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THREE NEW *C*-ALKYLATED FLAVONES FROM THE FLOWER OF *ROSA RUGOSA* AND THEIR BIOACTIVITY

Jing Li,[1] Huan-Huan Xing,[1,2] Wei-Song Kong,[1] Ye-Kun Yang,[1] Lin Ye,[1] Xin Liu,[1] Yan-Ping Li,[3] Gao-Xiong Rao,[3] Hai-Ying Yang,[2] Gang Du,[2] Min Zhou,[1] Qiu-Fen Hu,[1,2] Guang-Yu Yang,[1] Yun-Hua Qin,[1]* and Xue-Mei Li[1]*

[1] Key Laboratory of Tobacco Chemistry of Yunnan Province, China Tobacco Yunnan Industrial Co., Ltd, Kunming 650231, P.R. China. [2] Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University, Kunming 650031, P.R. China. [3] College of Pharmaceutic Science, Yunnan University of Traditional Chinese Medicine, Kunming 650500, P.R. China. E-mail: xmlikm@126.com, jszxtg_2015@163.com

Abstract – Three new *C*-alkylated flavones (**1-3**), together with three known *C*-alkylated flavones (**4-6**), were isolated from the flower of *Rosa rugosa*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compounds **1-6** were evaluated for their anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity. The results revealed that compound **1** showed good inhibition with inhibition zone diameter (IZD) of 13.4 ± 1.0 mm. Compounds **2-6** also showed weak inhibition with IZD in the range of 7.6 – 11.2 mm, respectively. Compounds **1-3** were also tested for the antioxidant activity, and they shows good antioxidant activity with an IC_{50} value of 4.2, 3.6 and 3.4 μg/mL, respectively.

Rosa rugosa Thunb. (Rosaceae) is a suckering shrub, which native to temperate and cold coastal areas of East Asia, but was introduced to Europe and North America as an ornamental plant.[1,2] Now, *R. rugosa* had widely cultivated in the Yunnan Province. The sweetly scented flowers of *R. rugosa* are used to make rose oils, a most widely used essential oil in perfumery.[3] In China, the flower buds and petals, after drying, are used to produce herbal tea.[4] Moreover, flower decoction is used as an agent for activating blood circulation to relieve blood stasis as well as in indigestion and for wounds due to its anti-inflammatory and astringent effects.[5]

Previous studies have shown the presence of tannins,[6,7] phenylethanoids,[8,9] terpenoids,[10,11] chromones,[12,13]

and flavonoids[14-16] in this plants. Flavones are a class of flavonoids based on the backbone of 2-phenylchromen-4-one mainly found in cereals, herbs, and microorganism.[17] The capacity of flavones to act as antioxidants and their role in the prevention of coronary heart diseases are the most important actions of flavones. The other actions are those against bacterium, fungus ulcers, viruses, inflammation, arthritis, osteoporosis and diarrhea.[17]

Continuing the search for novel and bioactive metabolites from medicinal plants, we now report the isolation and characterization of three new *C*-alkylated flavones (**1**-**3**), along with three known *C*-alkylated flavones (**4**-**6**), from the flower of *R. rugosa*. Described in this paper are the structure elucidation of compounds **1**-**6**, and the evaluation of the anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity of compounds **1**-**6** and antioxidant activity of compounds **1**-**3**.

