

## Short communication

Antimicrobial and anti-oxidant activities of quinoline alkaloids from *Pseudomonas aeruginosa* BCC76810

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## ABSTRACT

Twelve 4-hydroxyquinoline derivatives (**1–12**) and three phenazine alkaloids (**13–15**) have been isolated from *Pseudomonas aeruginosa* BCC76810. All these compounds, except compounds **6**, **7**, **9**, and **11**, are known. The new quinolines are 2-((*Z*)-undec-4'-enyl)-4-hydroxyquinoline (**6**), 2-(3'-(2'-hexylcyclopropyl)propyl)-4-hydroxyquinoline (**7**), 2-*n*-octyl-4-hydroxyquinoline *N*-oxide (**9**), and 2-((*Z*)-undec-4'-enyl)-4-hydroxyquinoline *N*-oxide (**11**). Their structures were elucidated based on the spectroscopic information, such as 1D, 2D NMR, UV, and HRESIMS data. 4-Hydroxyquinolines (**1–6**) and 4-hydroxyquinoline-*N*-oxides (**8–11**) exhibited antimalarial activity against *Plasmodium falciparum*, K1 strain (IC<sub>50</sub> 0.25–2.07 μg/mL) with moderate to weak cytotoxicity against cancerous (KB, MCF-7, NCI-H187) and non-cancerous (Vero) cells. In addition, 4-hydroxyquinoline *N*-oxides also displayed anti-*Bacillus cereus* and antioxidant activities.

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## 1. Introduction

Quinoline alkaloids are typically found in higher plants such as *Ruta chalepensis*, (Sayed et al., 2000) *Euodia rutaecarpa* (Zhao et al., 2015), *Waltheria indica* (Cretton et al., 2014), in fungi such as *Penicillium* sp. EPF-6 (Kakinuma et al., 2000), *Corginarius subtortus* (Teichert et al., 2008), and in Gram-negative bacteria such as *Cytophaga johnsonii* (Evans et al., 1978), *Pseudomonas methanica* (Kitamura et al., 1986), *P. aeruginosa*. (Royt et al., 2001) Quinolines possess a broad range of biological activities including antimalarial, anticancer, antimicrobial, antifungal, anthelmintic, anticonvulsant, anti-inflammatory, and analgesic activities. 2-alkyl-4-hydroxyquinolines (or 4-hydroxy-2-alkyl-quinolines, HAQs) was frequently found from various strains of *Pseudomonas* spp. (Hays et al., 1945; Lépine et al., 2004; Wells, 1952). Some HAQs showed antibacterial, anti-swimming, and anti-biofilm activities (Hays et al., 1945; Reen et al., 2015). HAQs have also been known as quorum sensing (QS) signal molecules, which involved in intercellular communication resulting in the cell population density (Fuqua et al., 2001).

In our continuing search for the bioactive secondary metabolites from rice-associated bacteria, we came across the bacterium, *Pseudomonas aeruginosa* BCC76810, isolated from the root tissues of Thai rice (*Oryza sativa* L.). The EtOAc crude extract exhibited antimalarial activity against *Plasmodium falciparum*, K-1 multidrug resistant strain with IC<sub>50</sub> value of 2.35 μg/mL and showed weak cytotoxicity against both cancerous and non-cancerous cells (IC<sub>50</sub> 11.8–48.7 μg/mL). Therefore, the chemical investigation of this strain was conducted which led to the isolation of quinoline derivatives **1–12** and phenazines **13–15**. Herein, we describe four new quinolines (**6**, **7**, **9**, and **11**) together with the <sup>1</sup>H and <sup>13</sup>C NMR spectral assignments of compounds **2–7** and **9–11**, being documented in Tables 1 and 2.

## 2. Results and discussion

The spectroscopic data, including <sup>1</sup>H, <sup>13</sup>C NMR, and UV data, of compounds **1–4** were almost identical and they can significantly be differentiated by the mass spectroscopic data. The <sup>1</sup>H NMR spectra showed a typical pattern of 1,2-disubstituted aromatic protons and one upfield methine proton. Together with the 2D NMR spectral analyses (COSY, HMQC, and HMBC), the spectroscopic information were in good agreement with 4-hydroxyquinoline moiety, whose UV spectra (λ<sub>max</sub> 236, 314, 324 nm) were the

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**Table 1**  
<sup>1</sup>H and <sup>13</sup>C NMR assignments of compounds **2–7** in CDCl<sub>3</sub>.

