

## Phenolic compounds from the rhizomes of *Zingiber pellitum*

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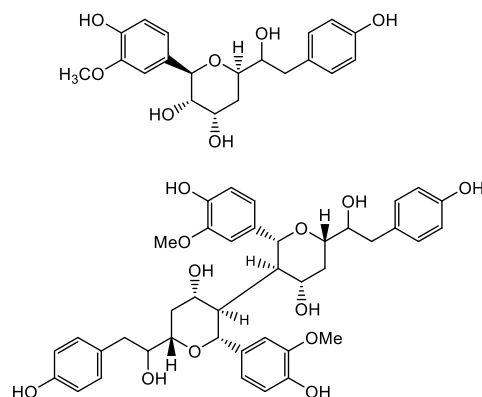
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### Abstract

Six new phenolic compounds have been isolated from the rhizome of *Zingiber pellitum*, including two new diarylheptanoids, zingiberpyrans A and B, rhamnocitrin-3-*O*-rhamnoside, meranzin hydrate, vanillin and (+)- $\alpha$ -viniferin. Their structures were elucidated by analyzing NMR, HRESIMS, and CD spectral evidences combining with the published data. Rhamnocitrin-3-*O*-rhamnoside (**3**) exhibited the strongest inhibition of nitric oxide production in LPS-stimulated RAW264.7 cells (IC<sub>50</sub> 49.6  $\mu$ M) while zingiberpyran A (**1**) was less active (IC<sub>50</sub> 71.0  $\mu$ M).



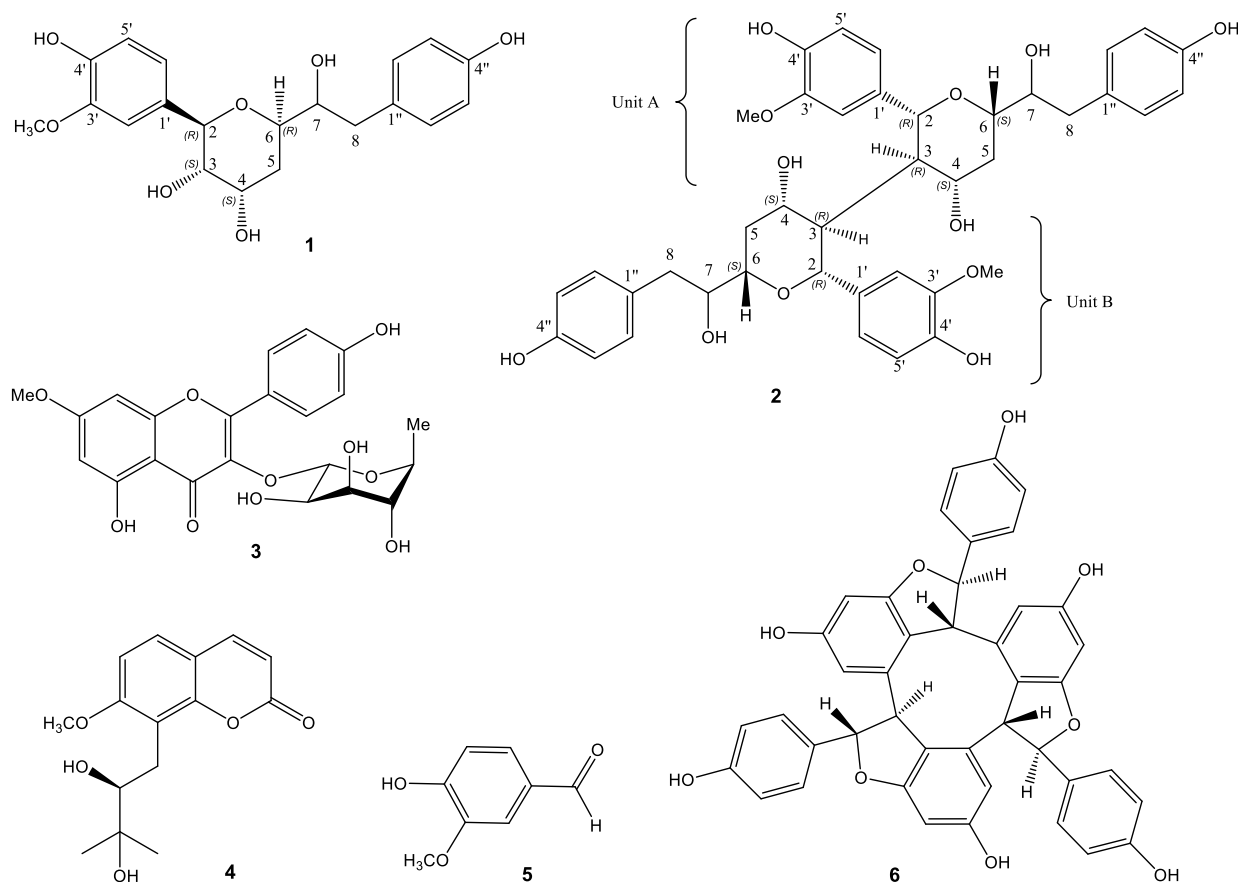
*Zingiber pellitum* Gagnep.



