, CH$_2$(CH$_2$)$_2$Br), 1.60–1.70 (m, 2H, ArO(CH$_2$)$_2$CH$_2$), 1.81–2.45 (m, 4H, ArOCH$_2$CH$_2$, CH$_2$CH$_2$Br), 3.44 (t, 2H, J = 6.7 Hz, CH$_2$Br), 4.02 (t, 2H, J = 6.3 Hz, ArOCH$_2$), 6.96 (d, 2H, J = 8.6 Hz, ArH), 7.40 (s, 1H, =CH), 7.60 (d, 2H, J = 8.6 Hz, ArH).

5-(4-((8-Bromooctyl)oxy)phenyl)-3H-1,2-dithiole-3-thione (3f). The title compound was obtained as a reddish brown solid (76%), mp 118–120 °C. IR (cm^{-1}, KBr): ν_{max} 596, 833, 1184, 1400, 1483, 1598. ^{1}H NMR (300 Hz, CDCl$_3$): δ 1.26–1.38 (m, 4H, CH$_2$CH$_2$(CH$_2$)$_3$Br), 1.56–1.72 (m, 4H, ArO(CH$_2$)$_2$CH$_2$, CH$_2$(CH$_2$)$_2$Br), 1.81–2.45 (m, 4H, ArOCH$_2$CH$_2$, CH$_2$CH$_2$Br), 3.42 (t, 2H, J = 6.7 Hz, CH$_2$Br), 4.02 (t, 2H, J = 6.4 Hz, ArOCH$_2$), 6.96 (d, 2H, J = 8.6 Hz, ArH), 7.40 (s, 1H, =CH), 7.60 (d, 2H, J = 8.6 Hz, ArH).

5-(4-((12-Bromododecyl)oxy)phenyl)-3H-1,2-dithiole-3-thione (3g). The title compound was obtained as a reddish brown solid (75%), mp 113–115 °C. IR (cm^{-1}, KBr): ν_{max} 597, 833, 1186, 1401, 1483, 1598. ^{1}H NMR (300 Hz, CDCl$_3$): δ 1.26–1.38 (m, 12H, 6 × CH$_2$(CH$_2$)$_3$Br), 1.43–1.62 (m, 4H, ArO(CH$_2$)$_2$CH$_2$, CH$_2$(CH$_2$)$_2$Br), 1.78–2.09 (m, 4H, ArOCH$_2$CH$_2$, CH$_2$CH$_2$Br), 3.41 (t, 2H, J = 6.8 Hz, CH$_2$Br), 4.02 (t, 2H, J = 6.5 Hz, ArOCH$_2$), 6.96 (d, 2H, J = 8.7 Hz, ArH), 7.40 (s, 1H, =CH), 7.60 (d, 2H, J = 8.7 Hz, ArH).

(±)-2-(1-Hydroxypentyl)benzoic acid (4). To a stirred solution of NBP (1.90 g, 10.00 mmol) in CH$_3$OH–H$_2$O (20 mL, 1 : 1 v/v) was added NaOH (0.80 g, 20.00 mmol). The reaction mixture was heated at 50 °C for 0.5 h. The solvent was removed under reduced pressure and dissolved in water (20 mL), followed by acidification with 5% HCl to pH 3–4 at −10 to 0 °C. The mixture was extracted with cold Et$_2$O (10 mL × 3) and quickly used for the next step without any purification.

(±)-2-(1-Acetoxypentyl)benzoic acid (5). A solution of acetyl chloride (1.06 mL, 15.00 mmol) in dry CH$_2$Cl$_2$ (10 mL) was added dropwise to a mixture of 4 (1.04 g, 5.00 mmol), Et$_3$N (2.09 mL, 15.00 mmol) and DMAP (0.50 mmol) in CH$_2$Cl$_2$ (80 mL) at −10 °C and the solution was left stirring at −10 °C for 5 h. The mixture was acidified with 1 M HCl to pH 2 and stirred for 1 h at room temperature. The organic layer was

