Bioorganic & Medicinal Chemistry 20 (2012) 6848–6855

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel nitric oxide-releasing isochroman-4-one derivatives: Synthesis and evaluation of antihypertensive activity

Renren Bai [a,b], Xue Yang [a,b], Yao Zhu [a,b], Zhiwen Zhou [a,b], Weijia Xie [a,b], Hequan Yao [a,b], Jieyun Jiang [c], Jie Liu [b,d,*], Mingqin Shen [e], Xiaoming Wu [a,b], Jinyi Xu [a,b,*]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China
[c] Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, 800 Rose Street, Lexington, KY 40536, USA
[d] Department of Organic Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China
[e] Jiangsu Provincial Institute of Traditional Chinese Medicine, Nanjing 210028, PR China

ARTICLE INFO

Article history:
Received 12 August 2012
Revised 18 September 2012
Accepted 19 September 2012
Available online 28 September 2012

Keywords:
Nitric oxide (NO)
Structure modification
Isochroman-4-one derivatives
Hybrids
Antihypertensive activity

ABSTRACT

By coupling nitric oxide (NO)-donor moieties with a natural antihypertensive product (±)-7,8-dihydroxy-3-methyl-isochroman-4-one [(±)-XJP] and its analogue (±)-XJP-B, a series of novel NO-releasing isochroman-4-one derivatives were designed and synthesized. The NO-releasing assay indicated that compounds **Ia**, **Id**, **IIIb** and **IIIe** released the maximum amount of NO. The maximum reductions of blood pressure of **Ia**, **IIIb** and **IIIe** in SHRs were nearly 40%, which was obviously superior to that of the lead compounds and comparable to that of reference drug captopril. These results suggested that NO-donor/natural product hybrids may provide a promising approach for the discovery of novel antihypertensive agents.

1. Introduction

Nitric oxide (NO), a free radical gas, is a multifunctional messenger molecule with diverse physiological functions, such as dilation of blood vessels, inhibition of platelet aggregation and suppression of smooth muscle cell proliferation.[1,2] Accumulated evidence suggests NO plays an active role in central regulation of sympathetic vasomotor tone and systemic arterial pressure (SAP).[3,4] Furthermore, NO is considered to be a major fine tuner by counterbalancing vasoconstrictors (sympathetic nervous activity, the renin–angiotensin system, and endothelin-1).[5,6] Though the relation between NO and hypertension is not fully clear, increasing evidences suggested that NO may be beneficial for the treatment of hypertension also by dilation of blood vessels.[7] During the development of new cardiovascular drugs, NO-donor hybrid compounds have been proved to show improved properties in many cases due to their NO-releasing ability. Several examples of this approach were recently reported, which are NO-donor moieties combined with angiotensin AT_1 receptor antagonists, β-adrenergic receptor antagonists or angiotensin converting enzyme inhibitors.[8–13]

Searching for the active natural products from plants is always an important strategy for development of new antihypertensive drugs.[14] Despite many kinds of drugs have been used for the treatment of hypertension in clinic, effective blood pressure control remains a major medical challenge and there has been a consistent demand for more novel and effective antihypertensive drugs. (±)-7,8-dihydroxy-3-methyl-isochroman-4-one [(±)-XJP] is just a novel and structurally unique natural polyphenolic compound, which was isolated from banana (*Musa sapientum* L.) peel by our group and displayed potent antihypertensive activity.[15] In both acute and therapeutic antihypertensive tests in renal hypertensive rats (RHRs), the maximum antihypertensive effect of (±)-XJP at the dose of 100 mg/kg was comparable to that of captopril at the dose of 25 mg/kg. The mechanism studies revealed that (±)-XJP has moderate ACE inhibitory activity, suggesting that ACE may be one of its targets.[16] By chiral separation, (−)-XJP and (+)-XJP were first obtained. CD calculations demonstrated that the absolute configuration of (+)-XJP is *S*-configured and the absolute configuration of (−)-XJP is *R*-configured. Antihypertensive evaluation proved that (±)-XJP isomers possess similar pharmacodynamic effects, but of different potency, and the *R*-(−)-XJP is more potent than that of *S*-(+)-XJP.[17]

In the further structure modification, (±)-XJP-B, an analogue of (±)-XJP (Fig. 1), was found somewhat more potent than (±)-XJP in spontaneously hypertensive rats (SHRs). However, the

* Corresponding authors. Tel.: +86 025 83271445 (J.X.).
E-mail addresses: cpu-jill@163.com (J. Liu), jinyixu@china.com (J. Xu).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.09.043

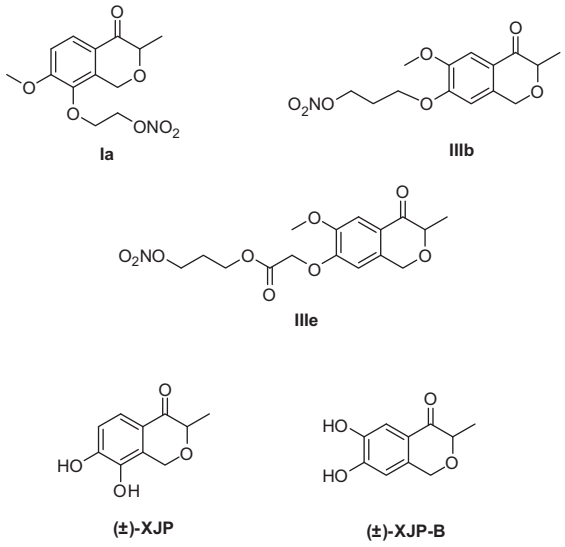


Figure 1. The structures of (±)-XJP and (±)-XJP-B.

antihypertensive effects of (±)-XJP and (±)-XJP-B are far from potent drug candidates, and they are also easily oxidized. Since the molecular weights of the two compounds are below 200, introducing other effective moieties using hybrid approach is quite advisable.

Inspired by the obtained interesting results of our previous studies, in which an NO-donor moiety was connected to a 'native' molecule for the purpose of enhancing its therapeutic impact,[18,19] we designed a series of NO-releasing isochroman-4-one derivatives, in which the lead compounds (±)-XJP and (±)-XJP-B were coupled with NO-donors via various linkers. Herein, we would like to report the synthesis and biological evaluation of these novel NO-releasing isochroman-4-one derivatives.

2. Results and discussions

2.1. Chemistry

Several synthetic routes of (±)-XJP have been reported by our group,[15,16] (±)-XJP-B was synthesized by the similar route. As shown in Scheme 1, the major intermediates **4**, **7** and **8** were obtained by using a catalytic amount of $AlCl_3$.

The preparation of the nitrate derivatives **Ia–c** followed the synthetic routes illustrated in Scheme 2.[7,20,21] An alkyl chain with a bromide group was introduced to intermediate **4** by reaction with dibromo alkanes to produce compounds **4a–c**. Subsequent reaction with $AgNO_3$ (silver nitrate) afforded target products **Ia–c**. Compounds **IIa–c** and **IIIa-c** were synthesized by the same way.

The synthesis of compounds **Id–f** was accomplished according to the general pathway illustrated in Scheme 3. Compound **4** was reacted with ethyl bromoacetate to give ester **9**. Subsequent hydrolysis of this compound and reaction with dibromo alkanes yielded the derivatives **11a–c**, which were subsequently converted to the nitro esters **Id–f** by treatment with $AgNO_3$ in anhydrous acetonitrile. Compounds **IId–f** and **IIId–f** were obtained by the same way.

