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Identification and evaluation of soft coral diterpenes as inhibitors of HIF-2α induced gene expression

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ABSTRACT

Kidney cancer was the cause of almost 13,000 deaths in the United States in 2009. Loss of function of the *VHL* tumor suppressor gene (von Hippel–Lindau disease) dramatically increases the risk of developing clear cell kidney cancer. The VHL protein is best understood for its regulation of hypoxia inducible factor (HIF). HIF responds to changes in oxygen levels in the cell and is responsible for mediating the transcriptional response to hypoxia. Of the three known HIFα gene products, HIF-2α appears to play a fundamental role in renal carcinoma. A high throughput screen was developed to identify small molecule inhibitors of HIF-2 gene expression. The screen was performed and yielded 153 confirmed active natural product extracts. Three of the active extracts were from marine soft corals of the order Alcyonacea: *Sarcophyton* sp., *Lobophytum sarcophytoides* and *Asterospicularia laurae.* Bioassay-guided fractionation led to the isolation of two new cembrane diterpenes, (4*Z*,8*S**,9*R**,12*E*,14*E*)-9-hydroxy-1-(prop-1-en-2-yl)-8,12-dimethyl-oxabicyclo[9.3.2]-hexadeca-4,12,14-trien-18-one (**1**), and (1*E*,3*E*,7*R**,8*R**,11*E*)-1-(2-methoxypropan-2-yl)-4,8,12-trimethyloxabicyclo[12.1.0]-pentadeca-1,3,11-triene (**7**), as well as eight known compounds, **2**–**6** and **8**–**10**.

Kidney cancer was the cause of almost 13,000 deaths in the United States in 2009, with clear cell carcinoma, the most common histological form of kidney cancer, responsible for a majority of deaths.[1] Loss of function of the *VHL* tumor suppressor gene (von Hippel–Lindau disease) dramatically increases the risk of developing clear cell kidney carcinoma, as well as other tumors.[2] Additionally, loss of VHL protein function is prevalent in clear cell renal carcinoma. The VHL protein has a number of functions, many of which appear to contribute to its role as a tumor suppressor, in particular its regulation of hypoxia inducible factor (HIF). HIF responds to changes in O_2 levels in the cell and is responsible for mediating the transcriptional response to hypoxia. Under hypoxic conditions, or in the absence of functional VHL, HIF enhances the transcription of a number of downstream genes thought to be important in cancer.[3] Of the three known HIFα gene products, HIF-2α appears to play a fundamental role in renal carcinoma.[4,5] Therefore, a high throughput screen (HTS) was developed to identify small molecule inhibitors of HIF-2 gene expression that could potentially modulate downstream effectors of tumorigenesis.[3] In the assay, HIF-2α transcription activity was monitored in the renal cell carcinoma cell line 786-O engineered with five copies of the minimal HIF-2α hypoxia responsive element (HRE) of the vascular endothelial growth factor (VEGF) linked to a luciferase reporter gene.[3] The screen was performed on 146,814 natural product extracts sourced from a diverse collection of marine invertebrates, plants and fungi from the Natural Products Repository of the National Cancer Institute and yielded 153 confirmed active extracts. Three of the active extracts were from marine soft corals of the order Alcyonacea: *Sarcophyton* sp.,[6] *Lobophytum sarcophytoides*[7] and *Asterospicularia laurae*.[8] Bioassay-guided fractionation led to the isolation of two new cembrane diterpenes, (4*Z*,8*S**,9*R**,12*E*,14*E*)-9-hydroxy-1-(prop-1-en-2-yl)-8,12-dimethyl-oxabicyclo[9.3.2]-hexadeca-4,12,14-trien-18-one (**1**), and (1*E*,3*E*,7*R**,8*R**,11*E*)-1-(2-methoxypropan-2-yl)-4,8,12-trimethyl-oxabicyclo[12.1.0]-pentadeca-1,3,11-triene (**7**), as well as eight known compounds, **2**–**6** and **8**–**10** (Fig. 1).

