European Journal of Medicinal Chemistry 115 (2016) 369–380

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Discovery of a ring-opened derivative of 3-n-butylphthalide bearing NO/H_2S-donating moieties as a potential anti-ischemic stroke agent

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ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form
16 March 2016
Accepted 17 March 2016
Available online 19 March 2016

Keywords:
Nitric oxide
Hydrogen sulfide
Anti-platelet aggregation
Ischemic stroke

ABSTRACT

To search for novel anti-ischemic stroke agents with higher potency than a known drug 3-n-butylphthalide (NBP), a series of ring-opened derivatives of NBP bearing both nitric oxide (NO) and hydrogen sulfide (H_2S)-donating moieties (NO/H_2S-NBP) (**8a-8o**) were designed, synthesized, and biologically evaluated. The most active compound **8d** was more potent than NBP and the corresponding H_2S-NBP **10** or NO-NBP **13** in inhibition of the ADP-induced platelet aggregation *in vitro*. In addition, **8d** produced moderate levels of NO and H_2S, which could be beneficial for improving cardiovascular and cerebral circulation. More importantly, in a rat model of transient focal cerebral ischemia, oral treatment with **8d** improved neurobehavioral function, reduced the infarct brain size and brain-water content, and enhanced the levels of brain antioxidant SOD, GSH and GSH-Px but diminished the level of oxidant MDA. These protective effects of **8d** against the ischemia/reperfusion (I/R)-related brain damage were greater than that of NBP, suggesting that **8d** may be a promising agent for further investigation.

1. Introduction

Ischemic stroke accounts for more than 80% of all strokes, and is a leading cause of death and long-term disability globally [1,2]. In the past years, remarkable progresses have been made in the understanding of the pathogenesis of ischemic stroke, including genetic high risk, thrombosis, and chronic inflammatory diseases such as hypertension, diabetes, and others [3–5]. To date, many drugs are available for the intervention of ischemic stroke, such as anti-platelet aggregation drugs, anticoagulant drugs, neuroprotective drugs, and thrombolytic drugs [6–9]. However, the efficacy of the current drugs is not satisfactory. Therefore, the development of novel and more effective drugs for the treatment of ischemic stroke will be of great significance.

The 3-n-butylphthalide (NBP, Fig. 1) was approved by the State Food and Drug Administration (SFDA) of China as a new drug for the treatment of ischemic stroke in 2002. Previous studies have shown that NBP possesses beneficial effects on stroke through multiple actions that affect different pathophysiological processes [10,11], including inhibiting platelet aggregation, reducing thrombus formation, improving microcirculation, regulating energy metabolism, decreasing brain infarct volume and oxidative damage. However, the efficacy of NBP is moderate. To improve its therapeutic effects, it is often administered by combination with other anti-platelet drug(s) [12]. In addition, a range of structural modification and related studies on NBP have been carried out. It is shown that the potassium salt of 2-(1-hydroxypentyl)-benzoate (HPBA, Fig. 1), a ring-opened NBP, has comparable potency to NBP and that a lot of HPBA derivatives have also been investigated [13–15]. However, the potency of these derivatives is still not satisfactory. Therefore, new derivatives of NBP with more potency are needed for the treatment of ischemic stroke.

Nitric oxide (NO), a free radical gas, is a messenger molecule with a variety of physiological roles, such as relaxing vascular smooth muscle, inhibiting platelet aggregation, reducing

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http://dx.doi.org/10.1016/j.ejmech.2016.03.044
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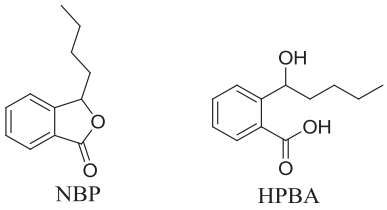


Fig. 1. Chemical structures of NBP and HPBA.

neutrophil adherence and activation, mitigating cell death, and promoting cell proliferation [16–21]. Thanks to these advantages, conjugation of NO donor with a known drug has been recognized as a means to improve activity of the anti-ischemic stroke drugs [22]. Recently, structurally diverse NO-donating derivatives of NBP have been reported by our group, and several derivatives exhibit greater anti-platelet and anti-thrombotic activities than NBP [22–24].

Hydrogen sulfide (H_2S) is known as the third gasotransmitter, following NO and carbon monoxide (CO). In central nervous system (CNS), H_2S is produced through the enzymes cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST) [25]. H_2S can regulate neurotransmission, acting as a neuroprotectant via its antioxidant, anti-inflammatory, and anti-apoptotic effects. Indeed, evidence accumulated over the past decades shows that H_2S has a potential therapeutic value in the treatment of several CNS diseases, including ischemic stroke, Alzheimer's disease, Parkinson's disease, etc [25–29]. More recently, our group has reported H_2S-based derivatives of NBP, some of which show better anti-platelet and anti-thrombotic activities than NBP [30].

Based on the above investigation, we hypothesized that NO/H_2S-donating derivatives of NBP (NO/H_2S-NBP) could display synergistic effects on ant-ischemic stroke with more potency than NBP, and corresponding NO-NBP or H_2S-NBP. To verify this hypothesis, a novel class of NO/H_2S-NBP were synthesized by using HPBA as a scaffold to which NO and H_2S donors were coupled. Nitrate (-(CH_2)$mONO_2$) was employed as an NO-donating moiety attached to HPBA through ferulic acid as a linker, which has anti-oxidant and anti-platelet aggregation activities [31]. In addition, 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (ADT-OH), a known H_2S-donor, in which 1,2-dithiole-3-thione is a H_2S-donating moiety [32], was introduced to the side chain of HPBA via a substituted acetate linkage. Herein, we report synthesis of these NO/H_2S-NBP, and their *in vitro* anti-platelet aggregative and *in vivo* anti-ischemic activities.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **8a-8o** was depicted in Scheme 1. Compounds **1a-1e**, **6**, **11** and **12** were synthesized as previously described [22,30,33]. Treatment of **1a-1e** with N-boc-piperazine gave protected intermediates **2a-2e**. The subsequent removal of the *t*-Boc protecting group by anhydrous HCl in ethyl acetate furnished H_2S donors **3a-3e**. The ferulic acid was converted into the corresponding bromo-substituted esters **4a-4c** using Et_3N in acetone. The S_N2 reaction of **4a-4c** with silver nitrate in CH_3CN afforded corresponding NO donors **5a-5c**. Compound **6** was coupled with **5a-5c** and methyl ferulate, respectively, in the presence of DCC/DMAP to yield corresponding esters **7a-7c** and **9**. Finally, compounds **3a-3e** were condensed with **7a-7c** to provide the target compounds **8a-8o**, respectively. In addition, compound **9** and **7a** were condensed with **3d** and **12** in the presence of Et_3N leading to **10** and **13** for comparison in biological evaluation where **10** was used as H_2S-NBP while **13** was used as NO-NBP.

2.2. Anti-platelet aggregation activity in vitro

The inhibitory effects of the target compounds on the adenosine diphosphate (ADP)-induced *in vitro* platelet aggregation in rabbit platelet rich plasma (PRP) were preliminarily evaluated using Born's turbidimetric method. NBP was used as a positive control. As shown in Fig. 2, all the target compounds displayed better inhibitory effects (19.74%–43.04%) than NBP (12.95%) at the same concentration (0.1 mM), among which **8d** (43.04%) was the most active.

Subsequently, the eight most potent compounds (**8d**, **8e**, **8g**, **8h**, **8j**, **8k**, **8m** and **8o**) were further assayed for their anti-platelet activity against the ADP induced platelet aggregation. Data were calculated and expressed as the IC_{50} values. As shown in Table 1, all of the tested compounds displayed higher inhibitory activity than NBP. Among them, **8d** was the most active inhibitor (IC_{50} = 0.14 mM), which was 5.2-fold more potent than NBP (IC_{50} = 0.74 mM). Therefore, **8d** was selected for the following investigations.

Given that **8d** was composed of two moieties (**3d** and **7a**), we further investigated their corresponding effects on the ADP-induced platelet aggregation *in vitro*. As shown in Fig. 3, **3d** and **7a** at 0.1 mM inhibited the ADP-induced platelet aggregation by 16.75% and 18.00%, respectively, which were lower than that of **8d** (40.95%), suggesting that these moieties may synergistically inhibit the ADP-induced platelet aggregation *in vitro*. To confirm that, **7a** and **3d** in combination were then tested for the inhibitory activity. As shown in Fig. 3, **8d** had a stronger anti-platelet aggregation activity than combination of **7a** with **3d**, which further support that both **3d** and **7a** displayed synergistic effects on anti-platelet aggregation of **8d**. Importantly, as shown in Fig. 3, the inhibitory activity of either **10** (27.81%) or **13** (19.75%) was significantly less than **8d** (40.95%), indicating that the NO/H_2S-NBP is more potent than the NO-NBP or H_2S-NBP alone in inhibition of the ADP-induced platelet aggregation. Furthermore, the effects of NO and H_2S on the antiplatelet aggregation activity of **8d** were investigated. The antiplatelet effect of **8d** was attenuated by treatment with 20 μM of carboxy-PTIO (a known NO scavenger [34]) or bismuth (III) subsalicylate (BSS, a reported H_2S scavenger [35]), from 40.95% to 29.15% and 40.95%–20.86%, respectively. These results further support the important roles of NO and H_2S-releasing groups for antiplatelet effect of **8d**.

