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## Two pairs of enantiomeric $\alpha$ -pyrone dimers from the endophytic fungus *Phoma* sp. YN02-P-3<sup>†</sup>

Xia-Nan Sang,<sup>ab</sup> Shao-Fei Chen,<sup>ab</sup> Gang Chen,<sup>ab</sup> Xiao An,<sup>ab</sup> Sheng-Ge Li,<sup>ab</sup> Xiao-Jie Lu,<sup>ab</sup> Dan Zhao,<sup>ab</sup> Jiao Bai,<sup>ab</sup> Hai-Feng Wang<sup>\*ab</sup> and Yue-Hu Pei<sup>\*ab</sup>

(±) Phomones A (1) and B (2), two pairs of novel enantiomeric  $\alpha$ -pyrone dimers from the endophytic fungus *Phoma* sp. YN02-P-3 are reported. Compounds 1 and 2 are the first examples of 6- $\alpha,\beta$ -unsaturated ester-2-pyrone dimers, and compound 1 possesses a novel 6/4/5/6 tetracyclic ring system. Their structures and stereochemistry were determined by the analysis of extensive spectroscopic data, ECD calculations and single-crystal X-ray diffraction data.

[2 + 2] Cycloaddition reactions that construct two new C–C bonds and establish up to four new stereogenic centers in a single step<sup>1–3</sup> are widely used in the synthesis of natural<sup>4,5</sup> and bioactive products and have been utilized to synthesize many kinds of important compounds.<sup>6</sup> The course of the addition reaction and the resulting regioselectivities have remained a topic of great interest in this area.<sup>7,8</sup>  $\alpha$ -Pyrone is a simple heterocyclic dienone system,<sup>9</sup> which is frequently used as the substrate of intramolecular photochemical reactions, in order to investigate the intermolecular cycloaddition reactivity.<sup>10–12</sup>

During our continuing search for novel bioactive secondary metabolites from endophytic fungi, *Phoma* sp. was obtained from the sample collected in the plant *Sumbaviopsis* J. J. Smith from Yunnan Province, China. Previous study of this fungus resulted in the isolation of six novel compounds phomeketale A–F,<sup>13</sup> one novel 3,4-dihydronaphthalen-1(2*H*)-one with spirobutyrolactone and a new isocoumarin.<sup>14</sup> Further investigation led to the discovery of two pairs of novel enantiomeric  $\alpha$ -pyrone dimers, (±) phomones A (1) and B (2), and a known compound rosellin (3).<sup>15</sup> Phomones A and B are the first examples of 6- $\alpha,\beta$ -unsaturated ester-2-pyrone dimers *via* intermolecular unsymmetrical [2 + 2] cycloaddition.<sup>16,17</sup> Their structures and stereochemistry were elucidated on the basis of the spectral data, single-crystal X-ray diffraction, and ECD analysis. Interestingly, it was found that phomone B (2) slowly transformed to phomone A (1) in MeOH over one month. The effect of H<sub>2</sub>O, pH- and temperature-dependent transformation between

compounds 1 and 2, as well as the structural elucidation, postulated biogenetic origin and biological evaluation of these metabolites are reported herein.

