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Efficient synthesis and structure–activity relationship of honokiol, a neurotrophic biphenyl-type neolignan

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Abstract—Honokiol, a biphenyl-type neolignan, which shows the remarkable neurotrophic effect in primary cultured rat cortical neurons, has been effectively synthesized in 21% yield over 14 steps starting from 5-bromosalicylic acid and *p*-hydroxybenzoic acid by utilizing Pd-catalyzed Suzuki–Miyaura coupling reaction as a key step. Additionally, the structure–activity relationship between neurite outgrowth-promoting activity and its O-methylated and/or its hydrogenated analogues was examined in the primary cultures of fetal rat cortical neurons, suggesting that 5-allyl and 4′-hydroxyl groups are essential for affecting the neurotrophic activity of honokiol.

1. Introduction

Since neurodegenerative diseases such as Alzheimer's disease have increasingly caused social problems as the swell of aged populations, it is the strong demand to develop some drugs for treatment of them. Several endogenous neurotrophic factors, such as the family of neurotrophins (NGF, BDNF, NT3, and NT4/5), are known to play key roles in neuronal survival, process outgrowth, and synaptic connectivity during development and nervous system plasticity in adults. Thus, they are likely to be useful agents for ameliorating neurodegeneration.[1,2] However, the peptidyl properties of neurotrophic factors have brought about some serious issues to overcome, for example, unable to cross the blood–brain barrier and to be easily metabolized, in clinical uses.[3,4] Small molecules, which can mimic functions of neurotrophic factors, might be promising alternatives for treatment of neurodegenerative diseases.[5,6] We have, therefore, been searching for neurotrophic small molecules in natural products by using the primary cultures of rat cortical neurons, resulting in the discovery of honokiol (**1**)[7] together with several active compounds from the bark of *Magnolia obovata* Thunb.[8] In previous papers, we reported that **1** has the significant neurotrophic property, that is, neurite outgrowth-promoting and neuroprotective effects in the primary cultures of fetal rat cortical neurons in two different serum-free media.[9] Moreover, we demonstrated that **1** increases the cytoplasmic free Ca^{2+} in rat cortical neurons and neuroblastoma SH-SY5Y cells.[10] These results compelled us to supply enough amount of **1** not only to clarify structure–activity relationships and the details of its neurotrophic effects, but also to examine its neurotrophic action in some in vivo animal models.

Herein, we report a highly efficient synthetic route[11] of **1** based on the Pd-catalyzed Suzuki–Miyaura protocol and structure–activity relationships between analogues **2a–c** and **3a–c** derived from **1** and their neurite outgrowth-promoting activity (Fig. 1).

2. Synthesis of honokiol (1) and its analogues 2a–c and 3a–c

As depicted in Scheme 1, a convergent synthetic route was envisioned to effectively produce some versatile analogues of **1** in future. Thus, two allyl groups would

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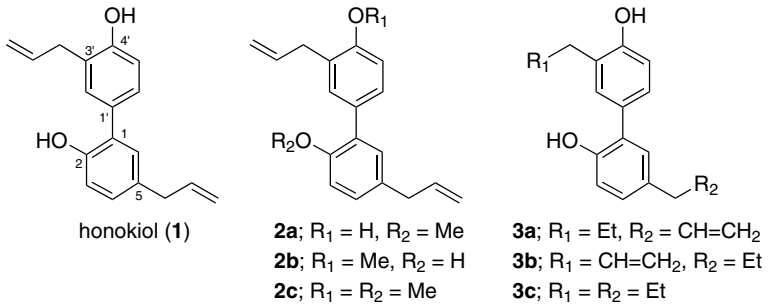
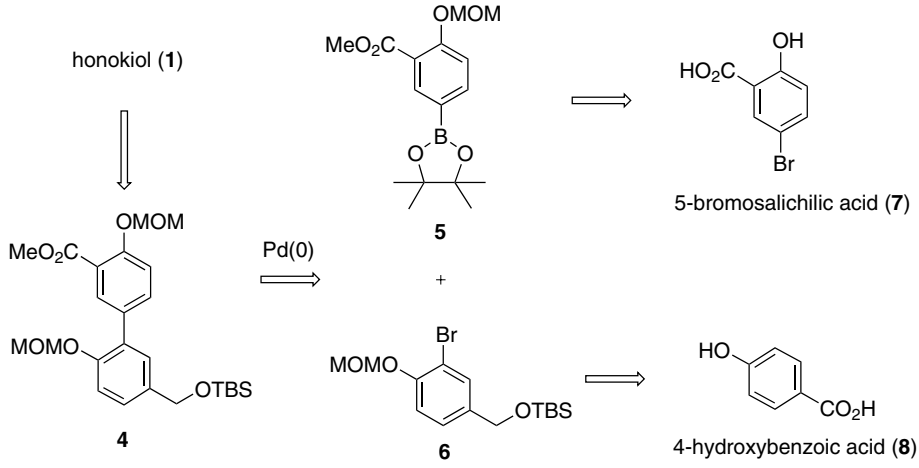


