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Daphmacromines K–O, alkaloids from *Daphniphyllum macropodum*

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ABSTRACT

Five new yuzurimine-type *Daphniphyllum* alkaloids, daphmacromines K–O (**1**–**5**), were isolated from the leaves and stems of *Daphniphyllum macropodum*. Their structures were elucidated by extensive spectroscopic techniques, including 2D NMR spectroscopy and mass spectrometry. Daphmacromine O (**5**) showed moderate cytotoxic activity against brine shrimp.

1. Introduction

Daphniphyllum alkaloids, elaborated by the plants of the genus *Daphniphyllum* (Daphniphyllaceae), are a family of natural products with diversified and complex polycyclic skeletons [1–4]. Their unique structural features have drawn much attention as challenging targets for total synthesis [5–9] and biosynthetic research [10,11] for several decades. In recent years, quite a number of new *Daphniphyllum* alkaloids have been isolated and identified, and some of them possessed novel skeletons [12–15]. In our continuing search for structurally unique and bioactive *Daphniphyllum* alkaloids from *Daphniphyllum macropodum*, five new yuzurimine-type ones, daphmacromines K to O (**1** to **5**) were obtained. We report herein the isolation, structural elucidation, and bioassay of these five alkaloids.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured with a Jasco P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for IR spectra as KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometer with TMS as internal standard. HRESIMS was performed on an API QSTAR time-of-flight spectrometer. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Waters X-Bridge C18 (4.6 × 250 mm) column. Column chromatography (CC) was performed using silica gel (200–300 mesh and 300–400 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. China) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden).

2.2. Plant material

The leaves and stems of *D. macropodum* were collected from Sichuan Province, People's Republic of China, in October 2010.

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The plant samples were identified by Prof. Liangke Song of the School of Life Science and Engineering, Southwest Jiaotong University (SWJTU). A voucher specimen (KIB H20101011) was deposited at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Science (CAS).

2.3. Extraction and isolation

The air-dried, powdered leaves and stems (34 kg) of *D. macropodum* were extracted three times with 95% EtOH. The extract was adjusted with saturated tartaric acid to pH 2–3 and then defatted with petroleum ether (PE). Next, the aqueous phase was adjusted to pH 10 with saturated Na_2CO_3 and extracted with $CHCl_3$ to obtain the crude alkaloid fraction (260 g). The total alkaloid was subjected to normal phase Si gel (200–300 mesh; $CHCl_3$/MeOH, 1:0 to 0:1) to obtain five major fractions (Fr 1–5). Fraction 2 (5 g) was further repeatedly subjected to Si, RP-18 and sephadex gel to produce compounds **1** (20 mg), **2** (5 mg), **4** (4 mg), and **5** (4 mg). Fr. 4 (30 g) was further chromatographed over a reverse phase medium pressure column (MeOH/H_2O, 1:1 to 1:0) to produce five fractions (Fr 4A–4E). Fraction 4B (3.0 g) was repeatedly subjected to Si, RP-18 and sephadex LH-20 gel to produce compound **3** (40 mg).

2.4. Daphmacromine K (**1**)

White amorphous powder. $[\alpha]_D^{22} = +46.4$ ($c = 0.23$, MeOH). UV (MeOH): 203 (3.00). IR (KBr): 3416, 2949, 2930, 2878, 2848, 1732, 1713, 1437, 1375, 1195, 1172, 1066 and 963 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 2 and 1. Positive ESIMS: *m/z* 384 $[M + H]^+$. Positive HRESIMS: $[M + H]^+$ *m/z* 384.2181, calcd 384.2175.

2.5. Daphmacromine L (**2**)

White amorphous powder. $[\alpha]_D^{21} = +25.1$ ($c = 0.26$, MeOH). UV (MeOH): 206 (2.92). IR (KBr): 3426, 1711, 1632, 1441, 1383, 1203, 1172, 1058 and 1014 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 2 and 1. Positive ESIMS: *m/z* 372 $[M + H]^+$. Positive HRESIMS: $[M + H]^+$ *m/z* 372.2168, calcd 372.2175.

