Strongylophorines: Natural Product Inhibitors of Hypoxia-Inducible Factor-1 Transcriptional Pathway

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Rapidly increasing experimental and clinical data provides evidence for the role of hypoxia inducible factor-1 (HIF-1) as a crucial mediator of tumor survival and progression. In our effort to identify inhibitors of the HIF-1 activation pathway, we screened fractions from marine invertebrates. Fractions from an extract of *Petrosia (Strongylophora) strongylata* potently inhibited the HIF-1 activation pathway. Strongylophorines 2, 3, and 8 isolated from the active fractions were found to be responsible for the HIF-1 inhibition with EC_{50} values of 8, 13, and 6 μM, respectively.

Introduction

A common characteristic of solid tumors is the presence of hypoxic regions (low concentrations of oxygen)[1,2] that are associated with resistance to both chemotherapy and radiation therapy.[3,4] Cell survival under hypoxic conditions is mediated in part by the heterodimeric transcriptional factor hypoxia inducible factor-1 (HIF-1[a]).[5–7] HIF-1 upregulates expression of genes associated with tumor growth and progression, such as those involved in angiogenesis, glycolysis, and metastasis.[8,9] Several inhibitors of the hypoxia-activated HIF-1 transcriptional pathway such as manassantin B,[10] laurenditerpenol,[11] YC-1,[12] 2-methoxyestradiol,[13] topotecan,[14] and PX-478 (*S*-2-amino-3-[4′-*N*,*N*-bis(2-chloroethyl)amino]phenylpropionic acid *N*-oxide dihydrochloride)[15] have been discovered. The search for inhibitors of the HIF-1 transcriptional pathway has been largely based on accumulating evidence that suggests selective HIF-1 inhibitors can be developed as molecular-targeted antitumor agents with fewer side effects.[10,16] Currently, patients expressing higher levels of HIF-1 are being enrolled for a phase I clinical trial of topotecan, a semisynthetic camptothecin analogue.[17] *S*-2-amino-3-[4′-*N*,*N*-bis(2-chloroethyl)amino]phenylpropionic acid *N*-oxide dihydrochloride, a second small molecule HIF-1 inhibitor, is expected to enter clinical trials soon.[17]

Historically, natural products have been a remarkable source of anticancer agents. From 1940 to date, 47% of 155 small molecule anticancer drugs have been either natural products or derivatives of natural products.[18] Previous work in our laboratory has focused on the discovery of molecular-targeted antitumor natural products from marine sources.[19–21] The marine environment offers rich chemical and biological diversity and has been the source of potent drug leads. We have used an integrated approach including an effective HP20 prefractionation method and a cell-based reporter assay to identify inhibitors of the HIF-1 transcriptional activation pathway. The goal of the present study was to identify promising marine-derived compounds that potently inhibit hypoxia-induced HIF-1 activation without significant cytotoxicity. A cell-based screen was established in a 96-well format to identify small molecule inhibitors of HIF-1 using genetically engineered U251 human glioma cells that stably express a luciferase reporter gene under the control of three copies of a canonical hypoxia responsive element (HRE).[14] A library of HP20 fractions (2,376) derived from marine invertebrate extracts was screened in the cell-based assay. Two HP20 fractions from the extract of *Petrosia* (*Strongylophora*) *strongylata* were found to potently inhibit HIF-1 activation by 55% and 75% at a concentration of 1 ppm with no significant cytotoxicity. Bioassay-guided separation led to the isolation of strongylophorines 8, 2, and 3 (**1**–**3**), respectively.

The strongylophorines are meroditerpenoids that were first reported from the sponge *Strongylophora durissima* collected in Papua New Guinea[22] and the Philippines.[23] They were previously reported to possess the following biological activities: antibacterial against *Micrococcus luteus* and *Salmonella typhii*,[24] antifungal against *Cladosporium cucumerinum*,[24] insecticidal against the neonate larvae of *Spodoptera littoralis*,[24] lethal toxicity to brine shrimp,[24] and inhibition of the maturation of starfish oocytes.[25] Compound **1** and strongylophorine 26 (structure not shown) were recently found to have potent anti-invasive activity using MDA-MB-231 breast cancer cells.[26] The present study is the first report on the effects of the strongylophorines on the HIF-1 transcriptional activation pathway.