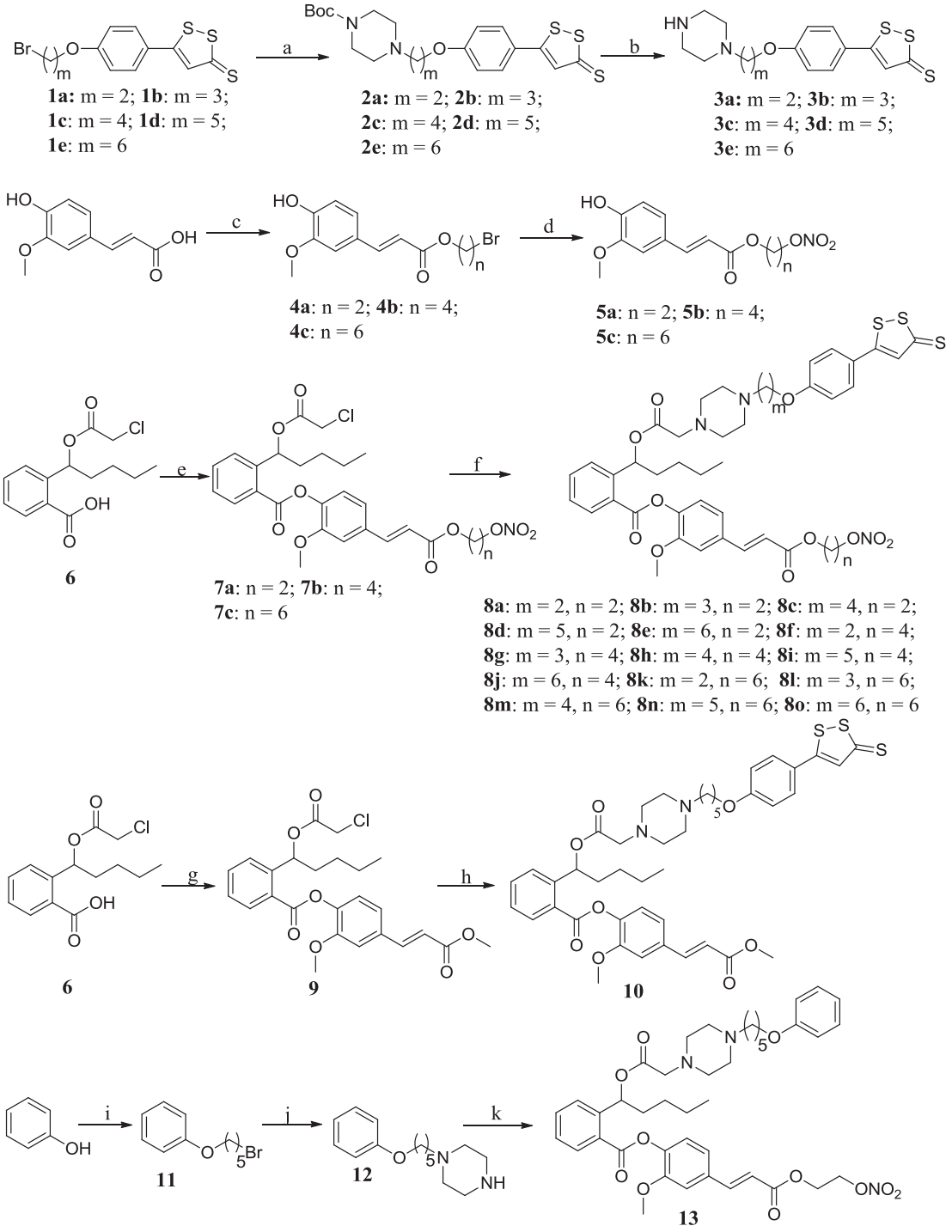
2.3. Assay of NO and H_2S release in vitro

In order to examine whether the target compounds could release both NO and H_2S, we first determined the levels of NO produced by **8d**, **8e**, **8g**, **8h**, **8j**, **8k**, **8m** and **8o** by Griess assay [36]. As shown in Fig. 4, the active compounds **8d**, **8o** and **8k** released moderate amount of NO (10.57, 10.22 and 9.57 μM, respectively). In contrast, the less active compounds **8h**, **8e**, **8j**, **8m** and **8g** under the same conditions released lower levels of NO (8.37, 7.51, 5.56, 5.38 and 3.32 μM, respectively).

Next, the levels of H_2S produced by **8d**, **8e**, **8g**, **8h**, **8j**, **8k**, **8m** and **8o** were measured using the method as previously reported [37]. As shown in Fig. 5, the active compounds **8d**, **8k** and **8o** released moderate levels of H_2S (4.4%, 3.82% and 3.32%, respectively) while the less active compounds **8h**, **8e**, **8j**, **8m** and **8g** released lower levels of H_2S (2.72%, 2.41%, 1.82%, 1.35% and 1.23%, respectively).

2.4. Anti-cerebral ischemic activity in rats

We further investigated the anti-cerebral ischemic activity of **8d**



Scheme 1. Synthesis of **8a-8o**, **10** and **13**. Reagents and conditions: (a) N-boc-piperazine, DMF, 70 °C, 8 h; (b) HCl/EtOAc, r.t.; (c) $Br(CH_2)_mBr$, Et_3N, acetone, reflux, 1–2 d; (d) $AgNO_3$, CH_3CN, reflux, 6 h; (e) **5a-5c**, DCC, DMAP, CH_2Cl_2, 0 °C to r.t.; (f) **3a-3e**, Et_3N, CH_2Cl_2, r.t., 12 h; (g) Methyl ferulate, DCC, DMAP, CH_2Cl_2, 0 °C to r.t.; (h) **3d**, Et_3N, CH_2Cl_2, r.t., 12 h; (i) $Br(CH_2)_5Br$, K_2CO_3, DMF, r.t., 24 h; (j) Piperazine, K_2CO_3, EtOH, 70 °C, 8 h; (k) **7a**, Et_3N, CH_2Cl_2, r.t., 12 h.

in a rat model of transient focal cerebral ischemia by intraluminal occlusion of the middle cerebral artery (MCAO) for 2 h followed by recirculation, which has been widely used to evaluate the protective effects of anti-stroke agents [38]. Male Sprague–Dawley rats were randomly and orally treated with 0.5% sodium carboxyl methyl cellulose (CMC-Na) (sham group and model group), NBP (80 mg/kg), or **8d** (80 mg/kg and 380 mg/kg, which were equal dose and molar to NBP, respectively) for seven days, and the rats were subjected to sham surgery or the MCA occlusion and reperfusion. The neurological functions of individual rats were assessed using Longa's method 24 h after reperfusion. As shown in Fig. 6, rats in the sham-operated group had no obvious neuronal abnormality, while neurological deficit scores in ischemia/reperfusion (I/R) model group were significantly higher ($P < 0.001$), and no significant difference in neurological deficit was found between the **8d**-treated group (80 mg/kg) and the model group. However, the

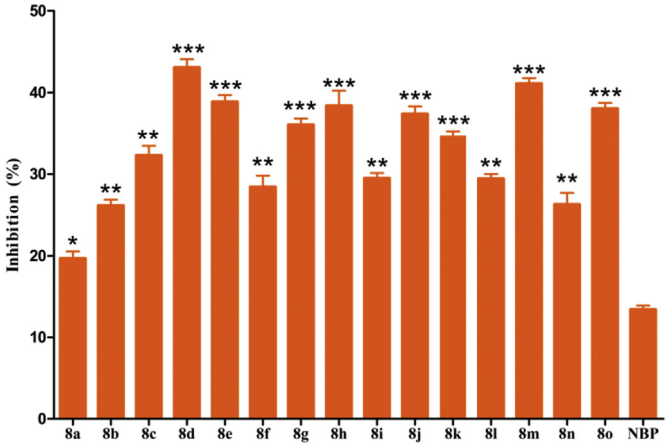


Fig. 2. The effects of compounds on the ADP (10 μM)-induced platelet aggregation *in vitro*. Rabbit platelet suspensions were preincubated with 0.1 mM of each compound tested at 37 °C for 5 min followed by the addition of ADP (10 μM). Data are expressed as the means (%) ± SD of each compound from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the NBP.

neurological deficit scores in the **8d**-treated I/R rats (380 mg/kg), but not in the NBP-treated group (80 mg/kg), were significantly reduced as compared with the model group ($P < 0.01$). These results suggested that **8d** displayed a better protective effect at an equal molar concentration of NBP on the surgical MCAO injury.

Similarly, the infarct size of individual rats were evaluated by 2,3,5-triphenyltetrazolium chloride (TTC) assay. In comparison with the model group, the cerebral infarct size in the **8d**-treated I/R rats was significantly reduced, particularly in rats treated with higher dose of **8d** ($P < 0.001$) (Fig. 7A). Additionally, administration of **8d** (380 mg/kg) markedly reduced the water content in I/R rat brain (Fig. 7B).

2.5. Antioxidative effects

In order to explore the mechanism underlying the anti-cerebral ischemic effects of **8d**, we evaluated the levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the brain tissues. In comparison with that in the sham group of rats, there was a significant elevation in MDA level (from 0.87 to 1.46 nmol/mg protein, Fig. 8A) and decline in GSH level (from 29.21 to 23.47 nmol/mg protein, Fig. 8B) in the model group. In contrast to the model group, pretreatment

Table 1
The IC_{50} values of compounds against platelet aggregation *in vitro*.

Compd.	IC_{50} (mM)
	ADP (10 μM)
Control	—
NBP	0.74 ± 0.20
8d	0.14 ± 0.08
8e	0.43 ± 0.12
8g	0.43 ± 0.04
8h	0.36 ± 0.02
8j	0.39 ± 0.03
8k	0.21 ± 0.02
8m	0.32 ± 0.02
8o	0.19 ± 0.03

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.

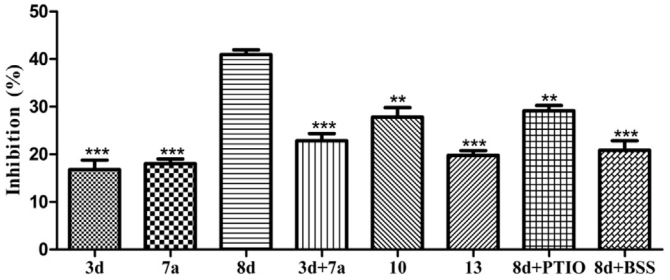


Fig. 3. Inhibition of ADP-induced rabbit platelet aggregation by selected compounds *in vitro*. Rabbit platelet suspensions were pre-incubated with tested compounds (0.1 mM) at 37 °C for 5 min followed by addition of ADP (10 μM). 20 μM of Carboxy-PTIO or BSS was added and incubated with the drug-platelet suspension in the indicated group. Data are expressed as the means (%) ± SD of each compound from three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. **8d**.

with both doses of **8d** (80 mg/kg and 380 mg/kg) remarkably decreased MDA level (from 1.46 to 1.07 nmol/mg protein and from 1.46 to 0.86 nmol/mg protein, respectively). Meanwhile, the GSH level in **8d**-treated group (380 mg/kg) was enhanced to 28.05 nmol/mg protein, which was somewhat greater than that in the NBP-

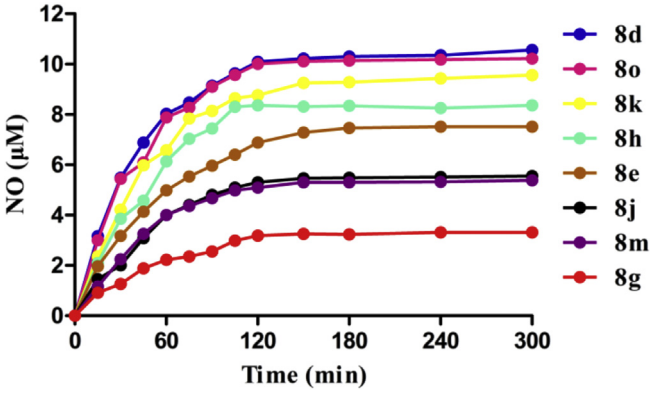


Fig. 4. NO-releasing assay of selected compounds *in vitro*. The levels of NO produced by selected compounds (0.1 mM) and presented as nitrite were determined by Griess assay. Individual compounds were incubated for the indicated periods and reacted with Griess reagent, followed by measuring at 540 nm. The levels of NO produced by individual compounds were calculated, according to the standard curve of nitrite. Data are expressed as the means of individual compounds tested at each time point and intra-group variations were less than 10%.

