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Novel neurotrophic phenylbutenoids from Indonesian ginger Bangle, *Zingiber purpureum*

Miwa Kubo [a,*], Minori Gima [a], Kohei Baba [a], Megumi Nakai [b], Kenichi Harada [a], Midori Suenaga [a], Yoichi Matsunaga [a], Eishin Kato [c], Shinya Hosoda [c], Yoshiyasu Fukuyama [a,*]

[a] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Yamashiro-cho, Tokushima 770-8514, Japan*
[b] *Department of Pharmacy, Kochi Medical Graduate School, 185-1, Okou-cho, Kohasu, Nankoku, Kochi 783-8505, Japan*
[c] *HOSODA SHC Co., Ltd, 3-2-21 Miyuki, Fukui 910-0854, Japan*

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ABSTRACT

Two new curcuminoids **1** and **2**, and a new phenylbutenoid dimer **3**, were isolated from Bangle (*Zingiber purpureum*). Their structures were determined on the basis of comprehensive spectroscopic data and their biogenetic pathway. Compounds **1** and **2** are the first example of curcumin coupled with phenylbutenoid. Compounds **1** and **2** promoted neurite outgrowth of NGF-mediated PC12 cells at concentrations ranging from 1 to 10 μM. In addition, compound **1** was found to accelerate the prevention of Aβ42 aggregation.

Neurotrophins play an essential role in the development of vertebrate nervous systems.[1] In particular, the neurotrophin nerve growth factor (NGF) has been recognized as an important regulatory substance in the nervous system,[2] making it potentially effective for the treatment of neurodegenerative diseases. However, this polypeptide cannot cross the brain–blood barrier because of its high molecular weight and easy metabolization by peptidases under physiological conditions.[3] To address this issue, considerable efforts have been deployed to find small molecules exhibiting neurotrophic activity. Our search for nonpeptide neurotrophic compounds in plants has provided numerous interesting natural products showing the desired properties, such as merrilactone A from *Illicium merrillianum*,[4] jiadifenin[5] and jiadifenolide[6] from *I. jiadifengpi*, and neovibsanins[7] from *Viburnum awabuki*.

Bangle, the rhizome of *Zingiber purpureum*, is a tropical ginger widely distributed in Southeast Asia. Used as a spice, this plant finds application in the traditional Indonesian medicine jamu. It has also been utilized to treat fever, headaches, stomach pain, rheumatism, and obesity and serves as an ingredient in post-partum herbal medicine.[8] Previous phytochemical studies on the rhizomes of *Zingiber cassumunar*, which is a synonym of *Zingiber purpureum*, reported the isolation of various types of phenylbutenoids, curcuminoids, and terpenoids.[9] In our previous study,[10] we successfully isolated compounds **4** and **5** as neurotrophic principles from the MeOH extract of the rhizomes of Bangle that exhibited neuritogenic activity in PC12 cells at 25 μg/mL.

Furthermore, chronic treatments with **4** and **5** were found to enhance hippocampal neurogenesis in dementia model OBX mice.[10] Our continuing search for the biologically active constituents of Bangle resulted in the isolation of curcuminoids **1** and **2** and the phenylbutenoid dimer **3**, named neocassumunarin A and B, and banglenol A.[11] Curcuminoids **1** and **2** are the first example of curcumin precursor coupled with phenylbutenoid. In this paper, we report the structures of **1**–**3** and their assesments for neurite outgrowth of PC12 cells and Aβ aggregation.

The MeOH extract of Bangle was fractionated by column chromatography on silica gel with hexane–EtOAc = 3:2 to give 10 fractions. Bioassay-guided fractionation using PC12 cells resulted in the isolation of three new compounds **1**–**3**, and a known compound **4** (Fig. 1).

Compound **1**[12] gave a molecular formula $C_{34}H_{34}O_8$, as deduced by high resolution (HR)-FABMS at *m*/*z* 609.1819 $[M+K]^+$. The ^1H

* Corresponding authors. Tel.: +81 88 602 8435; fax: +81 88 655 3051.
E-mail addresses: miwa-k@ph.bunri-u.ac.jp (M. Kubo), fukuyama@ph.bunri-u.ac.jp (Y. Fukuyama).

