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Marine Natural Products as Inhibitors of Hypoxic Signaling in Tumors

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Abstract

Marine natural products have become a major source of new chemical entities in the discovery of potential anticancer agents that potently suppress various antitumor molecular targets. As a consequence of insufficient vascularization, hypoxic regions form within rapidly growing solid tumor masses. Specific alterations of gene expression in these hypoxic tumor cells help facilitate the survival and metastatic spread of solid tumors. The transcriptional response to cellular hypoxia is primarily mediated by the transcription factor hypoxia-inducible factor-1 (HIF-1) that regulates the expression of more than 100 genes involved in cellular adaptation and survival under hypoxic stress. Clinical studies in cancer patients indicate that HIF-1 activation is directly correlated with advanced disease stages and treatment resistance. HIF-1 has emerged as an important tumor-selective molecular target for anticancer drug discovery. As a result, natural product-based inhibitors of HIF-1 activation have been identified from plants and microorganisms. Recently, structurally unique natural products from marine sponges, crinoids, and algae have been identified as HIF-1 activation inhibitors. The US National Cancer Institute's Open Repository of marine invertebrate and algae extracts has proven to be a valuable source of natural product HIF-1 inhibitors. Among the active compounds identified, certain marine natural products have also been shown to suppress the hypoxic induction of HIF-1 target genes such as vascular endothelial growth factor (VEGF). Some of these marine HIF-1 inhibitors act by interfering with the generation of mitochondrial signaling molecules in hypoxic cells. However, the precise mechanisms of action for many newly identified marine natural product HIF-1 inhibitors remain unresolved.

Keywords

cellular signaling; crinoids; gene expression; HIF-1 inhibitors; hypoxia-inducible factor-1; marine natural products; molecular-targeted antitumor agents; sponges; transcription factor; tumor hypoxia; tunicates

Introduction

Rapid tumor growth outstrips the capacity of existing blood vessels to supply oxygen and nutrients, and remove metabolic waste. As a result, hypoxia (reduced oxygen tension) and acidity are signature features of the tumor microenvironment (Fang et al., 2008; Tatum et al, 2006; Brown and Wilson, 2004). Hypoxia triggers tumor angiogenesis and the newly formed tumor blood vessels often fail to mature. The sluggish and irregular blood flow causes certain tumor regions to be under constant hypoxic stress. As a form of stress, hypoxia activates both survival and cell death programs. In oncogenically transformed cells, hypoxia provides a

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physiological pressure and selects for the cells with diminished apoptotic potential (Graeber et al, 1996). Hypoxic tumor cells that have adapted to oxygen and nutrient deprivation are associated with a more aggressive phenotype and poor prognosis (Fang et al., 2008; Tatum et al, 2006; Brown and Wilson, 2004). Hypoxic tumor cells are more resistant than normoxic tumor cells to radiation treatment and chemotherapy, and are considered an important contributor to disease relapse. Clinical studies have revealed that the extent of tumor hypoxia correlates with advanced disease stages, malignant progression, poor prognosis, and treatment resistance in cancer patients (Tatum et al, 2006; Brown and Wilson, 2004).

Current strategies to overcome tumor hypoxia (e.g. carbogen breathing, hyperbaric oxygenation with radiotherapy, and hypoxic cell radiation sensitizer) have only achieved limited success due to toxicity and poor response (Tatum et al, 2006; Brown and Wilson, 2004). Approaches that are under development include hypoxia-activated prodrugs, hypoxia-selective gene therapy, recombinant anaerobic bacteria, and inhibitors of hypoxia-inducible factor(s) (Tatum et al, 2006; Brown and Wilson, 2004). To date, only the bioreductive hypoxic cytotoxin tirapazamine (a prototype hypoxia-activated prodrug) has been extensively evaluated in clinical trials (Marcu and Olver, 2006). Improved efficacy was observed in some of the clinical studies when tirapazamine was combined with certain chemotherapeutic agents (von Pawel et al., 2000; Rischin et al., 2005; Covens et al. 2006; Maluf et al., 2006). It is clear that tumor hypoxia is an important unmet therapeutic need and deserves considerable drug discovery efforts.

Hypoxia-Inducible Factor-1

Numerous anticancer drug discovery efforts now focus on the discovery of small molecule inhibitors of the transcription factor hypoxia-inducible factor-1 (HIF-1) that mediates the important indirect effect of hypoxia – induction of genes that promote the adaptation, survival, malignant progression, and treatment resistance of hypoxic tumor cells (Tatum et al., 2006; Semenza, 2003, 2007a). Since its discovery in 1992 by Semenza and colleagues (Semenza and Wang, 1992), HIF-1 has been the subject of thousands of published studies. The key regulator of oxygen homeostasis HIF-1 is a heterodimer of the basic helix-loop-helix (bHLH) PER-ARNT-SIM (PAS) proteins HIF-1α and HIF-1β (also known as arylhydrocarbon receptor nuclear translocator or ARNT). In general, HIF-1α protein is degraded rapidly under normoxic conditions and is stabilized under hypoxic conditions, while HIF-1β protein is constitutively expressed. Upon hypoxic induction and activation, HIF-1 binds to the hypoxia response element (HRE) present in the promoters of target genes and activates transcription. The classical oxygen-dependent post-translational regulation of HIF-1α protein is summarized in Figure 1. Prolyl hydroxylases (PHD) [also known as HIF prolyl hydroxylases (HPH)] promote the degradation of HIF-1α protein and asparaginyl hydroxylase [also known as factor inhibiting HIF (FIH)] inactivates HIF-1α protein (Ivan et al., 2001; Jaakkola et al., 2001; Maxwell et al., 1999; Lando et al., 2002). Both PHDs and FIH require oxygen and iron for their activities. Thus, HIF-1 can be activated by either hypoxia or the addition of iron chelators/transition metals (chemical hypoxia). Recent studies have implicated other oxygen-independent pathways that also regulate HIF-1α degradation (Isaacs et al., 2002; Liu et al., 2007a).

