Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2039–2042

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Evaluation of a Spongistatin AB-Spiroketal Analogue

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Received 10 October 2001; accepted 11 March 2002

Abstract—The synthesis of a simplified analogue of the potent, cytotoxic tubulin-depolymerizing agent spongistatin 1, based on the AB spiroketal framework, is presented. The new structural analogue is an extension of a recently described spongistatin congener reported to disrupt microtubules in breast cancer cells in vitro and to alter the microtubule assembly reaction. Cytotoxicity data on the new structural analogue, as well as the parent congener, are reported. We found no significant cytotoxic or antitubulin activity with either compound.

Subsequent to the discovery and structural elucidation of the potent, tubulin-binding antitumor compounds comprising the spongipyran family,[1] intense effort has been directed at discerning the mode of action[2] of the spongistatins, as well as achieving total syntheses[3] of members of this exciting class of anticancer compounds. Owing to the impressive structural complexity of the spongistatins, however, in conjunction with their extreme scarcity, there is strong impetus for identification of simpler structural analogues which retain the remarkable cytotoxic profile common to members of the spongipyran family.

Recently, Uckun and co-workers reported the synthesis and biological evaluation of a spongistatin surrogate whose structure was conceived originally via rational design.[4] The design strategy entailed examination of the electron crystallographic structure of the zinc-induced tubulin sheets,[5] leading to identification of a putative spongistatin binding site on the α,β-tubulin heterodimer surface characterized by an array of hydrophobic amino acid residues. The proposed binding site was deemed favorable for spongistatin binding on the basis of the potential for stabilizing van der Waals interactions between the hydrophobic residues in the binding pocket and the spiroketal moieties embedded in spongistatin 1 (**1**) (Fig. 1). Molecular modeling using atomic coordinates for the α,β-tubulin hetereodimer derived from the electron crystallographic data suggested spiroketal-diol **3** would be a suitable target for synthesis and biological screening. The efficacy of the design rationale was tested via synthesis of **3**, subjection of **3** to in vitro assays to probe cytotoxicity against human breast cancer cells, and subsequent screening as a tubulin depolymerizing agent.[4] Spiroketal-diol **3** reportedly disrupted mitotic spindle formation in human breast cancer cells (BT-20 cells) at the 10 nanomolar level, disrupted microtubule assembly (MDA-MB-231 cells) at the 500 nanomolar level, and affected GTP-induced assembly of bovine brain microtubule protein.

We found the notable biological results for spiroketal-diol **3** intriguing, owing to the extreme structural simplicity. Therefore, via adaptation of our previously disclosed[3d] successful synthetic route culminating in the total synthesis of spongistatin 2 (**2**), we reasoned we could rapidly arrive at a potent, more-highly functionalized congener of **3** and effectively build upon the privileged

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0960-894X/02/$ - see front matter
PII: S0960-894X(02)00305-0

