# Rapid synthesis of ismine, a bioactive amaryllidaceae alkaloid

**Jing-jing Guo[a,b], Bi-juan Yang[b,c], Chen-xu Jing[b], Duo-zhi Chen[b]* and Xiao-jiang Hao[b]**

[a]*College of Traditional Chinese Medicine, Yunnan University of Traditional Chinese Medicine, Kunming 650500, P.R. China*

[b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P.R. China*

[c]*School of Chemical Science and Technology, Yunnan University, Kunming 650000, P.R. China*

Ismine (6-[2-(methylamino)phenyl]-1,3-benzodioxole-5-methanol, **1**), a biologically active alkaloid, has been synthesised by a rapid and simple four-step sequence. This synthesis involved a consecutive aryl–aryl and N–aryl coupling, leading to a phenanthridine derivative in a one-pot sequence, which employed a palladium catalyst and trifurylphosphine as the ligand. This synthesis gave an overall yield of 23%.

**Keywords:** amaryllidaceae alkaloid, palladium catalyst, aryl–aryl coupling, N–aryl coupling

Ismine (6-[2-(methylamino)phenyl]-1,3-benzodioxole-5-methanol, **1**, Fig. 1) is a natural bioactive alkaloid that was first isolated from an *Ismene* species in 1961.[1] During the ensuing 50 years, ismine was found to have neuroprotective, antibacterial, antifungal and cytotoxicity activities.[2–6] We have previously identified ismine as an activator of the Wnt signalling pathway that targets the DIX domain of the Axin protein and potentiates the Axin–LRP6 association, promoting Wnt signalling transduction.[7]

In the light of these results, structural modification of ismine is needed and larger amounts of material are required to confirm and further explore the potential activities of ismine compounds in bioassays, including *in vivo* assays. However, ismine cannot be readily isolated from plants. Therefore, we have designed a totally synthetic route for the production of ismine.

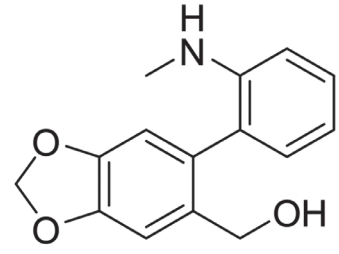

**Fig. 1** The structure of ismine (**1**).

## Results and discussion

According to the retrosynthetic analysis of compound **1**, we concluded that ismine could be obtained by reduction of 6-[2-(methylamino)phenyl]benzo[1,3]dioxole-5-carboxylic acid (**6**), which is the lactam ring-opening product of 5-methyl-[1,3]dioxolo-phenanthridin-6-one (**5**). We have previously developed a rapid synthetic method for obtaining phenanthridine compounds from halogenated benzamide analogues and iodobenzene by an efficient and consecutive aryl–aryl and N–aryl coupling process.[8] Therefore, this simple phenanthridine synthetic method could be employed to prepare the key intermediate **5**, which can be rapidly transformed into the target compound, ismine, by hydrolysis of lactam and hydrogenation (Fig. 2).

As shown in Fig. 3, ismine was synthesised from 2-bromo-4,5-(methylenedioxy)benzoic acid (**2**) in four steps. First, compound **2** was amidated with methylamine to obtain *N*-methyl-2-bromo-4,5-(methylenedioxy)benzamide (**3**), which was then coupled with iodobenzene (**4**) using a palladium catalyst to afford 5-methyl-[1,3]dioxolo-phenanthridin-6-one (**5**). This was followed by hydrolysis of the lactam group in 35% aqueous HCl to afford **6**. Finally, ismine (**1**) was obtained by reduction of **6** in the presence of lithium aluminium hydride (LAH).

*Reagents and conditions:* (a) $SOCl_2$, DMF, THF, 50 °C, 2 h; (b) $CH_3NH_2$ (30%), 5 °C, 1 h, 78% yield; (c) $K_2CO_3$, norbornene,