Soft coral specimens were repeatedly extracted with CH_2Cl_2–MeOH (1:1) and 100% MeOH according to the methodology outlined in McCloud[9] to give the organic solvent crude extracts. A portion of the crude organic *Sarcophyton* sp. extract (165 mg) was subjected to a solvent–solvent partition, with the activity

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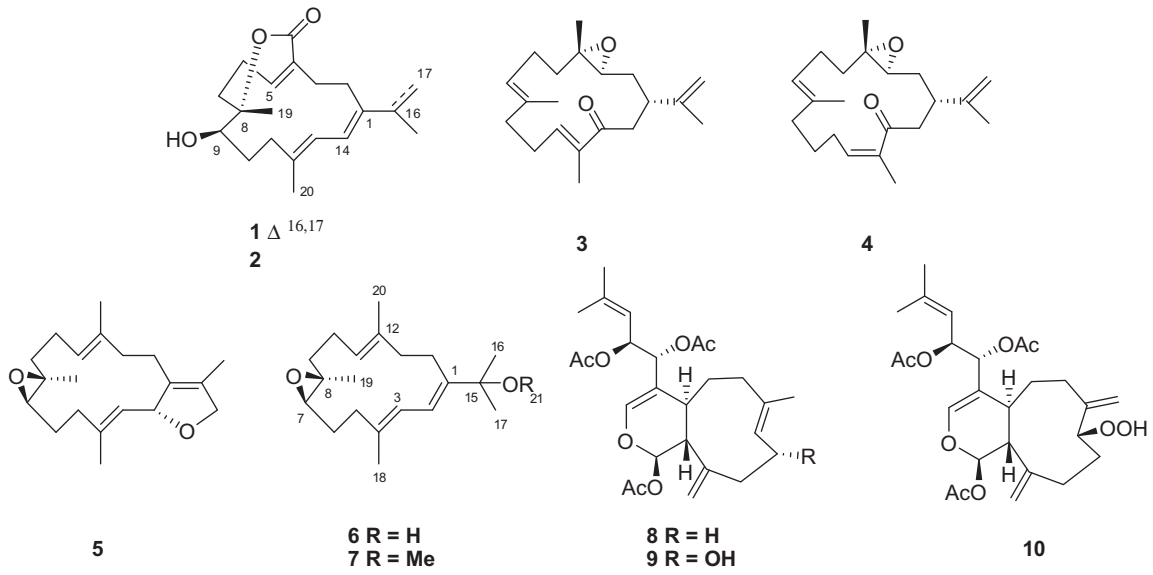

Figure 1. Structures of compounds **1–9**.

concentrated in the hexane and EtOAc fractions. The EtOAc fraction was subjected to size-exclusion LH-20 chromatography and semi-preparative C_{18} HPLC eluting with a gradient from MeCN–H_2O (60:40) to 100% MeCN to yield the new natural product **1** (7.2 mg, 4.4% crude extract weight) and a known cembrane **2**[10,11] (2.3 mg, 1.4% crude extract weight). The hexane fraction was subjected to reversed-phase C_8 flash chromatography followed by normal-phase SiO_2 flash chromatography to yield known compounds **3** (3.7 mg, 2.2% crude extract weight) and **4** (0.8 mg, 0.5% crude extract weight).[12]

HRESIMS data for compound **1** revealed a molecular formula of $C_{20}H_{28}O_3$, accounting for seven double bond equivalents. A comparison of the ^{1}H and ^{13}C NMR spectroscopic data[13] with those observed for the known natural product **2**,[11] suggested a common cembrane core containing an α–ß-unsaturated seven-membered lactone ring system. The major difference between compounds **1** and **2** centered on the C-1 isopropyl substituent, where the two doublet methyl resonances in **2** were replaced by a pair of broad singlets of an *exo*-methylene group (δ_H 4.91, δ_H 4.99; δ_C 112.1) and an allylic singlet methyl (δ_H 1.90; δ_C 21.2) in **1**. Interpretation of the 2D HMBC NMR data associated with the two resonances and the extra degree of unsaturation in **1** were all consistent with an isopropenyl substituent at C-1. The structure of compound **1** was therefore concluded to be the 16,17-dehydro derivative of **2**. The *EE* geometry of the $\Delta^{12,13}$ and $\Delta^{1,14}$ double bonds in **1** was confirmed upon the observation of a strong ROESY correlation between H-14 (δ_H 6.47, d, J = 11.6 Hz) and Me-20 (δ_H 1.83, s) as well as a 11.6 Hz coupling constant between H-13 (δ_H 5.72) and H-14 (δ_H 6.47), consistent with other related *EE* diene cembranes.[11,12,14] The relative stereochemistry at C-9 was established by comparison of the H-9 and Me-19 ^{1}H NMR chemical shifts with that of the known natural product (4*Z*,8*S*,9*R*,12*E*,14*E*)-**2** and the semi-synthetic C-9 epimer (4*Z*,8*S*,9*S*,12*E*,14*E*)-**2**. For the naturally-occurring (8*S*,9*R*)-**2**, the H-9 and Me-19 chemical shifts have been reported as δ_H 4.29 and δ_H 1.35, respectively,[11] whereas the C-9 epimer (8*S*,9*S*)-**2** had significantly upfield-shifted resonances for H-9 and Me-19 at δ_H 4.02 and δ_H 1.10, respectively.[14] With the observed H-9 and Me-19 chemical shifts of δ_H 4.25 and δ_H 1.38, in agreement with those reported for (4*Z*,8*S*,9*R*,12*E*,14*E*)-**2**, the stereochemistry of C-9 in **1** was concluded to be 9*R*. The H-9 resonance did not show a ROESY correlation to Me-19, which suggested that the two were on the opposite face of the molecule and that the configuration at C-8 was *S*. Therefore, the configuration of the new natural product was assigned as (4*Z*,8*S**,9*R**,12*E*,14*E*)-**1**. Once purified, compound **1** was found to be unstable and it decomposed in solution as well as within a month at −20 °C dry storage.