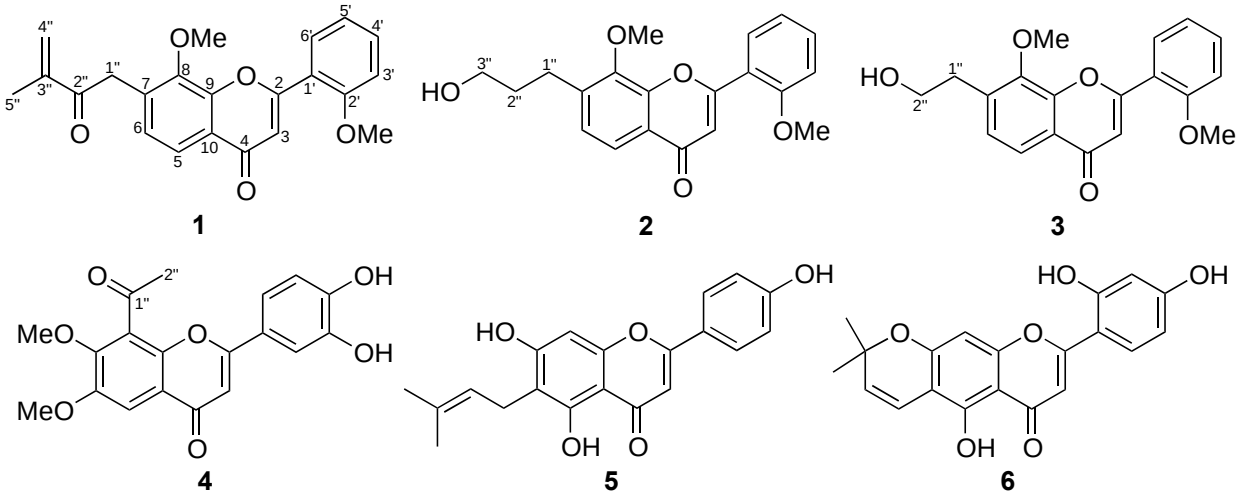

Figure 1. *C*-Alkylated flavones from the flower *Rosa rugosa*

The flowers of *R. rugosa* were extracted with 95% MeOH, followed by repeated column chromatography on silica gel, Sephadex LH-20 and RP-18 silica gel. Final purification by semi-preparative RP-HPLC afforded six *C*-alkylated flavones (**1**-**6**). The structures of **1**-**6** are shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1**-**3** are given in Table 1. The known compounds were identified as rugosaflavonoid B (**4**),[13] 6-prenylapigenin (**5**),[17] and cycloartocarpesin (**6**).[18]

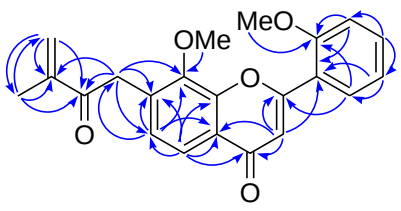

Figure 2. Key HMBC correlations (↷) of **1**

Compound **1** was obtained as an orange-yellow gum. It has the molecular formula $C_{22}H_{20}O_5$ from HRESIMS (*m/z*: 387.1215 $[M+Na]^+$, calcd 387.1208). Its IR spectral data showed the presence of carbonyl groups (1680 and 1665 cm^{-1}) and aromatic ring (1610, 1537, and 1469 cm^{-1}). The UV absorptions at 360, 258, and 210 nm also showed a substituted aromatic ring. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) along with analysis of the DEPT spectra displayed 22 carbon signals and 20 proton signals, respectively, corresponding to a

1,2,3,4-tetrasubstituted aromatic ring (C-5~C-10; H-5 and H-6), a 1,2-disubstituted aromatic ring (C-1′~C-6′; H-3′~H-6′), an *α,β*-unsaturated ketone (C-2~C-4; H-3), a 3-methyl-2-oxobut-3-enyl group (C-1″~C-5″; H_2-1″, H_2-4″, and H_3-5″),[19] and two methoxy groups (δ_C 61.5 q, 56.1 q, δ_H 3.84 s, 3.81 s). The typical NMR signal of two aromatic ring and *α,β*-unsaturated ketone indicated that **1** should be a flavone.[20] This was also supported by the HMBC correlations (Figure 2) from H-3 to C-2, C-4, C-10, C-1′, from H-5 to C-4, C-7, C-9, C-10, and from H-6′ to C-2. Since the nucleus of compound was determined, the additional signals (a 3-methyl-2-oxobut-3-enyl group and two methoxy groups) were accounted for the remaining substituents. The HMBC of correlations of two methoxy protons (δ_H 3.84, 3.81) with C-8 (δ_C 155.1) and C-2′ (δ_C 156.8) suggested the attachment position of the two methoxy groups at C-8 and C-2′, respectively. The 3-methyl-2-oxobut-3-enyl group located C-7 was supported by the HMBC of correlations from H_2-1″ (δ_H 4.61) to C-6 (δ_C 128.3), C-7 (δ_C 133.2), and C-8 (δ_C 155.1), and from H-6 (δ_H 6.72) to C-1″ (δ_C 38.5). Furthermore, the typical protons signals (H-5, H-6, H-3′~H-6′) also supported 6,7-disubstituted for ring A and 2′-monosubstituted for ring B on flavone nucleus. The structure of 8,2′-dimethoxy-7-(3-methyl-2-oxobut-3-enyl)flavone (**1**) was therefore established as shown in Figure 1.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1**-**3** ($CDCl_3$, 125 and 500 MHz)

