JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

Albopunctatone, an Antiplasmodial Anthrone-Anthraquinone from the Australian Ascidian *Didemnum albopunctatum*

Anthony R. Carroll,*[,†,‡,§] Brittney D. Nash,[†] Sandra Duffy,[‡] and Vicky M. Avery[‡]

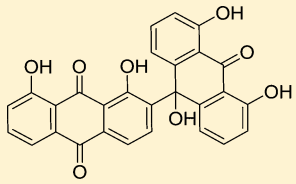
[†]School of Environment, Griffith University, Gold Coast, QLD 4222, Australia

[‡]Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia

[§]Environmental Futures Centre, Griffith University, Gold Coast, QLD 4222, Australia

Supporting Information

ABSTRACT: Chemical investigation of a MeOH extract of the Great Barrier Reef ascidian *Didemnum albopunctatum* has led to the isolation and identification of a new anthrone-anthraquinone, albopunctatone (**1**), together with the known 1,8-dihydroxy-9,10-anthraquinone (**2**). The structure of **1** was established from interpretation of 1D and 2D NMR spectroscopic and mass spectrometric data. The compounds were screened for antiplasmodial activity against chloroquine-resistant and -sensitive strains of the malaria parasite, *Plasmodium falciparum*. Albopunctatone (**1**) was moderately active against both strains (IC_{50} 5.3 and 4.4 ± 0.5 μM, respectively), while **2** was inactive at doses up to 40 μM. Both compounds were also inactive up to 40 μM when tested against a variety of cancerous and normal human cell lines and the kinetoplastid *Trypanosoma brucei brucei*, indicating selectivity for the malaria parasite, *P. falciparum*.



Albopunctatone

Antiplasmodial Activity

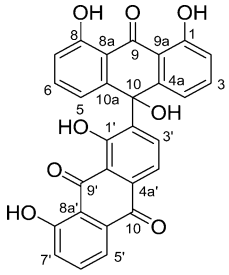
IC_{50} Dd2 = 5.3 μM
IC_{50} 3D7 = 4.4 μM

Australian ascidians have proven to be a rich source of diverse and biologically active natural products.[1] Over the last 30 years well over 140 compounds have been isolated, and this represents over 20% of the total ascidian compound diversity isolated worldwide. The majority of these compounds are alkaloids, as exemplified by the botryllamindes,[2,3] aplidiopsamine A,[4] and leptoclinidamines,[5] and peptides such as the virenamides[6] and patellins.[7] A selection of ascidians, mainly from the family Polyclinidae, have yielded non-nitrogenous compounds, exemplified by the rubrolides,[8] prunolides,[8] and longithorones,[9] but only a few species from the most species diverse family, the Didemnidae, have yielded non-nitrogenous compounds. We have used positive ESMS analysis of crude ascidian MeOH extracts to identify species to target for further analysis.[5] This method has proven to be a very effective tool to identify alkaloid- and peptide-containing extracts, but extracts that do not contain alkaloids or peptides can be overlooked if the natural products they contain do not ionize strongly by positive ESMS. To avoid overlooking non-alkaloid-containing extracts, we also analyzed those extracts that lack prominent positive ions by negative ESMS. In our routine analysis of extracts from Australian ascidians to find antiplasmodial-active compounds, we identified an extract from the Great Barrier Reef ascidian *Didemnum albopuntatum* that yielded an intense red-colored extract, which failed to generate any prominent ions by positive ESMS analysis. Subsequent negative ESMS showed a prominent ion at *m/z* 239 and another at *m/z* 479. Interestingly, the MeOH extract dramatically changed color to light yellow upon addition of acid, and this suggested that the extract contained phenolic compounds. Herein we report on the natural products chemistry and antiplasmodial activity of *D. albopunctatum*.

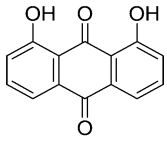
The freeze-dried ascidian was exhaustively extracted with MeOH, yielding an intensely dark red extract. Reversed-phase HPLC separation of the MeOH extract on C_{18} bonded silica with a gradient from 99% H_2O/1% TFA to 99% MeOH/1% TFA resulted in the isolation of 1,8-dihydroxy-9,10-anthraquinone (**2**). A later eluting fraction contained a mixture of **2** and another compound, and this was further purified by normal-phase HPLC using diol-bonded silica gel with a gradient from *n*-hexane to CH_2Cl_2 followed by a gradient to MeOH. This resulted in the isolation of larger amounts of **2** and a new compound that we have named albopunctatone (**1**) (3.0 mg, 0.002%). The structure of **2** was confirmed from analysis of 1D and 2D NMR data and by comparison with literature data.[10,11] Although the crude extract was dark red, colored fractions that eluted from the RP HPLC separation were all yellow, suggesting that the molecules contained pH-sensitive chromophores. These yellow fractions could be converted back to intensely red fractions by addition of base.