Pos.	2 <sup>a</sup>		3 <sup>a</sup>		4 <sup>b</sup>		5 <sup>a</sup>		6 <sup>a</sup>		7 <sup>b</sup>	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
2	–	155.2, qC	–	155.3, qC	–	154.2, qC	–	147.5, qC	–	155.4, qC	–	153.5, qC
3	6.23, s	108.5, CH	6.24, s	108.5, CH	6.21, s	108.5, CH	6.33, s	109.9, CH	6.25, s	108.5, CH	6.28, s	108.8, CH
4	–	179.2, qC	–	179.2, qC	–	179.0, qC	–	179.1, qC	–	179.2, qC	–	178.9, qC
4a	–	125.3, qC	–	125.2, qC	–	125.1, qC	–	125.3, qC	–	125.2, qC	–	125.1, qC
5	8.35, d (8.0)	125.6, CH	8.36, d (8.0)	125.6, CH	8.35, d (8.3)	125.7, CH	8.35, d (8.1)	126.0, CH	8.36, d (8.0)	125.6, CH	8.35, d (8.0)	125.9, CH
6	7.32, dd (8.0, 7.4)	123.8, CH	7.32, dd (8.0, 7.5)	123.8, CH	7.32, dd (8.3, 7.5)	123.5, CH	7.32, dd (8.1, 7.1)	123.9, CH	7.32, dd (8.0, 7.5)	123.8, CH	7.32, dd (8.0, 7.5)	123.5, CH
7	7.58, dd (7.4, 8.2)	132.0, CH	7.58, dd (7.5, 8.2)	132.0, CH	7.57, dd (7.0, 8.3)	131.8, CH	7.57, dd (7.1, 8.0)	132.2, CH	7.58, dd (7.5, 8.3)	132.0, CH	7.57, dd (7.3, 8.0)	131.8, CH
8	7.73, d (8.2)	118.6, CH	7.74, d (8.2)	118.6, CH	7.63, d (8.3)	117.9, CH	7.52, d (8.0)	118.0, CH	7.74, d (8.3)	118.7, CH	7.50, d (8.0)	117.4, CH
8a	–	140.8, qC	–	140.8, qC	–	140.3, qC	–	140.2, qC	–	140.8, qC	–	140.0, qC
1'	2.67, t (7.6)	34.6, CH <sub>2</sub>	2.68, t (7.6)	34.6, CH <sub>2</sub>	2.65, t (7.2)	34.5, CH <sub>2</sub>	6.29, d (12.0)	122.5, CH	2.71, t (7.7)	34.1, CH <sub>2</sub>	–0.34, q (5.16)/0.52–0.58, m	11.0, CH <sub>2</sub>
2'	1.69, quint (7.3)	29.4, <sup>c</sup> CH <sub>2</sub>	1.71, quint (7.5)	29.3, <sup>d</sup> CH <sub>2</sub>	1.70, quint, (6.9)	28.8, CH <sub>2</sub>	5.98, dt (12.0, 7.2)	141.7, CH	1.80, quint (7.5)	29.2, <sup>g</sup> CH <sub>2</sub>	0.58–0.68, m	15.8, CH
3'	1.20–1.33, m	29.5, <sup>c</sup> CH <sub>2</sub>	1.12–1.33, m	29.5, <sup>d</sup> CH <sub>2</sub>	1.15–1.34, m	29.7, CH <sub>2</sub>	2.36, dt, (14.4, 7.1)	29.7, <sup>f</sup> CH <sub>2</sub>	2.05, q, (7.0)	26.9, CH <sub>2</sub>	0.58–0.68, m	15.2, CH
4'	1.20–1.33, m	29.4, <sup>c</sup> CH <sub>2</sub>	1.12–1.33, m	29.5, <sup>d</sup> CH <sub>2</sub>	1.15–1.34, m	28.6, <sup>e</sup> CH <sub>2</sub>	1.35–1.47, m	29.6, <sup>f</sup> CH <sub>2</sub>	5.24, m	128.3, CH	1.18–1.48 m/1.40–1.50, m	28.7, CH <sub>2</sub>
5'	1.20–1.33, m	29.2, <sup>c</sup> CH <sub>2</sub>	1.12–1.33, m	29.6, <sup>d</sup> CH <sub>2</sub>	1.15–1.34, m	29.2, <sup>e</sup> CH <sub>2</sub>	1.15–1.30, m	29.4, <sup>f</sup> CH <sub>2</sub>	5.32, m	131.5, CH	1.83, quint (7.4)	28.1, CH <sub>2</sub>
6'	1.20–1.33, m	32.0, CH <sub>2</sub>	1.12–1.33, m	29.7, <sup>d</sup> CH <sub>2</sub>	1.15–1.34, m	29.6, <sup>e</sup> CH <sub>2</sub>	1.15–1.30, m	29.3, <sup>f</sup> CH <sub>2</sub>	1.91, q (6.6)	27.5, CH <sub>2</sub>	2.63–2.78, m	34.3, CH <sub>2</sub>
7'	1.20–1.33, m	22.8, CH <sub>2</sub>	1.12–1.33, m	32.1, CH <sub>2</sub>	1.15–1.34, m	29.6, <sup>e</sup> CH <sub>2</sub>	1.15–1.30, m	32.0, CH <sub>2</sub>	1.12–1.32, m	29.8, <sup>g</sup> CH <sub>2</sub>	1.18–1.48, m	29.3, <sup>h</sup> CH <sub>2</sub>
8'	0.82, t (6.9)	14.3, CH <sub>3</sub>	1.12–1.33, m	22.9, CH <sub>2</sub>	1.15–1.34, m	33.1, CH <sub>2</sub>	1.15–1.30, m	22.8, CH <sub>2</sub>	1.12–1.32, m	29.2, <sup>g</sup> CH <sub>2</sub>	1.18–1.48, m	29.1, <sup>h</sup> CH <sub>2</sub>
9'	–	–	0.83, t (6.9)	14.3, CH <sub>3</sub>	1.15–1.34, m	31.9, CH <sub>2</sub>	0.84, t (6.7)	14.3, CH <sub>3</sub>	1.12–1.32, m	32.0, CH <sub>2</sub>	1.18–1.48, m	30.1, <sup>h</sup> CH <sub>2</sub>
10'	–	–	–	–	1.15–1.34, m	22.7, CH <sub>2</sub>	–	–	1.12–1.32, m	22.9, CH <sub>2</sub>	1.18–1.48, m	31.9, CH <sub>2</sub>
11'	–	–	–	–	0.85, t (7.0)	14.1, CH <sub>3</sub>	–	–	0.83, t	14.3, CH <sub>3</sub>	1.18–1.48, m	22.7, CH <sub>2</sub>
12'	–	–	–	–	–	–	–	–	–	–	0.85, t (6.9)	14.1, CH <sub>3</sub>
4-OH	11.91, s	–	12.04, s	–	11.14, s	–	10.34, s	–	12.15, s	–	10.05, s	–