washed with water, dried, filtered, and evaporated to dryness. The residue was recrystallized from *n*-hexane to obtain 5 as a white crystal (0.85 g, 68%, over two steps). mp 65–66 °C. MS (ESI): *m/z* 249.1 [M − H]$^-$. IR (cm^{-1}, KBr): ν_{max} 1412, 1691, 1734, 2958, 3450. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.93 (t, 3H, CH$_3$, J = 8.5 Hz), 1.37–1.42 (m, 4H, 2 × CH$_2$), 1.88–1.91 (m, 2H, CH$_2$), 2.13–2.33 (m, 3H, COCH$_3$), 6.61–6.72 (m, 1H, OCHCH$_2$), 7.37–7.40 (m, 1H, ArH), 7.56–7.62 (m, 2H, ArH), 8.05 (d, 1H, ArH, J = 8.1 Hz), 10.98 (brs, 1H, COOH). ^{13}C NMR (75 Hz, CDCl$_3$): δ 172.0, 166.5, 140.8, 133.1, 130.3, 130.0, 127.1, 125.7, 74.8, 41.0, 36.3, 27.8, 22.4, 13.8.

(±)-2-[1-(2-Chloroacetoxy)pentyl]benzoic acid (6). Compound 6 was synthesized in the same manner as compound 5. Starting from compound 4 (1.04 g, 5.00 mmol) and 2-chloroacetyl chloride (2.08 mL, 15.00 mmol), compound 6 was obtained as a white crystal (0.93 g, 65%, over two steps). mp 67–68 °C. MS (ESI): *m/z* 283 [M − H]$^-$. IR (cm^{-1}, KBr): ν_{max} 1412, 1691, 1734, 2958, 3450. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.93 (t, 3H, CH$_3$, J = 4.2 Hz), 1.37–1.42 (m, 4H, 2 × CH$_2$), 1.88–1.91 (m, 2H, CH$_2$), 4.11–4.32 (m, 2H, COCH$_2$Cl), 6.71–6.78 (m, 1H, OCHCH$_2$), 7.36–7.42 (m, 1H, ArH), 7.56–7.62 (m, 2H, ArH), 8.08 (d, 1H, ArH, J = 8.1 Hz), 10.89 (brs, 1H, COOH). ^{13}C NMR (75 Hz, CDCl$_3$): δ 172.0, 166.5, 140.8, 133.1, 130.3, 130.0, 127.1, 125.7, 74.8, 41.0, 36.3, 27.8, 22.4, 13.8.

(±)-2-(1-(2-(Diethylamino)acetoxy)pentyl)benzoic acid (7a). To a solution of 6 (284 mg, 1.0 mmol) and K$_2$CO$_3$ (276 mg, 2.0 mmol) in acetone (20 mL) was added diethylamine (0.2 mL, 2.0 mmol), and the solution was heated to 65 °C and refluxed for 4 h. The solution was then filtered, and the filtrate was reconstituted in EtOAc (20 mL) and the solvent was removed under reduced pressure. The crude was purified by flash chromatography (CH$_2$Cl$_2$–MeOH = 100/1–50/1, v/v), to give 7a as a light yellow oil (231 mg, 72%). MS (ESI): *m/z* 322 [M + H]$^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.83 (t, 3H, CH$_3$, J = 7.5 Hz), 1.02 (t, 6H, 2 × NCH$_2$CH$_3$, J = 7.0 Hz), 1.29–1.37 (m, 4H, 2 × CH$_2$CH$_3$), 1.87–1.88 (m, 2H, CHCH$_2$), 2.94 (q, 4H, 2 × CH$_2$, J = 7.0 Hz), 3.62–3.76 (m, 2H, COCH$_2$N), 6.69 (m, 1H, CH), 7.21–7.26 (m, 1H, ArH), 7.34–7.43 (m, 2H, ArH), 7.80 (d, 1H, ArH, J = 7.5 Hz), 10.67 (brs, 1H, COOH).

(±)-2-(1-(2-(4-Methylpiperazin-1-yl)acetoxy)pentyl)benzoic acid (7b). Compound 7b was synthesized in the same manner as compound 7a. Starting from compound 6 (284 mg, 1.0 mmol) and *N*-methyl piperazine (0.22 mL, 2.0 mmol), compound 7b was obtained as a light yellow oil (271 mg, 78%). MS (ESI): *m/z* 349 [M + H]$^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.83 (t, 3H, CH$_3$, J = 7.5 Hz), 1.29–1.37 (m, 4H, 2 × CH$_2$CH$_3$), 1.87–1.88 (m, 2H, CHCH$_2$), 2.26 (t, 3H, NCH$_3$, J = 7.0 Hz), 2.45 (m, 8H, 4 × NCH$_2$), 3.62–3.76 (m, 2H, COCH$_2$N), 6.69 (m, 1H, CH), 7.21–7.26 (m, 1H, ArH), 7.34–7.43 (m, 2H, ArH), 7.80 (d, 1H, ArH, J = 7.5 Hz), 10.67 (brs, 1H, COOH).

