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Analysis of HIF-1 inhibition by manassantin A and analogues with modified tetrahydrofuran configurations

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ABSTRACT

We have shown that manassantin A downregulated the HIF-1α expression and inhibited the secretion of VEGF. We have also demonstrated that the 2,3-*cis*-3,4-*trans*-4,5-*cis*-configuration of the tetrahydrofuran is critical to the HIF-1 inhibition of manassantin A.

Molecular oxygen (O_2) is required for aerobic metabolism to maintain intracellular bioenergetics and to serve as an electron acceptor in many organic and inorganic reactions.[1] Hypoxia, usually defined as $\leqslant$2% of O_2, occurs in a variety of pathological conditions, including stroke, tissue ischemia, inflammation, and tumor growth.[2] Mammalian tissues have developed a number of essential mechanisms to cope with the stress of hypoxia. Among these coping mechanisms is the response mediated by the hypoxia-inducible transcription factor 1 (HIF-1). It is a basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) family protein that forms a heterodimer with its α and β subunits and acts as a transcription factor.[3] There are two additional HIF-1α-related bHLH-PAS proteins: HIF-2α and HIF-3α.[4] Like HIF-1α, they also bind to HIF-1β (ARNT) for activation. HIF-1 is a main regulator of hypoxia since it activates more than 60 genes involved in angiogenesis (VEGF), glucose transport (GLUT1), glycolytic pathways (LDHA), ROS signals (iNOS), and erythropoiesis (EPO), as well as a number of other processes.[5]

Through HIF-1, tumors adapt to hypoxia by increasing angiogenesis and metastatic potential, altering apoptosis, and regulating metabolism.[6] These adaptations make tumors more aggressive and treatment-resistant resulting in poor patient prognosis.[7] Immunohistochemical analyses have revealed that HIF-1 is overexpressed in many human cancers.[8] HIF-1 overexpression is not only involved in tumor progression but also associated with resistance to radiation[9] and chemotherapy.[10] It has been shown that inhibition of HIF-1 activity suppresses tumor growth, destroys blood vessels, enhances tumor apoptosis, and increases radiosensitivity,[11] making HIF-1 a good potential target for anti-cancer treatment.

Due to the importance of HIF-1 in tumor development and progression, a considerable amount of effort has been made to identify HIF-1 inhibitors for treatment of cancer.[12] A variety of anticancer drugs, most of which were not developed as HIF-1 inhibitors, have been reported to inhibit HIF-1. However, these compounds possess relatively low HIF-1 inhibitory activity ($\geqslant$micromolar range). In addition, most of them lack the desired selectivity for the HIF-1 signaling pathway or toxicity profiles required for a useful therapeutic agent. The dineolignans manassantin A (**1**), manassantin B (**2**), manassantin B_1 (**3**), and 4-*O*-demethylmanassantin B (**4**)

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(Fig. 1), isolated from the aquatic plant *Saururus cernuus* L. (Saururaceae), have been shown to be potent inhibitors of HIF-1.[13] However, their molecular mechanisms of action have yet to be established. Since manassantins may sensitize cancer cells to chemo- and/or radiotherapy by HIF-1 inhibition,[11c,14] use of manassantins in combination with other cytotoxic drugs and/or radiation has great potential for therapeutic applications.

In broad connection with our interest in the stereoselective synthesis of substituted tetrahydrofurans,[15,16] we recently completed the synthesis of **1** and **2** via a direct nucleophilic addition of an organozinc reagent to a 2-benzenesulfonyl cyclic ether to synthesize the 2,3-*cis*-3,4-*trans*-4,5-*cis*-tetrahydrofuran moiety of **1** and **2**.[17] In addition, we also showed that the (*R*)-configuration at C-7 and C-7‴ is not critical for HIF-1 inhibition and that the hydroxyl group at C-7 and C-7‴can be replaced with carbonyl group without significant loss of activity.

Herein, we present initial biological data demonstrating the significant potential of **1** as a potent HIF-1 inhibitor with little cytotoxicity. In addition, we describe the synthesis and conformation–activity relationship study of tetrahydrofuran core analogues of **1** to characterize the effect of tetrahydrofuran conformation on HIF-1 inhibitory activity.

