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## A concise synthesis and biological study of evodiamine and its analogues†‡

Jie-Dan Deng,<sup>§ab</sup> Shuai Lei,<sup>§a</sup> Yi Jiang,<sup>id a</sup> Hong-Hua Zhang,<sup>a</sup> Xiao-Ling Hu,<sup>a</sup> Huai-Xiu Wen,<sup>c</sup> Wen Tan<sup>\*a</sup> and Zhen Wang<sup>id \*ab</sup>

Efficient access to evodiamine and its analogues is presented via Lewis acid catalysis. In this reaction, three chemical bonds and two heterocyclic-fused rings are constructed in one step. The reaction shows good functional group tolerance and atom economy, and various heteroatom-containing evodiamine analogues are obtained in moderate to excellent yields even on a gram scale. An anti-tumor study *in vitro* demonstrates compound 2b possesses potent efficacy against hepatoma cell line (IC<sub>50</sub> = 5.7 μM).

Polycyclic heterocycles are crucial motifs that widely exist in drugs and alkaloids,<sup>1</sup> and many of them have shown superior bioactivity in anti-bacterial (roquefortine C), anti-tumor (camptothecin), and cerebral vasodilatory (vincoline) agents *etc.* according to the known reports (Fig. 1).<sup>2</sup> Thus, the rapid construction of polycyclic heterocycle skeletons is always a hotspot in synthetic chemistry.<sup>3</sup> Among them, evodiamine is one of the most typical polycyclic heterocycles, the synthesis and modification of which have aroused broad interest in both synthetic and pharmaceutical industries because of its diverse biological activities.<sup>4</sup> In the last decades, several groups have done elegant work in this domain. In 1997, Chen firstly employed evodiamine for anti-inflammatory purposes.<sup>5</sup> Subsequently, Kamiya demonstrated evodiamine's efficacy in anti-obesity in 2001.<sup>6</sup> After that, Zhang successfully applied evodiamine in a transgenic mouse model for Alzheimer's disease (AD) treatment.<sup>7</sup> Through chemical decorations, Sheng *et al.* discovered several evodiamine analogues as potent anti-cancer drug candidates in recent years.<sup>8</sup> The structure–activity relationships of evodiamine derivatives have been reported previously, in which R<sub>1</sub> and X follow the listed orders respectively.

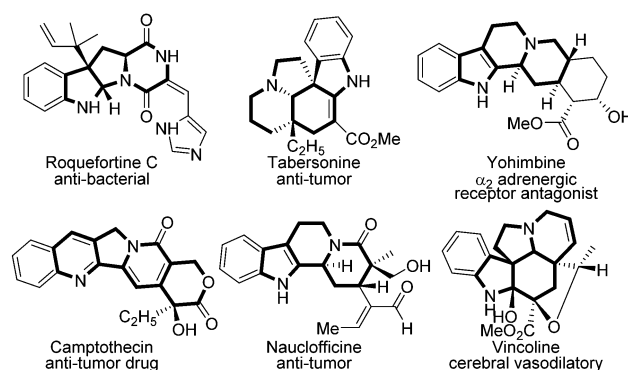


Fig. 1 Polycyclic heterocycle skeletons in drugs and natural products.

For R<sub>1</sub>: OH > I > F > OMe > Br > Cl or Me; for X: O, S > N-Me > CH > C=N. Meanwhile, when different positions of Ar<sub>2</sub> are substituted by different groups, the activity also varies.<sup>8b,d</sup> Despite evodiamine and its analogues having shown great potential benefits for human health, the approaches to synthesise these compounds are still limited.<sup>9</sup> Conventional routes to such kind of chemicals usually suffer from multi-step preparation and an imine intermediate needing to be synthesized stepwise, along with this process employing some toxic reagents as well as transition metals (Scheme 1).<sup>10</sup> In this context, methodologies to prepare evodiamine and its analogues concisely are highly desired in synthetic chemistry. Recently, Wu and co-workers developed an elegant work on the one-pot synthesis of evodiamine and its analogues; unfortunately, the substrates were merely constrained to *N*-methylisatoic anhydride accordingly.<sup>11</sup> Therefore, related works on one-step preparation of diversified heteroatom-containing evodiamine analogues still remain scarce. Herein, we report concise access to evodiamine and various heteroatom-containing evodiamine analogues *via* Lewis acid catalysis. In this protocol, different fused heterocycles are efficiently constructed within one step.

