

HETEROCYCLES, Vol. 91, No. 9, 2015, pp. 1816 - 1822. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 17th July, 2015, Accepted, 26th August, 2015, Published online, 2nd September, 2015
DOI: 10.3987/COM-15-13287

FLAVONES FROM THE LEAVES OF YUNNAN LOCAL SUN CURED TOBACCO AND THEIR CYTOTOXICITY

Yu-Ping Wu,^{1,2} Guang-Hui Kong,¹ Wei Li,¹ Neng-Jun Xiang,² Li-Xuang Liu,² Guo-Rong Yang,² Xiu-Ping Lu,^{1,*} and Qiang Liu^{2,*}

¹ Yunnan Academy of Tobacco Agricultural Sciences, Kunming, Yunnan 650031, PR China; ² Key Laboratory of Tobacco Chemistry of Yunnan Province, China tobacco yunnan industrial Co., Ltd, Kunming 650231, P.R. China; E-mail: liuq@ynzy-tobacco.com, xplu1970@163.com

Abstract – Two new flavones, 4',7-dimethoxy-6-hydroxy-8-isobutyrylflavone (**1**), 4',7-dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]flavone (**2**), together with four known flavones (**3-6**) were isolated from the leaves of Yunnan local sun cured tobacco. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1-6** were screened for the cytotoxic activity in a panel of human cancer cell lines, including NB4, A549, SHSY5Y, PC3, and MCF7. Compound **2** exhibited high cytotoxicity for A549 and SHSY5Y cells with IC₅₀ values of 1.6 and 2.8 μM, respectively. The other compounds also exhibited cytotoxicity with IC₅₀ values in the range of 4.3 – 9.5 μM, respectively.

Nicotiana tabacum L. is the most commonly grown of all plants in the *Nicotiana* genus, and its leaves are commercially grown in many countries to be processed into tobacco.^{1,2} In addition, *N. tabacum* is also used as insecticide, anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine because of it containing many useful chemical compounds.^{1,3,4} In previous work, a number of bioactive compounds, such as terpenoids,⁵⁻⁷ alkaloids,^{8,9} lignans,^{10,11} flavonoid,¹²⁻¹⁴ phenylpropanoids,^{15,16} chromanones,^{17,18} biphenyls,¹⁹ phenolic amides,²⁰ coumarins,^{21,22} and the homologous, were isolated from this plant. Therefore, the multipurpose utilization of this plant is an interesting topical and attracts more and more attentions. Motivated by search for more bioactive metabolites from this plant, an investigation on the chemical constituents of the leaves of Yunnan local sun cured tobacco (a variant of *N. tabacum*) was carried out. As a result, two new (**1-2**) and four known flavones (**3-6**) were isolated from this plant. In addition, the cytotoxicity of compounds **1-6** was evaluated. This article deals with the isolation,

structural elucidation and biological activities of these flavones.