Previously, we reported that **1** exhibited a highly potent level of HIF-1 inhibitory activity in a luciferase-reporter based assay (IC_{50} = 1–10 nM).[17] Based on the luciferase assay data, we used Western blots to further confirm the HIF-1 inhibitory activity of **1**. 4T1 cells, a mouse mammary carcinoma, were grown under hypoxic conditions (0.5% O_2) for 24 h with various concentrations (0, 1, 10, 100 nM, and 1 μM) of **1**. Western blots were performed on nuclear extracts as reported.[11b] A dose–response study revealed that exposure of 4T1 cells to **1** at concentrations higher than 10 nM for 24 h significantly inhibited hypoxia-induced expression of the HIF-1α protein (Fig. 2a).

To determine if **1** also inhibits chemically-induced HIF-1α expression,[18] we treated 4T1 cells with 240 μM of $CoCl_2$ for 24 h and carried out Western blots with nuclear extracts. HIF-1α expression induced by $CoCl_2$ was inhibited by **1** (100 nM) (Fig. 2b) indicating that **1** inhibits chemically induced HIF-1α expression as well as hypoxia-induced HIF-1α expression. It should be noted that **1** was reported to have no significant effect on iron chelator-induced HIF-1 activation in T47D cells (10 μM 1,10-phenanthroline, 16 h).[13c] To examine if the HIF-1 inhibition by **1** is cell-type specific, we carried out the same experiment with MDA-MB-231, a human breast cancer cell line, and observed the same inhibition effect, which showed that the HIF-1 inhibition effect by **1** is not cell-type specific (Fig. 2b).

As stated earlier, more than 60 genes have been identified as targets of HIF-1. Vascular endothelial growth factor (VEGF) is a gene that is highly involved in tumor progression as a pro-angiogenic factor. The effects of **1** on HIF-1 regulated VEGF secretion were examined in 4T1 cells using ELISA. Cells were incubated under hypoxia (0.5% O_2 for 24 h) with various concentrations (0, 1, 10, 100 nM, and 1 μM) of **1**. Cell culture supernates were collected, and VEGF levels in supernates were measured by a commercially available kit (R&D systems, Minneapolis, MN). As we observed from HIF-1 expression, VEGF induced by hypoxia was significantly inhibited by **1** at concentrations higher than 10 nM (Fig. 3).

Cytotoxicity of **1** was examined using the MTS assay, a standard colorimetric cytotoxicity assay (see Supplementary data for details). 4T1 cells were seeded in a 96-well plate and incubated with serially diluted **1** (0–10 μM) for 24 h. Up to the highest concentration examined (10 μM), cells had ~70% survival rate. Considering that **1** completely inhibited the expression of HIF-1 at the concentration ⩽100 nM, **1** possesses a significant therapeutic window ($IC_{50\ (cytotoxicity)}/IC_{50\ (HIF\text{-}1\ inhibition)} \geqslant 100$).

The configuration of **1** is largely determined by the 2,3-*cis*-3,4-*trans*-4,5-*cis*-configuration of the tetrahydrofuran core. We hypothesized that the overall conformation should be an important determinant for the binding mode and affinity toward molec-

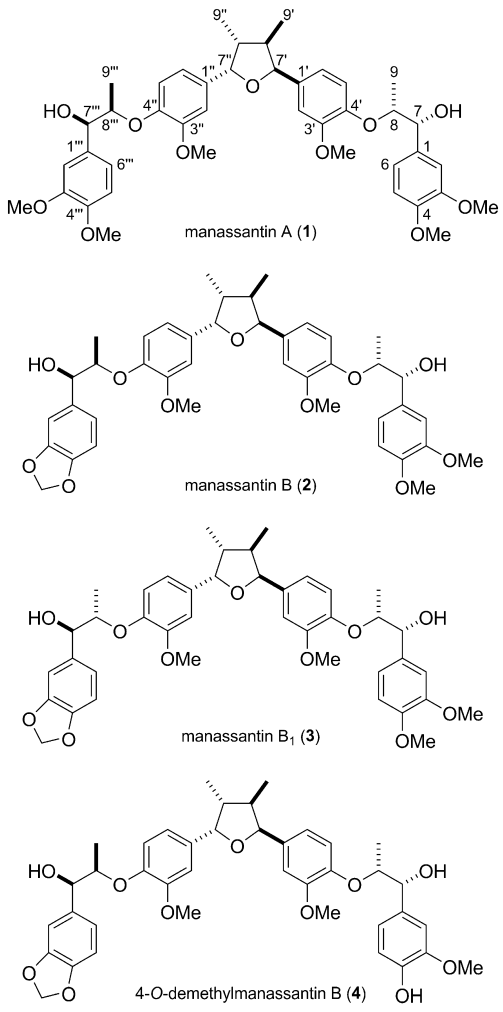


Figure 1. Structure of dineolignans from *Saururus cernuus*.

