ORGANIC LETTERS

2009
Vol. 11, No. 18
4048−4050

Synthesis of Antitumor Marine Alkaloid Discorhabdin A Oxa Analogues

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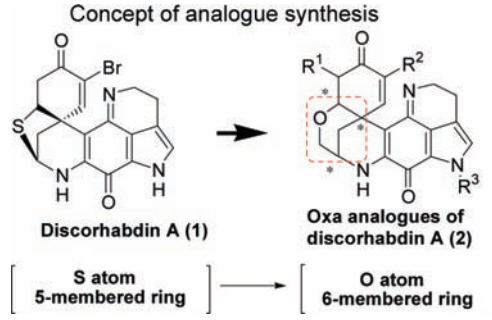
Received June 29, 2009

ABSTRACT



Discorhabdin A (1) exhibits a strong cytotoxic activity in vitro, but it is difficult to synthesize and handle due to the instability of its highly strained *N,S*-acetal structure. We then designed the analogues of discorhabdin A which also have strong cytotoxic activity and stability. The synthesis and examination of the biological activity of various types of stable discorhabdin A oxa analogues (2) were achieved.

The discorhabdin alkaloids discorhabdins A−X were isolated from marine sponges such as the New Zealand sponges, the Okinawan sponges, etc.[1] They have a peculiar structure incorporating azacarbocyclic spirodienone and pyrroloiminoquinone systems and have attracted much attention as new antitumor lead compounds due to their strong cytotoxicity.[2] Among the isolated discorhabdins, discorhabdin A (**1**) exhibits the strongest cytotoxic activity in vitro. It is suggested that the bridged sulfide structure plays an important role in expressing the activity based on the following results.[3] That is, discorhabdin E,[1j] which has no sulfur atom, exhibits a slightly weak activity compared to discorhabdin A (**1**) against mouse leukemia cell P388, while discorhabdin U,[1p] which has a sulfur atom, but lacks the sulfur ring, exhibits activity between discorhabdin E and discorhabdin A (**1**) (Figure 1).

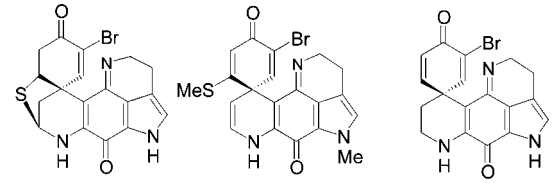


Figure 1. Cytotoxic activity against P388 tumor cell in vitro.

Up to now, many discorhabdin alkaloids have been isolated and synthesized, but biological studies including the structure−activity relationship or the mode of action of the discorhabdins have not been reported frequently. It is still a problem to have a large supply of the discorhabdin alkaloids for performing in vivo bioassays. Amassing a large amount of discorhabdin A is especially difficult due to its instability derived from the *N,S*-acetal unit despite its strong antitumor activity.

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10.1021/ol901471r CCC: $40.75
Published on Web 08/13/2009

Therefore, we intended to synthesize stable discorhabdin A analogues which can be easily synthesized and possess a strong cytotoxic activity. We first intended to stabilize the hindered *N*,*S*-acetal moiety which might be sensitive to acids, bases, and oxidants. We expected that the discorhabdin A oxa analogues (**2**) having the bridged six-membered oxacyclic structure might provide good lead compounds because of their improved stability by converting the hindered five-membered ring system into a six-membered one and by changing the labile *N*,*S*-acetal structure into a cyclic secondary amine (Figure 2).