(±)-Phomone A (1a/1b) was initially obtained as colorless block crystals. Its molecular formula was established to be C<sub>24</sub>H<sub>28</sub>O<sub>14</sub> (eleven degrees of unsaturation) on the basis of HRESIMS at *m/z* 563.1352 [M + Na]<sup>+</sup> (calcd 563.1371). Inspection of <sup>1</sup>H and <sup>13</sup>C NMR spectra (Table 1) indicated one 4-oxy- $\alpha$ -pyrone ring ( $\delta_C$  163.3, 110.0, 168.1, 116.3 and 156.3), one 4-oxy- $\alpha$ -dihydropyrone ring ( $\delta_C$  163.2, 107.4, 164.9, 53.1 and 82.6), four methoxyl groups ( $\delta_H$  3.54,  $\delta_C$  57.9;  $\delta_H$  3.65,  $\delta_C$  51.6;  $\delta_H$  4.03,  $\delta_C$  62.1 and  $\delta_H$  3.78,  $\delta_C$  51.7), five methylene groups ( $\delta_H$  4.28,  $\delta_C$  52.5;  $\delta_H$  4.12/4.29,  $\delta_C$  74.4;  $\delta_H$  4.35,  $\delta_C$  52.8;  $\delta_H$  2.53/2.85,  $\delta_C$  33.9 and  $\delta_H$  4.43/4.33,  $\delta_C$  52.2), three methine groups ( $\delta_H$  4.62,  $\delta_C$  79.8;  $\delta_H$  4.26,  $\delta_C$  40.8 and  $\delta_H$  3.92,  $\delta_C$  47.2) and two ester carbonyls ( $\delta_C$  170.5 and  $\delta_C$  169.2). The HMBC spectrum (Fig. 2) corroborated the presence of the 4-oxy- $\alpha$ -pyrone ring moiety based on correlations from methylene H<sub>2</sub>-10' ( $\delta_H$  4.35) to C-2' ( $\delta_C$  163.3), C-3' ( $\delta_C$  110.0) and C-4' ( $\delta_C$  168.1) and from H<sub>2</sub>-12' ( $\delta_H$  4.43/4.33) to C-4', C-5' ( $\delta_C$  116.3) and C-6' ( $\delta_C$  156.3), and due to the other HMBC correlations, the gross structure of 1 could not be established unambiguously. Fortunately, a crystal suitable for X-ray crystallographic study (CCDC 1504985) was obtained upon slow evaporation of MeOH by keeping the sample at room temperature for one month. The final refinement on the Cu K $\alpha$  data resulted the crystal of 1 had a *p*2<sub>1</sub>/*c* space group, indicating a racemic nature, which was in accordance with the lack of optical activity. Furthermore, the X-ray diffraction analysis (Fig. 3) allowed to unambiguously assign the absolute configurations of the two enantiomers of 1 to be (5*R*, 6*R*, 7*R*, 7'*S*, 8'*S*) and (5*S*\*, 6*S*\*, 7*S*\*, 7'*R*\*, 8'*R*\*) as Fig. 1, respectively. Separation by using chiral-phase HPLC yielded 1a ([ $\alpha$ ]<sub>D</sub><sup>20</sup> + 30 (c 0.20 MeOH)) and 1b ([ $\alpha$ ]<sub>D</sub><sup>20</sup> – 39 (c 0.20 MeOH)) in a ratio of 1 : 1, whose absolute configurations were established by comparing the calculated ECD spectra with the experimental spectra (Fig. 4). From the above evidence, the absolute stereochemistry for

<sup>a</sup>Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China. E-mail: wanghaifeng0310@163.com; peiyueh@vip.163.com

<sup>b</sup>School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China

<sup>†</sup> Electronic supplementary information (ESI) available: 1D and 2D NMR, HRESIMS, UV, IR, and ECD spectra of phomones and detailed experimental procedures. CCDC 1504985. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ra26319d



Table 1  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1** and **2**<sup>a</sup>

Position	<b>1</b>		<b>2</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (m, J in Hz)
2	163.2		166.5	
3	107.4		112.4	
4	164.9		169.0	
5	53.1		58.1	
6	82.6		80.1	
7	79.8	4.62 (dd, 9.6, 3.2)	141.4	7.31 (d, 15.6)
8	33.9	2.53 (dd, 9.6, 16.4), 2.85 (dd, 3.2, 16.4)	124.3	6.39 (d, 15.6)
9	170.5		167.7	
10	52.5	4.28 (s)	55.1	4.56 (s)
11	57.9	3.54 (s)	63.0	4.03 (s)
12	74.4	4.29(d, 9.6), 4.12 (d, 9.6)	62.6	3.90 (d, 11.4), 3.75 (d, 11.4)
13	51.6	3.65 (s)	52.4	3.78 (s)
2'	163.3		166.5	
3'	110.0		111.3	
4'	168.1		170.4	
5'	116.3		117.9	
6'	156.3		158.2	
7'	40.8	4.26 (d, 10.0)	37.2	4.20 (d, 11.4)
8'	47.2	3.92 (d, 10.0)	50.9	4.39 (d, 11.4)
9'	169.2		170.4	
10'	52.8	4.35(s)	55.4	4.56 (s)
11'	62.1	4.08 (s)	63.3	4.20 (s)
12'	52.2	4.43 (d, 12.4), 4.33 (d, 12.4)	54.6	4.55 (d, 12.4), 4.46 (d, 12.4)
13'	51.7	3.67 (s)	52.8	3.64 (s)

<sup>a</sup> Measured in DMSO-*d*<sub>6</sub> at 400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ .

**1a** (5*R*, 6*R*, 7*R*, 7'*S*, 8'*S*) and **1b** (5*S*\*, 6*S*\*, 7*S*\*, 7'*R*\*, 8'*R*\*) were unambiguously determined as shown in Fig. 1.

(±)-Phomone B (**2a/2b**) shared the same molecular formula of C<sub>24</sub>H<sub>28</sub>O<sub>14</sub> with compound **1** based on HRESIMS and  $^{13}\text{C}$  NMR data. Detailed comparison of its NMR data (Table 1) with those of compound **1** indicated that the main differentiation between compounds **2** and **1** was the absence of the methene [ $\delta_{\text{H}}$  2.53 (1H, dd, *J* = 16.4, 9.6 Hz),  $\delta_{\text{H}}$  2.85 (1H, dd, *J* = 16.4, 3.2 Hz)] and the methine [ $\delta_{\text{H}}$  4.62 (1H, dd, *J* = 9.6, 3.2 Hz)] and the presence of one trans-double bond signals [ $\delta_{\text{H}}$  6.39 (1H, d, *J* = 15.6 Hz),  $\delta_{\text{H}}$  7.31 (1H, d, *J* = 15.6 Hz)] in compound **2**, which suggested that the furan ring might be open loop to the double bond additive and the C-12 primary alcohol. The HMBC spectrum (Fig. 2) corroborated the presence of a dihydropyrone ring from H<sub>2</sub>-10 ( $\delta_{\text{H}}$  4.56) to C-2 ( $\delta_{\text{C}}$  166.5), C-3 ( $\delta_{\text{C}}$  112.4) and C-4 ( $\delta_{\text{C}}$  169.0) and from H<sub>2</sub>-12 ( $\delta_{\text{H}}$  3.75/3.90) to C-4, C-5 ( $\delta_{\text{C}}$  58.1) and C-6 ( $\delta_{\text{C}}$  80.1) and an  $\alpha$ -pyrone moiety based on correlations from H<sub>2</sub>-10' ( $\delta_{\text{H}}$  4.56) to C-2' ( $\delta_{\text{C}}$  166.5), C-3' ( $\delta_{\text{C}}$  111.3) and C-4' ( $\delta_{\text{C}}$  171.4) and from H<sub>2</sub>-12' ( $\delta_{\text{H}}$  4.55/4.46) to C-4', C-5' ( $\delta_{\text{C}}$  117.9) and C-6' ( $\delta_{\text{C}}$  158.2). Further HMBC correlations observed from H-7' ( $\delta_{\text{H}}$  4.20) to C-5 and C-8' ( $\delta_{\text{C}}$  50.9) and from H-8' ( $\delta_{\text{H}}$  4.39) to C-7' ( $\delta_{\text{C}}$  37.2), C-9' ( $\delta_{\text{C}}$  170.4), C-6 and C-7 ( $\delta_{\text{C}}$  141.4) demonstrated two  $\alpha$ -pyrone rings should be conjugated through a cyclobutane ring and the additional  $\alpha,\beta$ -unsaturated ester moiety was obviously attached to C-6 due to the confirmation of the HMBC correlations from H-

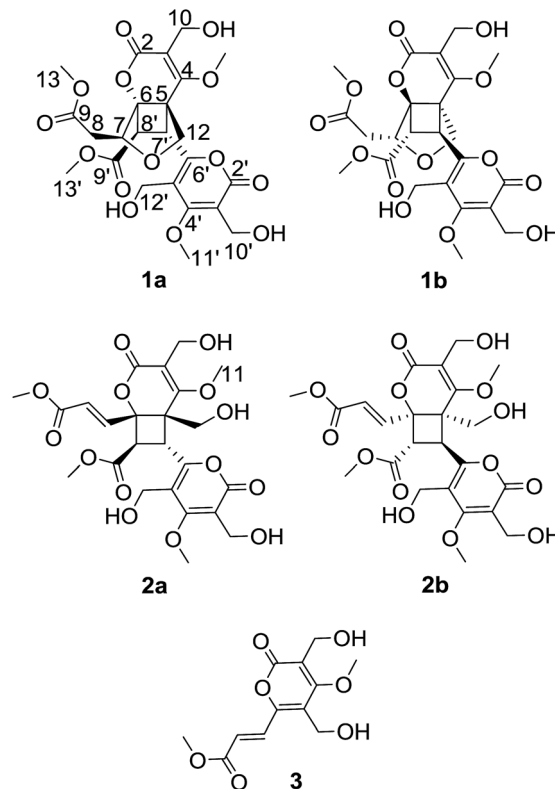


Fig. 1 Structures of compounds **1**–**3**.