<sup>a</sup>400 MHz for <sup>1</sup>H NMR and 100 MHz for <sup>13</sup>C NMR, <sup>b</sup>500 MHz for <sup>1</sup>H NMR and 125 MHz for <sup>13</sup>C NMR. c,d,e,f,g,h = exchangeable.

same as those earlier reported for 4-hydroxyquinolines (Hays et al., 1945). In addition, there were extra methylene carbons observed in the <sup>13</sup>C NMR spectra for compounds **2–4**, compared with that of **1**. For compounds **5** and **6**, there were additional sp<sup>2</sup> methine protons appeared in the <sup>1</sup>H NMR spectra, compared with those of compounds **1–4**. In compound **5**, the methine protons resonated at δ<sub>H</sub> 5.98 (H-2') and 6.29 (H-1'), which correlated in HMBC spectrum to C-2, C-4' and C-3, C-3', respectively. The evidence indicated the alkene position at C-1' and the NOESY spectrum showed cross-peak correlation between H-1' and H-3' (δ<sub>H</sub> 2.36), indicating "E" configuration. Thus, compound **5** was determined to be 2-(E)-non-1'-enyl-4-hydroxyquinoline, which previously isolated from *Pseudomonas aeruginosa* (Wells, 1952). HRESIMS data showed the mass ion peak at *m/z* 270.1858 [M+H]<sup>+</sup>, confirming the molecular formula C<sub>18</sub>H<sub>24</sub>NO.

Compound **6** showed the presence of twenty carbons in the <sup>13</sup>C NMR spectrum, which consisting of one methyl, eight methylene, seven sp<sup>2</sup> methine, and four quaternary carbons. Two additional methine protons resonating at δ<sub>H</sub> 5.24 and 5.32, which attributed to the sp<sup>2</sup> methine carbons at δ<sub>C</sub> 128.3 and 131.5 in the HMQC

spectrum. HMBC spectrum showed correlations from δ<sub>H</sub> 5.24 and 5.32 (H-4' and H-5') to C-3' and C-6'; and from δ<sub>H</sub> 2.71 (H-1') to C-2 and C-3. Moreover, COSY spectrum showed cross-peak correlations from H-1' to H-11'. The double bond configuration was assigned as "Z" based on the coupling constant (*J* = 10.7 Hz) and the chemical shifts of the adjacent methylene carbons resonating at δ<sub>C</sub> 26.9 (C-3') and δ<sub>C</sub> 27.5 (C-6') (Sugimoto et al., 1988). Therefore, compound **6** was 2-((Z)-undec-4'-enyl)-4-hydroxyquinoline. HRESIMS spectrum confirmed the molecular formula C<sub>20</sub>H<sub>27</sub>NO by giving the mass ion peak at *m/z* 298.2163 [M+H]<sup>+</sup>.

The <sup>1</sup>H NMR spectrum of compound **7** was similar to that of **4**, apart from the presence of high field protons resonating at δ<sub>H</sub> –0.34, 0.52–0.58, and 0.58–0.68. These high field protons were attributed in the HMQC spectrum to two sp<sup>3</sup> methine carbons at δ<sub>C</sub> 15.2 (C-3') and 15.8 (C-2') and to a methylene carbon at δ<sub>C</sub> 11.0 (C-1'), which indicated the existence of cyclopropane moiety (Evans et al., 1978). The COSY spectrum showed cross-peak correlations from H-1' to H-6' and from H-7' to H-12'. In addition, the HMBC spectrum showed correlations from H-4' and H-5' to C-3'; from H-4' to C-2'; from H-5' to C-2 and C-6'; from H-7' to C-2' and C-3'; and

**Table 2**  
The  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments of compounds **9–11** in  $\text{DMSO}-d_6$ .

Position	$9^a$		$10^b$		$11^a$	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
2	–	153.8, qC	–	153.9, qC	–	153.8, qC
3	5.93, s	106.0, CH	5.95, s	107.0, CH	5.92, s	108.1, CH
4	–	nd	–	171.5, <sup>e</sup> qC	–	177.4, qC
4a	–	125.3, qC	–	125.2, qC	–	125.1, qC
5	8.07, d (7.9)	125.3, CH	8.08, d (7.8)	125.4, CH	8.02, d (8.1)	125.2, CH
6	7.34, dd (7.9, 7.3)	123.6, CH	7.35, dd (7.8, 7.4)	123.8, CH	7.26, dd (8.1, 6.9)	123.2, CH
7	7.67, dd (8.4, 7.3)	132.1, CH	7.70, dd (7.4, 8.5)	132.2, CH	7.61, t (8.3)	131.9, CH
8	7.83, d (8.4)	115.6, CH	7.84, d (8.5)	115.5, CH	7.52, d (8.3)	118.3, CH
8a	–	141.0, qC	–	140.9, qC	–	140.6, qC
1'	2.69, t (7.4)	31.3, $\text{CH}_2$	2.71, t (7.6)	31.7, $\text{CH}_2$	2.58, t (7.3)	33.2, $\text{CH}_2$
2'	1.62, quint (7.0)	27.8, $\text{CH}_2$	1.63, quint (7.1)	27.8, $\text{CH}_2$	1.72, quint (7.5)	28.7, $\text{CH}_2$
3'	1.18–1.39, m	29.2, <sup>c</sup> $\text{CH}_2$	1.18–1.37, m	29.3, <sup>d</sup> $\text{CH}_2$	1.96, q (6.3)	26.5, $\text{CH}_2$
4'	1.18–1.39, m	29.1, <sup>c</sup> $\text{CH}_2$	1.18–1.37, m	29.2, <sup>d</sup> $\text{CH}_2$	5.38, t (3.8)	129.2, CH
5'	1.18–1.39, m	29.0, <sup>c</sup> $\text{CH}_2$	1.18–1.37, m	29.2, <sup>d</sup> $\text{CH}_2$	5.38, t (3.8)	130.9, CH
6'	1.18–1.39, m	31.7, $\text{CH}_2$	1.18–1.37, m	29.2, <sup>d</sup> $\text{CH}_2$	2.07, q, (6.3)	27.1, $\text{CH}_2$
7'	1.18–1.39, m	22.5, $\text{CH}_2$	1.18–1.37, m	31.7, $\text{CH}_2$	1.08–1.38, m	29.5, $\text{CH}_2$
8'	0.84, t (6.7)	14.4, $\text{CH}_3$	1.18–1.37, m	22.5, $\text{CH}_2$	1.08–1.38, m	29.5, $\text{CH}_2$
9'	–	–	0.84, t (6.4)	14.4, $\text{CH}_3$	1.08–1.38, m	31.5, $\text{CH}_2$
10'	–	–	–	–	1.08–1.38, m	22.5, $\text{CH}_2$
11'	–	–	–	–	0.83, t (7.0)	14.4, $\text{CH}_3$
4-OH	–	–	–	–	11.51, s	–

