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Discovery of a Potential Anti-Ischemic Stroke Agent: 3-Pentylbenzo[*c*]thiophen-1(3*H*)-one

Jing Wu,[†,‡,#] Jingjing Ling,[†,§,#] Xuliang Wang,[∥] Tingting Li,[†,§] Jingchao Liu,[†,‡] Yisheng Lai,[†,‡] Hui Ji,*[,†,§] Sixun Peng,[†,‡] Jide Tian,[⊥] and Yihua Zhang*[,†,‡]

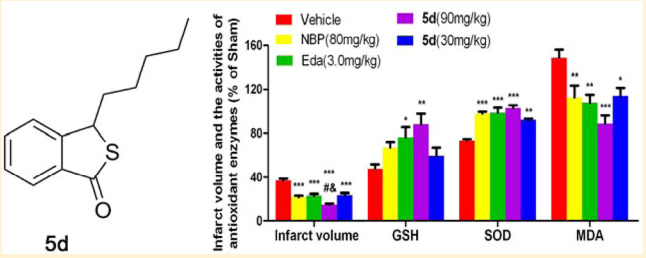
[†]State Key Laboratory of Natural Medicines, [‡]Center of Drug Discovery, and [§]Department of Pharmacology, China Pharmaceutical University, Nanjing 210009, P. R. China

[∥]CSPC Central Institute of Pharmaceutical Research, Shijiazhuang 050035, P. R. China

[⊥]Department of Molecular and Medical Pharmacology, University of California—Los Angeles, Los Angeles, California 90095, United States

Supporting Information

ABSTRACT: The development of novel antithrombotic agents with strong free radical scavenging activity is of great significance for the treatment of ischemic stroke. In the present study, 3-alkyl/arylalkyl-substituted benzo[*c*]thiophen-1(3*H*)-ones (**5a**–**h**) were designed and synthesized. The most active compound **5d** significantly inhibited the adenosine diphosphate (ADP) induced and arachidonic acid (AA) induced in vitro platelet aggregation, superior to clinically used antiplatelet drug aspirin (ASP) and anti-ischemic stroke drugs 3-*n*-butylphthalide (NBP) and edaravone (Eda). More importantly, in comparison with both NBP and Eda, **5d** exhibited stronger antithrombotic and free radical scavenging activities and better or comparable neuroprotective effects against ischemia/reperfusion (I/R) in rats by ameliorating neurobehavioral function, reducing infarct size and brain-water content, attenuating cerebral damage, and normalizing the levels of oxidative enzymes. Overall, our findings may provide an alternative strategy for the design of novel anti-ischemic stroke agents more potent than drugs like NBP and Eda.



INTRODUCTION

Ischemic stroke, the most common cerebrovascular disorder, accounts for more than 80% of all strokes and is one of the leading causes of morbidity and mortality worldwide.[1,2] Previous studies have demonstrated that there are three progressive stages involved in ischemic stroke:[3,4] stage 1, the formation of a thrombus usually initiated by blood vessel injury, which triggers platelet aggregation and adhesion to the vessel wall; stage 2, continued growth of the arterial thrombus, resulting in the blood vessel occlusion; stage 3, narrowing and obstruction of the microvasculature due to edema. Hence, the development of new drugs with antiplatelet aggregation and antithrombotic activities will be of great significance for the treatment of ischemic stroke. Despite the remarkable progress achieved in the past 2 decades in understanding the pathogenesis of ischemic stroke, there is only one FDA-approved medicine for the treatment of ischemic stroke, i.e., the serine protease tissue-type plasminogen activator (t-PA, a thrombolytic agent). Unfortunately, treatment of ischemic stroke with t-PA for thrombolysis may cause hemorrhage and t-PA has adverse effects on neuronal cell survival.[5−7]

Among multiple mechanisms implicated in the pathogenesis of ischemia/reperfusion (I/R) injury, increased oxidative stress has been considered as the primary cause because of free radical induced lipid peroxidation, and proteins and nucleic acid oxidation.[8] The oxidative stress promotes reactive oxygen species (ROS), which are extremely detrimental to the surrounding tissues. Although ROS can be neutralized by a defense system in normal tissues, increased production of ROS can lead to neuronal damage/death under an ischemic condition because the antioxidant defense system in ischemic tissues is interrupted. Along these lines, the efficacy of available antiplatelet aggregation and thrombolytic therapies for ischemic stroke in the clinic is limited. Thus, the development of novel antiplatelet aggregation and antithrombotic agents with free radical scavenging activity will be of importance in this field.

The racemic 3-*n*-butylphthalide (NBP, Figure 1) has been approved by the SFDA of China as a new drug mainly for the treatment of ischemic stroke since 2002. It is well-known that NBP can inhibit platelet aggregation and thrombosis, improve microcirculation, and reduce brain infarct volume, thus benefiting patients with ischemic stroke.[9−13] Despite these advantages, NBP is often administered with antioxidant and/or antiplatelet drug(s) to improve its therapeutic efficacy.[14] Therefore, new anti-ischemic stroke drugs with pronouncedly

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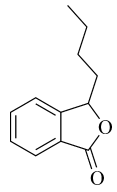


Figure 1. Structure of NBP.

inhibitory activities on both platelet aggregation and oxidation are clinically needed.

Previous studies have demonstrated that the brain vessel I/R-related oxidative stress is a key factor to secondary brain injury.[15,16] Many approaches have been documented to reduce ROS generation and to block the ROS-mediated neuronal cell death induced by ischemic insults, including utilization of antioxidants or inhibitors of cell death signaling molecules.[17−19] Given that a number of drug molecules containing sulfur exhibit a better efficacy in several aspects, especially for antioxidation, antifree radicals, and neuroprotection,[20−23] we therefore designed and synthesized a novel class of compounds by bioisosteric replacement of the oxygen atom at the 2-position of phthalide, the scaffold of NBP, with sulfur and, meanwhile, introduction of various lengths of alkyl, including straight/branch chain alkyl or arylalkyl groups, into 3-position of phthalide. It is anticipated that these novel compounds could synergistically inhibit thrombosis and oxidation and be more potent than NBP.

RESULTS AND DISCUSSION

Chemistry. The synthesis of target compounds **5a−h** is outlined in Scheme 1. Starting from isobenzofuran-1,3-dione **1**, benzo[*c*]thiophene-1,3-dione **2** was prepared in 92% yield by treatment with $Na_2S·9H_2O$ as described previously,[24] followed by reaction with the Grignard reagents **3a−h**, which were prepared in a two-step sequence from the corresponding bromoalkanes, to generate hydroxyl compounds **4a−h** in 33−73% yield. These compounds were converted to **5a−h** in 45−69% yield via one-pot reaction of dehydration and reduction by reaction with hydriodic acid. The target products **5a−h** were purified by column chromatography, and their structures were identified by MS, ^{1}H NMR, ^{13}C NMR, HRMS, and HPLC. The **5a−h** with a purity of >95% were used for subsequent biological experiments.