We initiated this study with 1a as the model substrate, and chose HC(OEt)<sub>3</sub> as the carbon source with Lewis acid AlCl<sub>3</sub> as the catalyst (Table 1). After screening various solvents, DMF was

<sup>a</sup> School of Pharmacy, Lanzhou University, West Donggang Road, No. 199, Lanzhou 730000, China. E-mail: tanwen@lzu.edu.cn, zhenw@lzu.edu.cn

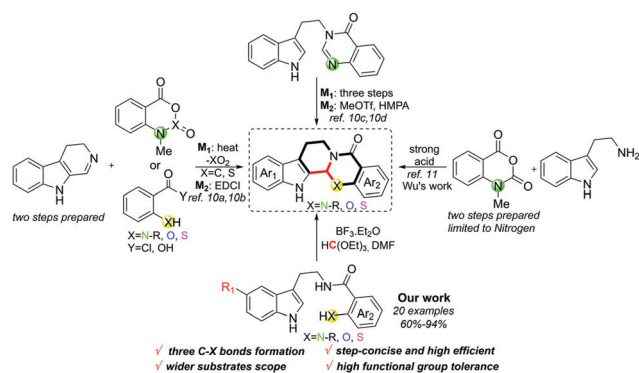
<sup>b</sup> Institution State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China

<sup>c</sup> Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, No. 23 Xining Road, Xining, P. R. China

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§ These authors contributed equally.



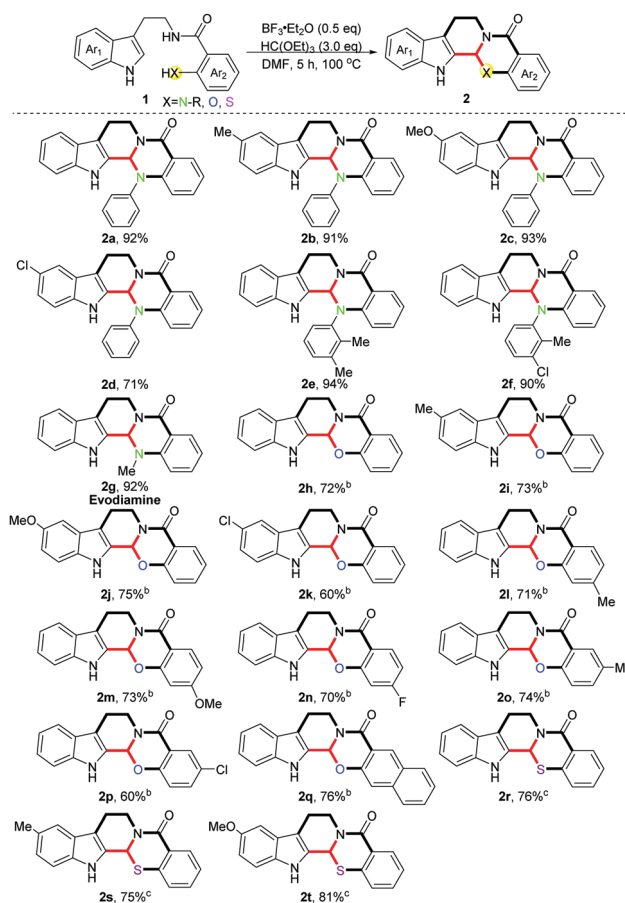
Scheme 1 Approaches to evodiamine and its analogues.

Table 1 Optimization of reaction conditions<sup>a</sup>

| Entry           | Solvent            | Lewis acid                         | HC(OEt) <sub>3</sub> (eq.) | Temp. (°C) | Yield <sup>b</sup> (%) |
|-----------------|--------------------|------------------------------------|----------------------------|------------|------------------------|
| 1               | DMF                | AlCl <sub>3</sub>                  | 3                          | 135        | 73                     |
| 2               | CH <sub>3</sub> CN | AlCl <sub>3</sub>                  | 3                          | 135        | 46                     |
| 3               | Toluene            | AlCl <sub>3</sub>                  | 3                          | 135        | 28                     |
| 4               | DMSO               | AlCl <sub>3</sub>                  | 3                          | 135        | N.R.                   |
| 5               | DMF                | ZnCl <sub>2</sub>                  | 3                          | 135        | 81                     |
| 6               | DMF                | ZnBr <sub>2</sub>                  | 3                          | 135        | 70                     |
| 7               | DMF                | SnCl <sub>4</sub>                  | 3                          | 135        | 45                     |
| 8               | DMF                | TiCl <sub>4</sub>                  | 3                          | 135        | 33                     |
| 9               | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 3                          | 135        | 93                     |
| 10 <sup>c</sup> | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 3                          | 135        | 91                     |
| 11 <sup>d</sup> | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 3                          | 135        | 75                     |
| 12              | DMF                | —                                  | 3                          | 135        | Trace                  |
| 13 <sup>c</sup> | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 1                          | 135        | 48                     |
| 14 <sup>c</sup> | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 3                          | 100        | 92                     |
| 15 <sup>c</sup> | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 3                          | 70         | 80                     |
| 16 <sup>c</sup> | DMF                | BF <sub>3</sub> ·Et <sub>2</sub> O | 3                          | 25         | N.R.                   |

