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# A Concise Synthesis and Biological Study of Evodiamine and Its Analogues

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**An 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, various heteroatom-contained Evodiamine analogues are obtained in moderate to excellent yields even on gram scale. Anti-tumor study *in vitro* demonstrates compound 2b possess potent efficacy against hepatoma cell line ($IC_{50}$=5.7 $\mu$M).**

Polycyclic heterocycles are crucial motifs widely exist in drugs and alkaloids,[1] many of them have shown superior bioactivity in anti-bacterials (Roquefortine C), anti-tumors (Camptothecin), cerebral vasodilatory (Vincoline) etc. according to the known reports (Figure 1).[2] Thus, making the rapid construction of polycyclic heterocycle skeletons is always the hotspot in synthetic chemistry.[3] Among them, Evodiamine as one of the most typical polycyclic heterocycles, the synthesis and modification of which have aroused broad interests in both synthetic and pharmaceutical industries because of its diverse biological activities.[4] In last decades,

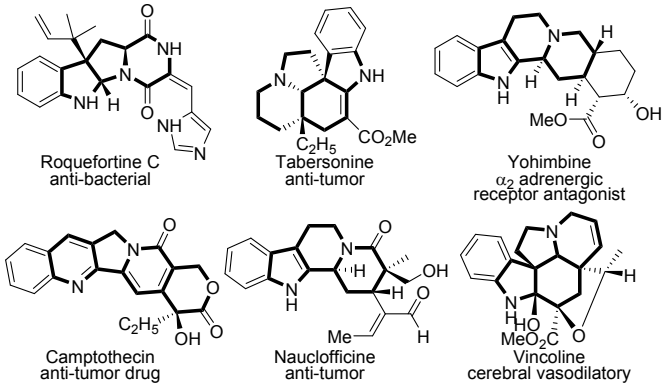
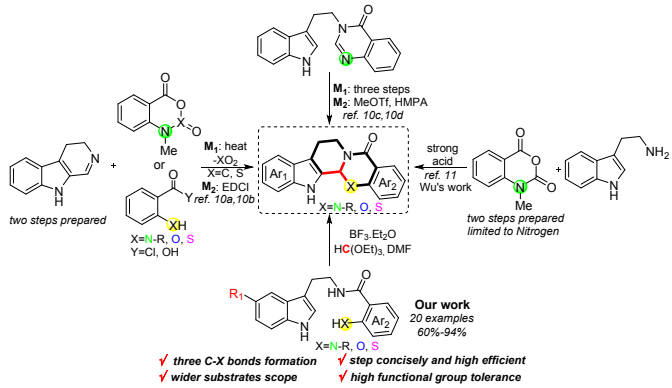



**Figure 1.** Polycyclic heterocycle skeletons in drugs and natural products.




**Scheme 1.** Approaches to Evodiamine and its analogues.

several groups have done elegant works in this domain, in 1997, Chen firstly employed Evodiamine for anti-inflammatory.[5] Subsequently, Kamiya demonstrated Evodiamine's efficacy in anti-obesity in 2001.[6] After that, Zhang successfully applied Evodiamine in transgenic mouse model for Alzheimer´s disease (AD) treatment.[7] Through chemical decorations, Sheng etc. discovered several Evodiamine analogues as potent anti-cancer drug candidates in recent years.[8] The structure–activity relationships of the evodiamine derivatives has been reported previously, in which $R_1$ and X follows the listed orders respectively. For $R_1$: OH > I > F > OMe > Br > Cl or Me; For X: 0, S > N-Me > CH > C=N. Meanwhile, when different positions of $Ar_2$ are substituted by different groups, the activity also varies.[8b,8d] Despite Evodiamine and its analogues having shown greatly potential benefits for human health, the approaches to these compounds are still limited.[9] Conventional methods to such kind of chemicals usually suffered from multi-step preparation and an imine intermediate had to be synthesized stepwise, along this process some toxic reagents as well as transition metals were also employed (Scheme 1).[10] In this context, the methodologies on how to prepare Evodiamine and its

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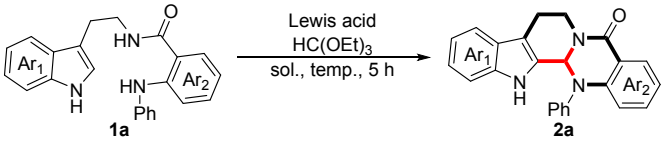
analogues concisely are highly desired in synthetic chemistry. Recently, Wu and co-workers developed an elegant work on one-pot synthesis of Evodiamine and its analogues, unfortunately, the substrates were merely constrained to N-methylisatoic anhydride accordingly.[11] Therefore, related works on one-step preparation of diversified heteroatom-contained Evodiamine analogues still remain blank. Herein, we report a concise access to Evodiamine and various heteroatom-contained Evodiamine analogues via Lewis acid catalysis, in this protocol, different fused heterocycles are efficiently constructed within one step.