Preclinical and clinical studies support HIF-1 as an important molecular target for anticancer drug discovery. At the molecular level, HIF-1 activates the expression of genes that promote hypoxic adaptation and survival by 1) increasing oxygen delivery through enhancing angiogenesis, erythropoiesis, and vasodilatation; 2) decreasing oxygen consumption through inducing genes involved in anaerobic metabolism and the reduction of mitochondrial oxygen consumption; and 3) promoting survival by expressing growth factors (Semenza, 2003, 2007a). In addition, HIF-1 also increases the expression of genes that confer drug resistance, metastasis, and dedifferentiation to assume a cancer stem cell-like phenotype (Semenza,

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2003, 2007a; Axelson et al., 2005). Numerous clinical studies indicate that the expression of HIF-1α protein is associated with advanced disease stage, metastasis, and poor prognosis in cancer patients (Tatum et al., 2006; Zhong et al., 1999; Bos et al., 2001; Birner et al., 2000, Mizokami et al., 2006). In tumor cells, the activation of oncogenes (e.g. *ras*, *src*, *myc*) and/or the loss of tumor suppressor genes (e.g. *PTEN*, *VHL*) also lead to the activation of HIF-1 (Brown and Wilson, 2004; Semenza, 2007a). In animal models, reduced tumor vascularity and retarded tumor growth were observed when HIF-1 was inhibited by methods that range from genetic manipulation to small molecules (Ryan et al., 2000; Rapisarda et al., 2004; Greenberger et al., 2008). Enhanced treatment outcomes were observed when HIF-1 inhibition was combined with chemotherapy or radiation (Unruh et al., 2003; Moeller et al., 2007; Li et al., 2006; Cairns et al., 2007). Studies conducted in kidney cancer models suggest that the transcription factor HIF-2 (a heterodimer of HIF-2α and HIF-1β/ARNT) may also be critical for hypoxia-induced gene expression (Raval et al., 2005). However, both *in vitro* and *in vivo* studies from independent groups support HIF-1 as the predominant mediator of hypoxia-induced gene expression in non-renal tumor cells (Sowter et al., 2003; Park et al., 2003; Greijer et al., 2005). In summary, clinical studies indicate that HIF-1 is a negative factor for cancer prognosis and results from multiple animal studies clearly demonstrate that blocking HIF-1 function suppresses tumor growth and enhances chemotherapy/radiation. Therefore, small molecule specific inhibitors of HIF-1 represent potential chemotherapeutic drugs that will suppress tumor growth and progression by inhibiting hypoxia-induced gene expression. As of October 2008, only two agents that inhibit HIF-1 have entered early phase clinical trials for cancer: EZN-2968 (a HIF-1α RNA antagonist) and PX-478 (a small molecule that decreases HIF-1α gene expression) (MedTRACK Biomedical Corporate Intelligence Database, 2008).

Hypoxia-Induced Mitochondrial Reactive Oxygen Species

Natural product-based inhibitors of the mitochondria electron transport chain (ETC) have recently been shown to inhibit hypoxia-induced HIF-1 activation (Baby et al., 2005; Guzy et al., 2005; Simon, 2006; Pan et al., 2007; Bell et al., 2007; Semenza, 2007b; Klimova and Chandel, 2008) Several seemingly conflicting theories have emerged to explain the role of mitochondria in the regulation of HIF-1 activation (Chandel et al., 1998; Vaux et al., 2001; Bell et al., 2008). Under hypoxic conditions, reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide are produced by the Q_p site of mitochondrial complex III (Figure 2). These hypoxia-induced ROS are believed to act as “signal molecules” that oxidize the catalytic Fe(II) in Fe(II)-dependent HIF-prolyl hydroxylases (i.e. PHD2 or HPH2) that are required for the initial steps in the ubiquitin-mediated proteasomal degradation of HIF-1α protein (Figure 1) (Gong and Agani, 2005; Baby et al., 2005; Guzy et al., 2005; Simon, 2006; Pan et al., 2007; Bell et al., 2007; Semenza, 2007b; Klimova and Chandel, 2008). Mitochondrial ROS may also inhibit the Fe(II)-dependent asparaginyl hydroxylase [a.k.a. factor inhibiting HIF, (FIH)] that has been shown to interfere with HIF-1 transcriptional activation by hydroxylating the N803 asparagine in the C-terminal transcriptional activation domain (C-TAD) of HIF-1α. Therefore, natural products that inhibit the mitochondrial ETC may suppress the production of the ROS signaling molecules in hypoxic tumor cells. This stabilizes HIF-1α protein by preventing proteasomal degradation under hypoxic conditions and activates HIF-1 by interfering with the ability of asparaginyl hydroxylase to suppress HIF-1 activation.

Natural Product-Based HIF-1 Inhibitors from Plants and Microorganisms

Natural products have been a major source of new drugs for centuries and statistics show that over 47% of approved anticancer agents are of natural origin (Newman and Cragg, 2007). The unrivaled chemical diversity of natural product-based drug discovery, empowered by functional bioassays, continues to play a key role in the discovery of chemotherapeutic agents,

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often with dissimilar modes of action. Small molecule HIF-1 inhibitors have served as important molecular probes to investigate the pathways that regulate HIF-1 activity (Nagle and Zhou, 2006a, b). Most of these are natural products (e.g. genistein, wortmannin, rapamycin, rotenone, geldanamycin, actinomycin D, cycloheximide) or natural product-derived synthetic compounds (e.g. PD98059, LY294002). Figure 3 illustrates some of the pathways that regulate HIF-1 and depicts where representative natural product-derived small molecule inhibitors interfere with the expression and activation of HIF-1.

As HIF-1 is recognized as an important molecular target for drug discovery, many laboratories have joined in the race to discover small molecule HIF-1 inhibitors (Nagle and Zhou, 2006a; Semenza, 2006; Melillo, 2007). Most laboratories use a synthetic or commercial compound library-based screening approach. Representative HIF-1 inhibitors discovered from compound library screening efforts include topotecan (Rapisarda et al., 2002), echinomycin (Kong et al., 2005), chetomin (Kung et al., 2004), a benzopyran derivative 103D5R (Tan et al., 2005), analogues of emetine and actinomycin D (Chau et al., 2005), a pyrroloquinoline derivative DJ12 (Jones and Harris, 2006), and a group of structurally diverse compounds including alkyliminophenylacetates that affect mitochondria function (Lin et al., 2008). Most of these compounds have a narrow window between HIF-1 inhibitory activity and cytotoxicity. Using a natural product chemistry-based approach, several groups have discovered chemically and mechanistically diverse HIF-1 inhibitors. Some of these HIF-1 inhibitors function at low nanomolar concentrations (e.g. manassantins) with a wide window between their HIF-1 inhibitory activity and cytotoxicity (Hodges et al., 2004; Hossain et al., 2005).

HIF-1 Inhibitors from Marine Algae

Marine natural products have proven to be a rich source of novel chemical entities in molecular-targeted anticancer drug discovery programs (Nagle and Zhou, 2004). Several research programs have recently begun to examine marine natural products as a potential source of HIF-1 activation inhibitors. This review provides an overview of all marine natural products that have been identified to be HIF-1 inhibitors, as of December 2008.