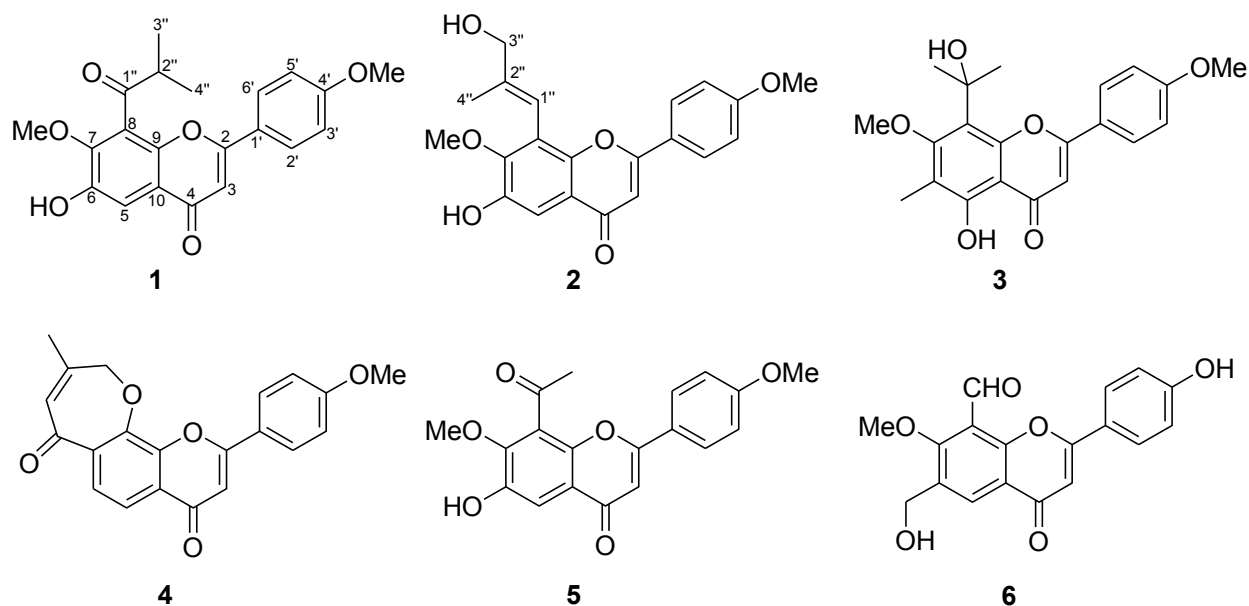


Figure 1. Flavones from the leaves of Yunnan local sun cured tobacco

A 70% aq. methanol extract prepared from the leaves of tobacco was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new flavones, 4',7-dimethoxy-6-hydroxy-8-isobutyrylflavone (**1**), 4',7-dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]flavone (**2**), and four known flavones (**3-6**). The structures of the compounds **1-6** were shown in Figure 1, and the ^1H and ^{13}C NMR data of **1** and **2** were listed in Table 1. The known compounds, compared with literature data, were identified as 4',7-dimethoxy-5-hydroxy-8-(2-hydroxypropan-2-yl)flavone (**3**),²³ tobaflavone E (**4**),² paranicflavone C (**5**),²⁴ and tabaflavone B (**6**).²⁵

Compound **1** was obtained as an orange gum. The molecular formula of $\text{C}_{21}\text{H}_{20}\text{O}_6$ was determined from the HRESIMS spectra showing the sodiated molecular ion at m/z 391.1166 $[\text{M}+\text{Na}]^+$ (calcd 391.1158), suggesting 12 degrees of unsaturation. The UV spectrum showed absorption maxima at 210, 254 and 362 nm, and the IR spectrum showed absorption bands at 3454, 1710, 1650, 1610, 1536, and 1455 cm^{-1} , indicating the presence of hydroxy group, carbonyl group, and aromatic ring. The ^1H and ^{13}C NMR spectrum of **1** (Table 1) along with analysis of the DEPT spectra displayed 21 carbon signals and 20 proton signals, respectively, corresponding to 4',6,7,8-tetrasubstituted flavones nucleus (Table 1, C-2 ~ C10 and C-1' ~ C-6'; H-3, H-5, H-2',6', and H-3',5'),²⁴ one isobutyryl group (δ_{C} 209.1 s, 38.0 d, and 20.2 q; δ_{H} 4.42 m and 1.31 (d) 6.6),²⁶ two methoxy groups (δ_{C} 61.1 q and 55.9 q; δ_{H} 3.89 s and 3.83 s), and one phenolic hydroxy proton (δ_{H} 10.75 s). The HMBC correlations (Figure 2) of H-3 (δ_{H} 6.56 s) with C-10 (δ_{C} 119.8), C-2 (δ_{C} 164.9), C-4 (δ_{C} 176.2), and C-1' (δ_{C} 122.7), of H-2',6' (δ_{H} 7.73) with C-2 (δ_{C} 164.9), of H-5 (δ_{H} 7.37) with C-4 (δ_{C} 176.2), C-6 (δ_{C} 143.2), C-7 (δ_{C} 159.0), C-9 (δ_{C} 148.0), and C-10 (δ_{C} 119.8),