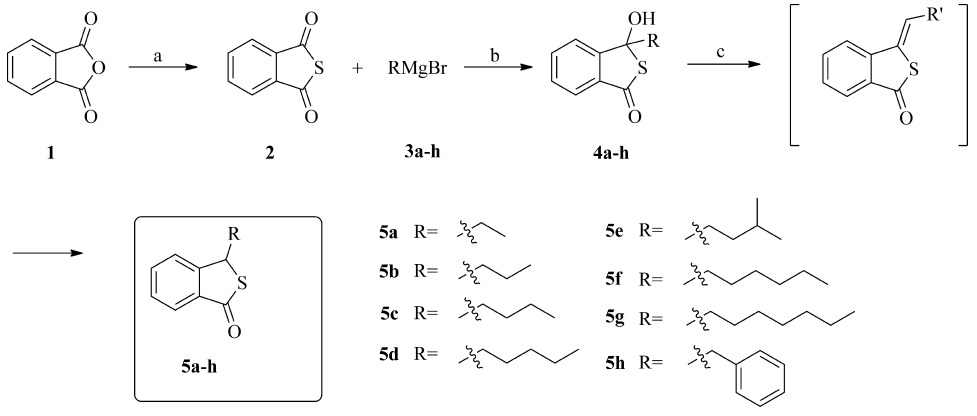
Pharmacology. In Vitro Antiplatelet Effect. The individual compounds were evaluated for inhibition of platelet aggregation in rabbit platelet rich plasma (PRP) in response to adenosine diphosphate (ADP, 10 μM) and arachidonic acid (AA, 1 mM) using Born's turbidimetric method.[25] For the ADP-induced platelet aggregation, three out of eight compounds displayed significant inhibitory effects, which were superior to one classical antiplatelet drug, aspirin (ASP), and two clinically used anti-ischemic stroke drugs, NBP and edaravone (Eda) at the same dosage. Eda, a free radical scavenger, has been demonstrated to have therapeutic effects on ischemic stroke in the clinic and has been marketed in Japan since June 2001.[26−28] As shown in Table 1, the inhibitory effects of **5d**, **5f**, and **5g** (IC_{50} of 0.42, 0.80, and 0.72 mM, respectively) on the ADP-induced platelet aggregation were stronger than those of Eda (IC_{50} = 0.85 mM), ASP (IC_{50} = 0.88 mM), and NBP (IC_{50} > 1 mM). For the AA-induced platelet activation, three compounds exerted much greater inhibition than NBP at the same concentration. Furthermore, **5b−d** (IC_{50} of 0.40, 0.35, and 0.21 mM, respectively) inhibited AA-induced

Table 1. Effects of 5a−h on the Inhibition of Platelet Aggregation Induced by ADP or AA in Vitro[a]

	IC_{50} (mM)	
compd	ADP (10 μM)	AA (1 mM)
control		
ASP	0.88	0.15
NBP	>1.0	0.48
Eda	0.85	0.25
5a	>1.0	>1.0
5b	>1.0	0.40
5c	>1.0	0.35
5d	0.42	0.21
5e	>1.0	>1.0
5f	0.80	0.83
5g	0.72	>1.0
5h	>1.0	>1.0

[a]Data are expressed as IC_{50} values (the dose achieving 50% inhibition of platelet aggregation).

Scheme 1. Synthesis of Compounds 5a−h[a]



[a]Reagents and conditions: (a) $Na_2S·9H_2O$, room temp, 5 h; (b) anhydrous ether, reflux, 2 h, then to room temp, 6 h; (c) AcOH, aqueous HI (57%), 125 °C, 1 h.

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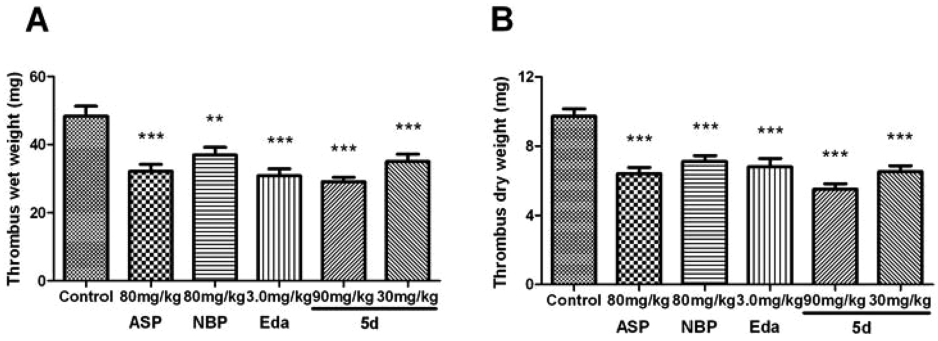

Figure 2. Effect of **5d** on the thrombus wet weight (A) and dry weight (B) in rats. Data are expressed as the mean ± SD of individual group of rats (n = 12) from two separate experiments. Data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test: (**) P < 0.01, (***) P < 0.001 vs the control group.

platelet aggregation more effectively than NBP (IC_{50} = 0.48 mM). Notably, the inhibitory effect of 0.1 mM **5d** (15.64%) on the ADP-induced platelet aggregation was 1.65-fold, 1.45-fold, and 1.36-fold stronger than that of NBP (9.50%), ASP (10.82%), and Eda (11.50%), whereas its inhibition (35.23%) on the AA-induced platelet activation was 2.02-fold more potent than that of NBP (17.43%) and comparable with that of ASP (37.20%) and Eda (36.12%). Interestingly, we found that these compounds did not affect the lengths of activated partial thromboplastin time (data not shown). These data suggest that these compounds inhibited platelet aggregation without affecting blood coagulation. These data also indicated that **5d** was a potent inhibitor of both the ADP- and AA-induced platelet aggregation. Therefore, **5d** was selected as the candidate compound for the following investigations.