<sup>a</sup>500 MHz for  $^1\text{H}$  NMR and 125 MHz for  $^{13}\text{C}$  NMR, <sup>b</sup>400 MHz for  $^1\text{H}$  NMR and 100 MHz for  $^{13}\text{C}$  NMR.

nd = cannot be seen in  $^{13}\text{C}$  NMR spectrum.

<sup>c,d</sup> = exchangeable.

<sup>e</sup> = observed in HMBC spectrum.

from H-3 to C-6'. Thus, from the spectroscopic evidence, compound **7** was 2-(3'-(2'-hexylcyclopropyl)propyl)-4-hydroxyquinoline (Fig. 1). HRESIMS spectrum confirmed the molecular formula  $\text{C}_{21}\text{H}_{30}\text{NO}$  by showing the mass ion peak at  $m/z$  312.2324  $[\text{M}+\text{H}]^+$ .

The  $^1\text{H}$  NMR spectra of compounds **8–11** were similar to those of compounds **1–4** and displayed the presence of 4-hydroxyquinoline moiety. The obvious different was that H-3 of compounds **8–11** (resonating at ca.  $\delta_{\text{H}}$  5.9) appeared at higher field than those in compounds **1–4** (resonating at ca.  $\delta_{\text{H}}$  6.2). HRESIMS data of compound **8** had 16 mass units higher than that of **1**. Therefore, compound **8** was determined to be *n*-heptyl-4-hydroxyquinoline *N*-oxide, confirmed by comparing with the published data of KF8940 (Kitamura et al., 1986). Due to the spectral similarity including  $^1\text{H}$ ,  $^{13}\text{C}$  and UV data of compounds **8–11**, therefore, it was suggested that compounds **9–11** must be 4-hydroxyquinoline *N*-oxide derivatives.

Compound **9** was obtained as a colorless solid, whose HRESIMS data revealed 14 mass units higher than that of **8**, indicating one additional methylene group in the molecule (Table 2). Moreover, an extra methylene carbon was observed in the  $^{13}\text{C}$  NMR spectrum. Therefore, compound **9** was determined to be 2-*n*-octyl-4-hydroxyquinoline *N*-oxide. Compound **9** is less polar than **8** as indicated by higher retention time ( $t_{\text{R}}$ ) in HPLC in the same solvent system. The same pattern was also noticed for compound **10**.

In the  $^1\text{H}$  NMR spectral data of compound **11** (Table 2), an additional signal at  $\delta_{\text{H}}$  5.38 and the high field protons at  $\delta_{\text{H}}$  1.96 and 2.07 were observed. In HMQC spectrum, these signals were attributed to two methine carbons at  $\delta_{\text{C}}$  129.2 and 130.9 and to two methylene carbons at  $\delta_{\text{C}}$  26.5 and 27.1, respectively. COSY spectrum showed cross-peak correlations of H-1'-H-4' and H-5'-H-10'. In addition, HMBC spectrum showed the correlations from  $\delta_{\text{H}}$  0.84 (10'- $\text{CH}_3$ ) to C-8' and C-9'; from  $\delta_{\text{H}}$  1.72 (H-2') to C-1', C-3', and C-4'; from  $\delta_{\text{H}}$  1.96 (H-3') and 2.07 (H-6') to C-4' and C-5'; from  $\delta_{\text{H}}$  2.58 (H-1') to C-2' and C-3'; from  $\delta_{\text{H}}$  5.38 (H-4' and H-5') to C-3' and C-6'; and from  $\delta_{\text{H}}$  5.92 (H-3) to C-2, C-4a, and C-1'. NOESY spectrum showed cross-peak correlation between H-3' and H-6' together with the allylic carbons of C-3' and C-6' resonating in the  $^{13}\text{C}$  NMR

spectrum at  $\delta_{\text{C}}$  26.5 and 27.1, respectively, indicating the “Z” configuration (Sugimoto et al., 1988). HRESIMS confirmed the molecular formula  $\text{C}_{20}\text{H}_{28}\text{NO}_2$  by giving the mass ion peak at  $m/z$  314.2125  $[\text{M}+\text{H}]^+$ . Compound **11** was then identified as 2-((Z)-undec-4'-enyl)-4-hydroxyquinoline *N*-oxide.

Although compounds **1–5**, **8**, and **10** were previously isolated from *Pseudomonas* spp. (Hays et al., 1945; Heeb et al., 2011; Lépine et al., 2004; Wells, 1952), only  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of compounds **1** and **8** were reported (Kitamura et al., 1986). Thus, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments of compounds **2–5** and **10** are herein provided in Tables 1 and 2, along with those of new compounds (**6**, **7**, **9**, and **11**).