2.6. Daphmacromine M (**3**)

White amorphous powder. $[\alpha]_D^{18} = +0.3$ ($c = 0.45$, MeOH). UV (MeOH): 203 (2.79). IR (KBr): 3426, 2974, 2945, 2503, 1726, 1631, 1456, 1376, 1346, 1245 and 1028 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 2 and 1. Positive ESIMS: *m/z* 400 $[M + H]^+$. Positive HRESIMS: $[M + H]^+$ *m/z* 400.2476, calcd 400.2487.

2.7. Daphmacromine N (**4**)

White amorphous powder. $[\alpha]_D^{22} = +21.5$ ($c = 0.12$, MeOH). UV (MeOH): 300 (3.21) and 202 (2.86); IR (KBr): 3439, 1685, 1629, 1437, 1385, 1270 and 1118 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 2 and 1. Positive ESIMS: *m/z* 386 $[M + H]^+$; Positive HRESIMS: $[M + H]^+$ *m/z* 386.2338, calcd 386.2331.

Table 1
^{13}C NMR spectroscopic data for daphmacromines K to O (**1** to **5**).

Position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
1	70.8	75.0	66.8	97.1	94.2
2	78.5	78.8	36.1	43.9	44.2
3	132.8	130.3	28.1	22.6	28.3
4	129.4	138.0	77.9	30.4	69.7
5	53.0	72.5	40.0	43.5	45.9
6	39.5c	41.2	35.8	38.9	35.6
7	51.7	54.2	56.9	59.5	59.0
8	43.2	47.5	47.3	51.5	49.6
9	146.9	144.7	143.3	150.4	83.5
10	134.8	136.0	137.1	152.3	73.2
11	26.6	26.7	25.8	32.1	27.0
12	27.6	25.8	28.2	26.3	28.1
13	39.5c	38.0	38.9	43.2	41.9
14	43.5	44.4	43.5	118.8	127.3
15	55.2	56.0	54.3	170.7	159.2
16	28.6	27.5	29.3	25.8	22.9
17	43.0	43.4	43.6	42.4	33.8
18	49.5	49.6	36.9	35.6	35.1
19	60.6	60.6	65.0	64.7	64.3
20	18.1	18.5	14.3	15.6	14.6
21	210.3		21.0	66.7	65.5
22	176.1	178.7	178.2	166.9	164.8
23	51.2	51.5		50.7	51.3
OAc			172.1		170.4
			20.9		21.0

[a] Recorded in Prydine-d_5 at 125 MHz.
[b] Recorded in Methanol-d_4 at 100 MHz.

2.8. Daphmacromine O (**5**)

White amorphous powder. $[\alpha]_D^{24} = +59.0$ ($c = 0.17$, MeOH). UV (MeOH): 239 (3.27). IR (KBr): 3440, 2956, 2927, 2878, 1743, 1716, 1632, 1249, 1118, 1029 and 843 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 2 and 1. Positive ESIMS: *m/z* 386 $[M + H]^+$. Positive HRESIMS: $[M + H]^+$ *m/z* 502.2429, calcd 502.2441.

3. Results and discussion

Daphmacromine K (**1**) was obtained as white amorphous powder, and its molecular formula $C_{23}H_{29}NO_4$ was established by positive HRESIMS (*m/z* 384.2181 $[M + H]^+$, calcd for $C_{23}H_{30}NO_4$, 384.2175), corresponding to 10 degrees of unsaturation. The IR absorption bands at 1732 and 1713 cm^{-1} suggested the presence of two carbonyl groups. The ^{13}C NMR and DEPT data (Table 1) revealed 23 carbon signals, and were distinguished as two methyls, seven sp^3 methylenes, five sp^3 methines, three sp^3 quaternary carbons, two double bonds (1,2-disubstituted one at δ_C 129.4 and 132.8, and tetrasubstituted one at δ_C 134.8 and 146.9), and two carbonyls (one isolated aldehyde at δ_C 210.3 and one ester at δ_C 176.1). Among them, two methylene groups (δ_C 51.7 and 60.6) and one methine group (δ_C 70.8) were ascribed to those bearing a nitrogen atom, while one quaternary carbon (δ_C 78.3) was attributed to it connected with an oxygen atom. The above data indicated that **1** possessed hexacyclic structure.

