ORGANIC LETTERS

2010
Vol. 12, No. 7
1416−1419

Antitumor Agents. 274. A New Synthetic Strategy for E-Ring SAR Study of Antofine and Cryptopleurine Analogues

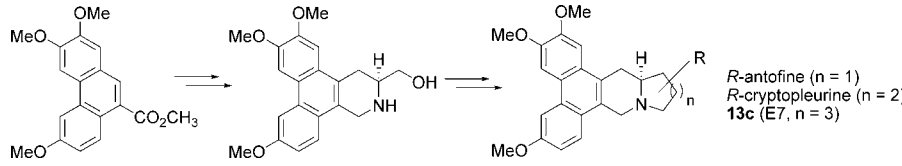
Xiaoming Yang,[†] Qian Shi,*[,†] Kenneth F. Bastow,[‡] and Kuo-Hsiung Lee*[,†]

Natural Products Research Laboratories and Division of Medicinal Chemistry and Natural Products, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7568

qshi1@email.unc.edu; khlee@unc.edu

Received December 7, 2009

ABSTRACT



A new versatile synthetic methodology for the synthesis of enantiomerically pure natural phenanthroindolizidines and phenanthroquinolizidines has been established and described. Natural products *R*-antofine and *R*-cryptopleurine, as well as a novel E-ring expanded analogue 13c (E7), 12-oxo-*S*-antofine (17), and 12*N*-methyl-12-aza-*S*-antofine (18) were synthesized with the new method. This strategy will greatly facilitate future SAR studies on the natural alkaloids with E-ring variations.

Phenanthroindolizidines and phenanthroquinolizidines are two series of natural alkaloids primarily found in the Asclepiadaceae and Moracea plant families. The leaves of these plants have been used since ancient times to treat asthma, bronchitis, rheumatism, etc.[1] To date, over 60 compounds have been isolated and characterized, such as *R*-tylophorine, *R*-antofine, and *R*-cryptopleurine (Figure 1), which are well-known representatives reported to have potent antitumor activity. Due to low natural abundance and interesting anticancer activity, total synthesis has attracted much attention to assist the development of both the natural compounds and new analogues.

To date, numerous synthetic strategies have been reported in the literature.[2] One representative strategy, first reported by Rapoport and co-worker, used building blocks such as proline, glutamic acid, aminoadipate, and pyroglutamate as the sources of the chiral center followed by intramolecular

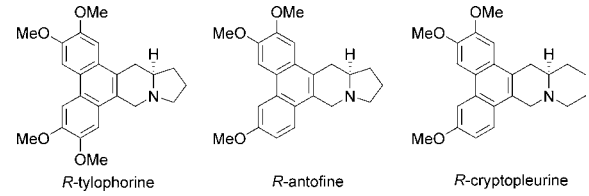


Figure 1. Representative structures of phenanthroindolizidines and phenanthroquinolizidines.

electrophilic addition.[3−7] Other strategies include a chiral auxiliary approach,[8] chiral allylic alcohol,[9] enantioselective catalysis for intramolecular alkene carboamination,[10] and enantioselective phase-transfer alkylation;[11] some of

[†] Natural Products Research Laboratories.
[‡] Division of Medicinal Chemistry and Natural Products.

(1) Gellert, E. *J. Nat. Prod.* **1982**, *45*, 50–73.
(2) For reviews, see: (a) Li, Z.; Jin, Z.; Huang, R. *Synthesis* **2001**, *16*, 2365–2378. (b) Chemler, S. R. *Curr. Bioact. Compd.* **2009**, *5*, 2.
(3) Buckley, T. F.; Rapoport, H. *J. Org. Chem.* **1983**, *48*, 4222–4232.
(4) Nordlander, J. E.; Njoroge, F. G. *J. Org. Chem.* **1987**, *52*, 1627–1630.
(5) Furstner, A.; Kennedy, J. W. J. *Chem.—Eur. J.* **2006**, *12*, 7398–7410.
(6) Jin, Z.; Li, S. P.; Wang, Q. M.; Huang, R. Q. *Chin. Chem. Lett.* **2004**, *15*, 1164.
(7) Faber, L.; Wiegrebe, W. *Helv. Chim. Acta* **1976**, *59*, 2201–2212.

10.1021/ol902819j
Published on Web 03/02/2010

them have also been used in the synthesis of seco structures.