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
2	163.1 s		163.7 s		163.5 s	
3	108.5 d	6.63 s	107.5 d	6.65 s	107.0 d	6.65 s
4	176.5 s		177.3 s		176.9 s	
5	125.2 d	7.43 (d) 8.2	124.7 d	7.52 (d) 8.2	124.9 d	7.57 (d) 8.2
6	128.3 d	6.72 (d) 8.2	126.5 d	6.79 (d) 8.2	126.1 d	6.82 (d) 8.2
7	133.2 s		130.9 s		132.1 s	
8	155.1 s		154.1 s		153.9 s	
9	152.1 s		151.3 s		150.8 s	
10	122.6 s		122.7 s		122.1 s	
1′	118.6 s		118.2 s		118.4 s	
2′	156.8 s		156.5 s		156.7 s	
3′	116.0 d	6.93 (d) 7.6	115.7 d	6.94 (d) 7.6	116.0 d	6.96 (d) 7.6
4′	131.3 d	7.32 (t) 7.6	131.6 d	7.34 (t) 7.6	131.4 d	7.38 (t) 7.6
5′	121.1 d	6.88 (t) 7.6	121.4 d	6.89 (t) 7.6	121.7 d	6.88 (t) 7.6
6′	129.0 d	7.89 (d) 7.6	128.4 d	7.86 (d) 7.6	128.5 d	7.85 (d) 7.6
1″	38.5 t	4.61 s	28.7 t	2.69 (t) 7.8	33.2 t	2.66 (t) 7.2
2″	200.4 s		36.8 t	1.85 m	62.8 t	3.56 (t) 7.2
3″	144.2 s		63.2 t	3.54 (t) 6.6		
4″	123.0 t	5.87, 6.13 s				
5″	18.9 q	1.92 s				
-OMe-8	61.5 q	3.84 s	61.4 q	3.83 s	61.2 q	3.81 s
-OMe-2′	56.1 q	3.81 s	56.1 q	3.80 s	55.9 q	3.78 s

The ^{1}H and ^{13}C NMR spectra of 7-(3-hydroxypropyl)-8,2′-dimethoxyflavone (**2**) were similar to those of

1. The marked differences between them were due to the inexistence of a 3-methyl-2-oxobut-3-enyl group signals, and appearance of a 3-hydroxypropyl group signals[21] (C-1″~C-3″; H_2-1″~H_2-3″) in compound **2**. This change indicated that the 3-methyl-2-oxobut-3-enyl group in **1** was replaced by a 3-hydroxypropyl group in compound **2**. The HMBC correlations from H_2-1″ (δ_H 2.69) to C-6 (δ_C 126.5), C-7 (δ_C 130.9), and C-8 (δ_C 154.1), from H_2-2″ (δ_H 1.85) to C-7 (δ_C 130.9), and from H-6 (δ_H 6.79) to C-1″ (δ_C 28.7) supported the 3-hydroxypropyl group located at C-7. In addition, the other substituents positions also determined by the further analysis of its HMBC correlations. Thus, the structure of **2** was determined as shown.