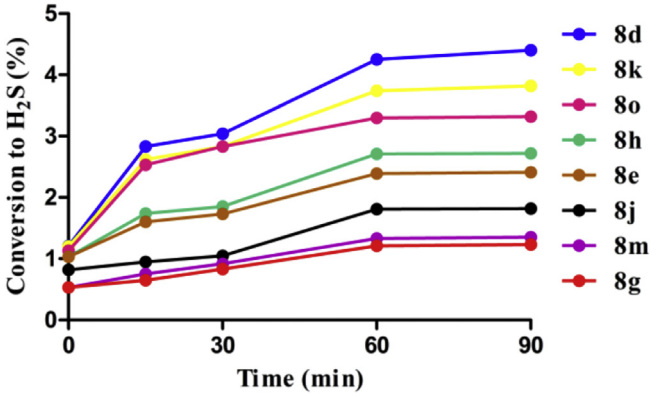


Fig. 5. Release of H_2S from selected compounds *in vitro*. Selected compounds (0.1 mM) was incubated (37 °C) for 90 min with fresh rat liver homogenate *in vitro*. H_2S conversion was determined. Data are expressed as accumulate % conversion of selected compounds to H_2S at each time point, $n = 6$.

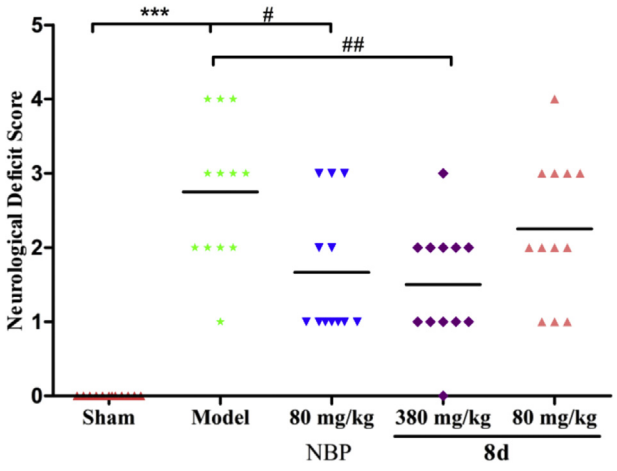


Fig. 6. Effects of **8d** and NBP on neurological deficit score in rats subjected to I/R ($n = 12$). Data are assessed 24 h after reperfusion and analyzed by Kruskal–Wallis test, followed by the Mann–Whitney *U* test. $^{***}P < 0.001$ vs. the sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the model group.

treated group (25.22 nmol/mg protein) and in vehicle group (23.47 nmol/mg protein).

Further analysis revealed that the activities of SOD and GSH-Px were obviously decreased in model group by 26.64% (from 119.10 to 87.37 U/mg protein, Fig. 8C) and 45.34% (from 28.21 to 15.42 U/mg protein, Fig. 8D), respectively, compared with that in the sham group. In contrast, pretreatment with **8d** at 80 and 380 mg/kg significantly enhanced the SOD activity by 23.07% (from 87.37 to 107.53 U/mg protein) and 32.02% (from 87.37 to 115.35 U/mg protein), respectively, compared with that in the model group. Moreover, the GSH-Px levels of **8d-**treated group (80 and 380 mg/kg) were elevated by 29.57% (from 15.42 to 19.98 U/mg protein) and 69.26% (from 15.42 to 26.10 U/mg protein), respectively. Notably, SOD and GSH-Px activities in the **8d**-treated group (380 mg/kg) were somewhat greater than those in the NBP group (24.36%, 108.65 U/mg protein; 63.55%, 25.22 U/mg protein, respectively).

3. Conclusion

In summary, a series of NO/H_2S-donating derivatives of NBP were designed, synthesized and biologically evaluated. The most active compound **8d** displayed the more potent inhibitory activity on the ADP-induced platelet aggregation *in vitro* than NBP, and the corresponding NO-NBP or H_2S-NBP. Furthermore, **8d** produced moderate levels of NO and H_2S *in vitro*, which could be beneficial for improving cardiovascular and cerebral circulation. Most importantly, treatment with **8d** improved neurobehavioral function, reduced the infarct brain size and brain-water content, thus

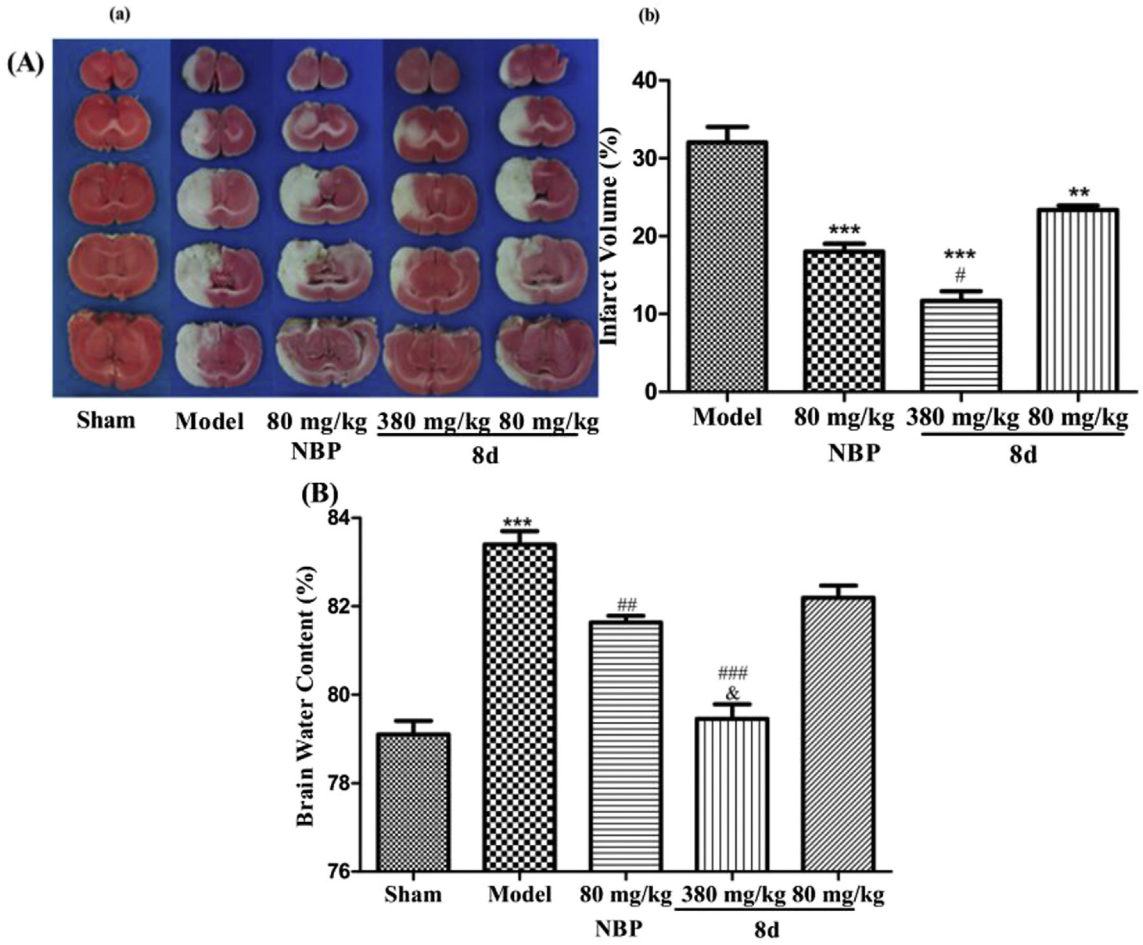


Fig. 7. (A) Effects of NBP and **8d** on infarction after cerebral I/R in rats. (a) TTC staining analysis of the infarcted brain regions. Data shown are representative examples from each treatment group of rats. (b) Quantitative analysis of the infarcted brain regions. The ratios of infarct area to whole brain areas were calculated. Data are expressed as the mean ± SD ($n = 6$) and analyzed by ANOVA followed by *post hoc* Tukey test. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the model group, $^{\#}P < 0.05$ vs. the NBP group. (B) Effects of NBP and **8d** on brain edema after cerebral I/R in rats. Data are expressed as the mean ± SD ($n = 6$) and analyzed by ANOVA followed by *post hoc* Tukey test. $^{***}P < 0.001$ vs. the sham group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the model group. $^{\&}P < 0.05$ vs. the NBP group.

