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PAPER

Design, synthesis and evaluation of nitric oxide releasing derivatives of 3-*n*-butylphthalide as antiplatelet and antithrombotic agents†

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Novel nitric oxide (NO) releasing derivatives (**7a–7l**) of 3-*n*-butylphthalide (NBP) were designed and synthesized. Compound **7e** inhibited the adenosine diphosphate (ADP), thrombin (TH) and arachidonic acid (AA)-induced *in vitro* platelet aggregation, superior to NBP and aspirin, released moderate levels of NO, and improved aqueous solubility relative to NBP. Furthermore, **7e** exhibited greater antithrombotic activity than NBP and aspirin in rats, and protected against collagen and adrenaline-induced thrombosis in mice. Therefore, NO-releasing NBP derivatives possessed potent antiplatelet aggregation and antithrombotic activity. Our findings may aid in the design of new therapeutic agents for the treatment of thrombosis-related ischemic stroke.

Introduction

Thromboembolic diseases, including myocardial infarction, acute atherosclerosis, pulmonary embolism and ischemic stroke, are the leading causes of morbidity and mortality worldwide.[1] The formation of a thrombus is usually initiated by blood vessel injury, which triggers platelet aggregation and adhesion of platelets to the vessel wall.[2] When acute thrombosis occurs in intracerebral arteries, the formed thrombus and embolus can block the cerebral blood flow (CBF), leading to the development of brain infarction and ischemic stroke.[3] Apparently, thrombosis is a key process in the pathogenesis of acute ischemic stroke. Hence, development of new drugs with antiplatelet aggregation and antithrombotic activities will be of great significance in treatment of ischemic stroke.

The racemic 3-*n*-butylphthalide (NBP) was approved by the State Food and Drug Administration (SFDA) of China as a new drug mainly for the treatment of ischemic stroke in 2002.[4] Previous studies have demonstrated that NBP can inhibit platelet aggregation and thrombosis, improve microcirculation, and reduce the brain infarct volume, providing clinical benefit to patients with ischemic stroke.[5] Unfortunately, NBP has poor aqueous solubility, limiting its clinical application,[4] Notably, the treatment of NBP with another antiplatelet drug improves the therapeutic efficacy.[6] Therefore, new derivatives of NBP may be more promising for the treatment of ischemic stroke.

Nitric oxide (NO) is a critical regulator of many physiological and pathological processes.[7] The endothelium-derived NO participates in the regulation of blood pressure, vascular tone, platelet function, neurotransmission and cerebral circulation in the cardiovascular and cerebrovascular systems.[8] In particular, NO can activate the soluble guanylyl cyclase (sGC) and increase in cGMP, which acts as the second messenger to induce vasodilatation and inhibit platelet aggregation.[9] Indeed, increasing NO production either from endothelial NOS (eNOS) or NO-releasing drugs (NO-donors) mimicking eNOS-derived NO has already been used as a therapeutic approach for thrombotic complications, such as myocardial infarction and ischemic stroke.[10] Furthermore, augmentation of NO production can improve CBF and promote angiogenesis, benefiting patients with cerebral ischemia.[11]

Accordingly, we hypothesized that new NO-releasing derivatives of NBP could display synergistic effects on platelet aggregation and thrombosis. Therefore, a series of novel NO-releasing derivatives of NBP (NO-NBP) were designed and synthesized, and their *in vitro* antiplatelet activities, NO-releasing ability, aqueous solubility and *in vivo* antithrombotic potency were evaluated.

Results and discussion

Strategy for the design of NO-NBP

Our previous studies and those of others have demonstrated that conjugation of an NO-donor moiety with a "native" molecule enhances therapeutic effect and/or reduces adverse effect of the native molecule.[12] In this study, we designed a novel class of compounds by connecting the NO-donors (nitrates) to 2-(1-hydroxypentyl) benzoic acid **1** through different linkers. Compound **1**, a ring-opening derivative of NBP, could be converted

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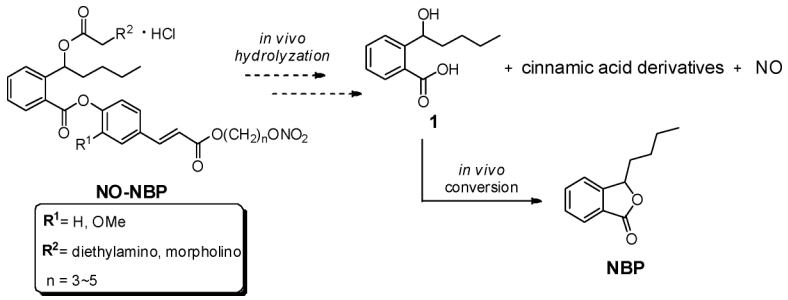
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of **6a–6f**, **7a–7l** and **8**, HPLC conditions chromatograms of **7a–7l**. See DOI: 10.1039/c1ob05478c



Scheme 1 Strategy for the design of NO-NBP.

to NBP very quickly *in vivo*.[13] The linkers included hydroxy-substituted cinnamic acid, like ferulic acid and *p*-hydroxyl cinnamic acid, which has both antioxidant and antiplatelet aggregation activity.[14] In addition, amines, such as morpholine and diethylamine, were introduced *via* a substituted acetate linkage to the side chain of **1**, respectively, to improve aqueous solubility of these derivatives. We expected that the ester bonds of NO-NBP would be cleaved *in vivo* by esterases to release **1**, which would subsequently undergo ring closure to generate NBP, and cinnamic acid derivatives as well as NO (Scheme 1). These bioactive compounds would synergistically inhibit platelet aggregation and thrombosis.

Chemistry

The synthesis of NO-NBP **7a–7l** is illustrated in Scheme 2. The bromo-substituted esters **4a–4f** were prepared as described previously,[15] and converted to the corresponding nitrates **5a–5f** using $AgNO_3$ in CH_3CN. NBP was generated by reacting commercially available 2-formylbenzoic acid **2** with Grignard reagent *n*-BuMgBr in 85% yield. Subsequently, the NBP was subjected to saponification and sequential acidification to pH 3–4 using dilute HCl solution at −10–0 °C to form **1** in 98% yield. Treatment of **1** with chloroacetyl chloride yielded the acylated compound **3**, which was esterified with **5a–5f** in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to offer esters **6a–6f**. Because **1** was found to be very easily lactonized to NBP at room temperature, it is necessary to handle **1** below 0 °C during the acidification and in the preparation of **3**. The esters **6a–6f** were condensed with diethylamine or morpholine, and treated with anhydrous ethereal HCl to generate the corresponding hydrochlorides **7a–7l** in 65–84% yields based on the two-step process. In addition, **3** was condensed with diethylamine to offer compound **8**, a NBP precursor. 1,4-Butanediol was treated with HNO_3 and Ac_2O in EtOAc to give its mononitrate **9**.[16] All of new compounds were further purified and characterized by IR, ESI-MS, ^{1}H NMR, ^{13}C NMR, and HRMS, and individual compounds with a purity of >95% were used for subsequent experiments.

In vitro antiplatelet effect of NO-NBP

The effects of individual 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.[17] In comparison with the reference drugs NBP and aspirin (ASP), a well-known antiplatelet agent, several compounds (*e.g.* **7d**, **7e** and **7k**) at 1.0 mM displayed more potent inhibition on platelet aggregation *in vitro* and **7e** inhibited platelet aggregation by 88.5% (Table 1).

Given that **7e** is composed of three moieties, NBP precurosor **8**, butyl ferulate (FAE), and organic nitrate **9**, we further characterized the inhibitory activity of each moiety on the ADP-induced platelet aggregation *in vitro*. We found that **8**, **9** and FAE at a concentration of 200 μM inhibited the ADP-induced platelet aggregation (36.3%, 32.5% and 16.0%, respectively) although each moiety was less potent than **7e** (51.1%, Fig. 1), suggesting that these three components in **7e** may synergistically inhibit the ADP-induced platelet aggregation *in vitro*.