**Keywords:** *Zingiber pellitum*, diarylheptanoids, zingiberpyrans A, zingiberpyrans B, anti-inflammation

## Introduction

*Zingiber pellitum* Gapnep. is an endemic species of Vietnam and is found in several regions of this country<sup>1</sup>. It is an herbaceous plant, reaching a height of 0.8-1.2 meters, with densely long hair covering the entire plant and a tuberous rhizome. This species is distinguished by its unique inflorescence type, producing terminal flowers on leafy stems<sup>1</sup>. Like other *Zingiber* species, *Z. pellitum* has a long history of use in traditional medicine to treat ailments such as cold, fever, and cough<sup>2</sup>. A number of compounds have been isolated from different *Zingiber* species, such as diarylheptanoids, gingerols, flavonoids and terpenoids, exhibiting a wide spectrum of bioactivities, such as anti-inflammatory, antioxidant, anti-microbial and anti-cancer<sup>2</sup>. However, previous reports on the chemical composition and biological activity of *Z. pellitum* predominantly focused on essential oil composition<sup>3-5</sup>. The present study, therefore, represents the first to report the isolation of six phenolic compounds (Figure 1), including two new diarylheptanoids, from *Z. pellitum* rhizomes, with their chemical structures determined through spectral analysis. Furthermore, the antioxidant and anti-inflammatory activities of the isolated compounds were also reported in this research.



**Figure 1.** Structures of the compounds isolated from *Z. pellitum* rhizomes.

## Results and Discussion

By using various chromatographic techniques, the methanol extract of *Z. pellitum* rhizomes was separated to obtain two new diarylheptanoids **1** and **2**, and four known compounds, rhamnocitrin-3-*O*-rhamnoside (**3**),<sup>6</sup> meranzin hydrate (**4**),<sup>7</sup> vanillin (**5**),<sup>8</sup> and (+)- $\alpha$ -viniferin (**6**).<sup>9</sup>