General procedure for the preparation of the target compounds 8a–g. To a solution of 5 (50 mg, 0.2 mmol) and K$_2$CO$_3$ (55 mg, 0.4 mmol) in acetone (10 mL) were added 3a–g (0.2 mmol), and the solution was heated to 65 °C and refluxed for 6–8 h. The solution was then filtered, and the filtrate was reconstituted in EtOAc (20 mL) and the solvent was removed

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under reduced pressure. The crude compounds were purified by flash chromatography (PE–EtOAc = 10/1–1 : 1, v/v), to give the title compounds (67–78%).

(±)-2-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)ethyl 2-(1-acetoxy-pentyl)benzoate (8a). The title compound was obtained as a reddish brown solid (75%), mp 86–88 °C. MS (ESI): m/z 503.1 $[M + H]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.9 Hz), 1.26–1.48 (m, 4H, CH_2CH_2CH$_3$), 1.74–1.83 (m, 2H, CHCH_2), 2.07 (s, 3H, OOCCH_3), 4.10 (t, 2H, ArOCH_2, J = 6.2 Hz), 4.41 (t, 2H, ArCOOCH_2, J = 6.2 Hz), 6.53 (dd, 1H, CH, J = 4.7 Hz, 8.0 Hz), 6.96 (d, 2H, ArH, J = 8.6 Hz), 7.27–7.32 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.52 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.6 Hz), 7.87 (d, 1H, ArH, J = 7.8 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 67.8, 64.6, 36.3, 28.1, 25.4, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{25}$H$_{26}$O$_5$S$_3$, $[M + H]^+$ 503.0942; found 503.1018.

(±)-3-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)propyl 2-(1-acet-oxypentyl)benzoate (8b). The title compound was obtained as a reddish brown solid (78%), mp 83–85 °C. MS (ESI): m/z 517.1 $[M + H]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.9 Hz), 1.26–1.48 (m, 4H, CH_2CH_2CH$_3$), 1.74–1.83 (m, 2H, CHCH_2), 1.98–2.04 (m, 2H, ArCOOCH$_2$CH_2), 2.07 (s, 3H, OOCCH_3), 4.10 (t, 2H, ArOCH_2, J = 6.2 Hz), 4.41 (t, 2H, ArCOOCH_2, J = 6.2 Hz), 6.53 (dd, 1H, CH, J = 4.7 Hz, 8.0 Hz), 6.96 (d, 2H, ArH, J = 8.6 Hz), 7.27–7.32 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.52 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.6 Hz), 7.87 (d, 1H, ArH, J = 7.8 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 67.8, 64.6, 36.3, 28.1, 25.4, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{26}$H$_{28}$O$_5$S$_3$, $[M + H]^+$ 517.1099; found 517.1297.

(±)-4-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)butyl 2-(1-acetoxy-pentyl) benzoate (8c). The title compound was obtained as a reddish brown oil (72%), MS (ESI): m/z 531.2 $[M + H]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.9 Hz), 1.26–1.48 (m, 4H, CH_2CH_2CH$_3$), 1.74–1.83 (m, 2H, CHCH_2), 2.00–2.06 (m, 4H, ArCOOCH$_2$(CH_2)$_2$), 2.07 (s, 3H, OOCCH_3), 4.10 (t, 2H, ArOCH_2, J = 6.2 Hz), 4.41 (t, 2H, ArCOOCH_2, J = 6.2 Hz), 6.53 (dd, 1H, CH, J = 4.7 Hz, 8.0 Hz), 6.96 (d, 2H, ArH, J = 8.6 Hz), 7.27–7.32 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.52 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.6 Hz), 7.87 (d, 1H, ArH, J = 7.8 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 67.8, 64.6, 36.3, 28.1, 25.8, 25.4, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{27}$H$_{30}$O$_5$S$_3$, $[M + H]^+$ 531.1255; found 531.1375.