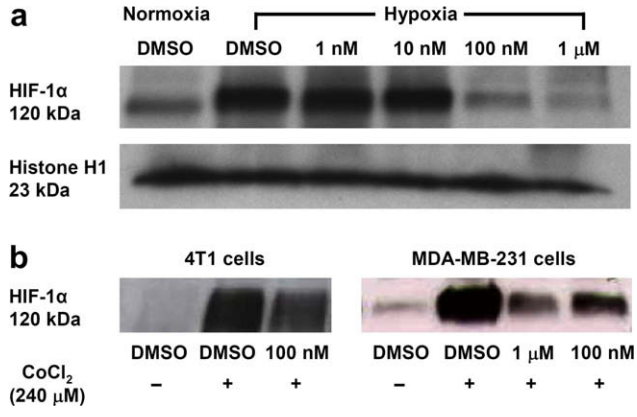


Figure 2. Inhibition of HIF-1α expression by **1**. (a) After treating 4T1 cells under hypoxia (0.5% O_2, 24 h) with and without **1**, HIF-1α expression was evaluated using Western blots. By comparison with loading control (histone H1), nuclear expression of HIF-1α was significantly inhibited by **1** at concentrations higher than 10 nM. (b) In 4T1 and MDA-MB-231 cells, HIF-1α expression induced by $CoCl_2$ (240 μM, 24 h) was also inhibited by **1**.

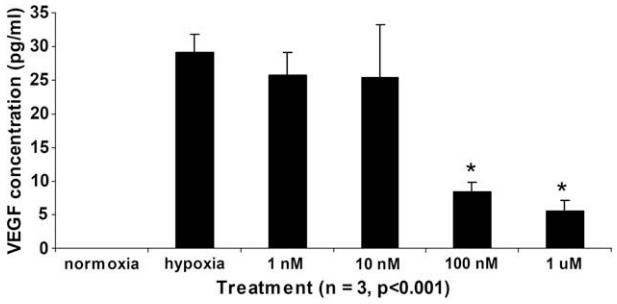


Figure 3. Inhibition of VEGF secretion by **1**. Inhibition of VEGF was determined in 4T1 cells after hypoxia treatment (0.5% O_2, 24 h). After treatment with and without **1**, cell culture supernates were collected, and VEGF secretion was measured using ELISA. Compound **1** significantly inhibited VEGF at concentrations higher than 10 nM ($p < 0.001$).

ular target(s), potency, and HIF-1 signaling specificity of **1**. To test the hypothesis, we prepared and evaluated manassantin A analogues with modifications in tetrahydrofuran configuration. These analogues were easily prepared through the procedures previously reported by our group (Scheme 1).[15,17]

The synthesis of manassantin A analogues with 2,3-*cis*-3,4-*trans*-4,5-*trans*- and 2,3-*trans*-3,4-*trans*-4,5-*trans*-tetrahydrofuran cores (**11** and **13**) was accomplished as described in Scheme 1. Briefly, the 2,3-*cis*-3,4-*trans*-4,5-*trans*-tetrahydrofuran **7a** was prepared via $BF_3{\cdot}OEt_2$-promoted deoxygenation of cyclic hemiketal **6**[15] followed by stereoselective reduction of the oxocarbenium ion intermediate. Deprotection of the Bn and TBS groups, BEMP-mediated coupling, and polymer-supported BH_4-reduction completed the synthesis of **11**.[19] Compound **13** was prepared via $BF_3{\cdot}OEt_2$-promoted epimerization/reductive deoxygenation followed by deprotection, BEMP-mediated coupling, and polymer-supported BH_4-reduction.[20]