2.2. Pharmacological evaluation

2.2.1. NO-releasing test of hybrid compounds Ia–f, IIa–f and IIIa–f in vitro

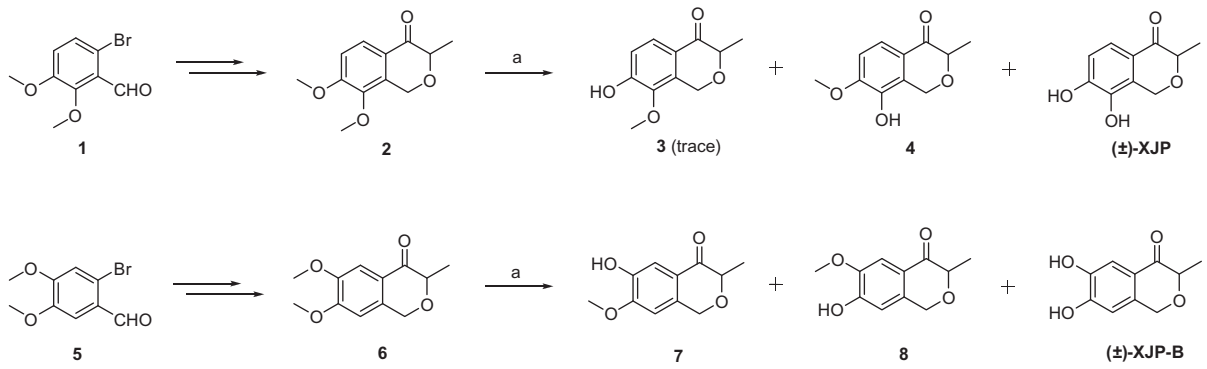
The NO-releasing results of the tested compounds indicated that the precursor compounds **4**, **7** and **8** did not release NO, but all the target NO-donating derivatives were found to release different amounts of NO (Table 1). In which, NO-donating derivatives of **8** were found to release the maximum amount of NO and the derivatives of **4** were the second. Among them, compounds **IIIb**, **IIIe**, **Ia** and **Id** showed the maximum releasing amount, while the NO-donating detivatives **IIa–f** released the least amount of NO. Generally, NO-donors bonded to phenolic hydroxyl or carboxyl of isochroman-4-one did not cause significant difference in the releasing amount of NO.

2.2.2. In vivo antihypertensive activity of compounds Ia, Id, IIIb and IIIe

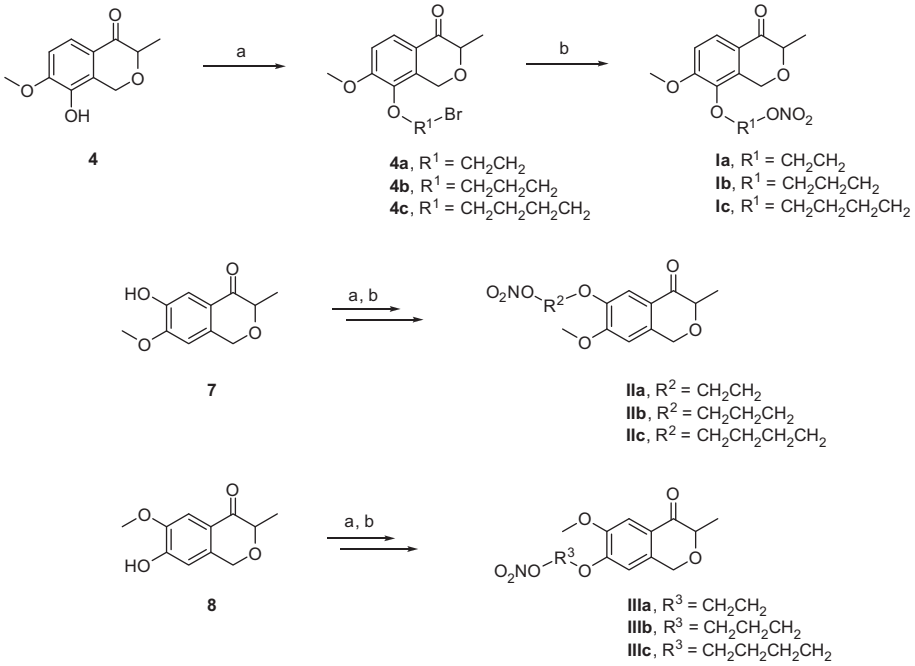
Compounds **Ia**, **Id**, **IIIb** and **IIIe**, which released much more amount of NO, were chosen for further antihypertensive evaluation in vivo in SHRs (Fig. 2). After oral administration of captopril (40 mg/kg), (±)-XJP, (±)-XJP-B, **Ia**, **Id**, **IIIb** and **IIIe** (80 mg/kg) to SHRs, the blood pressure and heart rate were determined from 0 to 24 h. The results showed that the average DAP of the SHRs treated with **Ia**, **IIIb** and **IIIe** was reduced much more than 30% from 6 to 12 h, which was obviously superior to the DAP reduction achieved by captopril (not more than 30%) and did not cause noticeable changes of HR. The maximum reductions of blood pressure of **Ia**, **IIIb** and **IIIe** were nearly 40% and the MAP changes of the four compounds exhibited more potent antihypertensive activities than captopril did. Moreover, the maximum DAP changes of **Ia**, **IIIb** and **IIIe**, were more significant than not only the lead compounds (±)-XJP and (±)-XJP-B, but also the positive control captopril. Overall, the results demonstrated that the nitric oxide-releasing isochroman-4-one derivatives could induce blood pressure of SHRs rapidly decline and remain for several hours.

2.2.3. Structure–activity relationships (SARs) discussion

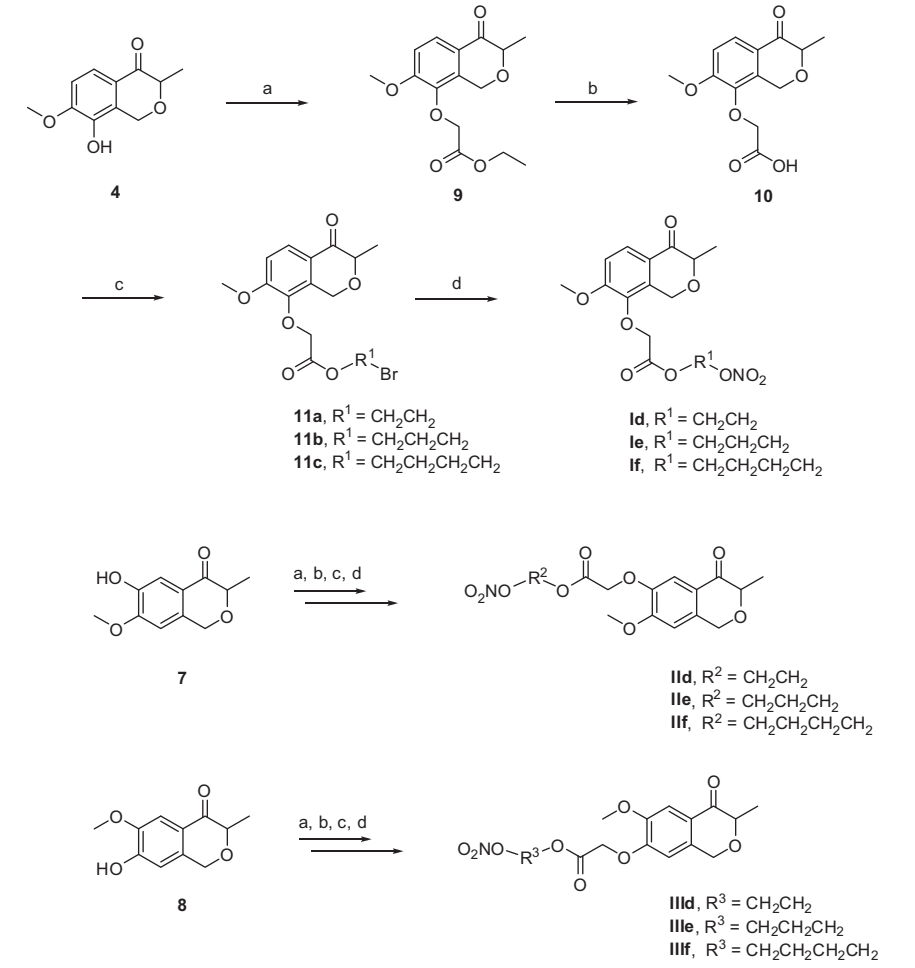
Generally, substituents at different positions affected the NO-releasing amount significantly. In which, 7-substituted NO-donating derivatives (**IIIa–IIIf**) were found to release the maximum amount of NO and the derivatives of substitution at 8-posotion (**Ia–If**) were