N.R. = no result. <sup>a</sup> Reaction conditions: **1a** (1 eq., 0.10 mmol), HC(OEt)<sub>3</sub> (3 eq., 0.30 mmol), Lewis acid (1 eq., 0.10 mmol), solvent (1 mL) under an Ar atmosphere for 5 h. <sup>b</sup> Isolated yields. <sup>c</sup> BF<sub>3</sub>·Et<sub>2</sub>O was used in 0.5 eq. <sup>d</sup> BF<sub>3</sub>·Et<sub>2</sub>O was used in 0.3 eq.

confirmed to be the optimal solvent and the desired product was obtained in 73% yield (entry 1). Considering the crucial role of Lewis acid in this transformation, different catalysts were checked subsequently (entries 5–9), and pleasingly, **2a** was generated in 93% yield when 1 equivalent BF<sub>3</sub>·Et<sub>2</sub>O was employed (entry 9). Inspired by this pleasing result, we tried to lower the amount of BF<sub>3</sub>·Et<sub>2</sub>O, and no obvious decrease in efficiency was observed when 0.5 equivalent BF<sub>3</sub>·Et<sub>2</sub>O was used (91%, entry 10). However, the yield reduced sharply on reducing the amount of BF<sub>3</sub>·Et<sub>2</sub>O further (75%, entry 11), and in the absence of Lewis acid merely trace **2a** was detected (entry 12). Meanwhile, a similar result was provided when we regulated the amount of HC(OEt)<sub>3</sub> (48%, entry 13). Lastly, we performed this reaction in different temperature gradients, and an excellent yield was provided at 100 °C (92%, entry 14), and no obvious effect was observed even at 70 °C (entry 15). However, this reaction was suppressed thoroughly at room temperature (entry 16).

Scheme 2 Investigation of substrate scope.<sup>a</sup> Standard conditions. <sup>b</sup> Reaction temperature was 135 °C. <sup>c</sup> CH<sub>2</sub>Cl<sub>2</sub> was used as the solvent, room temperature.

With the optimized conditions in hand, we investigated the scope of the reaction with regard to various substituents on the indole rings as well as the *ortho*-position of benzamides, and moderate to excellent yields were obtained in all cases (Scheme 2) (**2a–2t**, 60–94%). Different electron-donating substituents on the indole rings were well tolerated in this reaction (**2b**, 91%; **2c**, 93%). When an electron-withdrawing group was introduced the yield was reduced slightly (**2d**, 71%). Meanwhile, various aryl-substituted amidogens on the *ortho*-position of the benzamides provided the desired products in excellent yields (**2e**, 94%; **2f**, 90%). It is noteworthy that evodiamine **2g** was directly obtained within our protocol in 92% yield. Subsequently, various *ortho*-hydroxyl substituted benzamides were checked in this scenario (**1h–1q**), and electron-donating substituents showed no effects on the reaction while the electron-withdrawing ones gave a lower yield on the contrary (**2i–2k**, 60–75%), which is in accordance with the aforementioned results. Unexpectedly, diversified substituents on the *para*-position of *ortho*-hydroxyl substituted benzamides made no difference to the yields (**2l–2n**, 70–73%), no matter whether they were electron-donating or -withdrawing ones. Likewise, for the *meta*-substituted substrates, the same phenomenon was observed as the different electronic substituents on the indole rings (**2o**, 74%; **2p**, 60%). Lastly, three *ortho*-sulfhydryl benzamides were also tested in this reaction (**1r–1t**),

**Table 2** The cytotoxicity of the synthesized compounds on cancer cell lines and normal cell line ( $IC_{50}$ ,  $\mu M$ )<sup>a</sup>

| Compds     | CAL-27         | A549           | SMMC-7721      | WI-38          |
|------------|----------------|----------------|----------------|----------------|
| <b>2a</b>  | > 200          | 14.6 $\pm$ 2.9 | 12.6 $\pm$ 5.7 | 64.5 $\pm$ 7.8 |
| <b>2b</b>  | 75.1 $\pm$ 2.3 | 13.4 $\pm$ 0.9 | 5.7 $\pm$ 4.5  | 43.5 $\pm$ 5.0 |
| <b>2c</b>  | 9.9 $\pm$ 1.0  | > 200          | 12.9 $\pm$ 2.1 | > 200          |
| <b>2d</b>  | > 200          | 64.1 $\pm$ 1.8 | 16.8 $\pm$ 2.2 | 58.7 $\pm$ 2.0 |
| <b>2e</b>  | > 200          | 56.7 $\pm$ 2.8 | 43.3 $\pm$ 2.3 | 42.8 $\pm$ 5.7 |
| <b>2f</b>  | > 200          | 29.4 $\pm$ 3.2 | 9.2 $\pm$ 1.9  | 27.4 $\pm$ 2.1 |
| <b>2q</b>  | > 200          | > 200          | > 200          | 84.0 $\pm$ 3.6 |
| Vorinostat | 1.3 $\pm$ 0.3  | 3.1 $\pm$ 0.5  | 3.6 $\pm$ 0.5  | 10.2 $\pm$ 2.2 |