also suggested that **1** should be a flavone. The HMBC correlations of two methoxy protons (δ_{H} 3.83 and 3.89) with C-4' (δ_{C} 161.2) and C-7 (δ_{C} 159.0) concluded the linkage of the two methoxy groups at C-4' and C-7, respectively. The location of the phenolic hydroxy group was assigned to C-6 positions on the basis of HMBC correlations of the phenolic hydroxy proton signal (δ_{H} 10.75) with C-5 (δ_{C} 121.0), C-6 (δ_{C} 143.2) and C-7 (δ_{C} 159.0). Finally, the isobutyryl group located at C-8 was confirmed by the HMBC correlation of H-2'' (δ_{H} 4.42) with C-8 (δ_{C} 112.7). The typical proton signals of H-5 (δ_{H} 7.37), H-2',6' [δ_{H} 7.73 (d, $J = 8.6$)], and H-3',5' [δ_{H} 6.71 (d, $J = 8.6$)] also supported this substituents pattern. Thus, the structure of **1** was established as 4',7-dimethoxy-6-hydroxy-8- isobutyrylflavone.

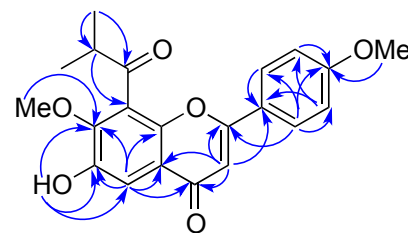


Figure 2. Key HMBC (\curvearrowright) correlations of **1**

Compound **2** was obtained as an orange gum. A molecular formula $\text{C}_{21}\text{H}_{20}\text{O}_6$ was assigned from HRESIMS (m/z : 391.1153 $[\text{M}+\text{Na}]^+$, calcd 391.1158). The ^1H and ^{13}C NMR data of **2** (Table 1) displayed 21 carbon and 20 proton signals, corresponding to a flavone nucleus²⁴ (Table 1, C-2 ~ C-10 and C-1' ~ C-6'; H-3, H-5, H-2',6', and H-3',5'), one (*E*)-3-hydroxy-2-methylprop-1-enyl moiety²⁷ (δ_{C} 125.8 d, 132.2 s, 58.9 t, 16.0 q; δ_{H} 6.61 (t, $J=5.6$), 4.41 and 4.44 (d) $J=5.6$, 1.85 s), two methoxy groups (δ_{C} 61.4 and 56.0, δ_{H} 3.90 and 3.86), and one phenolic hydroxy proton (δ_{H} 11.00). The ^1H and ^{13}C NMR spectral data of **2** were similar to those of **1**. The obvious chemical shift differences resulted from

Table 1. ^1H NMR and ^{13}C NMR Data (in $\text{C}_5\text{D}_5\text{N}$) of compounds **1** and **2**

No.	Compound 1		Compound 2	
	δ_{C} (m)	δ_{H} (m, J , Hz)	δ_{C} (m)	δ_{H} (m, J , Hz)
2	164.9 s		163.1 s	
3	105.7 d	6.56 s	104.2 d	6.52 s
4	176.2 s		177.1 s	
5	121.0 d	7.37 s	117.0 d	6.97 s
6	143.2 s		142.1 s	
7	159.0 s		156.6 s	
8	112.7 s		116.1 s	
9	148.0 s		147.4 s	
10	119.8 s		117.6 s	
1'	122.7 s		122.6 s	
2',6'	130.2 d	7.73 (d) 8.6	130.6 d	7.79 (d) 8.6
3',5'	116.0 d	6.71 (d) 8.6	115.3 d	6.81 (d) 8.6
4'	161.2 s		161.2 s	
1''	209.1 s		125.8 d	6.61 (t) 5.6
2''	38.0 d	4.42 m	132.2 s	
3''	20.2 q	1.31 (d) 6.6	58.9 t	4.41, 4.44 (d) 5.6
4''	20.2 q	1.31 (d) 6.6	16.0 q	1.85 s
-OMe-7	61.1 q	3.89 s	61.4 q	3.90 s
-OMe-4'	55.9 q	3.83 s	56.0 q	3.86 s
Ar-OH		10.75 s		11.00 s