The negative HRESIMS for **1** contained a pseudomolecular ion peak at *m/z* 479.07801 (Δ 3.9 ppm), allowing a molecular formula, $C_{28}H_{16}O_8$, to be assigned. The IR spectrum displayed absorbances at 3747, 1743, and 1707 cm^{-1}, indicating that the molecule contained hydroxy and carbonyl functionalities. The UV spectrum had absorbance maxima at 202, 225, 259, 289, 383, and 442 nm that underwent a bathochromic shift upon basification, indicating that the compound contained an

Received: January 24, 2012
Published: June 8, 2012



1



2

extended phenolic chromophore.[12] The ^{1}H NMR spectrum (Table 1) contained three downfield hydrogen-bonded phenolic signals (one at δ_H 12.18 integrated to double the intensity of the other two), eight additional aromatic multiplets, and a hydroxy proton singlet. The ^{13}C NMR spectrum contained 22 signals, and all but one (δ_C 69.5) were downfield of δ_C 110. Three signals, at δ_C 193.1, 192.3, and 181.1, were assigned to aromatic carbonyl groups. HSQC correlations from the eight aromatic proton signals allowed ^{13}C NMR chemical shifts and $^1J_{CH}$ to be assigned for each of their attached carbons. Three of the aromatic proton signals (δ_H 6.93, 7.51, and 6.76) were double the intensity of the other five aromatic signals, and this suggested that the molecule contained an element of symmetry. The multiplicity of the three double-intensity aromatic proton signals and the ^{13}C NMR chemical shifts of their attached carbons were also typical for a 2,3-disubstituted phenol, and this was confirmed from inspection of coupling constants and COSY and HMBC correlations. The remaining five aromatic signals were associated with two additional spin systems. COSY and HMBC correlations between the three signals at δ_H 7.30, 7.77, and 7.69 indicated that they too were part of a 2,3-disubstituted phenolic moiety, while correlations between the two doublets at δ_H 8.70 and 7.93 suggested that they were from a 2,3,6-trisubstituted phenol.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for 1

position	δ_C[a] ($^1J_{CH}$ in Hz[b])	δ_H[a] (J in Hz)	HMBC correlations
1, 8	161.1, C		
2, 7	116.5, CH (168)	6.93, d (8.2)	C-1, C-4, C-5, C-8, C-8a, C-9a
3, 6	137.1, CH (168)	7.51, dd (7.7, 8.2)	C-1, C-4a, C-8, C-10a
4, 5	119.3, CH (168)	6.76, d (7.7)	C-2, C-3, C8a, C-9a, C-10
4a, 10a	147.8, C		
8a, 9a	114.2, C		
9	192.3,[c] C		
10	69.5, C		
1′	157.6, C		
2′	141.6, C		
3′	132.9, CH (168)	8.70, d (8.0)	C-10, C-1′, C-4a′
4′	118.9, CH (168)	7.93, d (8.0)	C-2′, C9a′, C-10′
4a′	132.5, C		
5′	119.2, CH (168)	7.69, d (7.6)	C-7′, C-8a′, C-10′
6′	137.5, CH (168)	7.77, dd (7.6, 8.2)	C-8′, C-10a′
7′	124.3, CH (168)	7.30, d (8.2)	C-5′, C-8a′
8′	161.2, C		
8a′	116.0, C		
9′	193.1,[c] C		
9a′	115.7, C		
10′	181.1, C		
10a′	133.3, C		
1-OH, 8-OH		12.18, s	C-1, C-2, C-7, C-8, C8a, C-9a
10-OH		7.13, s	C-10, C-2′
1′-OH		12.00, s	C-1′, C-2′, C-9a′
8′-OH		11.60, s	C-7′, C-8′, C-8a′

[a]Spectra recorded in DMSO-d_6 at 30 °C. [b]$^1J_{CH}$ measured from a coupled HSQC spectrum. [c]These assignments are interchangeable.