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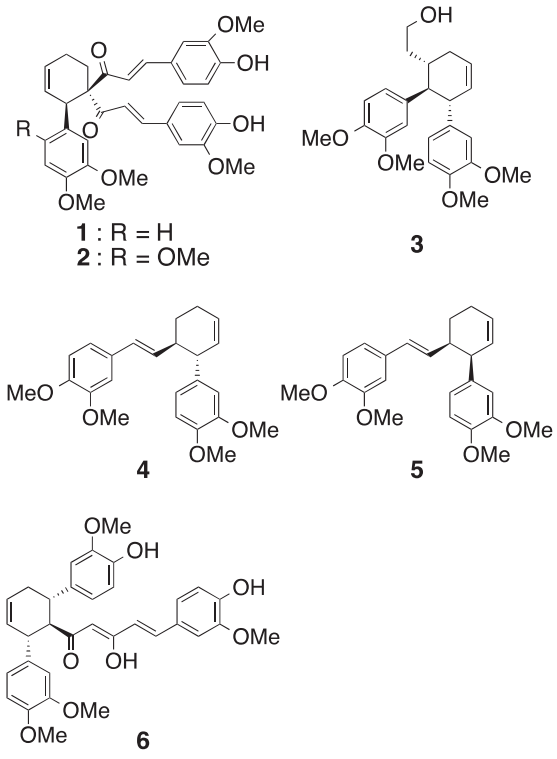

Figure 1. Structures of curcuminoids **1**, **2**, and **6** and phenylbutenoid dimers **3**–**5** isolated from *Zingiber purpureum*.

NMR data (600 MHz, C_6D_6, Table 1) of **1** indicated the presence of two methylenes [δ_H 2.73 (br dd, J = 14.5, 5.6) and 2.81 (ddd, J = 14.5, 12.3, 5.6), 2.07 (dddd, J = 18.1, 12.3, 5.6, 2.7), 2.17 (dt, J = 18.1, 5.3)], a methine [δ_H 5.04 (H, br d, J = 3.7)], four methoxy groups (δ_H 2.90, 2.96, 3.29, 3.32), one pair of *Z*-olefinic protons [δ_H 5.80 (m), 6.07 (m)], two pairs of *E*-olefinic protons [δ_H 7.08 (d, J = 15.5) and 7.62 (d, J = 15.5). 7.11 (d, J = 15.4) and 8.03 (d, J = 15.4)], and three trisubstituted benzene rings A–C [δ_H 6.52 (d, J = 8.4), 6.90 (d, J = 2.1), and 6.98 (dd, J = 8.4, 2.1). 6.73 (dd, J = 8.2, 1.8), 6.73 (d, J = 8.2), and 6.59 (d, J = 1.8). 6.73 (dd, J = 8.2, 1.8), 6.73 (d, J = 8.2), and 6.46 (d, J = 1.8)]. The ^{1}H NMR data obtained was similar to those of cassumunarin A (**6**).[13] The chemical shift value for methoxy groups were shifted higher upfield than criteria value, which is likely to be due to solvent effects of C_6D_6.[14] An extensive analysis of 2D COSY, HMQC provided four partial structures, as shown in Figure 2, and showed the presence of 12 quaternary carbons (δ_C 69.7, 127.1, 127.2, 132.5, 149.0, 149.3, 149.3, 146.9, 146.9, 148.9, 195.2, 196.1). The four phenolic methoxy group positions at C-9 and C-10 in aromatic ring A as well as at C-8′ and C-8″ in aromatic B and C, respectively, were determined by HMBC. The HMBC correlations of H-2/C-1, and H-6/C-1, C-8, and C-12 indicated that **1** had the same cyclohexene ring attached with a trisubstituted benzene ring as that of **6**. The connections of the two feruloyl moieties via C-1′ and C-1″ to C-1 of the cyclohexene ring were determined by HMBC correlations between the C-1′ carbonyl carbon (δ_C 196.1) and H-2, H-3′, and H-6 as well as those between the C-1″ carbonyl carbon (δ_C 195.2) and H-2 and H-3″. The above spectroscopic data revealed that the plane structure of **1** was the novel complex of phenylbutenoid and curcuminoid, as depicted in Figure 2. Compound **1** was optically inactive, and hence, comprised a racemate by chiral HPLC, indicating that the biogenetic formation of the chiral center (C-6) is not stereoselective. On the basis of the above spectral data, the structure of neocassumunarin A was represented as **1**.

