

HETEROCYCLES, Vol. 78, No. 8, 2009, pp. 2061 - 2065. © The Japan Institute of Heterocyclic Chemistry
Received, 17th February, 2009, Accepted, 13th April, 2009, Published online, 14th April, 2009.
DOI: 10.3987/COM-09-11684

**DIHYDROCHALCONE DESIGNED FROM
METHYLOPHIOPOGONANONE B STRONGLY INHIBITS
HYPOXIA-INDUCIBLE FACTOR (HIF)-1 α ACTIVITY**

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Abstract – Inhibition of hypoxia-inducible factor (HIF)-1 α activity of methylophiopogonanone B (**1**) and its derivatives were investigated. As the modification of the structure of **1**, dihydrochalcone (**11**) was led and showed one order higher activity (IC₅₀: 0.2 μ g/mL) than methylophiopogonanone B (**1**) (IC₅₀: 2.2 μ g/mL).

Angiogenesis is a key step during tumor progression, and the production of vascular endothelial growth factor (VEGF) plays central role during this step.¹ Transcription of the VEGF gene is enhanced under hypoxic conditions and controlled primarily by hypoxia response factor-1 (HRF-1).^{2,3} Hypoxia-inducible factor (HIF)-1 is a heterodimeric protein comprising HIF-1 α and HIF-1 β , both of which are basic helix-loop-helix transcription factors.⁴ Whereas HIF-1 β is constitutively expressed, HIF-1 α is stabilized under condition of hypoxia and activates hypoxia-inducible genes such as those for erythropoietin^{5,6} and VEGF.^{2,7} In the presence of oxygen, HIF-1 α is hydroxylated at specific proline residues and targeted for ubiquitination.⁸ Thus the level of HIF-1 α is critical to the expression of VEGF. Inhibitor of VEGF production are expected to be chemotherapeutic agents for cancer.^{9,10} In previous studies, we established

a high through-put assay system using a stable transformant of mammalian cells that has incorporated the luciferase gene under the control of hypoxia-response element.¹¹ Over 800 compounds including natural and synthetic flavonoids were applied to the reporter assay and methylpogonone B (**1**) (Figure 1) from *Ophiopogon tuberosus* (*Ophiopogon japonicus* KER-GAWLER) was found to be a strong inhibitor of HIF-1 α activity in hypoxia conditions.^{12,13} On the other hand, we established synthetic route for racemic **1** (*rac*-**1**) and found inhibition of the HIF-1 α activity of synthetic *rac*-**1** was as strong as natural **1**.¹⁴ In this study, we report developing stronger inhibitor of HIF-1 α activity by modification of the structure of **1**.