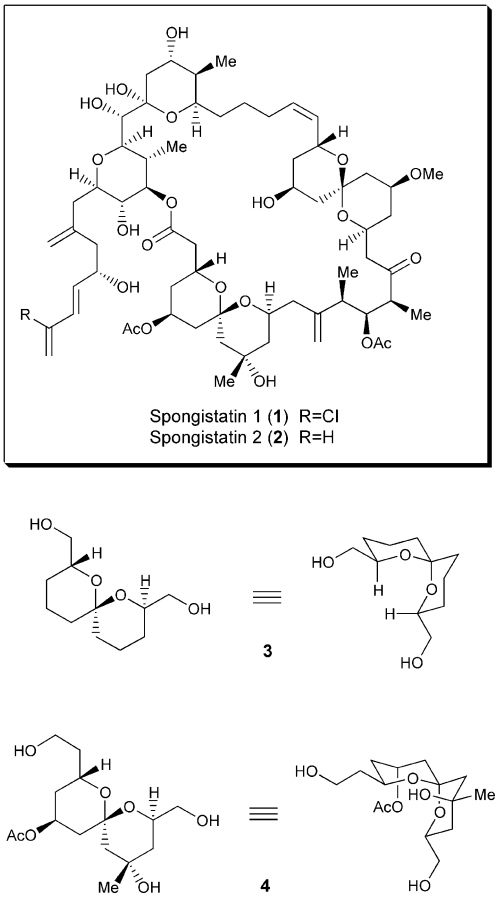
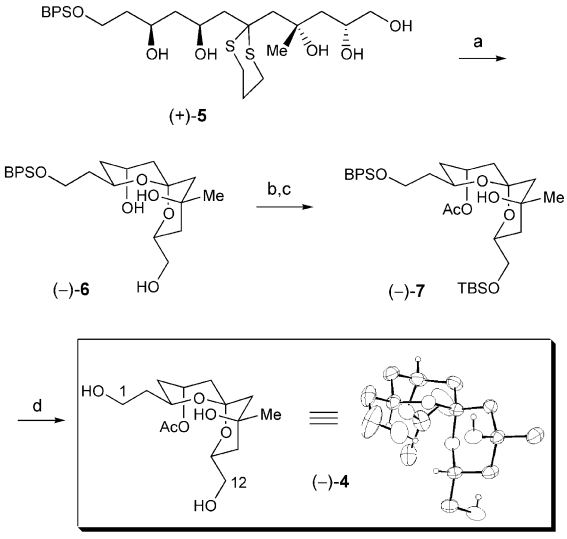


Figure 1. Structures of the potent, cytotoxic tubulin binders spongistatins 1 and 2, and structural analogues.

structural motif. We thus sought to obtain the spongistatin AB-spiroketal analogue **4** using material made available through our studies directed toward the synthesis of the spongistatins. In doing so, we intended to augment our ongoing program for identification of effective spongistatin structural analogues.[6]



Scheme 1. (a) (i) $HgClO_4 \cdot 4H_2O$, 2,6-lutidine, $MeCN/H_2O$ (10:1); (ii) $HClO_4$ (3.5% aq), CH_2Cl_2, 72%; (b) TBSCl (1.5 equiv), ImH (5 equiv), DMF, 67% (86% borsm); (c) Ac_2O (40 equiv), pyridine (80 equiv), DMAP (1 equiv), THF, 92%; (d) TBAF (3 equiv), THF, 93%.

Our synthesis of the spongistatin AB-spiroketal mimic **4** began (Scheme 1) with dithiane-penta-ol (+)-**5**,[7] derived via our one-pot unsymmetrical linchpin dithiane coupling of epoxides.[8] Dithiane removal[9] under buffered conditions followed by acid treatment afforded spiroketal (−)-**6** (72%). Selective silylation of the free primary hydroxyl group (86%, based on recovered starting material) and acetylation of the secondary axial hydroxyl group (92%) furnished bis-silyl ether (−)-**7**. The spongistatin AB-spiroketal mimic (−)-**4** was obtained in 91% yield upon exposure of (−)-**7** to tetrabutylammonium fluoride (TBAF) in THF. The structure of (−)-**4** was confirmed by single crystal X-ray analysis. Interestingly, the unit cell of (−)-**4** contained four different C(1)–C(2) rotational isomers of (−)-**4** (only one is depicted in Scheme 1).

With the AB-spiroketal spongistatin analogue (−)-**4** in hand, our first objective was to define the cytotoxicity via screening against a representative array of known human tumor lines. Since the design of (−)-**4** was inspired by spiroketal structure (−)-**3**, we deemed it also prudent to synthesize (−)-**3** as a control and subject both analogues to the same series of tests. Thus, (−)-**3** was prepared using the same route described previously;[4,10] (−)-**3** and (−)-**4** were then tested side-by-side against various human tumor cell lines. The results of the biological assay are presented in Table 1.