Antithrombotic Activity in Rats. Next, we investigated the antithrombotic activity of compound **5d** in a rat arteriovenous (A-V) shunt model.[29,30] Male SD rats were subjected to the surgical induction of A-V shunt, randomized, and treated orally with normal saline (negative control), ASP (80 mg/kg), NBP (80 mg/kg), Eda (3.0 mg/kg), and **5d** (30 and 90 mg/kg, respectively; the higher dose of **5d** was equimolar to that of NBP) for 5 consecutive days. Both of the wet and dry thrombus weights in individual rats were measured. As shown in Figure 2, treatment with 90 mg/kg **5d** reduced the weight of wet thrombus by 39.70% (from 48.31 ± 9.36 to 29.13 ± 3.92 mg) and the antithrombosis activity of **5d** was much greater than that of ASP (from 48.31 ± 9.36 to 32.15 ± 6.32 mg, 33.45%) and NBP (from 48.31 ± 9.36 to 36.92 ± 7.28 mg, 23.58%), and slightly higher than that of Eda (from 48.31 ± 9.36 to 30.85 ± 6.37 mg, 36.14%). In addition, treatment with **5d** at 30 mg/kg also lowered the thrombus wet weight by 27.35% (from 48.31 ± 9.36 to 35.01 ± 6.80 mg), which was more potent than NBP. Similarly, **5d** at 90 mg/kg decreased the thrombus dry weight by 43.33% (from 9.74 ± 1.36 to 5.52 ± 1.01 mg), which was much more effective than that of NBP (from 9.74 ± 1.36 to 7.12 ± 1.06 mg, 26.90%), Eda (from 9.74 ± 1.36 to 6.81 ± 1.52 mg, 30.08%), and ASP (from 9.74 ± 1.36 to 6.41 ± 1.16 mg, 34.19%). Moreover, treatment with 30 mg/kg **5d** also more effectively reduced the thrombus dry weight by 32.85% (from 9.74 ± 1.36 to 6.54 ± 1.03 mg), compared with that by NBP and Eda but not by ASP. These data clearly indicated that **5d** possessed a strong antithrombotic activity in vivo.

Free Radical Scavenging Activity in PC12 Cells. Various ROS can induce cellular and tissue damage following the I/R induction. Hydrogen peroxide (H_2O_2) can react with metal (i.e., copper ion) in biological systems via the Fenton reaction to produce hydroxyl radical (·OH), and H_2O_2 and ·OH can break DNA and have strong cytotoxicity.[31] We hypothesized that **5d** with sulfur-containing molecular structure might have free radical scavenging activity against ROS. To test this hypothesis, PC12 cells were treated with H_2O_2 or ·OH in the presence or absence of **5d** for the evaluation of its H_2O_2 and ·OH scavenging activities, and the cytotoxicity of H_2O_2 or ·OH against PC12 cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[32,33] Treatment with 10 μM **5d** remarkably increased the percentages of cell viability in the H_2O_2-treated or ·OH-treated PC12 cells to 77.23% or 86.08%, compared with 52.45% and 61.95% of H_2O_2-treated or ·OH-treated alone cells, respectively (Figure 3). The protective effect of **5d** was superior to that of NBP

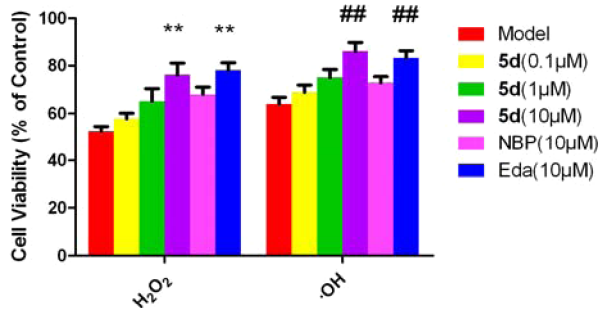

Figure 3. Effect of **5d** on H_2O_2-induced or ·OH-induced cytotoxicity in PC12 cells. Data are expressed as the mean ± SD of each group of cells from four separate experiments. Data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test: (**) P < 0.01 vs the H_2O_2-treated group; (##) P < 0.01 vs the ·OH-treated group.

(68.05%, 73.05%, respectively) and comparable with that of Eda (78.05%, 83.30%, respectively) at the same dosage. These findings suggested that **5d** might act as a scavenger of H_2O_2 and ·OH and be protected from H_2O_2 or ·OH-mediated cytotoxicity against PC12 cells in vitro.

Anticerebral Ischemic Activity in Rats. We further investigated the anticerebral ischemic activity of **5d** 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 antistroke agents.[34] SD rats were treated intragastrically (ig) with normal saline (negative control), NBP (80 mg/kg), Eda (3.0 mg/kg), or **5d** (30 and 90

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mg/kg; the higher dose of **5d** was equimolar to that of NBP) for 7 consecutive days, and the rats were subjected to sham surgery or the MCA occlusion and reperfusion. At 24 h after reperfusion, the neurological deficits of individual rats were assessed using Longa's method.[35] As shown in Table 2, no obvious neurological deficit was observed in sham-operated and saline-treated animals. Compared with that in the sham-operated group, the I/R model and saline-treated group of rats showed prominent neurological deficits 24 h after reperfusion ($P < 0.001$). In contrast, pretreatment with **5d** (30 or 90 mg/kg) significantly improved the neurobehavioral function in the rats, as evidenced by 25.09% and 37.45% reduction in neurological deficit scores ($P < 0.001$). The neuroprotective effect of higher dose of **5d** was more potent than that of NBP (31.47%) and Eda (34.46%).

Table 2. Effect of 5d on Neurological Deficit Score in the Rats Subjected to I/R[a]

group	dose (mg/kg)	neurological deficit scores
sham		0
model		2.67 ± 0.65***
NBP	80	1.83 ± 0.39###
Eda	3.0	1.75 ± 0.45###
5d	90	1.67 ± 0.49###
5d	30	2.00 ± 0.43###

[a]Data are expressed as the mean ± SD of each group of rats ($n = 12$): (***) $P < 0.001$ vs the sham group; (###) $P < 0.001$ vs the model group.

Reduction of Infarct Size and Brain-Water Content in Ischemic Brains. The infarct size of individual rats were evaluated by the 2,3,5-triphenyltetrazolium chloride (TTC) assay. There was no infarct damage in the sham group of rats. In comparison with that in the model group, the infarct size in the **5d**-treated rats was reduced by 35.50% (30 mg/kg) and 59.62% (90 mg/kg, Figure 4), respectively. Furthermore, the effect of **5d** at a higher dose (59.62%) was more pronounced than that of NBP (40.04%) and Eda (37.31%). No infarction damage was observed in the sham-operated animals. Additionally, treatment with **5d** (90 mg/kg) and Eda, but not **5d** (30 mg/kg) and NBP, also markedly reduced the water content in the brains from ischemic rats (Figure 5).

Attenuation of Cerebral Damage. Histopathological analysis revealed that treatment with **5d** considerably attenuated the cerebral damage of rats, as compared with that in the vehicle-treated model group. As shown in Figure 6, hematoxylin−eosin stain of the ipsilateral hemisphere of the ischemic brains showed the loss of hippocampal neurons, neuronal perikarya shrinkage, and macrophage infiltration, whereas an obviously smaller loss of hippocampal neurons occurred in the drug-treated groups, especially in the animals treated with 90 mg/kg **5d**.