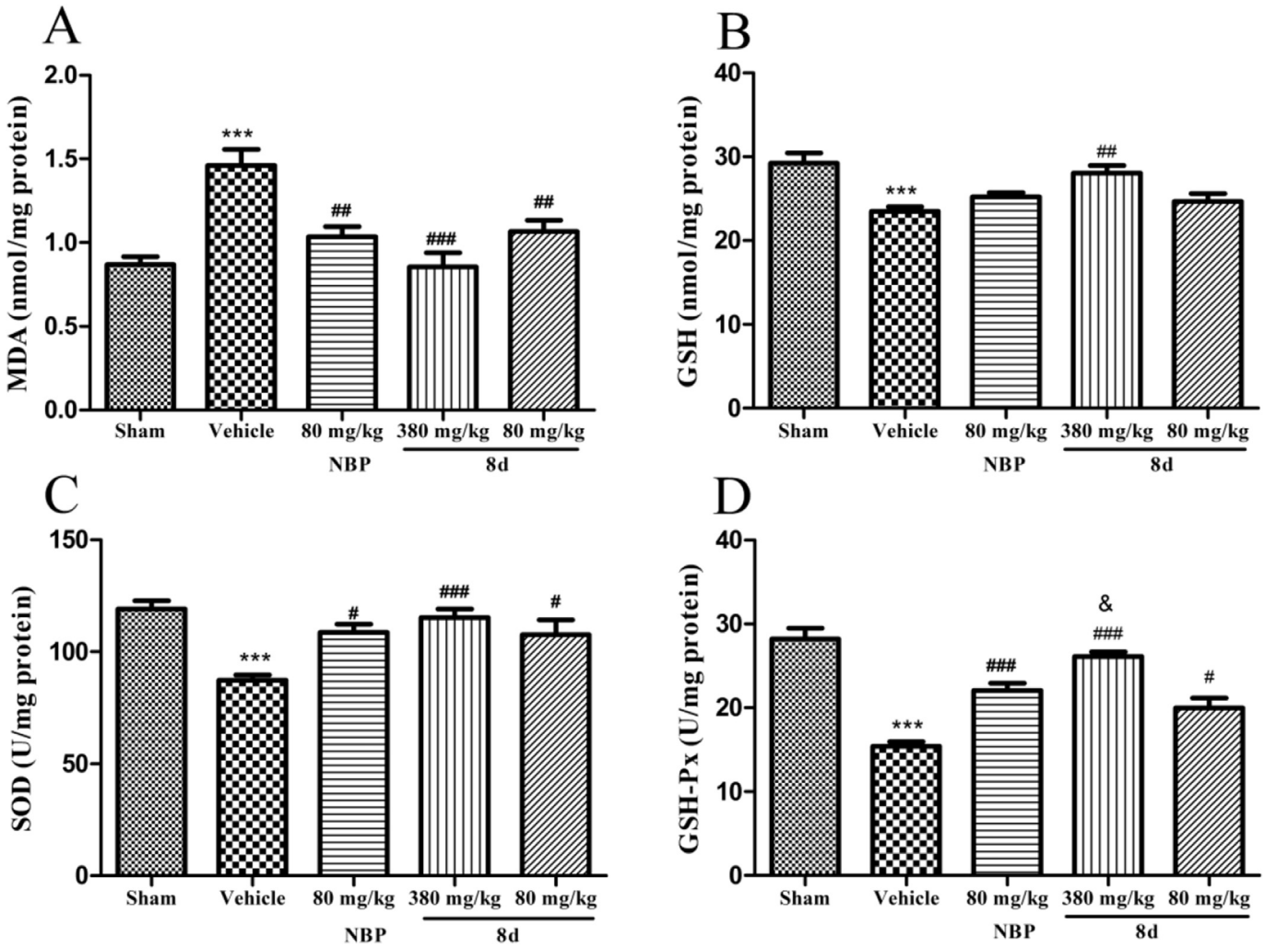


Fig. 8. Effect of **8d** on the levels of brain MDA (A), GSH (B), SOD activity (C) and GSH-Px activity (D) in rats after I/R in ischemic cerebral cortex. Data are expressed as the mean ± SD of individual groups of rats (n = 6) and were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* Turkey test. $^{***}P < 0.01$ vs. the sham group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the vehicle group. $^{\&}P < 0.05$ vs. the NBP group.

diminished cerebral damage in a rat model of MCAO. These protective effects of **8d** on the I/R-related brain damage were greater than that of NBP. In addition, treatment with **8d** significantly attenuated the I/R related oxidative stress in rat brains by increasing the levels of brain antioxidant SOD, GSH and GSH-Px but reducing the level of brain MDA, an index of reduced lipid peroxidation in the brains. Apparently, **8d** inhibited oxidative stress, which at least partially contributed to its anti-I/R brain injury in rats. Collectively, our findings suggest that **8d** may be a promising agent for further investigation.

4. Experimental protocols

4.1. Chemical analysis

Melting points of individual compounds were determined on a Mel-TEMP II melting point apparatus and uncorrected. ^{1}H NMR and ^{13}C spectra were recorded with a Bruker Avance 300 MHz spectrometer at 303 K, using TMS as an internal standard. All compounds were routinely checked by TLC and ^{1}H NMR. 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. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Solutions after reactions and extractions were concentrated using a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Compounds **1a**-**1e**, bromo-substituted esters **4a-4c**, corresponding nitrate **5a**-**5c**, **6**, **11** and **12** were synthesized as previously described [22,30,33,39,40].

4.1.1. General procedure for the preparation of ***2a-2e***

To a stirred solution of **1a-1e** (1.0 mmol) and N-boc-piperazine (0.224 g, 1.2 mmol) in DMF (20 mL) was added K_2CO_3 (0.415 g, 3.0 mmol). The mixture was then heated to 70 °C and stirred for 8 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was extracted with dichloromethane (20 mL) three times. The organic layer was separated and dried using anhydrous sodium sulfate, concentrated, purified by flash chromatography (petroleum ether/EtOAc, 1:1 v/v) to afford the title compounds.

*4.1.1.1. tert-Butyl 4-(2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) ethyl)piperazine-1- carboxylate (**2a**).* The title compound was obtained as a reddish brown oil, 65% yield. Analytical data for **2a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 8.8 Hz, 2H), 7.37 (s, 1H), 6.97 (d, J = 8.8 Hz, 2H), 4.16 (t, J = 5.6 Hz, 2H), 3.45 (t, J = 6.0 Hz, 4H), 2.85 (t,

J = 5.6 Hz, 2H), 2.53 (t, J = 6.0 Hz, 4H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.17, 173.05, 162.09, 154.77, 134.75, 128.69, 124.41, 115.62, 79.86, 66.37, 57.07, 53.50, 28.52; ESI-MS (*m/z*): 439.2 $[M+H]^+$.

*4.1.1.2. tert-Butyl 4-(3-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) propyl)piperazine-1- carboxylate (**2b**).* The title compound was obtained as a reddish brown oil, 68% yield. Analytical data for **2b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 8.8 Hz, 2H), 7.39 (s, 1H), 6.96 (d, J = 8.8 Hz, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.50–3.34 (m, 4H), 2.54 (t, J = 6.2 Hz, 2H), 2.45–2.35 (m, 4H), 2.07–1.93 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.22, 173.21, 162.50, 154.85, 134.72, 128.71, 124.20, 115.56, 79.83, 66.61, 55.02, 53.15, 28.55, 26.55; ESI-MS (*m/z*): 453.1 $[M+H]^+$.

*4.1.1.3. tert-Butyl 4-(4-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) butyl)piperazine-1- carboxylate (**2c**).* The title compound was obtained as a reddish brown oil, 73% yield. Analytical data for **2c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 8.7 Hz, 2H), 7.37 (s, 1H), 6.93 (d, J = 8.7 Hz, 2H), 4.03 (t, J = 6.1 Hz, 2H), 3.51–3.35 (m, 4H), 2.50–2.38 (m, 6H), 1.90–1.76 (m, 2H), 1.73–1.63 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.11, 173.21, 162.48, 154.78, 134.61, 128.67, 124.08, 115.49, 79.80, 68.14, 58.07, 52.94, 28.50, 27.06, 23.14; ESI-MS (*m/z*): 467.1 $[M+H]^+$.

*4.1.1.4. tert-Butyl 4-(5-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) pentyl)piperazine-1- carboxylate (**2d**).* The title compound was obtained as a reddish brown oil, 69% yield. Analytical data for **2d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 8.8 Hz, 2H), 7.37 (s, 1H), 6.93 (d, J = 8.8 Hz, 2H), 4.00 (t, J = 6.3 Hz, 2H), 3.51–3.37 (m, 4H), 2.51–2.33 (m, 6H), 1.92–1.73 (m, 2H), 1.64–1.33 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.13, 173.24, 162.55, 154.78, 134.61, 128.67, 124.05, 115.49, 79.81, 68.26, 58.44, 52.97, 29.00, 28.51, 26.30, 24.00; ESI-MS (*m/z*): 481.1 $[M+H]^+$.

*4.1.1.5. tert-Butyl 4-(6-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) hexyl)piperazine-1- carboxylate (**2e**).* The title compound was obtained as a reddish brown oil, 63% yield. Analytical data for **2e**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 8.6 Hz, 2H), 7.36 (s, 1H), 6.93 (d, J = 8.6 Hz, 2H), 3.99 (t, J = 6.2 Hz, 2H), 3.57–3.34 (m, 4H), 2.63–2.21 (m, 6H), 1.95–1.72 (m, 2H), 1.69–1.11 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.07, 173.25, 162.59, 154.75, 134.56, 128.64, 123.98, 115.49, 79.79, 68.36, 58.51, 52.91, 29.04, 28.49, 27.26, 26.42, 25.95; ESI-MS (*m/z*): 495.2 $[M+H]^+$.

*4.1.2. General procedure for the preparation of **3a-3e***

Compounds **2a-2e** (1.0 mmol) were dissolved in ethyl acetate (20 mL) and the solution was saturated with dry hydrogen chloride at 0 °C. The mixture was stirred for 3 h to complete the deprotection, and the solvent was evaporated. The resulting residue was extracted with dichloromethane (3 × 20 mL) and washed with saturated $NaHCO_3$ solution (10 mL). The organic layer was separated and dried using anhydrous sodium sulfate, concentrated, purified by flash chromatography (dichloromethane/methanol, 10:1 v/v) to afford the title compounds.

*4.1.2.1. 5-(4-(2-(Piperazin-1-yl)ethoxy)phenyl)-3H-1,2-dithiole-3-thione (**3a**).* The title compound was obtained as a reddish brown oil, 52% yield. Analytical data for **3a**: ^{1}H NMR (300 MHz, DMSO) δ 7.87 (d, J = 8.8 Hz, 2H), 7.77 (s, 1H), 7.08 (d, J = 8.9 Hz, 2H), 4.18 (t, J = 5.2 Hz, 2H), 3.15–3.02 (m, 4H), 2.78 (t, J = 5.1 Hz, 2H), 2.75–2.65 (m, 4H); ^{13}C NMR (75 MHz, DMSO) δ 214.84, 173.85, 161.88, 134.23, 129.07, 123.78, 115.62, 65.94, 56.01, 49.60; ESI-MS (*m/z*): 339.1 $[M+H]^+$.

*4.1.2.2. 5-(4-(3-(Piperazin-1-yl)propoxy)phenyl)-3H-1,2-dithiole-3-thione (**3b**).* The title compound was obtained as a reddish brown oil, 45% yield. Analytical data for **3b**: ^{1}H NMR (300 MHz, DMSO) δ 7.85 (d, J = 8.8 Hz, 2H), 7.74 (s, 1H), 7.05 (d, J = 8.8 Hz, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.13–3.03 (m, 4H), 2.64–2.53 (m, 4H), 2.51–2.43 (m, 2H), 1.91–1.86 (m, 2H); ^{13}C NMR (75 MHz, DMSO) δ 214.82, 173.92, 162.18, 134.18, 129.09, 123.66, 115.55, 66.25, 53.98, 49.46, 25.86; ESI-MS (*m/z*): 353.1 $[M+H]^+$.

