Hypoxia-Inducible Factor-1 Inhibitory Benzofurans and Chalcone-Derived Diels−Alder Adducts from *Morus* Species

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Hypoxia-inducible factor-1 (HIF-1) is the central mediator of cellular responses to low oxygen concentrations and vital to many aspects of cancer biology. Bioassay-guided fractionation of the chloroform-soluble extracts of *Morus* species using a hypoxia response element (HRE)-dependent reporter assay led to identification of six benzofurans (**1**−**6**) and two chalcone-derived Diels−Alder adducts (**7**, **8**) from Mori Cortex Radicis and three prenylated benzofurans (**9**−**11**) and four chalcone-derived Diels−Alder adducts (**12**−**15**) from *Morus bombycis*. The structure of the new 2-arylbenzofuran-type compound, moracin Q (**3**), was elucidated by spectroscopic methods, and the absolute configuration of **2** was determined for the first time. The selected compounds (**1**−**3**, **5**, **7**, **9**, **10**, and **12**) from the cell-based reporter assay were found to inhibit hypoxia-induced HIF-1α accumulation in a dose-dependent manner in human hepatocelluar carcinoma cell-line Hep3B cells. Furthermore, these compounds were also active against hypoxia-induced vascular endothelial growth factor (VEGF) secretion in Hep3B cells.

Mammalian tumor cells exhibit many adaptive responses to hypoxic conditions, such as the transcriptional activation of VEGF, erythropoietin, and other proteins in order to enhance oxygen delivery to cells.[1] This adaptation is mediated by a transcription factor, HIF-1, a heterodimer protein comprising HIF-1α and HIF-1β. HIF-1α protein is degraded rapidly under normoxic conditions and stabilized under hypoxic conditions, while HIF-1β protein is constitutively expressed.[2,3] Under hypoxia, HIF-1 binds to the hypoxia response element (HRE) in the promoter of target genes and activates transcription. The overexpression of the oxygen-regulated HIF-1α causes the proliferation of tumor cells and resistance against chemotherapy and radiotherapy in cancer treatment.[3,4] Accordingly, inhibition of HIF-1 represents a promising approach to cancer chemotherapy. At present, a number of HIF-1 inhibitors including natural antagonists are in clinical trials as anticancer agent.[5,6]

Mori Cortex Radicis, the root bark of some *Morus* species, has been used in Oriental medicine as antidiabetic, diuretic, expectorant, and laxative agents.[7] From these species, a series of prenylated flavonoids, benzofurans, and other phenolic compounds[7−9] with various biological activities including cytotoxicity[10] and inhibition of COX-1, COX-2, and NO production[11] have been reported.

As part of our program on small-molecule HIF-1 inhibitors, a cell-based HRE reporter assay was performed for natural products, among which the chloroform-soluble extracts of *Morus* species (Mori Cortex Radicis and *Morus bombycis*) were found to show inhibitory effects on HIF-1 activation by hypoxia. The bioassay-guided fractionation led to the isolation of eight compounds (**1**−**8**) from the chloroform-soluble extracts of Mori Cortex Radicis and seven compounds (**9**−**15**) from the chloroform-soluble extracts of *M. bombycis*, respectively. These compounds are 2-arylbenzofuran-based derivatives, including moracin O (**1**), moracin P (**2**),[12] moracin Q (**3**), moracin M (**4**),[13] mulberrofuran H (**5**),[14] mulberrofuran G (**6**),[8] albafuran A (**9**),[15] mulberrofurans D (**10**)[16] and W (**11**),[17] and chalcone-derived Diels−Alder-type adducts sanggenons O (**7**) and C (**8**)[18,19] and kuwanons J (**12**), Q (**13**), R (**14**), and V (**15**).[20] The structure of the new compound moracin Q (**3**) was identified by spectroscopic methods, and the (2″*R*) absolute configuration of moracin P (**2**) was determined for the first time by Mosher's method[21] (see Supporting Information). The known compounds were identified through the interpretation of their physical and spectroscopic data in comparison with those in the literature. The biological evaluation of the isolated compounds was carried out using a luciferase reporter assay followed by a HIF-1α accumulation assay and detection of secreted VEGF by ELISA in the human hepatocarcinoma Hep3B cell line. Herein, we describe the isolation, structure elucidation, and biological characterization of these compounds.