Using a T47D human breast tumor cell-based luciferase reporter assay to monitor HIF-1 activity, extracts from more than two thousand plants and marine organisms were evaluated for HIF-1 inhibitory activity (Hodges et al., 2004; Mohammed et al., 2004). Bioassay-guided fractionation of the lipid extract of a Jamaican collection of the red alga *Laurencia intricata* Lamouroux (Rhodomelaceae) yielded the first marine natural product that inhibited HIF-1 activation (Mohammed et al., 2004). The active compound was a structurally novel bicyclic diterpene called laurenditerpenol (**1**) that inhibited hypoxia (1% O_2)-induced HIF-1 activation in T47D cells (IC_{50} 0.4 μM). At similar concentrations, laurenditerpenol had little effect on iron chelator (1,10-phenanthroline)-induced HIF-1 activation. Laurenditerpenol was shown to inhibit HIF-1 activation by blocking hypoxia-induced HIF-1α protein accumulation. Respiration studies established that **1** suppresses mitochondrial oxygen consumption at ETC complex I (IC_{50} 0.8 μM). Further studies with laurenditerpenol were hindered by a lack of compound supply. However, a recently completed total synthesis has resolved the absolute configuration of laurenditerpenol (Chittiboyina et al., 2007) and together with other synthetic efforts (e.g. Jung and Im, 2008) may provide sufficient compound to allow further evaluation of laurenditerpenol as a potential anticancer agent.

Marine Echinoderm-Derived HIF-1 Inhibitors

As part of an effort to evaluate the US National Cancer Institute's (NCI) Open Repository of marine invertebrate extracts, the lipid extract from a Papua New Guinea collection of the crinoid *Comantheria rotula* (Comasteridae) was found to inhibit hypoxia-induced HIF-1 activation in T47D breast tumor cells. Bioassay-guided fractionation of the extract yielded

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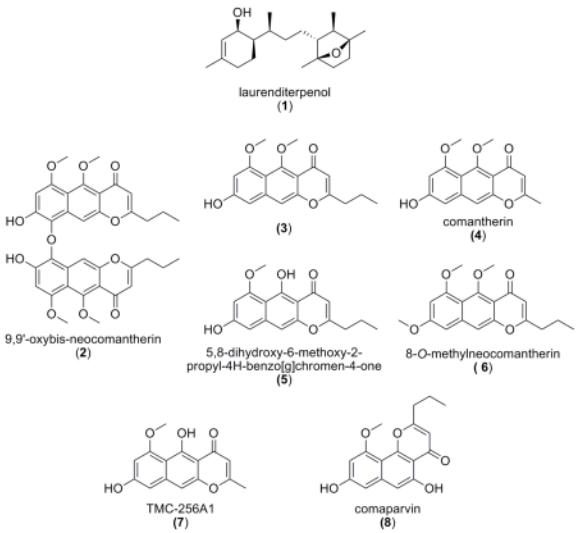
seven benzo[*g*]chromen-4-one and benzo[*h*]chromen-4-one pigments (**2**–**8**) (Dai et al., 2007a). The crinoid pigments significantly inhibited both hypoxia-induced (IC_{50} values 1.7 to 7.3 μM) and iron chelator-induced HIF-1-dependent luciferase reporter activity (IC_{50} values 0.6 to 3.0 μM) in T47D cells. Pigments **2**, **3**, **5**, and **7** also significantly suppressed HIF-1 activation in PC-3 prostate tumor cells. However, this inhibition of HIF-1 in the reporter assay did not translate into a significant decrease in the expression of a downstream HIF-1 target, secreted vascular endothelial growth factor (VEGF). Only compound **8** (10 μM) exhibited a marginal activity to suppress the hypoxia-induced production of secreted VEGF protein from T47D cells. The new benzo[*g*]chromenone dimer 9,9′-oxybis-neocomantherin (**2**) was found to inhibit tumor cell growth in the NCI 60-cell line panel (GI_{50} values of 1.6–18.2 μM), and compound **7** produced a unique pattern of tumor cell growth suppression. Five tumor cell lines that originated from different organs (Colon SW-620, Melanoma SK-MEL-5, Ovarian IGROV1, Renal 786-0, Breast HS 578T) were found to be hypersensitive to the cytostatic effects of **7** (GI_{50} values of 0.29–0.62 μM). Three other tumor cell lines (non-small cell lung cancer NCI-H522, melanoma SK-MEL-2, renal UO-31) were moderately sensitive (GI_{50} values of 2.2–5.1 μM). Compound **7** was considerably less effective at suppressing the growth of most other tumor cells lines in the NCI 60-cell line panel (GI_{50} values for most other cell lines ranged from 20 to 47 μM). Since hypoxia-induced HIF-1 signaling is believed to be regulated, at least in part, by the ROS species generated by mitochondrial ETC complex III, it was postulated that certain types of radical scavengers may be able to inhibit HIF-1 signaling. The crinoid compounds **2**–**8** were evaluated for their radical scavenging ability using a modified 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay (Dai et al., 2007a). Benzochromenones **2**–**5** and **7** were found to have about 40% of the DPPH radical scavenging capacity of Trolox, a commercially available standard radical scavenger, in a modified DPPH assay. Because the HIF-1 inhibitory activity of *Comantheria* pigments did not translate into a significant effect on the HIF-1 target genes (i.e. secreted VEGF), they were considered possible “nuisance compounds” and not further pursued.



Marine Sponge Metabolites as HIF-1 Inhibitors

Extracts of marine sponges from the NCI Open Repository have shown considerable activities in cell-based assays for inhibitors of HIF-1 activation. The lipophilic extract of a South African species of the marine sponge *Axinella* sp. inhibited hypoxia (1% O_2)-induced HIF-1 activation in T47D cells (Dai et al., 2006). The *Axinella* sp. extract yielded seven new sodwanone triterpenoids, two previously reported sodwanones and a related yardenone-type triterpene.

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Sodwanone V (**9**) inhibited both hypoxia- and iron chelator (1,10-phenanthroline)-induced HIF-1 activation in T47D cells with comparable potency (IC_{50} 15 μM). Compound **9** also inhibited HIF-1 activation in PC-3 prostate tumor cells (IC_{50} 15 μM). The triterpenes sodwanone A (**10**), 10,11-dihydrosodwanone B (**11**), 3-epi-sodwanone K (**12**), and sodwanone T (**13**) only weakly inhibited hypoxia-induced HIF-1 activation in T47D cells (IC_{50} values 20–25 μM).