Results and Discussion

The Effect of Compounds on Hypoxia-Induced Activation of the HIF-1 Transcriptional Pathway. To study the effect of compounds on the hypoxia-induced HIF-1 activation pathway, U251-HRE cells were incubated at 1% O_2 in the absence and presence of compounds. Compounds **1**–**3** inhibited HIF-1 transcriptional activity as determined by a luciferase reporter assay using a hypoxia-responsive reporter construct. To ensure that the inhibition of hypoxia-activated HIF-1 was due to inhibition of the HIF-1 transcriptional pathway versus cytotoxicity or inhibition of HIF-1-independent luciferase expression, we performed a parallel MTT cell viability assay and a luciferase reporter assay using U251-pGL3 cells (Promega), which contain the firefly luciferase coding sequence under the control of SV40 promoter and enhancer elements. To establish that the effect was hypoxia related, U251-HRE cells

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[a] Abbreviations: HIF-1, hypoxia-inducible factor-1; VEGF, vascular endothelial growth factor; NMR, nuclear magnetic resonance; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum coherence; MeOH, methanol; HPLC, high-performance liquid chromatography; HP20, diaion HP-20ss chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; NP-40, nonidet p-40; h, hour.

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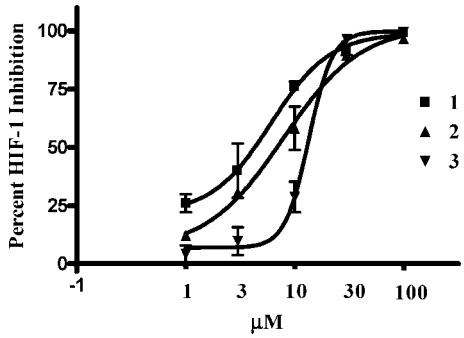


Figure 1. Dose–response studies performed against U251-HRE human glioma cells to determine the effects of **1**–**3** at 1, 3, 10, 30, and 100 μM concentrations on hypoxia-induced HIF-1 activation using a luciferase reporter assay.

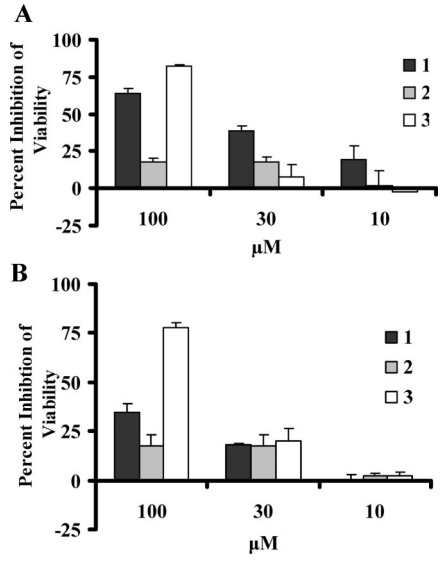


Figure 2. MTT assay performed using U251-HRE human glioma cells to determine the cytotoxicity of **1**–**3** at 10, 30, and 100 μM concentrations: (A) cytotoxicity under normoxic conditions; (B) cytotoxicity under hypoxic conditions.

were cultured under both hypoxic and normoxic conditions, and luminescence was compared. Hypoxic induction of 15-fold above normoxic luciferase expression was set as a validity standard for all experiments.

Our results demonstrate that all three compounds inhibited the HIF-1-dependent luciferase expression in U251-HRE cells with EC_{50} values much lower than their cytotoxic concentrations. Figure 1 shows that compounds **1**–**3** inhibited hypoxic induction of luciferase expression with EC_{50} values of 6, 8, and 13 μM, respectively, and did not inhibit luciferase expression in U251-pGL3 control cells at 30 μM, thus confirming HIF-1 selectivity. Additionally, HP20 fractions from *P.* (*S.*) *strongylata* did not show any effect in another luciferase reporter assay (data not shown) reaffirming the specificity of HIF-1 inhibition.

