# New Cytotoxic 1-Azaanthraquinones and 3-Aminonaphthoquinone from the Stem Bark of *Goniothalamus marcanii*

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Guided by brine shrimp toxicity and human tumor cell toxicity, fractionation of the alcoholic extract from the stem bark of *Goniothalamus marcanii* led to the isolation of four new 1-azaanthraquinones: marcanines B (**3**), C (**4**), D (**5**), and E (**6**), along with two known derivatives: marcanine A and dielsiquinone. A new 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**7**), a possible 1-azaanthraquinone biosynthetic precursor, was also isolated. The structures of the compounds were elucidated by spectroscopic analyses, mainly 1D and 2D NMR techniques (^{1}H, ^{13}C, NOEDS, COSY, HMQC, and HMBC), as well as comparison with literature data. All the compounds except **6** were evaluated for cytotoxic activity. They exhibited significant cytotoxicity against several human tumor cell lines, A-549, HT-29, MCF7, RPMI, and U251 with the $ED_{50}$ in the range of 0.04−3.03 $\mu$M.

In the course of our investigation for bioactive compounds from Thai natural resources, we have studied the stem bark of *Goniothalamus marcanii* Craib (Annonaceae). The plant, commonly called "Khao-Laam" in Thai, is a small tree growing wild in the north, northeast, and south of Thailand. Our preliminary screening of the methanolic extract of the bark revealed its strong toxicity in the brine shrimp lethality test (BST)[1] with $LD_{50}$ = 3.1 $\mu$g/mL and also showed cytotoxicity against human tumor cell lines (HTCL), A-549 (lung carcinoma), HT-29 (colon adenocarcinoma), MCF7 (breast carcinoma), RPMI (melanoma), and U251 (brain carcinoma) with $ED_{50}$ about 1 $\mu$g/mL. Most of previous phytochemical investigations of the genus *Goniothalamus* demonstrated the presence of several groups of natural chemicals, including 1-azaanthraquinones,[2] tetrahydrofuran acetogenins,[3−7] aporphine alkaloids,[8−10] and styryllactones.[11−13] However, no chemical and biological studies on this species have been reported to date. In this paper, we report the isolation, structure elucidation, and cytotoxicity of five new compounds found in this plant: 1-azaanthraquinones, marcanines B−E (**3−6**) and 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**7**), and the two known 1-azaanthraquinones, marcanine A (**1**) and dielsiquinone (**2**). The first 1-azaanthraquinone compound was found in mycelium of *Pyrenochaeta terrestris*, the fungus responsible for "pinkroot disease" of onions.[13] In higher plants, the 1-azaanthraquinones were reported only from Annonaceous plants, such as cleistopholine from *Cleistopholis patens*,[14] *Annona hayesii*,[15] and *Meiogyne virgata*;[16] dielsiquinone from *Gutteria dielsiana*;[17] and scorazanone from *G. scortechinii*.[2]

## Results and Discussion

The ethanolic extract of the stem bark of *G. marcanii* was subjected to partition between $H_2O$ and $CHCl_3$. The

CHCl₃ phase was further partitioned between 90% aqueous methanol and hexane. The residue of the 90% aqueous methanol layer showing pronounced activity in BST and HTCL cytotoxicity tests was, therefore, subjected to BST- and HTCL-guided fractionation using Si gel columns and TLC to yield two known 1-azaanthraquinones, marcanine A (**1**) and dielsiquinone (**2**), together with four new derivatives, marcanines B−E (**3−6**) in yields of 2.1 × 10⁻⁴, 8.6 × 10⁻⁵, 1.7 × 10⁻⁴, 2.9 × 10⁻⁴, 1.6 × 10⁻⁵, and 2.2 × 10⁻⁵% w/w of the dried bark, respectively. The new naphthoquinone derivative, 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**7**) was also isolated in yield of 3.2 × 10⁻⁵% w/w.
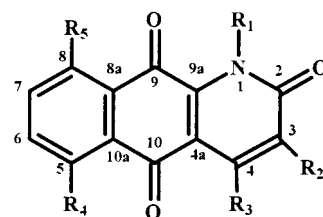