HMBC correlations observed from the three aromatic proton signals at δ_H 6.93, 7.51, and 6.76 and the phenolic proton signal at δ_H 12.18 allowed all of the nonprotonated aromatic carbons that were three bonds away from each proton to be assigned, and this confirmed that **1** contained two identical 2,3-disubstituted phenolic moieties. In addition an HMBC correlation from the aromatic doublet at δ_H 6.76 to a quaternary benzylic alcohol carbon at δ_C 69.5 and the downfield chemical shift of the phenolic proton signal at δ_H 12.18, indicative of a hydrogen-bonded hydroxy proton/carbonyl group, suggested that **1** contained a 1,8,9-trihydroxy-10-anthrone moiety. Analysis of HMBC correlations and comparison of ^{1}H and ^{13}C NMR chemical shifts for the remaining signals with those obtained for **2** suggested that **1** also contained a 1,8-dihydroxyanthraquinone moiety substituted at C-2. The proton at δ_H 8.70 (H-3′) from the anthraquinone also correlated to the oxygenated quaternary carbon at δ_C 69.5, indicating that a bond between C-9 of the 1,8,9-trihydroxy-10-anthrone and C-2 of the 1,8-dihydroxy-9,10-anthraquinone linked the two partial structures. This assignment was corroborated by the observation of an additional HMBC correlation from the hydroxy proton at δ_H 7.13 to C-2′ (δ_C 141.6), confirming that **1** is an anthrone-anthraquinone.

1,8-Dihydroxy-9,10-anthraquinone (**2**) is commonly known as dantron, danthron, dianthon, antrapurol, or chrysazin. Dantron can be synthetically produced by several processes,[13] but is also a naturally occurring substance, having been identified in several species of plants, fungi (both terrestrial and marine), and insects.[14−18] It has also been isolated from marine invertebrates such as the coral *Tubastrea micrantha* and the bryozoan *Dakaira subovoidea*.[19,20] It has been used as a laxative and as an intermediate for dyes.[21,22] However, on the basis of evidence of malignant tumor formation in multiple species of experimental animals, **2** is reasonably anticipated to be a human carcinogen and is now only administered to terminal cancer patients to alleviate the symptoms of constipation resulting from morphine use.[23] Albopuntatone (**1**) is related to anthrone-anthraquinones isolated from several plant species and is one of only eight 9,2′-anthrone-anthraquinone natural products that have been isolated to date.[24−27] It is the first anthrone-anthraquinone to be isolated from a marine source, all related metabolites being isolated from plants. It is also the first achiral anthrone-anthraquinone to be reported. Another two specimens of *D. albopuntatum* collected from different locations on the Great Barrier Reef were also analyzed for anthraquinone derivatives, and both contained similar concentrations of the two compounds.

Both **1** and **2** were tested for their ability to inhibit the blood borne protozoan parasite *Plasmodium falciparum*, responsible for malaria. Both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of the parasite were used to test the compounds.[28] 1,8-Dihydroxy-9,10-anthraquinone (**2**) was

