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

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Abstract – Inhibition of hypoxia-inducible factor (HIF)-1α activity of methylophiopogonanone B (**1**) and its derivatives were investigated. As the modification of the structure of **1**, dihydrochalcone (**11**) was leaded and showed one order higher activity (IC_{50}: 0.2 μg/mL) than methylophiopogonanone B (**1**) (IC_{50}: 2.2 μg/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-1α and HIF-1β, both of which are basic helix-loop-helix transcription factors.[4] Whereas HIF-1β is constitutively expressed, HIF-1α 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-1α is hydroxylated at specific proline residues and targeted for ubiquitination.[8] Thus the level of HIF-1α 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 methylophiopogonanone B (**1**) (Figure 1) from Ophiopogonis tubers (*Ophiopogon japonicus* KER-GAWLER) was found to be a strong inhibitor of HIF-1α 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-1α activity of synthetic *rac*-**1** was as strong as natural **1**.[14] In this study, we report developing stronger inhibitor of HIF-1α activity by modification of the structure of **1**.

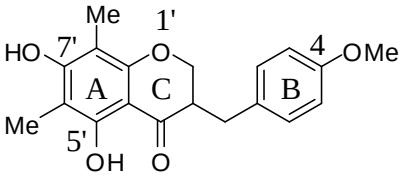
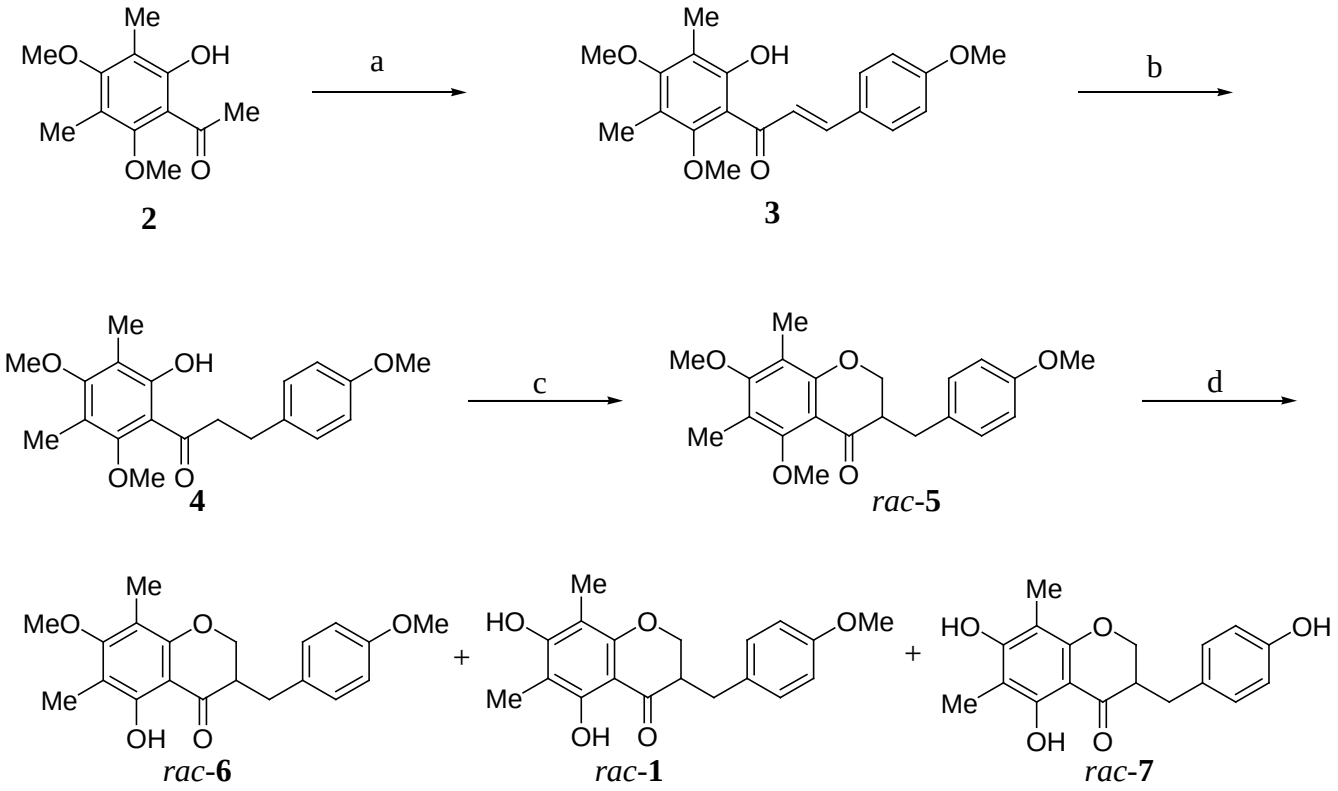