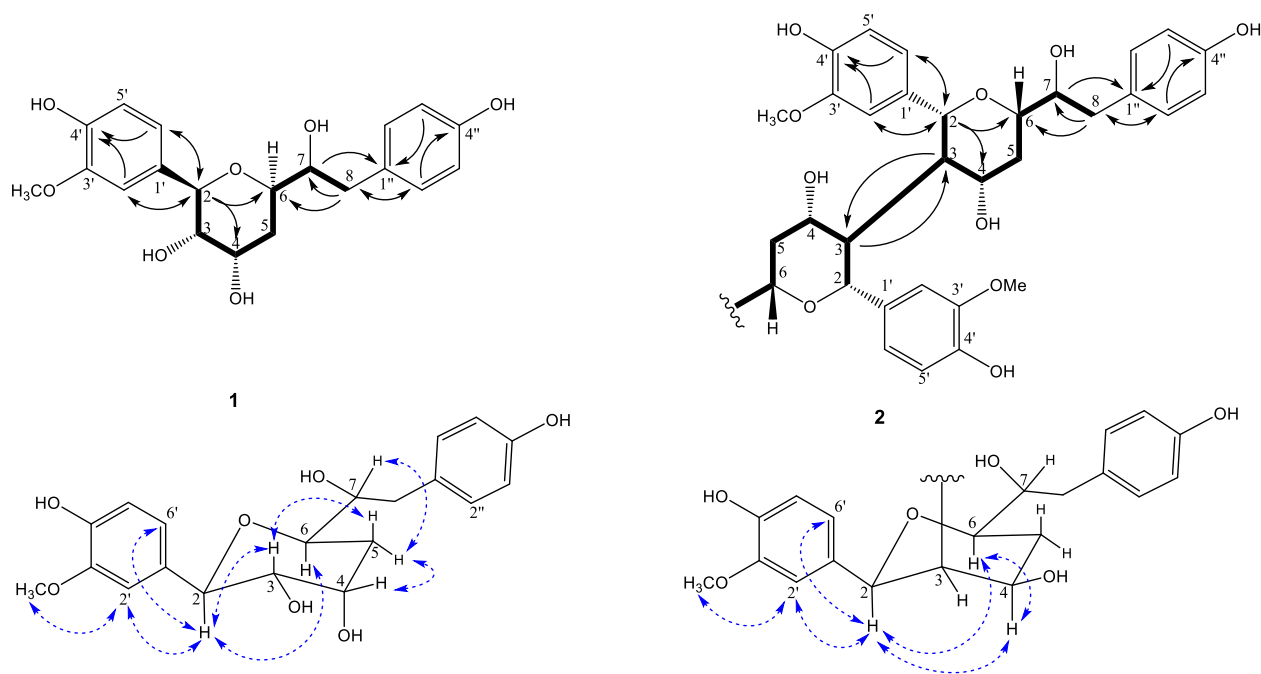
Compound **1** was obtained as an amorphous pale-yellow powder with the molecular formula C<sub>20</sub>H<sub>24</sub>O<sub>7</sub>, which was confirmed by the sodium adduct at  $m/z$  399.1379 [M + Na]<sup>+</sup> from the HRESIMS (calcd. for C<sub>20</sub>H<sub>24</sub>NaO<sub>7</sub>, 399.1420). The <sup>1</sup>H-NMR spectrum of **1** provided the information of 7 aromatic protons, therein 4 protons at  $\delta_H$  7.03 (2H, d,  $J$  = 8.4 Hz, H-2''/6'') and 6.70 (2H, d,  $J$  = 8.4 Hz, H-3''/5'') suggesting the presence of an AA'BB' spin system, and 3 proton signals at  $\delta_H$  6.81 (1H, d,  $J$  = 8.4 Hz, H-5'), 6.94 (1H, d,  $J$  = 1.8, 7.8 Hz, H-6'), 7.10 (1H, d,  $J$  = 1.8 Hz, H-2') were considered to belong to an ABX system (Table 1). The <sup>13</sup>C-NMR and HSQC demonstrated the presence of 20 carbon signals, regarding 12 signals belonging to two sets of aromatic systems, five oxymethines at  $\delta_C$  69.2, 73.7, 73.9, 75.8, and 79.4, one methoxy group at  $\delta_C$  56.5 and two methylene sp<sup>3</sup> carbons  $\delta_C$  35.4 and 39.8 (Table 2). In the COSY spectrum, the H-H spin-spin interactions were observed between H-2 ( $\delta_H$  4.39)  $\leftrightarrow$  H-3 ( $\delta_H$  3.53)  $\leftrightarrow$  H-4 ( $\delta_H$  4.17)  $\leftrightarrow$  H-5 ( $\delta_H$  1.77, 2.02)  $\leftrightarrow$  H-6 ( $\delta_H$  3.77)  $\leftrightarrow$  H-7 ( $\delta_H$  3.61)  $\leftrightarrow$  H-8 ( $\delta_H$  2.67, 2.83), which allowed for the identification of a continuous bond chain from C-2 to C-8 (Figure 2). The HMBC experiment showed the couplings from H-2 ( $\delta_H$  4.39) to C-1' ( $\delta_C$  133.5), C-2' ( $\delta_C$  112.7), C-6' ( $\delta_C$  122.0) and C-4 ( $\delta_C$  69.3) and interaction from H-8 ( $\delta_H$  2.67, 2.83) to C-1'' ( $\delta_C$  131.1), C-2''/6'' ( $\delta_C$  131.4) và C-6 ( $\delta_C$  73.9) allowing the identification of compound **1** as a diarylheptanoid with two benzene rings at C-2 and C-8 positions. Besides, HMBC couplings from H-2 ( $\delta_H$  4.39) to C-6 ( $\delta_C$  73.9) confirmed the presence of a cyclic pyran ring (Figure 2). The location of the methoxy group was assigned at C-3' based on the HMBC correlation from the methoxy signal ( $\delta_H$  3.90) to C-3' ( $\delta_C$  147.5).