(±)-5-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)pentyl 2-(1-acet-oxypentyl) benzoate (8d). The title compound was obtained as a reddish brown oil (74%), MS (ESI): m/z 545.3 $[M + H]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.9 Hz), 1.24–1.47 (m, 4H, CH_2CH_2CH$_3$), 1.67–1.73 (m, 2H, CHCH_2), 1.78–2.05 (m, 6H, ArCOOCH$_2$(CH_2)$_3$), 2.07 (s, 3H, OOCCH_3), 4.06 (t, 2H, ArOCH_2, J = 6.3 Hz), 4.36 (t, 2H, ArCOOCH_2, J = 6.2 Hz), 6.54 (dd, 1H, CH, J = 4.8 Hz, 8.1 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz),

7.28–7.33 (m, 1H, ArH), 7.39 (s, 1H, C=CH), 7.51–7.52 (m, 2H, ArH), 7.59 (d, 2H, ArH, J = 8.7 Hz), 7.88 (d, 1H, ArH, J = 7.8 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 68.0, 64.9, 36.6, 29.7, 28.4, 25.8, 25.4, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{28}$H$_{32}$O$_5$S$_3$, $[M + H]^+$ 545.1412; found 545.2204.

(±)-6-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)hexyl 2-(1-acet-oxypentyl)benzoate (8e). The title compound was obtained as a reddish brown oil (78%), MS (ESI): m/z 559.4 $[M + H]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.8 Hz), 1.26–1.45 (m, 4H, CH_2CH_2CH$_3$), 1.47–1.56 (m, 4H, ArCOO(CH$_2$)$_2$CH_2, ArO(CH$_2$)$_2$CH_2), 1.70–1.73 (m, 2H, CHCH_2), 1.78–1.85 (m, 4H, ArCOOCH$_2$CH_2, ArOCH$_2$CH_2), 2.07 (s, 3H, OOCCH_3), 4.03 (t, 2H, ArOCH_2, J = 6.3 Hz), 4.34 (t, 2H, ArCOOCH_2, J = 6.6 Hz), 6.54 (dd, 1H, CH, J = 4.8 Hz, 8.1 Hz), 6.96 (d, 2H, ArH, J = 8.7 Hz), 7.28–7.33 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.53 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.7 Hz), 7.88 (d, 1H, ArH, J = 7.6 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 68.3, 65.1, 36.7, 28.9, 28.6, 28.1, 25.8, 25.7, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{29}$H$_{34}$O$_5$S$_3$, $[M + H]^+$ 559.1568; found 559.1626.

(±)-8-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)octyl 2-(1-acetoxy-pentyl)benzoate (8f). The title compound was obtained as a reddish brown oil (70%), MS (ESI): m/z 587.2 $[M + H]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.8 Hz), 1.26–1.42 (m, 4H, CH_2CH_2CH$_3$), 1.47–1.68 (m, 12H, ArCOOCH$_2$(CH)$_6$), 1.70–1.73 (m, 2H, CHCH_2), 2.06 (s, 3H, OOCCH_3), 4.02 (t, 2H, ArOCH_2, J = 6.4 Hz), 4.32 (t, 2H, ArCOOCH_2, J = 6.7 Hz), 6.54 (dd, 1H, CH, J = 4.7 Hz, 8.0 Hz), 6.96 (d, 2H, ArH, J = 8.7 Hz), 7.28–7.33 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.52 (m, 2H, ArH), 7.59 (d, 2H, ArH, J = 8.8 Hz), 7.88 (d, 1H, ArH, J = 7.7 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 68.3, 65.1, 36.7, 29.6, 29.3, 29.1, 25.9, 25.8, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{29}$H$_{34}$O$_5$S$_3$, $[M + H]^+$ 587.2246; found 587.2324.