A portion of the *L. sarcophytoides* organic extract (1.02 g) was subjected to a solvent–solvent partitioning scheme, concentrating the HIF-2α activity into the MeOtBu fraction. The MeOtBu fraction was subjected to two rounds of size exclusion chromatography on Sephadex LH-20 (2:5:1 hexanes/CH_2Cl_2/MeOH; 1:1 CH_2Cl_2/MeOH) followed by reversed-phase C_{18} flash chromatography to yield **5** (32.6 mg, 3.2% crude extract weight) and **7** (1.2 mg, 0.1% crude extract weight).

The molecular formula for **7**, $C_{21}H_{34}O_2$, was derived from NMR and HRESIMS data. Analysis of the spectroscopic data for **7**,[15] and comparison with the reported data for **6**,[16] indicated they were closely related. The major chemical shift differences between **6** and **7** occurred around the tertiary alcohol. The presence of a methoxyl signal in **7**, the downfield shift of the quaternary oxygenated carbon (δ_C 78.0 in **7**; δ_C 74.2 in **6**), and the molecular formula for **7** all suggested that **7** was the methoxyl derivative of **6**. HMBC correlations confirmed the location and presence of the methoxyl group in **7**. Methanol and acetic acid were utilized in the isolation of **7**. Attempts to re-isolate **7** without using MeOH were unsuccessful; compound **6** was isolated when MeOH was not used (2.1 mg, 0.6% crude extract weight). Therefore, compound **7** appears to be an artifact of isolation.

A portion of the *A. laurae* organic extract (229 mg) was separated by two Diol SPE cartridges (2 g resin each), and the equivalent fractions were combined to give five total fractions; Fraction 1 = 9:1 hexanes/CH_2Cl_2, Fraction 2 = 20:1 CH_2Cl_2/EtOAc, Fraction 3 = EtOAc, Fraction 4 = 5:1 EtOAc/MeOH, Fraction 5 = MeOH. Size exclusion chromatography of fraction 2 on Sephadex LH-20 using hexanes/CH_2Cl_2/MeOH (2:5:1) yielded **9** (56.3 mg, 24.6% crude extract weight). Size exclusion chromatography of fraction 1 on Sephadex LH-20 using hexanes/CH_2Cl_2/MeOH (2:5:1) followed by reversed-phase C_4 flash chromatography yielded **8** (7.1 mg, 3.1% of crude extract weight) and **10** (7.6 mg, 3.3% of crude extract weight). The known compounds **2–6** and **8–10** were identified upon comparison of the $[\alpha]_D$, ^{1}H NMR, ^{13}C NMR and HRESIMS data with published values.[10–12,16–23]

Data on the in vitro HIF-2α activity[24] of compounds **1–10** is outlined in Table 1. Cembranes **1–7** showed poor HIF-2α inhibition, and were not evaluated further. The xenicin-type diterpenes **8–10** had the lowest IC_{50} values with low cytotoxicity. They are

Table 1
HIF-2α inhibitory activity of compounds **1**–**10**

Compound	% HIF Inhibition EC_{50} (μM)	% Cytotoxicity IC_{50} (μM)
1	Inactive[a]	Inactive[a]
2	Inactive[a]	Inactive[a]
3	107.5	147.3
4	Inactive[a]	Inactive[a]
5	183.3	291.2
6	Inactive[a]	Inactive[a]
7	148.9	134.2
8	3.4	5.2
9	6.2	26.3
10	11.8	14.2

[a] >300 μM.

currently undergoing further in vitro evaluation, the results of which will be published at a later date.