7 ( $\delta_{\text{H}}$  7.31) to C-6 and C-8 ( $\delta_{\text{C}}$  124.3), and C-9 ( $\delta_{\text{C}}$  167.7) established the planar structure of compound **2** as shown in Fig. 1.

The NOESY spectrum gave diagnostic correlations of H-7' with H<sub>2</sub>-12 and H-7' with H-7, which illustrated H-7', H<sub>2</sub>-12, H-7 oriented in the same direction, and analyses of the coupling constants placed H-8' on the opposite side of the cyclobutane ring. Subsequent chiral resolution of compound **2** was performed on a chiral column to yield **2a** and **2b** in a ratio of 1 : 1, which were virtually opposite in terms of their CD curves (Fig. 5). The final assignment of **2a** (5*S*, 6*S*, 7'*R*, 8'*R*) and **2b** (5*R*, 6*R*, 7'*S*, 8'*S*) was made by the comparison of the calculated electronic circular dichroisms (ECD) *via* a quantum method with the experimental data (Fig. 5).

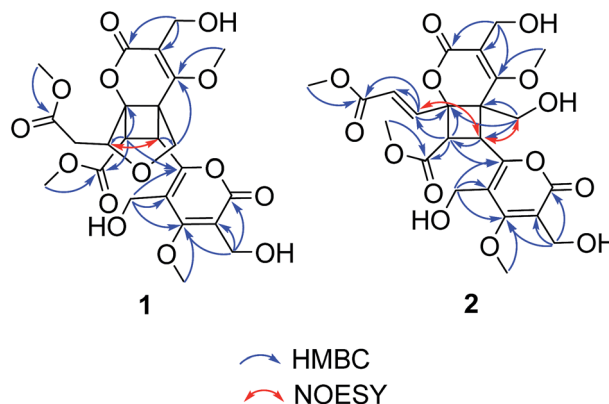


Fig. 2 Key HMBC and Noesy correlations of compounds **1** and **2**.



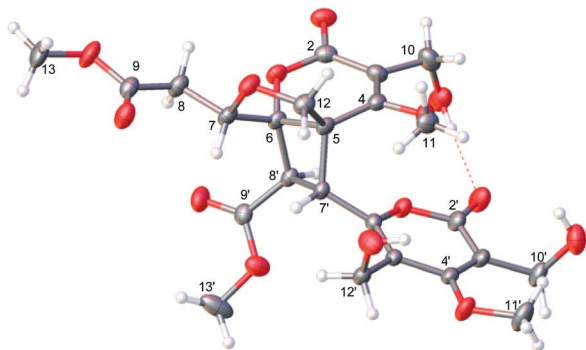


Fig. 3 X-ray crystallographic data for 2.

It was found that compound 2 slowly transformed to compound 1 in MeOH over one month, which indicated that a cyclization reaction was occurring. The effect of H<sub>2</sub>O-, pH- and temperature-dependent transformation between phomones A (1) and B (2) were further studied. As shown in Fig. S21,<sup>†</sup> the H<sub>2</sub>O-temperature heating experiment suggested that H<sub>2</sub>O could promote transformation and the epimerization was quite sensitive to H<sub>2</sub>O. The pH-dependent experiment revealed that the transformation was smothered by acid (Fig. S24<sup>†</sup>) and promoted by alkali (Fig. S22<sup>†</sup>). Meanwhile, it was not going to make the alkali promote retransformation successful (Fig. S23<sup>†</sup>). The variable-temperature heating experiment (Fig. S25<sup>†</sup>) revealed that the single compound 2 was stable in anhydrous ethanol solution below 50 °C for 12 hours. Based on the above results, epimerization of 2 and 1 could be induced by H<sub>2</sub>O and alkali. The main effect of these transformed observations was H<sub>2</sub>O, since H<sub>2</sub>O might come into being along with the fermentation. In our study, three pairs of enantiomers, (±) phomones A (1), B (2) and the acetylated products 4a/4b (Scheme 2) were successfully separated by HPLC employing a CHIRALPAK AD-H chiral column (250 × 4.6 mm, 5 μm) and using anhydrous ethanol as the mobile phase at a flow rate of 0.3 mL min<sup>-1</sup>.