(±)-12-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)dodecyl 2-(1-acetoxypentyl)benzoate (8g). The title compound was obtained as a reddish brown oil (67%), MS (ESI): m/z 665.2 $[M + Na]^+$. ^{1}H NMR (300 Hz, CDCl$_3$): δ 0.89 (t, 3H, CH$_3$, J = 6.8 Hz), 1.26–1.42 (m, 4H, CH_2CH_2CH$_3$), 1.45–1.66 (m, 20H, ArCOOCH$_2$(CH)$_{10}$), 1.70–1.73 (m, 2H, CHCH_2), 2.06 (s, 3H, OOCCH_3), 4.02 (t, 2H, ArOCH_2, J = 6.4 Hz), 4.32 (t, 2H, ArCOOCH_2, J = 6.7 Hz), 6.54 (dd, 1H, CH, J = 4.7 Hz, 8.0 Hz), 6.96 (d, 2H, ArH, J = 8.7 Hz), 7.28–7.33 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.52 (m, 2H, ArH), 7.59 (d, 2H, ArH, J = 8.8 Hz), 7.88 (d, 1H, ArH, J = 7.7 Hz). ^{13}C NMR (75 Hz, CDCl$_3$): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 68.3, 65.1, 36.7, 29.8, 29.7, 29.6, 29.3, 29.1, 25.9, 25.8, 22.5, 21.2, 14.0. HRMS (ESI): m/z calcd for C$_{35}$H$_{46}$O$_5$S$_3$, $[M + H]^+$ 643.2507; found 643.2601.

General procedure for the preparation of the target compounds 9a–f. To a solution of **7a** or **7b** (0.2 mmol) and K$_2$CO$_3$

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(55 mg, 0.4 mmol) in acetone (10 mL) were added **3c–e** (0.2 mmol), and the solution was heated to 65 °C and refluxed for 6–8 h. The solution was then filtered, and the filtrate was reconstituted in EtOAc (20 mL) and the solvent was removed under reduced pressure. The crude compounds were purified by flash chromatography (PE–EtOAc = 5/1–1 : 1, v/v), to give the title compounds (65–71%).

(±)-4-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)butyl 2-(1-(2-(diethylamino) acetoxy)pentyl)benzoate (**9a**). The title compound was obtained as a reddish brown oil (66%), MS (ESI): m/z 602.2 [M + H]⁺. ¹H NMR (300 Hz, CDCl₃): δ 0.89 (t, 3H, CH₃, J = 6.9 Hz), 1.06 (t, 6H, 2 × NCH₂CH₃, J = 7.2 Hz), 1.26–1.45 (m, 4H, CH₂CH₂CH₃), 1.76–1.88 (m, 2H, CHCH₂), 2.00–2.04 (m, 4H, ArCOOCH₂(CH₂)₂), 2.68 (q, 4H, 2 × NCH₂CH₃, J = 7.1 Hz), 3.42 (s, 2H, OCOCH₂N), 4.10 (t, 2H, ArOCH₂, J = 6.2 Hz), 4.41 (t, 2H, ArCOOCH₂, J = 6.2 Hz), 6.62 (dd, 1H, CH, J = 5.1 Hz, 7.8 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz), 7.27–7.32 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.52–7.55 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.8 Hz), 7.87 (d, 1H, ArH, J = 7.6 Hz). ¹³C NMR (75 Hz, CDCl₃): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 67.8, 64.6, 47.6, 36.3, 28.1, 25.8, 25.4, 22.5, 21.2, 14.0, 12.2. HRMS (ESI): m/z calcd for C₃₁H₃₉NO₅S₃, [M + H]⁺ 602.1990; found 602.2074.

(±)-5-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)pentyl 2-(1-(2-(diethylamino)acetoxy)pentyl)benzoate (**9b**). The title compound was obtained as a reddish brown oil (67%), MS (ESI): m/z 616.2 [M + H]⁺. ¹H NMR (300 Hz, CDCl₃): δ 0.89 (t, 3H, CH₃, J = 6.8 Hz), 1.06 (t, 6H, 2 × NCH₂CH₃, J = 7.1 Hz), 1.26–1.45 (m, 4H, CH₂CH₂CH₃), 1.60–1.67 (m, 2H, CHCH₂), 1.75–2.04 (m, 6H, ArCOOCH₂(CH₂)₃), 2.68 (q, 4H, 2 × NCH₂CH₃, J = 7.1 Hz), 3.42 (s, 2H, OCOCH₂N), 4.05 (t, 2H, ArOCH₂, J = 6.2 Hz), 4.36 (t, 2H, ArCOOCH₂, J = 6.4 Hz), 6.62 (dd, 1H, CH, J = 4.9 Hz, 8.0 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz), 7.28–7.32 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.54 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.8 Hz), 7.87 (d, 1H, ArH, J = 7.6 Hz). ¹³C NMR (75 Hz, CDCl₃): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 67.8, 64.6, 47.6, 36.7, 29.7, 28.7, 28.4, 22.6, 22.5, 21.2, 14.0, 12.2. HRMS (ESI): m/z calcd for C₃₂H₄₁NO₅S₃, [M + H]⁺ 616.2147; found 616.2230.