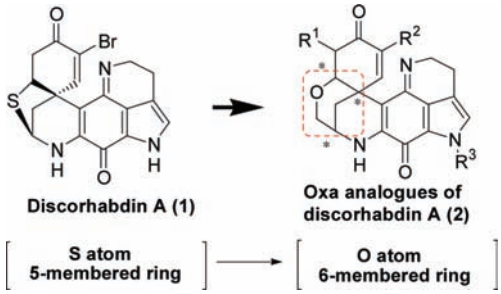


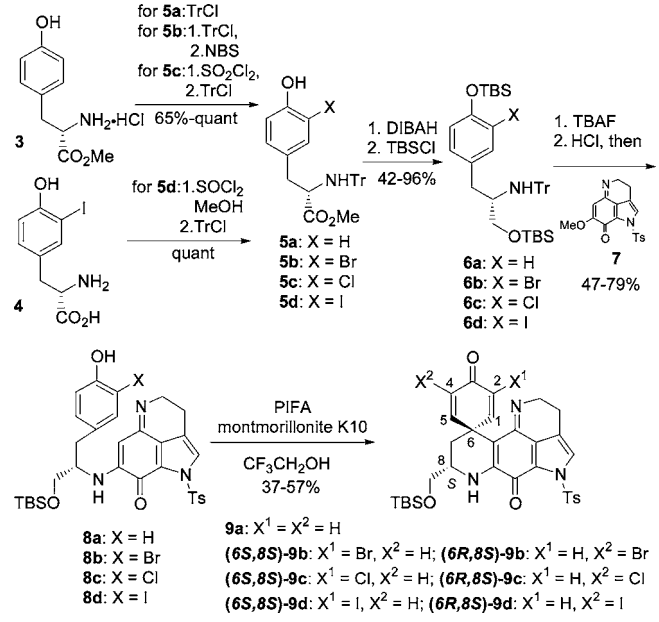
Figure 2. Concept for analogue design.

We now present the synthesis and biological activity of the discorhabdin A oxa analogues (**2**).

Recently, in our laboratory, the first asymmetric total synthesis of discorhabdin A was accomplished via spirodienone (6*S*,8*S*)-**9b** obtained by diastereoselective oxidative spirocyclization of **8b** using phenyliodine(III) bis(trifluoroacetate) (PIFA). For the synthesis of discorhabdin A, Br series compounds (**5b**, **6b**, **8b**, **9b**, and **10b**) in Schemes 1 and 2 were used.[4] We synthesized various types of stable discorhabdin A oxa analogues based on the discorhabdin A synthesis.

For the syntheses of the many kinds of discorhabdin A oxa analogues, the synthetic route through the spirodienone compounds **9a**−**d** was studied. The key starting spirodienone compounds **9a**−**d** were synthesized from tyrosine methyl ester hydrochloride (**3**) or 3-iodotyrosine (**4**) (Scheme 1).

Scheme 1. Synthesis of Spiro Compounds



Compounds **5a**−**c** were prepared from **3** as follows: tritylation for **5a**, tritylation followed by bromination for **5b**, and chlorination[5] followed by tritylation for **5c**. On the other hand, **5d** was obtained from **4** by esterification followed by tritylation. The reduction of **5a**−**d** with diisobutylaluminium hydride (DIBAH) followed by silylation of the resulting alcohols with *tert*-butyldimethylsilyl chloride (TBSCl) gave the corresponding bis-silylated compounds **6a**−**d**. Selective deprotection of the phenolic silyl group of **6a**−**d** with tetra-*n*-butylammonium fluoride (TBAF) and then detritylation with HCl aq gave the aminophenol compounds which were coupled with *N*-protected pyrroloiminoquinone **7** to produce **8a**−**d**, respectively. Compound **7** was prepared by our previously developed PIFA-induced pyrroloiminoquinone formation.[6,7] The PIFA-induced spirocyclization reaction of **8a**−**d** gave the spiro cyclohexadienones **9a**−**d**. Compound

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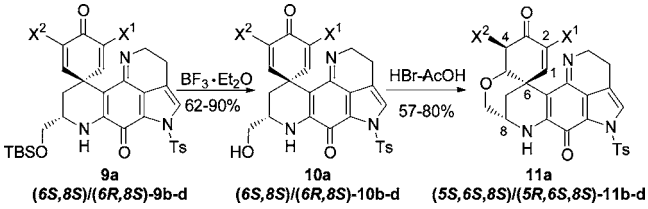
8a afforded the single compound **9a**. On the other hand, for the halo compounds **8b**−**d**, two diastereomeric isomers, the (6*S*,8*S*)-isomers and (6*R*,8*S*)-isomers **(6*S*,8*S*)-9b** and **(6*R*,8*S*)-9b** in a ratio of 1.5:1 from **8b**, **(6*S*,8*S*)-9c** and **(6*R*,8*S*)-9c** in a ratio of 1.4:1 from **8c**, and **(6*S*,8*S*)-9d** and **(6*R*,8*S*)-9d** in a ratio of 2.0:1 from **8d**, were obtained (Scheme 1).