Table 1. IC_{50} Values[a] (μM) of **1**−**15** on the Inhibition of HIF-1 Activity, VEGF Secretion, and Cell Viability

compound	HIF-1	VEGF	cell viability
moracin O (**1**)	0.14[b] ± 0.02	7.22 ± 2.13	>30
moracin P (**2**)	0.65[b] ± 0.13	10.6 ± 2.46	>30
moracin Q (**3**)	5.88 ± 0.54	9.28 ± 1.34	>30
moracin M (**4**)	na[d]	na	>30
mulberrofuran H (**5**)	140[b] ± 0.08	15.4 ± 1.52	>30
mulberrofuran G (**6**)	2.60 ± 0.74	28.6 ± 1.17	>30
sanggenon O (**7**)	1.03 ± 1.43	2.08 ± 0.63	8.75 ± 2.04
sanggenon C (**8**)	1.26 ± 2.01	3.20 ± 0.71	8.26 ± 1.32
albafuran A (**9**)	3.51 ± 1.65	2.62 ± 0.88	6.27 ± 1.74
mulberrofuran D (**10**)	4.05 ± 1.36	2.17 ± 1.17	8.76 ± 1.95
mulberrofuran W (**11**)	3.62 ± 1.61	3.05 ± 1.14	6.07 ± 1.88
kuwanon J (**12**)	4.10 ± 1.43	3.14 ± 1.45	8.55 ± 3.04
kuwanon Q (**13**)	3.80 ± 0.87	4.24 ± 1.32	5.94 ± 2.67
kuwanon R (**14**)	3.17 ± 1.27	3.51 ± 0.95	6.42 ± 1.25
kuwanon V (**15**)	8.32 ± 2.37	7.84 ± 2.66	9.54 ± 2.48
17-DMAG[c]	57.2[b] ± 0.22	79.5[b] ± 0.96	>30

[a] Data are means ± SD from three separate experiments. [b] Values in nM. [c] Positive control. [d] na: not active (>50 μM).

Results and Discussion

Compound **3** was obtained as a brown powder, and its positive HRFABMS showed an $[M + H]^+$ peak at *m*/*z* 357.1338, corresponding to the molecular formula $C_{20}H_{21}O_6$. Its UV absorptions resembled those of **1** and **2**, indicating a 2-arylbenzofuran-type skeleton. The ^1H spectrum of **3** showed the isopropyl signals [δ_H 1.16 (6H, d, J = 7.0 Hz, H-4‴, 5‴), 2.89 (1H, sept, J = 7.0 Hz, H-3‴)], two downfield singlets (δ_H 6.87 and 6.94, each 1H), a methoxy resonance (δ_H 3.77, 3H), and *meta*-coupled aromatic protons [δ_H 6.75 (2H, d, J = 2.0 Hz, H-2′, 6′) and 6.25 (1H, t, J = 2.0 Hz, H-4′)]. The ^{13}C and DEPT NMR data were similar to those

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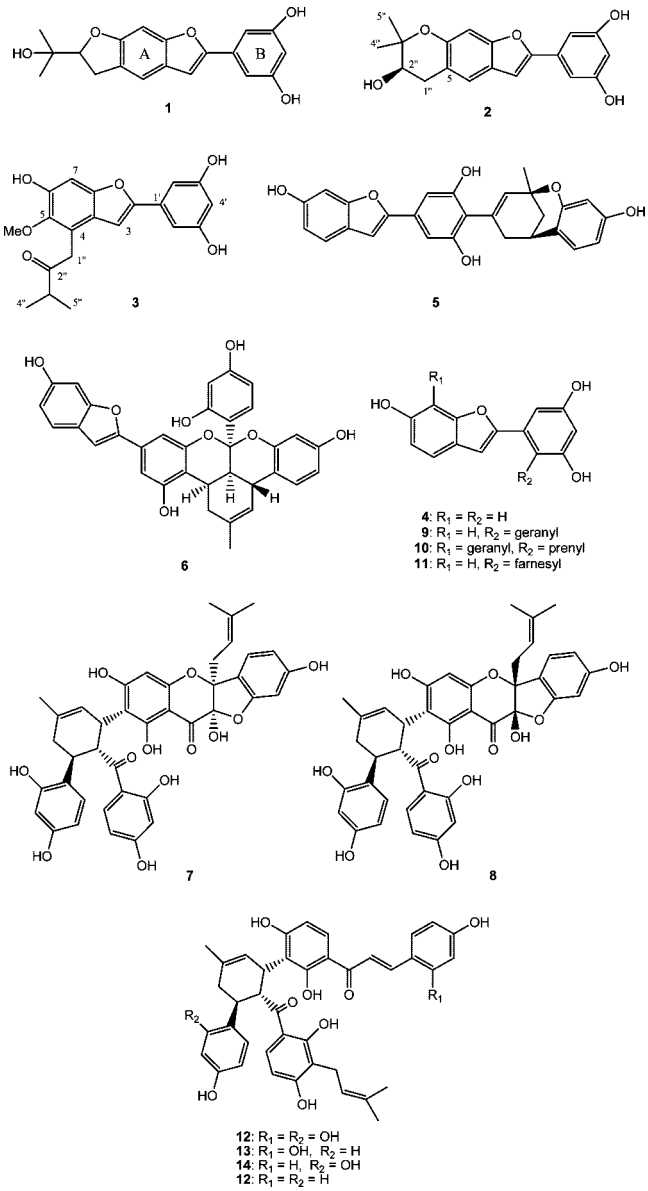