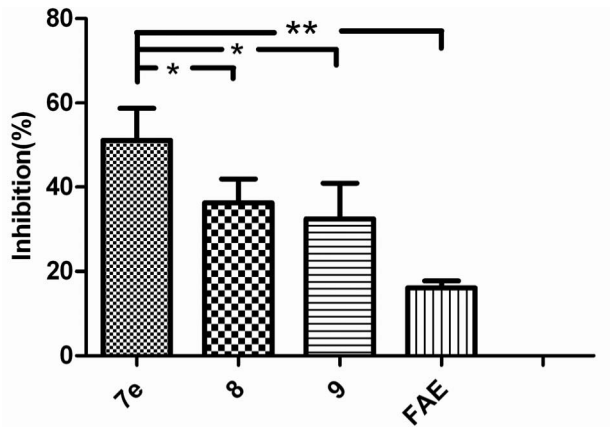
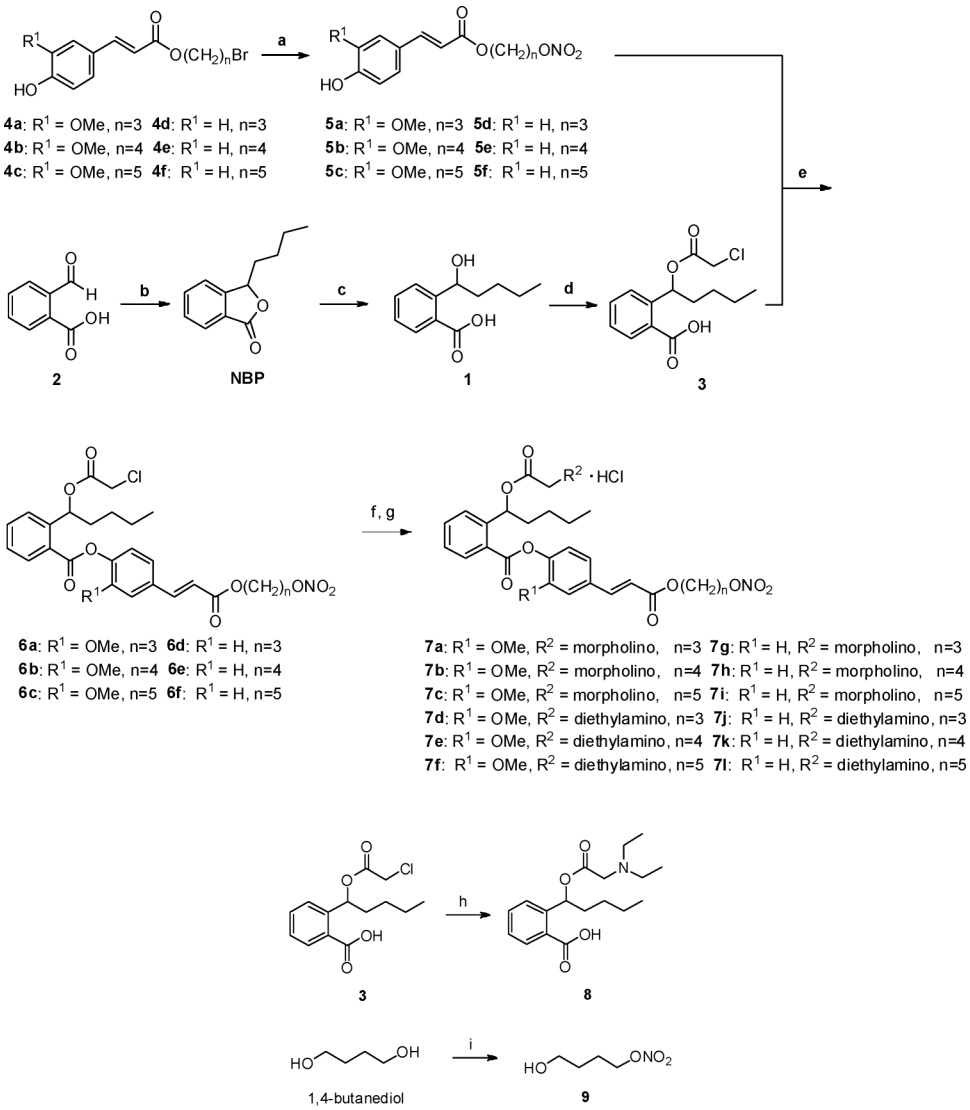


Fig. 1 Inhibition of **7e**, **8**, **9** and FAE on the ADP-induced platelet aggregation *in vitro*. Rabbit platelet suspensions were preincubated with testing compound (200 μM) at 37 °C for 5 min and exposed to 10 μM of ADP, followed by continually monitoring. Rabbit platelet suspensions that had been treated with vehicle and exposed to ADP were used as positive controls. Data are expressed as mean ± SD of each group ($n = 4$) for two separate experiments. * $P < 0.05$, ** $P < 0.01$ *vs.* **7e**, determined by Student's *t* test.

Next, we determined whether the NO-releasing ability of **7e** could contribute to its inhibitory activity on the ADP-induced platelet aggregation in rabbit PRP. Notably, the inhibitory effect of **7e** was enhanced upon treatment of 1 mM glutathione (GSH), which is known to promote the NO release from nitrates, suggesting that NO contributed to the inhibitory effect of **7e** on the ADP-induced platelet aggregation in PRP (Fig. 2). In contrast, treatment with 10 μM of haemoglobin (Hb), a known NO quencher,[18] significantly reduced the inhibition of **7e** on the ADP-induced platelet aggregation. Our data indicate that the nitrate moiety in **7e** is important for its strong inhibition of the



Scheme 2 Synthetic route of compounds **7a**–**7l**, **8** and **9**. *Reagents and conditions*: (a) $AgNO_3$, CH_3CN, 60 °C, 2 h; (b) (i) *n*-BuMgBr, Et_2O, −5 to 0 °C, 5 h; (ii) 1 M HCl, RT, 0.5 h; (c) (i) NaOH, CH_3OH–H_2O, reflux, 0.5 h; (ii) 1 M HCl, −10 to 0 °C; (d) $ClCH_2COCl$, Et_3N, DMAP, CH_2Cl_2, −10 °C; (e) DCC, DMAP, CH_2Cl_2, RT, 5 h; (f) diethylamine or morpholine, Et_3N, DMF, RT, 8 h; (g) 1 M ethereal HCl, 0 °C, 1 h; (h) diethylamine, Et_3N, CH_2Cl_2, RT, 12 h; (i) HNO_3, Ac_2O, EtOAc, 0 °C, 12 h.

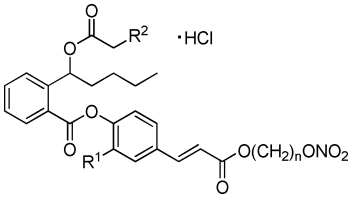
ADP-induced platelet aggregation. Given that NO can activate sGC and result in an increase in the levels of cGMP, inhibiting platelet aggregation, we further tested whether inhibition of sGC could modulate the inhibitory effect of **7e** on the ADP-induced platelet aggregation. We found that treatment with 25 μM of 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), a well known inhibitor of sGC, significantly mitigated the inhibitory effect of **7e** on the ADP-induced platelet aggregation. These data further supported that NO produced by **7e** contributed to its potent antiplatelet aggregation activity through the sGC pathway.

Further analysis revealed that **7e** inhibited the ADP, thrombin (TH), and arachidonic acid (AA)-induced platelet aggregation in PRP in a concentration-dependent manner and the inhibitory potency of **7e** on the platelet aggregation induced by these aggregators ranked in the order AA > ADP > TH, which was similar to that of NBP (Table 2, Fig. 3). In comparison with NBP and ASP, **7e** displayed much higher inhibition on the ADP- and TH-induced platelet aggregation, but comparable inhibition on the AA-induced platelet activation (Table 3), indicating that different activation mechanisms of the three aggregators inducing platelet aggregation are involved and affect the inhibitory activities of **7e**, which remain to be investigated.

Correlation of NO produced by NO-NBP with their antiplatelet aggregation activities

To examine the potential relationship between NO produced by NO-NBP and their antiplatelet aggregation activities, the levels of NO produced by **7e**, **7k**, **7d**, **7c** and **7i** were determined by Griess assay (Fig. 4).[19] We found that the levels of NO produced by **7e**, **7k**, **7d**, **7c** and **7i** were 0.3, 0.28, 0.2, 0.05, 0.04 μg ml^{-1}, respectively. Further analysis revealed that the levels of NO produced by these NO-NBP were correlated positively with the potencies of these compounds in inhibiting platelet aggregation *in vitro*

Table 1 The effect of compounds on the ADP (10 μM)-induced platelets aggregation *in vitro*[a]



Compd. (1.0 mM)	R^1	R^2	*n*	% inhibition
Aspirin	—	—	—	73.7
NBP	—	—	—	51.4
7a	OMe		3	23.4
7b	OMe		4	29.4
7c	OMe		5	18.5
7d	OMe		3	60.6
7e	OMe		4	88.5
7f	OMe		5	30.3
7g	H		3	21.4
7h	H		4	35.7
7i	H		5	19.8
7j	H		3	31.2
7k	H		4	70.5
7l	H		5	19.3

[a] Rabbit platelet suspensions were preincubated with 1.0 mM of each compound tested at 37 °C for 5 min followed by the addition of ADP (10 μM). Data are expressed as mean % of inhibition from two separate experiments.

($R^2 = 0.98$, $P < 0.01$, determined by logistic regression analysis). These, together with observation that treatment with the NO quencher, Hb, mitigated the inhibitory effect of **7e** on the ADP-induced platelet aggregation, clearly indicate that the moderate levels of NO produced by these NO-NBP are crucial for their antiplatelet aggregation activities.

Aqueous solubility of NO-NBP

Since poor solubility of NBP affects its clinical application and therapeutic efficacy, we further examined whether an increase in aqueous solubility of NO-NBP could be associated with their enhanced bioactivities. We determined the solubility of individual compounds as described previously.[20] As shown in Table 3, the saturated concentrations of compounds **7e**, **7d** and **7k** with strong antiplatelet aggregation activity were 0.98, 0.62 and 0.81 mM, respectively, which were higher than that of control NBP (0.53 mM). In contrast, the saturated concentrations of compounds **7c** and **7i** with poor antiplatelet aggregation activity were 0.36 mM and 0.39 mM, which were less than that of NBP. Further analysis indicated that the saturated concentrations of these compounds were correlated significantly with their antiplatelet aggregation activities ($R^2 = 0.96$, $P < 0.01$, determined

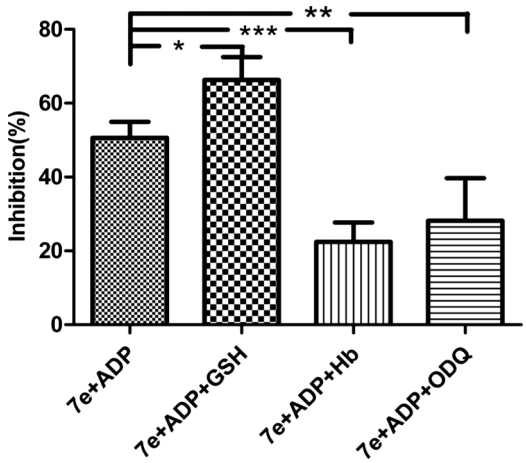


Fig. 2 The NO formed by **7e** contributes to the inhibition of **7e** on the ADP-induced platelet aggregation *in vitro*. Rabbit platelet rich plasma (PRP) were treated with **7e** (200 μM) in presence or absence of 1 mM of GSH, 10 μM of haemoglobin (Hb) or 25 μM of ODQ, respectively and exposed to ADP. The platelet aggregation was determined. Data are expressed as mean ± SD of each group ($n = 4$) from two separate experiments. * $P < 0.05$, ** $P < 0.01$ *vs.* **7e** alone, determined by Student's *t* test.

