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One-Pot Synthesis of Luotonin A and Its Analogues

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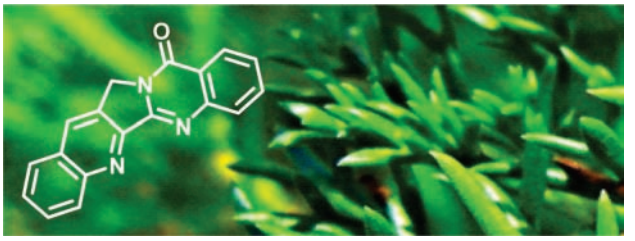
ABSTRACT



Starting with inexpensive reagents, a self-directed chemical process with the aid of a single metal triflate was readily achieved to concomitantly construct quinazoline and pyrroloquinoline cores to afford the synthesis of luotonin A and its analogues. Among all compounds prepared, 2c, 2d, and 3b exhibit more potent inhibitory activity than luotonin A against human topoisomerase I.

This paper reports the synthesis of luotonin A and its analogues in one pot. Many naturally occurring products have quinoline and/or quinazoline rings embedded in their structures, and luotonin A (**1**) carries both structural motifs (Figure 1).[1] Our group recently reported the application of metal triflates for use in the synthesis of natural and unnatural quinazoline containing alkaloids such as asperlicin C, circumdatin F, and sclerotigenin.[2] We were interested in further showcasing this Lewis acid catalyzed cyclization reaction for other natural product syntheses, particularly in an intramolecular fashion, and, for exactly this reason, specifically targeted luotonin A. Here, we report a synthetic strategy for the simultaneous construction of quinazoline and pyrroloquinoline cores (BCD rings) applied to the total synthesis of **1**. This concomitant formation of three rings leading to luotonin A preparation in one pot, to our knowledge, has not been reported in the literature and may potentially be useful for the synthesis of other complex natural products.

Luotonin A is a cytotoxic alkaloid first isolated in 1997 from the plant *Peganum nigellastrum*.[3] This plant has a history of use in Chinese medicine for the treatment of rheumatism and inflammation. Being experimentally demonstrated as a human DNA topoisomerase I (hTopI) poison to stabilize a DNA/enzyme binary complex using the same mechanism of action as camptothecin (CPT),[4] **1** displays useful cytotoxicity *in vitro* against the murine

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(1) For recent reviews, see: (a) Michael, J. P. *Nat. Prod. Rep.* **2008**, *25*, 166. (b) Michael, J. P. *Nat. Prod. Rep.* **2007**, *24*, 223. (c) Mhaske, S. B.; Argade, N. P. *Tetrahedron* **2006**, *62*, 9787. (d) Connolly, D. J.; Cusack, D.; O'Sullivan, T. P.; Guiry, P. J. *Tetrahedron* **2005**, *61*, 10153.

(2) (a) Tseng, M.-C.; Yang, H.-Y.; Chu, Y.-H. *Org. Biomol. Chem.* **2010**, *8*, 419. (b) Tseng, M.-C.; Lai, C.-Y.; Chu, Y.-W.; Chu, Y.-H. *Chem. Commun.* **2009**, 445. (c) Tseng, M.-C.; Chu, Y.-H. *Tetrahedron* **2008**, *64*, 9515.

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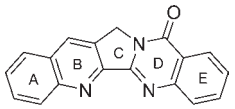
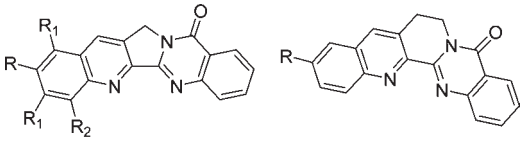


luotonin A (**1**)

Figure 1. Structure of luotonin A (**1**).