Upon further examination of the NCI Open Repository of marine invertebrate and algae extracts, the lipophilic extract of an Indonesian collection of *Lendenfeldia* sp. was found to inhibit hypoxia-induced HIF-1 activation in the previously described T47D cell-based reporter assay (Dai et al., 2007b). Chromatographic separation yielded the new substituted naphthalene dimer (*S*)-2,2′-dimethoxy-1,1′-binaphthyl-5,5′,6,6′-tetraol (**14**), a new furanolipid, and three known homoscalarane sesterterpenes. Compound **14** inhibited hypoxia-induced HIF-1 activation (IC_{50} 4.6 μM) without producing a similar level of tumor cell cytotoxicity (IC_{50} 19 μM) in the time frame used for the HIF-1 reporter assay (i.e. 16 h). However, upon extended incubation (i.e. 48 h), compound **14** reduced the viability of T47D (IC_{50} 8.7 μM) and MDA-MB- 231 (IC_{50} 6 μM) breast tumor cells. The general cytotoxicity of this compound precluded its further consideration as a HIF-1 inhibitor.

The lipid extract of the Papua New Guinea sponge *Diacarnus levii* suppressed hypoxia-induced HIF-1 activation and hypoxic tumor cell survival in T47D cells (Dai et al., 2007c). *Diacarnus levii* yielded four new norsesterterpene peroxides, diacarnoxides A–D. Rather than acting as inhibitors of HIF-1 activation, diacarnoxides appear to preferentially inhibit the growth/viability of hypoxic tumor cells. Diacarnoxide B (**15**) exhibited a significantly enhanced ability to suppress the growth of MCF-7 and MDA-MB-231 breast tumor cells under hypoxic conditions [MCF-7 IC_{50} values: 13.8 μM (normoxia), 6.6 μM (hypoxia); MDA-MB-231 IC_{50} values: 13.3 μM (normoxia), 5.6 μM (hypoxia)]. Diacarnoxide B is a novel class of hypoxic cytotoxin, which bears no structural similarity the extensively studied hypoxia-activated cytotoxin tirapazamine.

The extract of the sponge *Dendrilla nigra* yielded four new lamellarin-like phenolic pyrroles (Liu et al., 2007b). The compound 7-hydroxyneolamellarin A (**16**) inhibited both hypoxia- and iron chelator [1,10-phenanthroline (10 μM)]-induced HIF-1 activation (IC_{50} values 1.9 μM and 3.7 μM, respectively). Similarly, this newly identified *Dendrilla* metabolite inhibited hypoxia- and iron chelator-induced production of secreted VEGF protein in T47D cells at 10 μM. However, only 1 mg of **16** was isolated from the *D. nigra* extract and insufficient supply hampered further pharmacological evaluation of this compound.

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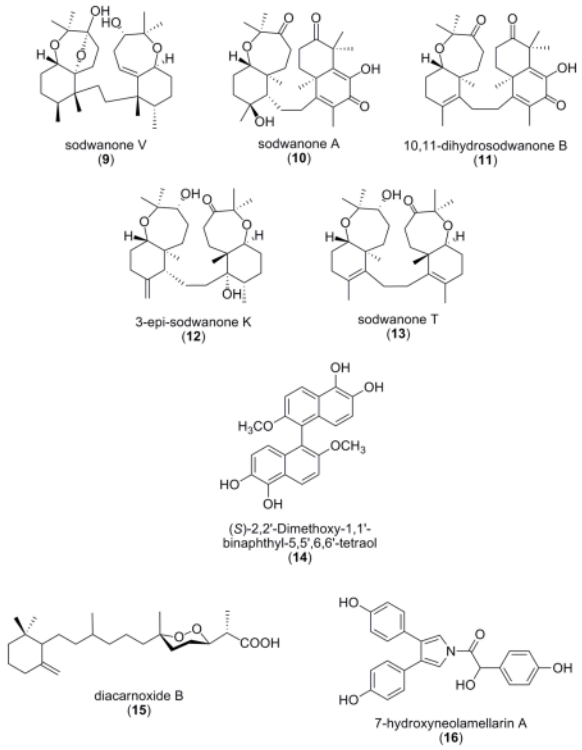

The macrolide latrunculin A (**17**), first isolated from the Red Sea sponge *Negombata magnifica* (Podospongiidae) (Kashman et al., 1980), disrupts actin polymerization and inhibits microfilament formation by reversibly binding to actin monomers (Spector et al., 1989). Actin microfilaments are essential for cell shape and organization, cellular adhesion, and cell migration (Nakaseko and Yanagida, 2001; Gachet et al., 2001). In terms of cancer biology, lactrunculin-mediated disruption of actin polymerization suppresses tumor cell viability, inhibits angiogensis, and interferes with the ability of tumor cells to metastasize (Newman and Cragg, 2004; El Sayed et al., 2006). Latrunculin A (**17**, IC_{50} 6.7 μM) and its 17-*O*-[*N*-(benzyl)-carbamate] (**18**, IC_{50} 29 μM) suppressed hypoxia-induced HIF-1 activation in T47D cells (El Sayed et al., 2008). Latrunculin A also displayed potent anti-invasive activity against human prostate cancer PC-3M cells. These findings suggest that the actin cytoskeleton may play an important, yet unrecognized, role in regulating HIF-1 activation.

Ireland and coworkers recently screened a library of 2,376 chromatographically purified fractions derived from marine invertebrate extracts (Mohammed et al., 2008) using genetically engineered U251 human glioma cells that stably express a luciferase reporter gene under the control of hypoxia responsive element (Rapisarda et al., 2002). Two fractions from an extract of a Papua New Guinea collection of the sponge *Petrosia* (*Strongylophora*) *strongylata* significantly inhibited HIF-1 activation at 1 μg/ml. Following bioassay-guided fractionation, three previously reported strongylophorine meroditerpenoids (Braekman et al., 1978; Salva and Faulkner, 1990) were identified to be responsible for the observed HIF-1 inhibition. Strongylophorines 2 (**19**), 3 (**20**), and 8 (**21**) inhibited hypoxia-induced HIF-1 activation in the U251 cell-based system with EC_{50} values of 8, 13, and 6 μM, respectively. Strongylophorine 8 (**21**) had previously been shown to possess potent anti-invasive activity in a MDA-MB-231 breast cancer cell-based invasion assay (Warabi et al., 2004). Compounds **19**–**21** (30 μM) inhibited the accumulation of hypoxia-induced HIF-1α protein and VEGF expression in U251-HRE human glioma cells.