**Table 1.** <sup>1</sup>H NMR data (mult.,  $J$  in Hz) of **1** and **2**

No	<b>1</b> (in CD <sub>3</sub> OD)	<b>2</b> (in CD <sub>3</sub> OD)	<b>2</b> (in DMSO- <i>d</i> <sub>6</sub> )
2	4.40 (1H, d, 10.2)	5.09 (1H, d, 6.0)	4.91 (1H, d, 4.8)
3	3.55 (1H, dd, 10.2, 3.0)	3.31 (1H, overlapped)	3.09 (1H, s)
4	4.17 (1H, q-like, 3.0)	4.68 (1H, q-like, 3.6)	4.44 (1H, q-like, 3.0)
5	2.05 (1H, ddd, 13.8, 12.0, 2.4)	2.04 (1H, ddd, 13.8, 7.8, 6.0)	1.84 (1H, m)
	1.79 (1H, ddd, 13.8, 3.6, 2.4)	1.82 (1H, m)	1.78 (1H, m)
6	3.78 (1H, dt, 12.0, 3.0)	3.32 (1H, overlapped)	3.20 (1H, s)
7	3.61 (1H, td, 7.2, 3.6)	3.46 (1H, m, H-7),	3.35 (1H, m, H-7),
8	2.85 (1H, dd, 13.2, 6.6)	2.60 (1H, dd, 13.8, 4.)	2.50 (1H, dd, 13.8, 4.2)
	2.69 (1H, dd, 13.2, 7.2)	2.53 (1H, dd, 13.8, 9.0)	2.39 (1H, dd, 13.8, 9.0)
2'	7.10 (1H, d, 1.8)	6.97 (1H, d, 1.8),	6.89 (1H, d, 1.2),
5'	6.81 (1H, d, 8.4)	6.82 (1H, d, 8.4)	6.75 (1H, d, 8.4)
6'	6.94 (1H, dd, 1.8, 7.8)	6.85 (1H, d, 8.4, 1.8)	6.72 (1H, d, 8.4, 1.2)
2''-6''	7.03 (2H, d, 8.4)	6.97 (2H, d, 8.4)	6.93 (2H, d, 8.4)
3''-5''	6.70 (2H, d, 8.4)	6.68 (2H, d, 8.4)	6.62 (2H, d, 8.4)
OCH <sub>3</sub>	3.90 (3H, br s)	3.85 (3H, br s)	3.74 (3H, br s)

This NMR data were almost identical to those of hedycoropyran A and B [11], except for the small difference in the pyran ring. The large coupling constant between H-2 and H-3 ( $J_{2,3}$  = 10.2 Hz) indicated that H-2 and H-3

were in di-axial relationship, while H-4 was in an equatorial orientation due to the small coupling constant with H-3 ( $J_{3,4} = 3.0$  Hz).<sup>10</sup> In the NOESY spectrum of **1**, an evident cross-peak between H-2 and H-6 confirmed the axial-orientation of H-6. For the absolute configuration identification, an ECD experiment was recorded. The 2*R*-configuration was determined based on the negative Cotton effect at 225 nm.<sup>11,12</sup> Consequently, (3*S*,4*S*,6*R*) absolute configurations were assigned. However, the configuration of C-6 could not be determined using the present spectroscopic data. Thus, compound **1** was determined to be (2*R*,3*S*,4*S*,6*R*)-6-(*-*-1-hydroxy-2-(4-hydroxyphenyl)ethyl)-2-(4-hydroxy-3-methoxyphenyl)tetrahydro-2*H*-pyran-3,4-diol, for which the name zingiberpyran A is proposed.



**Figure 2.** Key COSY (—), HMBC (→) and NOESY (<---->) correlations of compounds **1** and **2**.

Compound **2** was obtained as an amorphous pale-yellow powder. Its HRESIMS showed a pseudomolecular ion peak at  $m/z$  741.2852 [ $M + Na$ ]<sup>+</sup> compatible with the molecular formula  $C_{40}H_{46}O_{12}$  of **2**. The NMR data of **2** were similar to those of **1** with two aromatic AA'BB' and ABX systems, five aliphatic methines, two methylenes and one methoxy group. The detailed 2D NMR analysis of **2** (COSY, NOESY, HSQC and HMBC) allowed to assign the <sup>1</sup>H and <sup>13</sup>C NMR data as in Table 1. The appearance of the upfield-shifted resonance  $\delta_c$  58.4 (C-3) instead of  $\delta_c$  73.7 in **1** suggested that C-3 was not hydroxylated. Comparing with the NMR data of diarylheptanoids previously reported [13, 14], this chemical shift is compatible with the C–C connection. In combination with the molecular formula  $C_{40}H_{46}O_{12}$  as deduced from the HRESIMS experiment, two identical moieties, A and B, were proposed and linked via a C-3(A)—C-3(B) bridge. This connection was also supported by HMBC analysis which revealed a clear cross-peak between the proton at  $\delta_H$  3.31 (H-3) and the “itself” carbon atom at  $\delta_c$  58.4 (C-3) (Figure 2 and S19). It is noted that HMBC experiment gives heteronuclear long-range proton–carbon correlations but not direct one-bond correlations. Thus, the coupling from the proton at  $\delta_H$  3.31 to the carbon atom at  $\delta_c$  58.4 was assigned for the correlation from H-3 (A) to C-3(B) and vice versa.