| | $\underline{R_1}$ | $\underline{R_2}$ | $\underline{R_3}$ | $\underline{R_4}$ | $\underline{R_5}$ |
|---|---|---|---|---|---|
| **1** = | H | H | CH₃ | H | H |
| **2** = | H | OCH₃ | CH₃ | H | H |
| **3** = | CH₃ | OCH₃ | CH₃ | H | H |
| **4** = | CH₃ | OCH₃ | CH₂OH | H | H |
| **5** = | H | OCH₃ | CH₃ | OH | H |
| **6** = | CH₃ | OCH₃ | CH₃ | H | OH |
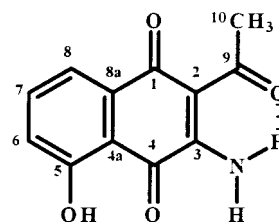


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**Table 1.** $^1$H NMR Data for Compounds **1**−**6** (500 MHz in CDCl$_3$)[a]

| proton(s) | 1 | 2 | 3 | 4 | 5 | 6[b] |
|---|---|---|---|---|---|---|
| 3 | 6.69 q (0.9) | | | | | |
| 5 | 8.19 dd (7.6, 1.2) | 8.16 dd (7.6, 0.9) | 8.07 dd (7.6, 1.2) | 8.09 dd (6.7, 2.3) | | 7.62 dd (7.6, 1.2) |
| 6 | 7.78 ddd (7.6, 7.6, 1.2) | 7.76 ddd (7.7, 7.7, 0.9) | 7.73 ddd (7.6, 7.6, 1.5) | 7.73 ddd (7.3, 6.9, 1.8) | 7.36 dd (8.2, 1.2) | 7.77 dd (8.5, 7.6) |
| 7 | 7.86 ddd (7.6, 7.6, 1.2) | 7.83 ddd (7.7, 7.7, 0.9) | 7.77 ddd (7.6, 7.6, 1.5) | 7.79 ddd (7.3, 6.9, 1.8) | 7.62 dd (8.2, 7.3) | 7.26 dd (8.5, 1.2) |
| 8 | 8.24 dd (7.6, 1.2) | 8.22 dd (7.7, 0.9) | 8.11 dd (7.3, 1.5) | 8.14 dd (6.7, 2.3) | 7.73 dd (7.3, 1.2) | |
| NH | 9.72 br s | | | | | |
| N-CH$_3$ | | | 3.96 s | 3.97 s | | 3.95 s |
| 3-OCH$_3$ | | 4.06 s | 4.01 s | 4.01 s | 4.06 s | 3.97 s |
| 4-CH$_3$ | 2.71 d (0.9) | 2.66 s | | | 2.65 s | 2.54 s |
| 4-CH$_2$OH | | | | 4.29 br s | | |
| 4-CH$_2$OH | | | | 3.90 br s | | |
| 5-OH | | | | | 12.55 s | |
| 8-OH | | | | | | 11.52 s |

[a] Chemical shifts ($\delta$) are in ppm relative to solvent peak. Observed splitting $J$ (Hz). [b] In acetone-$d_6$.