Table 2 The IC_{50} values of **7e**, aspirin, and NBP inhibiting rabbit platelet aggregation *in vitro*

	IC_{50} (mM)		
Compd.	ADP (10 μM)	TH (0.5 U/mL)	AA (1.0 mM)
Aspirin	0.82	> 1.0[b]	0.04
NBP	> 1.0[a]	> 1.0[c]	0.10
7e	0.24	0.41	0.16

[a] A 51.3% inhibition of platelet aggregation was observed at maximal concentration of 1.0 mM. [b] A 48.4% inhibition of platelet aggregation was observed at maximal concentration of 1.0 mM. [c] A 31.3% inhibition of platelet aggregation was observed at maximal concentration of 1.0 mM.

Table 3 The solubility of NBP and selected NO-NBP[a]

Compd.	Solubility (mM)	Compd.	Solubility (mM)
NBP	0.53	**7e**	0.98
7c	0.36	**7k**	0.81
7d	0.62	**7i**	0.39

[a] Data are expressed as mean concentration of individual compounds saturating in 5 ml saline at 25 °C.

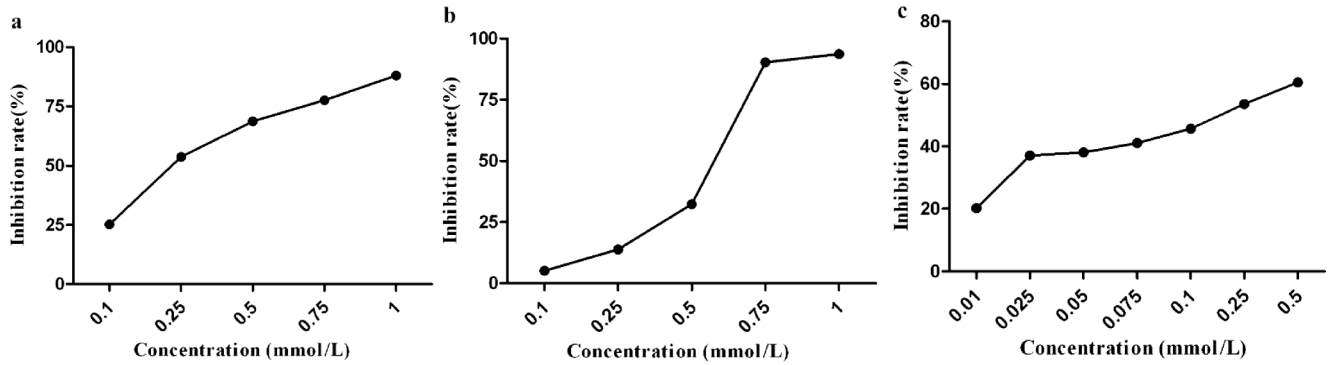


Fig. 3 Compound **7e** inhibits the ADP-, TH- and AA-induced platelet aggregation in a dose-dependent manner. PRP were treated in duplicate with vehicle alone or with the indicated doses of **7e** and exposed to 10 μM of ADP (a), 0.5 U mL^{-1} of TH (b), and 1.0 mM of AA (c), respectively. The platelet aggregation was determined. The PRP treated with vehicle and individual aggregators were used as positive controls and data are expressed as mean % of different doses of **7e** relative to the positive controls from two separate experiments. Intra-group variation was less than 7%. Treatment with 1 mM of **7e** did not cause detectable platelet aggregation (data not shown).

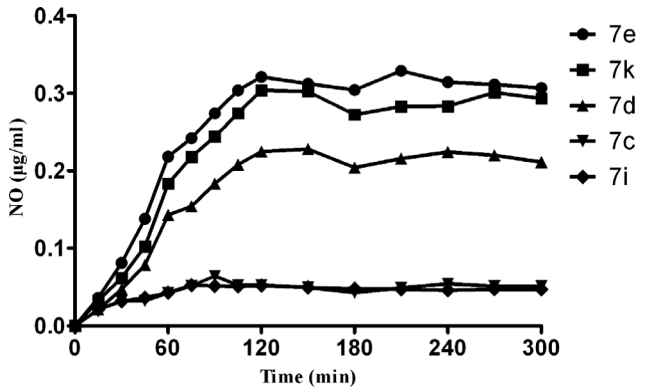


Fig. 4 Variable levels of NO were produced by selected NO-NBP *in vitro*. The levels of NO produced by selected NO-NBP were determined by Griess assay. Individual compounds were incubated for the indicated periods and reacted with Griess reagent at 30 °C for 10 min, followed by measuring at 540 nm. The levels of NO produced by individual compounds were calculated, according to the standards of different concentrations of nitrate. Data are expressed as means of individual compounds tested at each time point and intra-group variations were less than 10%.

by logistic regression analysis). These data clearly demonstrate that NO-NBP have better solubility, which contribute to their high antiplatelet aggregation activity. The improved solubility of these compounds may enhance their abilities to target the lesion areas and promote clinical applications.

Antithrombotic activities of 7e and 7k in rats

Next, we investigated the effects of compounds **7e** and **7k** on the formation of thrombus in a rat extra-corporeal circulation of arteriovenous (A-V) cannula model. Male SD rats were randomized and treated orally with 0.1% CMC-Na (negative control), ASP, NBP, **7e** and **7k** at equimolar doses (1.58×10^{-4} mol kg^{-1}) for 5 days. The formation of thrombus in these rats was induced by a surgical procedure and the thrombus weights in individual rats were measured. As shown in Fig. 5, the thrombus weights in the rats receiving **7e** (27.95 ± 7.32 mg) and **7k** (35.27 ± 4.85 mg) were significantly less than that of the negative control group (53.37 ± 7.35 mg, $P < 0.01$), and the thrombus weights in the

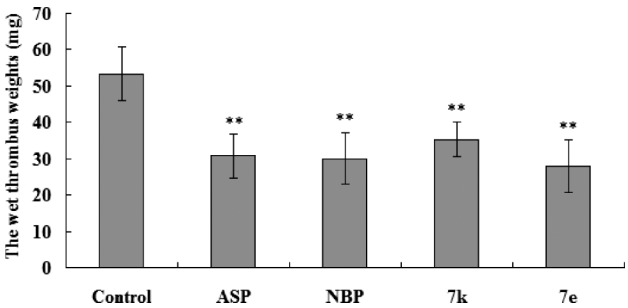


Fig. 5 Effect of ASP, NBP, **7e** and **7k** on the thrombus formation in rats. Male SD rats were randomized and treated orally with vehicle control, ASP, NBP, **7e**, and **7k** for consecutive 5 days and the formation of thrombus in individual rats was induced. Subsequently, the thrombus weights were measured. Data are expressed as mean ± SD of individual groups of rats (n = 10) from two separate experiments. Control and experimental groups of rats were tested simultaneously. $^{**}P < 0.01$ *vs.* the control group, determined by Student's t test.

7e treated rats were also slightly less than that in the NBP-treated rats (29.97 ± 7.01 mg) and ASP (30.73 ± 6.08 mg), indicating that **7e** has greater antithrombotic activity *in vivo*.

Inhibitory effect of 7e on the thrombosis in mice

In parallel, we finally evaluated the antithrombotic effect of **7e** on a mouse model of thromboembolism. Male Swiss mice were randomized and administered orally with vehicle (0.1% CMC-Na) alone, equimolar doses of **7e**, ASP, and NBP, respectively. Two hours after the treatment, the rats were injected intravenously with collagen and adrenaline for inducing thromboembolism and the development of hemiplegia and death in the rats were monitored within 15 min. Apparently, treatment with **7e** significantly reduced the onset of hemiplegia and death in mice and its protective effect was similar to that of NBP and Asp in this model (Table 4).

Discussion and conclusions

Analysis of structure and activity relationship (SAR) revealed that **7a–7l** with different linkers displayed variable antiplatelet aggregation activities. The varied length of carbon chain

Table 4 The effect of **7e** on the thromboembolism-induced death and hemiplegia in mice

Group	Dose (mg kg^{-1})	Number of mice	Number of death in 5 min and hemiplegia in 15 min	Protection rate (%)
Control	—	17	11	35.3
Aspirin	100	15	3	80.0[a]
NBP	100	17	5	70.6[a]
7e	342	18	5	72.2[a]

[a] $P < 0.05$ *vs.* the control group.

connecting to ferulic acid or *p*-hydroxyl cinnamic acid in those compounds appeared to affect their antiplatelet aggregation activities. Evidentially, **7b**, **7e**, **7h** and **7k** with a four-carbon linker displayed the strongest antiplatelet aggregation activity in the same subgroup. It is possible that varied linkers have different abilities to modulate the structure, stability, metabolism and penetrability of these compounds, which affect the NO production and NBP releasing, leading to various bioactivities of NO-NBP. Furthermore, the amino component substituted in the side-chain of NO-NBP was important for their bioactivities. The compounds (**7d–7f** and **7j–7l**) bearing a diethylamino side-chain exhibited stronger inhibitory effects than the compounds (**7a–7c** and **7g–7i**) bearing a morpholino side-chain. Indeed, **7e** containing a four-carbon linker and a diethylamino side-chain produced the highest levels of NO and displayed the best aqueous solubility and the strongest biological activities both *in vitro* and *in vivo* among the compounds tested. However, the precise mechanisms underlying the SAR of NO-NBP remain to be further investigated.