Scheme 1. Synthetic routes of key intermediates, (±)-XJP and (±)-XJP-B. Reagents and conditions: (a) $AlCl_3$, NaI, $CHCl_3$, reflux.



Scheme 2. Synthetic routes of compounds **Ia–c**, **IIa–c** and **IIIa–c**. Reagents and conditions: (a) BrR^1Br, acetone, K_2CO_3, reflux, yields 69–85%; (b) CH_3CN, $AgNO_3$, reflux, yields 75–82%.



Scheme 3. Synthetic routes of compounds **Id–f**, **IId–f** and **IIId–f**. Reagents and conditions: (a) $BrCH_2COOC_2H_5$, acetone, K_2CO_3, reflux, 84%; (b) (i) $MeOH/H_2O$, KOH, rt; (ii) 10% HCl, rt, 78%; (c) BrR^1Br, DMF, K_2CO_3, 69–80%; (d) CH_3CN, $AgNO_3$, reflux, 65–75%.

Table 1
The amount of NO released by the tested compounds

Compd	Molar concentration of NO-released (μmol/L)						
	10 min	30 min	60 min	80 min	100 min	120 min	150 min
4	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—
Ia	1.51 ± 0.21	1.78 ± 0.13	4.53 ± 0.28	7.29 ± 0.30	10.50 ± 0.47	14.61 ± 0.39	16.26 ± 1.02
Ib	7.42 ± 0.41	7.45 ± 0.32	9.23 ± 0.78	9.27 ± 1.33	9.40 ± 1.10	9.43 ± 0.82	9.47 ± 1.05
Ic	2.19 ± 0.25	2.46 ± 0.20	3.02 ± 0.41	3.84 ± 0.58	4.03 ± 0.49	4.01 ± 0.82	4.02 ± 0.44
Id	10.62 ± 0.73	10.84 ± 0.74	13.00 ± 0.78	13.12 ± 1.39	13.35 ± 2.19	13.27 ± 1.07	16.68 ± 1.53
Ie	0.84 ± 0.11	1.19 ± 0.20	2.27 ± 0.25	3.84 ± 0.37	3.95 ± 0.53	4.01 ± 1.44	4.86 ± 0.44
If	4.05 ± 0.23	5.08 ± 0.11	6.04 ± 0.78	6.27 ± 1.04	6.55 ± 0.78	6.59 ± 0.65	8.72 ± 0.82
IIa	5.56 ± 0.37	5.84 ± 0.26	6.04 ± 0.28	6.52 ± 0.75	7.56 ± 0.84	8.10 ± 1.10	10.06 ± 1.42
IIb	5.14 ± 0.28	5.59 ± 0.31	6.29 ± 0.99	6.77 ± 1.04	6.89 ± 1.12	7.01 ± 1.07	7.04 ± 0.76
IIc	4.81 ± 0.33	4.83 ± 0.37	4.87 ± 0.60	5.93 ± 0.71	6.05 ± 1.08	6.09 ± 0.84	7.54 ± 0.91
IId	8.43 ± 0.87	8.81 ± 0.38	10.07 ± 0.62	11.95 ± 1.50	12.09 ± 1.01	12.10 ± 1.35	13.99 ± 1.50
IIe	5.65 ± 0.79	5.76 ± 0.44	6.29 ± 0.88	7.77 ± 1.20	8.23 ± 0.69	8.60 ± 0.84	8.72 ± 1.01
IIf	5.73 ± 0.81	6.01 ± 0.43	7.13 ± 1.10	8.69 ± 1.52	8.90 ± 0.99	9.02 ± 1.00	10.81 ± 1.20
IIIa	10.12 ± 0.80	10.25 ± 0.57	11.24 ± 0.45	11.28 ± 1.45	12.09 ± 1.64	12.27 ± 1.90	14.72 ± 2.62
IIIb	17.45 ± 0.65	17.87 ± 0.96	18.12 ± 1.09	18.30 ± 1.67	18.81 ± 1.44	18.95 ± 2.17	28.58 ± 2.32
IIIc	4.81 ± 0.58	5.17 ± 0.52	7.72 ± 0.55	7.85 ± 0.41	8.31 ± 0.72	8.68 ± 1.51	12.99 ± 1.74
IIId	6.66 ± 1.11	6.94 ± 0.12	8.64 ± 0.91	11.11 ± 1.00	11.34 ± 1.33	12.94 ± 2.44	16.26 ± 1.53
IIIe	10.37 ± 0.88	10.41 ± 0.88	11.91 ± 1.28	14.70 ± 0.77	14.61 ± 2.28	17.61 ± 1.94	20.62 ± 1.89
IIIf	7.42 ± 0.97	7.45 ± 0.63	8.72 ± 1.04	11.70 ± 0.99	11.67 ± 1.34	11.69 ± 1.83	14.92 ± 1.74