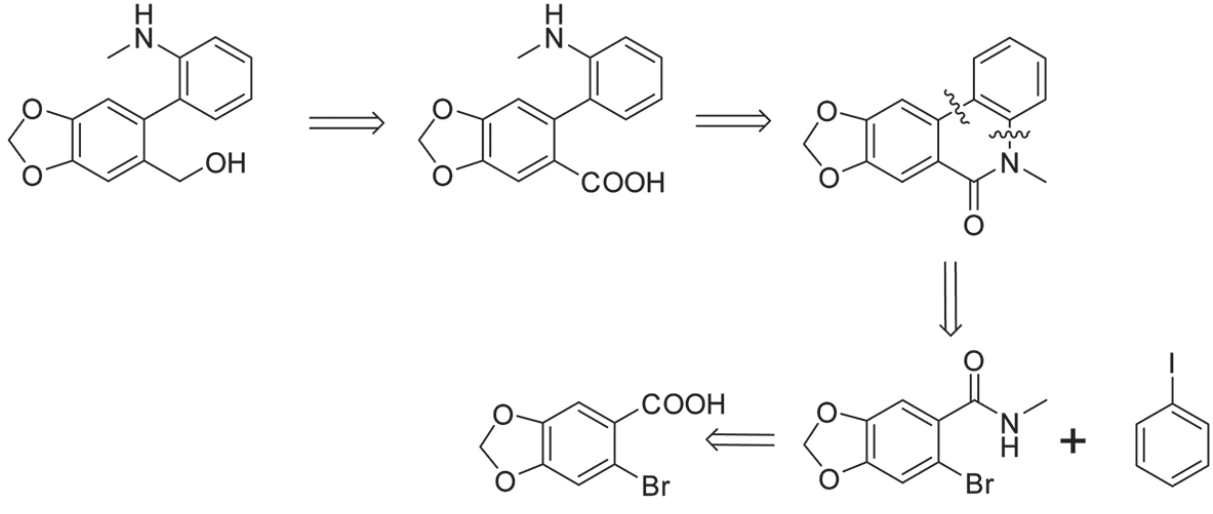

**Fig. 2** The retrosynthetic analysis of ismine.

* Correspondent. E-mail: chenduozhi@mail.kib.ac.cn

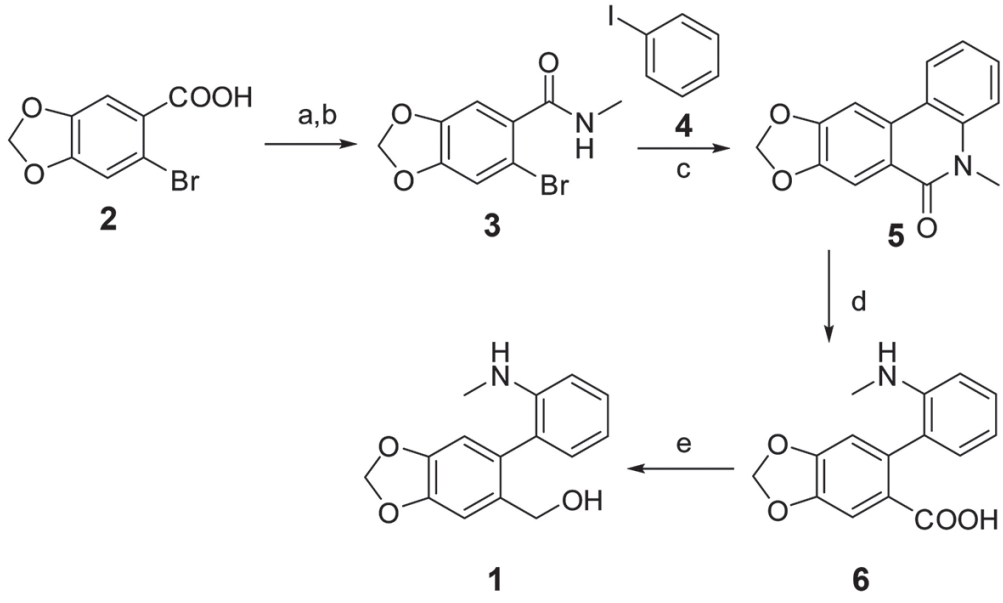


**Fig. 3** Total synthesis of ismine (**1**).

$Pd(OAc)_2$, TFP, MeCN, 95 °C, 12 h, 60% yield; (d) 35% HCl, 120 °C, 48 h, 75% yield; (e) LAH, $CH_2Cl_2$, −40 °C, 4 h, 65% yield.

An efficient and convenient synthetic route to ismine was developed for the production of sufficient quantities to enable its use in further bioassays. This new route hinges on an efficient palladium-catalysed coupling reaction involving consecutive aryl–aryl and N–aryl coupling. This relatively simple procedure, utilisation of cheap and readily available reagents, and overall yield (23%) of products are the main advantages of the present approach.