dx.doi.org/10.1021/np300074z | J. Nat. Prod. 2012, 75, 1206−1209

inactive up to a dose of 40 μM, while albopunctatone (**1**) was moderately active (IC_{50} of 4.4 ± 0.5 μM against 3D7 (n = 2) and 5.3 μM against Dd2 (n = 1)). The reference compound, puromycin, a general cytotoxic, showed an IC_{50} of 89.0 ± 10.2 nM against 3D7, while artemisinin, a well-documented antimalarial, gave an IC_{50} value of 7.2 ± 1.1 nM against 3D7, as expected. Compounds **1** and **2** were also tested for cytotoxicity against normal human embryonic kidney (HEK293) and various cancerous cell lines (MCF-10A, BT-474, MDA-M, Panc-1, Bx-*Pc*-3, SU-86-86), and both compounds were inactive at or below the highest concentration tested (40 μM) against all of these cell lines. In addition **1** and **2** were tested for inhibition against the protozoan parasite *Trypanosoma brucei brucei*, and both compounds were inactive up to 40 μM. These combined bioactivity data demonstrated that **1** is selective for the *Plasmodium* parasite and has a selectivity index of >9.1 toward a chloroquine-sensitive *Plasmodium* strain compared with the cancerous and non-cancerous cell lines. Related anthrone-anthraquinones have previously been shown to possess antiplasmodial activity against either chloroquine-sensitive or chloroquine-resistant strains of the parasite, and two compounds, chryslandicin and 10-(chysophanaol-7′-yl)-10-hydroxychrysophanol-9-anthrone, possessing two methyl substituents *meta* to one of the phenolic hydroxy groups on both the anthrone and anthraquinone moieties, were reported to be seven and four times more potent against the chloroquine-sensitive strain (3D7), respectively, compared to **1**, while also exhibiting minimal cell toxicity toward the cancerous KB cell line.[26,27] Interestingly these related compounds have not been tested for activity against a chloroquine-resistant strain, but on the basis of our results these compounds would be predicted to show comparable activity against a chloroquine-resistant strain.[27] The observation that compounds esterified at C-10 show similar antiplasmodial activity, but higher cytotoxicity, suggests that the ester group is a liability.[26] Likewise, the observation that replacement of the C-8 and C-8′ hydroxy groups with methyl groups does not affect the activity suggests that substitution at these positions may also not be necessary for antiplasmodial activity. Albopuntactone (**1**) is a simpler, achiral analogue of these compounds and as such would be a good starting point for further evaluation against *P. falciparum* to determine its mode of action.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded at 30 °C on a Varian 600 MHz spectrometer equipped with a triple-resonance cold probe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for DMSO-d_6 at δ_H 2.49 and δ_C 39.5 or the solvent peak for $CDCl_3$ at δ_H 7.26 and δ_C 77.0. LRESIMS and HRESIMS were recorded on a Applied Biosystems Mariner Biospectrometry TOF workstation using negative electrospray ionization and mobile phase 1:1 MeOH/H_2O. IR and UV spectra were recorded on a Bruker Tensor 27 spectrometer and a Shimadzu UV-1800 UV spectrophotometer, respectively. Alltech Davisil 30−40 μm 60 Å C_{18} bonded silica was used to adsorb the ascidian extract prior to HPLC separation. A Merck Hitachi L7100 pump equipped with a Merck Hitachi L7455 PDA detector and a Merck Hitachi L7250 autosampler were used for HPLC. A Betasil C_{18} 5 μm 120 Å column (21.2 mm × 150 mm) and a YMC diol bonded silica gel 5 μm 120 Å column (21.2 mm × 150 mm) were used for semipreparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered.

Animal Material. *Didemnum albopunctatum* was collected by scuba diving from the Swain Reefs in the Southern Great Barrier Reef in 2005. A voucher specimen (G325059) was deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried ascidian sample (137.1 g dry weight) was extracted exhaustively using MeOH (5 × 300 mL), yielding a deep red gum (3.55 g). This extract was dissolved in a small amount of MeOH (30 mL), and C_{18} silica gel (4.0 g) was added. The solvent was evaporated, and the extract adsorbed onto the gel was transferred to four refillable HPLC columns (10 mm × 20 mm). Two of these columns were connected in series to a C_{18} HPLC column, and the combined columns eluted with a linear gradient of H_2O containing 1% TFA to MeOH containing 1% TFA at a flow rate of 10 mL min^{-1} over 60 min. Sixty 1 min fractions were collected, and all fractions were analyzed by (−) LRESIMS and ^{1}H NMR spectroscopy. Fractions containing similar signals were combined. This process was repeated on the remaining extract. Pure 1,8-dihydroxy-9,10-anthraquinone (**2**) (65 mg) eluted in approximately 50% MeOH, and fractions eluting in approximately 60−65% MeOH were still a mixture of compounds. These were combined and separated further by normal-phase HPLC on diol-bonded silica gel. A gradient from n-hexane to CH_2Cl_2 over 60 min followed by a gradient to MeOH over 20 min were used. Eighty 1 min fractions were collected. Fraction 33 was pure albopunctatone (**1**) (3.0 mg, 0.002%), and fractions 12−15 were pure 1,8-dihydroxy-9,10-anthraquinone (**2**) (16 mg).

Albopunctatone (1): yellow solid; UV λ_{max} (MeOH) (log ε) 202 (4.42), 227 (4.00), 259 (3.68), 287 (3.44), 391 (3.40), 442 (3.25) nm; λ_{max} (MeOH/NH_4OH) 203 (4.67), 225 (3.93), 255 (3.72), 289 (3.44), 383 (3.30) 513 (3.17); IR ν_{max} (film) 3747, 2962, 2929, 2857, 1743, 1709, 1647, 1548, 1533, 1518, 1276, 1211, 1057 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Table 1; (−)-HRESIMS m/z 479.07801 (calcd for $C_{28}H_{15}O_8$, 479.07614).