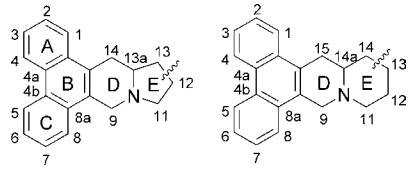


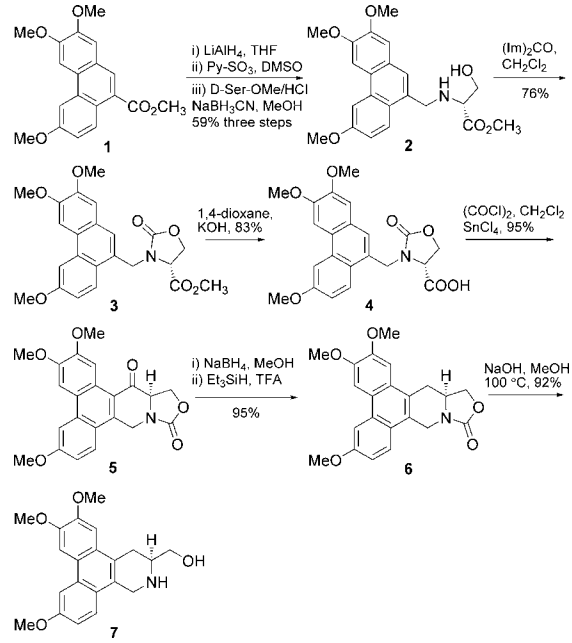
Figure 2. Ring and position numbering of phenanthroindozlidines and phenanthroquinolizidines.

It has been reported that a major side effect prohibiting the therapeutic use of these natural alkaloids is their CNS toxicity, such as disorientation and ataxia.[12] Analogues with higher polarity may be desirable to ameliorate such side effects by preventing the compounds from crossing the blood−brain barrier. However, only a few polar antofine analogues with a C-14 OH group have been synthesized.[13] No E-ring substituted analogues (at C11−C14) have been reported to date, despite the large pool of approaches presently available. As a result, current SAR study is still in a premature stage, and additional phenanthroindolizidine and phenanthroquinolizidine analogues with diverse structural features, especially polar functionalities on the E-ring, are urgently needed for a more extensive study of the biological properties.

In this paper, we report the design and synthesis of a key intermediate **7** that should prove to be a versatile precursor to a series of interesting E-ring modified analogues (Figure 2). Possible modifications include incorporation of heteroatoms, introduction of polar groups such as hydroxy and amino groups, and addition of multiple substitutions, all of which are not readily accessible by reported synthetic methods. Two natural products, *R*-antofine and *R*-cryptopleurine, were synthesized to verify the feasibility of this new strategy, and three new compounds, 7-membered analogue E7 (**13c**), 12-oxo-*S*-antofine (**17**), as well as 12*N*-methyl-12-aza-*S*-antofine (**18**) were synthesized for the first time to further corroborate the versatility of this method.

In our innovative approach, the E-ring is constructed after the D-ring has been conjugated. This synthetic strategy has the following merits: the size of the E-ring can be easily altered via ring-closing metathesis and various functional groups can be readily introduced during E-ring formation and subsequent reactions. The resulting new analogues would provide useful SAR information about the alkaloid E-ring. In contrast to Rapoport's lactam moiety, another novelty in our strategy is the use of an oxazolidinone moiety, which not only allows efficient formation of the D-ring but also is easily cleaved to provide the useful synthetic precursor **7**.

Scheme 1. Synthesis of the Key Intermediate **7**



As shown in Scheme 1, compound **1** was obtained via three steps as reported in the literature in 45% yield.[14] Subsequent reduction with $LiAlH_4$ was followed by oxidization with Py•SO_3/DMSO to yield an aldehyde, which was reductively aminated by using D-serine methyl ester hydrochloride to give **2** in an overall yield of 59% (three steps). Construction of the oxazolidinone ring system was accomplished by reaction of **2** with Im_2CO in CH_2Cl_2 to afford **3** in 76% yield. The D-ring was formed by acylation of acid **4** to yield **5** in 79% yield. Compound **6** was obtained from **5** by reduction of ketone to methylene in two steps. Refluxing **6** with 6 N NaOH (aq) in MeOH successfully cleaved the oxazolidinone to give the key intermediate **7** in 95% yield, from which a series of interesting modifications could be achieved.

E7 (**13c**) was first synthesized along with two natural products, *R*-antofine (**13a**) and *R*-cryptopleurine (**13b**), from **7** as described below. The amino group was protected with a Boc group to furnish **8** in 96% yield (Scheme 2). The hydroxy group was then oxidized by Py•SO_3 to give an aldehyde, which was converted to an alkene **9** by Wittig

(8) (a) Comins, D. L.; Chen, X.; Morgan, L. A. *J. Org. Chem.* **1997**, *62*, 7435–7438. (b) Ihara, M.; Takino, Y.; Tomotake, M.; Fukumoto, K. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2287–2292. (c) Suzuki, H.; Aoyagi, S.; Kibayashi, C. *J. Org. Chem.* **1995**, *60*, 6114–6122.