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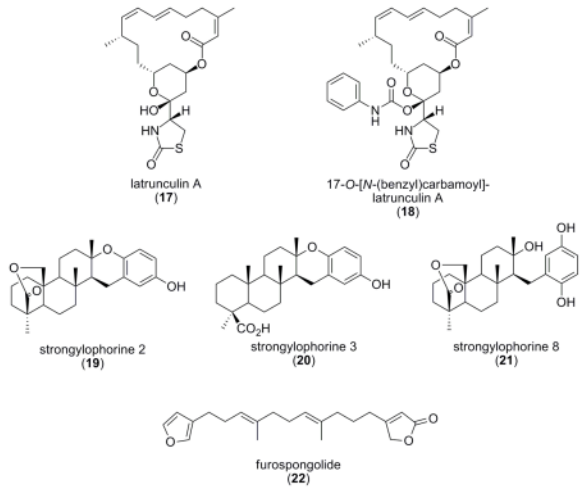
As part of the previously described molecular-targeted antitumor drug discovery program, 15,000 extracts of marine organisms and plants were evaluated for HIF-1 inhibitory activity (Liu et al., 2008). The lipid extract of a Saipan collection of marine sponge *Lendenfeldia* sp. inhibited hypoxia (1% O_2)-induced HIF-1 activation by 91% (5 μg/ml). Bioassay-guided isolation of this extract from the NCI Open Repository of marine invertebrate extracts yielded the terpene-derived furanolipid furospongolide (**22**) as the primary inhibitor of hypoxia-induced HIF-1 activation (IC_{50} 2.9 μM, T47D cells). Furospongolide (**22**) suppressed HIF-1 activation by inhibiting the hypoxic induction of HIF-1α protein. This terpene-derived furanolipid metabolite **22** was shown to block the hypoxia-induced production of the downstream HIF-1 target secreted VEGF. As previously described, the generation of mitochondrial ROS species at complex III in hypoxic cells is generally believed to play a vital role in HIF-1 regulation. Inhibitors of the mitochondrial ETC could block the production of ROS-mediated signaling processes that stabilize HIF-1α protein under hypoxic conditions. Mitochondrial respiration was measured in the same T47D cells that were used to examine HIF-1 activation with a Clark-type electrode system. Furospongolide (**22**) inhibited cellular oxygen consumption in a concentration-dependent manner (e.g. 38% inhibition at 10 μM). Additional mechanistic studies indicated that **22** inhibits hypoxia-induced HIF-1 activity by blocking NADH-ubiquinone oxidoreductase (complex I)-mediated mitochondrial electron transfer, thereby suppressing tumor cell respiration and hypoxic ROS generation (Figure 2). Therefore, while the two compounds have little to no structural homology, both the sponge metabolite furospongolide (**22**) and red alga (*Laurencia intricata*) natural product laurenditerpenol (**1**) inhibit hypoxia-induced HIF-1 activation in breast tumor cells by inhibiting NADH-ubiquinone oxidoreductase-mediated mitochondrial signaling pathways. While the molecular target of **22** may be associated mitochondrial-associated toxicity, it is envisioned that the moderately potent activity and relatively simple structure of this marine furanolipid provides an attractive target for structural modification and possible optimization of this compound.



Marine Tunicate-Derived HIF-1 Inhibitors

The antitumor agent Aplidin® (**23**, dehydrodidemnin B, plitidepsin, aplidine, APLD) was originally isolated from the Mediterranean tunicate *Aplidium albicans* and shown to have significant antitumor and antiangiogenic activity (Urdiales et al., 1996; Caers et al., 2008; Mitsiades et al., 2008). Aplidin has been shown to block tumor cell proliferation, inhibit cell cycle progression, induce apoptosis and suppress tumor-associated angiogenesis (Erba etal., 2002; González-Santiago et al., 2006; Muñoz-Alonso et al., 2008; Broggini et al., 2003). The antiangiogenic effects of **23** result from the ability of this cyclic depsipeptide to inhibit secreted

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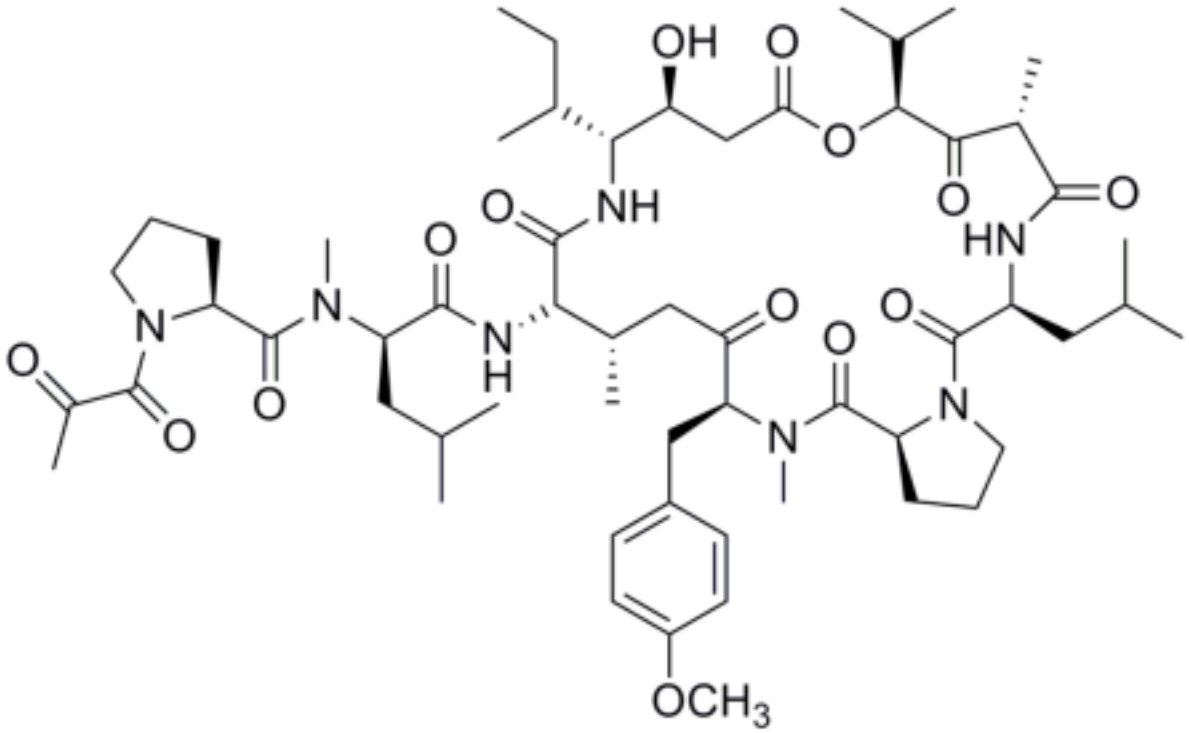
VEGF protein production, decrease VEGF receptor (VEGFR-1, FLT-1) expression, and deregulate the VEGF-VEGFR-1 autocrine signaling loop in human leukemia cells (Broggini et al., 2003). However, it was not clear precisely how **23** exerted these effects on VEGF, VEGFR-1 and other genes that affect tumor angiogenesis. Anaplastic thyroid cancer (ATC) is a rare, but highly metastatic and malignant form of thyroid cancer (Sipos and Mazzaferri, 2008). The effect of Aplidin on ATC growth was examined in a murine xenograft model (Straight et al., 2006). Mice (BALB/c nu/nu) were first injected with ARO-81 ATC cells and allowed to implant for 3 weeks. Both low dose (0.5 mg/kg/day) and high dose (1.0 mg/kg/day) intraperitoneal injections of Aplidin significantly inhibited ATC xenograft growth and induced ARO-81 cell apoptosis. Gene expression profiling of angiogenesis-associated genes by cDNA microarray analysis indicated that Aplidin treatment inhibited HIF-1A gene expression, as well as the expression of the HIF-1 target genes VEGF, transforming growth factor-β (TGFβ) and others that support tumor angiogenesis. While this study did not evaluate the effect of **23** on HIF-1 activation nor identify the mechanisms involved, it does indicate that anticancer/antiangiogenic therapy with Aplidin suppresses the expression of HIF-1 and angiogenesis-related HIF-1 target genes *in vivo*. The effect of Aplidin on HIF-1 regulation may be due to the ability of this marine metabolite to inhibit GTP-dependent elongation factor 1α and mammalian protein synthesis (Ahuja et al., 2000; Crews et al., 2004). Since the half-life of HIF-1α protein is relatively short, studies indicate that protein synthesis inhibitors such as the natural products cycloheximide and emetine potently block HIF-1α protein expression and inhibit hypoxia- and iron chelator-induced HIF-1 activation *in vitro* (Wang and Semenza, 1993; Chau et al., 2005; Zhou et al., 2005).