Figure 1. Structure of methylophiopogonanone B (**1**)

Previously, we reported the synthesis of methylophiopogonanone 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 - H_2O, 84%.
b; H_2, 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 IC_{50} for the inhibition of HIF-1α activity and cell viability were estimated as shown in Table (entry 1-8). Among them, inhibition of the HIF-1α activity of *rac*-**7** (IC_{50}: 0.8 μg/mL) and **4** (IC_{50}: 1.1 μg/mL) were comparatively stronger than that of *rac*-**1** (IC_{50}: 2.2 μg/mL). This suggests that dihydrochalcone **4** is a better leading compound for inhibition of the HIF-1α activity since the dihydrochalcone is synthesized much easier than homoisoflavanone.

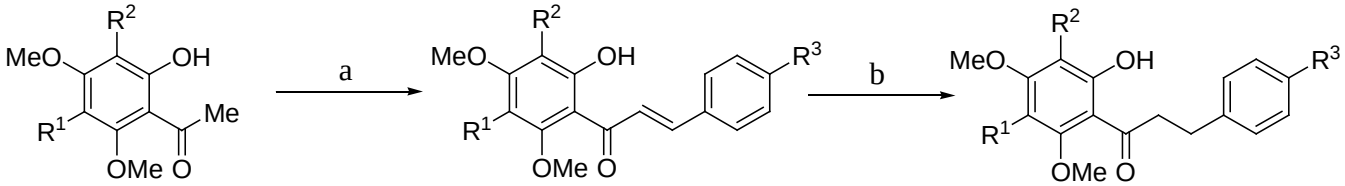
Table IC_{50} of inhibition of HIF1-α activity and cell viability for synthetic compounds

entry	compound	IC_{50} (μg/mL) for inhibition of HIF1-α activity	IC_{50} (μg/mL) for cell viability
1	*rac*-**1**	2.2	>10.0
2	**2**	>10.0	>10.0
3	**3**	3.2	>10.0
4	**4**	1.1	>10.0
5	*rac*-**5**	6.0	>10.0
6	*rac*-**6**	>10.0	>10.0
7	*rac*-**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-1α activity, next four dihydrochlacones 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** (IC_{50} for **8**: 5.8 μg/mL; IC_{50} for **9**: >10.0 μg/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** (IC_{50}: 7.0 μg/mL) showed weaker activity than **4**, compound **11** was found to be effective to inhibit the HIF-1α activity. Inhibition of the HIF-1α activity of **11** (IC_{50}: 0.2 μg/mL) showed one order higher than that of *rac*-**1** (IC_{50}: 2.2 μg/mL). Meanwhile, IC_{50} for structurally related chalcone **12** was >10.0 μg/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** (IC_{50}: 0.2 μg/mL) was stronger inhibitor than **4** (IC_{50}: 1.1

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



12: R^1 = Me, R^2 = Me, R^3 = H

8: R^1 = H, R^2 = H, R^3 = OMe (73%)
9: R^1 = Me, R^2 = H, R^3 = OMe (71%)
10: R^1 = Me, R^2 = Me, R^3 = NMe_2 (40%)
11: R^1 = Me, R^2 = Me, R^3 = H (55%)

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

In summary, reporter assay for inhibition of the HIF-1α 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-1α inhibitory activity and cyototoxicity 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-1α 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 ^{1}H and ^{13}C NMR of **7** and **8** were identical with those of reported value.[15] **10**: ^{1}H 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 $C_{21}H_{27}NO_4$: 357.1940. Found: 357.1950. **11**: ^{1}H 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 $C_{19}H_{22}O_4$: 314.1518. Found: 314.1517.

Measurement of HIF-1α inhibitory activity.

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

HIF-1-dependent luciferease (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 X 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]

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