(±)-6-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)hexyl 2-(1-(2-(diethylamino)acetoxy)pentyl)benzoate (**9c**). The title compound was obtained as a reddish brown oil (70%), MS (ESI): m/z 630.2 [M + H]⁺. ¹H NMR (300 Hz, CDCl₃): δ 0.89 (t, 3H, CH₃, J = 6.8 Hz), 1.07 (t, 6H, 2 × NCH₂CH₃, J = 7.1 Hz), 1.26–1.47 (m, 4H, CH₂CH₂CH₃), 1.60–1.66 (m, 2H, CHCH₂), 1.82–1.84 (m, 8H, ArCOOCH₂(CH₂)₄), 2.70 (q, 4H, 2 × NCH₂CH₃, J = 7.0 Hz), 3.44 (s, 2H, OCOCH₂N), 4.03 (t, 2H, ArOCH₂, J = 6.3 Hz), 4.34 (t, 2H, ArCOOCH₂, J = 6.6 Hz), 6.62 (dd, 1H, CH, J = 5.0 Hz, 7.8 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz), 7.28–7.32 (m, 1H, ArH), 7.38 (s, 1H, C=CH), 7.51–7.54 (m, 2H, ArH), 7.58 (d, 2H, ArH, J = 8.8 Hz), 7.87 (d, 1H, ArH, J = 7.6 Hz). ¹³C NMR (75 Hz, CDCl₃): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.9, 67.8, 64.6, 47.6, 36.7, 29.7, 28.9, 28.6, 28.1, 25.8, 25.6, 22.4, 14.0, 12.2. HRMS (ESI): m/z calcd for C₃₃H₄₃NO₅S₃, [M + H]⁺ 630.2303; found 630.2387.

(±)-4-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)butyl 2-(1-(2-(4-methylpiperazin-1-yl)acetoxy)pentyl)benzoate (**9d**). The title compound was obtained as a reddish brown oil (71%), MS (ESI): m/z 629.2 [M + H]⁺. ¹H NMR (300 Hz, CDCl₃): δ 0.89 (t, 3H, CH₃, J = 6.9 Hz), 1.25–1.42 (m, 4H, CH₂CH₂CH₃), 1.54–1.62 (m, 2H, CHCH₂), 1.80–1.86 (m, 4H, ArCOOCH₂(CH₂)₂), 2.28 (s, 3H, NCH₃), 2.48–2.58 (m, 8H, 4 × NCH₂), 3.24 (s, 2H, OCOCH₂N), 4.10 (t, 2H, ArOCH₂, J = 6.2 Hz), 4.40 (t, 2H, ArCOOCH₂, J = 6.4 Hz), 6.62 (dd, 1H, CH, J = 5.1 Hz, 7.7 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz), 7.26–7.31 (m, 1H, ArH), 7.39 (s, 1H, C=CH), 7.50–7.51 (m, 2H, ArH), 7.59 (d, 2H, ArH, J = 8.8 Hz), 7.86 (d, 1H, ArH, J = 8.0 Hz). ¹³C NMR (75 Hz, CDCl₃): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 73.1, 67.8, 64.6, 59.4, 54.8, 52.9, 45.9, 36.6, 29.7, 28.0, 25.4, 22.6, 14.0. HRMS (ESI): m/z calcd for C₃₂H₄₀N₂O₅S₃, [M + H]⁺ 629.2099; found 629.2183.