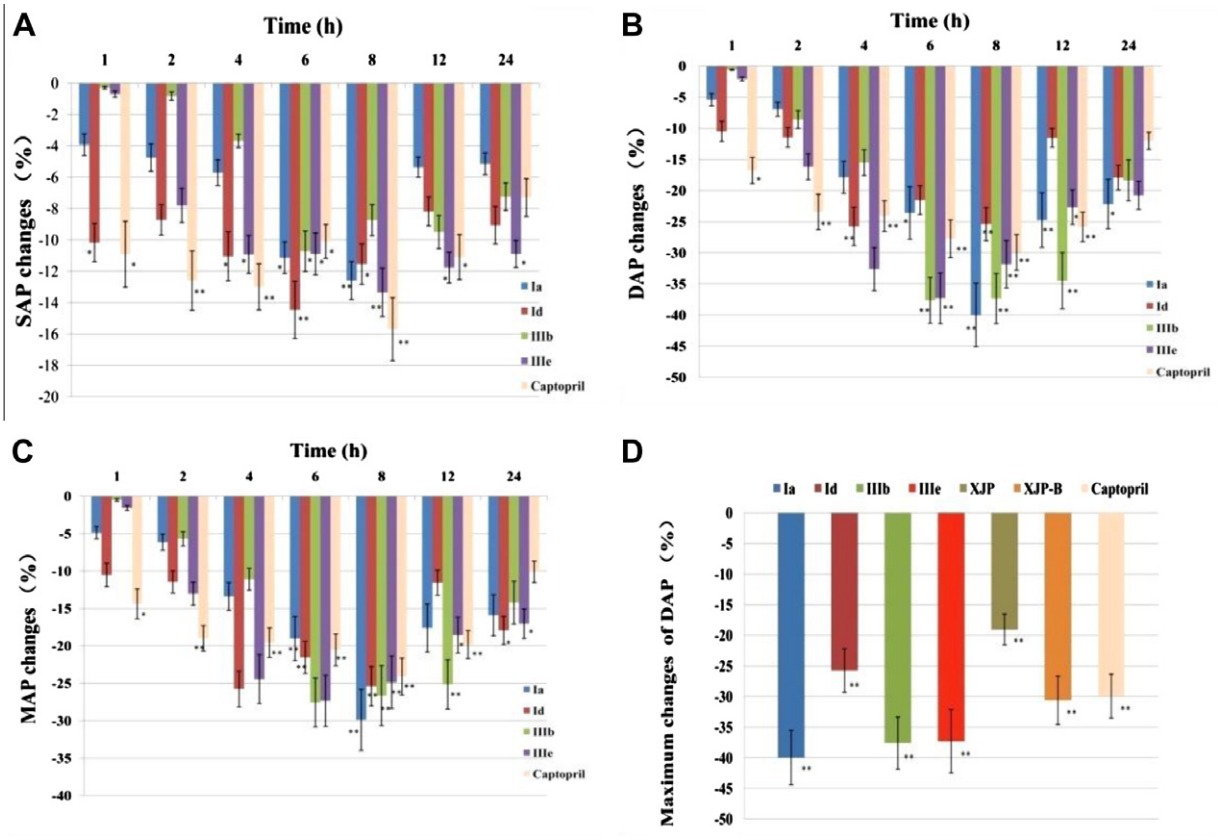


Figure 2. (A) The acute antihypertensive activities of **Ia**, **Id**, **IIIb**, **IIIe** and captopril in SHRs (SAP, systolic arterial pressure); (B) The acute antihypertensive activities of **Ia**, **Id**, **IIIb**, **IIIe** and captopril in SHRs (DAP, diastolic arterial pressure); (C) The acute antihypertensive activities of **Ia**, **Id**, **IIIb**, **IIIe** and captopril in SHRs (MAP, mean artery pressure); (D) The maximum changes of DAP of **Ia**, **Id**, **IIIb**, **IIIe**, (±)-**XJP**, (±)-**XJP-B** and captopril in SHRs. Data are represented as mean ± SEM (n = **8**). Significance levels $^{*}p$ <0.05 and $^{**}p$ <0.01 as compared with the respective control.

the second. However, 6-substituted ones (**IIa**–**f**) did not release as much as the above ones, suggesting the substituent position of the NO-donor moiety maybe mainly influence the NO-releasing amount. Moreover, the antihypertensive activity evaluation in vivo showed that 7-substituted NO-donating derivatives (for instance, **IIIb** and **IIIe**) and 8-substituted NO-donating derivatives (**Ia**) exhibited more stronger activity. We speculated that the releasing of NO contributed to antihypertensive activity and higher levels of NO releasing could produce stronger activity. Furthermore, when the NO-donors bonded to the phenolic hydroxyl, the activities seems somewhat better than the ones bonded to the carboxyl of isochroman-4-one (for instance, **Ia** > **Id**). Anyway, the

impacts of the length of carbon chain on the biological activities were not obvious.

3. Conclusions

By coupling NO-donor moieties with natural product (±)-XJP and its analogue (±)-XJP-B, a series of novel NO-releasing isochroman-4-one derivatives were designed and synthesized. The NO-releasing assay indicated variable levels of NO were produced by the target compounds. Among them, compounds **Ia**, **Id**, **IIIb** and **IIIe** were found to release the maximum amount of NO. The antihypertensive evaluation showed that the average DAP of the SHRs treated with **Ia**, **IIIb** and **IIIe** was reduced much more than 30% from 6 to 12 h, which was obviously superior to that of captopril and did not cause noticeable changes of HR. The maximum reductions of blood pressure of **Ia**, **IIIb** and **IIIe** (80 mg/kg) were nearly 40%, which were more potent than the lead compounds (±)-XJP (80 mg/kg), (±)-XJP-B (80 mg/kg) and positive control captopril (40 mg/kg). The improved activities of these hybrid molecules may make them promising candidates as antihypertensive agents.

4. Experimental

4.1. Chemistry

Most chemicals and solvents were of analytical grade and, when necessary, were purified and dried by standard methods. Melting points were taken on an XT-4 micro melting point apparatus and uncorrected. IR spectra were recorded in KBr on a Nicolet Impact 410 grating infrared spectrophotometer (ν_{max} in cm^{-1}) and ^1H NMR spectra were recorded with 300 MHz spectrometers in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. High-resolution mass spectra were recorded using Agilent QTOF 6520. Purity of all tested compounds was $\geqslant$95%, as estimated by HPLC analysis. The major peak of the compounds analyzed by HPLC accounted for $\geqslant$95% of the combined total peak area when monitored by a UV detector at 254 nm. Flash chromatography was done on Merck silica gel 60 (230-400 mesh).

4.1.1. General procedure for the preparation of compounds 4a-c

K_2CO_3 (0.276 g, 2 mmol) was added to a solution of compound **4** (0.208 g, 1 mmol) in anhydrous acetone (10–12 mL) and the mixture was refluxed for 30 min. Then dibromo alkane (3 mmol) was added and the mixture was refluxed for 3 h. After filtrated and concentrated under reduced pressure, followed by purified by flash column chromatography with *n*-hexane/ethyl acetate (8:1, v/v) as eluent, compounds **4a–c** were afforded as white solid in yield of 69–85%.

4.1.1.1. 8-(2-Bromoethoxy)-7-methoxy-3-methylisochroman-4-one (4a). White power, yield 85%, mp 123–125 °C; IR (KBr), cm^{-1}: 2973, 2830, 1693, 1595, 1494, 1400, 1281, 1269, 1226, 1081, 1061; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.6 Hz, $-CH_3$), 3.62 (t, 2H, J = 6.0 Hz, $-CH_2-$), 3.93 (s, 3H, $-OCH_3$), 4.16–4.22 (m, 1H, –CH–), 4.30–4.39 (m, 2H, $-CH_2-$), 4.83 (d, 1H, J = 15.7 Hz, $-CH_2-$), 5.25 (d, 1H, J = 15.7 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.7 Hz, Ar-H), 7.85 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) m/z: 315.1 $[M+H]^+$.

4.1.1.2. 8-(3-Bromopropoxy)-7-methoxy-3-methylisochroman-4-one (4b). White power, yield 80%, mp 90–92 °C; IR (KBr), cm^{-1}: 2985, 2938, 2825, 1671, 1601, 1513, 1400, 1356, 1276, 1207, 1109, 1039, 877; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.7 Hz, $-CH_3$), 2.27–2.33 (m, 2H, $-CH_2-$), 3.67 (t, 2H, J = 6.3 Hz, $-CH_2-$), 3.94 (s, 3H, $-OCH_3$), 4.09–4.13 (m, 2H, $-CH_2-$), 4.19 (q, 1H, J = 6.7 Hz, –CH–), 4.82 (d, 1H, J = 15.7 Hz, $-CH_2-$), 5.14 (d, 1H, J = 15.7 Hz, $-CH_2-$), 6.95 (d, 1H, J = 8.7 Hz, Ar-H), 7.85 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) m/z: 329.1 $[M+H]^+$.