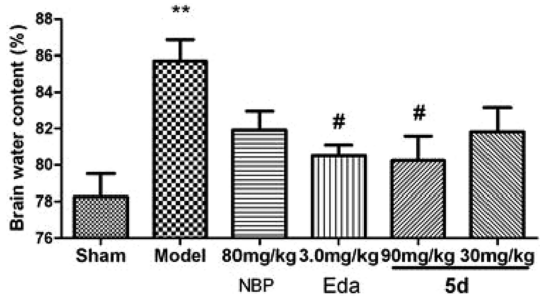

Figure 5. Effect of **5d** on brain edema after cerebral I/R in rats. Data are expressed as the mean ± SD of individual groups of rats ($n = 6$) and were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test: (**) $P < 0.01$ vs the sham-operated group; (#) $P < 0.05$ vs the model group.

Antioxidative Effect. To further explore the mechanism underlying the anticerebral ischemic effect of **5d**, we evaluated the levels of glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) in the brain tissues. In comparison with that in the sham group of rats, the levels of GSH in the model group were decreased by 52.70% (from 60.38 to 28.56 nmol/mg). However, pretreatment with **5d** (90 mg/kg) significantly enhanced GSH levels by about 82.67% to 52.17 nmol/mg (Figure 7A), which were slightly higher than that in the Eda-treated rats (58.33%, 45.22 nmol/mg) and significantly greater than that in the NBP-treated rats (40.83%, 40.22 nmol/mg).

Furthermore, the SOD activity in the ischemic brains from the model group of rats was significantly decreased by 26.77% (from 156.75 to 114.79 U/mg, Figure 7B) relative to that in the sham-operated rats. In contrast, treatment with 30 or 90 mg/kg **5d** significantly increased the SOD activities by 25.87% (from 114.79 to 144.49 U/mg) and 39.77% (160.44 U/mg), respectively, as compared with that in the model group. Notably, the SOD activity of the 90 mg/kg **5d**-treated rats was

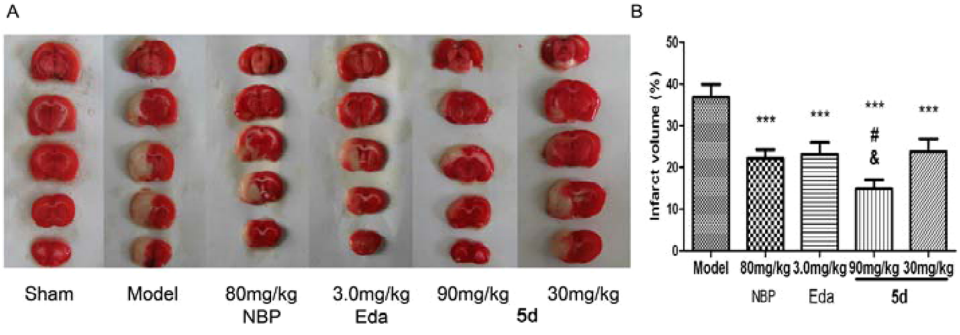

Figure 4. Effect of **5d** on infarction after cerebral I/R in rats. (A) TTC staining analysis of the infarcted brain regions. Data shown are representative examples from each treatment group of rats. (B) Quantitative analysis of the infracted brain regions. The ratios of infarct area to whole brain areas in individual rats were calculated. 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 Tukey test: (***) $P < 0.001$ vs the model group; (#) $P < 0.05$ vs the NBP group; (&) $P < 0.05$ vs the Eda group.

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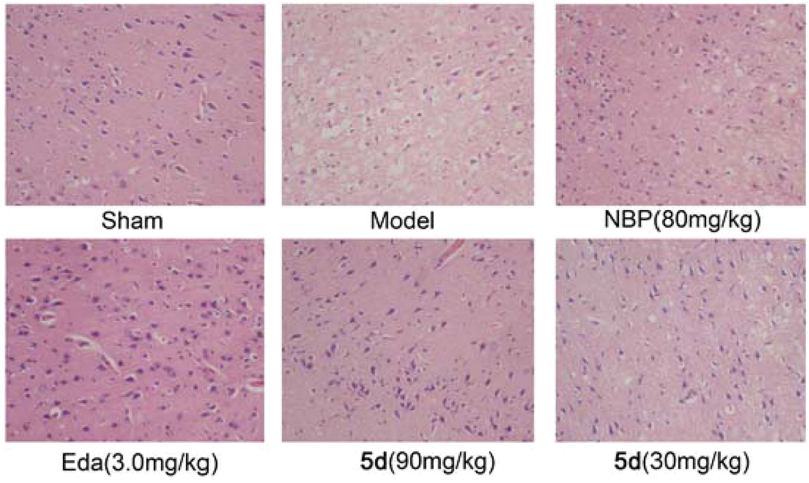

Figure 6. Effect of **5d** on neuronal injury in the ischemic cerebral cortex of rats. The rat brain tissue sections were stained by hematoxylin and eosin (HE) staining, and data shown are representative images (magnification, ×200) from individual groups of rats (n = 6).

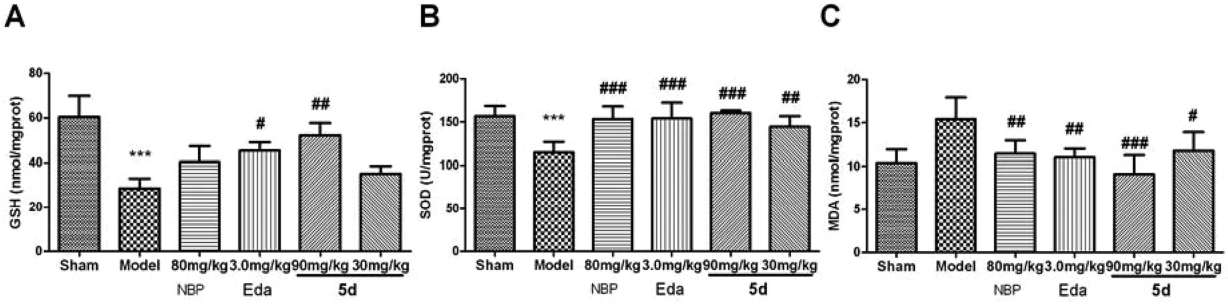

Figure 7. Effect of **5d** on the levels of brain GSH (A), SOD activity (B), and MDA (C) in rats after I/R in ischemic cerebral cortex. Data are expressed as the mean ± SD of individual groups of rats (n = 12) and were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test: (***) $P < 0.001$ vs the sham-operated group; (#) $P < 0.05$, (##) $P < 0.01$, (###) $P < 0.001$ vs the model group.

slightly greater than those in the NBP-treated (33.61%, 153.37 U/mg) or Eda-treated (34.20%, 154.06 U/mg) group.