Compound **3** was isolated as orange-yellow gum and it gave an $[M+Na]^+$ peak at *m/z* 349.1044, consistent with a molecular formula of $C_{19}H_{18}O_5$. Its ^1H and ^{13}C NMR spectroscopic data were similar to those of **2**, which suggested that compound **3** was structurally related to **2**. The marked differences between them were due to the disappearance of a 3-hydroxypropyl group, and appearance of a 2-hydroxyethyl group[22] (C-1″ and C-2″; H_2-1″ and H_2-2″) in compound **3**. These changes indicated that a 3-hydroxypropyl group in **2** was replaced by a 2-hydroxyethyl group in compound **3**. This was also supported by the HMBC correlations of the H_2-1″ (δ_H 2.66) to C-6 (δ_C 126.1), C-7 (δ_C 132.1), and C-8 (δ_C 153.9), from H_2-2″ (δ_H 3.56) to C-7 (δ_C 132.1), and from H-6 (δ_H 6.82) to C-1″ (δ_C 33.2). Morever two methoxy groups located at C-8 and C-2′ was supported by the HMBC correlations of the methoxy proton signals (δ_H 3.81, 3.78) with C-8 and C-2′, respectively. Based on the above findings, the structure of **3** was formulated as 7-(2-hydroxyethyl)-8,2′-dimethoxyflavone.

Compounds **1-6** were screened for anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity according to arbitrary criterion[23] with diameter of inhibition zone (IZD) as follow: very weak inhibition (with IZD of 6-8 mm), weak inhibition (with IZD of 8-12 mm), good inhibition (with IZD of 12-16 mm), and strong inhibition (with IZD of >16 mm) activities respectively. The IZD of the positive control was 32 mm and the negative control to zero. The results revealed that compound **1** showed good inhibition with IZD of 13.4 ± 1.0 mm. Compounds **2-6** showed weak inhibition with IZD of 9.5 ± 0.8, 8.2 ± 0.5, 11.2 ± 1.0, 7.6 ± 0.9, 8.8 ± 0.8 mm, respectively.

Compounds **1-3** were also tested for the antioxidant activity by the 2',7'-dichlorofluorescein diacetate (DCFH) method reported previously.[24] The results revealed that compound **1-3** shows good antioxidant activity with an IC_{50} value of 4.2, 3.6 and 3.4 μg/mL, respectively.

EXPERIMENTAL

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectra. 1D- and 2D-NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard.

Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% H_2SO_4 in ethanol and heating.

Plant Material. The air-dried flowers of *R. rugosa* were collected from Pingyin County, Shandong province, and harvested in June 2016. The species was identified by Prof. Chen Y. J. A voucher specimen (YNNI 16-9-54) was deposited in the herbarium of the Yunnan Minzu University.

Extraction and Isolation. The samples (4.6 kg) were crushed to 30 mesh, and the powders were extracted with 95% aqueous MeOH (4 × 8 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (322 g) was applied to a silica gel (150-200 mesh) column eluted with $CHCl_3$-MeOH gradients (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to afford six fractions (A-F). Further separation of fraction B (9:1, 13.8 g) by silica gel column chromatography, eluted with $CHCl_3$-Me_2CO (1:0-1:2), yielded subfractions B1–B7. Subfraction B2 (9:1, 2.87 g) was loaded on to another silica gel column using petroleum ether-EtOAc elution, and then separated semi-preparative HPLC (68% MeOH, flow rate 20 mL/min) to afford **1** (15.2 mg). Subfraction B3 (8:2, 5.28 g) was separated on a silica gel column eluted by petroleum ether-EtOAc, followed by semi-preparative HPLC (60% MeOH, flow rate 20 mL/min) to give **2** (18.6 mg), **3** (22.7 mg), **4** (48.5 mg), **5** (28.8 mg), and **6** (36.2 mg).

Anti-MRSA agar disc diffusion assay. The MRSA strain ZR11 was clinical isolated from infectious samples of critically ill patients in the Clinical Laboratory of the First People's Hospital of Yunnan Province, and confirmed by standard cefoxitin disk diffusion test following CLSI standard procedures.[23] The anti-MRSA activity of the compounds was evaluated via the disc diffusion method. The ZR11 strain was inoculated in Müeller Hinton Broth and were incubated at 37 °C for 24 h. The turbidity of bacterial suspension was adjusted to 0.5 McFarland standard which equals to 1.5×10^8 colny-forming units (CFU)/mL. Sterile filter paper discs (6 mm) were impregnated with 20 μL (50 μg) of each compound and placed on inoculated Müeller Hinton agar containing bacterial suspension which adjusted to 0.5 McFarland standard .The commercial available discs containing 30 μg Vancomycin were used as positive control whereas discs without samples (5% DMSO) acted as negative control . The inhibition zones including the diameter of the disc (mm) were measured and compared after incubation at 37 °C for 24 h. The tests were carried out in triplicate for each sample.