of mulberrofuran Y[10] except for the replacement of the geranyl chain by a 3-methyl-2-butanone moiety.[22] The HMBC correlations (see Supporting Information) indicated that the side chain was attached to C-4 and the methoxy group located at C-5. Thus, **3** was identified as a new 2-arylbenzofuran derivative, named moracin Q.

For the biological evaluation of the isolated compounds as inhibitors of HIF-1 activation, we carried out a luciferase reporter assay in human hepatocellular carcinoma cell-line Hep3B cells transfected with the HRE-luc reporter construct.[27] Of these compounds, **1**, **2**, and **5** potently inhibited HRE-dependent reporter expression under hypoxia in the range of nanomolar concentrations without cytotoxicity up to >30 μM (Table 1). The prenylated compounds (**9**−**11**) and chalcone-derived Diels−Alder adducts (**7**, **8**, **12**−**15**) also showed strong HIF-1 inhibitory effect in the Hep3B cell-based assay. However, moracin M (**4**), the backbone structure of the 2-arylbenzofuran compounds, was inactive.

To confirm whether these compounds inhibit the hypoxia-induced accumulation of HIF-1α protein, **1**−**3**, **5**, **7**, **9**, **10**, and **12** were selected to evaluate their effect on the HIF-1α protein levels in Hep3B cells by Western blot analysis. The results (Figure 2) showed that all the tested compounds dose-dependently inhibited the hypoxia-induced HIF-1α accumulation in Hep3B cells. For compounds **1**, **2**, and **5**, however, much higher concentrations were required for effective inhibition in this experiment in comparison with the effective concentrations in the HRE-dependent reporter assay. A possible explanation for this might be that these compounds interfere with the luciferase expression and/or luciferase-based assay system.[23−25] Therefore, the representative compound **1** was tested for its effect on the luciferase expression from the control construct (pGL3-control) and on the luciferase reaction.[18,19] As shown in Figure 2, compound **1** was found to inhibit the luciferase expression at an IC_{50} value of 1.79 nM and intervene in the luciferase reaction with an EC_{50} of 1.0 nM, respectively. These results indicate that the additional inhibitory effects of **1**, **2**, and **5** in the luciferase reporter assay may be responsible for the discrepancy in effective concentrations in the reporter assay versus Western blot analysis, which also serves as a caution to researchers in this field. This observation, therefore, shows the importance of a secondary confirmation assay, such as Western blot analysis for HIF-1α accumulation under hypoxia, for interpretation of primary HRE reporter assay data.

The expression of several HIF-1 target genes, such as VEGF, is induced by hypoxia in most cell types. In particular, VEGF stimulates new blood vessel formation, tumor growth, and metastasis. This makes hypoxic tumors highly pro-angiogenic tumors with a highly aggressive phenotype.[1] Accordingly, the effects of isolated compounds on VEGF production were further evaluated by ELISA assay to measure the secreted VEGF levels in the Hep3B cell culture media. As shown in Table 1, the compounds effective on HIF-1α accumulation, including **1**, were found to be active against hypoxia-induced VEGF secretion, and most of the compounds exhibited IC_{50} values comparable to the reporter assay results.