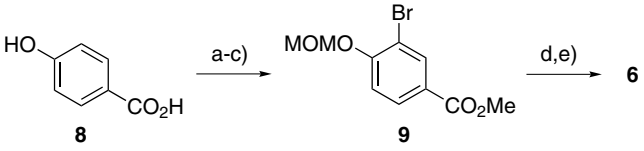
Figure 1. Structure of honokiol (**1**) and its analogues **2a**–**c** and **3a**–**c**.



Scheme 1. Retrosynthetic analysis of honokiol (**1**).

be elaborated from an ester and a hydroxymethyl groups of biaryl **4** at later stage of synthesis, respectively, so as to introduce some versatile functional groups for enhancing activity as well as preparing molecular probes needed to analyze the mechanism of action. The Pd-catalyzed Suzuki–Miyaura reaction[12] would be applied to construct biaryl **4** from borate **5** and bromide **6**. Both the borate **5** and the bromide **6** would be easily obtained from 5-bromosalicylic acid (**7**) and 4-hydroxybenzoic acid (**8**), respectively.

The synthesis of **1** commenced with the Fischer esterification of 4-hydroxybenzoic acid (**8**) followed by regioselective bromination[13] using *N*-bromosuccinimide and HBF_4 in MeCN and protection of phenolic hydroxyl group with methoxymethyl chloride to give the bromide **9** in good yield. Reduction of **9** with DIBAL-H in CH_2Cl_2 at $-78\,^{\circ}C$ followed by protection of a hydroxyl group with *tert*-butyldimethylsilyl chloride gave aryl bromide **6** quantitatively over two steps (Scheme 2).



Scheme 2. Preparation of one unit **6**. Reagents and conditions: (a) concd H_2SO_4, MeOH, reflux, overnight, 99%; (b) HBF_4, NBS, MeCN, $-20\,^{\circ}C$ to rt, 80%; (c) MOMCl, *i*-Pr_2NEt, DMF, rt, 3 h, 98%; (d) DIBAL-H, CH_2Cl_2, $-78\,^{\circ}C$, 2 h, 100%; (e) TBSCl, imid., DMF, rt, 100%.

The other unit **5** was prepared as followings: Fischer esterification of 5-bromosalicylic acid (**7**) followed by protection of a phenolic hydroxyl group with methoxymethyl chloride gave methoxymethyl ether **10** in quantitative yield and then **10** was subjected to Pd-catalyzed transformation into pinacol borate **5**. However, in the case of using pinacolborane with several palladium catalysts, none of **5** was obtained. Under the particular conditions, that is, bis(pinacolato)diborane (1.0 equiv)[14] with $PdCl_2$(dppf) (10 mol %), dppf (10 mol %), and AcOK (3.0 equiv) in 1,4-dioxane at $80\,^{\circ}C$, the coupling reaction smoothly proceeded to provide the desired arylboronate **5** in 86% yield. The critical coupling reaction between aryl bromide **6** and arylboronate **5** based on the Suzuki–Miyaura protocol[12] was accomplished by using 10 mol % of $PdCl_2$(dppf) with dppf (10 mol %) and K_3PO_4 (3.0 equiv) in 1,4-dioxane at $108\,^{\circ}C$, giving rise to the desired coupling adduct **4** in 87% yield. The methyl ester moiety of **4** was reduced to a hydroxymethyl group with $LiAlH_4$, and then *tert*-butyldimethylsilyl group was removed by 47% HF/pyridine/MeCN (1:3:5) in a high yield. Both the hydroxyl groups of diol **11** were converted to bromides