**Table 2.**  $^{13}\text{C}$  NMR data of **1** and **2**

No	<b>1</b> (in CD <sub>3</sub> OD)	<b>2</b> (in CD <sub>3</sub> OD)	<b>2</b> (in DMSO- <i>d</i> <sub>6</sub> )
2	79.4	81.1	78.1
3	73.7	58.4	56.6
4	69.3	79.8	77.5
5	35.4	36.6	34.8
6	73.9	72.4	70.5
7	75.8	76.2	73.9
8	39.8	40.0	38.4
1'	133.5	135.8	134.7
2'	112.7	111.7	110.7
3'	148.7	149.2	147.4
4'	147.2	147.4	145.6
5'	115.7	116.3	115.2
6'	122.0	120.8	118.7
1''	131.1	131.4	130.1
2''-6''	131.4	131.3	129.9
3''-5''	116.0	116.0	114.6
4''	156.7	156.5	155.1
OCH <sub>3</sub>	56.5	56.5	55.6

Similar to **1**, compound **2** exhibited the negative Cotton effect at 230 nm in the ECD spectrum, indicating 2*R*-configuration. For the relative configurations of the pyran ring, the coupling constants and NOESY correlations were analyzed. Because signals of H-3 and H-6 overlapped in the  $^1\text{H}$  NMR spectrum of **2** recorded in CD<sub>3</sub>OD, the NMR data in DMSO-*d*<sub>6</sub> of **2** is provided as well (see supplemental material). The smaller coupling constant between H-2 and H-3 ( $J_{2,3} = 6.0$  Hz in CD<sub>3</sub>OD and 4.8 in DMSO-*d*<sub>6</sub>) indicated that H-2 and H-3 were in an axial-equatorial relationship but not di-axial in case of **1**. The NOE correlations of H-2/H-4, H-2/H-6 and H-4/H-6 confirmed the same orientation of H-2, H-4 and H-6. From these evidences, compound **2** was newly elucidated to be (2*R*,2'*R*,3*R*,3'*R*,4*S*,4'*S*,6*S*,6'*S*)-6,6'-bis(1-hydroxy-2-(4-hydroxyphenyl)ethyl)-2,2'-bis(4-hydroxy-3-methoxyphenyl)octahydro-2*H*,2'*H*-[3,3'-bipyran]-4,4'-diol, for which the name zingiberpyran B is proposed.

All the isolated compounds were evaluated for their anti-inflammatory effect via inhibition of nitric oxide production. In RAW264.7 cells stimulated by lipopolysaccharide (LPS), rhamnocitrin-3-*O*-rhamnoside (**3**) exhibited the strongest effect with the IC<sub>50</sub> value of 49.6 ± 0.73 μM while zingiberpyran A (**1**) proved less active (IC<sub>50</sub> 71.0 μM). The other compounds showed any remark inhibition at 100 μM.

## Conclusions

Two new diarylheptanoid compounds named zingiberpyrans A and B, and four known compounds, vanillin, (+)- $\alpha$ -viniferin, rhamnocitrin-3-*O*-rhamnoside, and meranzin hydrate were isolated for the first time from the rhizomes of *Zingiber pellitum*. Their structures were elucidated by using NMR, HR-ESI-MS and CD spectral data. All isolated compounds were evaluated for the inhibition of NO production in the LPS-stimulated RAW264.7 cells. Compound **1** and **3** showed remarkable inhibitory activity with IC<sub>50</sub> value of 71.0 and 49.6 μM, respectively.

## Experimental Section

### General:

#### Plant material

*Zingiber pellium* was collected from Binh Chau-Phuoc Buu Nature Reserve, Ba Ria - Vung Tau, Vietnam in January 2023 and was authenticated by one of the authors, Dr. Nguyen Quoc Binh. A voucher specimen (NPH1.2023) is deposited at the herbarium of the Institute of Ecology and Biological Resources.