In summary, we designed and synthesized a series of NO-releasing derivatives of NBP and found that some NO-NBP had a strong inhibitory effect on platelet aggregation *in vitro*, particularly for **7e**, which was the most potent in inhibiting the ADP-, TH- and AA-induced platelet aggregations, superior to NBP and ASP. Furthermore, several NO-NBP produced moderate levels of NO *in vitro* and exhibited better solubility, which were correlated with their antiplatelet aggregation activities. Notably, our preliminary pharmacokinetic study showed that **7e** could generate the parent molecule NBP under physiological conditions, which is consistent with our working hypothesis.[21] Interestingly, all the three structural moieties (NBP precursor **8**, nitrate **9** and FAE) had moderate antiplatelet aggregation activity, and treatment with NO quencher or sGC inhibitor mitigated the inhibitory effect of **7e** on the ADP-induced platelet aggregation. Furthermore, **7e** exhibited greater antithrombotic activity in rats, and protected against the collagen and adrenaline induced thrombosis in mice. These data suggest that these structural moieties may act synergistically inhibiting platelet aggregation and thrombosis. Therefore, our findings may aid in the design of new therapeutic reagents for the treatment of thrombosis-related ischemic stroke and other vascular diseases.

Experimental section

General

Bromo-substituted esters **4a–4f**,[15] and NO donor moiety **9** were prepared as described previously.[16] Melting points were determined using a capillary apparatus (RDCSYI). All of the synthesized compounds were purified by column chromatography (CC) on silica gel 60 (200–300 mesh) or thin layer chromatography (TLC) on silica gel 60 F254 plates (250 μm; Qingdao Ocean Chemical Company, China). Subsequently, they were routinely analyzed by IR (Shimadzu FTIR-8400*S*), ^{1}H NMR and ^{13}C NMR (Bruker ACF-300Q, 300 MHz), and MS (Hewlett-Packard 1100 LC/MSD spectrometer). The purity of test compounds was characterized by HPLC analysis (Shimadzu LC-2010A HPLC system) and high resolution mass spectrometry (Agilent technologies LC/MSD TOF). Target compounds **7a–7l** with a purity of >95% were used for subsequent experiments (see the ESI†).

(±)-3-Butyl-1(3*H*)-isobenzofuranone (NBP)[22]

To a solution of magnesium (0.48 g, 20.0 mmol) in anhydrous Et_2O (5 mL) was added 1-bromobutane (2.74 g, 20.0 mmol) under nitrogen atmosphere until the Grignard reaction had started. After complete addition, the reaction mixture was refluxed for 1 h and allowed to cool to RT. A solution of **2** (1.50 g, 10.0 mmol) in anhydrous Et_2O (30 mL) was added dropwise to the Grignard solution at −5 °C. After completion by addition, the reaction mixture was stirred at RT for 5 h. The reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (10 mL). The mixture was acidified with 1 M HCl (15 mL) to pH 2 and stirred for 1 h at RT. 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 afford NBP as a light yellow oil (1.61 g, 85%). ESI-MS: *m*/*z* 213 $[M + Na]^+$. ^{1}H NMR ($CDCl_3$, 300 Hz, δ): 0.93(t, 3H, CH_3, J = 3.1 Hz), 1.37–1.45 (m, 4H, 2×CH_2), 1.86–1.89 (m, 2H, CH_2), 5.48–5.49 (m, 1H, CH), 7.37–7.39 (m, 1H, ArH), 7.56–7.5 (m, 2H, ArH), 8.05 (d, 1H, ArH, J = 6.5 Hz).

(±)-2-(1-Hydroxypentyl)benzoic acid (1)

To a solution of NBP (1.24 g, 6.52 mmol) in MeOH–H_2O (10 mL, 1 : 1 v/v) was added NaOH (0.28 g, 7.18 mmol). The reaction mixture was refluxed for 0.5 h. The solvent was removed under reduced pressure and dissolved in water (10 mL), followed by acidification with 1 M HCl to pH 3–4 at –5-0 °C. The mixture was extracted with cold Et_2O (5 mL × 3) and quickly used for the next step without any purification.

(±)-2-[1-(2-Chloroacetoxy)pentyl]benzoic acid (3)

To a solution of compound **1** (1.35 g, 6.52 mmol) and Et_3N (2.72 mL, 19.6 mmol) in CH_2Cl_2 (100 mL) was added 2-chloroacetyl chloride (2.72 mL, 19.6 mmol) at −10 °C and the solution was left stirring at −10 °C for 5 h. The mixture was acidified with 1 M HCl to pH 2 and stirred for 1 h at RT. The organic layer was separated and dried with anhydrous Na_2SO_4. After removal of solvent, the residue was recrystallized from

n-hexane to afford **3** as white crystals (1.11 g, 60%, over two steps). Mp 67–68 °C. ESI-MS: *m/z* 283 $[M - H]^-$. IR (KBr): 1412, 1691, 1734, 2958, 3450 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.93 (t, 3H, CH_3, J = 4.2 Hz), 1.37–1.42 (m, 4H, 2×CH_2), 1.88–1.91 (m, 2H, CH_2), 4.11 (m, 2H, $COCH_2Cl$), 6.78 (m, 1H, CH), 7.36–7.42 (m, 1H, ArH), 7.56–7.62 (m, 2H, ArH), 8.08 (d, ArH, J = 8.1 Hz), 10.89 (brs, 1H, COOH). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 172.0, 166.5, 140.8, 133.1, 130.3, 130.0, 127.1, 125.7, 74.8, 41.0, 36.3, 27.8, 22.4, 13.8.

General procedure for the synthesis of nitrate 5a–5f[23]

To a solution of corresponding bromo-substituted esters **4a–4f** (5.0 mmol) in CH_3CN (15 mL), was added $AgNO_3$ (7.5 mmol) at RT. The suspension was heated at 70° C for 2 h while being protected from light. The mixture was filtered and the filtrate concentrated. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water, brine, in turn, and dried with anhydrous Na_2SO_4, and finally concentrated to give the crude title compound (78–85%).

(*E*)-3-(Nitrooxy)propyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (5a)

The title compound was obtained as a pale yellow solid (78%), mp 60–61 °C. ESI-MS: *m/z* 298 $[M + H]^+$, 320 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 2.12–2.20 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.33 (t, 2H, CH_2ONO_2, J = 6.0 Hz), 4.61 (t, 2H, OCH_2, J = 6.3 Hz), 6.27 (d, 1H, CH =, J = 15.9 Hz), 6.91–6.93 (m, 1H, ArH), 7.03–7.09 (m, 2H, ArH), 7.59 (d, 2H, CH =, J = 16.0 Hz).

(*E*)-4-(Nitrooxy)butyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (5b)

The title compound was obtained as a pale yellow solid (80%), mp 63–64 °C. ESI-MS: *m/z* 312 $[M + H]^+$, 334 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 1.81–1.89 (m, 4H, 2×CH_2), 3.93 (s, 3H, OCH_3), 4.24 (t, 2H, CH_2ONO_2, J = 5.5 Hz), 4.52 (t, 2H, OCH_2, J = 6.0 Hz), 6.27 (d, 1H, CH═, J = 16.1 Hz), 6.91–6.93 (m, 1H, ArH), 7.03–7.09 (m, 2H, ArH), 7.61 (d, 1H, CH═, J = 16.0 Hz).

(*E*)-5-(Nitrooxy)pentyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (5c)

The title compound was obtained as a pale yellow solid (83%), mp 67–68 °C. ESI-MS: *m/z* 326 $[M + H]^+$, 348 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 1.50–1.56 (m, 2H, CH_2), 1.72–1.85 (m, 4H, 2×CH_2), 3.92 (s, 3H, OCH_3), 4.23 (t, 2H, CH_2ONO_2, J = 6.3 Hz), 4.48 (t, 2H, OCH_2, J = 6.5 Hz), 6.25 (d, 1H, CH═, J = 16.0 Hz), 6.91–6.93 (m, 1H, ArH), 7.03–7.09 (m, 2H, ArH), 7.62 (d, 1H, CH═, J = 15.9 Hz).

(*E*)-3-(Nitrooxy)propyl 3-(4-hydroxyphenyl)acrylate (5d)

The title compound was obtained as a pale yellow solid (80%), mp 68–69 °C. ESI-MS: *m/z* 268 $[M + H]^+$, 290 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 2.12–2.19 (m, 2H, CH_2), 4.33 (t, 2H, CH_2ONO_2, J = 6.1 Hz), 4.61 (t, 2H, OCH_2, J = 6.8 Hz), 6.28 (d, 1H, CH═, J = 16.0 Hz), 6.82–6.87 (m, 2H, ArH), 7.41–7.46 (m, 2H, ArH), 7.61 (d, 2H, CH═, J = 16.0 Hz).

(*E*)-4-(Nitrooxy)butyl 3-(4-hydroxyphenyl)acrylate (5e)

The title compound was obtained as a pale yellow solid (84%), mp 73–74 °C. ESI-MS: *m/z* 282 $[M + H]^+$, 304 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 1.86–1.88 (m, 4H, 2×CH_2), 4.26 (t, 2H, CH_2ONO_2, J = 5.5 Hz), 4.53 (t, 2H, OCH_2, J = 6.0 Hz), 6.26 (d, 1H, CH═, J = 16.1 Hz), 6.82–6.87 (m, 2H, ArH), 7.42–7.46 (m, 2H, ArH), 7.61 (d, 1H, CH═, J = 16.0 Hz).

(*E*)-5-(Nitrooxy)pentyl 3-(4-hydroxyphenyl)acrylate (5f)

The title compound was obtained as a pale yellow solid (87%), mp 75–76 °C. ESI-MS: *m/z* 296 $[M + H]^+$, 318 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 1.50–1.56 (m, 2H, CH_2), 1.72–1.85 (m, 4H, 2×CH_2), 4.23 (t, 2H, CH_2ONO_2, J = 6.3 Hz), 4.56 (t, 2H, OCH_2, J = 6.5 Hz), 6.25 (d, 1H, CH═, J = 16.0 Hz), 6.82–6.87 (m, 2H, ArH), 7.42–7.47 (m, 2H, ArH), 7.62 (d, 1H, CH═, J = 15.9 Hz).