2a; R = OCH_3, R_1, R_2 = H
2b; R = CH_3, R_1, R_2 = H
2c; R = Br, R_1, R_2 = H
2d; R = Cl, R_1, R_2 = H
2e; R, R_1 = H, R_2 = CH_3
2f; R = H, R_1 = CH_3, R_2 = H

3a; R = H
3b; R = OCH_3
3c; R = CH_3
3d; R = Br
3e; R = Cl

Figure 2. Structures of luotonin A analogues (**2a**−**f**) and C-ring expanded analogues (**3a**−**e**).

leukemia P-388 cell line with an IC_{50} value of 6.3 μM. Albeit it is not potent enough for cancer chemotheraphy, luotonin A is nevertheless a lead compound and has been the subject of a number of total syntheses and bioactivity investigations.[5,6] Many of the syntheses of **1** however involved lengthy synthetic procedures, harsh experimental conditions, long reaction hours, and low overall yields.[7] Among the syntheses reported, most completed **1** with the convergent construction of one or two rings (B, C, or D) from fragment compounds. Thus, further development of a practical and efficient, preferably one-pot, synthesis of **1** and its derivatives (**2a**−**f** and **3a**−**e**) would be of immense value for SAR study (Figure 2).

Scheme 1. One-Pot Synthesis of Luotonin A (**1**)

By retrosynthetic analysis of **1**, a simultaneous construction of BCD rings by quinazolinone formation (D ring) and an intramolecular aza-Diels−Alder (BC rings) reaction can in principle be achieved with the highest efficiency. Since both reactions were known to be catalyzed by Lewis acids,[2,8] we envisaged that a self-directed sequence of reactions—(i) imine formation, (ii) quinazolinone formation, (iii) intramolecular aza-Diels−Alder reaction, and finally (iv) dehydrogenation and (v) aromatization—activated and catalyzed by metal triflates could concomitantly form the desired structure **1**.

Scheme 1 illustrates our initial success of luotonin A synthesis of which the BCD rings were assembled from the appropriate aniline, a propargyl unit, and the glyoxal. The proposed reaction sequence did concomitantly occur in a one-step fashion under our experimental conditions. This total synthesis of **1** started from the commercial, inexpensive isatoic anhydride **4**. Upon reacting first with propargylamine in DMF at ambient temperature for 1 h, **4** was readily converted to a 2-aminobenzamide **5** in nearly quantitative yield.[9] After removing the solvent and excessive amine *in vacuo*, reflux of this 2-aminobenzamide **5** in *o*-xylene with aniline (10 equiv), $Yb(OTf)_3$ (20 mol %), and 40% aqueous glyoxal (15 equiv) for 12 h furnished the fluorescent product **1** with 35% isolated yield after column chromatography. The selection of 20 mol % $Yb(OTf)_3$ was based upon the screening optimization of 11 metal triflates available in our laboratory (Table S1, Supporting Information). This sequence of reactions was remarkably facile, and because of the air and water stability of the catalyst, the reaction required no special precautions. Of the metal triflates investigated, all of them catalyzed the reaction under the same experimental conditions; however, the isolated yields (14−26%) from other catalysts were inferior to that with $Yb(OTf)_3$ (Table S1, Supporting Information). Albeit $In(OTf)_3$ was known to be a superior Lewis acid for the hetero Diels−Alder reactions,[10] in our hands it

(4) Cagir, A.; Jones, S. H.; Gao, R.; Eisenhauer, B. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 13628.

(5) For a recent review on the synthesis and biological activity of luotonin A and related derivatives, see: Ma, Z.; Hano, Y.; Nomura, T. *Heterocycles* **2005**, *65*, 2203.

(6) Since its discovery in 1997, there have been 74 publications (SciFinder on January 7, 2011) on the synthesis and biological applications of luotonin A.