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References and notes

1. American Cancer Society. Cancer Facts & Figures 2009. Atlanta: American Cancer Society, 2009.
2. Linehan, W. M.; Bratslavsky, G.; Pinto, P. A.; Schmidt, L. S.; Neckers, L.; Bottarro, D. P.; Srinivasan, R. *Annu. Rev. Med.* **2010**, *61*, 329.
3. Woldemichael, G. M.; Vasselli, J. R.; Gardella, R. S.; McKee, T. C.; Linehan, W. M.; McMahon, J. B. *J. Biomol. Screening* **2006**, *11*, 678.
4. Löfstedt, T.; Fredlund, E.; Holmquist-Mengelbier, L.; Pietras, A.; Ovenberger, M.; Poellinger, L.; Påhlman, S. *Cell Cycle* **2007**, *6*, 919.
5. Qing, G.; Simon, M. C. *Curr. Opin. Genet. Dev.* **2009**, *19*, 60.
6. The soft coral *Sarcpohyton* sp. was collected by SCUBA in Darwin Harbour, Northern Territory, Australia (12 28.61S; 130 47.24E) by Dr. Alvarez de Glasby (Museum and Art Gallery of the Northern Territory) in May 2002 under contract through the Coral Reef Research Foundation for the National Cancer Institute. The specimens were immediately frozen and stored frozen until extraction as described by McCloud.[9] Taxonomic identification was by Phil Alderslade (University of Tasmania, Australia) and a voucher specimen (0M9H2084) was deposited at the Smithsonian Institution.
7. The soft coral *Lobophytum sarcophytoides* was collected by SCUBA near Luconia, Bombie, Sarawak, Malaysia (05 30.72N; 112 22.46E) by Dr. Steve Oakley (Coral Reef Research Foundation) in April 2003 under contract through the Coral Reef Research Foundation for the National Cancer Institute. The specimens were immediately frozen and stored frozen until extraction.[7] Taxonomic identification was by Leen van Ofwegen (Naturalis, National Museum of Natural History, Leiden, The Netherlands) and a voucher specimen (0M9I5636) was deposited at the Smithsonian Institution.
8. The soft coral *Asteropicularia laurae* was collected by SCUBA from a large reef west of Mabul (04 14.05N; 118 33.61E) in Malaysia by Dr. Nicholas J. Pilcher (Marine Research Foundation) in July 2004 under contract through the Coral Reef Research Foundation for the National Cancer Institute. The specimen was immediately frozen and stored until extraction.[7] Taxonomic identification was by Leen van Ofwegen (Naturalis, National Museum of Natural History, Leiden, The Netherlands) and a voucher specimen (0PHG1423) was deposited at the Smithsonian Institution.
9. McCloud, T. G. *Molecules* **2010**, *15*, 4526.
10. Bowden, B. F.; Coll, J. C.; Wills, R. H. *Aust. J. Chem.* **1982**, *35*, 621.
11. Gross, H.; Wright, A. D.; Beil, W.; König, G. M. *Org. Biomol. Chem.* **2004**, *2*, 1133.
12. Ravi, B. N.; Faulkner, D. J. *J. Org. Chem.* **1978**, *43*, 2127.