Comparison of the NMR data of **1** with those of daphhimalenine B [15] showed very close similarities, except for the following observation: one α-nitrogen methine

Table 2
^{1}H NMR spectroscopic data for daphmacromines K to O (**1** to **5**).

	1[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
1	3.42 (s)	3.48 (s)	3.57 (m)[c]		
2			2.75 (m)[c]	2.40 (m)	2.11 (m)[c]
3	6.23 (d, 10.5)		1.73 (m)	1.81 (m)	2.11 (m)[c]
				1.52 (td, 9.0, 7.0)	1.53 (m)
4	6.37 (d, 10.5)		4.97 (dd, 12.0, 6.0)	2.38 (m)	5.94 (m)
				1.59 (m)	
6	2.04 (m)	2.08 (m)	2.62 (m)	2.62 (m)	2.52 (m)
7	2.85 (m)	3.06 (m)	3.72 (d, 14.8)	3.39 (m)	3.20 (dd, 12.5, 9.0)
	2.85 (m)	3.06 (m)	3.57 (m)[c]	3.27 (d, 12.5)	2.97 (d, 12.5)
11	2.65 (m)	2.02 (m)	2.18 (m)	2.23 (m)	2.48 (dt, 3.0, 14.5)
	2.03 (m)	2.02 (m)	2.42 (m)	1.86 (m)	1.93 (d, 14.5)
12	1.91 (m)	2.70 (m)	2.07 (m)	2.95 (t, 14.5)	1.75 (t, 14.5)
	1.59 (m)	1.58 (dd, 9.5, 6.5)	1.64 (m)	1.92 (m)	1.33 (m)
13	3.83 (dd, 15.5, 9.0)	3.60 (m)	2.80 (m)	3.56 (m)	3.78 (d, 16.0)
	2.28 (m)	2.84 (d, 15.5)	2.10 (m)	3.43 (m)	3.58 (d, 16.0)
14	3.10 (dt, 3.5, 10.0)	2.95 (t, 8.0)	2.63 (m)		
15	3.75 (br s)	3.63 (m)	3.57 (m)[c]		
16	1.87 (m)	1.84 (dt, 14.4, 7.5)	1.93 (m)	2.69 (dd, 18.0, 6.0)	2.74 (m)
	1.42 (m)	1.35 (m)	1.48 (m)	2.49 (dd, 18.0, 7.0)	2.16 (m)
17	2.58 (m)	2.64 (m)	2.97 (m)	2.57 (m)	2.16 (m)
	2.32 (m)	2.35 (dd, 14.4, 9.0)	2.33 (m)	2.17 (m)	2.16 (m)
18	2.68 (m)	2.68 (m)	2.75 (m)[c]	3.03 (m)	2.88 (m)
19	3.38 (dd, 12.5, 9.5)	3.39 (dd, 12.1, 9.7)	3.95 (t, 10.8)	3.77 (m)	3.68 (m)
	2.35 (dd, 12.5, 4.0)	2.39 (dd, 12.1, 3.0)	2.87 (m)	2.28 (dd, 11.5, 6.0)	2.16 (m)
20	1.25 (d, 7.5)	1.30 (d, 7.5)	1.15 (m)[c]	1.08 (d, 9.0)	0.95 (d, 7.5)
21	10.82 (s)		1.15 (m)[c]	4.11 (d, 10.5)	4.51 (d, 12.0)
				3.82 (d, 10.5)	3.93 (d, 12.0)
23	3.58	3.56 (s)		3.70 (s)	3.69 (s)
OAc			2.08 (s)		2.08 (s)

[a] Recorded in Prydine-d_5 at 500 MHz.
[b] Recorded in Methanol-d_4 at 400 MHz.
[c] Overlapped.