To determine HIF-1 inhibitory activity of **11** and **13**, we used a luciferase-reporter based assay as a primary screen. For this assay, we used 4T1-ODD-Luc cells[21] stably transfected with the oxygen-dependent-degradation (ODD) domain of HIF-1α and a firefly luciferase reporter. This ODD-Luc reporter contains a CMV promoter, which is constitutively active. Since its ODD domain is identical to that of HIF-1, it enables us to directly detect the stability of HIF-1. Cells were seeded in the 24-well plate at a density of 10^5 cells/well. After 16-h incubation, cells were treated with 240 μM of $CoCl_2$ and serially diluted compounds for 24 h. Since luciferase requires O_2 for its activity but the ODD-Luc is highly sensitive to reoxygenation, we induced the HIF-1 expression by $CoCl_2$, not by hypoxia to accurately determine the effect of the compounds on HIF-1 stability. Luciferase signals were detected and quantified as relative light units (RLUs). The ODD-Luc assay to assess HIF-1 inhibitory activity of **11** and **13** revealed that **11** was nearly inactive and **13** was less active than **1** by 10-fold (IC_{50} = 47 nM) (Fig. 4). Based on these results, the 2,3-*cis*-3,4-*trans*-4,5-*cis*-configuration of the tetrahydrofuran core is critical for HIF-1 inhibition.

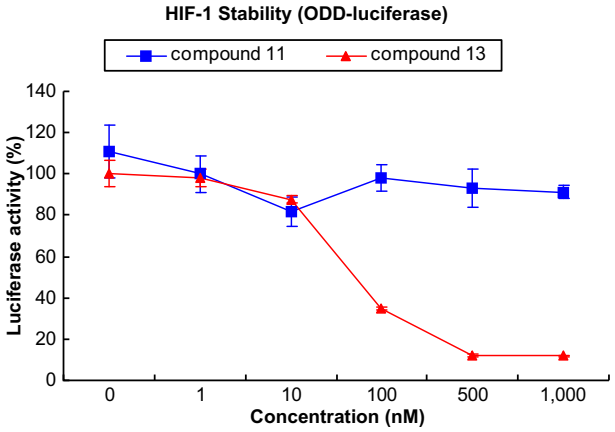
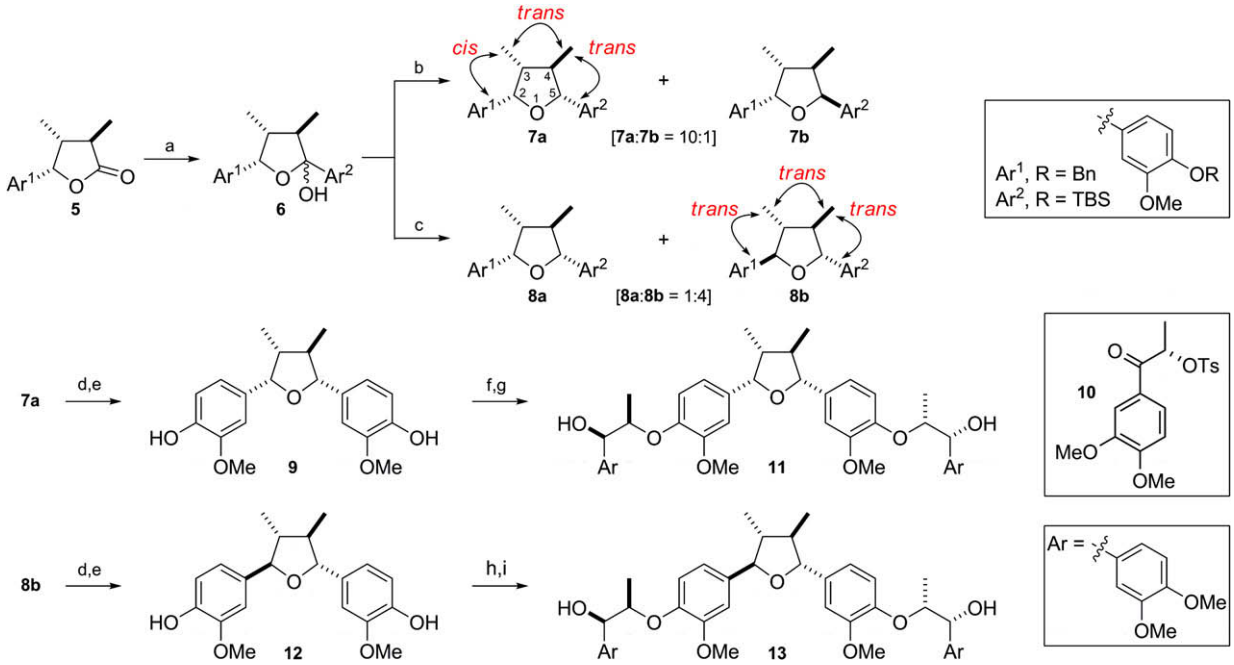


Figure 4. Inhibition of HIF-1 by **11** and **13**.