(9) Kim, S.; Lee, T.; Lee, E.; Lee, J.; Fan, G. J.; Lee, S. K.; Lee, D. *J. Org. Chem.* **2004**, *69*, 3144–3149.

(10) Zeng, W.; Chemler, S. R. *J. Org. Chem.* **2008**, *73*, 6045–6047.

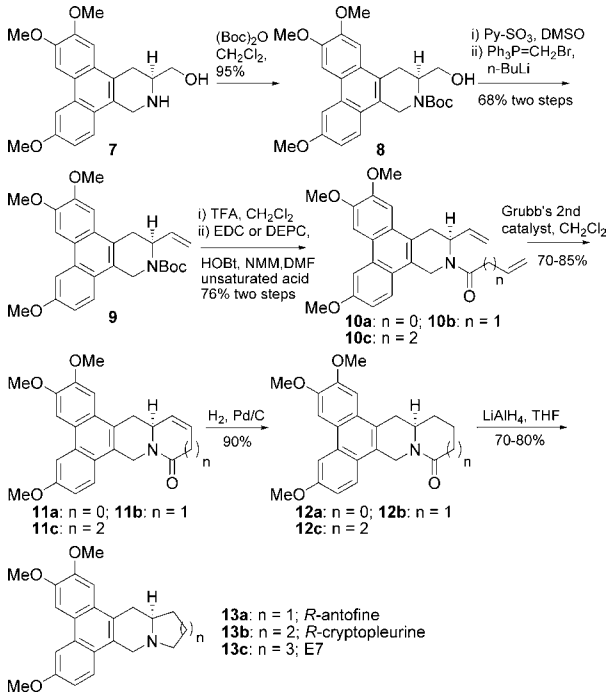
(11) Kim, S. H.; Lee, J.; Lee, T.; Park, H. G.; Kim, D. *Org. Lett.* **2003**, *5*, 2703–2706.

(12) Suffness, M.; Douros, J. *Anticancer Agents Based on Natural Product Models*; Academic Press: New York, 1980; pp 465−487.

(13) (a) Gao, W.; Busson, S.; Grill, S. P.; Gullen, E. A.; Hu, Y. C.; Huang, X.; Zhong, S.; kaczmarek, C.; Gutierrez, J.; Francis, S.; Baker, D. C.; Yu, S.; Cheng, Y. C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4338–4342. (b) Gao, W.; Lam, W.; Zhong, S.; kaczmarek, C.; Baker, D. C.; Cheng, Y. C. *Cancer Res.* **2004**, *64*, 678–688.

(14) Su, C. R.; Damu, A. G.; Chiang, P. C.; Bastow, K. F.; Morris-Natschke, S. L.; Lee, K. H.; Wu, T. S. *Bioorg. Med. Chem.* **2008**, *16*, 6233–6241.

Scheme 2. Synthesis of Antofine, Cryptopleurine, and E7

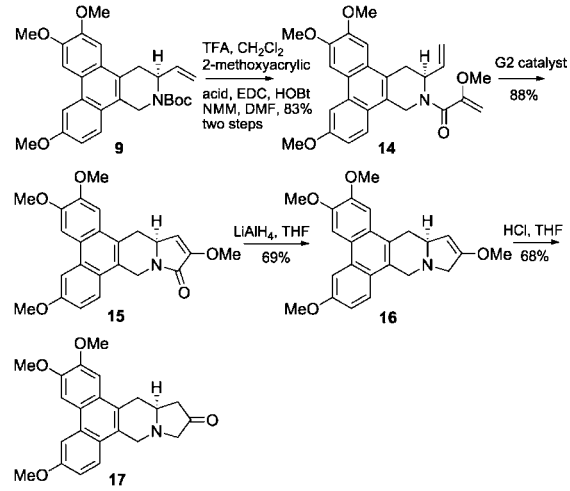


reagent in 68% yield over two steps. After the Boc group was removed, an appropriate unsaturated acid (e.g., **10a**, acrylic acid; **10b**, 3-butenoic acid; **10c**, 4-pentenoic acid) was introduced to afford **10** in an average yield of 80% using EDC or DEPC (for **10b**, double-bond isomerization occurred on the *N*-amide side chain using EDC/HOBt). Cycloalkene analogues (**11a**−**c**) were obtained by cyclization with Grubb's second-generation (G2) catalyst and then underwent hydrogenation with H_2/Pd/C in MeOH to afford **12a**−**c** in high yields. The desired target alkaloids *R*-antofine (**13a**) and *R*-cryptopleurine (**13b**) as well as an E-ring expanded analogue (**13c**) were obtained by reduction of amide to amine in an average yield of 70%. The analytical information obtained from our synthesized **13a** and **13b** agreed with the data reported in the literature.[4,9]