1,8-Dihydroxy-9,10-anthraquinone (2): yellow needles ($CHCl_3$), mp 193−195 °C (lit. mp 192−193 °C).[29]

Biological Activity. *Malaria Imaging Assay.* Antimalarial activity was determined using the method described by Duffy and Avery.[28] Briefly, compounds were incubated in the presence of 2% or 3% parasite (3D7 or Dd2) and 0.3% hematocrit in a total assay volume of 50 μL, for 72 h at 37 °C and 5% CO_2, in poly-D-lysine-coated CellCarrier Imaging plates. After incubation plates were stained with DAPI (4′,6-diamidino-2-phenylindole) in the presence of saponin and Triton X-100 and incubated for a further 5 h at RT in the dark before imaging on the OPERA HTS confocal imaging system. The digital images obtained were analyzed using the PerkinElmer Acapella spot detection software, where spots that fulfill the criteria established for a stained parasite are counted. The percent inhibition of parasite replication was calculated using DMSO and artemisinin control data.

Cytotoxicity Assay. Compounds were added to assay wells containing adherent cells (HEK293 or breast and pancreatic cancer cell lines) seeded at the appropriate densities according to their respective growth rates in 384-well black/clear tissue culture treated plates (Falcon, BD Biosciences) in an assay volume of 45 μL. The plates were incubated for 72 h at 37 °C and 5% CO_2. For the cancer cell lines, resazurin (Sigma-Aldrich) was added to a final concentration of 600 μM per well and incubated for 4 h before a measurement of fluorescent intensity was recorded on a Perkin-Elmer EnVision at 530/595 nm. For the HEK-293 cell line, the supernatant was removed after incubation, and 40 μL of 10% Presto Blue substrate (Sigma Aldrich) in DMEM plus 2 mM glutamax was added to each well. The plates were incubated for a further 3 h and measured on the Perkin-Elmer EnVision at 530/595 nm. The percent inhibition of growth was calculated in relation to the maximum and minimum inhibition of fluorescence caused by >10% DMSO or 20 μM puromycin (100% inhibition) and 0.4% DMSO (no inhibition). All experiments were performed in triplicate, n = 3. IC_{50} values were obtained by plotting percent inhibition against log dose using the Prizm4 graphing package using nonlinear regression with variable slope plot.

Trypanosome Viability Estimation Assay. The *T.b. brucei* viability assay was carried out as previously described by Sykes and Avery,[30] with a modification of the compound dilution medium. Briefly, 55 μL well^{-1} of 2000 cells mL^{-1} in HMI-9 medium supplemented with 10% FCS and 100 IU mL^{-1} penicillin/streptomycin were added to 384-well

plates with a Multidrop liquid handler (Thermo Scientific, USA). Plates were incubated for 24 h prior to addition of 5 μL of prediluted compounds or DMSO for control wells. Compounds or controls in 100% DMSO were prediluted 1:20 in high-glucose DMEM medium with L-glutamine supplemented with 1× nonessential amino acids and 1 mM sodium pyruvate (Invitrogen, USA), by a Minitrack robotic liquid handler (PerkinElmer, USA). Plates were incubated for a further 48 h at 37 °C and 5% CO_2 before addition of 10 μL of Alamar Blue (Biosource, USA). Plates were incubated for 2 h under the same conditions, then for a further 22 h at room temperature. Wells were read at excitation 535 nm, emission 590 nm on a Victor II Wallac plate reader (PerkinElmer, USA). Reference drugs included pentamidine (Sigma, USA), diminazene aceturate (Sigma, USA), and puromycin (Calbiochem, USA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra for albopunctatone and antiplasmodial concentration response curves for albopunctatone. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +61-7-5552-9187. Fax: +61-7-5552-7785 E-mail: a.carroll@girffith.edu.au.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. J. N. A. Hooper and his team at the Queensland Museum for collection and identification of the ascidian. We thank C. Lovitt, T. Shelper, and A. Jones for performing the biological evaluations against the breast and pancreatic cancer cell lines and trypanosomes, respectively. We gratefully acknowledge the Australian Red Cross Blood Bank for provision of the human erythrocytes for the culture of *P. falciparum*.