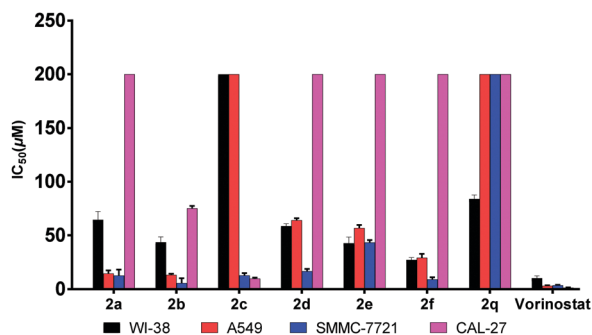
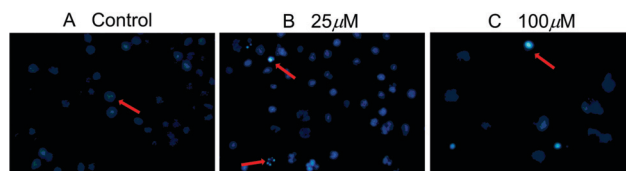
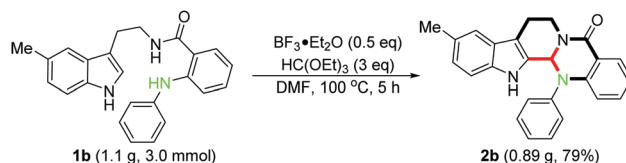
<sup>a</sup> All values are the mean  $\pm$  SEM ( $n = 3$ ).

since the stronger nucleophilicity of the sulphur atom compared with nitrogen and oxygen atoms enabled these reactions to be performed under milder conditions. Different electron-donating groups on the indole rings showed no effects on the yields (**2s**, 75%; **2t**, 81%).

According to previous studies,<sup>8</sup> since evodiamine analogues usually possess anti-tumor activity, these unreported compounds synthesized by us were evaluated for their cytotoxicity using the MTT method (Table 2 and Fig. 2). The results data showed that the compound **2b** exhibited higher activity than other compounds in the field of inhibiting proliferation of SMMC-7721 cells, and its  $IC_{50}$  value was 5.7  $\mu M$  which was close to the  $IC_{50}$  of vorinostat. Moreover, the selectivity of **2b** on both the tumor cell lines and normal cell line (WI-38) was also better than that of vorinostat.

The induced apoptosis in SMMC-7721 cells of compound **2b** was further confirmed by DAPI fluorescence after a 72 h treatment. Under a fluorescence microscope, the fluorescence of the cells in the control group was more diffuse and uniform, and the nucleus was obviously a regular ellipsoid shape (Fig. 3A). But when the cells were exposed to compound **2b** for 72 h, the nuclei were apparently damaged. Most cells showed condensed and fragmented nuclei (arrows) (Fig. 3B and C). This indicated that compound **2b** could induce significant cell apoptosis in SMMC-7721 cells. All data mentioned above suggests that these new compounds merit further research to identify promising features as antitumor candidates in the future.

To demonstrate the robust nature of this protocol, we performed a gram scale reaction using **1b** as the typical substrate, and the corresponding product **2b** was obtained in good yield (0.89 g, 79%), which could ensure a sufficient reagent supply in the following insightful bioactivity assessments (Scheme 3).

**Fig. 2** *In vitro* antitumor activity of the new compounds ( $IC_{50}$ ,  $\mu M$ ).**Fig. 3** Apoptosis of SMMC-7721 cells after compound **2b** treatment (200 $\times$ ). SMMC-7721 cells were incubated with compound **2b** for 72 h, and the nuclei were stained by DAPI.**Scheme 3** Gram scale experiment.

In summary, we have developed an efficient route to evodiamine and various heteroatom-containing analogues. In this transformation three chemical bonds and two fused rings were constructed within one step. The reaction showed good functional group tolerance, and all the expanded examples were obtained in good to excellent yields even on gram scales. Notably, the unreported evodiamine analogue **2b** synthesized in our protocol exhibited potent efficacy against SMMC-7721 ( $IC_{50} = 5.7 \mu M$ ). The fluorescence photomicrography results showed compound **2b** could particularly induce significant apoptosis in SMMC-7721 cells. More insightful bioactivity assays and applications of the reaction are in progress in our lab.

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## Conflicts of interest

The authors declare no competing financial interest.

## Notes and references

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