Scheme 1. Reagent and conditions: (a) Ar^2Li, THF, −78 °C, 40 min, 70%; (b) $BF_3{\cdot}OEt_2$, $NaBH_3CN$, CH_2Cl_2, −78 °C, 30 min, 99%; (c) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 to −20 °C, 2 h; then, $NaBH_3CN$, −78 °C, 30 min, 96%; (d) H_2, Pd/C, EtOAc/EtOH (3:1), 25 °C, 2 h; (e) TBAF, THF, 25 °C, 1 h, 88% for 2 steps; (f) **10**, BEMP, CH_2Cl_2, 25 °C, 18 h, 66%; (g) (polystyrylmethyl)trimethylammonium borohydride, MeOH, 25 °C 48 h, 75%; (h) **10**, BEMP, CH_2Cl_2, 25 °C, 20 h, 92%; (i) (polystyrylmethyl)trimethylammonium borohydride, MeOH, 25 °C, 30 h, 86%.

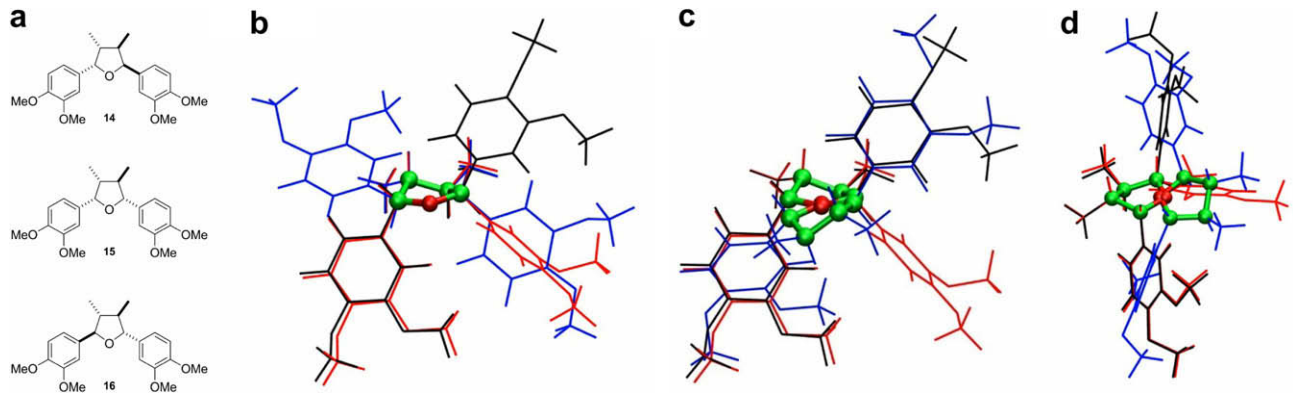


Figure 5. Optimized conformations of truncated structures of **1**, **11**, and **13** (a) truncated structures (**14**–**16**). (b) Overlay I. (c) Overlay II: front view. (d) Overlay II: side view (**14** in black, **15** in red, **16** in blue, and tetrahydrofurans in green).

To further characterize the effect of tetrahydrofuran conformation of **1**, **11**, and **13** on the HIF-1 inhibition, we optimized the conformations of truncated structures[22] using density functional theory (B3LYP)[23] at the 6-31G* level (GAUSSIAN 03, D.02 version[24]). As shown in Figure 5, compound **14** adopted a nearly linear conformation, but compound **15** adopted a bent-shaped conformation remarkably different from that of **14**. However, in one of the two possible orientations for **16** (Overlay II), the conformation was relatively close to that of **14** (Fig. 5c and d)[25] indicating that the linear-shaped conformation resulting from the 2,5-*trans*-configuration is critical to the HIF-1 inhibition. Thus, designing a ligand that mimics the overall conformation of **1** may improve the potency and selectivity toward the hypoxia signaling pathway.