■ REFERENCES

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2011**, *28*, 196−268.
(2) Mcdonald, L. A.; Swersey, J. C.; Ireland, C. M.; Carroll, A. R.; Coll, J. C.; Bowden, B. F.; Fairchild, C. R.; Cornell, L. *Tetrahedron* **1995**, *51*, 5237−5244.
(3) Yin, S. Y.; Cullinane, C.; Carroll, A. R.; Quinn, R. J.; Rohan A. Davis, R. A. *Tetrahedron Lett.* **2010**, *51*, 3403−3405.
(4) Carroll, A. R.; Duffy, S.; Avery, V. M. *J. Org. Chem.* **2010**, *75*, 8291−9294.
(5) Carroll, A. R.; Avery, V. M. *J. Nat. Prod.* **2009**, *72*, 696−699.
(6) Carroll, A. R.; Feng, Y.; Bowden, B. F.; Coll, J. C. *J. Org. Chem.* **1996**, *61*, 4059−4061.
(7) Carroll, A. R.; Coll, J. C.; Bourne, D. J.; MacLeod, J. K.; Zabriskie, T. M.; Ireland, C. M.; Bowden, B. F. *Aust. J. Chem.* **1996**, *49*, 659−667.
(8) Carroll, A. R.; Healy, P. C.; Quinn, R. J.; Tranter, C. J. *J. Org. Chem.* **1999**, *64*, 2680−2682.
(9) Davis, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 158−160.
(10) Crecely, K. M.; Crecely, R. W.; Goldstein, J. H. *J. Magn. Reson.* **1969**, *1*, 633−638.
(11) Arnone, A.; Fronza, G.; Mondelli, R. *J. Magn. Reson.* **1977**, *28*, 69−79.
(12) Retzow, A.; Wiegrebe, W. *Sci. Pharm.* **1985**, *53*, 209−21.
(13) Aoyama, S.; Morita, J. *J. Pharm. Soc. Jpn.* **1932**, *52*, 48−56.
(14) Metzger, W.; Reif, K. *J. Chromatogr., A* **1996**, *740*, 133−138.
(15) Fournier, G.; Bercht, C. A. L; Paris, R. R.; Paris, M. R. *Phytochemistry* **1975**, *14*, 2099.
(16) Qiao, L.; He, Liying; Li, Y.; He, Y.; Gao, J.. *Xibei Zhiwu Xuebao* **2008**, *28*, 1258−1260.
(17) Zhang, J.; Tao, L.; Liang, Y.; Chen, L.; Mi, Y.; Zheng, L.; Wang, F.; She, Z.; Lin, Y.; To, K. K. W.; Fu, L. *Mar. Drugs* **2010**, *8*, 1469−1481.
(18) Howard, D. F.; Phillips, D. W.; Jones, T. H.; Blum, M. S. *Naturwissenschaften* **1982**, *69*, 91−92.
(19) Sanduja, R.; Alam, M.; Wellington, G. M. *J. Chem. Res. (S)* **1986**, *12*, 450−451.
(20) Shindo, T.; Sato, A.; Kasanuki, N.; Hasegawa, K.; Sato, S.; Iwata, T.; Hata, T. *Experientia* **1993**, *49*, 177−178.
(21) Mascolo, N.; Capasso, R.; Capasso, F. *Phytother. Res.* **1998**, *12*, S143−S145.
(22) Sekar, N.; Dighe, R. *Colourage* **2007**, *54*, 72−78.
(23) Bennett, M.; Cresswell, H. *Palliat. Med.* **2003**, *17*, 418−422.
(24) Conner, J. M.; Gray, A. I.; Waterman, P. G. *J. Nat. Prod.* **1990**, *53*, 1362−1364.
(25) Qhotsokoane-LusunziA, A. M.; Karuso, P. *Aust. J. Chem.* **2001**, *54*, 427−430.
(26) Hou, Y.; Cao, S.; Brodie, P. J.; Callmander, M. W.; Ratovoson, F.; Rakotobe, E. A.; Rasamison, V. E.; Ratsimbason, M.; Alumasa, J. N.; Roepe, P. D.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2009**, *17*, 2871−2876.
(27) Wube, A. A.; Bucar, F.; Asres, K.; Gibbons, S.; Rattray, L.; Croft, S. *Phytother. Res.* **2005**, *19*, 472−476.
(28) Duffy, S.; Avery, V. M. *Am. J. Trop. Med. Hyg.* **2012**, *86*, 84−92.
(29) Cameron, D. W.; Feutrill, G. I.; McKay, P. G. *Aust. J. Chem.* **1982**, *35*, 2095−2109.
(30) Sykes, M. L.; Avery, V. M. *Am. J. Trop. Med. Hyg.* **2009**, *81*, 665−674.