Effect of Compounds on Cell Viability. Analysis of cell viability was performed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with U251 human glioma cells. The results showed that **1** was more cytotoxic to U251 human glioma cells than either **2** or **3** at 10 and 30 μM (Figure 2A) under normoxic conditions. However, **3** showed greater cytotoxicity than **1** or **2** at a concentration of 100 μM. Furthermore, this study revealed that **2** exhibited the greatest differential activity, showing HIF-1 inhibition with an EC_{50} of 8 μM and only 17% inhibition of cell viability at 100 μM under

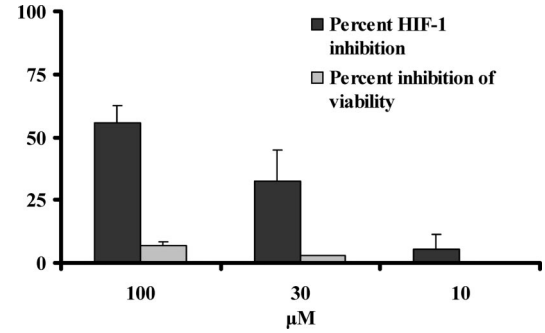
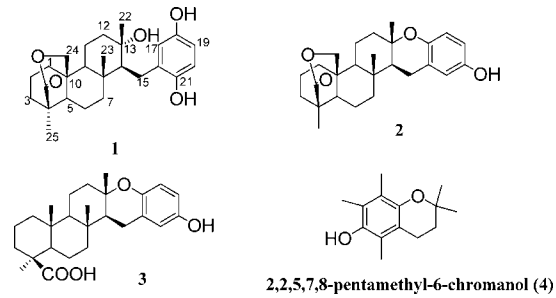


Figure 3. HIF-1 inhibition and cell viability assay results of **4** against U251-HRE human glioma cells.

Chart 1. Structures of Compounds Isolated from *P. (S.) strongylata* and a Commercially Available Chromanol **4**



normoxic conditions. Interestingly, **1** showed less cytotoxicity toward U251 human glioma cells under hypoxic conditions (24 h incubation) as compared to normoxic conditions (16 h incubation) (Figure 2).

Compound **1** is a hydroquinone with a tricyclic diterpenoid fragment (Chart 1). Compound **2** differs structurally from **1** in the absence of C-13 and C-21 hydroxyls which condense to form a chomanol ring. Compound **3** retains the chromanol system but lacks the A-ring lactone, which is replaced by a carboxyl at C-25 and a methyl at C-24. Interestingly, a previous study has reported the relative cytotoxicities of hydroquinones and chromanols.[27] The study showed that geranyl hydroquinone was more cytotoxic than its chromanol analogue cordiachromene A by a factor of 10. Our results indicated that in the strongylophorines the chromanols **2** and **3** were less cytotoxic than the hydroquinone **1**, consistent with the results observed for geranyl hydroquinone.

In an attempt to extend our investigation, the commercially available chromanol **4** was tested and as predicted showed little cytotoxicty at 100 μM concentration (Figure 3). Compounds **1**–**3** all possess a tricyclic diterpenoid fragment, and this moiety was found to be important for HIF-1 inhibition as evident from the lower HIF-1 inhibition by **4** compared to the three strongylophorines (Figure 3). Our results also showed that the chromanol moiety reduces cytotoxicity but with respect to the strongylophorines did not significantly affect the HIF-1 inhibition, suggesting a different mechanism for cytotoxicity and HIF-1 inhibition. For example, redox interconversion between the quinone and the hydroquinone may be involved in cytotoxicity but not involved in HIF-1 inhibition. Reduced cytotoxicity of **1** under hypoxic conditions as compared to normoxic conditions (Figure 2) suggests that oxygen-dependent redox cycling plays a role in cytotoxicity. As predicted, cytotoxicity of **2** and **3** did not change significantly under hypoxic conditions.