**Table 2.** $^{13}$C NMR Data for Compounds **1**−**6** (125 MHz in CDCl$_3$)[a]

| carbon | 1 | 2 | 3 | 4 | 5 | 6[b] |
|---|---|---|---|---|---|---|
| 2 | 160.4 | 156.8 | 158.6 | 159.1 | 156.5 | 155.4 |
| 3 | 127.7 | 152.8 | 151.0 | 151.0 | 152.5 | c |
| 4 | 139.8 | 134.7 | 135.9 | 135.6 | 137.1 | c |
| 4a | 116.1 | 119.7 | 120.4 | 119.9 | 116.7 | c |
| 5 | 126.7 | 126.5 | 126.5 | 126.8 | c | 119.3 |
| 6 | 133.7 | 133.6 | 133.6 | 134.2 | 126.8 | 137.7 |
| 7 | 135.8 | 135.4 | 134.3 | 134.5 | 135.9 | 124.1 |
| 8 | 127.6 | 127.5 | 126.4 | 126.7 | 119.5 | c |
| 8a | 133.3 | 130.0 | 132.5 | 132.1 | c | 114.9 |
| 9 | 181.4 | 182.0 | 182.9 | 184.2 | c | c |
| 9a | 152.2 | 137.6 | 139.5 | 140.2 | 141.8 | 140.9 |
| 10 | 178.0 | 177.4 | 180.3 | 179.9 | 176.6 | c |
| 10a | 130.0 | 128.9 | 132.2 | 132.1 | 112.1 | c |
| N-CH$_3$ | | | 35.2 | 35.4 | | 35.8 |
| 3-OCH$_3$ | | 60.0 | 59.7 | 60.9 | 59.9 | 59.7 |
| 4-CH$_3$ | 22.8 | 13.9 | 14.2 | | 13.9 | 14.2 |
| 4-CH$_2$OH | | | | 56.3 | | |

[a] Chemical shifts ($\delta$) are in ppm relative to solvent peak. Assigments were aided by HMQC and HMBC experiments. [b] In acetone-$d_6$. [c] Due to trace amount of sample, data could not be observed.

Marcanine A (**1**) was isolated as yellow needles and identified as 4-methyl-1$H$-1-aza-2,9,10-anthracenetrione by comparing its spectral data with the literature data.[18] Compound **1** was previously synthesized by the reactions of 2-amino-1,4-naphthoquinone with ethyl acetoacetate or 2,2,6-trimethyl-5$H$-1,3-dioxin-4-one.[18] However, this is the first report of **1** from a natural source with its complete $^1$H and $^{13}$C assignments on the basis of analyses of the HMQC and HMBC spectra (Tables 1 and 2).

Dielsiquinone (**2**) was isolated as a yellow amorphous powder and identified as 3-methoxy-4-methyl-1$H$-1-aza-2,9,10-anthracenetrione by comparing its spectral data with the literature data.[2] Compound **2** was previously isolated from the trunkwood of the Annonaceous plant *G. dielsiana*.[2] This is the first report of the isolation of **2** from the genus *Goniothalamus* as well as its complete $^{13}$C assignments on the basis of examination of the HMQC and HMBC spectra (Table 2).

Marcanine B (**3**) was obtained as an orange powder. The close similarity of the $^1$H and $^{13}$C NMR chemical shifts of **3** to those of **1** and **2** (Tables 1 and 2) indicated the common 4-methyl-1-aza-2,9,10-anthracenetrione nucleus. The IR spectrum confirmed this evidence by showing the quinone carbonyl and the lactam carbonyl absorptions at 1663 cm$^{-1}$ and 1648 cm$^{-1}$, respectively. The UV absorption bands ($\lambda_{max}$ 272, 299, 320 nm) also confirmed the presence of the conjugated quinonoid moiety. Accurate mass measurement by HREIMS of **3** exhibited the molecular ion at $m/z$ 283.0840, consistent with the molecular formula C$_{16}$H$_{13}$-NO$_4$. The difference in 14 mass units between **2** and **3** and the appearances of a three-proton singlet at $\delta$ 3.96 and a methyl carbon at $\delta$ 35.2 in the $^1$H and $^{13}$C NMR spectra of **3**, respectively, suggested an additional $N$-methyl group. The unchanged UV spectra of **3** upon addition of sodium hydroxide or sodium acetate supported the $N$-methyl-2-quinolone nucleus.[19,20] The $N$-methyl protons ($\delta$ 3.96) revealed the HMBC correlations to the lactam carbonyl carbon C-2 ($\delta$ 158.6) and the quaternary carbon C-9a ($\delta$ 139.5). The long-range HMBC correlations of 4-CH$_3$ ($\delta$ 2.63) to the oxygenated quaternary carbon C-3 ($\delta$ 151.0) and two quaternary carbons C-4 ($\delta$ 135.9) and C-4a ($\delta$ 120.4) confirmed the substitution of the methyl group at C-4. The signals of the carbonyl carbons of the quinone unit at $\delta$ 182.9 and 180.3 ppm were assigned as C-9 and C-10 by the long-range correlations to the signals of H-8 ($\delta$ 8.11) and H-5 ($\delta$ 8.07), respectively. The upfield shift of C-10 might result from transferring of electron density by cross conjugation between N-1 and C-10. The HMBC spectrum also revealed correlations of H-8 and H-5 to the quaternary carbons at C-10a ($\delta$ 132.2) and C-8a ($\delta$ 132.5), respectively. The chemical shifts of C-2, C-4, and C-9a were found relatively upfield compared with those of marcanine A, due to the effect of the 3-OCH$_3$. Compound **3** was, therefore, deduced as 3-methoxy-$N$,4-dimethyl-1-aza-2,9,10-anthracenetrione.