The versatility and usefulness of this strategy were further demonstrated by the synthesis of two new analogues: 12-oxo-*S*-antofine (**17**, Scheme 3) and 12*N*-methyl-12-aza-*S*-antofine (**18**, Scheme 4). Compounds **17** and **18** contain a ketone and an additional nitrogen, respectively, in the E-ring.

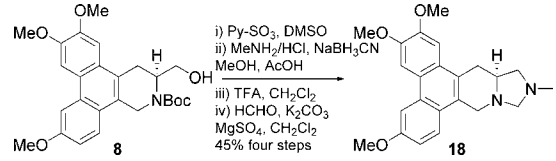
Compound **14** was obtained by reacting **9** with 2-methoxyacrylic acid after removal of the Boc group in 83% yield. The ring closure was accomplished using G2 catalyst to give compound **15** in 88% yield. Subsequent reduction by $LiAlH_4$ in THF and hydrolysis with HCl afforded **17** in 50% yield over two steps to generate a carbonyl functionality at C12 position. For the synthesis of compound **18**, the alcohol **8** was oxidized to the aldehyde, which underwent reductive amination to give an intermediate amine. After removal of the Boc group, both nitrogens were connected by HCHO to produce **18** in 45% yield over four steps. By virtue of similar strategies, we are able to synthesize a series of cryptopleurine

Scheme 3. Synthesis of 12-Oxo-*S*-antofine



analogues with different functional groups on the E ring for further derivatization (unpublished data). Moreover, this novel strategy is also applicable for other natural products in this family, such as tylophorine, which has different substitution patterns on the phenanthrene scaffold.

Scheme 4. Synthesis of 12*N*-Methyl-12-aza-*S*-antofine



The new compound **E7** was screened *in vitro* against a panel of human tumor cell lines including A549 (lung), KB (nasopharyngeal), DU-145 (prostate), and HCT-8 (colon), using *R*-antofine and *R*-cryptopleurine as a comparison. The results (GI_{50}) are listed in Table 1. Interestingly, compound E7 showed significant cytotoxic activity and was as potent as *R*-antofine against A549 cell growth but with improved selectivity relative to KB and DU145 tumor cell lines. This result suggests that the E-ring size may affect the interaction between the molecule and the binding site in the target, and

Table 1. Anticancer Activities (GI_{50}) of *R*-Antofine, *R*-Cryptopleurine, E7, **17**, and **18**

compd	A549 (nM)	DU145 (nM)	KB (nM)	HCT-8 (nM)
R-antofine	22	25	36	ND
R-cryptopleurine	1.38	1.59	1.51	1.09
E7	25	179	102	10
17	290	710	480	420
18	660	2030	1720	1000

the expansion of the E-ring may play a role in the anticancer selectivity. Although moderate to significant reduction in cytotoxicity was observed for compounds **17** and **18**, further modifications on the ketone and substituents on the imidazolidine nitrogen are of great interest to potentially improve the anticancer activity.

In conclusion, we have established a novel strategy to synthesize new phenanthroindolizidine and phenanthroquinolizidine analogues, which provides a useful tool for managing CNS toxicity and exploring the SAR profile, especially on the E-ring, for which no analogues have been reported so far, despite substantial progress in the development of their syntheses. Three new E-ring modified analogues E7, **17**, and **18** were first reported through our novel strategy detailed above to verify our method's feasibility and apparent versatility. Other modifications using *R*-cryptopleurine as template are being studied in our laboratories, aiming to discover interesting molecules with strong anticancer activity and reduced CNS toxicity. The detailed synthetic pathways to other analogues, their biological activity, and mechanistic study results will be reported in due time.

Acknowledgment. This study was supported by Grant No. CA17625-32 from the National Cancer Institute awarded to K.H.L.

Supporting Information Available: General experimental information, cytotoxicity assay, detailed experimental procedures, and copies of ^{1}H NMR and ^{13}C NMR spectra of key intermediates **3**, **5**−**7**, **9**, **11**, **12**, **15** and **16** and final products **13**, **17**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL902819J