the substituent group variation on the benzene ring. The appearance of one (*E*)-3-hydroxy-2-methylprop-1-enyl moiety signals and the disappearance of one isobutyryl group signals [δ_{C} 209.1 s, 38.0 d, 20.2 q, and 20.2 q; δ_{H} 4.42 m, and 1.31 (d) 6.6] were observed in compound **2**. These changes indicated that the isobutyryl group in **1** was replaced by a (*E*)-3-hydroxy-2-methylprop-1-enyl moiety in **2**. The (*E*)-3-hydroxy-2-methylprop-1-enyl moiety located at C-8, the phenolic hydroxy group located at C-6,

and two methoxy groups located at C-4' and C-7, respectively, were confirmed by further analysis of its HMBC correlations. In addition, the ROESY correlation of H-1'' with H-3'' indicated an *E*-configuration for the C-1'', C-2'' double bond. Thus, structure of 4',7-dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]flavone (**2**) was established.

The cytotoxicities of compounds **1-6** were tested using a previously reported procedure.^{28,29} The cytotoxic abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were shown in Table 2. The results revealed that compound **2** exhibited high cytotoxicity for A549 and SHSY5Y cells with IC₅₀ values of 1.6 and 2.8 μM. The other compounds also exhibited cytotoxicity with IC₅₀ values in the range of 4.3 – 9.5 μM, respectively.

Table 2. Cytotoxic activities of compounds **1-6**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	6.2	4.3	6.4	5.1	8.5
2	5.6	1.6	2.8	8.4	6.8
3	>10	8.2	8.8	>10	7.8
4	8.4	>10	6.9	7.2	>10
5	9.5	>10	6.8	>10	7.9
6	>10	8.2	>10	6.8	6.3
Taxol	0.03	0.02	0.1	0.1	0.05

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, Human prostate cancer cell; MCF7, Human breast adenocarcinoma cell.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard, and the chemical shifts (δ) were expressed in ppm. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C₁₈ (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H₂SO₄ in EtOH.

Plant material. The sun cured tobacco leaves (Dali Heqing tobacco, a variety of *Nicotiana tabacum*) were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in September 2013. The identification of the plant material was verified by Prof. H. W. Yang (School of Tobacco, Yunnan Agriculture University).

Extraction and Isolation. The air-dried and powdered tobacco leaves (4.8 kg) were extracted three times with 70% aqueous MeOH (3 × 6.0 L) at room temperature and filtered to yield a filtrate, which was

successively evaporated under reduced pressure to obtain a crude extract (325 g). This crude extract was applied to Si gel (200-300 mesh) column chromatography eluting with a CHCl₃-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A–F. The separation of fraction C (8:2, 20.5 g) was subjected to Si gel column chromatography eluting with CHCl₃-(Me)₂CO and then run on preparative HPLC (55% MeOH-H₂O, flow rate 12 mL/min) to yield compounds **1** (13.6 mg), **2** (10.2 mg), and **4** (12.6 mg). Fraction C (14.6 g) was subjected to Si gel column chromatography eluting with CHCl₃-(Me)₂CO and then run on preparative HPLC (46% MeOH-H₂O, flow rate 12 mL/min) to yield compounds **3** (12.2 mg), **5** (16.2 mg), and **6** (18.0 mg).

4',7-Dimethoxy-6-hydroxy-8-isobutyrylflavone (1): Obtained as orange gum; UV (CH₃OH), λ_{\max} (log ϵ) 210 (4.18) 254 (3.69), 362 (3.58), nm; IR (KBr) ν_{\max} 3454, 2927, 1710, 1650, 1610, 1536, 1455, 1376, 1236, 1176, 1068, 946, 818 cm⁻¹; ¹³C NMR and ¹H NMR data (in C₅D₅N, 500 and 125 MHz) see Table 1; positive ESIMS m/z 391 [M+Na]⁺; HRESIMS m/z 391.1166 [M+Na]⁺ (calcd for C₂₁H₂₀NaO₆, 391.1158).