(±)-5-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)pentyl 2-(1-(2-(4-methylpiperazin-1-yl)acetoxy)pentyl)benzoate (**9e**). The title compound was obtained as a reddish brown oil (70%), MS (ESI): m/z 643.2 [M + H]⁺. ¹H NMR (300 Hz, CDCl₃): δ 0.89 (t, 3H, CH₃, J = 6.9 Hz), 1.24–1.46 (m, 4H, CH₂CH₂CH₃), 1.52–1.56 (m, 2H, CHCH₂), 1.84–1.92 (m, 6H, ArCOOCH₂(CH₂)₃), 2.27 (s, 3H, NCH₃), 2.47–2.57 (m, 8H, 4 × NCH₂), 3.23 (s, 2H, OCOCH₂N), 4.06 (t, 2H, ArOCH₂, J = 6.2 Hz), 4.36 (t, 2H, ArCOOCH₂, J = 6.4 Hz), 6.62 (dd, 1H, CH, J = 5.0 Hz, 7.8 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz), 7.27–7.30 (m, 1H, ArH), 7.39 (s, 1H, C=CH), 7.50–7.51 (m, 2H, ArH), 7.59 (d, 2H, ArH, J = 8.8 Hz), 7.87 (d, 1H, ArH, J = 7.8 Hz). ¹³C NMR (75 Hz, CDCl₃): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.2, 67.3, 64.1, 58.5, 53.9, 52.0, 45.0, 35.7, 28.7, 28.0, 27.6, 22.6, 21.6, 14.0. HRMS (ESI): m/z calcd for C₃₃H₄₂N₂O₅S₃, [M + H]⁺ 643.2256; found 643.2339.

(±)-6-(4-(3-Thioxo-3H-1,2-dithiol-5-yl)phenoxy)hexyl 2-(1-(2-(4-methylpiperazin-1-yl)acetoxy)pentyl)benzoate (**9f**). The title compound was obtained as a reddish brown oil (65%), MS (ESI): m/z 657.0 [M + H]⁺. ¹H NMR (300 Hz, CDCl₃): δ 0.89 (t, 3H, CH₃, J = 6.8 Hz), 1.25–1.47 (m, 4H, CH₂CH₂CH₃), 1.50–1.56 (m, 2H, CHCH₂), 1.80–1.84 (m, 8H, ArCOOCH₂(CH₂)₄), 2.28 (s, 3H, NCH₃), 2.48–2.58 (m, 8H, 4 × NCH₂), 3.24 (s, 2H, OCOCH₂N), 4.03 (t, 2H, ArOCH₂, J = 6.4 Hz), 4.33 (t, 2H, ArCOOCH₂, J = 6.6 Hz), 6.62 (dd, 1H, CH, J = 5.0 Hz, 7.8 Hz), 6.96 (d, 2H, ArH, J = 8.8 Hz), 7.28–7.32 (m, 1H, ArH), 7.39 (s, 1H, C=CH), 7.49–7.50 (m, 2H, ArH), 7.59 (d, 2H, ArH, J = 8.8 Hz), 7.87 (d, 1H, ArH, J = 7.6 Hz). ¹³C NMR (75 Hz, CDCl₃): δ 173.1, 170.3, 166.9, 162.3, 143.7, 134.8, 134.6, 132.3, 131.3, 130.1, 128.6, 127.1, 126.2, 125.8, 124.1, 115.5, 92.6, 72.2, 67.3, 64.1, 58.5, 53.9, 52.0, 45.0, 35.7, 28.7, 28.0, 27.6, 24.8, 22.8, 22.4, 14.0. HRMS (ESI): m/z calcd for C₃₄H₄₄N₂O₅S₃, [M + H]⁺ 657.2412; found 657.2497.

4.2. Antiplatelet aggregation effect in vitro

Blood samples were withdrawn from rabbit carotid artery and mixed with 3.8% sodium citrate solution (9 : 1, v/v), followed