4-Hydroxyquinoline derivatives, except compound **7**, exhibited antimalarial against *P. falciparum*, K-1 strain with  $\text{IC}_{50}$  in a range of 0.25–2.07  $\mu\text{g}/\text{mL}$ , while phenazine derivatives **12–14** were inactive. Only 4-hydroxyquinoline *N*-oxide derivatives (**8–11**) displayed anti-*Bacillus cereus* ( $\text{IC}_{50}$  6.25–25  $\mu\text{g}/\text{mL}$ ) and anti-oxidant in a DPPH free radical scavenging activities (Table 3). In addition, compounds **9**, **11**, **13**, and **14** showed anti-TB activity at maximum tested concentration (MIC 50  $\mu\text{g}/\text{mL}$ ). All tested compounds showed moderate to weak cytotoxicity against both cancerous (KB, MCF-7, NCI-H187) and non-cancerous (Vero) cells (Table 3).

Compounds **1**, **3**, and **5** were earlier isolated from *Pseudomonas aeruginosa* and had strong antibacterial activity against Gram-positive bacteria (Hays et al., 1945). In addition, Compounds **1**, **8**, and **12** was isolated from *Pseudomonas methanica* KY4634 and was given the names of MY12-62c, KF8940, and MY12-62a, respectively (Kitamura et al., 1986). Only compound **8** showed selective inhibitor for 5-lipoxygenase, while the rest inhibited 5-lipoxygenase, 12-lipoxygenase, and cyclooxygenase. 2-Alkyl-4-hydroxyquinoline *N*-oxides was originally obtained from the culture filtrate of *P. aeruginosa* for the dihydrostreptomycin-antagonist activity against *Bacillus subtilis* and *Staphylococcus aureus* (Lightbown and Jackson, 1956). The result showed that the nitrogen oxide group was essential for the activity, as well as the length of the alkyl chain. 2-*n*-Nonyl-4-hydroxyquinoline *N*-oxide gave the best antagonist activity.

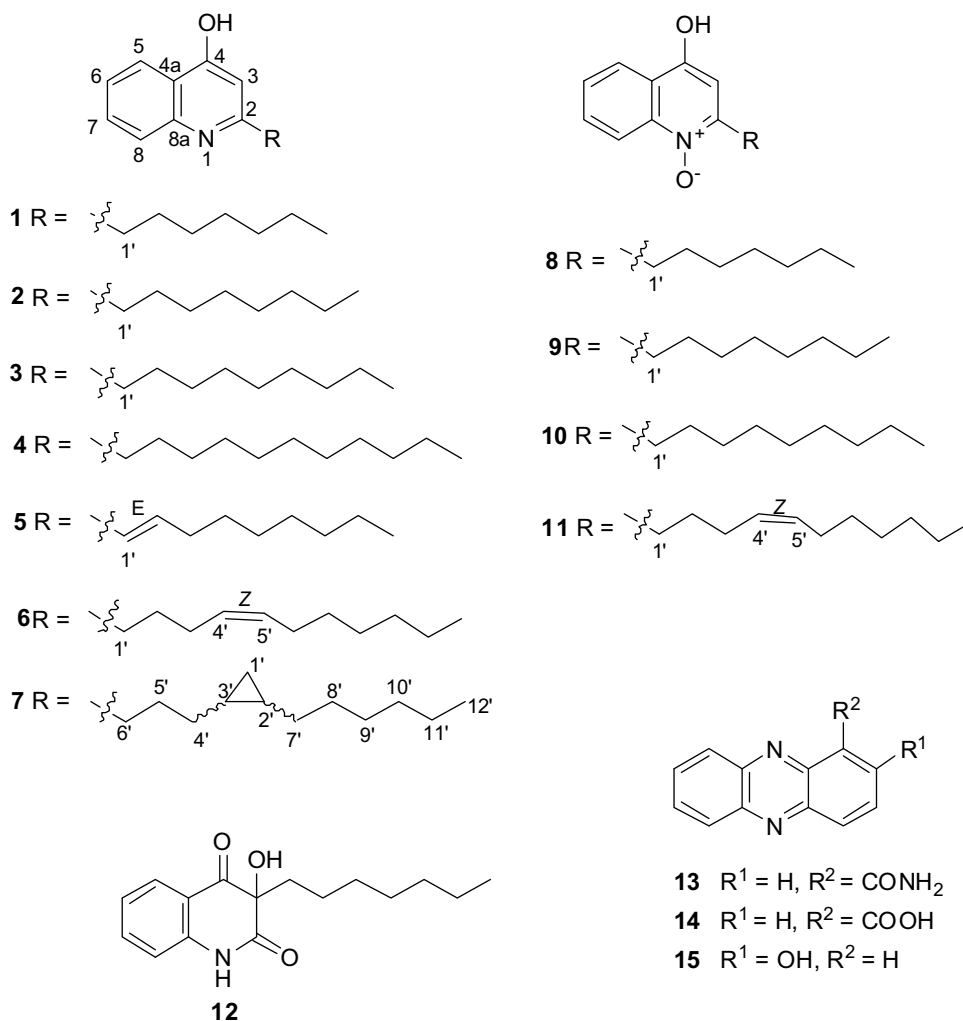


Fig. 1. Chemical structures of the isolated compounds (1–15).

Phenazine alkaloids (**13**, **14**, and **15**) were also isolated from *Pseudomonas aeruginosa* (Jayatilake et al., 1996; Mehnaz et al., 2013). Compounds **13** and **14** showed antibacterial activity against *B. cereus*, *Micrococcus luteus*, and *Staphylococcus aureus*, while compound **15** displayed moderate anti-*Mycobacterium tuberculosis* activity (MIC 32.9 µg/mL) with cytotoxicity against HCT-116 (human colon carcinoma) cells (IC<sub>50</sub> 15.6 µM).