Compounds **1** and **2** were approximately 2-fold more potent as HIF-1 inhibitors than compound **3**, suggesting that the A-ring

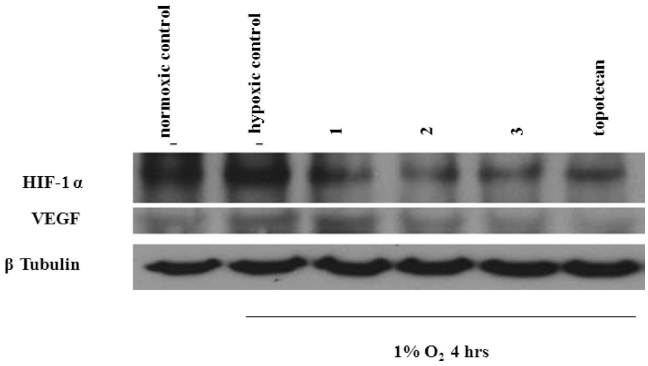


Figure 4. Western blot performed using U251-HRE human glioma cells to determine the effect of compounds on HIF-1α and VEGF expression at 30 μM.

lactone is also important for HIF-1 inhibition. A recent study found **1** to have anti-invasive activity with an EC_{50} of 7 μg/mL or 16 μM in an assay using MDA-MB-231 breast cancer cells.[26] The same study reported that removal of the A-ring lactone in compound **1** resulted in an analogue with no anti-invasion activity at concentrations below its EC_{50} for cytotoxicity. Our study shows that the A-ring lactone increases HIF-1 inhibition, but its absence does not eliminate the activity.

Strongylophorines Decreased the Protein Expression of HIF-1α and VEGF in U251-HRE Cells. Vascular endothelial factor (VEGF) is thought to be a key mediator in glioma growth and angiogenesis.[28,29] VEGF expression is regulated by HIF-1α under hypoxic condtions.[30] Therefore, we investigated the effect of **1**–**3** on HIF-1α and VEGF expression in U251-HRE cells under hypoxic conditions. Our results showed that HIF-1α and VEGF accumulated under hypoxic conditions (Figure 4, lane 2) compared to cells cultured under normoxic conditions (Figure 4, lane 1). Compounds **1**–**3** decreased protein levels of HIF-1α and VEGF under hypoxic conditions at 30 μM (Figure 4, lanes 3–5). Topotecan at 1 μM was used as a positive control (Figure 4, lane 6).

In summary, these data confirm that **1**–**3** inhibit the HIF-1 transcriptional pathway and also effectively decrease expression of VEGF, a downstream HIF-1 target gene. Our study has also identified **2** as an interesting lead compound which exhibited the greatest differential activity, showing HIF-1 inhibition with an EC_{50} of 8 μM and only 17% inhibition of cell viability at 100 μM. Further evaluation of its antitumor activity is underway. Overall, this work indicates that the presence of a chomanol in the strongylophorines reduces cytotoxicity while having little effect on the inhibition of HIF-1 transcriptional activation.

Materials and Methods

General Experimental Procedures. ^{1}H, ^{13}C, HMBC, and HSQC NMR spectra were recorded on a Varian Inova 500 MHz spectrometer. Accurate mass measurements were conducted on a Micromass Q-Tof Micro mass spectrometer using leucine-enkephalin as an internal standard. HPLC was performed on an Agilent 1100 series chromatography system with a photodiode array detector. Diaion HP-20ss and **4** were purchased from Sigma-Aldrich.

Sponge Sample, Extraction and Isolation. The sponge *Petrosia (Strongylophora) strongylata* Thiele, 1903 was collected from Milne Bay, Papua New Guinea (S 10° 15.492′ E 150° 43.577′). A voucher specimen, sample no. PNG05-2-031, is maintained at the University of Utah. Frozen sponge (100 g wet weight) was extracted with methanol, and the solvent was removed in vacuo. The crude extract was fractionated on a Diaion HP-20ss resin (100% H_2O to 100% 2-propanol in 11 steps, 40 mL fractions). The fractions eluting with 20% 2-propanol and 30% 2-propanol were individually subjected to semipreparative reversed-phase HPLC employing the following method: linear gradient from 60% MeOH/H_2O to 100% MeOH in 25 min, Phenomenex C18, 10 × 250 mm column (4.5 mL/min). Compound **1** (6.8 mg) eluted at 8.5 min, **2** (3.3 mg) at 13.9 min, and **3** (3.5 mg) at 17.9 min.

Compound 1: ^{1}H NMR and ^{13}C NMR data are identical to previously reported values;[23] HRESIMS $[M + Na]^+$ *m/z* 451.2479 (calcd 451.2460 for $C_{26}H_{36}O_5Na$).