13. *Compound* ***1***: clear oil; $[\alpha]_D$ = +90 (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 282 (3.87), 227 (3.90) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.47 (1H, d, *J* = 11.6 Hz, H-14), 6.07 (1H, t, *J* = 4.1 Hz, H-5), 5.72 (1H, d, *J* = 11.6 Hz, H-13), 4.25 (1H, dd, *J* = 10.0, 6.9 Hz, H-9), 3.20 (1H, m, H-3a), 2.55 (1H, m, H-6a), 2.52 (2H, m, H-2), 2.45 (1H, m, H-11a), 2.41 (1H, m, H-6b), 2.28 (1H, m, H-10a), 2.22 (1H, m, H-7a), 2.10 (1H, m, H-11b), 2.04 (1H, m, H-7b), 1.95 (3H, br s, H-17), 1.85 (1H, m, H-3b), 1.83 (3H, br s, H-20), 1.54 (1H, m, H-10b), 1.38 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.1 (C-18), 143.1 (C-15), 140.5 (C-5), 137.7 (C-1), 136.5 (C-12), 133.4 (C-4), 122.8 (C-14), 121.5 (C-13), 112.1 (C-16), 83.6 (C-8), 66.1 (C-9), 37.4 (C-3), 34.5 (C-7), 31.7 (C-11), 27.3 (C-6), 26.3 (C-10), 24.9 (C-2), 21.9 (C-19), 21.2 (C-17), 19.4 (C-20); HRESIMS *m*/*z* $[M+H]^+$ 317.2117 (calcd for $C_{20}H_{29}O_3$, 317.2111).
14. Uchio, Y.; Nitta, M.; Nakayama, M.; Iwagawa, T.; Hase, T. *Chem. Lett.* **1983**, 613.
15. *Compound* ***7***: $[\alpha]_D$ = −24.0 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 245 (3.22), 203 (3.51) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.22 (1H, d, *J* = 10.7, H-2), 5.95 (1H, dq, *J* = 10.7, 1.4, H-3), 5.12 (1H, br t, *J* = 7.0, H-11), 3.03 (3H, s, H-21), 2.87 (1H, dd, *J* = 6.5, 3.8, H-7), 2.33 (1H, m, H-5a), 2.25 (4H, m, H-5b, H-13a, H-14), 2.12 (2H, m, H-10), 2.06 (2H, m, H-9a, H-13b), 1.91 (1H, m, H-6a), 1.78 (3H, br s, H-18), 1.63 (3H, br s, H-20), 1.61 (1H, m, H-6b), 1.31 (3H, s, H-16), 1.31 (3H, s, H-16), 1.30 (3H, s, H-17), 1.26 (3H, s, H-19), 1.21 (1H, m, H-9b); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 145.2 (C-1), 136.5 (C-12), 124.4 (C-11), 121.6 (C-2), 120.2 (C-3), 78.0 (C-15), 62.7 (C-7), 60.0 (C-8), 50.4 (C-21), 41.6 (C-13), 38.9 (C-9), 35.3 (C-5), 26.2 (C-6), 26.0 (C-16), 25.5 (C-17), 25.4 (C-14), 23.4 (C-10), 18.0 (C-18), 16.7 (C-19), 15.8 (C-20); HRESIMS *m*/*z* $[M+H]^+$ 319.2646 (calcd for $C_{21}H_{35}O_2$, 319.2632).
16. Coll, J. C.; Hawes, G. B.; Liyanage, N.; Oberhänsli, W.; Wells, R. J. *Aust. J. Chem.* **1977**, *30*, 1305.
17. Kashman, Y.; Zadock, E.; Néeman, I. *Tetrahedron* **1974**, *30*, 3615.
18. Tursch, B. *Pure Appl. Chem.* **1976**, *48*, 1.
19. Bowden, B. F.; Coll, J. C.; Hicks, W.; Kazlauskas, R.; Mitchell, S. J. *Aust. J. Chem.* **1978**, *31*, 2707.
20. Frincke, J. M.; McIntyre, D. E.; Faulkner, D. J. *Tetrahedron Lett.* **1980**, *21*, 735.
21. Bowden, B. F.; Coll, J. C.; Heaton, A.; König, G.; Bruck, M. A.; Cramer, R. E.; Klein, D. M.; Scheuer, P. J. *J. Nat. Prod.* **1987**, *50*, 650.
22. Braekman, J. C.; Daloze, D.; Tursch, B.; Declercq, J. P.; Germain, G.; Van Meerssche, M. *Bull. Soc. Chim. Belg.* **1979**, *88*, 71.
23. Bowden, B. F.; Cusack, B. J.; Dangel, A. *Mar. Drugs* **2003**, *1*, 18.
24. HIF-2α inhibition was assessed as previously described.[3] In brief, the assay used the renal cell carcinoma cell line 786-O engineered with five copies of the HIF-2 hypoxia responsive element (HRE) linked to a luciferase reporter gene. 5000 cells/well were plated in 384 well white plates and left to attach overnight. The following day, cells were treated with test samples or controls and the plates were incubated for a day to allow for HIF-2 signal to decrease. Following the incubation, luciferin was added to the cells and the luciferase signal measured. Treated cells were monitored in parallel for sample cytotoxicity using XTT colorimetric metabolic dye.