### 3. Experimental

#### 3.1. General experimental procedures

Melting points were measured on a MP90 melting point apparatus from Mettler Toledo. UV spectra were recorded in MeOH using Spekol 1200 from Analytik Jena. FT-IR spectra were obtained on a Bruker ALPHA spectrometer. Optical rotations were measured using a JASCO P-1030 digital polarimeter. NMR spectra were acquired on either a Bruker Avance 500 NMR spectrometer (at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C) or a Bruker Avance III 400 spectrometer (at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C), using either CDCl<sub>3</sub> or DMSO-*d*<sub>6</sub> as an internal standard. HRESIMS data were obtained from a Bruker MicrOTOF spectrometer. Column chromatography was performed on Sephadex LH-20 column or silica gel 60 column (230–400 mesh ASTM from Merck). HPLC was performed on a Dionex – Ultimate 3000 series, which equipped

with a binary pump, an autosampler, and a diode array detector. Preparative HPLC was equipped with a Sunfire C18 column from Waters (10 µm, diam. 19 mm × 250 mm).

#### 3.2. Biological material

*Pseudomonas aeruginosa* BCC76810 was isolated from the root of rice (*Oryza sativa*) at the basic vegetative growth phase, collected in flooded area at Suphan Buri province, Thailand. The root sample was cleaned with running tap water and cut into small pieces. Surface sterilization was performed using 10% (v/v) sodium hypochlorite. The sample was then rinsed with sterilized distilled water 5 times and ground using a mortar and pestle. Ground tissues were placed in glass bottles containing sterilized distilled water and shaken on a rotary shaker to obtain bacterial suspension. Serial dilutions of the suspension was prepared up to the 10<sup>-3</sup> concentration and plated on nutrient agar (NA), consisting of (% w/v): 1% meat extract, 1% peptone, 0.12% NaCl, 1.5% agar in distilled water, pH 7.0. The isolation plate was incubated at 30 °C for 48 h, and then the strain was re-purified on NA. Control plate was obtained by plating the water that was used for the final rinse on NA plate.

Bacterial DNA of the strain BCC76810 was obtained by using a GF-1 bacterial DNA extraction Kit (Vivantis, Malaysia) and amplified the 16S rRNA gene with the universal primers, which

**Table 3**  
Biological activities of the isolated compounds (**1–6** and **8–14**).

Compound <sup>a</sup>	Anti <i>B. cereus</i> <sup>b</sup> (MIC, µg/ mL)	Anti-tubercular <sup>c</sup> (MIC, µg/ mL)	Antimalarial <sup>d</sup> (IC <sub>50</sub> , µg/ mL)	Anti-oxidant <sup>e</sup> IC <sub>50</sub> (µg/ mL)	Cytotoxicity (IC <sub>50</sub> , µg/mL) <sup>c</sup>			
					Vero	KB	MCF- 7	NCI-H187
<b>1</b>	>25.0	>50.0	2.07	>1000	10.06	>50.0	>50.0	19.59
<b>2</b>	>25.0	>50.0	0.52	>1000	16.06	>50.0	>50.0	>50.0
<b>3</b>	>25.0	25.0	0.56	>1000	8.69	>50.0	>50.0	20.18
<b>4</b>	>25.0	50.0	0.25	nt	18.41	>50.0	>50.0	8.12
<b>5</b>	>25.0	>50.0	0.35	nt	13.45	>50.0	>50.0	18.63
<b>6</b>	>25.0	25.0	0.50	>1000	4.47	15.94	>50.0	4.28
<b>8</b>	25.0	>50.0	0.56	78.44	1.59	>50.0	>50.0	27.59
<b>9</b>	25.0	50.0	0.38	139.35	12.46	>50.0	>50.0	17.59
<b>10</b>	25.0	>50.0	0.60	953.53	13.87	>50.0	>50.0	24.21
<b>11</b>	6.25	50.0	0.67	nt	17.42	>50.0	>50.0	8.80
<b>12</b>	>25.0	>50.0	>10.0	>1000	>50.0	>50.0	>50.0	>50.0
<b>13</b>	>25.0	50.0	>10.0	>1000	8.14	>50.0	>50.0	23.29
<b>14</b>	>25.0	50.0	>10.0	nt	>50.0	36.84	>50.0	28.85
Vancomycin	2.00	–	–	–	–	–	–	–
Ethambutol	–	0.94	–	–	–	–	–	–
Isoniazid	–	0.05	–	–	–	–	–	–
Ofloxacin	–	0.78	–	–	–	–	–	–
Rifampicin	–	0.03	–	–	–	–	–	–
Streptomycin	–	1.25	–	–	–	–	–	–
Dihydroartemisinin	–	–	7.79 × 10 <sup>-4</sup>	–	–	–	–	–
Mefloquine	–	–	0.012	–	–	–	–	–
Ellipticine	–	–	–	–	1.23	2.04	–	3.79
Doxorubicin	–	–	–	–	–	0.398	7.13	0.11
Tamoxifen	–	–	–	–	–	–	6.95	–
BHT	–	–	–	88.73	–	–	–	–

<sup>a</sup> nt = compounds were not tested due to inadequate material.

<sup>b</sup> Maximum tested concentration was at 25.00 µg/mL.

<sup>c</sup> Maximum tested concentration was at 50.00 µg/mL.

<sup>d</sup> Maximum tested concentration was at 10.00 µg/mL.