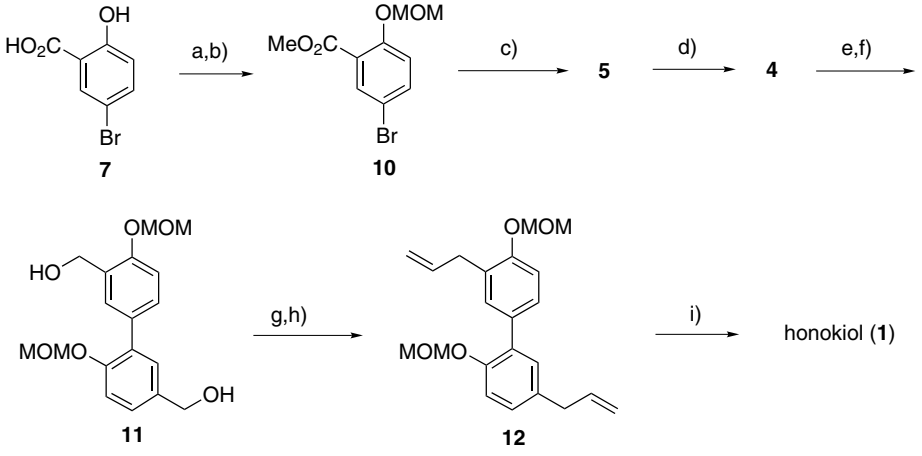
by treatment of *N*-bromosuccinimide and triphenylphosphine, followed by the reaction with vinylcuprate to give diallyl compound **12** in a moderate yield. Finally, treatment of **12** with 2 M HCl afforded honokiol (**1**), all spectra data[15] (^{1}H NMR, ^{13}C NMR, IR, HR-EIMS) of which were superimposed on those of natural product. Thus, we have established the efficient synthesis of honokiol (**1**) in a 21% overall yield (Scheme 3).

Next, six analogues **2a**–**c** and **3a**–**c** were prepared as follows: Treatment of **1** with TMS–diazomethane in MeOH yielded a mixture of methylated products, which were separated by a flash silica gel chromatography to give methylated analogues **2a**–**c** in 13%, 13%, and 47% yield, respectively. On the other hand, **1** was subjected to hydrogenation with Wilkinson's catalyst in EtOH to afford dihydrogenated analogues **3a**–**c** in 12%, 7%, and 18% yield, respectively.

3. Structure–activity relationship

The neuronal cultures and neurite outgrowth-promoting assay were performed with slight modifications in serum-free medium and cell density according to the way as previously described.[9] Briefly, the neurons were isolated from cerebral cortex of pregnant day 18 SD rat fetal (SLC, Japan) and cultured in Dulbecco's modified eagle's medium (DMEM) (Gibco BRL, USA) supplemented with 10% of fetal bovine serum (FBS) and 50 IU mL^{-1} penicillin-50 μg mL^{-1} streptomycin at a density of 5000 cells cm^{-2} on poly-L-lysine-coated 24-well plates for 24 h. Then the medium was changed to the serum-free Neurobasal™ medium (NB) plus 2% B27 supplement containing the tested sample. The treatment continued for 144 h. Then the neurons were fixed with 4% paraformaldehyde (in PBS) for 20 min, and permeabilized with 0.1% Triton X-100 in PBS for 20 min after endogenous peroxidase activity was blocked by freshly prepared 0.3% H_2O_2 for 20 min. For demonstration of morphology, the neurons were incubated in primary antibody anti-MAP2 (1:1000) overnight in 4 °C followed by incubation with horse peroxidase-conjugated second antibody [rabbit anti-mouse IGg (1:2)] for 1 h, then peroxidase was developed with 200 μL substrate Simple Stain DAB solution. Images of neuron in random fields were captured with inverse microscope equipped with Macintosh computer and imaging software LuminaVision 1.0 (Mitani Corp., Fukui, Japan). The length of the primary (longest) neurite on each neuron was manually measured with software MACSCOP 2.6 (Mitani Corp., Fukui, Japan). The average length of at least 100 neurons in each group was expressed as mean ± SEM. Statistical analysis was performed with origin 7.0 (OriginLab, USA).