Compound 2: ^{1}H NMR and ^{13}C NMR data are identical to previously reported values;[23] HRESIMS $[M + Na]^+$ *m/z* 433.2367 (calcd 433.2355 for $C_{26}H_{34}O_4Na$).

Compound 3: ^{1}H NMR and ^{13}C NMR data are identical to previously reported values;[23] HRESIMS $[M + Na]^+$ *m/z* 435.2520 (calcd 435.2511 for $C_{26}H_{36}O_4Na$).

Cell Culture and Reagents. Cells were cultured in RPMI 1640 (Whittaker Bioproducts, Walkersville, MD). The culture media was supplemented with 5% heat-inactivated fetal bovine serum, 50 U/mL penicillin, streptomycin (50 μg/mL), 2 mM glutamine, and 100 μg/mL G-418.

Cell Lines. Stably transfected U251-HRE and U251-pGL3 human glioma cells were generous gifts from Dr. Giovanni Melillo from the Developmental Therapeutics Program of the National Cancer Institute at Frederick, MD.

Cell-Based Assay. U251-HRE cells (100 μL) were plated in 96-well white bottom plates (Costar catalog no. 3917) at 10000 cells/well and incubated for 24 h at 37 °C, 5% CO_2. Prior to addition, fractions and pure compounds were dissolved in DMSO and 2-propanol, respectively, and appropriate concentrations were made in 100 μL of medium. After 16 h of incubation under hypoxic conditions (1% O_2, 5% CO_2, 94% N_2) in a modular incubator chamber (Billups Rothenberg, MIC 101), the cells were lysed with 25 μL of 1× reporter lysis buffer (Promega). Finally, 25 μL of Bright-Glo luciferase reagent (Promega) was added before measuring luminescence using a microtiter plate luminometer (Veritas microplate luminometer, Turner Biosystems). Those fractions that inhibited hypoxia-induced HIF-1 by greater than 70% were considered leads and subjected to secondary assays including an MTT cytotoxicity assay and a luciferase reporter assay performed using U251-pGL3 cells to exclude compounds that inhibit luciferase expression in a HIF-1 independent manner. Topotecan (Sigma) and doxorubicin hydrochloride (Sigma) were used as positive controls. All data were normalized to DMSO and 2-propanol.

MTT Assay. Cells were plated at a density of 10000 cells/well in a 96-well plate. Twenty-four hours after plating, they were exposed to medium containing compounds for 16 h at 37 °C for normoxic conditions and were incubated in a hypoxic incubator for 24 h at 37 °C for hypoxic conditions. After exposure of cells to the compounds, media were aspirated, and 100 μL of fresh culture medium containing 20 μL of MTT (1.5 mg/mL) was added to each well for 3 h at 37 °C. A total of 100 μL of DMSO per well was added to solubilize the formazan crystals. The absorbance was measured by a microplate reader at 570 nm. The percent cytotoxicity was calculated by comparison of the A_{570} reading from treated versus control cells. Makaluvamine C (10 μM) was used as the positive control.

Western Blot. U251-HRE cells (1×10^5) were plated in six well plates. After 24 h, cells were treated with strongylophorines (30 μM), topotecan (1 μM), and compound-free medium for 4 h under hypoxic conditions. Cells were then washed with PBS and lysed in buffer containing 1× PBS and 0.1% NP-40. Whole cell extracts were prepared by sonicating for 10 s on ice. Cell lysates were then centrifuged at 14000 rpm for 10 min, supernatants were collected, and protein concentration was determined by Bio-Rad Dc protein assay. Protein (100 μg) was separated on 12.5% polyacrylamide gels. Blots were blocked in PBST (PBS and 0.25% tween 20) for 15 min at room temperature. Monoclonal anti-HIF-1α and anti-VEGF (Novus Biologicals) at a dilution of 1:500 and β-tubulin (Cell Signaling) at 1:10000 were used as primary antibodies. After being washed three times in PBST, membranes were incubated for 1 h at room temperature with an antimouse HRP

conjugate antibody (Cell Signaling, diluted 1:5000). Membranes were washed three times in PBST, and chemiluminescence detection was performed using ECL reagents (Amersham Biosciences, Piscataway, NJ).