*4.1.2.3. 5-(4-(4-(Piperazin-1-yl)butoxy)phenyl)-3H-1,2-dithiole-3-thione (**3c**).* The title compound was obtained as a reddish brown oil, 47% yield. Analytical data for **3c**: ^{1}H NMR (300 MHz, DMSO) δ 7.85 (d, J = 8.8 Hz, 2H), 7.75 (s, 1H), 7.06 (d, J = 8.8 Hz, 2H), 4.07 (t, J = 6.2 Hz, 2H), 3.16–2.99 (m, 4H), 2.59 (s, 4H), 2.47–2.34 (m, 2H), 1.84–1.65 (m, 2H), 1.62–1.44 (m, 2H); ^{13}C NMR (75 MHz, DMSO) δ 214.78, 173.90, 162.16, 134.13, 129.05, 123.57, 115.51, 67.83, 56.81, 49.27, 26.24, 22.29; ESI-MS (*m/z*): 367.1 $[M+H]^+$.

*4.1.2.4. 5-(4-((5-(Piperazin-1-yl)pentyl)oxy)phenyl)-3H-1,2-dithiole-3-thione (**3d**).* The title compound was obtained as a reddish brown oil, 40% yield. Analytical data for **3d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 8.8 Hz, 2H), 7.32 (s, 1H), 6.90 (d, J = 8.8 Hz, 2H), 3.97 (t, J = 6.2 Hz, 2H), 3.26–3.06 (m, 4H), 2.64 (s, 4H), 2.50–2.30 (m, 2H), 1.86–1.70 (m, 2H), 1.60–1.38 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.84, 173.29, 162.46, 134.37, 128.57, 123.86, 115.39, 68.14, 57.81, 49.82, 28.81, 26.13, 23.67; ESI-MS (*m/z*): 381.1 $[M+H]^+$.

*4.1.2.5. 5-(4-((6-(Piperazin-1-yl)hexyl)oxy)phenyl)-3H-1,2-dithiole-3-thione (**3e**).* The title compound was obtained as a reddish brown oil, 41% yield. Analytical data for **3e**: ^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, J = 8.8 Hz, 2H), 7.76 (s, 1H), 7.06 (d, J = 8.8 Hz, 2H), 4.05 (t, J = 6.3 Hz, 2H), 3.13–2.94 (m, 4H), 2.61–2.53 (m, 4H), 2.40–2.28 (m, 2H), 1.79–1.65 (m, 2H), 1.50–1.13 (m, 6H); ^{13}C NMR (75 MHz, DMSO) δ 214.78, 173.91, 162.20, 134.12, 129.07, 123.54, 115.50, 68.02, 57.35, 49.33, 28.50, 26.51, 25.86, 25.35; ESI-MS (*m/z*): 395.1 $[M+H]^+$.

*4.1.3. General procedure for the preparation of **7a-7c***

To a solution of compound **6** (2.1 mmol) and the corresponding nitrates **5a**-**5c** (2.0 mmol) in anhydrous CH_2Cl_2 (25 mL) was added DMAP (0.1 mmol) and the solution was left stirring at room temperature for 10 min. DCC (2.1 mmol) was then added and the solution was solution was left stirring at room temperature for 6 h. The solution was then filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 5:1 v/v) to afford the title compounds.

*4.1.3.1. (±)-(E)-2-Methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-chloroacetoxy)pentyl)benzoate (**7a**).* The title compound was obtained as a colorless oil, 85% yield. Analytical data for **7a**: ^{1}H NMR (300 MHz, $CDCl_3$): 8.17 (d, J = 7.7 Hz, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.61–7.59 (m, 2H), 7.49–7.33 (m, 1H), 7.25–7.15 (m, 3H), 6.71–6.67 (m, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.76–4.73 (m, 2H), 4.56–4.44 (m, 2H), 4.10 (s, 2H), 3.89 (s, 3H), 1.95–1.85 (m, 2H), 1.53–1.15 (m, 4H), 0.85 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.56, 166.37, 164.62, 151.68, 145.46, 143.58, 141.87, 133.26, 131.10, 127.75, 127.28, 126.33, 123.60, 121.61, 117.24, 111.47, 75.02, 70.71, 60.39, 56.04, 41.15, 36.61, 27.97, 22.50, 14.03; ESI-MS (*m/z*): 572.1 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{26}H_{28}ClNO_{10}Na$ $[M+Na]^+$ 572.1294, found 572.1306.

*4.1.3.2. (±)-(E)-2-Methoxy-4-(3-(4-(nitrooxy)butoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-chloroacetoxy)pentyl)benzoate (**7b**).* The title compound was obtained as a colorless oil, 88% yield. Analytical data

for **7b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 7.6 Hz, 1H), 7.64 (d, J = 15.9 Hz, 1H), 7.59–7.52 (m, 2H), 7.39–7.34 (m, 1H), 7.21–7.06 (m, 3H), 6.71–6.67 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.45 (t, J = 5.9 Hz, 2H), 4.19 (t, J = 5.5 Hz, 2H), 4.08 (s, 2H), 3.84 (s, 3H), 1.95–1.71 (m, 6H), 1.46–1.19 (m, 4H), 0.82 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.43, 166.31, 164.36, 151.38, 144.07, 143.29, 141.38, 133.26, 133.01, 130.78, 127.49, 127.01, 126.04, 123.23, 121.11, 117.91, 111.19, 74.64, 72.63, 63.45, 55.71, 40.91, 36.33, 27.69, 24.85, 23.44, 22.19, 13.76; ESI-MS (*m/z*): 600.2 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{28}H_{32}ClNO_{10}Na$ $[M+Na]^+$ 600.1607, found 600.1625.

*4.1.3.3. (±)-(E)-2-Methoxy-4-(3-((6-(nitrooxy)hexyl)oxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-chloroacetoxy)pentyl)benzoate (**7c**).* The title compound was obtained as a colorless oil, 80% yield. Analytical data for **7c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.62–7.57 (m, 2H), 7.50–7.33 (m, 1H), 7.26–7.11 (m, 3H), 6.71–6.67 (m, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.47–4.42 (m, 2H), 4.23–4.19 (m, 2H), 4.10 (s, 2H), 3.88 (s, 3H), 1.92–1.88 (m, 2H), 1.85–1.73 (m, 4H), 1.47–1.39 (m, 4H), 1.39–1.18 (m, 4H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.89, 166.48, 164.58, 151.56, 144.10, 143.49, 141.53, 133.55, 133.18, 131.01, 127.68, 127.26, 126.25, 123.44, 121.33, 118.37, 111.32, 74.94, 73.26, 64.40, 55.95, 41.09, 36.56, 28.53, 27.90, 26.69, 25.61, 25.40, 22.43, 13.97; ESI-MS (*m/z*): 628.2 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{30}H_{36}ClNO_{10}Na$ $[M+Na]^+$ 628.1920, found 628.1937.

*4.1.4. General procedure for the preparation of **8a-8o***

To a solution of compounds **7a-7c** (1.0 mmol) and Et_3N (0.122 g, 1.2 mmol) in anhydrous dichloromethane (10 mL) was added **3a-3e** (1.0 mmol), and the mixture was stirred at room temperature for 12 h. The dichloromethane was removed in vacuo, and the resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1 v/v) to afford the title compounds.

*4.1.4.1. (±)-(E)-2-Methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)ethyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8a**).* The title compound was obtained starting from **3a** and **7a**. Reddish brown oil, 21% yield. Analytical data for **8a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.64–7.54 (m, 4H), 7.44–7.36 (m, 2H), 7.26–7.14 (m, 3H), 6.96 (d, J = 8.8 Hz, 2H), 6.65–6.61 (m, 1H), 6.41 (d, J = 16.0 Hz, 1H), 4.77–4.74 (m, 2H), 4.52–4.49 (m, 2H), 4.17–4.10 (m, 2H), 3.88 (s, 3H), 3.34–3.19 (m, 2H), 2.85 (t, J = 5.6 Hz, 2H), 2.64 (s, 8H), 1.91–1.79 (m, 2H), 1.39–1.27 (m, 4H), 0.84 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.25, 173.08, 169.54, 166.39, 164.68, 162.16, 151.75, 145.52, 144.39, 141.98, 134.79, 133.27, 133.09, 131.08, 128.70, 127.51, 127.34, 126.38, 124.43, 123.69, 121.63, 117.24, 115.66, 111.54, 73.34, 70.71, 66.30, 60.43, 59.47, 56.92, 56.11, 53.43, 52.88, 36.73, 28.14, 22.55, 14.08; ESI-MS (*m/z*): 852.2 $[M+H]^+$, 874.2 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{41}H_{46}N_3O_{11}S_3$ $[M+H]^+$ 852.2289, found 852.2305.

*4.1.4.2. (±)-(E)-2-Methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(3-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)propyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8b**).* The title compound was obtained starting from **3b** and **7a**. Reddish brown oil, 23% yield. Analytical data for **8b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 16.0 Hz, 1H), 7.63–7.51 (m, 4H), 7.44–7.34 (m, 2H), 7.23–7.13 (m, 3H), 6.95 (d, J = 8.8 Hz, 2H), 6.65–6.61 (m, 1H), 6.41 (d, J = 16.0 Hz, 1H), 4.76–4.73 (m, 2H), 4.51–4.48 (m, 2H), 4.10–4.04 (m, 2H), 3.88 (s, 3H), 3.33–3.19 (m, 2H), 2.60–2.50 (m, 10H), 2.03–1.96 (m, 2H), 1.88–1.83 (m, 2H), 1.42–1.31 (m, 4H), 0.83 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.22, 173.20, 169.58, 166.37, 164.67, 162.54, 151.76, 145.49, 144.41, 141.99, 134.67, 133.26, 133.05, 131.05, 128.67, 127.48, 127.36, 126.39, 124.16, 123.68, 121.61, 117.24, 115.59, 111.55, 73.27, 70.70, 66.74, 60.42, 59.53, 56.10, 54.89, 53.08, 52.99, 36.72, 28.12, 26.58, 22.53, 14.07; ESI-MS (*m/z*): 866.2 $[M+H]^+$, 888.2 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{42}H_{48}N_3O_{11}S_3$ $[M+H]^+$ 866.2445, found 866.2468.