## Experimental

ESI and HREIMS were recorded using a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. Melting points were measured using X-4 apparatus (Yingyu Yuhua Instrument Factory, Gongyi, Henan Province, China). NMR experiments were carried out on a Bruker AM-400 spectrometer, a DRX-500 spectrometer or an Avance III 600 spectrometer with the solvents $CDCl_3$, DMSO-$d_6$ and $Me_4Si$ as internal standard. Column chromatography was performed on silica gels (60–80 mesh, 200–300 mesh, 300–400 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China). Pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany) was used for TLC. Semi-preparative HPLC was performed on a Hypersil Gold RP-C18 column (i.d. 10 × 250 mm; Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) eluted with $CH_3CN$–$H_2O$ at room temperature. All regular solvents and reagents were reagent grade and were purchased from Aldrich-Sigma Chemical Co., Acros Organics or J&K Scientific. The purities of all compounds were over 95% as determined by HPLC. All yields reported are for dry compounds that require no further purification for use in other reactions.

N-*Methyl-2-bromo-4,5-(methylenedioxy)benzamide* (**3**)

Compound **2** (260 mg, 1 mmol) was dissolved in THF (20 mL), to which DMF (0.1 mL) and $SOCl_2$ (0.5 mL, 4mmol) were added. The solution was stirred for 2 h at 50 °C and then concentrated to remove THF. The residue was then added to a 30% solution of methylamine in water (20 mL) at 5 °C and filtered. The cake was purified by column chromatography to give **3** as: Pale yellow solid; m.p. 184–185 °C; yield 200 mg (78%); $^1$H NMR (500 MHz, $CDCl_3$): δ 7.03 (s, 1H), 6.98 (s, 1H), 6.01 (s, 2H), 2.99 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 167.7 (C), 149.7 (C), 147.5 (C), 131.0 (C), 113.2 (CH), 110.6 (C), 109.7 (CH), 102.3 ($CH_2$), 26.8 ($CH_3$); HREIMS *m/z*: 256.9684 $[M]^+$ (calcd for $C_9H_8BrNO_3$, 256.9688).

*5-Methyl-[1,3]dioxolo-phenanthridin-6-one* (**5**)

A flask was charged under nitrogen with $Pd(OAc)_2$ (5.0 mg, 0.02 mmol), tri-2-furylphosphine (10 mg, 0.035 mmol), $K_2CO_3$ (72.3 mg, 0.52 mmol), the amide **3** (0.26 mmol), a solution of norbornene (40 mg, 0.4 mmol) in anhydrous solvent (7 mL), and iodobenzene (**4**, 0.26 mmol). The mixture was heated with stirring at 95 °C for 12 h and then cooled to room temperature. After the addition of saturated $NH_4Cl$ (30 mL) and extraction with EtOAc (3 × 20 mL), the combined organic extracts were washed with brine (50 mL) and dried over $Na_2SO_4$. Removal of the solvent under reduced pressure gave the crude product, which was purified by flash chromatography on silica gel to furnish **5** as: Colourless powder; m.p. 237–239 °C; yield 52 mg (80%); $^1$H NMR (500 MHz, $CDCl_3$): δ 8.10 (dd, *J* = 8.7, 1.5 Hz, 1H), 7.89 (s, 1H), 7.67 (s, 1H), 7.45–7.36 (m, 2H), 7.30 (t, *J* = 7.3 Hz 1H), 6.15 (s, 2H), 3.13 (s, 3H); $^{13}$C NMR (500 MHz, $CDCl_3$): δ 160.8 (C), 152.2 (C), 148.3 (C), 137.3 (C), 130.5 (C), 128.7 (CH), 122.8 (CH), 122.3 (CH), 121.1 (C), 119.1 (C), 115.2 (CH), 106.9 (CH), 101.8 ($CH_2$), 100.3 (CH), 30.2 ($CH_3$); HREIMS *m/z*: 253.0731 $[M]^+$ (calcd for $C_{15}H_{11}NO_3$, 253.0739).

*6-(2-(Methylamino)phenyl)benzo[d][1,3]dioxole-5-carboxylic acid* (**6**)

Compound **5** (29 mg, 0.1 mmol) was dissolved in DMF (1 mL). The solution was then cooled to −5 °C and aqueous HCl (35%, 5 mL) was added. The mixture was then heated to 120 °C and stirred for 48 h. It was then diluted with saturated $NaHCO_3$ (10 mL). The solution was extracted with $CH_2Cl_2$ (2 × 25 mL), and the organic layer was washed with brine, concentrated and then purified by column chromatography using chloroform:methanol (30:1) as the eluent to give **6** as: Pale yellow powder; m.p. 197–199 °C; yield 21 mg (75%); $^1$H NMR (500 MHz, $CDCl_3$): δ 8.02 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.82 (s, 1H), 7.60 (s, 1H), 7.36–7.32 (m, 2H), 7.26 (t, *J* = 7.7 Hz, 1H), 6.07 (s, 2H), 3.05 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 163.2 (C), 152.3 (C), 148.5 (C), 142.3 (C), 137.0 (C), 133.0 (CH), 131.8 (C), 130.6 (C), 127.3 (CH), 122.4 (CH), 121.9 (CH), 106.7 (CH), 102.2 ($CH_2$), 100.8 (CH), 29.7 ($CH_3$); HREIMS *m/z*: 271.0839 $[M]^+$ (calcd for $C_{15}H_{13}NO_4$, 271.0845).