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by centrifuging at 500 rpm for 10 min at room temperature. After the resulting platelet-rich plasma (PRP) supernatant was collected, the residue was centrifuged at 3000 rpm for another 10 min at room temperature to obtain platelet-poor plasma (PPP). The PRP was adjusted with PPP in order to obtain platelet counts of $400–450 \times 10^9$ Pl L^{-1}. Platelet aggregation was determined by Born's turbidimetric method using a four-channel aggregometer (LG-PABER-I Platelet-Aggregometer, Beijing, China) within 3 h after blood collection. Briefly, PRP (240 μL) was pre-incubated with vehicle, positive control or different concentrations (0.1, 0.2, 0.4, 0.8 and 1.6 mM) of individual compounds (30 μL) for 5 min at 37 °C, followed by the addition of 10 μM of the adenosine 5′-diphosphate sodium salt (ADP, Sigma-Aldrich, USA) or 0.33 mM of the arachidonic acid (AA, Cayman Chemical, USA) respectively to induce the platelet aggregation. The maximum aggregation rate (MAR) was recorded within 5 min at 37 °C. The inhibition rate of the tested compounds on platelet aggregation was calculated with the following formula: Inhibition rate (%) = (100% − MAR of tested compound/MAR of vehicle). The IC_{50} value of each compound was calculated accordingly.

4.3. Inhibitory effect on the thrombosis in mice

Male ICR mice (30–35 g) were purchased from B&K Universal Group Limited, Shanghai, China. Animals were kept under standard laboratory conditions and maintained in a 12 h light–dark cycle with free access to food and water. All procedures were performed following institutional approval in accordance with the NIH Guide for the Care and Use of Laboratory Animals. A model of acute systemic vascular thromboembolism induced by infusion of a mixture of collagen and adrenaline was performed as described previously.[30] Briefly, the mice were divided into groups randomly and treated orally with vehicle dimethyl sulfoxide (DSMO), aspirin (160 mg kg^{-1}), NBP (160 mg kg^{-1}), or **8e** (160 or 470 mg kg^{-1}) by gavage daily for seven days. Two hours after the last administration, the mice were injected intravenously with a mixture of 0.21 mg kg^{-1} collagen and 44.5 μg kg^{-1} adrenaline. Mice were observed for thrombosis-related death within 15 min. The results are expressed as the percentage of mice alive as a function of time. The protective rates against vehicle are also calculated.

4.4. Antithrombotic activity assay in rats

Male Sprague-Dawley (SD) rats (250–280 g) were randomized and divided into six groups (n = 12 per group): (1) control group (0.5% CMC-Na), (2) vehicle group (A-V + 0.5% CMC-Na), (3) A-V + NBP (80 mg kg^{-1}) group, (4) A-V + aspirin (80 mg kg^{-1}) group, (5) A-V + **8e** (80 mg kg^{-1}) group, and (6) A-V + **8e** (235 mg kg^{-1}) group. The 3–6 groups of rats were treated by gavage with the indicated dose of the compound in saline daily for consecutive 7 days. Subsequently, the rats were anesthetized by intraperitoneal (i.p.) injection of pentobarbital (50 mg kg^{-1}). The control group of rats received a sham surgery while the remaining rats were subjected to the arteriovenous (A-V) shunt procedure.[31] Briefly, individual rats were inserted with an A-V shunt tube that directly connected the right carotid artery with the left jugular vein of rats. The polyethylene tube (14 cm, containing 6 cm long 4 braided silk threads) was filled with saline before installation. After 15 min circulation of blood through the shunt tube, both ends of the tubing were pinched, and the cotton thread was removed from the shunt tube. The wet weight of the cotton thread was measured immediately and the cotton thread was dried at the room temperature for 6 h, followed by measuring the dry weight. The wet and dry weights of thrombus formed on the cotton threads were determined by subtracting the pre-experiment weight of the dry 6 cm long threads.

4.5. Assay of hydrogen sulfide release *in vitro*

Compound **8e** (100 μM) was incubated (37° C) for timed intervals (0–90 min) with phosphate buffer or fresh rat liver homogenate (430 μL) and the concentration of released H_2S was measured as described previously.[32] After incubation, zinc acetate (1% w/v, 250 μL) was injected followed by trichloroacetic acid (10% w/v, 250 μL) to stop the reaction. Subsequently, N,N-dimethyl-p-phenylenediamine sulfate (20 μM; 133 μL) in 7.2 M HCl was added followed by $FeCl_3$ (30 μM, 133 μL) in 1.2 M HCl. The resulting solution was determined 10 min thereafter using a microplate reader at 670 nm. The H_2S concentration of **8e** was calculated against a standard curve of NaHS (3.125, 6.25, 12.5, 25, 50, 100 μM). Results are expressed as total % conversion of **8e** to H_2S at each time point.

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