Marcanine C (**4**) was obtained as an orange amorphous powder and had the molecular formula C$_{16}$H$_{13}$NO$_5$ as deduced by HREIMS, showing an abundant molecular ion at $m/z$ 299.0792. The structure of **4** was closely related to **3** based on $^1$H NMR, $^{13}$C NMR, IR, and UV spectral data. However, **4** had 16 mass units more than **3**, indicating the presence of one extra oxygen atom, and its IR spectrum showed the presence of a hydroxyl group (3480 cm$^{-1}$). The $^1$H NMR spectrum of **4**, showing methylene protons as a broad singlet at $\delta$ 4.92 and an exchangeable proton as a broad signal at $\delta$ 3.90, suggested the presence of a hydroxymethyl group in **4** which was consistent with a hydroxymethyl carbon at $\delta$ 56.3 in the $^{13}$C NMR spectrum. The COLOC experiment supported the substitution of the hydroxymethyl group at C-4 by the correlations of C-3 ($\delta$ 151.0), C-4 ($\delta$ 135.6), and C-4a ($\delta$ 119.9) to the protons at $\delta$ 4.92. These results led to the structure of 3-methoxy-4-hydroxymethyl-$N$-methyl-1-aza-2,9,10-anthracenetrione for **4**.

**Table 3.** $^1H$ (500 MHz) and $^{13}C$ (125 MHz) NMR Data for Compound **7** in CDCl$_3$[a]

| position | $^{13}C$ | $^1H$ | HMBC correlations |
|---|---|---|---|
| 1 | 180.4 | | |
| 2 | 124.7 | | |
| 3 | 152.5 | | |
| 4 | 184.8 | | |
| 4a | 114.0 | | |
| 5 | 161.9 | | |
| 6 | 122.2 | 7.21 dd (7.9, 1.8) | C-4a, C-5, C-8 |
| 7 | 139.1 | 7.73 | C-5, C-6, C-8a |
| 8 | 119.7 | 7.76 | C-1, C-8, C-4a |
| 8a | 133.7 | | |
| 9 | 202.2 | | |
| 10 | 33.1 | 2.73 s | C-9 |
| 3-NH$_2$ | | 7.12 br s, 10.69 br s | |
| 5-OH | | 11.38 s | C-6, C-5, C-4a |

[a] Chemical shifts ($\delta$) are in ppm relative to solvent peak. Observed splitting $J$ (Hz).

Marcanine D (**5**) was isolated as a yellow powder. The HREIMS of **5** exhibited the molecular ion peak at $m/z$ 285.0637, leading to the molecular formula C$_{15}$H$_{11}$NO$_5$. The IR spectrum of **5** exhibited the presence of quinone carbonyl (1677 cm$^{-1}$), lactam carbonyl (1641 cm$^{-1}$), and hydroxyl (3484 cm$^{-1}$) groups. The $^1H$ and $^{13}C$ NMR spectra of **5** indicated that the lactam part had the same substitutions as **2**, but the aromatic part was different. The $^1H$ NMR spectrum of **5** showed a chelated hydroxyl proton as a sharp singlet at $\delta$ 12.55 and three adjacent aromatic protons at $\delta$ 7.73 (dd, $J = 7.3$, 1.2 Hz, H-8), 7.62 (dd, $J = 8.2$, 7.3 Hz, H-7), and 7.36 (dd, $J = 8.2$, 1.2 Hz, H-6). NOE enhancement observed for the hydroxyl proton (2.5%) by irradiation of the 4-methyl protons ($\delta$ 2.65) in the NOE difference spectrum (NOEDS) of **5** permitted us to place the hydroxyl group at C-5. Compound **5** was then assigned as 3-methoxy-5-hydroxy-4-methyl-1$H$-1-aza-2,9,10-anthracenetrione. Due to trace amount of **5**, some of $^{13}C$ assignments (Table 2) were obtained by comparing with the former compounds and analysis of its HMBC spectrum as well.