Antioxidant assay. The antioxidant activity was tested by 2',7'-dichlorofluorescein diacetate (DCFH) method reported previously.[24] Myelomonocytic HL-60 cells (1×10^6 cells/mL, ATCC) were suspended in RPMI 1640 medium with 10% FBS and antibiotics at 37 °C in 5% CO_2: 95% air. 125 μL of the cell suspension was added to each well of a 96-well plate. After treatment with a different concentration of the test material for 30 min, the cells were stimulated with 100 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma) for 30 min. Then the cells were incubated for 15 min after the addition of 5 μg/mL DCFH-DA (Mole-Cular Probes). DCFH-DA is a non-fluorescent probe that diffuses into cells. Cytoplasmic esterases hydrolyse DCFH-DA to 2',7'-dichlorofluorescein (DCFH), and the reactive oxygen species (ROS) generated within HL-60 cells oxidize DCFH to the fluorescent dye 2',7'-dichlorfluorescein (DCF). The ability of the test materials to inhibit exogenous cytoplasmic ROS-catalysed oxidation of DCFH in HL-60 cells was measured by PMA tred control incubations with and without the test materials. The levels of DCF were measured using a CytoFluor 2350, fluorescence measurement system (Millipore) with an excitation wavelength at 485 nm (bandwidth 20 nm) and emission at 530 nm (band width 25 nm).

8,2′-Dimethoxy-7-(3-methyl-2-oxobut-3-enyl)flavone (1): $C_{22}H_{20}O_5$, Pale yellow gum; UV (MeOH) λ_{max} (log ε) 360 (3.69), 258 (3.87), 210 (4.32) nm; IR (KBr): ν_{max} 2967, 2854, 1680, 1665, 1610, 1537, 1469, 1356, 1139, 1055 cm^{-1}; ^1H and ^{13}C NMR data (in $CDCl_3$, 500 and 125 MHz), see Table 1; ESIMS *m/z* 387; HRESIMS *m/z* 387.1215 $[M+Na]^+$ (calcd $C_{22}H_{20}NaO_5$ for 387.1208).

7-(3-Hydroxypropyl)-8,2′-dimethoxyflavone (2): $C_{20}H_{20}O_5$, pale yellow gum; UV (MeOH) λ_{max} (log ε) 354 (3.63), 252 (3.82), 210 (4.29) nm; IR (KBr): ν_{max} 3342, 2836, 1662, 1612, 1557, 1432, 1162, 1254, 1070 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 363; HRESIMS *m/z* 363.1202 $[M+Na]^+$ (calcd $C_{20}H_{20}NaO_5$ for 363.1208).

7-(2-Hydroxyethyl)-8,2′-dimethoxyflavone (3): $C_{19}H_{18}O_5$; obtained as orange-yellow gum; UV (MeOH) λ_{max} (log ε) 365 (3.70), 262 (3.92), 210 (4.38), nm; IR (KBr) ν_{max} 3445, 1665, 1615, 1558, 1426, 1396, 1157, 1064 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 349 $[M+Na]^+$; HRESIMS *m/z* 349.1044 $[M+Na]^+$ (calcd for $C_{19}H_{18}NaO_5$, 349.1052).

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REFERENCES

1. *The Dictionary of Roses in Colour*, ed. by S. M. Gault and P. M. Synge, Ebury Press, London, 1971.