*4.1.4.3. (±)-(E)-2-Methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(4-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)butyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8c**).* The title compound was obtained starting from **3c** and **7a**. Reddish brown oil, 15% yield. Analytical data for **8c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 7.6 Hz, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.61–7.57 (m, 4H), 7.45–7.37 (m, 2H), 7.24–7.16 (m, 3H), 6.95 (d, J = 8.7 Hz, 2H), 6.66–6.63 (m, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.80–4.71 (m, 2H), 4.58–4.47 (m, 2H), 4.05–4.02 (m, 2H), 3.89 (s, 3H), 3.33–3.19 (m, 2H), 2.60–2.40 (m, 10H), 1.85–1.81 (m, 4H), 1.69–1.67 (m, 2H), 1.46–1.27 (m, 4H), 0.84 (t, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.23, 173.23, 169.60, 166.39, 164.68, 162.58, 151.78, 145.53, 144.43, 142.01, 134.69, 133.27, 133.07, 131.07, 128.70, 127.50, 127.38, 126.42, 124.15, 123.71, 121.63, 117.25, 115.57, 111.57, 73.28, 70.71, 68.28, 60.44, 59.58, 58.10, 56.12, 53.06, 36.75, 28.14, 27.16, 23.88, 22.55, 14.08; ESI-MS (*m/z*): 880.2 $[M+H]^+$, 902.2 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{43}H_{50}N_3O_{11}S_3$ $[M+H]^+$ 880.2602, found 880.2618.

*4.1.4.4. (±)-(E)-2-Methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(5-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)pentyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8d**).* The title compound was obtained starting from **3d** and **7a**. Reddish brown oil, 21% yield. Analytical data for **8d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 7.8 Hz, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.62–7.54 (m, 4H), 7.43–7.35 (m, 2H), 7.25–7.14 (m, 3H), 6.97–6.92 (m, 2H), 6.66–6.63 (m, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.77–4.74 (m, 2H), 4.52–4.49 (m, 2H), 4.03–4.01 (m, 2H), 3.89 (s, 3H), 3.33–3.19 (m, 2H), 2.60–2.35 (m, 10H), 1.90–1.76 (m, 4H), 1.65–1.50 (m, 4H), 1.38–1.22 (m, 4H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.06, 173.18, 169.54, 166.29, 164.60, 162.56, 151.69, 145.41, 144.35, 141.92, 134.53, 133.18, 133.00, 130.97, 128.62, 127.41, 127.28, 126.35, 123.99, 123.61, 121.54, 117.18, 115.47, 111.49, 73.17, 70.66, 68.30, 60.35, 59.54, 58.53, 56.05, 53.11, 36.66, 28.99, 28.49, 28.06, 26.54, 22.47, 14.01; ESI-MS (*m/z*): 894.3 $[M+H]^+$, 916.3 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{44}H_{52}N_3O_{11}S_3$ $[M+H]^+$ 894.2758, found 894.2771.

*4.1.4.5. (±)-(E)-2-Methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(6-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)hexyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8e**).* The title compound was obtained starting from **3e** and **7a**. Reddish brown oil, 24% yield. Analytical data for **8e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 7.8 Hz, 1H), 7.69 (d, J = 16.0 Hz, 1H), 7.63–7.49 (m, 4H), 7.42–7.30 (m, 2H), 7.23–7.10 (m, 3H), 6.93 (d, J = 8.8 Hz, 2H), 6.64–6.60 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.75–4.72 (m, 2H), 4.50–4.47 (m, 2H), 3.99 (t, J = 6.4 Hz, 2H), 3.87 (s, 3H), 3.32–3.18 (m, 2H), 2.63–2.58 (m, 8H), 2.45–2.34 (m, 2H), 1.92–1.74 (m, 4H), 1.59–1.28 (m, 10H), 0.82 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.08, 173.24, 169.51, 166.34, 164.64, 162.61, 151.72, 145.46, 144.34, 141.95, 134.59, 133.23, 133.07, 131.02, 128.66, 127.47, 127.31, 126.37, 124.03, 123.65, 121.58, 117.21, 115.53, 111.52, 73.29, 70.68, 68.37, 60.39, 59.41, 58.44, 56.08, 52.92, 52.59, 36.69, 29.03, 28.09, 27.24, 26.39, 25.93, 22.50, 14.04; ESI-MS (*m/z*): 908.3 $[M+H]^+$, 930.3 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{45}H_{54}N_3O_{11}S_3$ $[M+H]^+$ 908.2915, found 908.2935.

4.1.4.6. (±)-(E)-2-Methoxy-4-(3-(4-(nitrooxy)butoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)

*phenoxy)ethyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8f**).* The title compound was obtained starting from **3a** and **7b**. Reddish brown oil, 19% yield. Analytical data for **8f**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 16.0 Hz, 1H), 7.63–7.51 (m, 4H), 7.44–7.34 (m, 2H), 7.24–7.16 (m, 3H), 6.97 (d, J = 8.8 Hz, 2H), 6.66–6.62 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.53 (t, J = 5.9 Hz, 2H), 4.26–4.18 (m, 2H), 4.16 (t, J = 5.6 Hz, 2H), 3.89 (s, 3H), 3.34–3.20 (m, 2H), 2.85 (t, J = 5.5 Hz, 2H), 2.64 (s, 8H), 1.87 (s, 6H), 1.39–1.28 (m, 4H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.16, 173.03, 169.51, 166.77, 164.66, 162.16, 151.69, 144.49, 144.34, 141.75, 134.70, 133.45, 133.02, 131.02, 128.65, 127.46, 127.35, 126.35, 124.35, 123.60, 121.39, 118.08, 115.63, 111.49, 73.26, 72.74, 66.36, 63.70, 59.47, 56.91, 56.07, 53.43, 52.90, 36.70, 28.09, 25.18, 23.83, 22.50, 14.04; ESI-MS (m/z): 880.3 $[M+H]^+$, 902.2 $[M+Na]^+$; ESI-HRMS (m/z): calculated for $C_{43}H_{50}N_3O_{11}S_3$ $[M+H]^+$ 880.2602, found 880.2619.

*4.1.4.7. (±)-(E)-2-Methoxy-4-(3-(4-(nitrooxy)butoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(3-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)propyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8g**).* The title compound was obtained starting from **3b** and **7b**. Reddish brown oil, 24% yield. Analytical data for **8g**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.63–7.51 (m, 4H), 7.44–7.34 (m, 2H), 7.23–7.16 (m, 3H), 6.95 (d, J = 8.7 Hz, 2H), 6.66–6.62 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.53 (t, J = 5.9 Hz, 2H), 4.26 (t, J = 5.5 Hz, 2H), 4.08 (t, J = 6.0 Hz, 2H), 3.88 (s, 3H), 3.36–3.21 (m, 2H), 2.66 (s, 10H), 2.09–2.04 (m, 2H), 1.93–1.78 (m, 6H), 1.38–1.28 (s, 4H), 0.83 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 214.98, 173.17, 169.50, 166.72, 164.60, 162.43, 151.58, 144.42, 144.29, 141.64, 134.49, 133.36, 133.00, 130.96, 128.58, 127.40, 127.20, 126.28, 124.00, 123.52, 121.33, 117.99, 115.46, 111.37, 73.18, 72.72, 66.62, 63.65, 59.40, 55.99, 54.80, 52.94, 52.86, 36.63, 28.03, 26.44, 25.10, 23.73, 22.44, 14.00; ESI-MS (m/z): 894.3 $[M+H]^+$, 916.3 $[M+Na]^+$; ESI-HRMS (m/z): calculated for $C_{44}H_{52}N_3O_{11}S_3$ $[M+H]^+$ 894.2758, found 894.2782.

*4.1.4.8. (±)-(E)-2-Methoxy-4-(3-(4-(nitrooxy)butoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(4-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)butyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8h**).* The title compound was obtained starting from **3c** and **7b**. Reddish brown oil, 28% yield. Analytical data for **8h**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 16.0 Hz, 1H), 7.63–7.52 (m, 4H), 7.48–7.34 (m, 2H), 7.24–7.16 (m, 3H), 6.95 (d, J = 8.8 Hz, 2H), 6.66–6.62 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.53 (t, J = 6.0 Hz, 2H), 4.27 (t, J = 5.7 Hz, 2H), 4.04 (t, J = 6.1 Hz, 2H), 3.89 (s, 3H), 3.34–3.20 (m, 2H), 2.63–2.49 (m, 10H), 1.97–1.81 (m, 8H), 1.73–1.71 (m, 2H), 1.40–1.28 (m, 4H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 215.09, 173.25, 169.49, 166.81, 164.68, 162.49, 151.68, 144.53, 144.32, 141.73, 134.58, 133.46, 133.10, 131.05, 128.68, 127.51, 127.30, 126.36, 124.09, 123.60, 121.4, 118.09, 115.52, 111.47, 73.33, 72.79, 68.12, 63.73, 59.31, 57.82, 56.07, 52.70, 52.46, 36.70, 29.78, 28.11, 27.03, 25.19, 23.82, 22.51, 14.07; ESI-MS (m/z): 908.3 $[M+H]^+$; ESI-HRMS (m/z): calculated for $C_{45}H_{54}N_3O_{11}S_3$ $[M+H]^+$ 908.2915, found 908.2941.

*4.1.4.9. (±)-(E)-2-Methoxy-4-(3-(4-(nitrooxy)butoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(5-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)pentyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8i**).* The title compound was obtained starting from **3d** and **7b**. Reddish brown oil, 28% yield. Analytical data for **8i**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.60–7.56 (m, 4H), 7.43–7.32 (m, 2H), 7.23–7.15 (m, 3H), 6.93 (d, J = 8.8 Hz, 2H), 6.65–6.61 (m, 1H), 6.39 (d, J = 16.0 Hz, 1H), 4.52 (t, J = 6.0 Hz, 2H), 4.25 (t, J = 5.7 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 3.88 (s, 3H), 3.32–3.18 (m, 2H), 2.60–2.53 (m, 8H), 2.41–2.33 (m, 2H), 1.94–1.74 (m, 8H), 1.60–1.43 (m, 4H), 1.31–1.27 (m, 4H), 0.83 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.15, 173.23, 169.56, 166.80, 164.68, 162.58, 151.69, 144.52, 144.39, 141.75, 134.61, 133.44, 133.04, 131.03, 128.66, 127.45, 127.33, 126.37, 124.05, 123.61, 121.41, 118.07, 115.51, 111.47, 73.25, 72.76, 68.31, 63.71, 59.55, 58.44, 56.07, 53.03, 52.98, 36.72, 29.02, 28.11, 26.50, 25.19, 24.04, 23.83, 22.52, 14.06; ESI-MS (m/z): 922.3 $[M+H]^+$, 944.3 $[M+Na]^+$; ESI-HRMS (m/z): calculated for $C_{46}H_{56}N_3O_{11}S_3$ $[M+H]^+$ 922.3071, found 922.3082.