In summary, we have shown that **1** downregulated the HIF-1α expression and inhibited the secretion of VEGF. We have also demonstrated that the 2,3-*cis*-3,4-*trans*-4,5-*cis*-configuration of the tetrahydrofuran is critical to the HIF-1 inhibition of **1**. This conformation–activity relationship study may help to identify structural motifs required for HIF-1 inhibition and allow structural modifications to increase specificity and decrease off-target effects.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.071.

References and notes

1. Semenza, G. L.. *Nat. Rev. Cancer* **2003**, *56*, 721.
2. Bertout, J. A.; Patel, S. A.; Simon, M. C. *Nat. Rev. Cancer* **2008**, *12*, 967.
3. Wang, G. L.; Jiang, B. H.; Rue, E. A.; Semenza, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5510.
4. Flamme, I.; Frolich, T.; Risau, W. *Mol. Pharmacol.* **2006**, *70*, 1469.
5. Lisy, K.; Peet, D. J. *Cell Death Differ.* **2008**, *15*, 642.
6. Vaupel, P. *Semin. Radiat. Oncol.* **2004**, *14*, 198.
7. (a) Brizel, D. M.; Sibley, G. S.; Prosnitz, L. R.; Scher, R. L.; Dewhirst, M. W. *Int. J. Radiat. Oncol. Biol. Phys.* **1997**, *38*, 285; (b) Hockel, M.; Schlenger, K.; Aral, B.; Mitze, M.; Schaffer, U.; Vaupel, P. *Cancer Res.* **1996**, *56*, 4509.
8. Semenza, G. L. *Drug Discovery Today* **2007**, *12*, 853.
9. Aebersold, D. M.; Burri, P.; Beer, K. T.; Laissue, J.; Djonov, V.; Greiner, R. H.; Semenza, G. L. *Cancer Res.* **2001**, *61*, 2911.
10. Koukourakis, M. I.; Giatromanolaki, A.; Sivridis, E.; Simopoulos, C.; Turley, H.; Talks, K.; Gatter, K. C.; Harris, A. L. *Int. J. Radiat. Oncol. Biol. Phys.* **2002**, *53*, 1192.
11. (a) Kung, A. L.; Wang, S.; Klco, J. M.; Kaelin, W. G.; Livingston, D. M. *Nat. Med.* **2002**, *6*, 1335; (b) Zhang, X.; Kon, T.; Wang, H.; Li, F.; Huang, Q.; Rabbani, Z. N.; Kirkpatrick, J. P.; Vujaskovic, Z.; Dewhirst, M. W.; Li, C. Y. *Cancer Res.* **2004**, *64*, 8139; (c) Moeller, B. J.; Cao, Y.; Li, C. Y.; Dewhirst, M. W. *Cancer Cell* **2004**, *5*, 429.
12. (a) Giaccia, A.; Siim, B. G.; Johnson, R. S. *Nat. Rev. Drug Disc.* **2003**, *10*, 803; (b) Lin, X.; David, C. A.; Donnelly, J. B.; Michaelides, M.; Chandel, N. S.; Huang, X.; Warrior, U.; Weinberg, F.; Tormos, K. V.; Fesik, S. W.; Shen, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 174; (c) Zhang, H.; Qian, D. Z.; Tan, Y. S.; Lee, K.; Gao, P.; Ren, Y. R.; Rey, S.; Hammers, H.; Chang, D.; Pili, R.; Dang, C. V.; Liu, J. O.; Semenza, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 19579.
13. (a) Rao, K. V.; Alvarez, F. M. *Tetrahedron Lett.* **1983**, *24*, 4947; (b) Hodges, T. W.; Hossain, C. F.; Kim, Y.-P.; Zhou, Y.-D.; Nagle, D. G. *J. Nat. Prod.* **2004**, *67*, 767; (c) Hossain, C. F.; Kim, Y.-P.; Baerson, S. R.; Zhang, L.; Bruick, R. K.; Mohammed, K. A.; Agarwal, A. K.; Nagle, D. G.; Zhou, Y.-D. *Biochem. Biophys. Res. Commun.* **2005**, *333*, 1026.