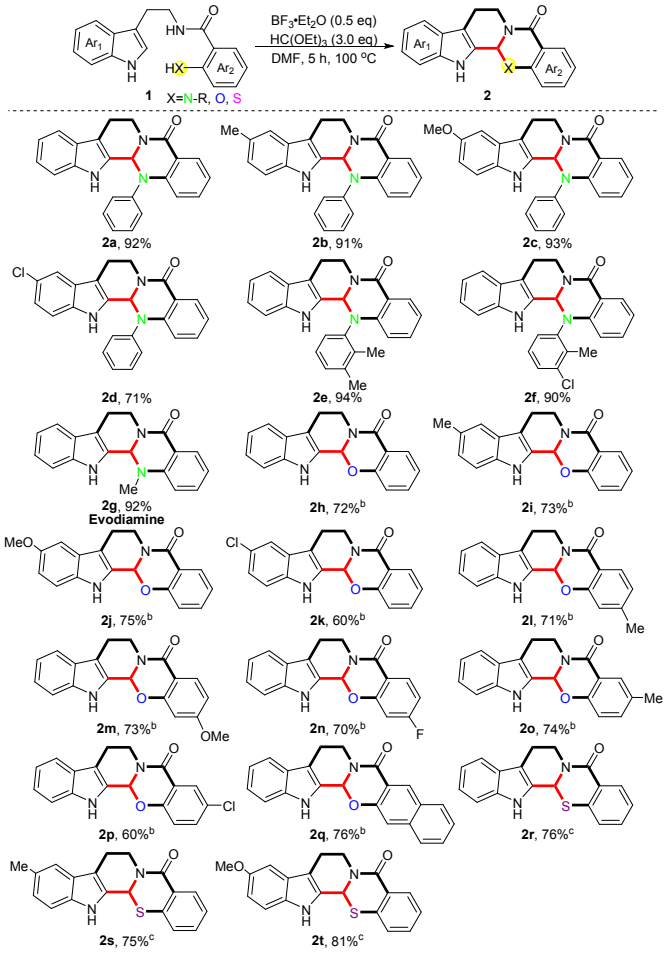



| Entry | Solvent | Lewis acid | $HC(OEt)_3$ (eq) | Temp. (°C) | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | DMF | $AlCl_3$ | 3 | 135 | 73 |
| 2 | $CH_3CN$ | $AlCl_3$ | 3 | 135 | 46 |
| 3 | Toluene | $AlCl_3$ | 3 | 135 | 28 |
| 4 | DMSO | $AlCl_3$ | 3 | 135 | N.R. |
| 5 | DMF | $ZnCl_2$ | 3 | 135 | 81 |
| 6 | DMF | $ZnBr_2$ | 3 | 135 | 70 |
| 7 | DMF | $SnCl_4$ | 3 | 135 | 45 |
| 8 | DMF | $TiCl_4$ | 3 | 135 | 33 |
| 9 | DMF | $BF_3•Et_2O$ | 3 | 135 | 93 |
| 10[c] | DMF | $BF_3•Et_2O$ | 3 | 135 | 91 |
| 11[d] | DMF | $BF_3•Et_2O$ | 3 | 135 | 75 |
| 12 | DMF | / | 3 | 135 | trace |
| 13[c] | DMF | $BF_3•Et_2O$ | 1 | 135 | 48 |
| 14[c] | DMF | $BF_3•Et_2O$ | 3 | 100 | 92 |
| 15[c] | DMF | $BF_3•Et_2O$ | 3 | 70 | 80 |
| 16[c] | DMF | $BF_3•Et_2O$ | 3 | 25 | N.R. |

**Table 1.** Optimization of reaction conditions.[a] [a]Reaction conditions: **1a** (1 eq, 0.10 mmol), $HC(OEt)_3$ (3 eq, 0.30 mmol), Lewis acid (1 eq, 0.10 mmol), solvent (1 mL) under Ar atmosphere for 5h. [b]Isolated yields. [c]$BF_3•Et_2O$ was used in 0.5 eq. [d]$BF_3•Et_2O$ was used in 0.3 eq. N.R.=no result.

We initiated this study with **1a** as the model substrate, and choosing $HC(OEt)_3$ as the carbon source with Lewis acid $AlCl_3$ as the catalyst. After screening of various solvents, DMF was confirmed to the optimal solvent and the desired product was obtained in 73% yield (entry 1). Considering the curial role of Lewis acid in this transformation, different catalysts were checked subsequently (entry 5-9), delightedly, **2a** was generated in 93% when 1 equivalent $BF_3•Et_2O$ was employed (entry 9). Inspired by this delighting result, we tried to lower the amount of $BF_3•Et_2O$, and no obviously decreased efficiency was observed when 0.5 equivalent $BF_3•Et_2O$ used (91%, entry 10). While the yield reduced sharply accompanying with reduced amount of $BF_3•Et_2O$ further (75%, entry 11), in the absence of Lewis acid merely trace of **2a** was detected (entry 12). Meanwhile, similar result was provided when we regulated the amount of $HC(OEt)_3$ (48%, entry 13). Lastly, we preformed this reaction in different temperature gradients, and excellent yield was provided at 100 °C (92%, entry 14), and no obvious effect was observed even at 70 °C (entry 15). Whereas, this reaction was suppressed thoroughly at room temperature (entry 16).

