SYNTHETIC APPROACH TOWARD THE MINIMAL ACTIVE STRUCTURE OF PHOMOPSIN-USTILOXIN CLASS OF ANTIBIOTICS: CONSTRUCTION OF 13-MEMBERED CYCLIC PEPTIDE SKELETON

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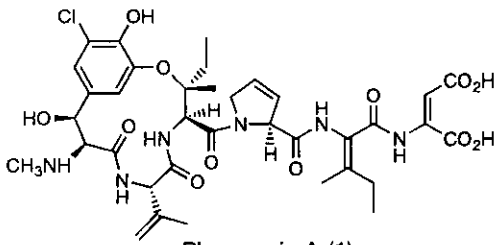
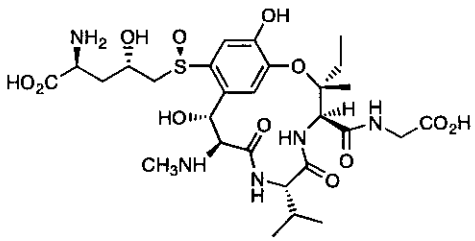
Abstract The 13-membered cyclic peptides (**5a** and **5b**), the core structure of phomopsin-ustiloxin class of antibiotics, have been synthesized. The final macrolactamization was overcome by the intramolecular aminolysis of corresponding ω-amino pentafluorophenyl ester.

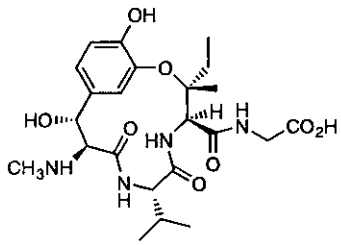
There are a number of antimitotic agents that interfere with the microtubule function by binding to tubulin. Maytansine,[1] rhizoxin,[2] dolastatin 10,[3] phomopsin A (**1**)[4] and ustiloxin A (**2**)[5] are known to share the same binding site (rhizoxin site).[6, 7] However, their structural diversity remains it difficult to find their common



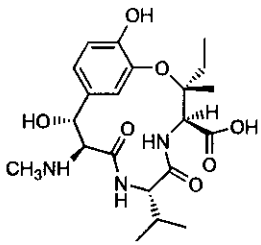
Phomopsin A (**1**)



Ustiloxin A (**2**)



Ustiloxin D (**3**)



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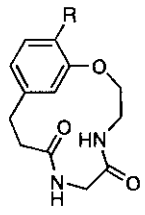
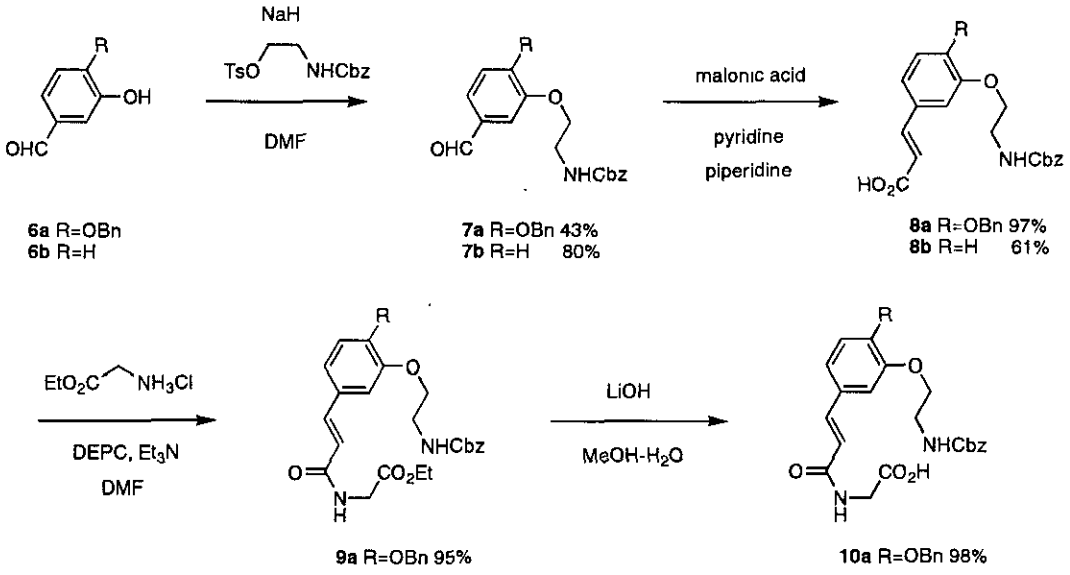


5a: R = OH
5b: R = H

structural elements to recognize the same binding site on tubulin. Among them, phomopsin A and ustiloxin A have a common 13-membered cyclic structure, and the newly isolated simplest ustiloxin congener, ustiloxin D (**3**),[7] also exhibits potent *anti*-tubulin activity. Since we regarded ustiloxin D (**3**) as the promising candidate to elucidate the structural requirement for the rhizoxin site ligands, we planned to synthesize a series of 13-membered cyclic peptides such as **4** or the variants with other amino acid units to find the minimal structure responsible for *anti*-tubulin activity. Construction of 13- membererd cyclic peptide skeleton, formed through a *meta*-phenylene substitution, is a challenging synthetic task. In this article, synthesis of *N*-protected seco ω-amino acids (**10a**) and (**10b**), and their macrolactamization to simple 13-membered cyclic peptides (**5a**) and (**5b**) are described.