Table 1. Results of biological screening of spiroketals (−)-**3** and (−)-**4** against representative human cancer cell lines (GI_{50} values in μg/mL)

Cell type (cell line)	(−)-**3**	(−)-**4**
Pancreas-a (BXPC-3)	>10	>10
Breast adn (MCF-7)	>10	>10
CNS Gliobl (SF268)	>10	>10
Lung-NSC (NCI-H460)	>10	>10
Colon (KM20L2)	>10	>10
Prostate (DU-145)	>10	>10

To our surprise, neither (−)-**3** nor (−)-**4** displayed detectable growth inhibitory effects against any of the cell lines tested. We were particularly concerned by the complete ineffectiveness of (−)-**3** and (−)-**4** to inhibit growth in the breast cancer cell line MCF-7 since (−)-**3** was previously reported[4a] to disrupt the spindle microtubules of BT-20 breast cancer cells at the 10 nM level and overall microtubule organization in MDA-MB-231 breast cancer cells at a 500 nM concentration. We also treated the latter cell line with 500 nM (−)-**3**, but we observed abundant microtubules. The untreated cells and the treated cells were indistinguishable. Unfortunately, our results also indicate that (−)-**3** possesses no cytotoxicity against several of the cell lines commonly used by the National Cancer Institute as standards for detecting cancer cell growth inhibitory activity.

We also examined (−)-**3** and (−)-**4** in our standard tubulin polymerization assay (Fig. 2A). In accord with our cell data, both compounds failed to affect tubulin assembly at concentrations up to 200 μM. This assay employs a highly purified bovine brain tubulin preparation,[11] and the compounds were added at concentrations ranging

from 200 nM to 200 μM. In all cases the reactions with both (−)-**3** and (−)-**4** could be superimposed on the control reaction without drug, as shown in Figure 2A for the 200 μM reaction mixtures. In contrast, as described previously,[2a] spongistatin 1 (**1**) caused progressive inhibition of assembly as the concentration was increased from 2 to 10 μM.

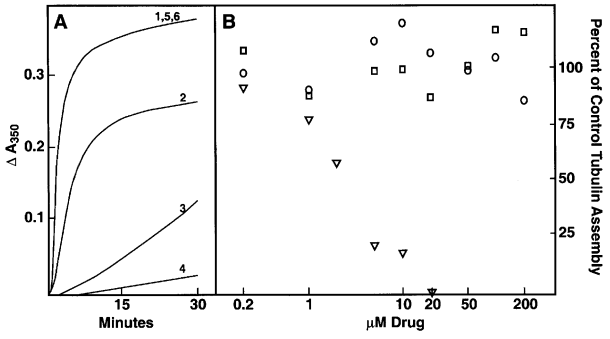


Figure 2. Inability of compounds (−)-**3** and (−)-**4** to inhibit either the glutamate-induced assembly of purified tubulin (panel A) or glycerol-enhanced assembly of microtubule protein (panel B). Tubulin and microtubule protein were prepared as described previously.[11] Tubulin assembly was followed turbidimetrically at 350 nm in either Gilford 250 (A) or Beckman DU7400/7500 (B) spectrometers equipped with electronic temperature controllers. For the panel A experiments, reaction mixtures contained tubulin (1.0 mg/mL = 10 μM), 0.8 M monosodium glutamate (pH 6.6, adjusted with HCl in 2 M stock solution), 0.4 mM GTP, 4% (v/v) dimethyl sulfoxide (the drug solvent), and drug as follows: none (curve 1), spongistatin 1 (**1**) at 2, 5, or 10 μM (curves 2, 3, and 4, respectively); compound (−)-**3** at 200 μM (curve 5); compound (−)-**4** at 200 μM (curve 6). Following a 15-min tubulin-drug pre-incubation at 30 °C without GTP, the reaction mixtures were placed on ice and GTP was added. They were transferred to cuvettes at 0 °C, and baselines were established. Incubation was at 30 °C. Curves 5 and 6 were essentially identical to curve 1, and the three curves are shown superimposed. For the panel B experiments, the reaction conditions of Uckun et al.[4a] were used, except that leupeptin and aprotinin were not included, and the microtubule protein (1.0 mg/mL) was prepared in the laboratory rather than purchased. All components were mixed at 0 °C. Reaction mixtures were placed in cuvettes at 5 °C, and baselines were established. Samples were held at 5 °C for 3 min and then incubated for 20 min at 37 °C (four readings per min). The 20-min turbidity readings in the presence of the indicated concentrations of drugs as a percentage of a simultaneously obtained control reaction are plotted. No significant drug effects were observed in the tracing except with spongistatin 1. Drugs are indicated as follows: ▽, spongistatin 1 (**1**); □, compound (−)-**3**; ○, compound (−)-**4**.