(δ_C 70.8) and one deshielded sp^3 quaternary carbon (δ_C 78.5) bearing one hydroxyl group for **1** were displayed by sp^3 methine and an amino ketal quaternary carbon of the latter. All the data suggested that they likely shared the same planar structural moiety at rings *B*, *C*, and *D*. The hydroxyl group was assigned to C-2 at δ_C 78.5 on the basis of the HMBC correlations the HMBC J^2 correlations from H-1, H-18 (δ_H 2.68, m) and H-3 (δ_H 1.25, d, 7.5) to C-2 (δ_C 78.5), and J^3 correlations from H-1 (δ_H 3.42, s) to C-3 (δ_C 132.8) and C-18 (δ_C 49.5). The gross structure of **1** was finally established from its 2D NMR spectra (HSQC, ^{1}H – ^{1}H COSY, and HMBC) as shown in Fig. 1. Four partial structures, a: H_3-20/H-18/H_2-19, b: H-3/H-4, c: H_2-7/H-6/H_2-12/H_2-11, and d: H_2-13/H-14/H-15/H_2-16/H_2-17 (Fig. 2A), were revealed by the ^{1}H – ^{1}H COSY spectrum. By the analysis of the HMBC spectrum (Fig. 1), the linkage of the four structural fragments a–d could be established *via* the nitrogen atom and five quaternary carbons (C-2, C-5, C-8, C-9, and C-10), and the aldehyde and ester group were assigned at C-21 and C-22, respectively. The relative configuration of **1** was elucidated to be the same as that of daphhimalenine B, as judged from the ROESY spectrum in Fig. 2B. The structure of daphmacromine K (**1**) was thereby elucidated as **1**.

Daphmacromine L (**2**), was established as $C_{22}H_{29}NO_4$ by positive HRESIMS (*m/z* 372.2168 $[M + H]^+$, calcd for $C_{22}H_{30}NO_4$, 372.2175) with nine degrees of unsaturation. The ^{13}C NMR spectrum displayed 22 carbon resonances attributed to two methyls, seven sp^3 methylenes, five sp^3 methines, three sp^3 quaternary carbons, two double bonds, (1,2-disubstituted one at 130.3 and 138.0, and tetrasubstituted one at δ_C 144.7 and 136.0), and one carbonyl group. Comparing the ^{1}H and ^{13}C NMR chemical shifts (Tables 2 and 1) of **2** with those of **1**, the major differences were the presence of a hydroxylated quaternary carbon (δ_C 72.5) in the former and the absence of the quaternary carbon at C-5 and the isolated aldehyde at C-21 in the latter. The hydroxylated quaternary carbon was assigned at C-5 (δ_C 72.5) by the HMBC correlations from H-3 (δ_H 6.09, dd, 10.0, 2.0), H-4 (δ_H 5.97, d, 10.0), H-6 (δ_H 2.04, m), H-7 (δ_H 3.06, m), and H-12 (δ_H 2.70, m; δ_H 1.58, d, 9.5, 6.5) to C-5. The remaining structure and relative configuration of **2** were identical to that of **1** as determined by the HMBC and ROESY experiments. Hence, the structure of **2** was established as shown in Fig. 1.

Daphmacromine M (**3**) was obtained as white powder. Its molecular formula, $C_{24}H_{33}NO_4$, was established by HRESIMS (*m/z* 400.2476 $[M + H]^+$, calcd for $C_{24}H_{34}NO_4$, 400.2487). The ^{1}H and ^{13}C NMR data of **3** (Tables 2 and 1) were analogous to those of yunnandaphnine B [16], except for the presence of acetyl signals (qC δ_C 172.1; $-CH_3$, δ_C 20.9, δ_H 2.08, s), instead of methoxyl in the latter. The location of the acetyl group at C-4 was deduced from the HMBC correlation from H-4 (δ_H 4.97, dd, 12.0, 6.0) to the acetyl carbonyl (δ_C 172.1). The structure of **3** was then verified by the combination of the HSQC, ^{1}H – ^{1}H COSY, HMBC and ROESY spectra as shown in Fig. 1.