Aplidin® (aplidine, APLD, dehydrodidemnin B, plitidepsin)
(**23**)

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Conclusions

Since oxygen is vital to the survival of metazoans, it comes as no surprise that HIF-1, a key mediator of oxygen homeostasis, is regulated by a network of signaling pathways and influenced by diverse small molecules. In terms of drug discovery, the challenge lies in finding small molecule HIF-1 inhibitors that are highly effective at suppressing tumor cells while exerting only minimal effects on normal cells.

One of the intended applications of natural product-based HIF-1 inhibitors is as adjunct agents to be used in combination with other cancer treatment options. This is supported by findings from preclinical studies that combined HIF-1 inhibition with radiation and chemotherapy (Unruh et a., 2003; Moeller et al., 2005; Li et al., 2006; Cairns et al., 2007). While the exploration of HIF-1 targeted antitumor natural products is a relatively new area of research, the discovery of marine derived HIF-1 activation inhibitors has begun to produce exciting new lead compounds. It is envisioned that marine natural products will play a crucial role in the future development of new classes of potent clinically useful HIF-1 inhibitors for the treatment of cancer.

Acknowledgments

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Abbreviations

4EBP1	eIF4E-binding protein-1
17-AAG	17-allylamino-17-demethoxygeldanamycin
17-DMAG	17-(dimethylaminoethylamino)-17-demethoxygeldanamycin
AKT	protein kinase B
APLD	aplidin®; aplidine; dehydrodidemnin B; plitidepsin
ARNT	arylhydrocarbon receptor nuclear translocator
AMP	adenosine-5′-monophosphate
ATC	anaplastic thyroid cancer
ATP	adenosine-5′-triphosphate
bHLH	basic helix-loop-helix
C-TAD	C-terminal transcriptional activation domain
CBP	CREB-binding protein; (cAMP-response element-binding protein)-binding protein
DPPH	1,1-diphenyl-2-picrylhydrazyl; 1, 1-diphenyl-2-picrylhydrazyl radical
ETC	electron transport chain
FIH	factor inhibiting HIF
FLT-1	Fms-related tyrosine kinase 1; vascular endothelial growth factor/vascular permeability factor receptor; VEGFR-1
GTP	guanosine-5′-triphosphate
HIF-1	hypoxia-inducible factor-1
HPH	HIF prolyl hydroxylase

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HRE	hypoxia response element
Hsp90	heat-shock protein 90
MAPK	mitogen-activated protein (MAP) kinase
MEK-1	meiosis-specific serine/threonine protein kinase
mTOR	mammalian target of rapamycin
NCI	National Cancer Institute
NO	nitric oxide
p300	E1A-binding protein, 300 kD
PAS	PER-ARNT-SIM
PHD	prolyl hydroxylase
PI3K	phosphoinositol 3-kinase
PTEN	phosphatase and tensin homolog
ROS	reactive oxygen species
RTK	receptor tyrosine kinases
S6K	ribosomal p70 S6 kinase
TGFβ	transforming growth factor-β TSC, tuberous sclerosis complex
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VHL	von Hippel-Lindau tumor suppressor