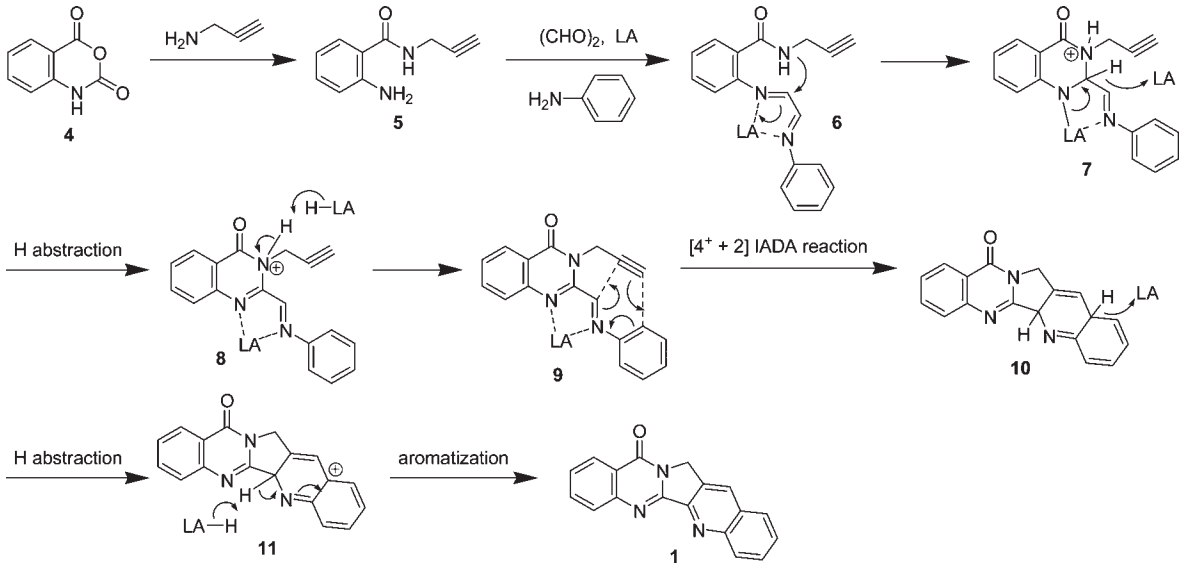
(7) For recent studies of luotonin A syntheses, see: (a) Ju, Y.; Lu, F., F.; Li, C. *Org. Lett.* **2009**, *11*, 3582. (b) Liang, Y.; Jiang, X.; Yu, Z.-X. *Org. Lett.* **2009**, *11*, 5302. (c) Sridharan, V.; Ribelles, P.; Ramos, M. T.; Menendez, J. C. *J. Org. Chem.* **2009**, *74*, 5715. (d) Nacro, K.; Zha, C.; Guzzo, P. R.; Herr, R. J.; Peace, D.; Friedrich, T. D. *Bioorg. Med. Chem.* **2007**, *15*, 4237. (e) Zhou, H.-B.; Liu, G.-S.; Yao, Z.-J. *J. Org. Chem.* **2007**, *72*, 6270. (f) Twin, H.; Batey, R. A. *Org. Lett.* **2004**, *6*, 4913. (g) Mhaske, S. B.; Argade, N. P. *J. Org. Chem.* **2004**, *69*, 4563. (h) Dallavalle, S.; Merlini, L.; Beretta, G. L.; Tinelli, S.; Zunino, F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5757. (i) Cagir, A.; Jones, S. H.; Eisenhauer, B. M.; Gao, R.; Hecht, S. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2051. (j) Cagir, A.; Eisenhauer, B. M.; Gao, R.; Thomas, S. J.; Hecht, S. M. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 6287. (k) Chavan, S. P.; Sivappa, R. *Tetrahedron* **2004**, *60*, 9931. (l) Harayama, T.; Hori, A.; Serban, G.; Morikami, Y.; Matsumoto, T.; Abe, H.; Takeuchi, Y. *Tetrahedron* **2004**, *60*, 10645. (m) Toyota, M.; Komori, C.; Ihara, M. *ARKIVOC* **2003**, 15. (n) Lee, E. S.; Park, J.-G.; Jahng, Y. *Tetrahedron Lett.* **2003**, *44*, 1883. (o) Osborne, D.; Stevenson, P. J. *Tetrahedron Lett.* **2002**, *43*, 5469. (p) Yadav, J. S.; Reddy, B. V. S. *Tetrahedron Lett.* **2002**, *43*, 1905. (q) Dallavalle, S.; Merlini, L. *Tetrahedron Lett.* **2002**, *43*, 1835. (r) Toyota, M.; Komori, C.; Ihara, M. *Heterocycles* **2002**, *56*, 101. (s) Ma, Z.-Z.; Hano, Y.; Nomura, T.; Chen, Y.-J. *Heterocycles* **1999**, *51*, 1593. (t) Kelly, T. R.; Chamberland, S.; Silva, R. A. *Tetrahedron Lett.* **1999**, *40*, 2723. (u) Wang, H.; Ganesan, A. *Tetrahedron Lett.* **1998**, *39*, 9097.