Moracins O (**1**) and P (**2**), with an additional oxygenated heterocycle coupled to the A ring, presumably derived from moracin M (**4**) via 5-prenylation and subsequent cyclization, showed potent activity in the reporter assay, while compounds lacking this heterocycle (**3**−**6**) did not. This observation suggests that an oxygenated heterocycle attached to the A ring of the 2-arylbenzofuran skeletons may be key to both inhibitory effects on HIF-1 activation and interference with luciferase activity in the HRE-dependent luciferase assay. However, the strong intrinsic HIF-1 inhibitory effects of **1**, **2**, and **5** were confirmed by Western blot analysis for the HIF-1α accumulation and by ELISA assay for the VEGF secretion (Figure 1 and Table 1). On the other hand, **4** did not show any inhibitory activity, while isoprenoid-substituted compounds (**9**−**11**) showed stronger effects on HIF-1 activity and VEGF secretion. These results suggest that the prenyl, geranyl, and/or farnesyl moieties of the 2-arylbenzofuran compounds are essential for the HIF-1 inhibitory activity.

In summary, although a variety of biological activities have been reported for the chemical constituents of *Morus* species, the potent HIF-1 inhibitory effects of the benzofuran- and chalcone-derived Diels−Alder-type compounds isolated from *Morus* species are reported for the first time. Further studies are in progress to comprehend the mechanism of action of the compounds inhibiting hypoxia-induced HIF-1 activation, as well as their effects on tumor growth *in vivo*.

Experimental Section

General Experimental Procedures. Optical rotation was measured on a JASCO P-1020 polarimeter. UV spectra were recorded on a UV-1601 spectrometer. NMR experiments were performed on a Varian^{unity} Ionva-400 instrument. ESIMS and HRFABMS spectra were recorded on a Finnigan Navigator LC/MS/DS system and a JMS-HX/HX 110A tandem mass spectrometer, respectively. Preparative HPLC was performed using a Waters system with a 515 pump and a 2996 photodiode array detector.

Plant Material. The dry material of Mori Cortex Radicis (30 kg) and *Morus bombycis* (2.5 kg) was purchased from the herbal medicine store in Daejeon, Korea, and identified by one of us (Y.H.K.). Voucher

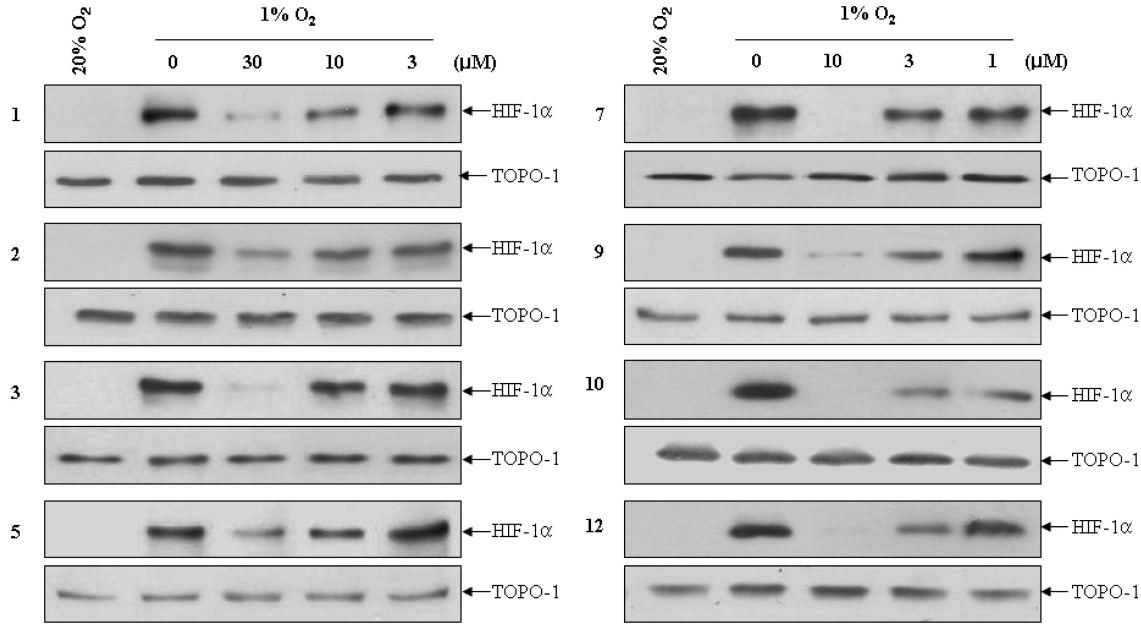

Figure 1. Effect of compounds on the HIF-1α accumulation. Hep3B cells were exposed to various concentrations of compounds for 30 min and then incubated for 12 h under normoxic or hypoxic conditions. The relative levels of HIF-1α protein in nuclear extract were determined by Western blot analysis using mouse monoclonal anti-HIF-1α antibody. TOPO-1 was detected for loading control.