*4.1.4.10. (±)-(E)-2-Methoxy-4-(3-(4-(nitrooxy)butoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(6-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)hexyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8j**).* The title compound was obtained starting from **3e** and **7b**. Reddish brown oil, 29% yield. Analytical data for **8j**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.60–7.56 (m, 4H), 7.45–7.32 (m, 2H), 7.23–7.15 (m, 3H), 6.94 (d, J = 8.8 Hz, 2H), 6.65–6.61 (m, 1H), 6.39 (d, J = 16.0 Hz, 1H), 4.52 (t, J = 5.9 Hz, 2H), 4.25 (t, J = 5.6 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 3.88 (s, 3H), 3.32–3.18 (m, 2H), 2.59–2.42 (m, 8H), 2.38–2.30 (m, 2H), 1.94–1.73 (m, 8H), 1.57–1.26 (m, 10H), 0.83 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.19, 173.24, 169.58, 166.81, 164.69, 162.65, 151.72, 144.54, 144.40, 141.79, 134.63, 133.46, 133.04, 131.04, 128.67, 127.46, 127.37, 126.40, 124.06, 123.63, 121.42, 118.09, 115.55, 111.51, 73.27, 72.76, 68.43, 63.72, 59.58, 58.58, 56.09, 53.05, 52.99, 36.73, 29.08, 28.12, 27.34, 26.72, 26.00, 25.22, 23.86, 22.53, 14.06; ESI-MS (m/z): 936.3 $[M+H]^+$, 958.3 $[M+Na]^+$; ESI-HRMS (m/z): calculated for $C_{47}H_{58}N_3O_{11}S_3$ $[M+H]^+$ 936.3228, found 936.3251.

*4.1.4.11. (±)-(E)-2-Methoxy-4-(3-((6-(nitrooxy)hexyl)oxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(2-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)ethyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8k**).* The title compound was obtained starting from **3a** and **7c**. Reddish brown oil, 15% yield. Analytical data for **8k**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.61–7.56 (m, 4H), 7.44–7.35 (m, 2H), 7.24–7.14 (m, 3H), 6.96 (d, J = 8.8 Hz, 2H), 6.66–6.61 (m, 1H), 6.41 (d, J = 16.0 Hz, 1H), 4.46 (t, J = 6.6 Hz, 2H), 4.22 (t, J = 6.5 Hz, 2H), 4.15 (t, J = 5.6 Hz, 2H), 3.88 (s, 3H), 3.34–3.19 (m, 2H), 2.85 (t, J = 5.6 Hz, 2H), 2.64 (s, 8H), 1.91–1.70 (m, 6H), 1.53–1.28 (m, 8H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.27, 173.03, 169.54, 166.99, 164.72, 162.22, 151.74, 144.36, 144.23, 141.72, 134.79, 133.63, 133.02, 131.07, 128.69, 127.49, 126.41, 124.46, 123.62, 121.42, 118.47, 115.69, 111.52, 73.33, 73.30, 66.41, 64.49, 59.52, 56.95, 56.11, 53.46, 52.94, 36.75, 28.66, 28.13, 26.85, 25.73, 25.52, 22.54, 14.06; ESI-MS (m/z): 930.3 $[M+Na]^+$; ESI-HRMS (m/z): calculated for $C_{45}H_{53}N_3O_{11}S_3Na$ $[M+Na]^+$ 930.2734, found 930.2758.

*4.1.4.12. (±)-(E)-2-Methoxy-4-(3-((6-(nitrooxy)hexyl)oxy)-3-oxoprop-1-en-1-yl)phenyl2-(1-(2-(4-(3-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)propyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8l**).* The title compound was obtained starting from **3b** and **7c**. Reddish brown oil, 12% yield. Analytical data for **8l**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 7.7 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.61–7.57 (m, 4H), 7.45–7.37 (m, 2H), 7.23–7.16 (m, 3H), 6.96 (d, J = 8.8 Hz, 2H), 6.66–6.62 (m, 1H), 6.41 (d, J = 15.9 Hz, 1H), 4.47 (t, J = 6.6 Hz, 2H), 4.22 (t, J = 6.5 Hz, 2H), 4.08 (t, J = 6.2 Hz, 2H), 3.88 (s, 3H), 3.34–3.20 (m, 2H), 2.61–2.53 (m, 10H), 2.02–1.93 (m, 2H), 1.89–1.75 (m, 6H), 1.57–1.43 (m, 4H), 1.39–1.22 (m, 4H), 0.84 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.10, 172.88, 166.93, 164.68, 161.47, 151.60, 144.05, 141.54, 134.76, 133.63, 133.21, 131.07, 128.69, 127.72, 127.32, 126.23, 124.74, 123.45, 121.34, 118.50, 115.41, 111.46, 73.29, 64.47, 56.02, 36.48, 28.55, 27.97, 26.73, 25.63, 25.41, 23.79, 22.40, 13.94; ESI-MS (m/z): 922.3 $[M+H]^+$; ESI-HRMS (m/z): calculated for $C_{46}H_{56}N_3O_{11}S_3$ $[M+H]^+$ 922.3071, found 922.3089.

*4.1.4.13. (±)-(E)-2-Methoxy-4-(3-((6-(nitrooxy)hexyl)oxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(4-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)butyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8m**).* The title compound was obtained starting from **3c** and **7c**. Reddish brown oil, 17% yield. Analytical data for **8m**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.62–7.50 (m, 4H), 7.43–7.33 (m, 2H), 7.23–7.15 (m, 3H), 6.94 (d, J = 8.8 Hz, 2H), 6.66–6.61 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.46 (t, J = 6.6 Hz, 2H), 4.22 (t, J = 6.5 Hz, 2H), 4.03 (t, J = 6.2 Hz, 2H), 3.88 (s, 3H), 3.33–3.24 (m, 2H), 2.76–2.27 (m, 10H), 1.83–1.60 (m, 10H), 1.48–1.28 (m, 8H), 0.83 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.20, 173.24, 169.57, 167.01, 164.71, 162.55, 151.69, 144.40, 144.24, 141.69, 134.67, 133.59, 133.05, 131.06, 128.69, 127.48, 127.36, 126.39, 124.12, 123.60, 121.42, 118.42, 115.55, 111.44, 73.31, 68.24, 64.49, 59.53, 58.06, 56.08, 52.99, 36.75, 28.64, 28.13, 27.13, 26.81, 25.72, 25.51, 23.30, 22.55, 14.08; ESI-MS (*m/z*): 936.3 $[M+H]^+$; ESI-HRMS (*m/z*): calculated for $C_{47}H_{58}N_3O_{11}S_3$ $[M+H]^+$ 936.3228, found 936.3231.

*4.1.4.14. (±)-(E)-2-Methoxy-4-(3-((6-(nitrooxy)hexyl)oxy)-3-oxoprop-1-en-1-yl)phenyl2-(1-(2-(4-(5-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)pentyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8n**).* The title compound was obtained starting from **3d** and **7c**. Reddish brown oil, 18% yield. Analytical data for **8n**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.59–7.56 (m, 4H), 7.43–7.32 (m, 2H), 7.23–7.13 (m, 3H), 6.93 (d, J = 8.8 Hz, 2H), 6.68–6.55 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.45 (t, J = 6.6 Hz, 2H), 4.21 (t, J = 6.5 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 3.87 (s, 3H), 3.31–3.17 (m, 2H), 2.68–2.42 (m, 8H), 2.38–2.33 (m, 2H), 1.89–1.70 (m, 8H), 1.62–1.45 (m, 8H), 1.39–1.25 (m, 4H), 0.83 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.13, 173.20, 169.56, 166.94, 164.67, 162.58, 151.67, 144.37, 144.19, 141.68, 134.59, 133.55, 133.00, 131.00, 128.65, 127.43, 127.36, 126.37, 124.04, 123.57, 121.37, 118.40, 115.51, 111.43, 73.27, 73.23, 68.32, 64.44, 59.57, 58.46, 56.05, 53.05, 36.71, 29.02, 28.61, 28.09, 26.78, 26.57, 25.68, 25.47, 24.04, 22.50, 14.04; ESI-MS (*m/z*): 950.3 $[M+H]^+$, 972.3 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{48}H_{60}N_3O_{11}S_3$ $[M+H]^+$ 950.3384, found 950.3363.

*4.1.4.15. (±)-(E)-2-Methoxy-4-(3-((6-(nitrooxy)hexyl)oxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1-(2-(4-(6-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy)hexyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**8o**).* The title compound was obtained starting from **3e** and **7c**. Reddish brown oil, 27% yield. Analytical data for **8o**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.61–7.51 (m, 4H), 7.41–7.31 (m, 2H), 7.21–7.10 (m, 3H), 6.93 (d, J = 8.7 Hz, 2H), 6.68–6.55 (m, 1H), 6.40 (d, J = 16.0 Hz, 1H), 4.45 (t, J = 6.5 Hz, 2H), 4.20 (t, J = 6.5 Hz, 2H), 3.99 (t, J = 6.1 Hz, 2H), 3.87 (s, 3H), 3.32–3.18 (m, 2H), 2.64–2.32 (m, 10H), 1.93–1.61 (m, 8H), 1.49–1.18 (m, 14H), 0.83 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.15, 173.21, 169.55, 166.95, 164.68, 162.59, 151.68, 144.37, 144.20, 141.68, 134.61, 133.56, 133.01, 131.02, 128.66, 127.44, 127.37, 126.37, 124.06, 123.58, 121.38, 118.41, 115.52, 111.44, 73.28, 73.26, 68.32, 64.45, 59.55, 58.44, 56.07, 53.04, 52.97, 36.71, 29.02, 28.62, 28.52, 28.10, 26.79, 26.51, 25.69, 25.47, 24.04, 22.51, 14.05; ESI-MS (*m/z*): 964.3 $[M+H]^+$, 986.3 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{49}H_{62}N_3O_{11}S_3$ $[M+H]^+$ 964.3541, found 964.3544.