Marcanine E (**6**) was obtained as an orange powder. The HREIMS of **6** exhibited the molecular ion at $m/z$ 299.0785, corresponding to the molecular formula C$_{16}$H$_{13}$NO$_5$. The $^1H$ NMR spectrum in CDCl$_3$ of **6** indicated the presence of the $N$-methyl protons ($\delta$ 4.01), 3-methoxy protons ($\delta$ 4.03), and 4-methyl protons ($\delta$ 2.60) in the lactam part and the presence of unresolved aromatic protons ($\delta$ 7.63–7.67). However, the $^1H$ NMR of **6** in acetone-$d_6$ clearly showed the presence of three adjacent aromatic protons at $\delta$ 7.77 (dd, $J = 8.5$, 7.6 Hz, H-6), 7.62 (dd, $J = 7.6$, 1.2 Hz, H-5), and 7.26 ($J = 8.5$, 1.2 Hz, H-7) and the chelated hydroxy proton at $\delta$ 11.52. The IR spectrum of **6** confirmed the hydroxyl absorption at 3502 cm$^{-1}$. The placement of the hydroxyl group at C-8 was assured by the NOEDS of **6** in CDCl$_3$. Irradiation of the $N$-methyl protons ($\delta$ 4.01) resulted in 1.9% NOE enhancement of the chelated hydroxy proton ($\delta$ 11.74). Due to the trace amount of **6**, some of $^{13}C$ assignments (Table 2) were determined by comparing with the former compounds. Compound **6** was, therefore, assigned as 3-methoxy-8-hydroxy-$N$,4-dimethyl-1-aza-2,9,10-anthracenetrione.

Compound **7** was identified as 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone mainly on the analyses of its $^1H$ and $^{13}C$ NMR spectra with the aid of HMQC and HMBC experiments (Table 3). The molecular formula C$_{12}$H$_9$NO$_4$ was established for **7** by the HREIMS giving the molecular ion at $m/z$ 231.0536. The IR spectrum of **7** indicated the presence of amino and hydroxyl (3260–3159 cm$^{-1}$), conju-

**Table 4.** Cytotoxic Activity of the Compounds from *G. marcanii*

| compound | ED$_{50}$ in $\mu$M | | | | |
|---|---|---|---|---|---|
| | A-549 | HT-29 | MCF7 | RPMI | U251 |
| **1** | 0.42 | 0.42 | 0.42 | 0.42 | 0.84 |
| **2** | 0.11 | 1.12 | 0.11 | 0.11 | 0.37 |
| **3** | 0.35 | 2.12 | 0.18 | 0.70 | 1.40 |
| **4** | 1.00 | 0.33 | 1.00 | 0.67 | nt[a] |
| **5** | 0.04 | 0.35 | 0.08 | 0.08 | 0.28 |
| **7** | 2.60 | nd[b] | 2.60 | 3.03 | 3.03 |

[a] nt = The sample was not tested. [b] nd = The activity was not detected at the concentration <10 $\mu$g/mL.