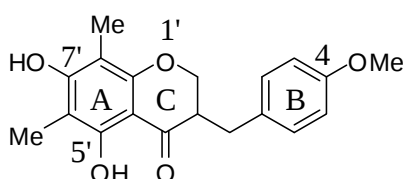
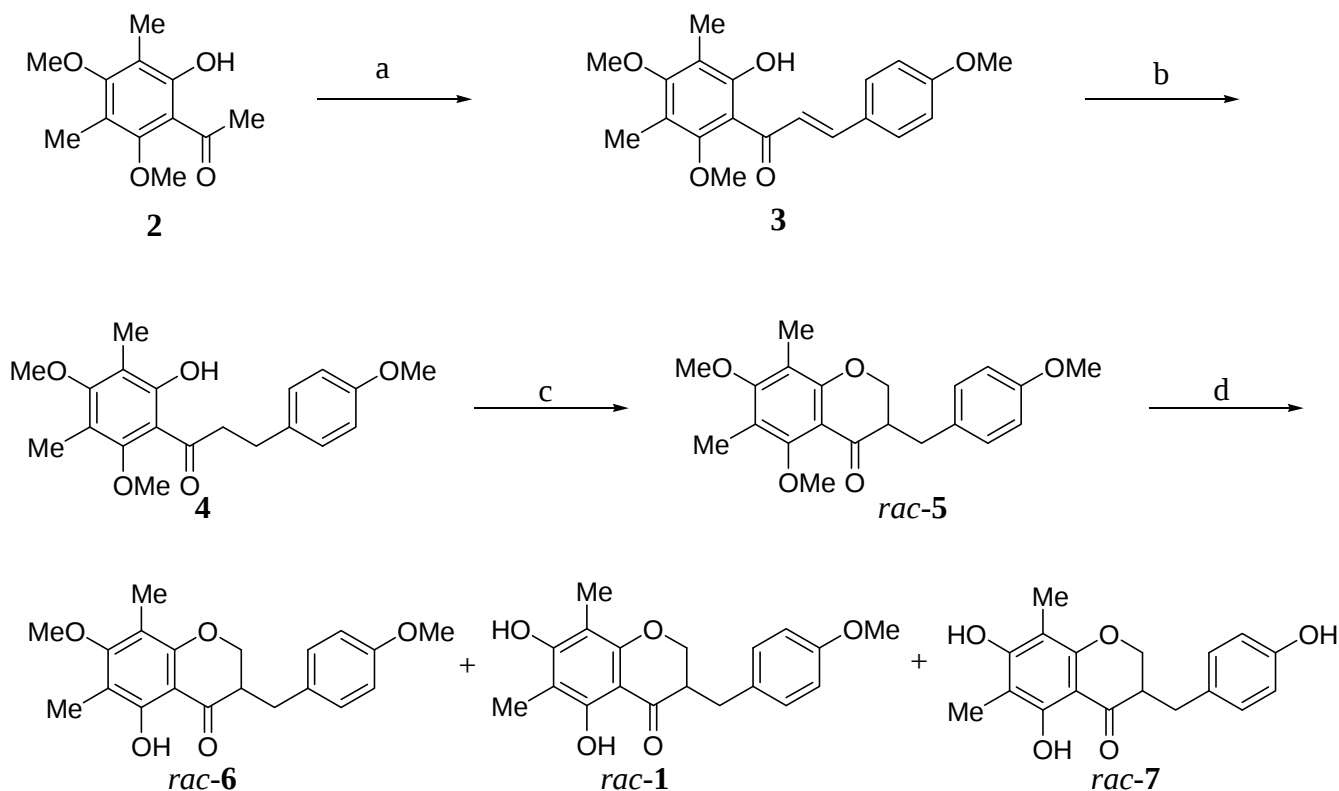


Figure 1. Structure of methylpogonone B (**1**)

Previously, we reported the synthesis of methylpogonone B (*rac*-**1**) from acetophenone derivative **2**.¹⁴ In this procedure, chalcone **3**, dihydrochalcone **4** and homoisoflavanones (*rac*-**5**, *rac*-**6** and *rac*-**7**) were also obtained as intermediates or by-products (Scheme 1).



Scheme 1 Conditions: a; anisaldehyde, KOH, MeOH - H₂O, 84%.
b; H₂, Pd/C, 94%. c; formaldehyde, diethylamine, 94%.
d; TMSI, *rac*-**6**: 18%, *rac*-**1**: 80%, *rac*-**7**: 1.6%.

The compounds, **2**, **3**, **4**, *rac*-**5**, *rac*-**6** and *rac*-**7**, were applied to the reporter-assay, and IC₅₀ for the inhibition of HIF-1 α activity and cell viability were estimated as shown in Table (entry 1-8). Among them, inhibition of the HIF-1 α activity of *rac*-**7** (IC₅₀: 0.8 μ g/mL) and **4** (IC₅₀: 1.1 μ g/mL) were comparatively stronger than that of *rac*-**1** (IC₅₀: 2.2 μ g/mL). This suggests that dihydrochalcone **4** is a better leading compound for inhibition of the HIF-1 α activity since the dihydrochalcone is synthesized much easier than homoisoflavanone.