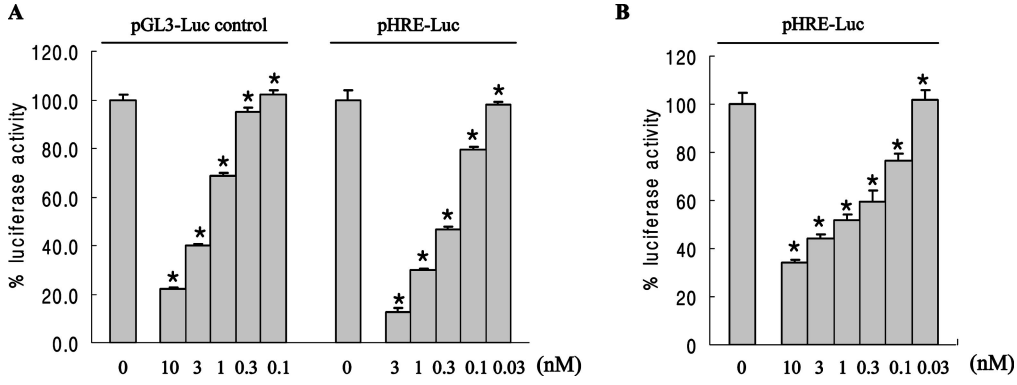

Figure 2. Effect of **1** on the luciferase expression and luciferase activity measurement. (A) Relative luciferase activities of **1** on the pGL3-Luc and pHRE-Luc constructs. Hep3B cells were transfected with pGL3-Luc or pHRE-Luc vectors. After 48 h, cells were treated with **1** in hypoxic conditions for 16 h; then the cells were lysed and luciferase activities were measured.[25,27] (B) Transfected Hep3B cells were exposed to hypoxic conditions for 16 h and then cell lysates were prepared. Various concentrations of **1** were directly added to the cell lysates, and the luciferase activity was immediately measured.[26] Bars represent standard deviations from three experiments. Asterisks (*) indicate a significance of $p < 0.05$ when compared to the untreated control.

specimens have been deposited in the Molecular Anticancer Research Center, Korean Research Institute of Biosciences and Biotechnology, Daejeon, Korea.

Extraction and Isolation. The dry material of Mori Cortex Radicis (30 kg) was powdered and extracted with MeOH (3 × 30 L). The MeOH extract was concentrated, and the residue (1670 g) was suspended in H_2O (5 L) and partitioned with *n*-hexane (3 × 2 L) and $CHCl_3$ (3 × 3 L), successively. The $CHCl_3$-soluble extract was concentrated *in vaccuo* to give a brown solid (939 g), which was then chromatographed on a silica gel column (1000 g) using a $CHCl_3$−MeOH gradient as a mobile phase to afford four fractions, A1−A4. Repeated silica column chromatography of A3 using 30:1 $CHCl_3$−MeOH as a mobile phase resulted in two fractions, B1 and B2. Fraction B1 was subjected to a Sephadex LH-20 column, eluted by 1:1 $CHCl_3$−MeOH, to give four subfractions, C1−C4. Of these, C3 was eluted over a reversed-phase column (ODS-60-I40/63, YMC, Japan) and then purified by preparative HPLC (YMC Pack Pro C18, 250 × 10 mm, 20% CH_3CN in H_2O, flow rate 4 mL/min) to afford **1** (12.7 mg) and **2** (42 mg). Compound **4** (2.6 mg) was obtained from fraction C2 by preparative HPLC (J'sphere ODS-H80, 250 × 10 mm, 35% CH_3CN in H_2O, flow rate 10 mL/min). Fraction B2 was chromatographed on a Sephadex LH-20 column, resulting in six subfractions, D1−D6. Preparative HPLC (J'sphere ODS-H80, 250 × 10 mm, 40% CH_3CN in H_2O, flow rate 10 mL/min) gave rise to **3** (2.5 mg) from subfraction D2 and **5** (5.5 mg) and **6** (8.0 mg) from subfraction D6. Repeated similar preparative HPLC of fraction A4 afforded **7** (70 mg) and **8** (10 mg).