As phomone A (1) and B (2) are 6- $\alpha,\beta$ -unsaturated ester-2-pyrone dimers, a close biosynthetic relationship could be

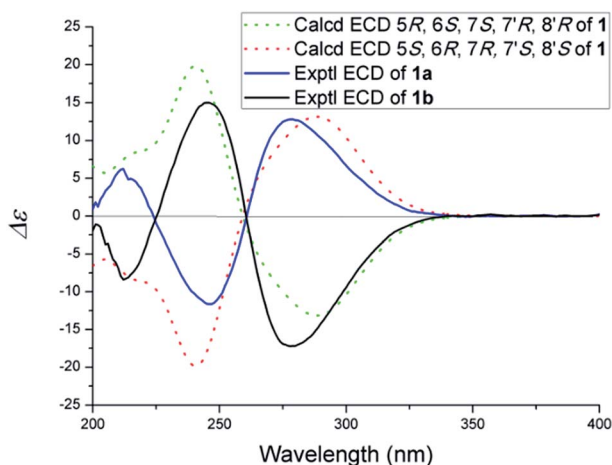


Fig. 4 Experimental and calculated ECD spectra of 1.

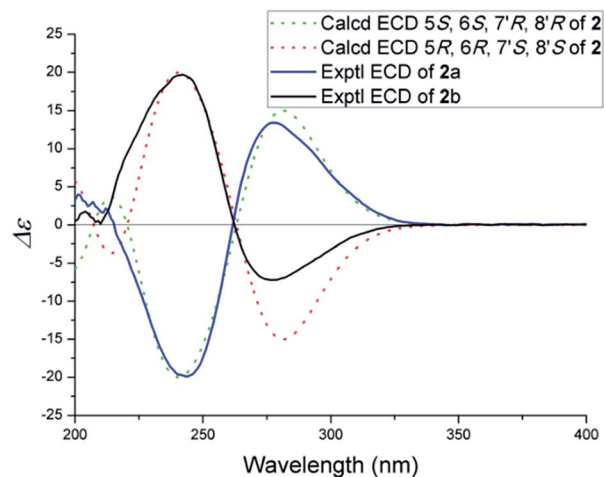
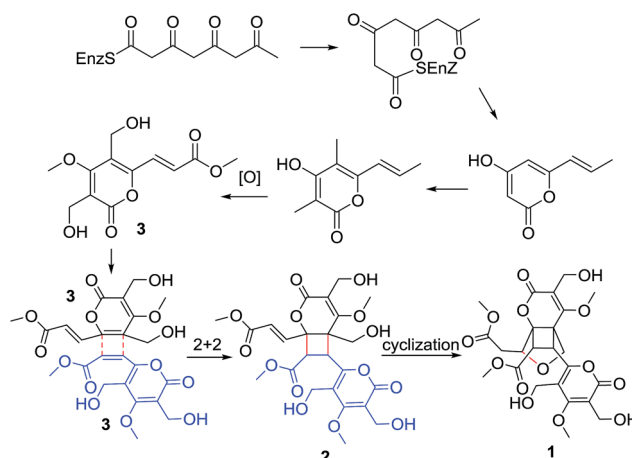
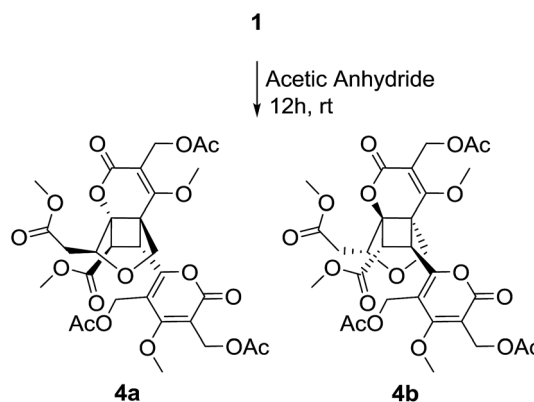


Fig. 5 Experimental and calculated ECD spectra of 2.