Statistical analysis. All data were analyzed using Microsoft Excel 2000 and Graph pad Prism 4.0 software. Error bars represent standard error. All experiments were performed in triplicate.

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References

(1) Nordsmark, M.; Bentzel, S. M.; Overgaard, J. Measurement of human tumor oxygenation status by a polarographic needle electrode. An analysis of inter and intratumour heterogeneity. *Acta Oncol.* **1994**, *33*, 383–389.

(2) Harris, A. L. Hypoxia - a key regulatory factor in tumour growth. *Nat. Rev. Cancer* **2002**, *2*, 38–47.

(3) Brizel, D. M.; Scully, S. P.; Harrelson, J. M.; Layfield, L. J.; Bean, J. M.; Prosnitz, L. R.; Dewhirst, M. W. Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma. *Cancer Res.* **1996**, *56*, 941–943.

(4) Reynolds, T. Y.; Rockwell, S.; Glazer, P. M. Genetic instability induced by the tumor microenvironment. *Cancer Res.* **1996**, *56*, 5754–5757.

(5) Semenza, G. L.; Wang, G. L. A nuclear factor induced by hypoxia via de novo protein synthesis binds to human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol. Cell. Biol.* **1992**, *12*, 5447–5454.

(6) Semenza, G. L.; Wang, G. L. Characterization of hypoxia - inducible factor 1 and regulation of DNA binding activity by hypoxia. *J. Biol. Chem.* **1993**, *268*, 21513–21518.

(7) Semenza, G. L.; Wang, G. L. Purification and characterization of hypoxia-inducible factor 1. *J. Biol. Chem.* **1995**, *270*, 1230–1237.

(8) Ratcliffe, P. J.; O'Rourke, J. F.; Maxwell, P. H.; Pugh, C. W. Oxygen sensing, hypoxia-inducible factor-1 and the regulation of mammalian gene expression. *J. Exp. Biol.* **1998**, *201*, 1153–1162.

(9) Blancher, C.; Harris, A. L. The molecular basis of the hypoxia response pathway: tumour hypoxia as a therapy target. *Cancer Metastasis Rev.* **1998**, *17*, 187–194.

(10) Hodges, T.; Hossain, C.; Kim, Y-P.; Zhou, Y-D.; Nagle, D. G. Molecular-targeted antitumor agents: the *Saururus cernuus* dineolignans manassantin B and 4-*O*-demethylmanassantin B are potent inhibitors of hypoxia-activated HIF-1. *J. Nat. Prod.* **2004**, *67*, 767–771.

(11) Mohammed, K. A.; Hossain, C. F.; Zhang, L.; Bruick, R. K.; Zhou, Y-D.; Nagle, D. G. Laurenditerpenol, a new diterpene from the tropical marine alga *Laurencia intricata* that potently inhibits HIF-1 mediated hypoxic signaling in breast tumor cells. *J. Nat. Prod.* **2004**, *67*, 2002–2007.

(12) Yeo, E. J.; Chun, Y. S.; Cho, Y. S.; Kim, J.; Lee, J. C.; Kim, M. S.; Park, J. W. YC-1: a potential anticancer drug targeting hypoxia-inducible factor-1. *J. Natl. Cancer Inst.* **2003**, *95*, 516–525.

(13) Ricker, J. L.; Chen, Z.; Yang, X. P.; Pribluda, V. S.; Swartz, G. M.; Van Waes, C. 2 - Methoxyestradiol inhibits hypoxia-inducible factor 1 alpha tumor growth, and angiogenesis and augments paclitaxel efficacy in head and neck squamous cell carcinoma. *Clin. Cancer Res.* **2004**, *10*, 8665–8673.

(14) Rapisarda, A.; Uranchimeg, B.; Scudiero, D. A.; Selby, M.; Sausville, E. A; Shoemaker, R. H.; Melillo, G. Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res.* **2002**, *62*, 4316–4324.

(15) Welsh, S.; Williams, R.; Kirkpatrick, D. L.; Paine-Murrieta, G.; Powis, G. Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1α. *Mol.Cancer Ther.* **2004**, *3*, 233–244.

(16) Powis, G.; Kirkpatrick, D. L. Hypoxia inducible factor-1α as a cancer drug target. *Mol. Cancer Ther.* **2004**, *3*, 647–654.