(8) (a) Kouznetsov, V. V. *Tetrahedron* **2009**, *65*, 2721. (b) Buonora, P.; Olsen, J.-C.; Oh, T. *Tetrahedron* **2001**, *57*, 6099.

(9) The 2-aminobenzamide **4** intermediate could be isolated in 97% yield, when needed.

(10) Ali, T.; Chauhan, K. K.; Frost, C. G. *Tetrahedron Lett.* **1999**, *40*, 5621.

Scheme 2. Possible Mechanism for the Synthesis of Luotonin A (**1**)[a]



[a] LA, Lewis acid; IADA, intramolecular aza-Diels–Alder.

catalyzed to produce **1** only with 24% isolated yield. These results might be ascribed to the just right Lewis acidity of $Yb(OTf)_3$ for the cascade reactions. More $Yb(OTf)_3$ did not improve the yield of **1** (Table S1, Supporting Information). If this reaction was performed at ambient temperature, no trace of **1** was observed.

While insufficient mechanistic data exist at present, a plausible mechanism for the synthesis of **1** is presented in Scheme 2. First, propargylamine reacted with isatoic anhydride **4** to form an isolable intermediate **5** followed by the Lewis acid mediated formation of the imine **6**, concomitantly leading to a ring-closed product **7**. Subsequently, a dehydrogenation of **7**→**8**→**9** forms the stable D ring in **9**, and later, an aromatization of **10**→**11**→**1** could readily proceed through Lewis acid catalyzed H-abstraction and deprotonation of the intermediary cyclohexadienyl cation. This aromatization of cyclohexadiene **10** and related hydrocarbons promoted by Lewis acids is well documented in literature.[11] As one of the key steps in this proposed mechanism, the process of **9**→**10** could be rationalized (elegantly proposed by Yu et al.[7b]) using a Lewis acid catalyzed, inverse electron-demand aza-Diels–Alder [$4^+ + 2$] cycloaddition reaction in the intramolecular fashion (IADA) between *N*-chelated *N*-phenyliminium azadiene and the electron-rich alkyne dienophile in **9**.

The success of the preparation of **1** using the $Yb(OTf)_3$ catalyst prompted us to further pursue utilizing this one-pot synthesis for other target compounds with greater diversity (Tables S2 and S3, Supporting Information). Previous studies on luotonin A derivatives were focused on the introduction of substituents on ring E.[12]

The results of the activity assay showed that E-ring substituents contributed to the stabilization of, but were not essential for targeting, the enzyme–DNA binary complex.[12] In this work, we focused on the synthesis of the A- and C-ring analogues of **1**, using readily available substituted anilines and but-3-yn-1-amine. Results show that, regardless of the nature of substituents, target compounds **2a**–**f** and **3a**–**e** were all readily synthesized in one-pot from their corresponding reagents (Tables S2 and S3, Supporting Information). In our hands, compounds **2d** and **3b** gave the highest (40%) and lowest (17%) isolated yields, respectively. It is worth noting that compounds **3a** and **3b** could be considered as the B ring-expanded analogues of natural products rutaecarpine and hortiacine, respectively.[13] The successful application of our one-pot protocol to access fused pyrrolo- and pyridoquinazolinoquinolines demonstrates the utility of this new method.