Daphmacromine N (**4**) has a molecular formula of $C_{23}H_{31}NO_4$, as deduced from the HRESIMS (*m/z* 386.2338 $[M + H]^+$, calcd for $C_{23}H_{32}NO_4$, 386.2331),with 9 degrees of unsaturation. The UV absorption band at 300 nm (log ε 3.21)

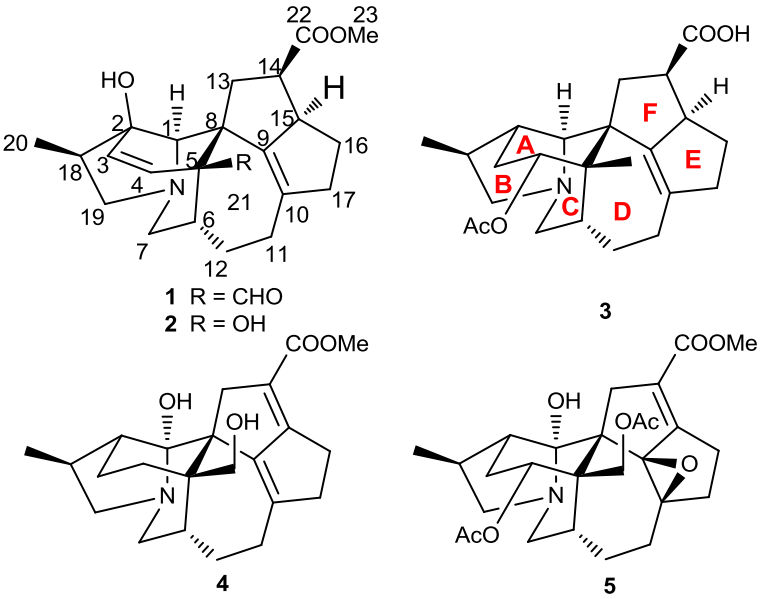

Fig. 1. Structures of **1** to **5**.

and IR absorptions at 1685, 1646, and 1629 cm^{-1} were features of a typical ester carbonyl conjugated with two double bands compared to literature data [17,18]. The ^{13}C and ^{1}H NMR data (Tables 2 and 1) showed that alkaloid **4** was a derivative of calycinine A [19], and the only difference was the presence of an OH group at C-21 (δ_C 68.1) in **4**, as judged by the HMBC correlations from H_2-21 (δ_H 4.11, d, 10.5; δ_H 3.82, d, 10.5) to C-4 (δ_C 30.4), C-5 (δ_C 43.5), C-6 (δ_C 38.9), and C-8 (δ_C 51.5). The relative configuration of **4** was further verified by ROESY data.

Daphmacromine O (**5**) was assigned as $C_{27}H_{35}NO_8$, as deduced from the HRESIMS (*m/z* 502.2429 $[M + H]^+$, calcd for $C_{27}H_{36}NO_8$, 502.2441). The ^{13}C (Table 1) and ^{1}H (Table 2) NMR data suggested that **5** was an analogue of daphtenidine D [20]. Two oxygenated quaternary carbons at δ_C 83.5 and 73.2 were respectively assigned to C-9 and C-10, which formed an epoxide, as in the case of caldaphnidine I [21] by the mutual HMBC correlations from H-13 (δ_H 3.58, d, 16.0) to C-9 (δ_C 83.5) and H-12 (δ_H 1.33, m) to C-10 (δ_C 73.2) (Fig. 3A). The relative configuration of **5** was elucidated by ROESY spectrum analysis, and the co-facial oriented protons H_3-20 (δ_H 0.95, d, 7.5), H-6 (δ_H 2.52, m), H_2-21 (δ_H 4.51, d, 12.0; δ_H 3.93, d, 12.0), H-4 (δ_H 5.94, m) and H-2 (δ_H 2.10, m) were artificially assigned as β orientation by ROESY cross peaks H_3-20/H-19β (δ_H 2.16, m)/H-6/H_2-21/H-4/H-2 (Fig. 3B). Hence, the structure of **5** was established as shown in Fig. 1.