4',7-Dimethoxy-6-hydroxy-8-[(E)-3-hydroxy-2-methylprop-1-enyl]flavone (2): Obtained as orange gum; UV (CH₃OH), λ_{\max} (log ϵ) 210 (4.22) 254 (3.69), 364 (3.58), nm; IR (KBr) ν_{\max} 3438, 2932, 2875, 1652, 1605, 1574, 1527, 1462, 1358, 1241, 1156, 1038, 962, 826, 768 cm⁻¹; ¹³C NMR and ¹H NMR data (in C₅D₅N, 500 and 125 MHz) see Table 1; positive ESIMS m/z 391 [M+Na]⁺; HRESIMS m/z 391.1153 [M+Na]⁺ (calcd for C₂₁H₂₀NaO₆, 391.1158).

ACKNOWLEDGEMENTS

This research was supported by the Foundation of Yunnan Tobacco Company (2014YN16), the National Natural Science Foundation of China (No. 31360081), the Applied Fundamental Foundation of Yunnan Province (No. 2013FB097), and the Basic Research Foundation of Yunnan Tobacco Industry Co. Ltd (2012JC01).

REFERENCES

1. The Editorial Committee of the Administration Bureau of Flora of China, 'Flora of China,' 67 vols. Beijing Science and Technology Press, Beijing, 2005.
2. M.-M. Miao, L. Li, Q.-P. Shen, C.-B. Liu, Y.-K. Li, T. Zhang, F.-M. Zhang, P. He, K.-M. Wang, R.-Z. Zhu, Y.-K. Chen, and G.-Y. Yang, *Fitoterapia*, 2015, **103**, 260.
3. A. Rodgman and T. A. Perfetti, 'The Chemical Components of Tobacco and Tobacco Smoke,' CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2008.
4. A. P. Cavender and M. Alban, *J. Ethnobiol. Ethnomed.*, 2009, **5**, 3.
5. X. Feng, Xin, J.-S. Wang, J. Luo, and L.-Y. Kong, *J. Asian Nat. Prod. Res.*, 2010, **12**, 252.