Further analysis revealed that the content of brain MDA, an index of lipid peroxidation, was significantly elevated by 47.80% (from 10.44 to 15.43 nmol/mg) in the model group, as compared with that in the sham-operated animals. However, pretreatment with **5d** (30 or 90 mg/kg) remarkably decreased levels of brain MDA by 23.66% (from 15.43 to 11.78 nmol/mg) and 40.90% (9.12 nmol/mg), compared with that in the model group (Figure 7C). More importantly, the effect of **5d** at a higher dose on the brain lipid peroxidation was much stronger than that of NBP (25.73%, 11.46 nmol/mg) or Eda (28.52%, 11.03 nmol/mg).

CONCLUSIONS

Analysis of structure and antiplatelet aggregation activity relationship (SAR) revealed that the target compounds **5a–h** bearing various alkyl groups displayed variable antiplatelet aggregation activity. Significantly, the various lengths of side chain at the 3-posion of those compounds appeared to affect their antiplatelet aggregation activity: (1) For the inhibition of ADP-induced platelet aggregation, generally, the longer the alkyl side chains of those compounds are, the stronger is the activity, with the exception of **5d** (15.64%), which was superior to both NBP (9.50%) and Eda (11.50%). An increase in steric hindrance at the terminus of side chains, however, greatly reduced the antiplatelet aggregation activity, for example, **5a** (6.94%) vs **5h** (3.14%), and **5d** (15.64%) vs **5e** (6.52%). (2) For the inhibition of AA-induced platelet aggregation, compounds **5b–d** bearing three- to five-carbon side chains showed stronger activity (19.43%, 25.24%, 35.23%) than NBP (17.43%), among which **5d** had antiplatelet aggregation activity, similar to that of Eda (36.12%), and interestingly, the inhibitory activity of those compounds was correlated with the lengths of their side chains. When compounds have the lengths of alkyl side chains with less than three or greater than five carbons, their antiplatelet aggregation activities were dramatically diminished (**5a**, **5e–h**). Besides, no obvious difference in the antiplatelet aggregation activity was observed between **5h** (1.83%) with benzyl at the 3-posion and **5a** (1.82%) with ethyl group at the same position; notably, the introduction of the methyl moiety into the second end carbon in the alkyl side chain of **5d** (35.23%) greatly impaired its activity (**5e**, 2.54%). These results suggest that different activation mechanisms may be involved in platelet aggregation induced by these two aggregators, leading to various inhibitory activities of the target compounds. We are interested in preparing more derivatives of **5d** by introducing electron withdrawing and/or electron donating group(s) into the benzene ring and in chiral synthesizing the enantiomers of **5d** to intensively reveal the SAR in future.

Our previous investigations have showed that several NBP ring-opening compounds inhibit platelet aggregation in vitro and thrombosis in vivo.[36,37] In this study, we designed and synthesized a series of novel 3-alkyl/arylalkyl-substituted benzo[*c*]thiophen-1(3*H*)-ones (**5a–h**) and found that **5d** not

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only significantly inhibited ADP- and AA-induced platelet aggregation in vitro and reduced both the wet and dry thrombus weights in vivo but also exhibited a potent free radical scavenging activity against the ROS-mediated cytotoxicity in PC12 cells. More importantly, the antiplatelet aggregation, antithrombosis, and free radical scavenging activities of **5d** were superior to those of NBP or Eda or even both of them. Furthermore, treatment with **5d** improved neurobehavioral function, reduced the infarct brain size and brain-water content, and cerebral damage in a rat model of MCAO, and the effect of **5d** on inhibiting the brain I/R injury was similar to that of Eda but greater than that of NBP. In addition, treatment with **5d** significantly mitigated the I/R-related oxidative stress in rat brains by increasing the levels of brain antioxidant SOD and GSH but reducing the levels of brain MDA, an index of reduced lipid peroxidation in the brains. Apparently, **5d** inhibited oxidative stress, which at least partially contributed to its anti-I/R brain injury in rats. Our data suggest that **5d** may be a potential agent for the intervention of ischemic stroke and such 3-substituted benzo[*c*]thiophen-1(3*H*)-ones may represent a novel class of compounds for the treatment of ischemic stroke and other vascular diseases. We recognized that while many compounds have therapeutic effects on ischemic stroke in animal models, only few have been demonstrated to benefit patients with ischemic stroke in the clinic. We also understand that our studies had limitations, including the lack of systemic pharmacokinetic and pharmacological studies of **5d**, particularly for the mechanisms underlying the therapeutic action of **5d**. Therefore, further studies are necessary to reveal other pharmacological activities of **5d** and elucidate the molecular mechanism(s) underlying the anti-ischemic stroke activity of **5d**.

EXPERIMENTAL SECTION

Chemistry. ^{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 spectrometer. Analytical and preparative TLC was 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 SPD-10Avp UV detector) and HRMS (Agilent Technologies LC/MSD TOF). **2** was synthesized as previously described.[24] The target compounds **5a−h** with a purity of >95% were used for subsequent experiments (see the ESI results).

All animal experiments were performed in accordance with the Animal (Scientific Procedures) Act 2009 (P. R. China), and all animal experimental protocols were approved by the Animal Research Protection Committee of China Pharmaceutical University, Nanjing, China.

Benzo[*c*]thiophene-1,3-dione (2).[24] Isobenzofuran-1,3-dione (15.0 g, 0.1 mol) was mixed with $Na_2S{\cdot}9H_2O$ (30.0 g, 0.125 mol) by mechanical stirring at room temperature for 5 h. The reaction was quenched by addition of H_2O (200 mL), and then the mixture was acidified with 2 M HCl (100 mL). The solid crude product was collected by filtration, as described in the workup procedure, and 14.9 g (92%) of **2** was obtained as a white powder solid. Mp 112−114 °C. ESI-MS: *m*/*z* 187 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74−7.79 (m, 2H, ArH), 7.92−7.95 (m, 2H, ArH).

General Procedure for the Preparation of 4a−h. The corresponding bromoalkanes (20.0 mmol) were added to a solution of magnesium (0.48 g, 20.0 mmol) in anhydrous Et_2O (5 mL) under nitrogen atmosphere until the Grignard reaction started. The reaction mixture was stirred at room temperature for 2 h, and then the Grignard solution was added dropwise to a solution of **2** (1.65 g, 10.0 mmol) in anhydrous Et_2O (30 mL) at −5 °C. The reaction mixture was stirred at room temperature for 5 h. Subsequently, the reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (10 mL), acidified with 2 M HCl (15 mL) to pH 2, and stirred for 1 h at room temperature. The solution was extracted with Et_2O (50 mL × 3), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The resulting residue was purified by column chromatography (petroleum ether−EtOAc, 15: 1 v/v) to give the title compounds.