Upon reviewing the report from Uckun and collaborators,[4a] however, we noted that in their studies on inhibition of tubulin assembly they used reaction conditions substantially different from our standard condition. They used a commercial bovine brain microtubule protein preparation (tubulin unresolved from microtubule-associated proteins), with further enhancement of assembly by addition of 2.5 M glycerol. In addition, they specifically reported inhibition of assembly by (−)-**3** at the relatively low concentration of 200 nM. We therefore further examined the effects of spongistatin 1 (**1**), (−)-**3**, and (−)-**4** with microtubule protein under the reaction conditions described by Uckun and collaborators.[4a] As shown in Figure 2B, no significant inhibitory effect was observed with either (−)-**3** or (−)-**4** across a broad concentration range (200 nM to 200 μM), while spongistatin 1 (**1**) again showed progressive inhibition that was nearly complete by 5 μM. However, it was possible that the major effect of (−)-**3** was on microtubule disassembly as opposed to inhibition of polymerization, since Uckun et al.[4a] reported an unusual loss of turbidity in a reaction mixture of microtubule protein containing 0.2 μM (−)-**3** held at 37 °C upon addition of GTP. To evaluate this possibility, we did two types of experiments. First, microtubule protein was assembled with GTP, and (−)-**3** or spongistatin 1 was added. We found no effect with (−)-**3**, whereas slow disassembly, as manifested by decline in turbidity, occurred with spongistanin 1 (data not presented). Second, we set up experiments similar to those described by Uckun et al.[4a] as shown in Figure 3. Reaction mixtures containing all components except GTP were placed in cuvettes at 0 °C, and at zero time the temperature was set at 37 °C, reaching that temperature in about 75 s. The increasing temperature caused a decrease in turbidity in all reaction mixtures. This had been attributed to disassembly of pre-existing ring oligomers composed of tubulin + microtubule-associated proteins.[12] Within 3 min, turbidity in the reaction mixtures began to rise at an almost linear rate (curve 0, no GTP), probably resulting from slow tubulin aggregation. Addition of GTP and mixing it into the reaction mixture (this took about 10 s), both without drug (curve G) or with different concentrations of (−)-**3** (curves 1, 2 and 3), caused a small drop in turbidity, which was followed by a rapid rise in turbidity, representing microtubule assembly. The data obtained form the reaction mixtures containing (−)-**3** are indistinguishable from the control. We thus do not observe any effect of (−)-**3** in this reaction system. In contrast, with spongistatin 1 (curve S), in the absence of GTP, there was a small rise in the turbidity relative to the control. Addition and mixing of GTP resulted in a similar loss in turbidity, but the rapid microtubule assembly reaction did not occur. Thus, taken together, our cytotoxicity and tubulin studies call