*6-[2-(Methylamino)phenyl]-1,3-benzodioxole-5-methanol (ismine)* (**1**)

A solution of **6** (30 mg, 0.1 mmol) in THF (5 mL) and $CH_2Cl_2$ (10 mL) was added to LAH (30 mg) at −5 °C. The reaction was stirred for 6 h at room temperature and then quenched using $H_2O$ (5 mL). The mixture was then extracted with $Et_2O$ (2 × 20 mL). The organic phase was washed with brine and concentrated, and the residue was purified by

column chromatography to give **1** as: Colourless solid; m.p. 98–99 °C; yield 17 mg (65%); $^{1}$H NMR (400 MHz, $CDCl_3$): δ 7.29 (t, *J* = 1.5 Hz, 1H), 7.02 (s, 1H), 7.00 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.83 (dd, *J* = 14.8, 7.6 Hz, 1H), 6.75 (d, *J* = 8.2Hz, 1H), 6.69 (s, 1H), 6.01 (s, 2H), 4.27 (d, *J* = 12.0, 1H), 4.21 (d, *J* = 12.0, 1H), 2.75 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 147.6 (C), 147.5 (C), 146.6 (C), 133.9 (C), 131.1 (C), 129.9 (CH), 129.0 (CH), 127.4 (C), 118.4 (CH), 111.0 (CH), 110.2 (CH), 109.9 (CH), 101.3 ($CH_2$), 63.8 ($CH_2$), 30.8 ($CH_3$); HREIMS *m/z*: 257.1055 $[M]^+$ (calcd for $C_{15}H_{15}NO_3$, 257.1052).[9]

## Acknowledgements

This research was supported financially by the national Natural Science Foundation of China (81402828 and 21432010), the "Light of Western" foundation of the Chinese Academy of Sciences, the Natural Science Fund of Yunnan Province (2016FB015) and the Foundation of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2015-ZZ14).

## Electronic Supplementary Information

The ESI is available through:
stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

*Received 9 October 2016; accepted 18 February 2017*
*Paper 1604366*
*https://doi.org/10.3184/174751917X14894997017496*
*Published online: 29 March 2017*

## References

1 R.J. Highet, *J. Org. Chem.*, 1961, **26**, 4767.
2 C. Iannello, J. Bastida, F. Bonvicini, F. Antognoni, G.A. Gentilomi and F. Poli, *Nat. Prod. Res.*, 2014, **28**, 704.
3 T. Morikawa, K. Ninomiya, H. Kuramoto, I. Kamei, M. Yoshikawa and O. Muraoka, *J. Nat. Med.*, 2016, **70**, 89.
4 L. Cahlikova, M. Hrabinova, A. Kulhankova, N. Benesova, J. Chlebek, D. Jun, Z. Novak, K. Macakova, J. Kunes, K. Kuca and L. Opletal, *Nat. Prod. Commun.*, 2013, **8**, 1541.
5 Z. Luo, F. Wang, J. Zhang, X. Li, M. Zhang, X. Hao, Y. Xue, Y. Li, F.D. Horgen, G. Yao and Y. Zhang, *J. Nat. Prod.*, 2012, **75**, 2113.
6 J.E. Ortiz, S. Berkov, N.B. Pigni, C. Theoduloz, G. Roitman, A .Tapia, J. Bastida and G.E. Feresin, *Molecules*, 2012, **17**, 13473.
7 S. Wang, J.L. Yin, D.Z. Chen, F. Nie, X.M. Song, C. Fei, H.F. Miao, C. Jing, B.W. Ma, J.L. Wang, S.C. Xie, C. Li, R. Zeng, W.J. Pan, X.J. Hao and L. Li, *Nat. Chem. Biol.*, 2013, **9**, 579.
8 C.X. Jing, J.Y. Cai, Y. Zhang, D.Z. Chen and X.J. Hao, *J. Chem. Res.*, 2015, **39**, 247.
9 D.T.A. Youssef, *J. Nat. Prod.*, 2001, **64**, 839