(±)-3-Ethyl-3-hydroxybenzo[*c*]thiophen-1(3*H*)-one (4a). The title compound was obtained as a light yellow solid, 46% yield. Mp 92−94 °C. ESI-MS: *m*/*z* 193 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (t, 3H, CH_3, *J* = 7.1 Hz), 2.11−2.48 (m, 2H, CH_2), 3.49 (s, 1H, OH), 7.48 (m, 1H, ArH), 7.59−7.68 (m, 3H, ArH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 195.36, 152.63, 134.97, 134.20, 129.85, 123.64, 123.29, 96.38, 37.09, 9.89. ESI-HRMS (*m*/*z*): $[M - H]^-$ calcd for $C_{10}H_9O_2S$, 193.0323; obsd 193.0325.

(±)-3-Hydroxy-3-propylbenzo[*c*]thiophen-1(3*H*)-one (4b). The title compound was obtained as a light yellow solid, 46% yield. Mp 78−80 °C. ESI-MS: *m*/*z* 207 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (t, 3H, CH_3, *J* = 7.1 Hz), 1.23−1.34 (m, 2H, CH_2), 2.03−2.43 (m, 2H, CH_2), 3.25 (s, 1H, OH), 7.52 (m, 1H, ArH), 7.62−7.73 (m, 3H, ArH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.15, 152.70, 134.79, 134.17, 129.88, 123.69, 123.34, 95.56, 44.80, 19.14, 13.92. ESI-HRMS (*m*/*z*): $[M - H]^-$ calcd for $C_{11}H_{11}O_2S$, 207.0480; obsd 207.0482.

(±)-3-Butyl-3-hydroxybenzo[*c*]thiophen-1(3*H*)-one (4c). The title compound was obtained as a light yellow solid, 47% yield. Mp 65−67 °C. ESI-MS: *m*/*z* 221 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, CH_3, *J* = 7.1 Hz), 1.11−1.72 (m, 4H, 2 × CH_2), 2.03−2.41 (m, 2H, CH_2), 3.94 (s, 1H, OH), 7.45 (m, 1H, ArH), 7.58−7.65 (m, 3H, ArH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 195.57, 152.96, 134.74, 134.19, 129.74, 123.70, 123.31, 95.82, 42.66, 29.68, 22.67, 13.84. ESI-HRMS (*m*/*z*): $[M - H]^-$ calcd for $C_{12}H_{13}O_2S$, 221.0636; obsd 221.0638.

(±)-3-Hydroxy-3-pentylbenzo[*c*]thiophen-1(3*H*)-one (4d). The title compound was obtained as a light yellow solid, 43% yield. Mp 61−63 °C. ESI-MS: *m*/*z* 235 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, CH_3, *J* = 6.9 Hz), 1.07−1.70 (m, 6H, 3 × CH_2), 2.00−2.42 (m, 2H, CH_2), 3.39 (s, 1H, OH), 7.46 (m, 1H, ArH), 7.60−7.68 (m, 3H, ArH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 195.32, 152.95, 134.78, 134.26, 129.90, 123.72, 123.36, 95.78, 42.61, 31.62, 25.41, 22.41, 13.96. ESI-HRMS (*m*/*z*): $[M - H]^-$ calcd for $C_{13}H_{15}O_2S$, 235.0793; obsd 235.0795.

(±)-3-Hydroxy-3-isopentylbenzo[*c*]thiophen-1(3*H*)-one (4e). The title compound was obtained as a light yellow oil, 45% yield. ESI-MS: *m*/*z* 235 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (m, 6H, 2 × CH_3), 1.23−1.52 (m, 3H, CH_2CH), 2.07−2.44 (m, 2H, CH_2), 3.55 (br s, 1H, OH), 7.47 (m, 1H, ArH), 7.53−7.63 (m, 2H, ArH), 7.78 (d, 1H, ArH, *J* = 7.8 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 195.52, 152.93, 134.72, 134.11, 129.66, 123.60, 123.15, 95.89, 40.54, 34.41, 27.97, 22.41, 22.28. ESI-HRMS (*m*/*z*): $[M - H]^-$ calcd for $C_{13}H_{15}O_2S$, 235.0793; obsd 235.0795.

(±)-3-Hexyl-3-hydroxybenzo[*c*]thiophen-1(3*H*)-one (4f). The title compound was obtained as a light yellow solid, 43% yield. Mp 56−58 °C. ESI-MS: *m*/*z* 249 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H, CH_3, *J* = 7.1 Hz), 1.07−1.72 (m, 8H, 4 × CH_2), 2.05−2.43 (m, 2H, CH_2), 3.36 (s, 1H, OH), 7.48 (m, 1H, ArH), 7.60−7.69 (m, 3H, ArH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 195.36, 152.97, 134.76, 134.18, 129.79, 123.75, 123.32, 95.79, 42.67, 31.45, 29.11, 25.70, 22.47, 13.97. ESI-HRMS (*m*/*z*): $[M - H]^-$ calcd for $C_{14}H_{17}O_2S$, 249.0949; obsd 249.0952.

(±)-3-Heptyl-3-hydroxybenzo[*c*]thiophen-1(3*H*)-one (4g). The title compound was obtained as a light yellow solid, 33% yield. Mp 56−58 °C. ESI-MS: *m*/*z* 263 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, CH_3, *J* = 7.2 Hz), 1.31−1.60 (m, 10H, 5 × CH_2), 2.02−2.44 (m, 2H, CH_2), 3.56 (s, 1H, OH), 7.45 (m, 1H, ArH), 7.52−7.62 (m, 2H, ArH), 7.77 (d, 1H, ArH, *J* = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.87, 152.70, 135.14, 133.70, 130.12, 124.38, 122.59, 96.07, 42.67, 29.71, 29.43, 29.02, 25.60, 22.58, 14.03.

ESI-HRMS (m/z): $[M - H]^-$ calcd for $C_{15}H_{19}O_2S$, 263.1106; obsd 263.1109.

(±)-3-Benzyl-3-hydroxybenzo[*c*]thiophen-1(3*H*)-one (4h). The title compound was obtained as a light yellow solid, 73% yield. Mp 126−128 °C. ESI-MS: m/z 255 $[M - H]^-$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27−3.61 (m, 2H, CH_2Ar), 3.79 (s, 1H, OH), 7.25−7.37 (m, 5H, ArH), 7.48 (m, 1H, ArH), 7.60−7.71 (m, 3H, ArH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 194.71, 152.35, 135.45, 134.99, 133.97, 130.82, 129.98, 128.55, 128.20, 127.99, 127.00, 124.34, 123.38, 94.96, 49.08. ESI-HRMS (m/z): $[M - H]^-$ calcd for $C_{15}H_{11}O_2S$, 255.0480; obsd 255.0482.