The dry bark of *M. bombycis* (2.5 kg) was powdered and extracted with MeOH (3 × 5 L). The MeOH extract was concentrated, and the residue (72 g) was suspended in H_2O (1 L) and partitioned with *n*-hexane (3 × 0.5 L) and $CHCl_3$ (3 × 0.5 L), successively. The $CHCl_3$-soluble extract was concentrated *in vacuo* to give a brown solid (15.5 g), which was then chromatographed on a silica gel column (1000 g) using a $CHCl_3$−MeOH gradient as a mobile phase to afford two fractions, A1 and A2. Fraction A2 was subjected to preparative HPLC (YMC Pack Pro C18, 250 × 10 mm, 60% CH_3CN in H_2O, flow rate 10 mL/min) to afford **9** (7.5 mg), **11** (7.2 mg), **12** (80.0 mg), **13** (2.5 mg), and **14** (8.7 mg). Fraction A1 was chromatographed on a silica column eluted with an *n*-hexane−EtOAc gradient to give five subfractions, B1−B5. The preparative HPLC (YMC Pack Pro C18, 250 × 10 mm, 60% CH_3CN in H_2O, flow rate 10 mL/min) of fractions B4 and B5 gave **15** (41.3 mg) and **10** (160.0 mg), respectively.

(−)-Moracin O (1): brown powder; $[\alpha]_D^{28}$ −4.2 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 219.1 (3.58), 320.8 (3.64), 335.0 (3.60);

ESIMS *m/z* 325.3 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[12]

(−)-Moracin P (2): brown powder; $[\alpha]_D^{28}$ −15.9 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 219.1 (3.83), 321.4 (3.88), 338.5 (3.85); ESIMS *m/z* 325.3 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[12]

Moracin Q (3): brown, amorphous powder; UV (MeOH) λ_{max} (log ϵ) 219.0 (3.32), 322.0 (3.38), 335.4 (3.23); ^{1}H (CD_3OD, 400 MHz) δ 1.16 (6H, d, J = 7.0 Hz, H-4″, 5″), 2.89 (1H, sept, J = 7.0 Hz, H-3″), 3.77 (3H, br s, O*CH*$_3$), 4.06 (2H, br s, H-1″), 6.25 (1H, t, J = 2.0 Hz, H-4′), 6.75 (2H, d, J = 2.0 Hz, H-2′, 6′), 6.87 (1H, s, H-3), 6.94 (1H, s, H-7); ^{13}C NMR (CD_3OD, 100 MHz) δ 214.8 (C, C-2″), 160.1 (C, C-3′, 5′), 156.6 (C, C-2), 152.6 (C, C-7a), 150.0 (C, C-6), 144.5 (C, C-5), 133.7 (C, C-1′), 123.4 (C, C-3a), 121.2 (C, C-4), 104.0 (CH, C-2′, 6′), 103.7 (CH, C-4′), 101.2 (CH, C-3), 98.8 (CH, C-7), 61.2 (CH_3, O*CH*$_3$), 41.2 (CH, C-3″), 40.8 (CH_2, C-1″), 19.0 (CH_3, C-4″, 5″); negative ESIMS *m/z* 355.4 [M − H]$^-$; positive HRFABMS *m/z* 357.1338 (calcd for $C_{20}H_{21}O_6$, 357.1335).

Moracin M (4): brown powder; UV (MeOH) λ_{max} (log ϵ) 218 (3.658), 316 (3.66); ESIMS *m/z* 241.3 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[13]

(+)-Mulberrofuran H (5): brown powder; $[\alpha]_D^{28}$ + 4.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (3.92), 290 (3.61), 322 93.92); ESIMS *m/z* 443.5 [M + H]$^+$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[14]

(+)-Mulberrofuran G (6): brown powder; $[\alpha]_D^{28}$ + 58.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.89), 285 (3.47), 320 (3.65); ESIMS *m/z* 563.6 [M + H]$^+$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[8]

(+)-Sanggenon C (7): yellow powder; $[\alpha]_D^{28}$ + 265.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (4.16), 308 (3.97); ESIMS *m/z* 709.4 [M + H]$^+$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[18,19]

(−)-Sanggenon O (8): yellow powder; $[\alpha]_D^{28}$ −15.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (3.67), 287 (3.24), 309 (3.22); ESIMS *m/z* 709.4 [M + H]$^+$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[18,19]