6. Y.-K. Chen, C.-Y. Meng, Z.-B. Su, L. Wang, G.-Y. Yang, and M.-M. Miao, *Asian J. Chem.*, 2014, **26**, 2246.
7. G. Y. Yang, W. Zhao, Y.-K. Chen, Z.-Y. Chen, Q.-F. Hu, and M.-M. Miao, *Asian J. Chem.*, 2013, **25**, 4932.
8. X. C. Wei, S. C. Sumithran, A. G. Deaciuc, H. R. Burton, L. P. Bush, L. P. Dwoskin, and P. A. Crooks, *Life Sci.*, 2005, **78**, 495.
9. T. Braumann, G. Nicolaus, W. Hahn, and H. Elmenhorst, *Phytochemistry*, 1990, **29**, 3693.
10. Y.-K. Chen, X.-S. Li, G.-Y. Yang, Z.-Y. Chen, Q.-F. Hu, and M.-M. Miao, *J. Asian Nat. Prod. Res.*, 2012, **14**, 450.
11. X.-M. Gao, X.-S. Li, X.-Z. Yang, H.-X. Mu, Y.-K. Chen, G.-Y. Yang, and Q.-F. Hu, *Heterocycles*, 2012, **85**, 147.
12. Z.-Y. Chen, J.-L. Tan, G.-Y. Yang, M.-M. Miao, Z.-Y. Chen, and T.-F. Li, *Phytochem. Lett.*, 2012, **5**, 233.
13. Y.-K. Li, Y.-L. Zhao, N.-J. Xiang, L. Yang, F. Wang, G.-Y. Yang, and Z.-Y. Wang, *Heterocycles*, 2014, **89**, 2771.
14. Y. Wang, C.-B. Liu, Q.-P. Shen, F.-M. Zhang, P. He, Z.-H. Liu, H.-B. Zhang, X.-D. Yang, M.-M. Miao, and G. Y. Yang, *Heterocycles*, 2015, **91**, 1198.
15. H.-Q. Leng, J.-X. Chen, Y. Hang, Y.-X. Duan, G.-Y. Yang, Y.-K. Chen, Y.-D. Guo, Q.-F. Hu, and M.-M. Miao, *Chem. Nat. Compd.*, 2014, **49**, 1028.
16. J.-L. Tan, Z.-Y. Chen, G.-Y. Yang, M.-M. Miao, Y.-K. Chen, and T.-F. Li, *Heterocycles*, 2011, **83**, 2381.
17. D.-R. Mou, W. Zhao, T. Zhang, L. Wan, G.-Y. Yang, Y.-K. Chen, Q.-F. Hu, and M.-M. Miao, *Heterocycles*, 2012, **85**, 2485.
18. G.-Y. Yang, W. Zhao, T. Zhang, Y.-X. Duan, Z.-H. Liu, M.-M. Miao, and Y.-K. Chen, *Heterocycles*, 2014, **89**, 183.
19. S.-Z. Shang, W.-X. Xu, P. Lei, W. Zhao, J.-G. Tang, M.-M. Miao, H.-D. Sun, J.-X. Pu, Y.-K. Chen, and G.-Y. Yang, *Fitoterapia*, 2014, **99**, 35.
20. S.-Z. Shang, Y.-X. Duan, X. Zhang, J.-X. Pu, H.-D. Sun, Z.-Y. Chen, M.-M. Miao, G.-Y. Yang, and Y.-K. Chen, *Phytochem. Lett.*, 2014, **7**, 413.
21. S.-Z. Shang, W.-X. Xu, L. Li, J.-G. Tang, W. Zhao, P. Lei, M.-M. Miao, H.-D. Sun, J.-X. Pu, Y.-K. Chen, and G.-Y. Yang, *Phytochem. Lett.*, 2015, **11**, 53.
22. C. Lei, W.-X. Xu, J.-Wu, S.-J. Wang, J.-Q. Sun, Z.-Y. Chen, and G.-Y. Yang, *Chem. Nat. Compd.*, 2005, **51**, 43.
23. S. Nazreen, G. Kaur, M. M. Alam, S. Shafi, H. Hamid, and M. Ali, *Fitoterapia*, 2012, **83**, 1623.

24. W. Zhao, L. Li, P. Lei, L. Yang, S.-Z. Shang, J.-G. Tang, G.-Y. Yang, Y.-K. Chen, and M.-M. Miao, [*Phytochem. Lett.*, 2015, **12**, 125.](#)
25. L. Yuan, W.-Z. Huang, C.-M. Zhang, N.-J. Xiang, C.-B. Liu, G.-Y. Yang, Y.-K. Chen, M.-M. Miao, and Y.-H. Ma, [*Phytochem. Lett.*, 2015, **12**, 75.](#)
26. G. V. Ccana-Ccpatinta, E. D. Stolz, P. F. Costa, S. M. K. Rates, and G. L. Poser, [*J. Nat. Prod.*, 2015, **78**, 1112.](#)
27. A. Andolfi, A. Boari, M. Evidente, A. Cimmino, M. Vurro, G. Ash, and A. Evidente, *J. Nat. Prod.*, 2015, **78**, 623.
28. M. Zhou, K. Zhou, X.-M. Gao, Z.-Y. Jiang, J.-J. Lv, Z.-H. Liu, G.-Y. Yang, M.-M. Miao, C.-T. Che, and Q.-F. Hu, [*Org. Lett.*, 2015, **17**, 2638.](#)
29. M. Zhou, M.-M. Miao, G. Du, S.-Z. Shang, W. Zhao, Z.-H. Liu, G.-Y. Yang, C.-T. Che, Q.-F. Hu, and X.-M. Gao. [*Org. Lett.*, 2014, **16**, 5016.](#)