General Procedure for the Preparation of 5a−h. To a solution of **4a−h** (1.0 mol) in AcOH (10 mL) was added aqueous 57% HI (5 mL). After complete addition, the reaction mixture was refluxed for 0.5 h and allowed to cool to room temperature. Then a solution of 5% $NaHSO_3$ (50 mL) was added to the mixture. The solution was extracted with Et_2O (50 mL × 3), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The resulting residue was purified by column chromatography (petroleum ether−EtOAc, 50:1 v/v) to give the title compounds.

(±)-3-Ethylbenzo[*c*]thiophen-1(3*H*)-one (5a). The title compound was obtained as a yellow oil, 46% yield. ESI−MS: m/z 179 $[M + H]^+$, 201 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (t, 3H, CH_3, J = 7.2 Hz), 1.85−2.38 (m, 2H, CH_2), 4.83 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.52 (d, 1H, ArH, J = 7.5 Hz), 7.64 (m, 1H, ArH), 7.80 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.26, 151.08, 136.17, 133.25, 128.13, 125.06, 123.61, 52.85, 29.19, 11.62. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{10}H_{11}OS$, 179.0531; obsd 179.0532.

(±)-3-Propylbenzo[*c*]thiophen-1(3*H*)-one (5b). The title compound was obtained as a yellow oil, 55% yield. ESI−MS: m/z 193 $[M + H]^+$, 215 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (t, 3H, CH_3, J = 7.5 Hz), 1.44−1.61 (m, 2H, CH_2), 1.73−2.30 (m, 2H, CH_2), 4.84 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.52 (d, 1H, ArH, J = 7.5 Hz), 7.65 (m, 1H, ArH), 7.79 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 196.57, 151.44, 135.96, 133.20, 128.10, 125.09, 123.67, 51.32, 38.62, 21.35, 13.80. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{11}H_{13}OS$, 193.0687; obsd 193.0689.

(±)-3-Butylbenzo[*c*]thiophen-1(3*H*)-one (5c). The title compound was obtained as a yellow oil, 67% yield. ESI−MS: m/z 207 $[M + H]^+$, 229 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, 3H, CH_3, J = 7.2 Hz), 1.35−1.56 (m, 4H, 2 × CH_2), 1.74−2.35 (m, 2H, CH_2), 4.83 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.53 (d, 1H, ArH, J = 7.5 Hz), 7.65 (m, 1H, ArH), 7.79 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.30, 151.47, 136.05, 133.22, 128.13, 125.10, 123.71, 51.55, 36.22, 30.12, 22.48, 13.86. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{12}H_{15}OS$, 207.0844; obsd 207.0846.

(±)-3-Pentylbenzo[*c*]thiophen-1(3*H*)-one (5d). The title compound was obtained as a yellow oil, 65% yield. ESI-MS: m/z 221 $[M + H]^+$, 243 $[M+Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H, CH_3, J = 6.9 Hz), 1.32−1.57 (m, 6H, 3 × CH_2), 1.75−2.33 (m, 2H, CH_2), 4.83 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.52 (d, 1H, ArH, J = 7.5 Hz), 7.62 (m, 1H, ArH), 7.79 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.30, 151.44, 135.90, 133.26, 128.10, 125.14, 123.57, 51.60, 36.44, 31.49, 27.70, 22.41, 13.96. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{13}H_{17}OS$, 221.1000; obsd 221.1002.

(±)-3-Isopentylbenzo[*c*]thiophen-1(3*H*)-one (5e). The title compound was obtained as a yellow oil, 69% yield. ESI-MS: m/z 221 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (m, 6H, 2 × CH_3), 1.22−1.30 (m, 2H, CH_2), 1.48 (m, 1H, CH), 1.74−2.35 (m, 2H, CH_2), 4.83 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.53 (d, 1H, ArH, J = 7.5 Hz), 7.63 (m, 1H, ArH), 7.79 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.10, 151.30, 135.89, 133.13, 128.00, 125.02, 123.50, 51.64, 36.82, 34.23, 27.86, 22.53, 22.13. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{13}H_{17}OS$, 221.1000; obsd 221.1002.

(±)-3-Hexylbenzo[*c*]thiophen-1(3*H*)-one (5f). The title compound was obtained as a yellow oil, 59% yield. ESI-MS: m/z 235 $[M + H]^+$; 257 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, CH_3, J = 7.2 Hz), 1.30−1.58 (m, 8H, 4 × CH_2), 1.74−2.34 (m, 2H, CH_2), 4.83 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.52 (d, 1H, ArH, J = 7.5 Hz), 7.62 (m, 1H, ArH), 7.79 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 199.96, 156.37, 140.22, 133.18, 127.98, 126.10, 123.65, 49.81, 37.78, 31.62, 29.85, 29.04, 22.59, 14.07. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{19}OS$, 235.1155; obsd 235.1157.

(±)-3-Heptylbenzo[*c*]thiophen-1(3*H*)-one (5g). The title compound was obtained as a yellow oil, 45% yield. ESI-MS: m/z 249 $[M + H]^+$, 271 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 3H, CH_3, J = 7.2 Hz), 1.27−1.58 (m, 10H, 5 × CH_2), 1.74−2.34 (m, 2H, CH_2), 4.83 (m, 1H, CH), 7.45 (m, 1H, ArH), 7.52 (d, 1H, ArH, J = 7.5 Hz), 7.62 (m, 1H, ArH), 7.79 (d, 1H, ArH, J = 7.5 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 197.12, 151.43, 135.92, 132.86, 128.06, 125.14, 123.55, 51.57, 36.49, 31.70, 29.29, 29.02, 28.02, 22.58, 14.04. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{21}OS$, 249.1313; obsd 249.1315.

(±)-3-Benzylbenzo[*c*]thiophen-1(3*H*)-one (5h). The title compound was obtained as a yellow solid, 62% yield. Mp 118−120 °C. ESI-MS: m/z 241 $[M + H]^+$, 263 $[M + Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.02−3.59 (m, 2H, CH_2), 5.07 (m, 1H, CH), 7.24−7.36 (m, 5H, ArH), 7.47 (m, 2H, ArH), 7.62 (m, 1H, ArH), 7.80 (d, 1H, ArH, J = 8.1 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ 196.71, 150.46, 137.99, 136.28, 133.14, 129.14, 129.14, 128.67, 128.67, 128.42, 127.23, 125.49, 123.82, 52.65, 42.95. ESI-HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{13}OS$, 241.0687; obsd 241.0689.