<sup>e</sup> Maximum tested concentration was at 1000.00 µg/mL.

was purified by using a Gel/PCR purification (Favorgen Biotech Corp, Taiwan). Then, PCR product was sequenced to obtain an almost complete 16S rRNA gene sequence (1421 nucleotides), which had the DDBJ accession number as LC075732. The sequence was compared its similarity by using the EzTaxon-e database and indicated its identity to *Pseudomonas aeruginosa* JCM5962T (BAMA01000316).

### 3.3. Fermentation and isolation

The strain was grown on NA at 37 °C and then the agar was cut into pieces (1 × 1 cm<sup>2</sup>), which was transferred into the seed medium. The seed culture was inoculated in 250 mL Erlenmeyer flask, which each contained 100 mL of nutrient broth (NB), at 37 °C on a rotary shaker (200 rpm). After 24 h, the seed culture (20 flasks) was transferred into 80 × 1 L Erlenmeyer flasks, which each contained 250 mL of NB. The production culture (20 L) was cultivated for 4 days at 37 °C on rotary shakers (200 rpm). Then, the whole culture was extracted three times with an equal volume of EtOAc. EtOAc was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to yield a brown gum (10.9 g). The gum was fractionated through a Sephadex LH-20 column, eluted with 100% MeOH, to give four main fractions (F1–F4). Fraction F1 (3.19 g) was subjected to another Sephadex LH-20 column, eluted with 100% MeOH, to obtain two subfractions (F11–F12). The subfraction F11 (1.72 g) was purified by a silica gel column, eluted with 60% EtOAc in CH<sub>2</sub>Cl<sub>2</sub>, to give four subfractions (F111–F114). Subfraction F111 afforded compound **12** (0.11 g) and subfraction F112 (72.3 mg) was further purified by a preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compound **5** (4.6 mg). Subfraction F113 (0.36 g) was further purified by a

preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compounds **1** (47.6 mg), **2** (47.0 mg), **3** (0.13 g), **6** (15.9 mg), **7** (2.5 mg), and **4** (4.1 mg), respectively. Subfraction F114 (81.7 mg) was purified by a preparative HPLC, eluted with a linear gradient system 70–90% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compounds **1** (23.0 mg), **2** (3.0 mg), and **3** (8.1 mg). Subfraction F12 (56.0 mg) was purified by a preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to afford compounds **12** (7.4 mg) and **8** (2.0 mg).

Fraction F2 (3.48 g) was subjected to a Sephadex LH-20 column, eluted with 100% MeOH, to give two subfractions (F21–F22). Subfraction F21 (3.08 g) was purified by a silica gel column, eluted with 40% EtOAc in CH<sub>2</sub>Cl<sub>2</sub> to afford three subfractions (F211–F213). Subfractions F211 contained compound **12** (0.14 g) and subfraction F212 (54.8 mg) was further purified by a preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compounds **2** (1.0 mg) and **5** (10.8 mg). Subfraction F213 (22.6 mg) was further purified by a preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compounds **1** (4.0 mg) and **3** (1.8 mg). Subfraction F22 (52.6 mg) was purified by a preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compounds **1** (2.0 mg) and **8** (3.0 mg).

Fraction F3 (0.79 g) was subjected to a Sephadex LH-20 column, eluted with 100% MeOH, to give two subfractions (F31–F32). Subfraction F31 (27.1 mg) was further purified by a preparative HPLC, eluted with a linear gradient system of 50–65% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to yield compounds **8**

(2.3 mg), **9** (1.0 mg), and **10** (3.0 mg). Subfraction F32 (0.37 g) was further purified by a preparative HPLC, eluted with a linear gradient system of 35–60% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to afford compounds **1** (4.1 mg), **8** (68.5 mg), **9** (8.0 mg), **10** (12.1 mg) and **11** (4.5 mg).

Fraction F4 (0.18 g) was subjected to a Sephadex LH-20 column, eluted with 100% MeOH, and then purified by preparative HPLC, eluted with a linear gradient system of 30–60% CH<sub>3</sub>CN in H<sub>2</sub>O over 30 min at a flow rate of 15 mL/min, to afford compounds **13** (24.2 mg), **14** (3.7 mg), and **15** (3.1 mg).

### 3.3.1. 2-n-Octyl-4-hydroxyquinoline-4-hydroxyquinoline (**2**)

Colorless solid; mp 128.5–129.1 °C; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 217 (3.99), 236 (4.15), 314 (3.95), 324 (3.90); FTIR (ATR)  $\nu_{\max}$ : 2925, 2854, 1637, 1594, 1551, 1504, 1472, 1443, 1354, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1; HRESIMS  $m/z$  258.1858 [M+H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>24</sub>NO, 258.1852).

### 3.3.2. 2-n-Nonyl-4-hydroxyquinoline (**3**)

Colorless solid; mp 138.5–139.2 °C; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 217 (3.92), 236 (4.06), 314 (3.85), 324 (3.82); FTIR (ATR)  $\nu_{\max}$ : 2953, 2922, 2851, 1639, 1594, 1553, 1503, 1473, 1441, 1355, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1; HRESIMS  $m/z$  272.2017 [M+H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>26</sub>NO, 272.2009).

### 3.3.3. 2-n-Undecyl-4-hydroxyquinoline (**4**)

Colorless solid; mp 144.8–145.3 °C; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 216 (3.89), 237 (4.04), 314 (3.78), 324 (3.72); FTIR (ATR)  $\nu_{\max}$ : 2923, 2853, 1637, 1592, 1550, 1504, 1472, 1443, 1354, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1; HRESIMS  $m/z$  300.2324 [M+H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>30</sub>NO, 300.2322).