(17) Garber, K. New drugs target hypoxia response in tumors. *J. Natl. Cancer Inst.* **2005**, *97*, 1112–1114.

(18) Newman, D. J.; Cragg, G. M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **2007**, *70*, 461–477.

(19) Verbitski, S. M.; Mullally, J. E.; Fitzpatrick, F. A.; Ireland, C. M. Punaglandins, chlorinated prostaglandins, function as potent Michael receptors to inhibit ubiquitin isopeptidase activity. *J. Med. Chem.* **2004**, *47*, 2067–2070.

(20) James, R. D.; Jones, D. A.; Aalbersberg, W.; Ireland, C. M. Naamidine A intensifies the phosphotransferase activity of extracellular signal-regulated kinases causing A-431 cells to arrest in G1. *Mol. Cancer Ther.* **2003**, *2*, 747–751.

(21) Marshall, K. M.; Matsumoto, S. S.; Holden, J. A.; Concepcion, G. P.; Tasdemir, D.; Ireland, C. M.; Barrows, L. R. The anti-neoplastic and novel topoisomerase II-mediated cytotoxicty of neoamphimedine. *Biochem. Pharmacol.* **2003**, *66*, 447–458.

(22) Braekman, J. C.; Daloze, D.; Hulot, G.; Tursch, B.; Declercq, J. P.; Germain, G.; Van Meerssche, M. Chemical studies of marine invertebrates. XXXVII. Three novel meroditerpenoids from the sponge *Strongylophora durissima*. *Bull. Soc. Chim. Belg.* **1978**, *87*, 917–926.

(23) Salva, J.; Faulkner, D. J. Metabolites of the sponge *Strongylophora durissima* from Maricaban Island, Philippines. *J. Org. Chem.* **1990**, *55*, 1941–1943.

(24) Balbin-Oliveros, M.; Edrada, R.; Proksch, P.; Wray, V.; Witte, W. M.; Van Soest, R. W. M. A new meroditerpenoid dimer from an undescribed Philippine marine sponge of the genus *Strongylophora*. *J. Nat. Prod.* **1998**, *61*, 948–952.

(25) Liu, H.; Namikoshi, M.; Akano, K.; Kobayashi, H.; Nagai, H.; Yao, X. Seven new meroditerpenoids, from the marine sponge *Strongylophora strongylata*, that inhibited the maturation of starfish oocytes. *J. Asian Nat. Prod. Res.* **2005**, *7*, 661–670.

(26) Warabi, K.; McHardy, L. M.; Matainaho, L.; Van Soest, R. W. M.; Roskelley, C. D.; Roberge, M.; Andersen, R. J. Strongylophorine-26, a new meroditerpenoid isolated from the marine sponge *Petrosia (Strongylophora) corticata* that exhibits anti-invasion activity. *J. Nat. Prod.* **2004**, *67*, 1387–1389.

(27) Benlimane, A. F.; Pouches, Y. F.; Le Boterff, J.; Verbist, J. F.; Roussakis, C.; Monniot, F. Substances cytotoxiques et antibacteriennes de l'ascidie *Aplidium antillense*. *J. Nat. Prod.* **1988**, *51*, 582–583.

(28) Berkman, R. A.; Merrill, M. J.; Reinhold, W. C.; Monacci, W. T.; Saxena, A.; Clark, W. C.; Robertson, J. T.; Ali, I. U.; Oldfield, E. H. Expression of the vascular permeability factor /vascular endothelial factor gene in central nervous system neoplasms. *J. Clin. Invest.* **1993**, *91*, 153–159.

(29) Ke, L. D.; Shi, Y. X.; Im, S. A.; Chen, X.; Yung, W. K. The relevance of cell proliferation, vascular endothelial growth factor and basic fibroblast growth factor production to angiogenesis and tumorigenicity in human glioma cell lines. *Clin. Can. Res.* **2000**, *6*, 2562–2572.

(30) Forsythe, J. A.; Jiang, B. H.; Iyer, N. V.; Agani, F.; Leung, S. W.; Koos, R. D.; Semenza, G. L. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.* **1996**, *16*, 4604–4613.

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