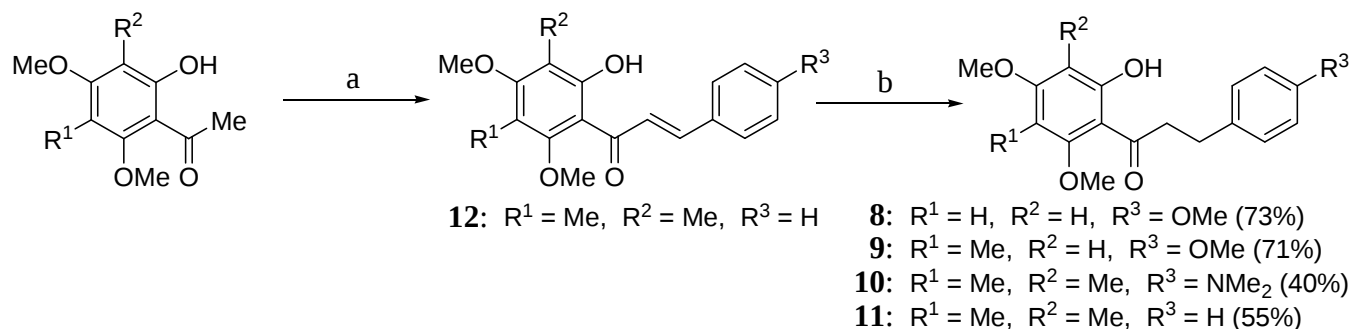
Table IC₅₀ of inhibition of HIF1- α activity and cell viability for synthetic compounds

entry	compound	IC ₅₀ (μ g/mL) for inhibition of HIF1- α activity	IC ₅₀ (μ g/mL) for cell viability
1	<i>rac</i> - 1	2.2	>10.0
2	2	>10.0	>10.0
3	3	3.2	>10.0
4	4	1.1	>10.0
5	<i>rac</i> - 5	6.0	>10.0
6	<i>rac</i> - 6	>10.0	>10.0
7	<i>rac</i> - 7	0.8	7.4
8	8	5.8	>10.0
9	9	>10.0	>10.0
10	10	7.0	>10.0
11	11	0.2	7.5
12	12	>10.0	>10.0

Since dihydrochalcone **4** showed strong inhibition of the HIF-1 α activity, next four dihydrochalcones shown in Scheme 2 were synthesized. In order to investigate the effects of C-methyl group on the A-ring to the activity, dihydrochalcones **8** and **9**, which were replaced one or two C-methyl moieties on A-ring of **4** to hydrogen, respectively, were tested. However the activities of these compounds were much weaker than that of **4** (IC₅₀ for **8**: 5.8 μ g/mL; IC₅₀ for **9**: >10.0 μ g/mL). These results showed the importance of both of two C-methyl groups on A-ring of **4**. On the other hand, dihydrochalcones **10** and **11** were tested to the reporter assay to investigate the effect of substituent at 4-position of B-ring of **4**. While compound **10** (IC₅₀: 7.0 μ g/mL) showed weaker activity than **4**, compound **11** was found to be effective to inhibit the HIF-1 α activity. Inhibition of the HIF-1 α activity of **11** (IC₅₀: 0.2 μ g/mL) showed one order higher than that of *rac*-**1** (IC₅₀: 2.2 μ g/mL). Meanwhile, IC₅₀ for structurally related chalcone **12** was >10.0 μ g/mL.

Several information for structure–activity relationship of **1** were obtained from above results. Firstly, C-ring of **1** is supposed not to be necessary for the strong activity, since dihydrochalcones **4** show much higher activity than structurally similar homoisoflavanone *rac*-**5**. Next, two C-methyl groups on A-ring might be important considering from weaker activity of **8** and **9** than **4**. Substituent on B-ring might be preferred smaller group since unsubstituted **10** (IC₅₀: 7.0 μ g/mL) was stronger inhibitor than **4** (IC₅₀: 1.1

$\mu\text{g/mL}$) possessing methoxy group and **9** (IC_{50} : $>10.0 \mu\text{g/mL}$) possessing dimethylamino group and *rac*-**7** (IC_{50} : $0.8 \mu\text{g/mL}$) possessing hydroxyl group was weaker than *rac*-**1** (IC_{50} : $2.2 \mu\text{g/mL}$) possessing methoxy group. Then B-ring is considered to be important to inhibit HIF-1 α activity, since **2** (Scheme 1) did not show high activity.