### 3.3.4. 2-(E)-Non-1'-enyl-4-hydroxyquinoline (**5**)

Colorless solid; mp 111.1–111.4 °C; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 216 (3.92), 236 (4.06), 314 (3.85), 324 (3.82); FTIR (ATR)  $\nu_{\max}$ : 2954, 2924, 2857, 1633, 1590, 1541, 1502, 1473, 1440, 1354, 759 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1; HRESIMS  $m/z$  270.1858 [M+H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>24</sub>NO, 270.1852).

### 3.3.5. 2-(Z)-undec-4-enyl-4-hydroxyquinoline (**6**)

Colorless solid; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 216 (3.94), 237 (4.12), 314 (3.89), 325 (3.86); FTIR (ATR)  $\nu_{\max}$ : 2953, 2925, 2854, 1638, 1594, 1551, 1504, 1472, 1443, 1354, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1; HRESIMS  $m/z$  298.2163 [M+H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>28</sub>NO, 298.2165).

### 3.3.6. 2-(3'(2'-Hexylcyclopropyl)propyl)-4-hydroxyquinoline (**7**)

Colorless solid;  $[\alpha]_D^{27} - 0.67$  (c 0.125, CHCl<sub>3</sub>); UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 216 (3.96), 236 (4.10), 315 (3.86), 325 (3.84); FTIR (ATR)  $\nu_{\max}$ : 2924, 2853, 1639, 1594, 1553, 1504, 1472, 1443, 1354, 761 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1; HRESIMS  $m/z$  312.2324 [M+H]<sup>+</sup> (calcd for C<sub>11</sub>H<sub>30</sub>NO, 312.2322).

### 3.3.7. 2-n-Octyl-4-hydroxyquinoline-4-hydroxyquinoline N-oxide (**9**)

Colorless solid; mp 129.1–129.8 °C; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 217 (3.86), 236 (3.93), 315 (3.61), 327 (3.64); FTIR (ATR)  $\nu_{\max}$ : 2954, 2926, 2854, 1594, 1554, 1488, 1467, 1435, 1378, 757 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>)

spectroscopic data, see Table 2; HRESIMS  $m/z$  274.1798 [M+H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>24</sub>O<sub>2</sub>N, 274.1802).

### 3.3.8. 2-n-Nonyl-4-hydroxyquinoline N-oxide (**10**)

Colorless solid; mp 125.5–125.9 °C; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 217 (4.10), 237 (4.23), 316 (3.89), 327 (3.89); FTIR (ATR)  $\nu_{\max}$ : 2954, 2925, 2854, 1632, 1591, 1551, 1505, 1472, 1443, 1356, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) spectroscopic data, see Table 2; HRESIMS  $m/z$  288.1956 [M+H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>26</sub>NO<sub>2</sub>, 288.1958).

### 3.3.9. 2-(Z)-undec-4'-enyl)-4-hydroxyquinoline N-oxide (**11**)

Colorless solid; UV  $\lambda_{\max}^{\text{MeOH}}$  (nm) (log  $\epsilon$ ): 216 (4.14), 236 (4.28), 316 (3.97), 325 (3.95); FTIR (ATR)  $\nu_{\max}$ : 2954, 2926, 2855, 1634, 1594, 1555, 1508, 1471, 1443, 1357, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>) spectroscopic data, see Table 2; HRESIMS  $m/z$  314.2125 [M+H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>28</sub>NO<sub>2</sub>, 314.2115).

## 3.4. Biological tests

Antimalarial assay against *Plasmodium falciparum* (K-1, multi-drug resistant strain) was performed by using the microculture radioisotope technique (Desjardins et al., 1979). Dihydroartemisinin and mefloquine were used as the positive controls. Antibacterial activity against *Bacillus cereus* was tested by resazurin microplate assay (REMA) (Sarker et al., 2007) and vancomycin was used as a positive control. The green fluorescent protein microplate assay (GFPMA) was used for evaluation of cytotoxicity against Vero cell (African green monkey kidney fibroblasts, ATCC CCL-81) and of anti-*Mycobacterium tuberculosis* strain H37Ra (Changsen et al., 2003). Ellipticine was used as positive control for cytotoxicity against Vero cell. Isoniazid, ofloxacin, rifampicin, streptomycin, and ethambutol were used as positive controls for antitubercular activity. Cytotoxicity against KB (oral human epidermoid carcinoma, ATCC CCL-17), MCF-7 (human breast cancer, ATCC HTC-22), and NCI-H187 cells (human small-cell lung cancer, ATCC CRL-5804) were evaluated by using the resazurin microplate assay (REMA) (O'Brien et al., 2000). Ellipticine and doxorubicin were used as the positive controls for cytotoxicity against KB cell. Tamoxifen and doxorubicin were used as the positive controls for cytotoxicity against MCF-7 cell. Doxorubicin was used as the positive control for cytotoxicity against NCI-H187 cell. IC<sub>50</sub> represents the concentration that caused 50% reduction in cell growth. Minimum inhibitory concentration (MIC) represents the lowest concentration that inhibited 90% growth of bacterium. Antioxidant activity was evaluated by using DPPH free radical scavenging method as described by Brand-Williams et al. (Brand-Williams et al., 1995). The tested compounds were diluted with methanol (6.25, 12.5, 25, 50, 100, 250, 500, 600 and 1000 µg/mL). The sample solution (100 µL) was mixed with 100 µL of 0.2 mM DPPH in methanol. After 30 min at room temperature in the dark, absorbance was measured at 517 nm using a spectrophotometer. IC<sub>50</sub> value reported means the amount of sample (expressed on the weight basis of the pure compounds) necessary to decrease the initial DPPH radical concentration by 50%. Dibutylhydroxytoluene (BHT) was used as the positive control for anti-oxidant activity.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.phytol.2016.07.007>.

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