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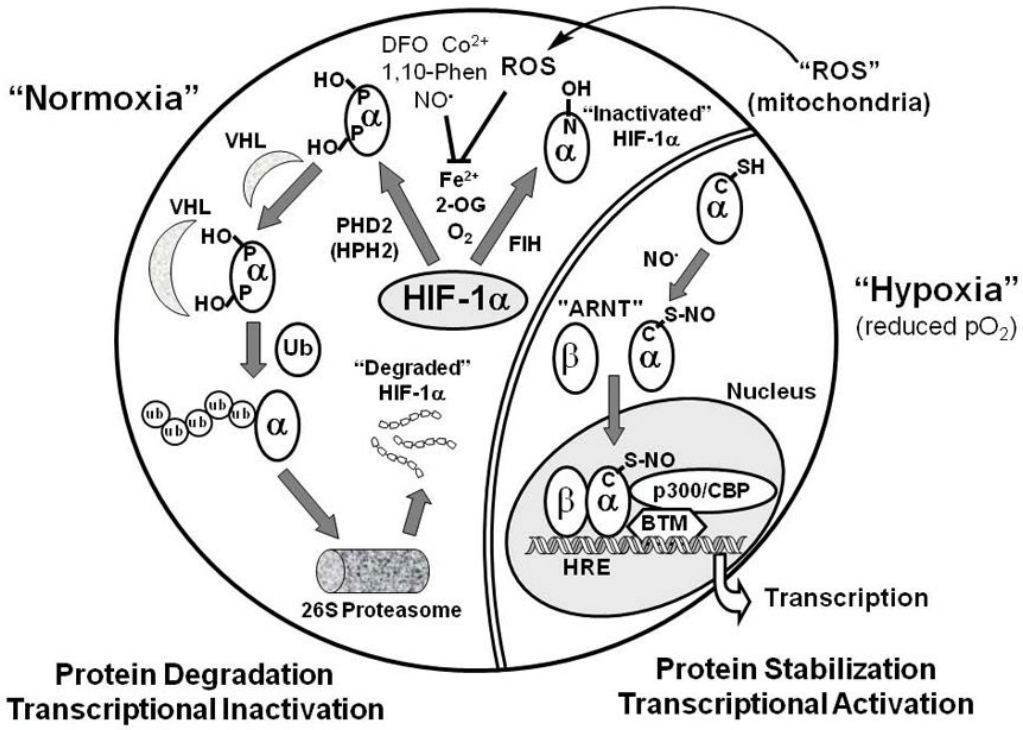

Figure 1. Hypoxic Regulation of Hypoxia-Inducible Factor-1 (HIF-1)

The two subunits that constitute the transcription factor HIF-1 are HIF-1α that is regulated by cellular oxygen levels and HIF-1β that is constitutively expressed. In the presence of oxygen (normoxic conditions), the HIF-1α protein is hydroxylated by Fe^{+2}/2-oxoglutarate/O_2-dependent prolyl hydroxylases (i.e. PHD2) at specific proline residues. This prolyl-hydroxylation 'tags' HIF-1α protein for polyubiquitination mediated by the von Hippel-Lindau tumor suppressor protein (pVHL) E3 ubiquitin ligase complex, followed by rapid degradation through a 26S proteasome-dependent mechanism. Another level of oxygen-dependent inactivation of HIF-1α protein coincides with the hydroxylation of an asparagine residue in the C-terminal transcriptional activation domain. This asparagyl-hydroxylation executed by a Fe^{+2}/2-oxoglutarate/O_2-dependent asparagyl hydroxylase [known as 'Factor Inhibiting HIF' (FIH)], disrupts the interaction between HIF-1 and the co-activator CBP/p300 and thus suppresses transcriptional initiation. The HIF-1α protein can be stabilized by the depletion of oxygen (hypoxic conditions), addition of iron chelators, transition metals, or nitric oxide radical (NO·). These inducing conditions inactivate the PHD2 that tags HIF-1α protein for ubiquitination and degradation, and the FIH that inhibits HIF-1 activation. In addition, NO can enhance the binding between HIF-1 and CBP/p300 by directly nitrosylating a sulfhydryl moiety in HIF-1α protein. Under hypoxic conditions, the lack of oxygen and the oxidation of the Fe^{+2} in the catalytic sites by reactive oxygen species (ROS) generated by hypoxic mitochondria lead to the inhibition of these hydroxylases that inactivate HIF-1. Natural product-based inhibitors of the mitochondrial electron transport chain (ETC) inhibit HIF-1 activation by decreasing the ROS produced by hypoxic mitochondria, thus allowing the PHD2-mediated degradation and FIH-facilitated inactivation of HIF-1α protein. Reproduced with the permission of D.G. Nagle (© 2008) at the University of Mississippi.