Scheme 1 Suggested biosynthetic pathway of 1 and 2.



Scheme 2 Acetylated of phomone A (1).

speculated. Phomone B (2) may be derived through a intermolecular unsymmetrical [2 + 2] cycloaddition reaction of two ethylenic bonds between two rosellisin (3) molecules phomone A (1) could be formed through an intramolecular cyclization reaction of phomone B (2) (Scheme 1).



Compounds **1** and **2** showed no activity ( $IC_{50} > 50 \mu M$ ) when evaluated for their cytotoxic activity against three human cancer cell lines, including human leukemia HL-60, human prostatic carcinoma PC-3 and human colon cancer HCT-116, using 5-fluorouracil as positive control. The acetylated products (**4a/4b**) of compound **1** showed moderate cytotoxic activity against HL-60 cell line with  $IC_{50}$  value of 11.05  $\mu M$  and 14.18  $\mu M$ , respectively.

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## Notes and references

- H. I. Omar, T. Shimo and K. Somekawa, *J. Mol. Struct.: THEOCHEM*, 2006, **763**, 115–121.
- Y. Yanagisawa, Y. Nishiyama, H. Tanimoto, T. Morimoto and K. Kakiuchi, *Tetrahedron Lett.*, 2014, **55**, 2123–2126.
- A. Furutani, K. Tsutsumi, H. Nakano, T. Morimoto and K. Kakiuchi, *Tetrahedron Lett.*, 2004, **45**, 7621–7624.
- M. Zhou, X. R. Li, J. W. Tang, Y. Liu, X. N. Li, B. Wu, H. B. Qin, X. Du, L. M. Li, W. G. Wang, J. X. Pu and H. D. Sun, *Org. Lett.*, 2015, **17**, 6062–6065.
- C. S. Yang, X. B. Wang, J. S. Wang, J. G. Luo, J. Luo and L. Y. Kong, *Org. Lett.*, 2011, **13**, 3380–3383.
- T. Sunazuka and S. Omura, *Chem. Rev.*, 2005, **105**, 4559–4580.
- H. I. Omar, Y. Odo, Y. Shigemitsu, T. Shimo and K. Somekawa, *Tetrahedron*, 2003, **59**, 8099–8105.
- T. Obata, T. Shimo, M. Yasutake, T. Shinmyozu, M. Kawaminami, R. Yoshida and K. Somekawa, *Tetrahedron*, 2001, **57**, 1531–1541.
- Y. Li, D. Z. Ye, X. L. Chen, X. H. Lu, Z. Z. Shao, H. Zhang and Y. S. Che, *J. Nat. Prod.*, 2009, **72**, 912–916.
- T. Suishu, T. Shimo and K. Somekawa, *Tetrahedron*, 1997, **53**, 3545–3556.
- T. Shimo, M. Matsushita, H. I. Omar and K. Somekawa, *Tetrahedron*, 2005, **61**, 8059–8064.
- T. Shimo, S. Ueda, T. Suishu and K. Somekawa, *J. Heterocycl. Chem.*, 1995, **32**, 727–730.
- X. N. Sang, S. F. Chen, G. Chen, X. An, S. G. Li, X. N. Li, B. Lin, J. Bai, H. F. Wang and Y. H. Pei, *RSC Adv.*, 2016, **6**, 64890–64894.
- X. N. Sang, S. F. Chen, X. An, G. Chen, H. F. Wang and Y. H. Pei, *J. Asian Nat. Prod. Res.*, 2016, **7**, 1–8.
- M. S. R. Nair and S. T. Carey, *Tetrahedron Lett.*, 1975, **41**, 3517–3518.
- T. Sagawa, Y. Takaishi, Y. Fujimoto, C. Duque, C. Osorio, F. Ramos, C. Garzon, M. Sato, M. Okamoto, T. Oshikawa and S. U. Ahmed, *J. Nat. Prod.*, 2005, **68**, 502–505.
- I. Ortmann, S. Werner, C. Kruger, S. Mohr and K. Schaffner, *J. Am. Chem. Soc.*, 1992, **114**, 5048–5054.