The morphological effects of honokiol (**1**) and its analogues **2a**–**c** and **3a**–**c** on the cultured rat cortical neurons were evaluated by the anti-MAP2 staining method. Honokiol (**1**) (Fig. 2b), 2-*O*-methylhonokiol (**2a**) (Fig. 2c), and 8′-dihydrohonokiol (**3a**) (Fig. 2d) had striking effects on the morphology of cortical neurons in comparison with the cortical culture (Fig. 2a) as shown in Figure 2. The other analogues **2b**, **2c**, **3b**, and **3c** showed no enhancement of the neurite extension. As shown in Figure 3, the quantitative analysis of the longest neurite length affected by each compound at concentrations of 0.1 and 1 μM indicates that **2a** (neurite length: 132.7 ± 5.7 and 122.6 ± 6.2 μM) and **3a** (127.5 ± 5.6 and 150.5 ± 8.1 μM) also have as high potential of enhancing the neurite extension in the cultured rat cortical neurons as **1** (138.9 ± 6.3 and 142.1 ± 5.3 μM), whereas other analogues **2b** (113.1 ± 4.5 and 114.4 ± 6.5 μM), **2c** (111.6 ± 5.8, 111.0 ± 5.4 μM), **3b** (102.6 ± 5.0, 120.1 ± 6.1 μM), and **3c** (100.9 ± 5.3, 102.3 ± 6.1 μM) diminish neurotrophic efficiency. Thus, these results suggest that 4′-hydroxy group and 5-allyl group are responsible for honokiol-mediated neurite outgrowth-promoting activity, but 3′-allyl group is not essential for that.



Scheme 3. Total synthesis of honokiol (**1**). Reagents and conditions: (a) concd H_2SO_4, MeOH, reflux, overnight, 100%; (b) MOMCl, *i*-Pr_2NEt, DMF, rt, 3 h, 100%; (c) bis(pinacolato)diborane, 10 mol% $PdCl_2$(dppf), dppf, AcOK, 1,4-dioxane, 80 °C, 86%; (d) **6**, 10 mol% $PdCl_2$(dppf), dppf, K_3PO_4, dioxane, reflux, 87%; (e) $LiAlH_4$, THF, 0 °C, 99%, (f) 47% HF/pyridine/MeCN (1/3/5), rt, 1 h, 98%; (g) NBS, PPh_3, CH_2Cl_2, 80%; (h) H_2C=C(H)MgBr, CuI, THF, −26 °C, 52%; (i) 2 M HCl, MeOH (5/1), rt, 44 h, 89%.