gated carbonyl (1645 cm$^{-1}$), and quinone carbonyl (1623 cm$^{-1}$) groups. The $^1H$ NMR spectrum of **7** in CDCl$_3$ showed three adjacent aromatic proton signals at $\delta$ 7.21, 7.73, and 7.76. The splitting patterns of these protons were clarified by the experiment in acetone-$d_6$ showing the signals at $\delta$ 7.23 (dd, $J = 8.4$, 1.1 Hz, H-6), 7.70 (dd, $J = 7.6$, 1.1 Hz, H-8), and 7.83 (dd, $J = 8.4$, 7.6 Hz, H-7). The three-proton signal at $\delta$ 2.73 was assigned as the methyl protons of the aceto group at C-2. In addition, the spectrum showed two exchangeable protons at $\delta$ 7.12 (br s) and 10.69 (br s), assignable to the primary amine protons at C-3, and one chelated hydroxyl proton at $\delta$ 11.38. The 0.9% intensity enhancement of the H-8 signal ($\delta$ 7.76) upon irradiation of the methyl protons ($\delta$ 2.73) in the NOEDS of **7** in CDCl$_3$ was observed. This observation assured the placement of the chelated hydroxyl proton at C-5. Compound **7** is proposed as an intermediate in the biosynthesis of the hydroxylated 1-azaanthraquinones by undergoing the formation of the pyridone ring via incorporation of one acetate unit. The shikimate–acetate pathway to the 1-azaanthraquinones had been previously described by Goulart et al.[17] and Arango et al.[21]

Compounds **1**–**5** and **7** were evaluated for their cytotoxicity against a panel of human tumor cell lines as summarized in Table 4. All 1-azaanthraquinones were comparably cytotoxic as Adriamycin. Compounds **1**, **3**, and **4** showed cytotoxicity in all cell lines, with the ED$_{50}$ in the range of 0.18 to 2.12 $\mu$M, while **2** and **5** were more active than the other marcanines in A-549, MCF7, and RPMI cells, with the ED$_{50}$ in the range of 0.04 to 0.11 $\mu$M. The 3-aminonaphthoquinone (**7**) was less cytotoxic than the 1-azaanthraquinones.

### Experimental Section

**General Experimental Procedures.** All solvents were redistilled. Column chromatography was carried out with SiO$_2$ gel 60 (Merck, 230–400 mesh). UV spectra were obtained on a Milton Roy Spectronic 3000 array single-beam spectrometer. IR spectra were measured on a Shimadzu IR-440 infrared spectrometer. LRMS were recorded on a Finnigan 4023 GC–MS with INCOS 2000 data system and a Finnigan MAT with INCOS 50 data. HRMS were measured on a VG 70–250S or a Kratos MS-50 mass spectrometer. $^1H$ and $^{13}C$ NMR spectra were recorded on either a JEOL JMN-A 500 or a Bruker AM-500 spectrometer. Chemical shifts were reported in parts per million ($\delta$) with solvent signal as the internal reference. The NMR solvents were CDCl$_3$ and acetone-$d_6$ with the reference chemical shifts for $^1H$ NMR at $\delta$ 7.24 and 2.05 ppm and the reference chemical shifts for $^{13}C$ NMR at $\delta$ 77.0 and 206.0 ppm, respectively.

**Plant Material.** The stem bark of *G. marcanii* was collected from Phuroe District, Loei Province, Thailand, in December 1989. Authentication of the plant was performed by comparison with the herbarium specimens (no. Kerr 8563 and no. Maxwell 71-234) in the Botany Section, Technical Division,

Department of Agriculture, Ministry of Agriculture and Co-operatives, Bangkok, Thailand. Voucher specimens of the plant (Cupcog 891201) have been deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

**Bioassays.** The brine shrimp larvae lethality bioassay was performed by the method of Meyer et al.[1] The cytotoxic activity bioassay against five human tumor cell lines, such as A-549 (human nonsmall cell lung carcinoma), HT-29 (human colon adrenocarcinoma), MCF7 (human breast carcinoma), RPMI (melanoma), and U251 (human brain carcinoma) was performed at the Cell Culture Service, Department of Pathology, College of Medicine, the Ohio State University. The assay was carried out by seeding cell-line aliquots from stock solutions into individual wells of microtiter plates. The plates were then incubated for 24 h to allow the cells to stabilize. Test agents were added to the cells at concentrations that represent 5-log dilutions. The cells were then incubated in the presence of the drug for a further 48 h. The cells were fixed to the plates by means of trichloroacetic acid, and after a number of washes, the cell layer was treated with a protein-binding dye, sulforhodamine B. The optical density, which was proportional to the protein mass, was read by automated spectrophotometric plate readers at a wavelength of 515 nm. The reference drug was Adriamycin.