Antiplatelet Aggregation in Vitro. Blood was drawn from rabbit carotid artery and mixed with 3.8% sodium citrate (9:1, v/v). After centrifugation at 500*g* 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 preincubated in duplicate for 5 min at 37 °C with vehicle, the individual compounds, or reference drugs (ASP, NBP, and Eda) 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) or AA (1 mM), 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 individual compounds that inhibited the platelet aggregation to 50% (IC_{50}) were calculated.

In Vitro Free Radical Scavenging Assay. The free radical scavenging capacities of **5d**, NBP, and Eda were tested against H_2O_2 and ·OH-mediated cytotoxicity against PC12 cells. PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated horse serum (Hyclone), 5% fetal bovine serum (GIBCO), 1.0 mM sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere (Thermo Scientific, 3110, OH, U.S.). During the exponential phase of growth, PC12 cells (20 000 cells/well) were cultured in 96-well plates that had been coated with poly-L-lysine for 24 h. The cells were treated in triplicate with different concentrations (0.1−10 μM) of the tested compounds for 2 h and exposed to 800 μM H_2O_2 or 1 mM H_2O_2/20 μM Fe^{2+} (pH 7.4) for 1 h. After replacement with fresh medium, the cells were incubated for 14 h and the cell viability was determined by MTT, as described previously.[38]

Antithrombotic Activity Assay in Rats. Male Sprague−Dawley (SD) rats (250−280 g) were purchased from B&K Universal Group, Shanghai, China. All procedures were performed following institutional approval in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Rats were randomized and divided into six groups (n = 12 per group): (1) control group, (2) model group (A-V), (3) A-V + **5d** (30 mg/kg) group, (4) A-V + **5d** (90 mg/kg) group, (5) A-V + NBP (80 mg/kg) group, (6) A-V + Eda (3.0 mg/kg) group. Groups 3−6 were treated by gavage with the indicated dose of the compound in saline daily for 5 consecutive days. Subsequently, the rats were anesthetized by intraperitoneal (ip) injection of pentobarbital (50 mg/kg). The control group of rats received a sham surgery, while the

remaining rats were subjected to the A-V shunt procedure. Briefly, individual rats were inserted with an arteriovenous (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 of four braided silk threads) was filled with saline before installation. After 15 min of 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 measurement of 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.

Focal Cerebral Ischemia. Rats were anesthetized ip with 350 mg/kg chloral hydrate (Sinopharm Chemical Reagent, Beijing, China) and subjected to a procedure of the middle cerebral artery occlusion (MCAO), as described previously with minor modification. Briefly, the right common carotid artery, internal carotid artery (ICA), and external carotid artery (ECA) of individual rats were surgically exposed. The MCAO was achieved by inserting a 4−0 monofilament nylon suture (Beijing Sunbio Biotech, Beijing, China) with a round tip into the ICA through the ECA stump and gently advancing to the MCA. After 2 h of MCAO, the suture was withdrawn to restore blood flow (reperfusion) in the MCA. The distal 5 mm of the suture is coated with silicone to a diameter of 0.26 mm. During the experiment, the cerebral blood flow (CBF) was monitored by laser Doppler flowmetry (LDF 100C, BIOPAC Systems) to ensure that occlusion of the MCA with the specific suture resulted in >80% decline in CBF. The control rats received a sham surgery. The core body temperature of individual rats was monitored with a rectal probe and maintained at 37 ± 0.5 °C during the whole procedure.

Rats were randomly divided into six groups (n = 12 per group): (1) sham group, (2) model group (I/R), (3) I/R + **5d** (30 mg/kg, ig) group, (4) I/R + **5d** (90 mg/kg, ig) group, (5) I/R + NBP (80 mg/kg, ig) group, (6) I/R + Eda (3.0 mg/kg, ig) group. The tested compound **5d** (30 and 90 mg/kg), reference drugs NBP (80 mg/kg), and Eda (3.0 mg/kg) were dissolved in normal saline and administrated orally to rats for 7 successive days before ischemia.

Evaluation of the Neurological Deficit. The neurological deficits of individual rats were evaluated by Longa's method with minor modification in a blinded manner at 24 h after reperfusion. The neurologic deficits were scored on a five-point scale: 0, normal function; 1, flexion of the torso and contralateral forelimb on lifting the animal by the tail; 2, circling to the contralateral side but normal posture at rest; 3, reclination to the contralateral side at rest; 4, absence of spontaneous motor activity.

Measurement of Infarct Size. At 24 h after reperfusion, the rats were sacrificed and their brains were rapidly dissected on ice and sliced into 2-mm-thick coronal sections. The brain sections were stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) for 30 min at 37 °C, followed by overnight immersion in 10% formalin in PBS. The infarcted tissues remained unstained (white), whereas normal tissues were stained red. The infarct zone was demarcated and analyzed by an image processing software. The infarct areas were calculated as a percentage of the total area of whole brain.

Measurement of Brain-Water Content. At 24 h after reperfusion, rats were decapitated and the brains were rapidly removed. Some brain samples were weighed immediately (wet weight) and then dried at 105 °C for 24 h for measurement of the dry weight. The percentage of water content was calculated with the following formula: [(wet weight − dry weight)/(wet weight)] × 100.

Histopathological Examination. At 24 h after reperfusion, the rats were deeply anesthetized with chloral hydrate and perfused with heparinized PBS, followed by perfusion with 4% paraformaldehyde in PBS. The brain tissue samples were cut coronally into three blocks at the level of the optical chiasm to the infundibular stem of the hypophysis. The middle blocks were embedded in paraffin. The paraffin-embedded tissues were sectioned at 5 μm, and the brain tissue sections were stained with hematoxylin and eosin (HE). Histopathological evaluation was performed by pathologists in a blinded manner.

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 and SOD 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.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Inhibition of platelet aggregation, table of compound purity by HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For H.J.: phone, +86-25-86021369; fax, +86-25-86635503; e-mail, huijicpu@163.com. For Y.Z.: phone, +86-25-83271015; fax, +86-25-83271015; e-mail, zyhtgd@ hotmail.com.

Author Contributions

#These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADP, adenosine diphosphate; AA, arachidonic acid; NBP, 3-*n*-butylphthalide; Eda, edaravone; ASP, aspirin; I/R, ischemia/reperfusion; ROS, reactive oxygen species; PRP, platelet rich plasma; PPP, platelet-poor plasma; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MCAO, middle cerebral artery occlusion; TTC, 2,3,5-triphenyltetrazolium chloride; GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde; ICA, internal carotid artery; ECA, external carotid artery

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