14. (a) Moeller, B. J.; Dreher, M. R.; Rabbani, Z. N.; Schroeder, T.; Cao, Y.; Li, C. Y.; Dewhirst, M. W. *Cancer Cell* **2005**, *8*, 99; (b) Moeller, B. J.; Batinic-Haberle, I.; Spasojevic, I.; Rabbani, Z. N.; Anscher, M. S.; Vujaskovic, Z.; Dewhirst, M. W. *Int. J. Radiat. Oncol. Biol. Phys.* **2005**, *63*, 545; (c) Viola, R. J.; Provenzale, J. M.; Li, F.; Li, C. Y.; Yuan, H.; Tashjian, J.; Dewhirst, M. W. *Am. J. Roentgenol.* **2008**, *191*, 1779; (d) Tarnawski, A. S.; Jones, M. K. *J. Mol. Med.* **2003**, *81*, 627; (e) Macpherson, G. R.; Figg, W. D. *Cancer Biol. Ther.* **2004**, *3*, 503; (f) Dang, D. T.; Chen, F.; Gardner, L. B.; Cummins, J. M.; Rago, C.; Bunz, F.; Kantsevoy, S. V.; Dang, L. H. *Cancer Res.* **2006**, *66*, 1684.
15. Kim, H.; Wooten, C. M.; Park, Y.; Hong, J. *Org. Lett.* **2007**, *9*, 3965.
16. Kim, H.; Baker, J. B.; Lee, S.-U.; Park, Y.; Bolduc, K. L.; Park, H.-B.; Dickens, M. G.; Lee, D.-S.; Kim, Y.-C.; Kim, S. H.; Hong, J. *J. Am. Chem. Soc.* **2009**, *131*, 3192.
17. Kim, H.; Kasper, A. C.; Moon, E. J.; Park, Y.; Wooten, C. M.; Dewhirst, M. W.; Hong, J. *Org. Lett.* **2009**, *11*, 89.
18. Wang, G. L.; Semenza, G. L. *Blood* **1993**, *82*, 3610.
19. For **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80–7.09 (m, 12 H), 5.13 (d, J = 8.4 Hz, 1 H), 4.63 (d, J = 8.0 Hz, 1 H), 4.62 (d, J = 8.0 Hz, 1 H), 4.42 (d, J = 9.2 Hz, 1 H), 4.06–4.14 (m, 2 H), 4.09 (br s, 2 H), 3.85–3.92 (m, 18 H), 2.23–2.30 (m, 1 H), 1.76–1.84 (m, 1 H), 1.16 (d, J = 6.0 Hz, 3 H), 1.15 (d, J = 6.0 Hz, 3 H), 1.08 (d, J = 6.4 Hz, 3 H), 0.67 (d, J = 7.2 Hz, 3 H); LRMS (FAB) found 732.4 [calcd for $C_{42}H_{52}O_{11}$ $(M)^+$ 732.4].
20. For **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96–7.01 (m, 4 H), 6.91 (s, 2 H), 6.90 (dd, J = 8.4, 1.2 Hz, 4 H), 6.81 (d, J = 8.0 Hz, 2 H), 4.65 (d, J = 9.2 Hz, 2 H), 4.62 (d, J = 8.4 Hz, 2 H), 4.05–4.13 (m, 4 H), 3.92 (s, 6 H), 3.86 (s, 6 H), 3.85 (s, 6 H), 1.78–1.81 (m, 2 H), 1.14 (d, J = 6.4 Hz, 6 H), 1.06 (d, J = 5.6 Hz, 6 H); HRMS (FAB) found 732.3492 [calcd for $C_{42}H_{52}O_{11}$ $(M)^+$ 732.3510].
21. Li, F.; Sonveaux, P.; Rabbani, Z. N.; Liu, S.; Yan, B.; Huang, Q.; Vujaskovic, Z.; Dewhirst, M. W.; Li, C. Y. *Mol. Cell* **2007**, *26*, 63.
22. To avoid any unnecessary complications and/or exaggeration by the flexible side chains, truncated structures of **1**, **11**, and **13** were used instead of the full structures. Initial geometries were determined by conformational search based on molecular mechanics (MMFF).
23. (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785; (b) Vosko, S.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200; (c) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098; (d) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
24. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C., et al GAUSSIAN *03, Revision D.02*; Gaussian: Wallingford, CT, 2004.
25. Optimization of the full structures of **1**, **11**, and **13** using density functional theory (B3LYP) at the 6-31G* level provided the same conclusion (see Supplementary data for details).