Scheme 2 Conditions: a. ArCHO, KOH, MeOH. b. H_2 , Pd/C, AcOEt

In summary, reporter assay for inhibition of the HIF-1 α activity was applied to methylophiopogonanone B (*rac*-**1**) and its derivatives and the activity of dihydrochalcone **11** possessing simple structure showed one order higher than that of *rac*-**1**. Considering from the results of the assay for dihydrochalcones **4**, **10**, and **11**, HIF-1 α inhibitory activity and cytotoxicity were strongly affected by kind of substituent on B ring. Further modification of **11**, especially on B ring, for the purpose of reducing cytotoxicity and enhancing the inhibition of HIF-1 α activity were underwent for discovery of new anticancer agent.

EXPERIMENTAL

Materials

Compound 8, 9, 10 and 11: These compounds were synthesized by condensation of the corresponding acetophenone derivatives and benzaldehyde derivatives as following the reported procedure.¹⁵ The ^1H and ^{13}C NMR of **7** and **8** were identical with those of reported value.¹⁵ **10:** ^1H NMR: δ 2.13 (s, 3H), 2.14 (s, 3H), 3.20 (t, 2H, $J = 7.6$ Hz) 3.42 (t, 2H, $J = 7.6$ Hz), 3.66 (s, 3H), 3.73 (s, 3H), 7.16–7.30 (m, 5H), 13.00 (s, 1H). ^{13}C NMR: δ 8.7, 9.2, 30.6, 44.6, 60.0, 61.7, 111.5, 115.3, 115.6, 126.0, 128.4 (2C), 128.4 (2C), 141.3, 158.8, 161.0, 163.5. IR ν : 2871 (br.), 1613, 1586, 1520, 1408, 1136, 1108 cm^{-1} . EI-HR-MASS calcd for $\text{C}_{21}\text{H}_{27}\text{NO}_4$: 357.1940. Found: 357.1950. **11:** ^1H NMR: δ 2.12 (s, 3H), 2.13 (s, 3H), 2.90 (s, 6H), 2.92 (t, 2H, $J = 7.7$ Hz) 3.37 (t, 2H, $J = 7.7$ Hz), 3.66 (s, 3H), 3.72 (s, 3H), 6.69 (d, 2H, $J = 8.4$ Hz), 7.10 (d, 2H, $J = 8.4$ Hz), 13.04 (s, 1H). ^{13}C NMR: δ 8.7, 9.2, 29.8, 40.9 (2C), 45.1, 60.0, 61.7, 111.5, 113.0 (2C), 115.2, 115.5, 129.0 (2C), 148.4, 149.1, 158.8, 161.0, 163.4. IR ν : 2870 (br.), 1614, 1586, 1519, 1407, 1136, 1108 cm^{-1} . EI-HR-MASS calcd for $\text{C}_{19}\text{H}_{22}\text{O}_4$: 314.1518. Found: 314.1517.

Measurement of HIF-1 α inhibitory activity.

Cell Culture: A stable transformant of CHO cells (clone A4-4) was established by the transfection of

HIF-1-dependent luciferase (5XHRE/pGL3/VEGF/E1b) and neomycin-resistant genes as described previously.¹⁶

Methods: Cells were plated into 96-well tissue culture plates (Falcon) at a density of 1×10^4 cells/well, and treated with synthetic compounds 16 h later. They were incubated further 48 h under hypoxic conditions, and harvested for the determination of luciferase activity. The assay was carried out using a kit provided by Promega Corp. (Madison, WI, USA) following the manufacturer's manual. The cell viability was estimated by the MTT method previously reported.¹⁶

ACKNOWLEDGEMENTS

We thank Mr. Takuya Genkai, Mr. Shintaro Koike and Mr. Kijyu Konno (Showa University) for their contribution to this research.

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