4.1.1.3. 8-(4-Bromobutoxy)-7-methoxy-3-methylisochroman-4-one (4c). White power, yield 69%, mp 88–90 °C; IR (KBr), cm^{-1}: 3067, 2949, 2849, 1690, 1594, 1400, 1283, 1222, 1118, 1074, 948, 791; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.49 (d, 3H, J = 6.7 Hz, $-CH_3$), 1.87–1.96 (m, 2H, $-CH_2-$), 2.05–2.17 (m, 2H, $-CH_2-$), 3.51 (t, 2H, J = 6.6 Hz, $-CH_2-$), 3.93 (s, 3H, $-OCH_3$), 3.97–4.05 (m, 2H, $-CH_2-$), 4.19 (q, 1H, J = 6.7 Hz, –CH–), 4.76 (d, 1H, J = 15.7 Hz, $-CH_2-$), 5.12 (d, 1H, J = 15.7 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.7 Hz, Ar-H), 7.83 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) m/z: 343.1 $[M+H]^+$.

4.1.2. General procedure for the preparation of compounds Ia–c, IIa–c and IIIa–c

A solution of derivatives **4a–c** (0.05 mmol) in dry acetonitrile (8–10 mL) was treated with a solution of $AgNO_3$ (0.15 mmol) in dry acetonitrile (3–5 mL) and the whole mixture was refluxed for 2–3 h. The mixture was then filtered and concentrated, and the residue was purified by flash column chromatography with *n*-hexane/ethyl acetate (5:1, v/v) as eluent to give compounds **Ia–c** as white solid in yield of 75–82%. Compounds **IIa–c** and **IIIa–c** were prepared by the above method.

4.1.2.1. 2-(7-Methoxy-3-methyl-4-oxoisochroman-8-yloxy) ethyl nitrate (Ia). White power, yield 70%, mp 90–92 °C; IR (KBr), cm^{-1}: 2973, 2849, 1692, 1627, 1595, 1493, 1280, 1077, 1053, 905, 866; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.6 Hz, $-CH_3$), 3.93 (s, 3H, $-OCH_3$), 4.19 (q, 1H, J = 6.6 Hz, –CH–), 4.27–4.36 (m, 2H, $-CH_2-$), 4.75–4.77 (m, 2H, $-CH_2-$), 4.79 (d, 1H, J = 15.9 Hz, $-CH_2-$), 5.13 (d, 1H, J = 15.9 Hz, $-CH_2-$), 6.95 (d, 1H, J = 8.7 Hz, Ar-H), 7.85 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) m/z: 298.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{13}H_{16}NO_7[M+H]^+$ 298.0921, found 298.0929.

4.1.2.2. 3-(7-Methoxy-3-methyl-4-oxoisochroman-8-yloxy) propyl nitrate (Ib). White power, yield 75%, mp 72–74 °C; IR (KBr), cm^{-1}: 2985, 2873, 1694, 1605, 1283, 1076, 1036, 941, 827; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 2.14–2.23 (m, 2H, $-CH_2-$), 3.92 (s, 3H, $-OCH_3$), 4.05–4.11 (m, 2H, $-CH_2-$), 4.19 (q, 1H, J = 6.7 Hz, –CH–), 4.72–4.76 (m, 2H, $-CH_2-$), 4.78 (d, 1H, J = 15.9 Hz, $-CH_2-$), 5.10 (d, 1H, J = 15.9 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.7 Hz, Ar-H), 7.85 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) m/z: 312.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{14}H_{17}NNaO_7[M+Na]^+$ 334.0897, found 334.0893.

4.1.2.3. 4-(7-Methoxy-3-methyl-4-oxoisochroman-8-yloxy) butyl nitrate (Ic). White power, yield 79%, mp 54–56 °C; IR (KBr), cm^{-1}: 3103, 2979, 2819, 1695, 1624, 1592, 1400, 1279, 1078, 880; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.7 Hz, $-CH_3$), 1.84–2.03 (m, 4H, $-CH_2-$, $-CH_2-$), 3.93 (s, 3H, $-OCH_3$), 3.96–4.05 (m, 2H, $-CH_2-$), 4.19 (q, 1H, J = 6.7 Hz, –CH–), 4.57 (t, 2H, J = 6.0 Hz, $-CH_2-$), 4.78 (d, 1H, J = 15.6 Hz, $-CH_2-$), 5.10 (d, 1H, J = 15.6 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.7 Hz, Ar-H), 7.83 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) m/z: 326.2 $[M+H]^+$. HRMS (ESI) m/z: calcd for $C_{15}H_{19}NNaO_7[M+Na]^+$ 348.1054, found 348.1052.

4.1.2.4. 2-(7-Methoxy-3-methyl-4-oxoisochroman-6-yloxy) ethyl nitrate (IIa). White power, yield 82%, mp 112–114 °C; IR (KBr), cm^{-1}: 2997, 2938, 2843, 1673, 1633, 1596, 1515, 1445, 1363, 1279, 1246, 1114, 906, 869; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 3.93 (s, 3H, $-OCH_3$), 4.22 (q, 1H, J = 6.7 Hz, –CH–), 4.34 (t, 2H, J = 4.4 Hz, $-CH_2-$), 4.81–4.92 (m, 4H, $-CH_2-$, $-CH_2-$), 6.63 (s, 1H, Ar-H), 7.48 (s, 1H, Ar-H); MS (ESI)

m/z: 298.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{13}H_{15}NNaO_7$ $[M+Na]^+$ 320.0741, found 320.0738.

4.1.2.5. 3-(7-Methoxy-3-methyl-4-oxoisochroman-6-yloxy) propyl nitrate (IIb). White power, yield 77%, mp 96–98 °C; IR (KBr), cm^{-1}: 2996, 2932, 2825, 1675, 1635, 1623, 1516, 1399, 1277, 1116, 951, 886. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 2.23–2.31 (m, 2H, $-CH_2-$), 3.92 (s, 3H, $-OCH_3$), 4.16 (t, 2H, J = 5.9 Hz, $-CH_2-$), 4.22 (q, 1H, J = 6.7 Hz, –CH–), 4.69 (t, 2H, J = 6.2 Hz, $-CH_2-$), 4.86 (s, 2H, $-CH_2-$), 6.61 (s, 1H, Ar-H), 7.49 (s, 1H, Ar-H); MS (ESI) *m/z*: 312.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{14}H_{17}NNaO_7[M+Na]^+$ 334.0897, found 334.0904.

4.1.2.6. 4-(7-Methoxy-3-methyl-4-oxoisochroman-6-yloxy) butyl nitrate (IIc). White power, yield 78%, mp 100–102 °C; IR (KBr), cm^{-1}: 2979, 2932, 2873, 1682, 1621, 1601, 1515, 1397, 1358, 1274, 1113, 962, 897, 879; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 1.95–1.96 (m, 4H, $-CH_2-$, $-CH_2-$), 3.92 (s, 3H, $-OCH_3$), 4.10 (s, 2H, $-CH_2-$), 4.22 (q, 1H, J = 6.7 Hz, –CH–), 4.57–4.59 (m, 2H, J = 6.1 Hz, $-CH_2-$), 4.86 (s, 2H, $-CH_2-$), 6.60 (s, 1H, Ar-H), 7.47 (s, 1H, Ar-H); MS (ESI) *m/z*: 326.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{15}H_{19}NNaO_7[M+Na]^+$ 348.1054, found 348.1056.