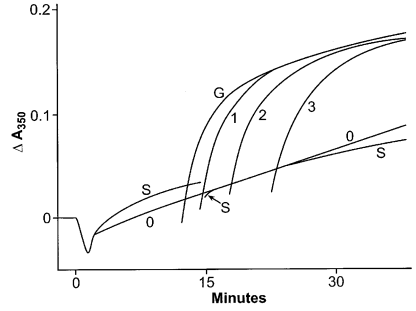
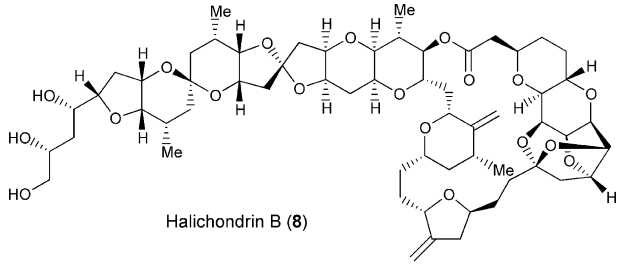


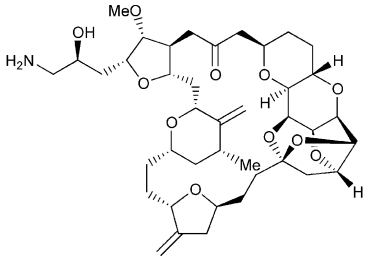
Figure 3. Reaction mixtures contained the components described by Uckun et al.,[4a] except for leupeptin and aprotinin, and we used our own preparation of microtubule protein. All components except GTP were added on ice, and the reaction mixtures were transferred to cuvettes held at 0 °C in Gilford 250 spectrophotometers. At zero time the temperature controller was set to 37 °C. Curves G and 0–3 were identical. At the indicated times GTP was added to the reaction mixtures represented by curves G, S, and 1–3 (no addition for curve 0). The reaction mixture represented by 0 and G contained no drug, while S contained 5 μM spongistatin 1 (**1**), and 1–3 contained (−)-**3** at 0.2, 2, and 50 μM, respectively. At the end of the experiment the reaction mixtures were chilled to 0 °C (not shown), and this resulted in substantial decrease in turbidity in the reaction mixtures represented by curves G and 1–3 but not in the reaction mixtures represented by curves 0 and S, indicating no microtubule assembly in the latter two reaction mixtures.

into question the original claims[4] that (−)-**3** disrupts breast cancer cell microtubules and interferes with the assembly of microtubule protein.

Although the work presented here makes it unlikely that such simple compounds as (−)-**3** and (−)-**4** represent the pharmacophore of the spongistatins, it is nonetheless possible that only a portion of the structure of these highly active compounds will turn out to be responsible for their biological activities. For example, the biological activity of halichondrin B (**8**), an antitumor compound with structural and biological similarities to the spongistatins, can be duplicated in analogues less than 2/3 the size of the parent compound. Compound **9** has potent antitumor activity in vivo[13a] and is nearly indistinguishable from halichondrin B (**8**) in its interactions with tubulin.[13b]



Halichondrin B (**8**)



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In conclusion, we have carried out a short synthesis of a highly functionalized spongistatin AB-spiroketal mimic (−)-**4** starting from material available from our total synthesis of spongistatin 2. Neither (−)-**4** nor the parent compound (−)-**3** was active against several lines of human cancer cells regularly utilized in drug cytotoxicity trials. Additionally, neither (−)-**3** nor (−)-**4** inhibited polymerization of tubulin or caused polymer disassembly using several assays.

Acknowledgements

Support was provided by the National Institutes of Health (National Cancer Institute) through grant CA-70329, contract N01-CO-56000, and a postdoctoral fellowship, CA-81337 to R.M.C.