#### Chemical and apparatus

Thin layer chromatography (TLC) was conducted on pre-coated silica gel (60 F254, Merck) and detected under ultraviolet light and sprayed with aqueous sulfuric acid 10%, vanillin 10% or ceric sulfate, then heated at 105°C until the spots appear clearly. Column chromatography (CC) was performed in silica gel (230-400 mesh ASTM, Merck), and Diaion HP 20 (Merck). HPLC system was used as Agilent HPLC 1100 series, coupling with detector DAD, and a semi-preparative process was performed on YMC-pack ODS-A 250x10 mm, 5µm, 20 nm column at a flow rate of 3.0 mL/min.

NMR spectra were recorded on a Bruker AvanceNEO 600 MHz and a Bruker 500MHz spectrometer using TMS-tetramethyl silane as an internal standard. HR-MS were collected on the Agilent 6530 Accurate-Mass Q-TOF LC/MS. Optical rotations were recorded on a Jasco P-2000 Digital Polarimeter.

#### Extraction and isolation

1.3 kg of dried powder of *Zingiber pellium* (ZP) was extracted exhaustively with methanol. Three methanol extractions (1.5L/time) were filtered, combined and concentrated under reduced pressure to obtain a concentrated total extract. This was partitioned with *n*-hexane and methanol to give the corresponding extracts: *n*-hexane extract (15.93 g) and methanol extract (39.13 g).

The methanol extract was loaded on the Diaion HP-20 CC with stepwise elution water, methanol 40%, methanol 100% and acetone 100% in order to collect 4 fractions M0, M40, M100 and A100 respectively. M100 fraction was chromatographed on silica gel CC and eluted with gradient elution of dichloromethane/methanol (10/1 – 1/1, v/v) to collect 8 fractions (from F1.1 to F1.8). Fraction F1.3 was separated by silica gel CC with hexane/ethyl acetate (4/1, v/v) followed by preparative HPLC (30-100% MeOH in H<sub>2</sub>O in 120 min) to afford compound **5** (3.6 mg). Fraction F1.6 was separated on a column with silica gel (stationary phase) and dichloromethane/acetone (5/1, v/v) (mobile phase), to produce 8 fractions, denoted as F2.1 to F2.8. Fraction F2.6 was continually chromatographed on HPLC (40-80% MeOH in H<sub>2</sub>O (HPLC grade) in 180 min) to yield compounds **1** (9.3 mg) and **2** (4.1 mg). Compound **6** was separated from fraction F2.8 by preparative HPLC eluting (30-80% MeOH in H<sub>2</sub>O (HPLC grade) in 200 min). Compounds **3** (3.0 mg) and **4** (2.7 mg) were isolated from fraction F1.7 by semi-preparative HPLC eluting with gradient solvent from 30 to 70% MeOH in 180 min.

Zingiberpyran A (**1**): amorphous pale yellow powder;  $[\alpha]_D^{28} = -19.6$  (c 0.10, MeOH); ECD (MeOH)  $\lambda_{max}$  ( $\Delta\epsilon$ ) 202 (–14.8), 225 (–1.22); HR-ESI-MS  $m/z = 399.1379$  [M + Na]<sup>+</sup> (calcd. 399.1420 for C<sub>20</sub>H<sub>24</sub>O<sub>7</sub>Na). <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD), see Table 1 and 2.

Zingiberpyran B (**2**): amorphous pale yellow powder;  $[\alpha]_D^{28} = -17.4$  (c 0.03, MeOH); ECD (MeOH)  $\lambda_{max}$  ( $\Delta\epsilon$ ) 206 (–5.6), 230 (–4.96); HR-ESI-MS  $m/z = 741.2852$  [M + Na]<sup>+</sup> (calcd. 741.2887 for C<sub>40</sub>H<sub>46</sub>NaO<sub>12</sub>). <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD and DMSO-*d*<sub>6</sub>) and <sup>13</sup>C-NMR (150 MHz, CD<sub>3</sub>OD and DMSO-*d*<sub>6</sub>), see Table 1 and 2.

#### Inhibition assay for nitric oxide production

The inhibitory effect of four isolated compounds was determined using a previously reported procedure<sup>15</sup> with dexamethasone used as a positive control (IC<sub>50</sub> 13.3 µM). All experiments were performed in triplicate.

## Acknowledgements

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## Supplementary Material

Full experimental detail including NMR, HRESIMS and ECD spectra of compounds **1** and **2** can be found via the “Supplementary information” section of this article’s webpage.

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