**Extraction and Isolation.** Ground, dry stem bark (6.8 kg) of *G. marcanii* was exhaustively macerated with 95% ethanol at room temperature, filtered, and concentrated to give a brown crude extract (453 g). The crude extract was partitioned between $CHCl_3$ and $H_2O$. The lower layer was concentrated to dryness under reduced pressure to give the $CHCl_3$ extract (198 g), which was then partitioned between hexane and 90% aqueous MeOH to afford the methanolic extract (151 g) and the hexane extract (42 g) after removal of solvent in vacuo. All fractions were evaluated for lethality to brine shrimp larvae and for cytotoxicity against HTCL. The methanolic extract showed strong toxicity in BST with $LD_{50} = 3.1$ $\mu$g/mL and also revealed cytotoxicity against HTCL with $ED_{50}$ about 1 $\mu$g/mL. Therefore, the methanolic extract (150 g) was adsorbed onto 150 g of Kieselguhr and fractionated by quick column chromatography over a scintered glass filter column of Si gel (800 g, 10 × 20 cm column) using increasing amounts of MeOH in $CHCl_3$ as eluent (1% MeOH to 50% MeOH) to yield 13 fractions (F008–F020). Fraction F009 (14.5 g) showed toxicity in BST with $LD_{50} = 4.8$ $\mu$g/mL and cytotoxicity against HTCL with $ED_{50} = 10^{-1}$ $\mu$g/mL. A portion of F009 (6.7 g) was further chromatographed on a Si gel flash column (250 g) eluting with gradients of hexane in $CH_2Cl_2$ and MeOH in $CH_2Cl_2$ to yield 15 fractions (F065–F079). Fraction F076 (36 mg) was recrystallized from dichloromethane to afford marcanine A (**1**) (6.5 mg) as yellow needles. Fraction F074 (52 mg) was further purified by Si gel TLC plates (250 $\mu$m, 1% MeOH in $CH_2Cl_2$) to yield two yellow powders, dielsiquinone (**2**) (2.7 mg) and marcanine D (**5**) (0.5 mg). Fraction F070 (34 mg) was purified by Si gel TLC plates (250 $\mu$m, with subsequent double developing with 1% MeOH in $CH_2Cl_2$ and toluene–EtOAc–HOAc, 94:6:1) to provide two orange compounds, marcanine B (**3**) (5.3 mg) and marcanine E (**6**) (0.7 mg). Fraction F077 (44 mg) was purified by Si gel TLC plates (250 $\mu$m, 2% MeOH in $CH_2Cl_2$) to afford marcanine C (**4**) (9.0 mg) as an orange powder. Purification of fraction F067 (50 mg) was performed by Si gel TLC plates (250 $\mu$m, subsequent double developing with 2% MeOH in $CH_2Cl_2$ and 10% $Et_2O$ in $CH_2Cl_2$) to yield 5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone (**7**) (1.0 mg) as a yellow powder.

**Marcanine B (3):** orange powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 272 (4.36), 299 sh (4.08), 320 sh (3.86), 419 (3.30) nm; UV (MeOH + 2.5 N NaOH) $\lambda_{max}$ (log $\epsilon$) 272 (4.38), 301 sh (4.07), 320 sh (3.88), 419 (3.32) nm; UV (MeOH + NaOAc) $\lambda_{max}$ (log $\epsilon$) 272 (4.38), 301 sh (4.07), 320 sh (3.88), 415 (3.33) nm; IR (film) $\nu_{max}$ 2921, 1663, 1648, 1587, 1419, 1384 cm$^{-1}$; $^1$H NMR data, Table 1; $^{13}$C NMR data, Table 2; EIMS (70 eV) $m/z$ 283 [M]$^+$ (100), 268 (61), 255 (7), 254 (38), 240 (69), 239 (22), 226 (10), 212 (24); HREIMS $m/z$ 283.0840 (calcd for $C_{16}H_{13}NO_4$, 283.0841).