2. *Flora of China*, Vol. 37, ed. by L. Putian and Y. Jiang, Chinese Science press, Beijing, 1985, p 401.
3. R. Bhat and K. R. N. Reddy, *Food Chem.*, 2017, **215**, 425.
4. M. Olech, R. Nowak, R. Los, J. Rzymowska, A. Malm, and K. Chrusciel, *Cent. Eur. J. Biol*., 2012, **7**, 172.
5. B. Lu, M. Li, and R. Yin, *Crit. Rev. Food Sci. Nutr*., 2016, **56**, 130.
6. M. Olech, R. Nowak, L. Pecio, R. Los, A. Malm, and J. Rzymowska, *Nat. Prod. Res*., 2017, **31**, 667.
7. S. Ochir, B. Park, M. Nishizawa, T. Kanazawa, M. Funaki, and T. Yamagishi, *J. Nat. Med*., 2010, **64**, 383.
8. Y. K. Li, J. J. Ma, L. Y. Yang, L. D. Shu, Y. Q. Shen, Q. F. Hu, and Z. Y. Xia, *Asian J. Chem*., 2013, **25**, 6457.
9. X.-M. Gao, L.-D. Shu, L.-Y. Yang, Y.-Q. Shen, Y.-J. Zhang, and Q.-F. Hu, *Bull. Korean Chem. Soc*., 2013, **34**, 246.
10. L. Feng, C. Chen, T. Li, M. Wang, J. Tao, D. Zhao, and L. Sheng, *Plant Physiol. Bioch*., 2014, **75**, 80.
11. H.-J. An, I.-T. Kim, H.-J. Park, H.-M. Kim, J.-H. Choid, and K.-T. Lee, *Int. Immunopharm*., 2011, **11**, 504.
12. L. Yang, J. Yao, L. Shu, Y. Shen, X. Gao, and Q.-F. Hu, *Asian J. Chem*., 2013, **25**, 8355.
13. Q.-F. Hu, B. Zhou, J.-M. Huang, Z.-Y. Jiang, X.-Z. Huang, L.-Y. Yang, X.-M. Gao, G.-Y. Yang, and C.-T. Che, *J. Nat. Prod*., 2013, **76**, 1866.
14. X. Gao, L. Yang, L. Shu, Y. Shen, Y. Zhang, and Q. Hu, *Heterocycles*, 2012, **85**, 1925.
15. G. Du, J.-M. Han, W.-S. Kong, W. Zhao, H.-Y. Yang, G.-Y. Yang, X.-M. Gao, and Q.-F. Hu, *Bull. Korean Chem. Soc*., 2013, **34**, 1263.
16. X.-M. Gao, L.-Y. Yang, X.-Z. Huang, L.-D. Shu, Y.-Q. Shen, Q.-F. Hu, and Z.-Y. Chen, *Heterocycles*, 2013, **87**, 583.
17. B. M. Abegaz, B. T. Ngadjui, E. Dongo, and H. Tamboue, *Phytochemistry*, 1998, **49**, 1147.
18. A. D. Pendse, R. Pendse, A. V. Rama Rao, and K. Venkataraman, *Ind. J. Chem*., 1976, **14b**, 69.
19. M. Zhou, K. Zhou, Y.-L. Zhao, N.-J. Xiang, T.-D. Zhang, C.-M. Zhang, Y-D. Wang, W. Dong, B.-K. Ji, L.-M. Li, J. Lou, G.-P. Li, and Q.-F. Hu, *Heterocycles*, 2015, **91**, 604.
20. Y. Yang, L. Li, J. Lou, Y. Cheng, Y. Wang, D. Shu, L. Shi, X.-M. Gao, P. Ning, and Q. Hu, *Heterocycles*, 2015, **91**, 375.
21. M. Zhou, K. Zhou, P. He, K.-M. Wang, R.-Z. Zhu, Y.-D. Wang, W. Dong, G.-P. Li, H.-Y. Yang, Y.-Q. Ye, G. Du, X.-M. Li, and Q.-F. Hu, *Planta Med*., 2016, **82**, 414.
22. H.-Y. Yang, Y.-H. Gao, D.-Y. Niu, L.-Y. Yang, X.-M. Gao, G. Du, and Q.-F. Hu, *Fitoterapia*, 2013,

91, 189.

23. Clinical and Laboratory Standards Institute (CLSI), 3rd edn., Wayne, Pennsylvania, 2008.

24. S. Takamatsu, A. M. Galal, S. A. Ross, D. Ferreira, M. A. Elsohly, A.-R. S. Ibrahim, and F. S. El-Feraly, *Phytother. Res.*, 2003, **17**, 963.