General procedure for the synthesis of compounds 6a–6f

To a solution of acylated compound **3** (2.1 mmol) and the corresponding nitrates **5a–5f** (2.0 mmol) in anhydrous CH_2Cl_2 (25 mL) was added DMAP (0.1 mmol) and the solution was left stirring at 0 °C for 10 min. DCC (2.1 mmol) was then added and the solution was left stirring at RT for 6 h. The solution was then filtered, and the filtrate washed successively with 1 M HCl, a saturated aqueous solution of $NaHCO_3$, water, and brine. The solution was then dried with anhydrous Na_2SO_4, filtered, and solvent removed under reduced pressure. The resulting residue was purified by flash chromatography (petroleum ether–EtOAc 5 : 1 v/v) to give the title compounds (70–83%).

(±)-(*E*)-2-(1-Chloroacetoxypentyl)benzoic acid {2-methoxy-4-[2-(3-nitrooxypropoxycarbonyl)-vinyl]}phenyl ester (6a)

The title compound was obtained as a colourless waxy solid (75%), mp 63–65 °C. ESI-MS: *m/z* 581 $[M + NH_4]^+$, 586 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.85 (t, 3H, CH_3, J = 6.9 Hz), 1.26–1.46 (m, 4H, 2×CH_2), 1.86–1.95 (m, 2H, CH_2), 2.12–2.20 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.09 (m, 2H, $COCH_2Cl$), 4.33 (t, 2H, CH_2ONO_2, J = 6.0 Hz), 4.62 (t, 2H, OCH_2, J = 6.0 Hz), 6.40 (d, 1H, CH═, J = 15.9 Hz), 6.67–6.71 (m, 1H, CH), 7.19–7.26 (m, 3H, ArH), 7.42 (m, 1H, ArH), 7.60 (m, 2H, ArH) 7.68 (d, 1H, CH═, J = 16.0 Hz), 8.15 (d, 1H, ArH, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.5, 166.4, 164.5, 151.6, 144.8, 143.5, 141.7, 133.3, 133.1, 131.0, 127.6, 127.2, 126.2, 123.5, 121.4, 117.7, 111.4, 74.9, 69.9, 60.5, 55.9, 41.0, 36.5, 27.9, 26.5, 22.4, 13.9. HRMS for $C_{27}H_{30}NO_{10}ClNa$ ($[M + Na]^+$) calcd: 586.1456, Found: 586.1466.

(±)-(*E*)-2-(1-Chloroacetoxypentyl)benzoic acid {2-methoxy-4-[2-(4-nitrooxybutoxycarbonyl)-vinyl]}phenyl ester (6b)

The title compound was obtained as a colourless waxy solid (74%), mp 62–64 °C. ESI-MS: *m/z* 595 $[M + NH_4]^+$, 600 $[M + Na]^+$. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.85 (t, 3H, CH_3, J = 7.1 Hz), 1.24–1.47 (m, 4H, 2×CH_2), 1.86–2.04 (m, 6H, 3×CH_2), 3.89 (s, 3H, OCH_3), 4.10 (m, 2H, $COCH_2Cl$), 4.26 (t, 2H, CH_2ONO_2, J = 6.0 Hz), 4.52 (t, 2H, OCH_2, J = 6.0 Hz), 6.41 (d, 1H, CH═, J = 15.9 Hz), 6.67–6.71 (m, 1H, CH), 7.20–7.26 (m, 3H, ArH), 7.42 (m, 1H, ArH), 7.61 (m, 2H, ArH) 7.68 (d, 1H, CH═, J = 16.0 Hz), 8.15 (d, 1H, ArH, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.7, 166.4,

164.5, 151.6, 144.4, 143.5, 141.6, 133.4, 133.1, 131.0, 127.6, 127.3, 126.2, 123.5, 121.3, 118.0, 111.4, 74.9, 63.6, 55.9, 41.0, 36.5, 27.9, 25.1, 23.7, 22.4, 13.9. HRMS for $C_{28}H_{32}NO_{10}ClNa$ ([M + Na]$^+$) calcd: 600.1612, Found: 600.1624.

(±)-(*E*)-2-(1-Chloroacetoxypentyl)benzoic acid {2-methoxy-4-[2-(5-nitrooxypentoxycarbonyl)-vinyl]}phenyl ester (6c)

The title compound was obtained as a colourless waxy solid (70%), mp 60–61 °C. ESI-MS: *m/z* 609 [M + NH_4]$^+$, 614 [M + Na]$^+$. ^{1}H NMR ($CDCl_3$, 300 Hz, *δ*): 0.85 (t, 3H, CH_3, *J* = 7.2 Hz), 1.29–1.44 (m, 4H, 2×CH_2), 1.52–1.60 (m, 2H, CH_2), 1.75–1.93 (m, 6H, 3×CH_2), 3.89 (s, 3H, OCH_3), 4.09 (m, 2H, $COCH_2Cl$), 4.24 (t, 2H, CH_2ONO_2, *J* = 6.4 Hz), 4.48 (t, 2H, OCH_2, *J* = 6.4 Hz), 6.42 (d, 1H, CH═, *J* = 15.9 Hz), 6.68 (m, 1H, CH), 7.19–7.26 (m, 3H, ArH), 7.42 (m, 1H, ArH), 7.60 (m, 2H, ArH), 7.67 (d, 1H, CH═, *J* = 16.0 Hz), 8.15 (d, 1H, ArH, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 300 Hz, *δ*): 166.8, 166.4, 164.5, 151.5, 144.2, 143.5, 141.5, 133.5, 133.1, 131.0, 127.6, 127.3, 126.2, 123.4, 121.3, 118.2, 111.3, 74.9, 73.0, 64.1, 55.9, 41.0, 36.5, 28.3, 27.9, 26.5, 22.5, 22.4, 13.9. HRMS for $C_{29}H_{34}NO_{10}ClNa$ ([M + Na]$^+$) calcd: 614.1769, Found: 614.1780.

(±)-(*E*)-2-(1-Chloroacetoxypentyl)benzoic acid {4-[2-(3-nitrooxypropoxycarbonyl)-vinyl]}phenyl ester (6d)

The title compound was obtained as a colourless waxy solid (79%), mp 61–62 °C. ESI-MS: *m/z* 551 [M + NH_4]$^+$. ^{1}H NMR ($CDCl_3$, 300 Hz, *δ*): 0.87 (t, 3H, CH_3, *J* = 6.9 Hz), 1.26–1.50 (m, 4H, 2×CH_2), 1.87–1.95 (m, 2H, CH_2), 2.12–2.20 (m, 2H, CH_2), 4.08 (m, 2H, $COCH_2Cl$), 4.33 (t, 2H, CH_2ONO_2, *J* = 6.0 Hz), 4.61 (t, 2H, OCH_2, *J* = 6.0 Hz), 6.42 (d, 1H, CH═, *J* = 16.0 Hz), 6.68 (m, 1H, CH), 7.28–7.31 (m, 2H, ArH), 7.36 (m, 1H, ArH), 7.40–7.63 (m, 4H, ArH), 7.72 (d, 1H, CH═, *J* = 16.0 Hz), 8.15 (d, 1H, ArH, *J* = 8.1 Hz). ^{13}C NMR ($CDCl_3$, 300 Hz, *δ*): 166.6, 166.5, 164.9, 152.3, 144.3, 143.7, 133.4, 132.2, 130.8, 129.4, 129.4, 127.7, 127.2, 126.4, 122.5, 122.5, 117.7, 74.8, 69.9, 60.5, 41.0, 36.3, 27.9, 26.5, 22.4, 13.9. HRMS for $C_{26}H_{28}NO_9ClNa$ ([M + Na]$^+$) calcd: 556.1350, Found: 556.1358.

(±)-(*E*)-2-(1-Chloroacetoxypentyl)benzoic acid {4-[2-(4-nitrooxybutoxycarbonyl)-vinyl]}phenyl ester (6e)

The title compound was obtained as a colourless waxy solid (83%), mp 60–62 °C. ESI-MS: *m/z* 565 [M + NH_4]$^+$. ^{1}H NMR ($CDCl_3$, 300 Hz, *δ*): 0.86 (t, 3H, CH_3, *J* = 6.9 Hz), 1.26–1.52 (m, 4H, 2×CH_2), 1.85–1.94 (m, 6H, 4×CH_2), 4.00 (m, 2H, $COCH_2Cl$), 4.26 (t, 2H, CH_2ONO_2, *J* = 5.8 Hz), 4.53 (t, 2H, OCH_2, *J* = 6.0 Hz), 6.42 (d, 1H, CH═, *J* = 15.9 Hz), 6.68 (m, 1H, CH), 7.26–7.30 (m, 2H, ArH), 7.43 (m, 1H, ArH), 7.55–7.63 (m, 4H, ArH), 7.70 (d, 1H, CH═, *J* = 16.0 Hz), 8.15 (d, 1H, ArH, *J* = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 300 Hz, *δ*): 166.7, 166.6, 164.9, 152.3, 144.0, 143.6, 133.3, 132.3, 130.8, 129.4, 129.4, 127.7, 127.3, 126.4, 122.4, 122.4, 118.0, 74.8, 72.6, 63.6, 41.0, 36.3, 27.9, 25.1, 23.7, 22.4, 13.9. HRMS for $C_{27}H_{30}NO_9ClNa$ ([M + Na]$^+$) calcd: 570.1507, Found: 570.1516.