4.1.2.7. 2-(6-Methoxy-3-methyl-4-oxoisochroman-7-yloxy) ethyl nitrate (IIIa). White power, yield 81%, mp 114–116 °C; IR (KBr), cm^{-1}: 2996, 2973, 1682, 1626, 1602, 1514, 1356, 1273, 1108, 921, 880, 791; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 3.90 (s, 3H, $-OCH_3$), 4.22 (q, 1H, J = 6.7 Hz, –CH–), 4.36–4.37 (m, 2H, $-CH_2-$), 4.80–4.89 (m, 4H, $-CH_2-$, $-CH_2-$), 6.61 (s, 1H, Ar-H), 7.51 (s, 1H, Ar-H); MS (ESI) *m/z*: 298.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{13}H_{16}NO_7[M+H]^+$ 298.0921, found 298.0916.

4.1.2.8. 3-(6-Methoxy-3-methyl-4-oxoisochroman-7-yloxy) propyl nitrate (IIIb). White power, yield 78%, mp 76–78 °C; IR (KBr), cm^{-1}: 2979, 2943, 2825, 1675, 1622, 1609, 1597, 1508, 1352, 1293, 1266, 1110, 979, 885; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 2.26–2.34 (m, 2H, $-CH_2-$), 3.90 (s, 3H, $-OCH_3$), 4.15–4.22 (m, 2H, $-CH_2-$), 4.24 (q, 1H, J = 6.7 Hz, –CH–), 4.70 (t, 2H, J = 6.1 Hz, $-CH_2-$), 4.79–4.90 (m, 2H, $-CH_2-$), 6.61 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H); MS (ESI) *m/z*: 312.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{14}H_{18}NO_7[M+H]^+$ 312.1078, found 312.1082.

4.1.2.9. 4-(6-Methoxy-3-methyl-4-oxoisochroman-7-yloxy) butyl nitrate (IIIc). White power, yield 82%, mp 82–84 °C; IR (KBr), cm^{-1}: 2991, 2961, 2896, 2837, 1676, 1642, 1622, 1601, 1512, 1352, 1278, 1111, 986, 869; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.7 Hz, $-CH_3$), 1.95–2.04 (m, 4H, $-CH_2-$, $-CH_2-$), 3.90 (s, 3H, $-OCH_3$), 4.09–4.11 (m, 2H, $-CH_2-$), 4.22 (q, 1H, J = 6.7 Hz, –CH–), 4.56–4.60 (m, 2H, J = 6.1 Hz, $-CH_2-$), 4.79–4.90 (m, 2H, $-CH_2-$), 6.59 (s, 1H, Ar-H), 7.49 (s, 1H, Ar-H); MS (ESI) *m/z*: 326.2 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{15}H_{19}NNaO_7[M+Na]^+$ 348.1054, found 348.1051.

4.1.3. Procedure for the preparation of 2-(7-methoxy-3-methyl-4-oxoisochroman-8-yloxy)acetic acid (10)

To a solution of compound **4** (0.208 g, 1 mmol) in dry acetone (10 mL), K_2CO_3 (0.276 g, 1 mmol) and ethyl bromoacetate (0.28 mL, 2.5 mmol) were added, and the mixture was refluxed for 3 h. After filtrated and concentrated under reduced pressure, the residue was dissolved in methanol/H_2O (1:1, v/v), and KOH (0.11 g, 2 mmol) was added. The whole mixture was stirred at room temperature for 4 h and the pH was adjusted to 1 with hydrochloric acid. The mixture was filtrated and the filter cake was evaporated to dryness at 70 °C in vacuo to provide compound **10**.

White power, yield 78%, mp 112–114 °C; IR (KBr), cm^{-1}: 3132, 2986, 2950, 2851, 1745, 1689, 1599, 1491, 1400, 1298, 1272, 1214, 1097, 1058; ^1H NMR (DMSO, 300 MHz) δ: 1.33 (d, 3H, J = 6.7 Hz, $-CH_3$), 3.90 (s, 3H, $-OCH_3$), 4.27 (q, 1H, J = 6.7 Hz, –CH–), 4.66 (d, 2H, J = 1.4 Hz, $-CH_2-$), 4.83 (d, 1H, J = 15.7 Hz, $-CH_2-$), 5.35 (d, 1H, J = 15.7 Hz, $-CH_2-$), 7.17 (d, 1H, J = 8.7 Hz, Ar-H), 7.68 (d, 1H, J = 8.7 Hz, Ar-H), 12.87 (s, 1H, –COOH); MS (ESI) *m/z*: 267.1 $[M+H]^+$.

4.1.4. General procedure for the preparation of compounds Id–e, IId–e and IIId–e

K_2CO_3 (0.276 g, 2 mmol) and dibromo alkanes (3 mmol) were added to a solution of compound **10** (0.208 g, 1 mmol) in anhydrous DMF (10 mL) and stired for 4 h at 0 °C. Water (10 mL) was added and extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure, followed by purification by flash column chromatography with *n*-hexane/ethyl acetate (8:1, v/v) as eluent to give compounds **11a–c** as white solid in yield of 69–80%.

A solution of derivatives **11a–c** (0.05 mmol) in dry acetonitrile (8–10 mL) was treated with a solution of $AgNO_3$ (0.15 mmol) in dry acetonitrile (3–5 mL) and the whole mixture was refluxed for 2–3 h. After filtered and concentrated, the residue was purified by flash column chromatography with *n*-hexane/ethyl acetate (5:1, v/v) to afford compounds **Id–e** as white solid in yield of 65–75%. Compounds **IId–e** and **IIId–e** were prepared by the above method.

4.1.4.1. 2-(Nitrooxy)ethyl 2-(7-methoxy-3-methyl-4-oxoisochroman-8-yloxy)acetate (Id). White power, yield 68%, mp 75–77 °C; IR (KBr), cm^{-1}: 2943, 2837, 1754, 1689, 1637, 1598, 1491, 1295, 1269, 1199, 1094, 1061, 917, 840; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.49 (d, 3H, J = 6.6 Hz, $-CH_3$), 3.94 (s, 3H, $-OCH_3$), 4.20 (q, 1H, J = 6.6 Hz, –CH–), 4.45–4.48 (m, 2H, $-CH_2-$), 4.68–4.70 (m, 2H, $-CH_2-$), 4.70–4.78 (m, 2H, $-CH_2-$), 4.86 (d, 1H, J = 16.0 Hz, $-CH_2-$), 5.32 (d, 1H, J = 16.0 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.6 Hz, Ar-H), 7.84 (d, 1H, J = 8.6 Hz, Ar-H); MS (ESI) *m/z*: 356.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{15}H_{18}NO_9[M+H]^+$ 356.0976, found 356.0975.