Compound **2**[15] gave a molecular formula $C_{35}H_{36}O_9$, as deduced by HR-EIMS at m/z 600.2356 $[M]^+$. Compound **2** was also found optically inactive by chiral HPLC. The spectral data of **2** were similar to those of **1**, except for the presence of a 1,2,4,5-tetrasubstituted benzene ring, instead of a 1,3,4-trisubstituted benzene ring, and an additional methoxy group. The HMBC analysis showed that three methoxy groups were bound to C-9, C-10, and C-12 in aromatic ring A. Additionally, the other HMBC correlations were consistent with the same structure framework as that of **1**. On the basis of the above spectral data, the structure of neocassumunarin B was represented as **2**.

Compound **3**[16] gave a molecular formula, $C_{24}H_{30}O_5$, as determined by HR-EI-MS at m/z 398.2096. Chiral HPLC analysis demonstrated that compound **3** was optically inactive. The ^{1}H NMR and physical data of **3** revealed the presence of a hydroxy group (3511 cm^{-1}), four methoxy groups (δ_H 3.67, 3.82, 3.74, and 3.79), a disubstituted double bond [δ_H 5.74 (br d, 10.8), 5.92 (m)], an oxymethylene [δ_H 3.55 (m), 3.60 (m)], and two trisubstituted benzenes [δ_H 6.43 (br s), 6.61 (d, 8.2), 6.37 (dd, 8.2, 2.1), and 6.30 (d, 2.1), 6.68 (d, 8.2), and 6.44 (br d, 8.2)]. These spectral data were similar to those of **4**. Extensive COSY and HMQC analysis led to three partial structures *A*–*C*, as shown in Figure 3. The HMBC correlations of H-4/C-6′, C-1′, and C-1″ suggested that both the 3- and 4-positions of the cyclohexene ring were attached to the trisubstituted benzene rings *B* and *C*. The plane structure of **3** was determined, as shown in Figure 3. The relative stereochemistry of **3** was elucidated by NOESY, as shown in Figure 4. The large J value ($J_{H\text{-}5ax,\ H\text{-}4ax} = J_{H\text{-}4ax,\ H\text{-}3ax} = 10.3$ Hz) obtained for the H-4 axial proton and NOESY correlations between H-4_{ax} and H-6_{ax} and between H-6_{eq} and H-5_{ax} indicated that the benzene ring at C-4 took a β orientation. NOESY correlations were observed between H-5β and H-6β and H-3β, between H-6α and H-7, as well as between H-4α and H-2″, implying that the hydroxyethyl group at C-5 and the benzene ring at C-3 both adopted an α orientation.

Thus, on the basis of the aforementioned spectral data, the structure of banglenol A was elucidated to be **3**.

The following plausible biosynthetic routes were proposed for **1**–**3**, and **6**: the formation of curcuminoids would start with the combination of feruloyl-CoA and malonyl-CoA into the curcumin precursor ***b***, leading to curcumin by subsequent decarboxylation.[17] Alternatively, the reduction and dehydroxylation of ***b*** would give rise to exomethylene intermediate ***c***, which would produce **1** or **2** by Diels–Alder reaction with phenylbutenoid diene ***d***. Conversely, the Diels–Alder reaction between curcumin enolate ***e*** and phenylbutenoid diene ***d*** would generate **6**. The Diels–Alder reaction between ***d*** and ***f*** would lead to **3** (Scheme 1).

Compounds **1**–**3** were isolated from the active fractions that promoted neurite outgrowth from PC12. Therefore, their ability to induce neurite outgrowth in these cells was evaluated as described previously. The obtained results were compared with those of **4**.[7,10] No compounds except **4** displayed any morphological effects on the cells in the absence of NGF. However, the curcuminoids neocassumunarin A (**1**) and B (**2**) significantly promoted neurite outgrowth from PC12 cells in a dose-dependent manner in the presence of NGF, as shown Figure 5. In the control group (0.5% EtOH), only a few cells had neurites. NGF (2 ng/mL) significantly induced neurite sprouting and outgrowth in PC12 cells. Similarly, compound **4** significantly induced the neurite sprouting in PC12 cells. On the other hands, in the presence of NGF, compound **1** significantly promoted neurite outgrowth at a concentration 1 μM, but showed toxicity against PC12 cells above 10 μM. Compound **2** significantly promoted neurite outgrowth at concentrations of 1 μM and 10 μM, but showed toxicity against PC12 cells at concentrations higher than 30 μM. The phenylbutenoid dimer **3** exhibited