(±)-(*E*)-2-(1-Chloroacetoxypentyl)benzoic acid {4-[2-(5-nitrooxypentoxycarbonyl)-vinyl]}phenyl ester (6f)

The title compound was obtained as a colourless waxy solid (81%), mp 57–59 °C. ESI-MS: *m/z* 579 [M + NH_4]$^+$. ^{1}H NMR ($CDCl_3$, 300 Hz, *δ*): 0.86 (t, 3H, CH_3, *J* = 6.9 Hz), 1.30–1.45 (m, 4H, 2×CH_2), 1.52–1.59 (m, 2H, CH_2), 1.73–2.03 (m, 6H, 3×CH_2), 4.09 (m, 2H, $COCH_2Cl$), 4.23 (t, 2H, CH_2ONO_2, *J* = 6.3 Hz), 4.48 (t, 2H, OCH_2, *J* = 6.3 Hz), 6.43 (d, 1H, CH═, *J* = 16.0 Hz), 6.68 (m, 1H, CH), 7.26–7.30 (m, 2H, ArH), 7.43 (m, 1H, ArH), 7.60–7.63 (m, 4H, ArH), 7.69 (d, 1H, CH═, *J* = 16.0 Hz), 8.14 (d, 1H, ArH, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 300 Hz, *δ*): 166.9, 166.6, 164.9, 152.2, 143.8, 143.7, 133.4, 132.3, 130.8, 129.4, 129.4, 127.7, 127.2, 126.4, 122.4, 122.4, 118.0, 74.8, 73.0, 64.1, 41.0, 36.3, 28.3, 27.9, 26.5, 23.7, 22.5, 22.4, 13.9. HRMS for $C_{28}H_{32}NO_9ClNa$ ([M + Na]$^+$) calcd: 584.1663, Found: 584.1664.

General procedure for the synthesis of compounds 7a–7l

To a solution of esters **6a–6f** (0.45 mmol) and Et_3N (1 mL) in DMF (5 mL) was added the appropriate amine (morpholine or diethylamine, 1.35 mmol) and the solution was left stirring at RT for 8 h. The solution was then filtered, and the filtrate was reconstituted in EtOAc (20 mL) and water (20 mL). The organic phase was washed with water and brine. The solution was then dried with anhydrous Na_2SO_4, filtered, and solvent removed under reduced pressure. The amino derivatives were purified by flash chromatography (petroleum ether–EtOAc 3 : 1 v/v), to which EtOAc (0.5 mL) and 1 M ethereal HCl (0.5 mL) was added dropwise at 0 °C. The solution was left stirring at room temperature for 1 h and filtered to give the title compounds (65–84%).

(±)-(*E*)-2-[1-(Morpholinoacetoxy)pentyl]benzoic acid {2-methoxy-4-[2-(3-nitrooxypropoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7a)

The title compound was obtained as a white solid (75%, for two steps), mp 103–105 °C. ESI-MS: *m/z* 615 [M + H]$^+$. IR (KBr): 759, 1035, 1641, 1739, 2955, 3069 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 Hz, *δ*): 0.85 (t, 3H, CH_3, *J* = 6.3 Hz), 1.31 (m, 4H, 2×CH_2), 1. 92 (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 3.36 (m, 4H, 2×NCH_2), 3.90 (s, 3H, OCH_3), 3.94 (m, 2H, $COCH_2N$), 4.04 (m, 4H, 2×OCH_2), 4.34 (t, 2H, CH_2ONO_2, *J* = 6.0 Hz), 4.62 (t, 2H, $COOCH_2$, *J* = 6.3 Hz), 6.42 (d, 1H, CH═, *J* = 16.0 Hz), 6.75 (m, 1H, CH), 7.17–7.27 (m, 3H, ArH), 7.43–7.67 (m, 3H, ArH), 7.70 (d, 1H, CH═, *J* = 15.9 Hz), 8.18 (d, 1H, ArH, *J* = 7.8 Hz), 13.78 (br s, 1H, NH$^+$). ^{13}C NMR ($CDCl_3$, 300 Hz, *δ*): 166.5, 164.5, 164.3, 151.5, 144.6, 142.6, 141.5, 133.5, 133.4, 131.1, 128.0, 127.2, 126.3, 123.4, 121.4, 117.8, 111.4, 75.5, 70.0, 64.1, 64.1, 60.6, 56.0, 55.6, 51.1, 51.1, 36.2, 27.8, 26.5, 22.3, 13.9. HRMS for $C_{31}H_{39}N_2O_{11}$ ([M + H]$^+$) calcd: 615.2554, found: 615.2568.

(±)-(*E*)-2-[1-(Morpholinoacetoxy)pentyl]benzoic acid {2-methoxy-4-[2-(4-nitrooxybutoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7b)

The title compound was obtained as a white solid (77%, for two steps), mp 102–104 °C. ESI-MS: *m/z* 629 [M + H]$^+$. IR (KBr): 760, 1038, 1632, 1741, 2872, 2958, 3064 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 Hz, *δ*): 0.85 (t, 3H, CH_3, *J* = 6.3 Hz), 1.31 (m, 4H, 2×CH_2), 1. 88 (m, 6H, 3×CH_2), 3.45 (m, 4H, 2×NCH_2), 3.90 (s, 3H, OCH_3), 4.01 (m, 4H, 2×OCH_2), 4.15 (m, 2H, $COCH_2N$), 4.26 (t, 2H, CH_2ONO_2, *J* = 6.0 Hz), 4.53 (t, 2H, $COOCH_2$, *J* = 6.0 Hz), 6.42 (d, 1H, CH═, *J* = 15.9 Hz), 6.77 (m, 1H, CH), 7.17–7.28 (m, 3H, ArH),

7.43–7.65 (m, 3H, ArH), 7.67 (d, 1H, CH═, J = 15.9 Hz), 8.19 (d, 1H, ArH, J = 7.8 Hz), 13.50 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.6, 164.5, 163.8, 151.5, 144.3, 142.4, 141.4, 133.5, 133.4, 131.1, 128.1, 127.2, 126.4, 123.4, 121.4, 118.2, 111.4, 75.7, 72.6, 63.8, 63.8, 63.6, 56.0, 55.1, 50.8, 50.8, 36.2, 27.8, 25.1, 23.7, 22.3, 13.9. HRMS for $C_{32}H_{41}N_2O_{11}$ ([M + H]$^+$) calcd: 629.2710, found: 629.2723.

(±)-(*E*)-2-[1-(Morpholinoacetoxy)pentyl]benzoic acid {2-methoxy-4-[2-(5-nitrooxypentoxycarbonyl)-vinyl]}phenyl ester (7c)

The title compound was obtained as a white solid (70%, for two steps), mp 102–103 °C. ESI-MS: m/z 643 [M + H]$^+$. IR (KBr): 759, 1032, 1636, 1742, 2951, 3068 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.85 (t, 3H, CH_3, J = 6.6 Hz), 1.31 (m, 4H, 2×CH_2), 1.55 (m, 2H, CH_2), 1.73–1.85 (m, 4H, 2×CH_2), 1.92 (m, 2H, CH_2), 3.43 (m, 4H, 2×NCH_2), 3.90 (s, 3H, OCH_3), 4.03 (m, 6H, 2×OCH_2 and $COCH_2N$), 4.23 (t, 2H, CH_2ONO_2, J = 6.3 Hz), 4.53 (t, 2H, $COOCH_2$, J = 6.3 Hz), 6.43 (d, 1H, CH═, J = 15.9 Hz), 6.76 (m, 1H, CH), 7.18–7.20 (m, 3H, ArH), 7.43–7.65 (m, 3H, ArH), 7.67 (d, 1H, CH═, J = 15.9 Hz), 8.18 (d, 1H, ArH, J = 8.1 Hz), 13.40 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.8, 164.5, 163.9, 151.5, 144.1, 142.4, 141.3, 133.6, 133.4, 131.1, 128.1, 127.2, 126.4, 123.3, 121.3, 118.4, 111.4, 75.7, 73.0, 64.1, 63.8, 63.8, 56.0, 55.1, 50.9, 50.9, 36.2, 28.2, 27.8, 26.5, 22.3, 22.3, 13.9. HRMS for $C_{33}H_{43}N_2O_{11}$ ([M + H]$^+$) calcd: 643.2867, found: 643.2881.

(±)-(*E*)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid {2-methoxy-4-[2-(3-nitrooxypropoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7d)

The title compound was obtained as a white solid (78%, for two steps), mp 109–110 °C. ESI-MS: m/z 601 [M + H]$^+$. IR (KBr): 759, 1252, 1631, 1731, 2953, 3078 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.86 (t, 3H, CH_3, J = 6.9 Hz), 1.36 (m, 7H, 2×CH_2 and CH_3), 1.48 (t, 3H, CH_3, J = 7.2 Hz), 1.92 (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 3.21–3.42 (m, 4H, 2×NCH_2), 3.89 (s, 3H, OCH_3), 3.99 (m, 2H, $COCH_2N$), 4.32 (t, 2H, CH_2ONO_2, J = 6.0 Hz), 4.62 (t, 2H, $COOCH_2$, J = 6.3 Hz), 6.42 (d, 1H, CH═, J = 16.0 Hz), 6.77 (m, 1H, CH), 7.18 (m, 3H, ArH), 7.47–7.65 (m, 3H, ArH), 7.69 (d, 1H, CH═, J = 15.9 Hz), 8.21 (d, 1H, ArH, J = 7.8 Hz), 12.78 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.4, 164.5, 164.4, 151.5, 144.6, 142.6, 141.4, 133.5, 133.4, 131.3, 128.1, 127.1, 126.1, 123.4, 121.4, 117.9, 111.4, 75.3, 70.0, 60.6, 56.0, 48.6, 48.5, 48.2, 36.1, 27.9, 26.5, 22.3, 13.9, 10.1, 10.0. HRMS for $C_{31}H_{41}N_2O_{10}$ ([M + H]$^+$) calcd: 601.2761, found: 601.2773.