*4.1.5. (±)-(E)-2-Methoxy-4-(3-methoxy-3-oxoprop-1-en-1-yl) phenyl 2-(1-(2-chloroacetoxy)pentyl)benzoate (**9**)*

To a solution of compound **6** (2.1 mmol) and methyl ferulate (2.0 mmol) in anhydrous CH_2Cl_2 (25 mL) was added DMAP (0.1 mmol) and the solution was left stirring at room temperature for 6 h. The solution was then filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 5:1 v/v) to afford **9** as a colorless oil, 85% yield. Analytical data for **9**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16 (d, J = 7.7 Hz, 1H), 7.67 (d, J = 16.0 Hz, 1H), 7.59 (d, J = 3.4 Hz, 2H), 7.42–7.36 (m, 1H), 7.22–7.11 (m, 3H), 6.72–6.68 (m, 1H), 6.41 (d, J = 16.0 Hz, 1H), 4.09 (s, 2H), 3.86 (s, 3H), 3.79 (s, 3H), 1.95–1.85 (m, 2H), 1.46–1.26 (m, 4H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.20, 166.43, 164.52, 151.50, 144.15, 143.45, 141.48, 133.48, 133.12, 130.96, 127.63, 127.20, 126.19, 123.39, 121.24, 118.03, 111.32, 74.87, 55.88, 51.70, 41.03, 36.50, 27.86, 22.37, 13.92; ESI-MS (*m/z*): 497.1 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{25}H_{27}ClO_7Na$ $[M+Na]^+$ 497.1338, found 497.1343.

*4.1.6. (±)-(E)-2-Methoxy-4-(3-methoxy-3-oxoprop-1-en-1-yl) phenyl 2-(1-(2-(4-(5-(4-(5-Thioxo-2,5-dihydrothiophen-3-yl) phenoxy)pentyl)piperazin-1-yl)acetoxy)pentyl)benzoate (**10**)*

To a solution of compound **9** (1.0 mmol) and Et_3N (0.122 g, 1.2 mmol) in anhydrous dichloromethane (10 mL) was added **3d** (1.0 mmol), and the mixture was stirred at room temperature for 12 h. The dichloromethane was removed in vacuo, and the resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1 v/v) to afford **10** as a reddish brown oil, 35% yield. Analytical data for **10**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.60–7.52 (m, 4H), 7.41–7.32 (m, 2H), 7.22–7.10 (m, 3H), 6.93 (dd, J = 6.9, 4.9 Hz, 2H), 6.64–6.60 (m, 1H), 6.39 (d, J = 16.0 Hz, 1H), 3.98 (t, J = 6.3 Hz, 2H), 3.86 (s, 3H), 3.79 (s, 3H), 3.34–3.19 (m, 2H), 2.68 (s, 8H), 2.54–2.47 (m, 2H), 1.90–1.73 (m, 4H), 1.69–1.54 (m, 2H), 1.49–1.42 (m, 2H), 1.39–1.23 (m, 4H), 0.82 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.04, 173.20, 169.36, 167.31, 164.62, 162.47, 151.58, 144.24, 141.58, 134.53, 133.49, 133.06, 131.00, 128.62, 127.46, 127.24, 126.27, 124.01, 123.51, 121.31, 118.07, 115.45, 111.38, 73.36, 68.10, 59.11, 57.97, 56.00, 52.54, 51.96, 51.82, 36.63, 28.83, 28.06, 25.57, 23.83, 22.46, 14.02; ESI-MS (*m/z*): 819.3 $[M+H]^+$, 841.3 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{43}H_{50}N_2O_8S_3Na$ $[M+Na]^+$ 841.2621, found 841.2611.

*4.1.7. (±) (E)-2-methoxy-4-(3-(2-(nitrooxy)ethoxy)-3-oxoprop-1-en-1-yl)phenyl 2-(1- (2-(4-(5-phenoxypentyl)piperazin-1-yl) acetoxy)pentyl)benzoate (**13**)*

To a solution of compound **7a** (1.0 mmol) and Et_3N (0.122 g, 1.2 mmol) in anhydrous dichloromethane (10 mL) was added **12** (1.0 mmol), and the mixture was stirred at room temperature for 12 h. The dichloromethane was removed in vacuo, and the resulting residue was purified by flash chromatography (petroleum ether/EtOAc, 1:1 v/v) to afford **13** as a colorless oil, 73% yield. Analytical data for **13**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 7.8 Hz, 1H), 7.71 (d, J = 16.0 Hz, 1H), 7.58 (d, J = 3.9 Hz, 2H), 7.42–7.36 (m, 1H), 7.30–7.14 (m, 5H), 6.94–6.86 (m, 3H), 6.66–6.62 (m, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.75–4.72 (m, 2H), 4.51–4.48 (m, 2H), 3.94 (t, J = 6.4 Hz, 2H), 3.88 (s, 3H), 3.33–3.19 (m, 2H), 2.60–2.50 (m, 8H), 2.44–2.33 (m, 2H), 1.95–1.74 (m, 4H), 1.59–1.27 (m, 8H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.56, 166.33, 164.61, 159.06, 151.66, 145.46, 144.42, 141.88, 133.16, 133.05, 130.99, 129.46, 127.41, 127.20, 126.33, 123.62, 121.57, 120.55, 117.13, 114.48, 111.40, 73.19, 70.66, 67.64, 60.33, 59.55, 58.52, 56.02, 53.00, 36.69, 29.22, 28.09, 26.57, 24.11, 22.49, 14.05; ESI-MS (*m/z*): 762.4 $[M+H]^+$, 784.4 $[M+Na]^+$; ESI-HRMS (*m/z*): calculated for $C_{41}H_{52}N_3O_{11}$ $[M+H]^+$ 762.3596, found 762.3612.

4.2. Biological assays

4.2.1. Anti-platelet aggregation in vitro

Blood was drawn from rabbit carotid artery and mixed with 3.8% sodium citrate (9:1, v/v). After centrifugation at 500g for 10 min at room temperature to obtain platelet-rich plasma (PRP), the remaining blood was further centrifuged at 3000 g for another

10 min to obtain platelet-poor plasma (PPP). Platelet aggregation was measured by Born's turbidimetric method in a platelet aggregometer (LG-PABER-I platelet aggregometer, Beijing) at 37 °C within 3 h after blood collection. Briefly, PRP (240 μL) was pre-incubated in duplicate for 5 min at 37 °C with vehicle, the individual compounds, or reference drugs (ASP, NBP, and Ticlid) at the same concentrations (0.1, 0.2, 0.4, 0.8, and 1.6 mM) and the platelet aggregation in individual PPR samples was induced by ADP (final concentration of 10 μM), followed by recording of light transmission at maximal aggregation within 5 min. The inhibition rate of the tested individual compounds on the platelet aggregation was calculated as the following formula: inhibition rate (%) = 100 × [(1 − (the platelet aggregation in samples with the tested compound)/(the platelet aggregation in control samples)]. In addition, the concentrations of selected compounds that inhibited the platelet aggregation to 50% (IC_{50}) were calculated.

4.2.2. In vitro nitric oxide release assay

In vitro NO formation of individual compounds was determined by Griess assay. Briefly, 0.1 mM of each compound in phosphate buffer solution (PBS) containing 2% dimethyl sulfoxide and 5.0 mM L-cysteine at pH 7.4 was incubated at 37 °C for 15–300 min and were sampled every 15 min for 120 min and then every 30 min for the remaining time. The collected samples (2 mL) were mixed with 0.5 ml of Griess reagent and incubated at 37 °C for 10 min, followed by measuring at 540 nm. The different concentrations of nitrite were used as standards to calculate the concentrations of NO formed by individual compounds.

4.2.3. In vitro hydrogen sulfide release assay

Individual compounds (100 μM) were incubated (37 °C) for timed intervals (0–90 min) with fresh rat liver homogenate (430 μL) and the concentration of released H_2S was measured as described previously [37]. 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 individual compounds 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 individual compounds to H_2S at each time point.

4.2.4. The cerebral ischemia/reperfusion (I/R) model in rats

Male SD rats (280–320 g) were randomly divided into five groups (n = 12 per group): (1) sham (0.5% CMC-Na), (2) model (I/R + 0.5% CMC-Na), (3) I/R + NBP (80 mg/kg), (4) I/R + **8d** (380 mg/kg), (5) I/R + **8d** (80 mg/kg). The rats were treated with the indicated compound at the indicated dose by gavage daily for seven days. Two hours after the last treatment, the rats were anesthetized with chloral hydrate (300 mg/kg, ip) and subjected to a middle cerebral artery occlusion (MCAO), as described previously with some modification. Briefly, the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed. A 40 monofilament nylon suture (Beijing Sunbio Biotech, Beijing, China) was carefully inserted from the ECA stump into the ICA and was gently advanced to occlude the origin of the right middle cerebral artery (MCA) until a light resistance was felt (18–20 mm from CCA bifurcation). Two hours later, the suture was withdrawn to restore blood flow (reperfusion). The sham group of rats received a sham operation by inserting the filament into the ICA and immediately withdrawing it. The core body temperature was monitored and maintained at 37 ± 0.5 °C with a heating pad and warm light throughout surgical procedures until the rats completely recovered from anesthesia.

4.2.4.1. Neurological deficit scores. Neurological deficits of individual rats were evaluated at 24 h post reperfusion by the Longa's method in a blinded manner. Tests were always performed between 10 and 11 a.m. to exclude the possible interference of circadian rhythm-related behavioral changes.

4.2.4.2. Measurement of infarct size. At 24 h post reperfusion, some rats (n = 6) from each group were sacrificed and their brains were rapidly removed onto ice and coronally sectioned (2 mm). The sections were immediately incubated in 2% TTC for 30 min at 37 °C and fixed in10% formalin overnight. The infarct areas (white) in individual sections were measured and analyzed using Image-Pro Plus (Version 6.0). The infarct size was expressed as the percentage size in the total area of whole brain.

4.2.4.3. Measurement of brain-water content. At 24 h post reperfusion, some rats (n = 6) form each group were sacrificed and their brains were rapidly removed on to ice, followed by isolating the ischemic hemispheres. The tissue samples were immediately weighed to obtain the wet weight. The brains were then dried at 100 °C for 24 h and determined for its dry weight. The percentage of brain water content was calculated as (wet weight − dry weight)/wet weight × 100%.

4.2.5. In vivo antioxidant activity assay

At 24 h after reperfusion, the rats were sacrificed and their brains were rapidly removed, rinsed with 0.9% cold saline, and blotted with filter paper before being kept at −70 °C. After quantification of protein concentrations, the levels of GSH, SOD and GSH-Px activities and the levels of MDA content in brain tissues were determined using commercial analysis kits (Jiancheng Institute of Biotechnology, Nanjing, China) according to the manufacturer's protocol.

Acknowledgments

This study was financially supported by grants from the National Natural Science Foundation of China (No. 81373419 and 21502071) and the Ph.D. Program Foundation of the Ministry of Education of China, China Pharmaceutical University (No. 20130096110005).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.03.044.

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