Compounds **1** to **5** were assayed *in vitro* for cytotoxicity against brine shrimp (*Artemia salina*) by the previously described Microwell method [22,23], and against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW-480) using the MTT method with cisplatin and paclitaxel

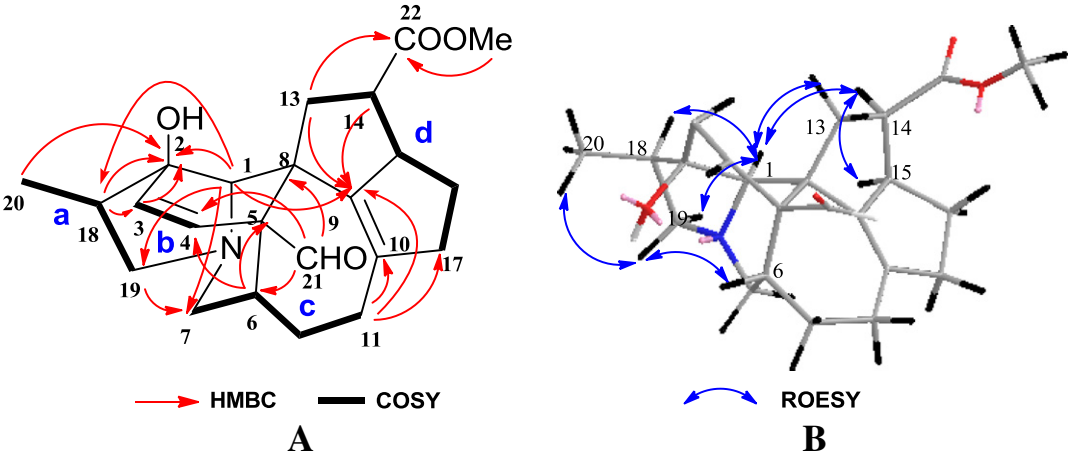

Fig. 2. (A) $^{1}H-^{1}H$ COSY (bold) and key HMBC correlations (arrow, H → C) of **1**. (B) Key ROESY correlations of **1**.

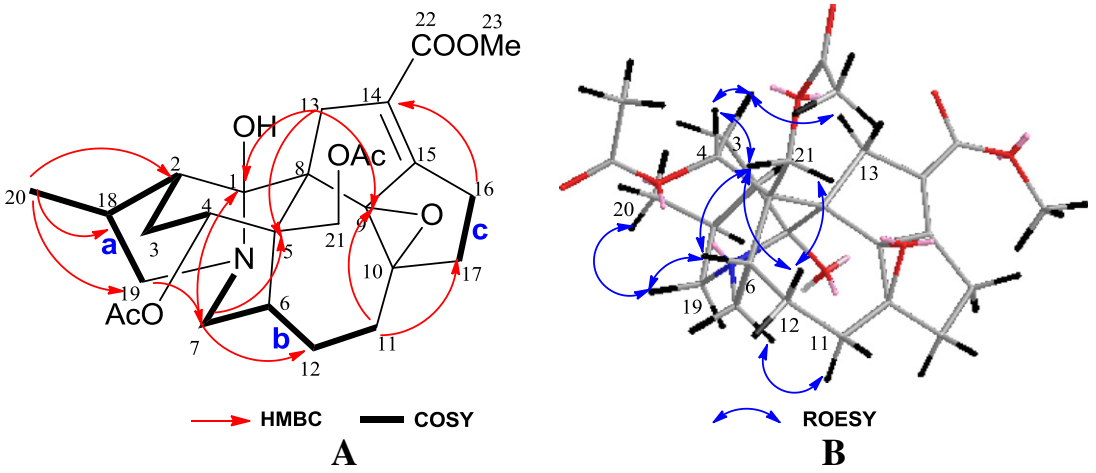

Fig. 3. (A) $^{1}H-{}^{1}H$ COSY (bold) and key HMBC correlations (arrow, H → C) of **5**. (B) Key ROESY correlations of **5**.

as positive controls [24], in which compound **5** exhibited corrected mortality of 76.6% against brine shrimp at 100 ppm.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.06.005.

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