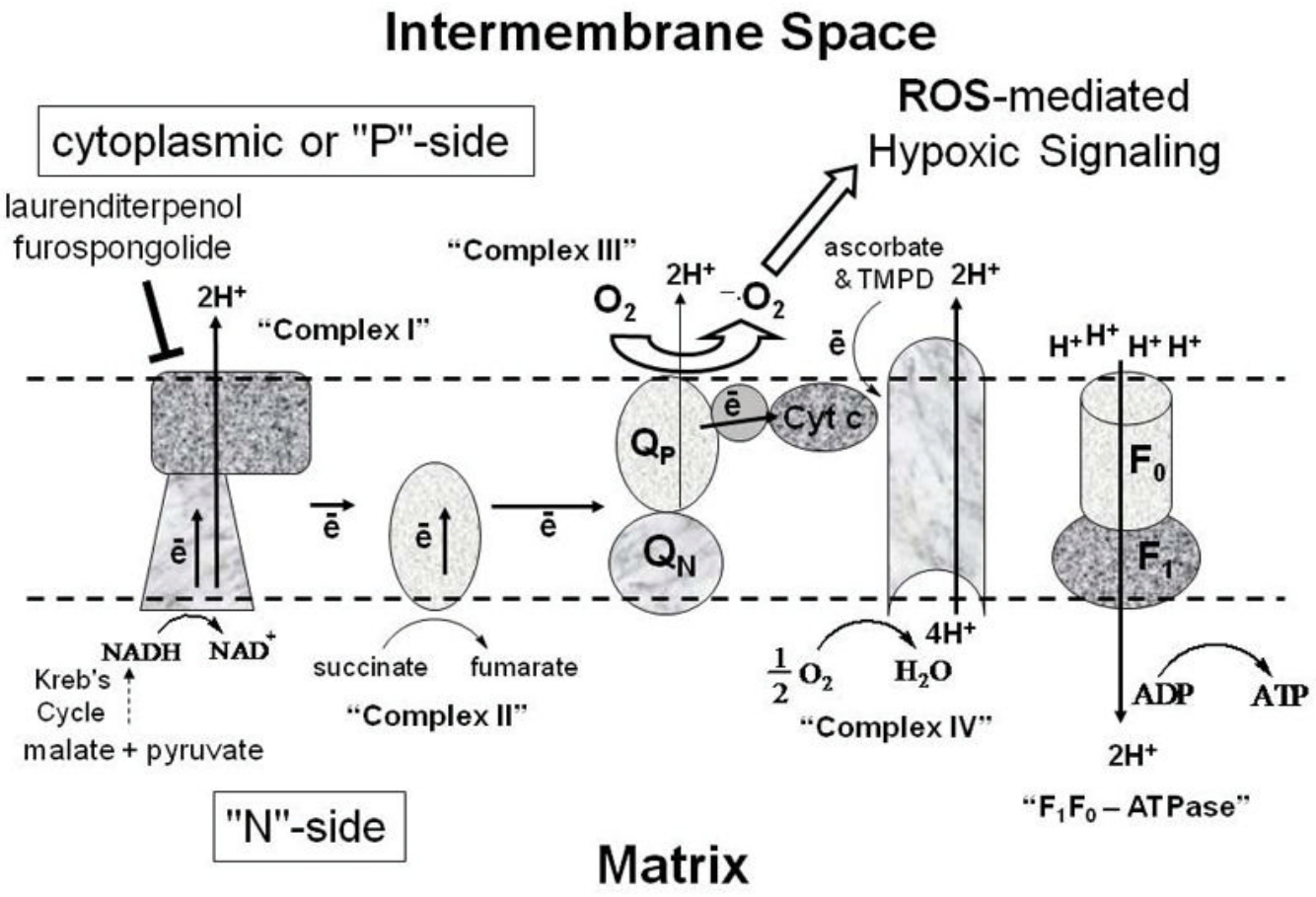

Figure 2. Probes of Mitochondrial Electron Transport Chain (ETC) and Oxidative Phosphorylation

The mitochondria of hypoxic cells release superoxide from the Q_p site of mitochondrial complex III (ubiquinol-cytochrome C oxidoreductase). This prevents the transfer of electrons required to drive the hypoxia-induced production of reactive oxygen species at complex III. The marine natural products laurenditerpenol (**1**) and furospongolide (**22**) inhibit the mitochondrial electron transport chain at the NADH-ubiquinone oxidoreductase (complex I) site. Superoxide anion and its metabolites (i.e. hydrogen peroxide, etc.) are believed to serve as cellular signaling molecules for the hypoxic regulation of HIF-1. Reproduced with the permission of D.G. Nagle (© 2008) at the University of Mississippi.

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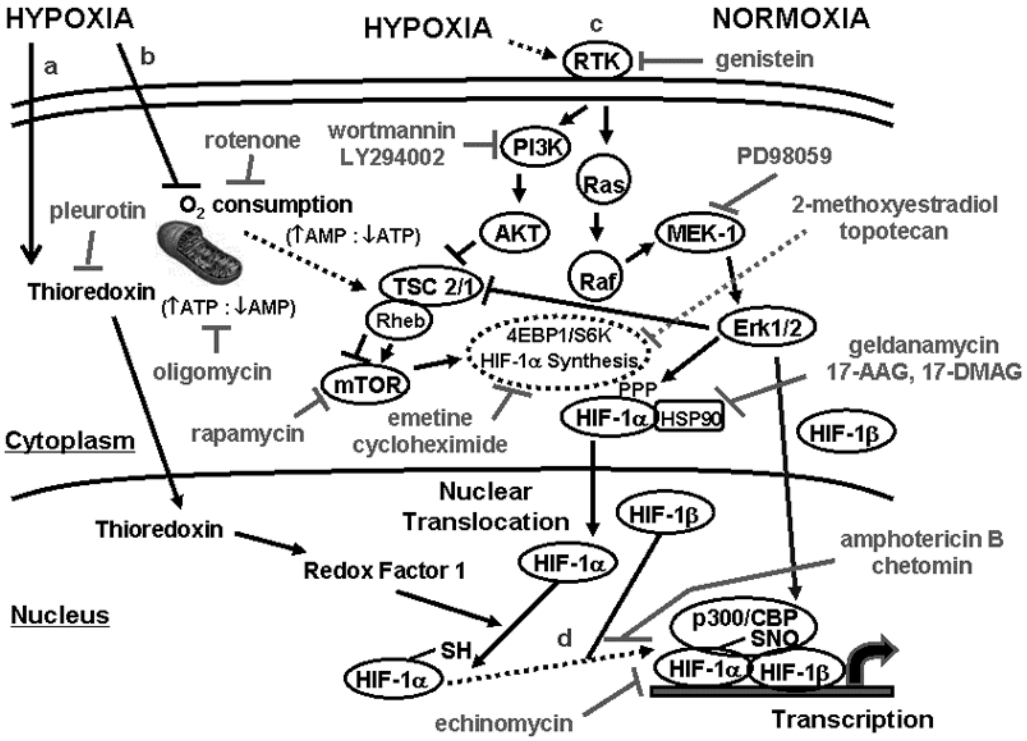

Figure 3. Natural Products Regulate HIF-1 Expression and Transcriptional Activation under Hypoxic and Normoxic Conditions

Cellular redox factors regulate the transcriptional activation of HIF-1 and the expression of HIF-1α is stringently regulated under hypoxic and normoxic conditions. a) The mushroom metabolite pleurotin inhibits the enzyme thioredoxin reductase which reduces a sulfhydryl moiety in HIF-1α. This enhances the binding between HIF-1 and the transcriptional co-activator CBP/p300 complex under hypoxic conditions; b) Hypoxic conditions and F_1F_0-ATPase (ATP synthase) inhibitors (e.g. oligomycin) increase cellular AMP:ATP ratios. This activates the TSC2-TSC1 tumor suppressor complex that suppresses mTOR activation, which blocks HIF-1α translation by inhibiting eukaryotic initiation factor 4E-binding protein-1 (4EBP1) and ribosomal p70 S6 kinase (S6K). Rapamycin and the protein translation inhibitors emetine and cycloheximide block HIF-1α synthesis. The antitumor agents topotecan and 2-methoxyestradiol may directly interfere with the synthesis HIF-1α or inhibit mitochondria-mediated hypoxic signaling; c) Following activation of receptor tyrosine kinases (RTKs), HIF-1α translation is regulated through PI3K/AKT and MAPK-mediated signaling pathways. Genistein and other natural product-derived compounds interfere with the translation of HIF-1α by inhibiting RTKs and MEK-1, respectively. The natural product-based PI3K inhibitors wortmannin and LY294002, and the natural product-derived MAPK inhibitor PD98059 interfere with HIF-1α translation and transcriptional activation. By interfering with Hsp90, Geldanamycin and semisynthetic geldanamycin derivatives [i.e. 17-allylamino-17-demethoxygeldanamycin (17-AAG), 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG)] destabilize HIF-1α and facilitate its proteasomal degradation by inhibiting the heat shock protein Hsp90; d) The antifungal agent amphotericin B and fungal metabolite chetomin directly interfere with the binding between HIF-1 and the co-activator CBP/p300. The *Streptomyces echinatus* metabolite echinomycin blocks the transcriptional activation of HIF-1 by disrupting the binding of HIF-1 to the hypoxia-response elements within the promoters of HIF-responsive genes. Reproduced with the permission of D.G. Nagle (© 2008) at the University of Mississippi.