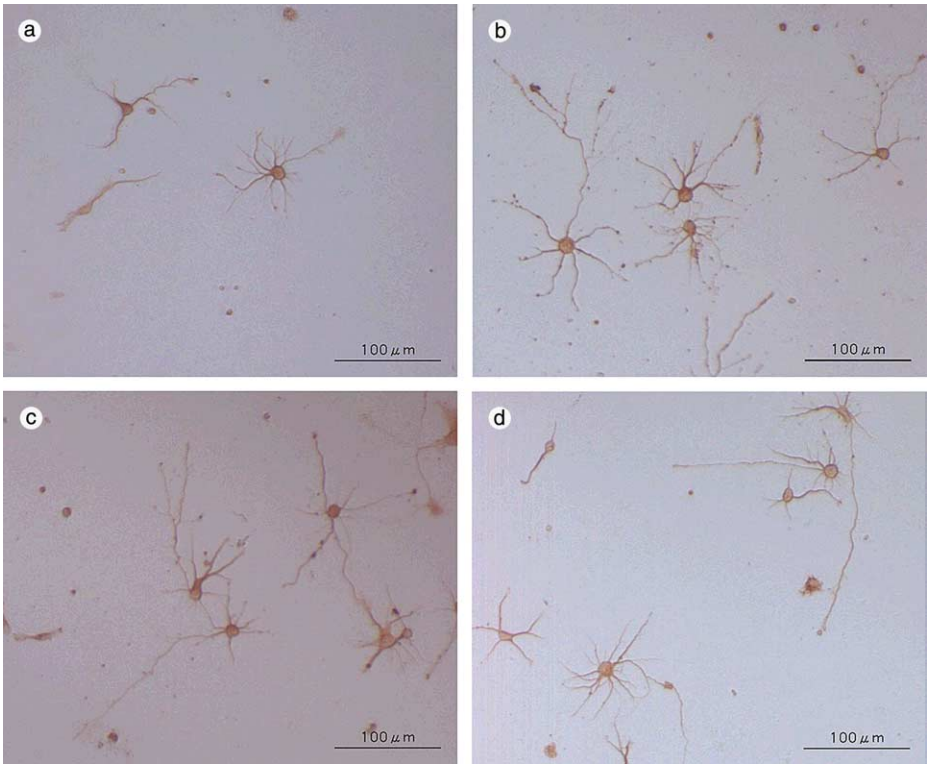


Figure 2. Morphology of cultured rat cortical neurons demonstrated with anti-MAP2 immunochemical staining. After 1-day pre-culture, neurons were treated for 3 days, and then stained by anti-MAP2 as described in the text. (a) Neurons in the presence of 0.5% ethanol as vehicle control; (b) neurons in the presence of 0.1 μM **1**; (c) neurons in the presence of 1 μM **2a**; (d) neurons in the presence of 0.1 μM **3a**.

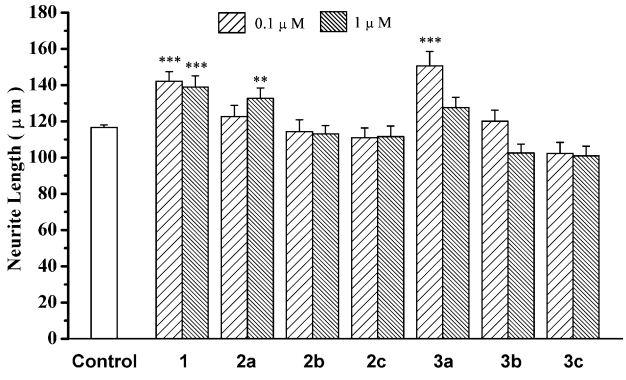


Figure 3. Quantitative analysis of anti-MAP2 immunochemically stained processes affected by honokiol (**1**) and its analogues **2a**–**c** and **3a**–**c**. In each group, the average length of the primary processes were determined from 100 neurons selected in random fields. **, $P < 0.01$; ***, $P < 0.001$ compared with control. Data presented here are derived from one of two repeated experiments with similar results.

In conclusion, we have achieved the efficient synthesis of honokiol (**1**) by utilizing Suzuki–Miyaura coupling reaction in 21% yield over 14 steps and then have prepared the six analogues of **1**. According to the morphological analyses of these analogues in the primary cultured rata cortical neurons together with the previous results,[16] the biaryl structure bearing a hydroxyl and a allyl groups at the 4′- and 5-positions, respectively, is shown to be at least essential for honokiol-mediated neurite outgrowth-promoting activity. Further research for enhancing and clarifying neurotrophic potential and mechanism caused by honokiol currently is undergoing based on the present data.

Acknowledgements

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15. Spectral data of **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (1H, dd, $J = 7.8$, 2.1 Hz), 7.21 (1H, s), 7.20 (1H, d, $J = 2.1$ Hz), 7.05 (1H, dd, $J = 7.8$, 2.1 Hz), 6.92 (1H, d, $J = 7.8$ Hz), 6.90 (1H, d, $J = 7.8$ Hz), 6.04 (1H, ddt, $J = 16.8$, 10.2, 6.6 Hz), 5.97 (1H, ddt, $J = 16.8$, 9.9, 6.9 Hz), 5.2 (1H, dq, $J = 9.9$, 1.5 Hz), 5.14 (1H, br s), 5.15 (1H, dq, $J = 16.8$, 1.5 Hz), 5.10 (1H, br s), 5.08 (1H, dq, $J = 16.8$, 1.5 Hz), 5.05 (1H, dq, $J = 10.2$, 1.2 Hz), 3.46 (1H, d, $J = 6.6$ Hz), 3.35 (1H, d, $J = 6.3$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.9, 150.7, 137.8, 135.9, 132.2, 131.1, 130.2, 129.6, 128.8, 128.5, 127.7, 126.3, 116.9, 116.6, 115.5, 39.4, 35.2; FT-IR (neat) 3434, 1637, 1607 cm^{-1}; HR-EIMS calcd for $C_{18}H_{18}O_2$ (M^+) 266.1307; found 266.1294.
16. 2-Allyphenol and 4-allyphenol are already known to be toxic against the rat cortical neurons at 1 μM, indicating that the biaryl structure in honokiol makes a contribution to express its neurotrophic activity.