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 the cases (**2a**-**2t**, 60%-94%). Different electron-donating substituents on




**Scheme 2.** Investigation of substrates scope.[a] [a]Standard conditions. [b]Reaction temperature was 135°C. [c]$CH_2Cl_2$ was used as the solvent, room temperature.

the indole rings were well tolerant in this reaction (**2b**, 91%; **2c**, 93%), when electron-withdrawing group 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%). Noteworthily, Evodiamine **2g** was directly obtained within our protocol in 92% yield. Subsequently, various *ortho*-hydroxyl substituted benzamides were checked in this scenario (**1h**-**1q**), electron-donating substituents showed no effects on the reaction while the electron-withdrawing one gave a lower yield on the contrary (**2i**-**2k**, 60%-75%), which in accordance with the aforementioned results. Unexpectedly, diversified substituents on the *para*-position of *ortho*-hydroxyl substituted benzamides made no difference on the yields (**2l**-**2n**, 70%-73%), no matter for the electron-donating or -withdrawing ones. Likewise, as for the *meta*-substituted substrates, the same phenomenon was

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

**Table 2.** The cytotoxicity of the synthesized compounds on cancer cell lines and normal cell line ($IC_{50}$, $\mu$M).[a] [a]All values are the mean ± SEM (n =3).

observed as the different electronical substituents on the indole rings (**2o**, 74%; **2p**, 60%). Lastly, three *ortho*-sulfydryl benzamides were also tested in this reaction (**1r**-**1t**), since stronger nucleophilicity of sulphur atom compared with Nitrogen and Oxygen atom which enabled these reactions to be performed under milder conditions. And different electron-donating groups on the indole rings showed no effects on the yields (**2s**, 75%; **2t**, 81%).

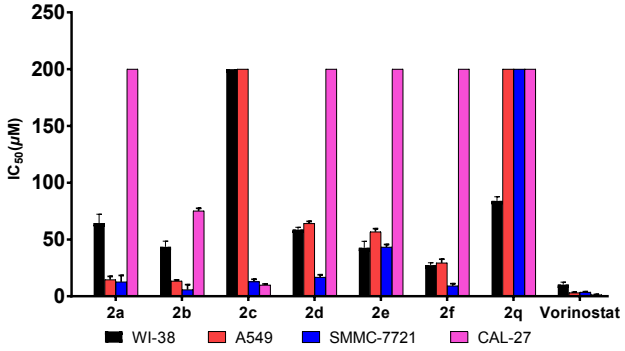



**Figure 2.** In Vitro Antitumor Activity of the new compounds ($IC_{50}$, $\mu$M)

According to previous studies,[8] since Evodiamine analogues usually possess anti-tumor activity, these unreported compounds synthesized by us were evaluated for their cytotoxicity using MTT method (table 2, figure 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 were close to $IC_{50}$ of Vorinostat. Moreover, the selectivity of **2b** on both the tumor cell lines and normal cell line (WI-38) were also better than that of Vorinostat.

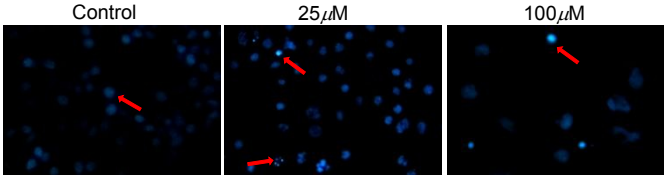
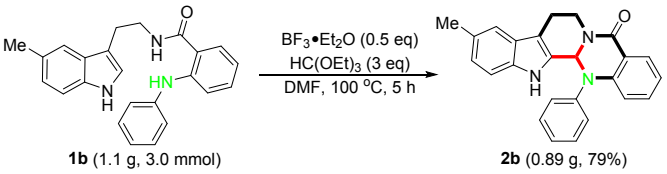



**Figure 3.** Apoptosis of SMMC-7721 cells after compound **2b** treatment (200×). SMMC-7721 cells were incubated with compound **2b** for 72 h, and the nuclei were stained by DAPI.

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




**1b** (1.1 g, 3.0 mmol) **2b** (0.89 g, 79%)

**Scheme 3.** Gram scale experiment.

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 sufficient reagent supplement in the following insightful bioactivity assessments.

In summary, we have developed an efficient access to Evodiamine and various heteroatom-contained 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 photomicrograph results showed compound **2b** could particularly induce a significant apoptosis in SMMC-7721 cells. More insightful bioactivity assays and applications of the reaction are under progress in our lab.

Dedicated to Lanzhou University for her 110th birthday. We thank Prof. Quan-Yi Zhao and Prof. Dian He for helpful discussions. Financial support was provided by the Recruitment Program of Global Experts (1000 Talents Plan).

## Conflicts of interest

The authors declare no competing financial interest.

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