(±)-(*E*)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid {2-methoxy-4-[2-(4-nitrooxybutoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7e)

The title compound was obtained as a white solid (84%, for two steps), mp 106–108 °C. ESI-MS: m/z 615 [M + H]$^+$. IR (KBr): 759, 1254, 1630, 1747, 2861, 2956, 3444 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.86 (t, 3H, CH_3, J = 6.6 Hz), 1.37 (m, 7H, 2×CH_2 and CH_3), 1.48 (t, 3H, CH_3, J = 7.2 Hz), 1.78 (m, 6H, 2×CH_3), 3.26–3.41 (m, 4H, 2×NCH_2), 3.89 (s, 3H, OCH_3), 4.00 (m, 2H, $COCH_2N$), 4.27 (t, 2H, CH_2ONO_2, J = 5.4 Hz), 4.53 (t, 2H, $COOCH_2$, J = 5.7 Hz), 6.42 (d, 1H, CH═, J = 15.9 Hz), 6.76 (m, 1H, CH), 7.19 (m, 3H, ArH), 7.44–7.65 (m, 3H, ArH), 7.68 (d, 1H, CH═, J = 16.0 Hz), 8.21 (d, 1H, ArH, J = 7.8 Hz), 12.73 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.4, 164.5, 164.4, 151.5, 144.2, 142.6, 141.4, 133.5, 133.4, 131.2, 128.1, 127.1, 126.1, 123.3, 121.3, 118.2, 111.4, 75.3, 72.7, 63.6, 55.9, 48.6, 48.5, 48.2, 36.1, 27.9, 25.1, 23.7, 22.3, 13.9, 10.1, 10.0. HRMS for $C_{32}H_{43}N_2O_{10}$ ([M + H]$^+$) calcd: 615.2918, found: 615.2931.

(±)-(*E*)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid {2-methoxy-4-[2-(5-nitrooxypentoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7f)

The title compound was obtained as a white solid (80%, for two steps), mp 106–107 °C. ESI-MS: m/z 629 [M + H]$^+$. IR (KBr): 758, 1253, 1633, 1733, 2872, 2953, 3073 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.86 (t, 3H, CH_3, J = 6.7 Hz), 1.37 (m, 7H, 2×CH_2 and CH_3), 1.48 (t, 3H, CH_3, J = 7.2 Hz), 1.56 (m, 2H, CH_2), 1.73–1.86 (m, 4H, 2×CH_2), 1. 93 (m, 2H, CH_2), 3.22–3.43 (m, 4H, 2×NCH_2), 3.89 (s, 3H, OCH_3), 4.00 (m, 2H, $COCH_2N$), 4.24 (t, 2H, CH_2ONO_2, J = 6.3 Hz), 4.49 (t, 2H, $COOCH_2$, J = 6.3 Hz), 6.42 (d, 1H, CH═, J = 15.9 Hz), 6.77 (m, 1H, CH), 7.20 (m, 3H, ArH), 7.40–7.65 (m, 3H, ArH), 7.70 (d, 1H, J = 16.0 Hz), 8.20 (d, 1H, ArH, J = 7.5 Hz), 12.79 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.7, 164.5, 164.4, 151.5, 144.0, 142.6, 141.3, 133.6, 133.4, 131.2, 128.1, 127.2, 126.1, 123.3, 121.3, 118.4, 111.4, 75.3, 73.0, 64.1, 55.9, 48.6, 48.5, 48.2, 36.1, 28.2, 27.9, 26.5, 22.4, 22.3, 13.9, 10.1, 10.0. HRMS for $C_{33}H_{45}N_2O_{10}$ ([M + H]$^+$) calcd: 629.3074, found: 629.3086.

(±)-(*E*)-2-[1-(Morpholinoacetoxy)pentyl]benzoic acid {4-[2-(3-nitrooxypropoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7g)

The title compound was obtained as a white solid (70%, for two steps), mp 100-102 °C. ESI-MS: m/z 585 [M + H]$^+$. IR (KBr): 856, 1166, 1631, 1739, 2859, 2951, 3069 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.86 (t, 3H, CH_3, J = 6.9 Hz), 1.33 (m, 4H, 2×CH_2), 1.91 (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 3.44 (m, 4H, 2×NCH_2), 3.59–4.02(m, 4H, 2×OCH_2), 4.22 (m, 2H, $COCH_2N$), 4.34 (t, 2H, CH_2ONO_2, J = 6.0 Hz), 4.62 (t, 2H, $COOCH_2$, J = 5.7 Hz), 6.43 (d, 1H, CH═, J = 15.9 Hz), 6.78 (m, 1H, CH), 7.27–7.30 (m, 2H, ArH), 7.47 (m, 1H, ArH), 7.60–7.69 (m, 4H, ArH), 7.73 (d, 1H, CH =, J = 16.0 Hz), 8.18 (d, 1H, ArH, J = 8.1 Hz), 13.54 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.5, 164.8, 163.9, 152.2, 144.2, 142.8, 133.7, 132.3, 131.0, 129.4, 129.4, 128.2, 127.0, 126.5, 122.4, 122.4, 117.9, 75.5, 70.0, 63.8, 63.8, 60.5, 55.0, 50.7, 50.7, 36.1, 27.8, 26.5, 22.3, 13.9. HRMS for $C_{30}H_{37}N_2O_{10}$ ([M + H]$^+$) calcd: 585.2448, found: 585.2460.

(±)-(*E*)-2-[1-(Morpholinoacetoxy)pentyl]benzoic acid {4-[2-(4-nitrooxybutoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7h)

The title compound was obtained as a white solid (65%, for two steps), mp 101–103 °C. ESI-MS: m/z 599 [M + H]$^+$. IR (KBr): 856, 1166, 1631, 1739, 2859, 2951, 3068 cm^{-1}. ^1H NMR ($CDCl_3$, 300 Hz, δ): 0.86 (t, 3H, CH_3, J = 6.3 Hz), 1.34 (m, 4H, 2×CH_2), 1.90 (m, 6H, 3×CH_2), 3.42 (m, 4H, 2×NCH_2), 4.01(m, 2H, $COCH_2N$), 4.07 (m, 4H, 2×OCH_2), 4.26 (t, 2H, CH_2ONO_2, J = 6.1 Hz), 4.53 (t, 2H, $COOCH_2$, J = 6.0 Hz), 6.43 (d, 1H, CH═, J = 16.0 Hz), 6.77 (m, 1H, CH), 7.27–7.30 (m, 2H, ArH), 7.47 (m, 1H, ArH), 7.62–7.68 (m, 4H, ArH), 7.73 (d, 1H, CH═, J = 16.0 Hz), 8.18 (d, 1H, ArH, J = 8.1 Hz), 13.50 (br s, 1H, NH^+). ^{13}C NMR ($CDCl_3$,

300 Hz, δ): 166.7, 164.8, 164.1, 152.1, 143.8, 142.8, 133.7, 132.4, 131.0, 129.4, 129.4, 128.1, 127.0, 126.6, 122.4, 122.4, 118.2, 75.4, 72.6, 63.9, 63.9, 63.6, 55.3, 51.0, 51.0, 36.1, 27.8, 25.1, 23.7, 22.3, 13.9. HRMS for $C_{31}H_{39}N_2O_{10}$ ([M + H]$^+$) calcd: 599.2605, found: 599.2616.

(±)-(*E*)-2-[1-(Morpholinoacetoxy)pentyl]benzoic acid {4-[2-(5-nitrooxypentoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7i)

The title compound was obtained as a white solid (75%, for two steps), mp 100–101 °C. ESI-MS: *m/z* 613 [M + H]$^+$. IR (KBr): 857, 1162, 1638, 1739, 28569, 2954, 3071 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 Hz, δ): 0.86 (t, 3H, CH_3, J = 6.8 Hz), 1.33 (m, 4H, 2×CH_2), 1.50–1.60 (m, 2H, CH_2), 1.73–1.83 (m, 4H, 2×CH_2), 1.91 (m, 2H, CH_2), 3.44 (m, 4H, 2×NCH_2), 4.05(m, 4H, 2×OCH_2), 4.11 (m, 2H, $COCH_2N$), 4.23 (t, 2H, CH_2ONO_2, J = 6.3 Hz), 4.49 (t, 2H, $COOCH_2$, J = 6.5 Hz), 6.43 (d, 1H, CH═, J = 16.0 Hz), 6.78 (m, 1H, CH), 7.26–7.29 (m, 2H, ArH), 7.47 (m, 1H, ArH), 7.61–7.67 (m, 4H, ArH), 7.70 (d, 1H, CH═, J = 16.0 Hz), 8.17 (d, 1H, ArH, J = 8.1 Hz), 13.50 (br s, 1H, NH$^+$). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.8, 164.8, 163.9, 152.1, 143.6, 142.8, 133.7, 132.4, 131.0, 129.4, 129.4, 128.1, 127.0, 126.5, 122.3, 122.3, 118.4, 75.5, 73.0, 64.1, 63.8, 63.8, 55.1, 50.9, 50.9, 36.1, 28.2, 28.0, 27.8, 22.3, 22.3, 13.9. HRMS for $C_{32}H_{41}N_2O_{10}$ ([M + H]$^+$) calcd: 613.2761, found: 613.2772.

(±)-(*E*)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid {4-[2-(3-nitrooxypropoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7j)

The title compound was obtained as a white solid (83%, for two steps), mp 107–110 °C. ESI-MS: *m/z* 571 [M + H]$^+$. IR (KBr): 885, 1171, 1635, 1739, 2858, 2955, 3069 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 Hz, δ): 0.87 (t, 3H, CH_3, J = 6.9 Hz), 1.36 (m, 7H, 2×CH_2 and CH_3), 1.48 (t, 3H, CH_3, J = 7.2 Hz), 1.92 (m, 2H, CH_2), 2.17 (m, 2H, CH_2), 3.13–3.49 (m, 4H, 2×NCH_2), 4.00 (m, 2H, $COCH_2N$), 4.34 (t, 2H, CH_2ONO_2, J = 6.0 Hz), 4.62 (t, 2H, $COOCH_2$, J = 6.3 Hz), 6.43 (d, 1H, CH═, J = 16.0 Hz), 6.78 (m, 1H, CH), 7.27–7.30 (m, 2H, ArH), 7.48 (m, 1H, ArH), 7.57–7.69 (m, 4H, ArH), 7.73 (d, 1H, CH═, J = 15.9 Hz), 8.20 (d, 1H, ArH, J = 7.5 Hz), 12.75 (br s, 1H, NH$^+$). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.5, 164.7, 164.5, 152.1, 144.1, 142.9, 133.7, 132.3, 131.1, 129.4, 129.4, 128.2, 127.0, 126.3, 122.3, 122.3, 117.9, 75.2, 70.0, 60.6, 48.6, 48.5, 48.3, 36.0, 27.9, 26.5, 22.3, 13.9, 10.1, 10.0. HRMS for $C_{30}H_{39}N_2O_9$ ([M + H]$^+$) calcd: 571.2656, found: 571.2668.