4.1.4.2. 3-(Nitrooxy)propyl 2-(7-methoxy-3-methyl-4-oxoisochroman-8-yloxy)acetate (Ie). White power, yield 69%, mp 41–43 °C; IR (KBr), cm^{-1}: 2991, 2943, 2855, 1760, 1691, 1624, 1596, 1399, 1285, 1270, 1197, 1171, 1060, 986, 868; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.6 Hz, -CH_3), 2.11 (t, 2H, J = 6.0 Hz, $-CH_2-$), 3.93 (s, 3H, $-OCH_3$), 4.22 (q, 1H, J = 6.6 Hz, –CH–), 4.21 (t, 2H, J = 6.6 Hz, $-CH_2-$), 4.30 (t, 2H, J = 6.0 Hz, $-CH_2-$), 4.69–4.80 (m, 2H, $-CH_2-$), 4.86 (d, 1H, J = 15.9 Hz, $-CH_2-$), 5.32 (d, 1H, J = 15.9 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.7 Hz, Ar-H), 7.84 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) *m/z*: 370.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{16}H_{19}NNaO_9[M+Na]^+$ 392.0952, found 392.0946.

4.1.4.3. 4-(Nitrooxy)butyl 2-(7-methoxy-3-methyl-4-oxoisochroman-8-yloxy)acetate (If). White power, yield 70%, mp 50–52 °C; IR (KBr), cm^{-1}: 2949, 2884, 2831, 1749, 1691, 1624, 1596, 1492, 1400, 1285, 1198, 1057, 969, 876; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.6 Hz, $-CH_3$), 1.79–1.80 (m, 4H, $-CH_2-$, $-CH_2-$), 3.93 (s, 3H, $-OCH_3$), 4.20–4.33 (m, 3H, –CH–, $-CH_2-$), 4.47 (m, 2H, $-CH_2-$), 4.73 (m, 2H, $-CH_2-$), 4.87 (d, 1H, J = 15.9 Hz, $-CH_2-$), 5.32 (d, 1H, J = 15.9 Hz, $-CH_2-$), 6.94 (d, 1H, J = 8.7 Hz, Ar-H), 7.84 (d, 1H, J = 8.7 Hz, Ar-H); MS (ESI) *m/z*: 384.1 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{17}H_{21}NNaO_9[M+Na]^+$ 406.1109, found 406.1112.

4.1.4.4. 2-(Nitrooxy)ethyl 2-(7-methoxy-3-methyl-4-oxoisochroman-6-yl)oxyacetate (IId). White power, yield 73%, mp 116–118 °C; IR (KBr), cm^{-1}: 2973, 2855, 1765, 1680, 1636, 1600, 1508, 1400, 1282, 1193, 1113, 862, 777; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.6 Hz, -CH_3), 3.96 (s, 3H, –OCH_3), 4.22 (q, 1H, J = 6.6 Hz, –CH–), 4.50–4.53 (m, 2H, –CH_2–), 4.68–4.71 (m, 2H, –CH_2–), 4.82–4.85 (m, 4H, –CH_2–, –CH_2–), 6.66 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H); MS (ESI) m/z: 356.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{15}H_{17}NNaO_9[M+Na]^+$ 378.0796, found 378.0798.

4.1.4.5. 3-(Nitrooxy)propyl 2-(7-methoxy-3-methyl-4-oxoisochroman-6-yloxy)acetate (IIe). White power, yield 65%, mp 85–87 °C; IR (KBr), cm^{-1}: 2997, 2870, 1758, 1685, 1629, 1603, 1510, 1314, 1279, 1197, 1159, 1044, 955, 896, 775; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.50 (d, 3H, J = 6.6 Hz, –CH_3), 2.10 (t, 2H, J = 6.0 Hz, –CH_2–), 3.96 (s, 3H, –OCH_3), 4.22 (q, 1H, J = 6.6 Hz, –CH–), 4.32 (t, 2H, J = 6.0 Hz, –CH_2–), 4.48 (t, 2H, J = 6.2 Hz, –CH_2–), 4.79-4.90 (m, 4H, –CH_2–, –CH_2–), 6.66 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H); MS (ESI) m/z: 370.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{16}H_{19}NNaO_9[M+Na]^+$ 392.0952, found 392.0955.

4.1.4.6. 4-(Nitrooxy)butyl 2-(7-methoxy-3-methyl-4-oxoisochroman-6-yloxy)acetate (IIf). White power, yield 71%, mp 96–98 °C; IR (KBr), cm^{-1}: 2981, 2870, 1755, 1672, 1630, 1515, 1400, 1357, 1279, 1205, 1114, 884, 790; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.26 (d, 3H, J = 6.6 Hz, –CH_3), 1.50–1.52 (m, 4H, –CH_2-, –CH_2–), 3.81 (s, 3H, –OCH_3), 4.21–4.26 (m, 3H, –CH_2–, –CH–), 4.44–4.45 (m, 2H, –CH_2–), 4.78–4.90 (m, 4H, –CH_2–, –CH_2–), 6.66 (s, 1H, Ar-H), 7.38 (s, 1H, Ar-H); MS (ESI) m/z: 384.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{17}H_{21}NNaO_9[M+Na]^+$ 406.1109, found 406.1114.

4.1.4.7. 2-(Nitrooxy)ethyl 2-(6-methoxy-3-methyl-4-oxoisochroman-7-yloxy)acetate (IIId). White power, yield 70%, mp 109–111 °C; IR (KBr), cm^{-1}: 3010, 2825, 2778, 1765, 1685, 1627, 1400, 1278, 1206, 1111, 862; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.6 Hz, –CH_3), 3.94 (s, 3H, –OCH_3), 4.22 (q, 1H, J = 6.6 Hz, –CH–), 4.50–4.53 (m, 2H, –CH_2–), 4.67–4.71 (m, 2H, –CH_2–), 4.81–4.85 (m, 4H, –CH_2–, –CH_2–), 6.53 (s, 1H, Ar-H), 7.53 (s, 1H, Ar-H); MS (ESI) m/z: 356.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{15}H_{17}NNaO_9[M+Na]^+$ 378.0796, found 378.0793.

4.1.4.8. 3-(Nitrooxy)propyl 2-(6-methoxy-3-methyl-4-oxoisochroman-7-yloxy)acetate (IIIe). White power, yield 69%, mp 55–57 °C; IR (KBr), cm^{-1}: 2979, 2926, 2831, 1754, 1685, 1623, 1603, 1511, 1400, 1384, 1282, 1201, 1110, 854, 775; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.6 Hz, –CH_3), 2.10 (t, 2H, J = 6.0 Hz, –CH_2–), 3.94 (s, 3H, –OCH_3), 4.22 (q, 1H, J = 6.6 Hz, –CH–), 4.33 (t, 2H, J = 6.0 Hz, –CH_2–), 4.48 (t, 2H, J = 6.0 Hz, –CH_2–), 4.79–4.89 (m, 4H, –CH_2–, –CH_2–), 6.53 (s, 1H, Ar-H), 7.53 (s, 1H, Ar-H); MS (ESI) m/z: 370.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{16}H_{20}NO_9[M+H]^+$ 370.1133, found 370.1138.

4.1.4.9. 4-(Nitrooxy)butyl 2-(6-methoxy-3-methyl-4-oxoisochroman-7-yloxy)acetate (IIIf). White power, yield 75%, mp 92–94 °C; IR (KBr), cm^{-1}: 2973, 2937, 2843, 1755, 1672, 1632, 1600, 1515, 1356, 1279, 1206, 1114, 884, 790; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.51 (d, 3H, J = 6.6 Hz, –CH_3), 1.78–1.79 (m, 4H, –CH_2–, –CH_2–), 3.94 (s, 3H, –OCH_3), 4.21–4.26 (m, 3H, -CH_2–, –CH–), 4.44–4.45 (m, 2H, –CH_2–), 4.78–4.90 (m, 4H, –CH_2–, –CH_2–), 6.52 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H); MS (ESI) m/z: 384.1 $[M+H]^+$; HRMS (ESI) m/z: Calcd for $C_{17}H_{21}NNaO_9[M+Na]^+$ 406.1109, found 406.1108.