Albafuran A (9): yellow powder; UV (MeOH) λ_{max} (log ϵ) 215 (4.02), 310 (3.65); ESIMS *m/z* 377.4 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[15]

Mulberrofuran D (10): yellow powder; UV (MeOH) λ_{max} (log ϵ) 210 (4.15), 310 (3.92); ESIMS *m/z* 445.5 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[16]

Mulberrofuran W (11): yellow powder; UV (MeOH) λ_{max} (log ϵ) 215 (4.09), 295 (3.68), 372 (3.97); ESIMS *m/z* 445.5 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[17]

(+)-Kuwanon J (12): yellow powder; $[\alpha]_D^{28}$ + 26.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.62), 305 (4.35), 388 (4.49); ESIMS *m/z* 677.5 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[20]

(+)-Kuwanon Q (13): yellow powder; $[\alpha]_D^{28}$ + 104.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.10), 304 (3.89), 385 (3.96); ESIMS *m/z* 661.5 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[20]

(+)-Kuwanon R (14): yellow powder; $[\alpha]_D^{28}$ + 45.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.31), 305 (4.02), 370 (4.17); ESIMS *m/z* 661.5 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[20]

(+)-Kuwanon V (15): yellow powder; $[\alpha]_D^{28}$ + 87.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.57), 302 (4.26), 370 (4.41); ESIMS *m/z* 645.5 [M − H]$^-$; ^{1}H and ^{13}C NMR data were in agreement with those previously published.[20]

Cell Culture. Human hepatocellular carcinoma cell-line Hep3B cells (American Type Culture Collection, Manassas, VA) were cultured in DMEM (Invitrogen, Grand Island, NY) supplemented with 10% (V/V) fetal bovine serum (Hyclone, Logan, UT), penicillin, and streptomycin in a humidified 5% CO_2 atmosphere at 37 °C. Hypoxic cultures were kept in a gas-controlled chamber (Thermo Electron Corp., Marietta, OH) maintained at 1% O_2, 94% N_2, and 5% CO_2 at 37 °C.

Reporter Assay. The ability of compounds to inhibit HIF-1 was determined by a reporter assay as previously described.[27] Cells were transiently transfected with the vectors for pGL3-HRE-luciferase plasmid, which contains six copies of HREs derived from the human VEGF gene, and pRL-CMV (Promega, Madison, WI) using Lipofectamine Plus reagent according to the instructions of the manufacturer (Invitrogen). pGL3-luciferase construct was used to check nonspecific effect on luciferase activity. Luciferase activity was determined, and the results were normalized to the activity of *Renilla* luciferase expressed by co-transfected *Rluc* gene under the control of a constitutive promoter. 17-DMAG (Calbiochem, La Jolla, CA) was used as a positive control.[3]

Cytotoxicity Assay. Cell viability was evaluated by the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) uptake method. Cells were seeded in a 96-well plate at 4×10^4 cells/well and incubated at 37 °C for 3 h, and then cells were treated with various concentrations of test compounds. After 24 h incubation at 37 °C, 10 μL of 5 mg mL^{-1} MTT solution was added to each well and incubated for another 4 h. After removing the supernatant, formazan crystals were dissolved in 100 μL of DMSO and the optical density values were measured at 570 nm with a microplate reader.

Western Blot Analysis. To evaluate the level of HIF-1α proteins, total cell lysates were analyzed as described[27] by Western blotting using anti-HIF-1α monoclonal antibody. Protein loading was controlled by probing the membranes for topoisomerase-1 (TOPO-1) protein with an antitopoisomerase-1 antibody.

VEGF ELISA. Cells were plated in a 96-well plate at a density of 2×10^5 cells per well and treated with various concentrations of compounds for 16 h under normoxic or hypoxic conditions. The VEGF levels in culture supernatant of hypoxia-stimulated Hep3B cells were determined by using the DuoSet ELISA Ddevelopment kit (R&D Systems, Inc., Minneapolis, MN) according to the manufacture's instruction as previously described.[27]

Statistical Analysis. Each experiment was performed at least three times, and representative data are shown. Data in the table were given as mean values ± standard deviation from separate experiments. Means were checked for statistical differences by using the Student's *t*-test with error probabilities of $p < 0.05$.

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Supporting Information Available: Mosher's method for the determination of absolute configuration of **2**, key HMBC correlations of **3**, and the effect of solvent extracts of *Morus* species on HIF-1 activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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