(±)-(*E*)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid {4-[2-(4-nitrooxybutoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7k)

The title compound was obtained as a white solid (85%, for two steps), mp 106–108 °C. ESI-MS: *m/z* 585 [M + H]$^+$. IR (KBr): 885, 1165, 1630, 1738, 2858, 2957, 3064 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 Hz, δ): 0.87 (t, 3H, CH_3, J = 6.9 Hz), 1.36 (m, 7H, 2×CH_2 and CH_3), 1.48 (t, 3H, CH_3, J = 7.2 Hz), 1.86–1.93 (m, 6H, 3×CH_2), 3.22–3.50 (m, 4H, 2×NCH_2), 4.01 (m, 2H, $COCH_2N$), 4.27 (t, 2H, CH_2ONO_2, J = 5.7 Hz), 4.53 (t, 2H, $COOCH_2$, J = 5.7 Hz), 6.43 (d, 1H, CH═, J = 15.9 Hz), 6.78 (m, 1H, CH), 7.27–7.30 (m, 2H, ArH), 7.49 (m, 1H, ArH), 7.58–7.68 (m, 4H, ArH), 7.72 (d, 1H, CH═, J = 15.9 Hz), 8.20 (d, 1H, ArH, J = 7.5 Hz), 12.70 (br s, 1H, NH$^+$). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.6, 164.7, 164.5, 152.1, 143.8, 142.9, 133.7, 132.4, 131.1, 129.4, 129.4, 128.2, 127.0, 126.3, 122.3, 122.3, 118.2, 75.2, 72.6, 63.6, 48.6, 48.5, 48.3, 36.0, 27.9, 25.1, 23.7, 22.3, 13.9, 10.1, 10.0. HRMS for $C_{31}H_{41}N_2O_9$ ([M + H]$^+$) calcd: 585.2812, found: 585.2823.

(±)-(*E*)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid {4-[2-(5-nitrooxypentoxycarbonyl)-vinyl]}phenyl ester hydrochloride (7l)

The title compound was obtained as a white solid (81%, for two steps), mp 104–106 °C. ESI-MS: *m/z* 599 [M + H]$^+$. IR (KBr): 885, 1158, 1635, 1738, 2857, 2961, 3070 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 Hz, δ): 0.87 (t, 3H, CH_3, J = 6.6 Hz), 1.36 (m, 7H, 2×CH_2 and CH_3), 1.48 (t, 3H, CH_3, J = 6.9 Hz), 1.57 (m, 2H, CH_2), 1.73–1.86 (m, 4H, 2×CH_2), 1.92 (m, 2H, CH_2), 3.30–3.49 (m, 4H, 2×NCH_2), 4.00 (m, 2H, $COCH_2N$), 4.24 (t, 2H, CH_2ONO_2, J = 6.4 Hz), 4.49 (t, 2H, $COOCH_2$, J = 6.3 Hz), 6.43 (d, 1H, CH═, J = 15.9 Hz), 6.78 (m, 1H, CH), 7.19–7.29 (m, 2H, ArH), 7.48 (m, 1H, ArH), 7.57–7.67 (m, 4H, ArH), 7.71 (d, 1H, CH═, J = 15.9 Hz), 8.20 (d, 1H, ArH, J = 7.8 Hz), 12.75 (br s, 1H, NH$^+$). ^{13}C NMR ($CDCl_3$, 300 Hz, δ): 166.8, 164.7, 164.5, 152.0, 143.6, 142.9, 133.7, 132.5, 131.1, 129.4, 129.4, 128.2, 127.0, 126.3, 122.3, 122.3, 118.4, 75.2, 73.0, 64.1, 48.6, 48.5, 48.3, 36.0, 28.2, 27.9, 26.5, 22.4, 22.3, 13.9, 10.1, 10.0. HRMS for $C_{32}H_{43}N_2O_9$ ([M + H]$^+$) calcd: 599.2969, found: 599.2980.

(±)-2-[1-(Diethylaminoacetoxy)pentyl]benzoic acid (8)

To a solution of acylated compound **3** (0.71 g, 2.5 mmol) and Et_3N (1 mL) in CH_2Cl_2 (15 mL) was added diethylamine (0.55, 7.5 mmol) and the solution was left stirring at room temperature for 12 h. The solvent was removed under reduced pressure and purified by flash chromatography (MeOH–EtOAc 1 : 10 v/v) to give the **8** as a colourless waxy solid (0.36 g, 45%). Mp 69–70 °C. ESI-MS: *m/z* 322 [M + H]$^+$. ^{1}H NMR ($CDCl_3$, 500 Hz, δ): 0.83 (t, 3H, CH_3, J = 7.5 Hz), 1.14–1.77 (m, 6H, 2×CH_3, J = 7.0 Hz), 1.29–1.37 (m, 4H, 2×CH_2), 1.87–1.88 (m, 2H, CH_2), 2.91–2.96 (q, 4H, 2×CH_2, J = 7.0 Hz), 3.62–3.76 (m, 2H, $COCH_2N$), 6.69 (m, 1H, CH), 7.21–7.26 (m, 1H, ArH), 7.34–7.43 (m, 2H, ArH), 7.80 (d, 1H, ArH, J = 7.5 Hz), 10.67 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$, 500 Hz, δ): 172.2, 168.1, 140.8, 133.1, 130.3, 130.0, 127.1, 125.7, 74.7, 51.8, 47.0, 47.0, 36.3, 27.8, 22.3, 13.8, 10.5, 10.5. HRMS for $C_{18}H_{28}NO_4$ ([M + H]$^+$) calcd: 322.2018, found: 322.2022.

Platelet aggregation *in vitro*

Blood samples were withdrawn from rabbit carotid artery and mixed with 3.8% trisodium citrate (9 : 1 v/v), followed by centrifuging at 500 rpm for 10 min. The supernatants were collected and used as platelet rich plasma (PRP). Additional samples were centrifuged at 3000 rpm for 10 min and the supernatants were collected as platelet poor plasma (PPP). The effect of individual compounds on the ADP-, TH- and AA-induced platelet aggregation was measured by Born's turbidimetric method using a Platelet-Aggregometer (LG-PABER-I Platelet-Aggregometer, Beijing). Briefly, PRP (240 µl) was pre-treated in duplicate with vehicle, different concentrations of individual compounds or the reference drugs for 5 min and exposed to 10 µM of ADP, 0.5 U mL^{-1} of TH, or 1.0 mM of AA incubated at 37 °C for 5 min, respectively. The formation of platelet aggregation was monitored longitudinally by optical density. Platelet aggregation was induced by ADP (final concentration 10 µM), TH (final concentration

0.5 U mL^{-1}) or AA (final concentration 1.0 mM). Compounds under study or vehicle alone were added to the PRP samples 5 min before addition of the aggregating agent. The antiplatelet aggregatory activity of individual compounds was evaluated as percent inhibition of platelet aggregation compared to positive controls that had been pre-treated with vehicle alone and exposed with the inducer samples. The IC_{50} values of most compounds were determined by nonlinear regression analysis and the percent inhibition at the maximal concentration tested (1.0 mM) was calculated.

Antithrombotic activity assay in rats

The antithrombotic activities of individual compounds were tested in male SD rats, as previously described.[24] Briefly, after anesthesia, rats were subjected to surgical exposure of their neck areas and inserted with an 8 cm polyethylene tube connecting the left jugular vein and right carotid artery. The saline-filled shunt was assembled by connecting two cannulae with a 6 cm slightly curved Tygon tubing containing a 5 cm long cotton thread. The rats were maintained on extracorporeal circulation for 15 min, during which a thrombus formed and adhered to the cotton thread. The shunt was then removed and the thread with the associated thrombus was collected and immediately weighed. The wet weight of a thrombus was calculated by subtracting the weight of the 5 cm dry cotton from the total weight and the antiplatelet aggregation activities of individual compounds were expressed as percent inhibition related to untreated controls.

Effect of compounds on the thrombosis in mice

Male Swiss mice (30–35 g, from ICR animal colony) were randomized and treated orally with vehicle alone or compounds at indicated concentrations for two hours, respectively. Subsequently, the mice were injected intravenously with a mixture of 1.0 mg mL^{-1} of collagen and 44.5 μg mL^{-1} of adrenaline (Sigma, St Louis, USA), and observed for thrombosis-related death for 5 min and hemiplegia for 15 min. The antithrombotic effects of individual compounds were calculated as percent protection relative to controls injected with vehicle alone. The experimental protocols of animal studies were approved by the Animal Research Care Committee of China Pharmaceutic University.

In vitro nitric oxide release assay

In vitro NO formation of individual compounds was determined by Griess assay. Briefly, 0.1mM 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.

Aqueous solubility assays

Individual compounds at *ca.* 1 mg were dissolved in 10 ml of methanol and the maximum UV absorption of each compound was determined in a UV755B spectrophotometer, eventually diluting the solution (with MeOH) as necessary. A saturated solution of each compound was then prepared by stirring magnetically a small volume of normal saline in the presence of excess compound for 5 h. The saturated solution was filtered with a Millipore 0.45-μm filter to remove solid compound and measured by UV-spectrometry at the wavelength determined. Total solubility was determined by the relationship: $C' = A'CA^{-1}$, where C = the concentration of standard solution (mg mL^{-1}), A = absorbance of standard solution, A' = absorbance of saturated solution, and C' = concentration of saturated solution (mg mL^{-1}).

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