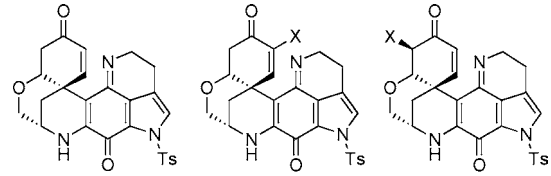
The spiro cyclohexadienones **9a** and **(6*S*,8*S*)-** and **(6*R*,8*S*)-9b**−**d** were desilylated by BF_3•Et_2O to produce the corresponding amino alcohols **10a** and **(6*S*,8*S*)-** and**(6*R*,8*S*)-10b**−**d**. Since longer treatment of the spiro cyclohexadienones with BF_3•Et_2O gave poor results, we changed the acid, BF_3•Et_2O, to 30% HBr−AcOH and succeeded in obtaining the bridged ether analogues **11a** and **(5*S*,6*S*,8*S*)-** and **(5*R*,6*S*,8*S*)-11b**−**d** in good yields. As expected, the oxa analogues **11a** and **(5*S*,6*S*,8*S*)-** and**(5*R*,6*S*,8*S*)-11b**−**d** are very stable. For example, we preserved them for 1 month at room temperature. However, we could not observe their decomposition on the basis of their TLC and ^{1}H NMR spectra (Scheme 2).

Scheme 2. Synthesis of Oxa Analogues **11**



With the discorhabdin A oxa analogues **11a**−**d** in hand, we examined their antitumor activities in vitro against five kinds of tumor model cells, WiDr, HCT-116, DU-145, P388, and L1210. As a reference, the IC_{50} values of discorhabdin A in vitro for each cell are shown: WiDr = 0.03 μM, HCT-116 = 0.03 μM, DU-145 = 0.09 μM, P388 = 0.03 μM, and L1210 = 0.04 μM. All oxa analogues exhibited good IC_{50} values. In particular, (5*S*,6*S*,8*S*)-**11b** and its diastereomer (5*R*,6*R*,8*S*)-**11b** gave the best results. Their IC_{50} values against HCT-116 (0.04, 0.05 μM) are almost the same as those of discorhabdin A (0.03 μM). To our surprise, their IC_{50} values against L1210 (0.01, 0.02 μM) are stronger than that of discorhabdin A (0.06 μM) (Table 1).

Table 1. Activities of Analogues Against Tumor Cells



		IC_{50} values (μM)				
compd	X	HCT-116	WiDr	DU-145	P388	L1210
11a		0.06	0.22	0.18	0.1	0.14
(5*S*,6*S*,8*S*)-11b	Br	0.04	0.08	0.15	0.1	0.01
(5*R*,6*S*,8*S*)-11b	Br	0.05	0.06	0.38	0.1	0.02
(5*S*,6*S*,8*S*)-11c	Cl	0.05				
(5*R*,6*S*,8*S*)-11c	Cl	0.07				
(5*S*,6*S*,8*S*)-11d	I	0.05				
(5*R*,6*S*,8*S*)-11d	I	0.06				
discorhabdin A		0.03	0.03	0.09	0.03	0.04

In conclusion, we prepared various stable discorhabdin A oxa analogues with the oxygen cross-linked spiro-fused ring system and found that they exhibit a strong activity against tumor model cells in vitro and some of them are the same as discorhabdin A. A detailed study of the activity of these compounds including in vivo testing will be performed in due course.

Acknowledgment. This work was financially supported by a Grant-in-Aid for Scientific Research (A) and (B) and a Grant-in-Aid for Scientific Research for Exploratory Research from the Japan Society for the Promotion of Science. Y.W. thanks the Japan Society for the Promotion of Science (JSPS) for a Research Fellowship for Young Scientists. We thank Drs. Tomoyuki Shibata and Kousaku Fujiwara in Shinagawa R&D Center, Daiichi Sankyo Co., Ltd., for biological assays.

Supporting Information Available: Experimental details and detailed spectroscopic data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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