References and Notes

1. (a) Pettit, G. R.; Tan, R.; Gao, F.; Williams, M. D.; Doubek, D. L.; Boyd, M. R.; Schmidt, J. M.; Chapuis, J.-C.; Hamel, E.; Bai, R.; Hooper, J. N. A.; Tacket, L. P. *J. Org. Chem.* **1993**, *58*, 2538. (b) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R. *J. Chem. Soc., Chem. Commun.* **1993**, 1166. (c) Fusetani, N.; Shinoda, K.; Matsunaga, S. *J. Am. Chem. Soc.* **1993**, *115*, 3977. (d) Kobayashi, M.; Aoki, S.; Sakai, K.; Kawazoe, K.; Kihara, N.; Sakai, T.; Kitigawa, I. *Tetrahedron Lett.* **1993**, *34*, 2795. (e) Kobayashi, M.; Aoki, S.; Kitigawa, I. *Tetrahedron Lett.* **1994**, *35*, 1243.
2. (a) Bai, R.; Cichacz, Z.; Herald, C. L.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1993**, *44*, 757. (b) Luduena, R. F.; Roach, M. C.; Prasad, V.; Pettit, G. R.; Cichacz, Z. A.; Herald, C. L. *Drug Develop. Res.* **1995**, *35*, 40. (c) Bai, R. L.; Taylor, G. F.; Cichacz, Z. A.; Herald, C. L.; Kepler, J. A.; Pettit, G. R.; Hamel, E. *Biochemistry* **1995**, *34*, 9714.
3. (a) Evans, D. A.; Trotter, B. W.; Côté, B.; Coleman, P. J.; Dias, L. C.; Tyler, A. N. *Angew. Chem.* **1997**, *109*, 2957, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2744. (b) Evans, D. A.; Trotter, B. W.; Coleman, P. J.; Côté, B.; Dias, L. C.; Rajapakse, H. A. *Tetrahedron* **1999**, *55*, 8671. (c) Hayward, M. M.; Roth, R. M.; Duffy, K. J.; Dalko, P. I.; Stevens, K. L.; Guo, J.; Kishi, Y. *Angew. Chem.* **1998**, *110*, 202, *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 192. (d) Smith, A. B., III; Lin, Q.; Doughty, V. A.; Zhuang, L.; McBriar, M. D.; Kerns, J. K.; Brook, C. S.; Murase, N.; Nakayama, K. *Angew. Chem.* **2001**, *113*, 202 , *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 196.
4. (a) Uckun, F. M.; Mao, C.; Vassilev, A. O.; Huang, H.; Jan, S.-T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 541. (b) Huang, H.; Mao, C.; Jan, S.-T.; Uckun, F. M. *Tetrahedron Lett.* **2000**, *41*, 1699.
5. Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199.
6. Smith, A. B., III; Lin, Q.; Pettit, G. R.; Chapuis, J.-C.; Schmidt, J. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 567.
7. Lin Q., University of Pennsylvania, unpublished data.
8. Smith, A. B., III; Boldi, A. M. *J. Am. Chem. Soc.* **1997**, *119*, 6925.
9. Fujita, E.; Nagao, Y.; Kaneko, K. *Chem. Pharm. Bull.* **1978**, *26*, 3743.
10. Spiroketal (−)-**3** had spectroscopic properties (^{1}H, ^{13}C NMR, IR, optical rotation) identical to those reported previously.[4b]
11. Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173.
12. Spann, U.; Renner, W.; Mandelkow, E.-M.; Bordas, J.; Mandelkow, E. *Biochemistry* **1987**, *26*, 1123.
13. (a) Towle, M. J.; Salvato, K. A.; Budrow, J.; Wels, B. F.; Kuznetsov, G.; Aalfs, K. K.; Welsh, S.; Zheng, W.; Seletsk, B. M.; Palme, M. H.; Habgood, G. J.; Singer, L. A.; Dipietro, L. V.; Wang, Y.; Chen, J. J.; Quincy, D. A.; Davis, A.; Yoshimatsu, K.; Kishi, Y.; Yu, M. J.; Littlefield, B. A. *Cancer Res.* **2001**, *61*, 1013. (b) Verdier-Pinard, P.; Bai, R.; Yu, M. J.; Littlefield, B. A.; Hamel, E. *Mol. Biol. Cell* **2000**, *11*, 188a.