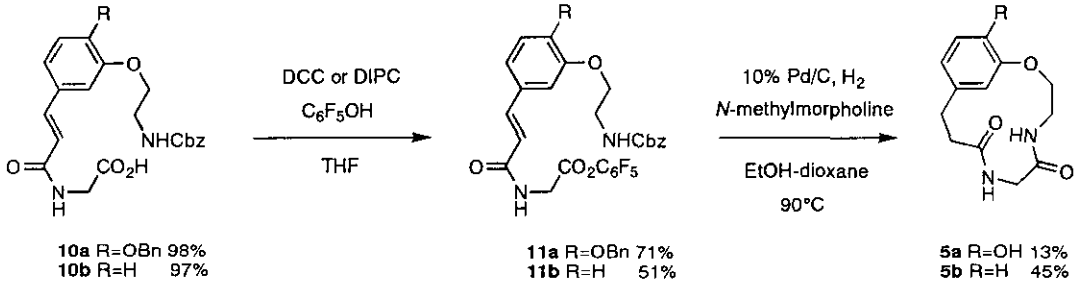
Synthesis of **10a** and **10b** are achieved as shown in **Scheme 1**. Aryl alkyl ethers (**7a**) and (**7b**) were synthesized by the alkylation of **6a**[8] and **6b** with sodium hydride and *N*-carbobenzyloxyaminoethyl *p*-toluenesulfonate in 43% and 80% of respective yields. Knoevenagel condensation of **7a** and **7b** with malonic acid gave *m*-coumaric acid derivatives (**8a**) and (**8b**). Coupling of **8a** and **8b** with glycine ethyl ester (DEPC (diethylphosphoryl cyanide), Et_3N/DMF) followed by basic hydrolysis (LiOH/MeOH-H_2O) gave **10a** and **10b** in high yield.

Scheme 1



Macrolactamization of **10a** and **10b** was accomplished successfully according to the following procedure (**Scheme 2**). An activation of ω-*N*-protected seco amino acid with dialkylcarbodiimide (DCC (dicyclohexylcarbodiimide) for **10a**; DIPC (diisopropylcarbodiimide) for **10b**) and pentafluorophenol afforded pentafluorophenyl esters (**11a**) and (**11b**). Final cyclization reactions were achieved by hydrogenation condition (Pd/C, H_2) at elevated temperature (90°C) under high dilution condition.[9] Under this reaction condition, hydrogenation of unsaturated carbon-carbon double bond, deprotection of *N*-Cbz group and macrolactamization took place in one pot to give **5a** and **5b**.[10] On the other hand, macrolactamization of saturated ω-amino acid of **10b** with PyBroP (bromotrispyrrolidinophosphonium hexafluorophosphate) or DPPA (diphenylphosphoryl azide) failed. For macrolactamization to occur, the elevated temperature seems to be necessary to increase the conformational flexibility of the molecules.

Scheme 2



10a R=OBn 98%
10b R=H 97%

11a R=OBn 71%
11b R=H 51%

5a R=OH 13%
5b R=H 45%

Our successful synthesis of **5a** and **5b** would provide a basis of future synthesis directed to the variously functionalized analogs of phomopsin-ustiloxin class of antibiotics. Currently, synthesis of cyclic peptides with additional functional groups are in progress in this laboratory for further approach to the minimal bioactive structure of phomopsin-ustiloxin class of antibiotics.[11]

ACKNOWLEDGEMENT

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10. All new compounds gave satisfactory spectroscopic and analytical data. Representative ^{1}H-nmr data (500 MHz, $CDCl_3$) for selected compounds.

    5a: δ 6.87 (d, J = 8.1 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 6.68 (dd, J = 2.0 Hz, J' = 8.1 Hz, 1H), 6.57 (br, 1H), 5.76 (br, 1H), 5.58 (br 1H), 4.41 (m, 2H), 3.74 (d, J = 6.3 Hz, 2H), 3.67 (m, 2H), 2.92 (m, 2H), 2.38 (m, 2H).

    5b: δ 7.21 (dd, J, J' = 7.8-8.0 Hz, 1H), 6.87 (br, 1H), 6.81 (dd, J = 2.5 Hz, J' = 8.2 Hz, 1H), 6.78 (d, J = 7.3 Hz, 1H), 6.62 (br, 1H), 5.87 (br, 1H), 4.32 (m, 2H), 3.69 (d, J = 6.3 Hz, 2H), 3.64 (m, 2H), 2.98 (m, 2H), 2.40 (m, 2H).
11. Although ustiloxin D exhibited strong *anti*-tubulin activity (IC_{50} = 6.6μM)[7], both **5a** and **5b** did not inhibit the microtubule assembly (IC_{50} > 100μM). An inhibitory activity was determined as described previously. M. Takahashi, S. Iwasaki, H. Kobayashi, S. Okuda, T. Murai, Y. Sato, T. Haraguchi-Hiraoka, and H. Nagano, *J. Antibiot.*, 1987, **40**, 66.

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