All compounds synthesized in this work were assayed for biological activities (Figure 3). In literature, only limited numbers of luotonin A derivatives with substituents on ring A and C were reported and showed promising biological activities.[14] Inhibitory activities of compounds **1**, **2a**–**f**, and **3a-e** to hTopI were determined by assaying the relaxation of a supercoiled pBlueScript SK+ plasmid DNA (for experiments, see Supporting Information).[15]

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(13) For a recent review, see: Lee, S. H.; Son, J.-K.; Jeong, B. S.; Jeong, T.-C.; Chang, H. W.; Lee, E.-S.; Jahng, Y. *Molecules* **2009**, *13*, 272.

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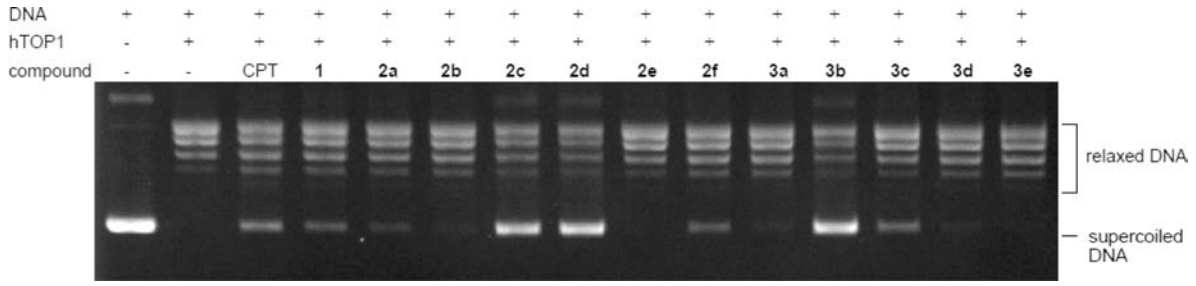

Figure 3. Analysis of hTop1 inhibition by luotonin A (**1**) and its analogues (**2a**−**f**, **3a**−**e**). For experimental conditions, see the Supporting Information.

As shown in Figure 3, our preliminary result demonstrated that, to our delight, compounds **2c**, **2d**, and **3b** exhibited more potent inhibitory activities than luotonin A (**1**) and the control CPT against hTopI: 48%, 54%, 54%, 18%, and 25% inhibition, respectively, at a concentration of 5 μM. It is also noted that compounds **2e** and **3e** did not show any inhibitory activity against hTopI. This preliminary data on the SAR study clearly indicated that compounds **2d** and **3b** at 5 μM possess a 2.2 times stronger inhibitory activity on hTop1 compared to CPT (5 μM); the result shown in Figure 3 likely could serve as a useful starting point for hit identification in inhibitor discovery. Using the convenient one-pot method developed in this work, a combinatorial approach on the design and synthesis of luotonin A templated libraries is in progress and the results on the identification of potent inhibitors will be reported in due course.

In summary, we have developed a one-pot protocol for the synthesis of luotonin A (**1**) and its analogues (**2a**−**f** and **3a**−**e**) under Lewis acid catalysis. Our method avoids the need for harsh basic or acidic conditions and allows concomitant construction of multiple rings (B, C, and D). This is the shortest synthesis for luotonin A. Compared with elegant syntheses previously developed for luotonin A (**1**) by research groups of Batey[7f] (10% yield in eight steps) and Yao[7e] (47% yield in five steps), our approach presents the advantages of a one-pot route, moderate but acceptable isolated yield (especially for the key concomitant three-ring cyclization step), and no need for the isolation and purification of any intermediates. The chemistry presented in this work efficiently achieves the synthesis of the luotonin A natural product as well as the preparation of its analogues and should contribute to the SAR study in this family of antitumor agents to further identify luotonin A analogues with more potent anticancer activity.

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Supporting Information Available. Tables S1−S3, experimental conditions and procedures, ^{1}H and ^{13}C NMR data and spectra of all compounds **1**, **2a**−**f**, and **3a**−**e**. This material is available free of charge via the Internet at http://pubs.acs.org.