4.2. Pharmacological evaluation

4.2.1. NO-releasing test: nitrate/nitrite measurement in vitro

The levels of nitrate/nitrite formed from individual compounds were determined by the colorimetric assay using the nitrate/nitrite colorimetric assay kit. 0.1 mM of each compound in phosphate buffer solution (PBS) containing 2% dimethyl sulfoxide and 5.0 mM L-cysteine at pH 7.4 was incubated at 37 °C for 10–150 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.[8,19,22,23]

4.2.2. Antihypertensive effects in SHRs

Male SHRs were purchased from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). After one week of acclimation, 64 SHRs (10-weeks-old, 180-200 g body weight) were randomly divided into eight groups, namely the SHR model group, captopril control group, (±)-XJP control group, (±)-XJP-B control group and the compounds **Ia**, **Id**, **IIIb**, **IIIe** control groups. After oral administration with saline water, captopril (40 mg/kg), (±)-XJP, (±)-XJP-B, **Ia**, **Id**, **IIIb** and **IIIe** (80 mg/kg) to SHRs respectively, the SAP, DAP and heart rate (HR) were measured using the tail-cuff method with a blood pressure monitor (BP-2000, Visitech Systems, Inc., US) from 0 to 24 h.[24–26]

Acknowledgments

This study was financially supported by a grant from 'Eleventh Five-Year' Major Innovation Projects for New Drug Candidates (No. 2009ZX09103-128), Project for Research and Innovation of Graduates in Colleges and Universities of Jiangsu Province (CXZZ11-0798), Fundamental Research Funds for the Central Universities (JKY2011027) and Project Program of State Key Laboratory of Natural Medicines, China Pharmaceutical University (JKGQ201115).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Moncada, S.; Higgs, A. N. *Engl. J. Med.* **2002**, *1993*, 329.
2. Higashi, Y.; Sasaki, S.; Nakagawa, K.; Kimura, M.; Noma, K.; Sasaki, S.; Hara, K.; Matsuura, H.; Goto, C.; Oshima, T.; Chayama, K. *J. Am. Coll. Cardiol.* **2003**, *42*, 256.
3. Hu, H.; Zharikov, S.; Patel, J. M. *Peptides* **2012**, *35*, 78.
4. Tai, M.-H.; Wang, L.-L.; Wu, K. L. H.; Chan, J. Y. H. *Free Radic. Biol. Med.* **2005**, *38*, 450.
5. Fiorucci, S.; Antonelli, E.; Morelli, A. *Dig. Liver Dis.* **2003**, *35*, S61.
6. Lovich, M. A.; Bruno, N. K.; Plant, C. P.; Wei, A. E.; Vasquez, G. B.; Johnson, B. J.; Fine, D. H.; Gilbert, R. J. *Nitric Oxide* **2011**, *24*, 204.
7. Bhandari, S. V.; Bothara, K. G.; Patil, A. A.; Chitre, T. S.; Sarkate, A. P.; Gore, S. T.; Dangre, S. C.; Khachane, C. V. *Bioorg. Med. Chem.* **2009**, *17*, 390.
8. Li, Y.-Q.; Ji, H.; Zhang, Y.-H.; Shi, W.-B.; Meng, Z.-K.; Chen, X.-Y.; Du, G.-T.; Tian, J.-D. *Eur. J. Pharmacol.* **2007**, *577*, 100.
9. Breschi, M. C.; Calderone, V.; Digiacomo, M.; Martelli, A.; Martinotti, E.; Minutolo, F.; Rapposelli, S.; Balsamo, A. *J. Med. Chem.* **2004**, *47*, 5597.
10. Jayachandran, M.; Hayashi, T.; Sumi, D.; Thakur, N. K.; Kano, H.; Ignarro, L. J.; Iguchi, A. *Biochem. Bioph. Res. Commun.* **2001**, *280*, 589.
11. Villarroya, M.; López, M. G.; de Pascual, R.; García, A. G. *Cardiovasc. Drug Rev.* **2005**, *23*, 149.
12. Visentin, S.; Rolando, B.; Di Stilo, A.; Fruttero, R.; Novara, M.; Carbone, E.; Roussel, C.; Vanthuyne, N.; Gasco, A. *J. Med. Chem.* **2004**, *47*, 2688.
13. Iwanaga, Y.; Gu, Y.; Dieterle, T.; Presotto, C.; Soldato, P.; Peterson, K. L.; Ongini, E.; Condorelli, G.; Ross, J. *FASEB. J.* **2004**, *18*, 587.

14. Li, Y. C. *Curr. Opin. Invest. Drugs* **2007**, *8*, 750.
15. Qian, H.; Huang, W. L.; Wu, X. M.; Zhang, H. B.; Zhou, J. P.; Ye, W. C. *Chin. Chem. Lett.* **2007**, *18*, 1227.
16. Liu, J.; Ren, H.; Xu, J. Y.; Bai, R. R.; Yan, Q.; Huang, W. L.; Wu, X. M.; Fu, J. H.; Wang, Q. J.; Wu, Q.; Fu, R. *Bioorg. Med. Chem. Lett.* **1822**, *2009*, 19.
17. Bai, R. R.; Liu, J.; Zhu, Y.; Yang, X.; Yang, C.; Kong, L. Y.; Wang, X. B.; Zhang, H. Y.; Yao, H. Q.; Shen, M. Q.; Wu, X. M.; Xu, J. Y. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6490.
18. Li, D. H.; Wang, L.; Cai, H.; Zhang, Y. H.; Xu, J. Y. *Molecules* **2012**, *17*, 7557.
19. Li, Y.; Wang, X. L.; Fu, R.; Yu, W. Y.; Wang, X. L.; Lai, Y. S.; Peng, S. X.; Zhang, Y. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4210.
20. Zou, X.-Q.; Peng, S.-M.; Hua, C.-P.; Tan, L.-F.; Yuan, Q.; Deng, H.-W.; Li, Y.-J. *Bioorg. Med. Chem.* **2010**, *18*, 3020.
21. Liu, W. K.; Liu, C. Z.; Gong, C. G.; Lin, W. Y.; Guo, C. C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1647.
22. Tang, X.; Gu, X.; Ai, H.; Wang, G.; Peng, H.; Lai, Y.; Zhang, Y. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 801.
23. Ling, Y.; Ye, X.; Zhang, Z.; Zhang, Y.; Lai, Y.; Ji, H.; Peng, S.; Tian, J. *J. Med. Chem.* **2011**, *54*, 3251.
24. Huang, W.-H.; Sun, J.; He, H.; Dong, H.-W.; Li, J.-T. *Food Chem.* **2011**, *128*, 968.
25. Yang, N.-C.; Jhou, K.-Y.; Tseng, C.-Y. *Food Chem.* **2012**, *132*, 1796.
26. Ng, C. F.; Koon, C. M.; Cheung, D. W. S.; Lam, M. Y.; Leung, P. C.; Lau, C. B. S.; Fung, K. P. *J. Ethnopharmacol.* **2011**, *137*, 1366.