**Marcanine C (4):** orange powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 272 (4.23), 306 sh (3.97), 323 sh (3.82), 419 (3.19) nm; UV $\lambda_{max}$ (MeOH + 2.5 N NaOH) (log $\epsilon$) 272 (4.29), 305 sh (4.1), 325 sh (3.97), 415 (3.58) nm; UV (MeOH + NaOAc) $\lambda_{max}$ (log $\epsilon$) 273 (4.23), 305 sh (3.99), 324 sh (3.82), 415 (3.22) nm; IR (KBr) $\nu_{max}$ 3720–3234, 2929, 1657, 1584, 1514, 1462, 1397 cm$^{-1}$; $^1$H NMR data, Table 1; $^{13}$C NMR data, Table 2; EIMS (70 eV) $m/z$ 299 [M]$^+$ (83), 284 (100), 270 (7), 256 (21), 239 (16), 211 (8), 210 (11), 183 (4), 182 (6); HREIMS $m/z$ 299.0792 (calcd for $C_{16}H_{13}NO_5$, 299.0790).

**Marcanine D (5):** yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 275 (4.20), 295 (4.05) nm; IR (film) $\nu_{max}$, 3484–3352, 2954, 2919, 2851, 1677, 1641, 1522, 1289 cm$^{-1}$; $^1$H NMR data, Table 1; $^{13}$C NMR data, Table 2; EIMS (70 eV) $m/z$ 285 [M]$^+$ (100), 270 (19), 256 (47), 242 (22), 228 (14), 214 (58), 186 (12); HREIMS $m/z$ 285.0637 (calcd for $C_{15}H_{11}NO_5$, 285.0637).

**Marcanine E (6):** orange powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 274 (4.13), 300 (3.95) nm; IR (film) $\nu_{max}$ 3502, 2955, 2919, 2855, 1660, 1652, 1647, 1635, 1455 cm$^{-1}$; $^1$H NMR data in acetone-$d_6$, Table 1; $^{13}$C NMR data in acetone-$d_6$, Table 2; $^1$H NMR (CDCl$_3$) $\delta$ 11.74 (1H, br s, 8-OH); 7.67–7.63 (2H, H-5, H-6), 4.03 (3H, s, O–CH$_3$), 4.01 (3H, s, N–CH$_3$), 2.60 (3H, s, 4-CH$_3$); EIMS (70 eV) $m/z$ 299 [M]$^+$ (100), 284 (37), 270 (19), 256 (12), 242 (6), 228 (14); HREIMS $m/z$ 299.0785 [M]$^+$ (calcd for $C_{16}H_{13}$-NO$_5$, 299.0790).

**5-Hydroxy-3-amino-2-aceto-1,4-naphthoquinone (7):** yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 260 (4.31), 296 (4.08) nm; IR (film) $\nu_{max}$ 3260, 3159, 2965, 2924, 1645, 1623, 1450 cm$^{-1}$; $^1$H and $^{13}$C NMR data in CDCl$_3$, Table 3; $^1$H NMR (acetone-$d_6$, 500 MHz) $\delta$ 11.40 (1H, s, 8-OH), 10.71 (1H, br s, N–H), 8.17 (1H, br s, N–H), 7.83 (1H, dd, $J = 8.4$, 7.6 Hz, H-7), 7.70 (1H, dd, $J = 7.6$, 1.1 Hz, H-8), 7.23 (1H, dd, $J = 8.4$, 1.1 Hz, H-6), 2.62 (3H, s, H-10); EIMS (70 eV) $m/z$ 231 [M]$^+$ (100), 216 (57), 203 (8), 188 (26), 160 (4); HREIMS $m/z$ 231.0536 (calcd for $C_{12}H_9NO_4$, 231.0532).

**References and Notes**

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