Letters

Biological Activity of the Tryprostatins and Their Diastereomers on Human Carcinoma Cell Lines

Shuo Zhao,[†] Kirsten S. Smith,[‡] Amy Morin Deveau,[‡] Christine M. Dieckhaus,[‡] Michael A. Johnson,[‡] Timothy L. Macdonald,[‡] and James M. Cook*,[†]

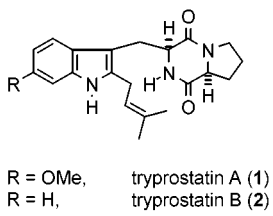
Department of Chemistry, University of Wisconsin−Milwaukee, Milwaukee, Wisconsin 53201, and Department of Chemistry, University of Virginia, McCormick Road, Charlottesville, Virginia 22904

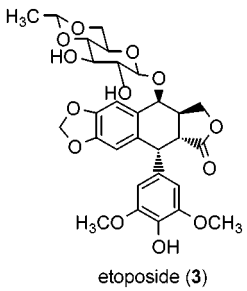
Received December 19, 2001

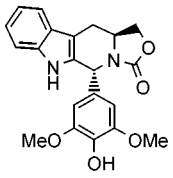
Abstract: Tryprostatin A **1** and B **2** are indole alkaloid-based fungal products that act in the G2/M phase of the cell cycle. Tryprostatin A and B as well as their two enantiomers and four diastereomers have been synthesized via a common strategy. As a measure of cytotoxicity, these eight stereoisomers were assayed for their growth inhibitory properties in human breast, prostate, and lung cancer cell lines. The ability of the tryprostatins and the tryprostatin stereoisomers to induce topoisomerase II-mediated DNA relaxation or to inhibit tubulin polymerization was also examined. Although none of the stereoisomers were significantly active in topoisomerase II- or tubulin-based assays, ds2-try B **11** was found to exihibit a cytotoxicity profile more potent than etoposide **3** in the human cancer cell lines examined. In addition, ds2-try B **11** is comprised of an L-tryptophan derivative coupled to a D-proline moiety, the latter stereochemistry of which may enhance the activity of **11** and potential analogues in vivo.

Tryprostatins A **1** and B **2** (Figure 1) have been isolated as secondary metabolites from the fermentation broth of a marine fungal strain of *Aspergillus fumigatus* BM939. It was found that tryprostatins A **1** and B **2** completely inhibited cell cycle progression of tsFT210 cells in the G2/M phase at a final concentration of 50 μg/mL of **1** and 12.5 μg/mL of **2**, respectively.[1−3] Tryprostatins A **1** and B **2** contain a 2-isoprenyltryptophan moiety and a proline residue, the latter of which is fused to the diketopiperazine unit.

The biological activity of these alkaloids has stimulated research on their total synthesis.[4−10] The interest in tryprostatin A and B in the present work stems from the desire to determine whether these alkaloids inhibit topoisomerase II or tubulin polymerization. The structures of the tryprostatins resemble an indole-based class of topo II inhibitors that includes azatoxin **4**,[11] a dual topo II/tubulin inhibitor designed as a structure-based hybrid of etoposide **3** and ellipticine **5** by Macdonald et al.[12] Etoposide **3**, a topoisomerase II inhibitor, is one of the most commonly used agents in cancer chemotherapy[13] and has improved significantly the treatment







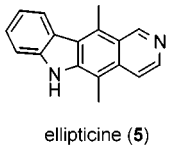


Figure 1.

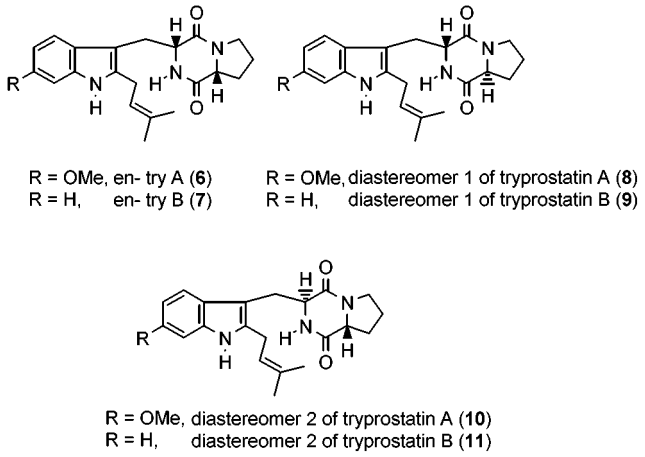


Figure 2.

of leukemia, lymphomas, and many solid tumors, including testicular and ovarian cancers. Ellipticine **5**, a linear tetracyclic azasubstituted aromatic compound, is the anticancer active indole alkaloid which intercalates strongly with DNA.[14] The similarities in the structures of the tryprostatins and azatoxin **4** led us to investigate topoisomerase II inhibition and tubulin inhibitory activity of the tryprostatins. A synthetic route to tryprostatin A **1** and B **2** as well as their enantiomers (**6** and **7**)[7] was extended to the mismatched pairs **8**−**11** in order to assess the effect of the stereochemistry of the diketopiperazine structure on the biological activity (Figure 2). The eight diastereomers were evaluated for their ability to inhibit topoisomerase II (G2 phase) or tubulin binding protein (M phase). The ultimate goals of this research are to shed light on the mechanism of action of the tryprostatins and employ this knowledge to design agents active against human carcinoma cell lines.

Chemistry. The synthesis of ds2-try B **11** is described here and serves as an example of the preparation of

* To whom correspondence should be addressed. Tel: 414-229-5856. Fax: 414-229-5530. E-mail: capncook@uwm.edu.
[†] University of Wisconsin−Milwaukee.
[‡] University of Virginia.

10.1021/jm0155953 CCC: $22.00 © 2002 American Chemical Society
Published on Web 03/15/2002

Scheme 1

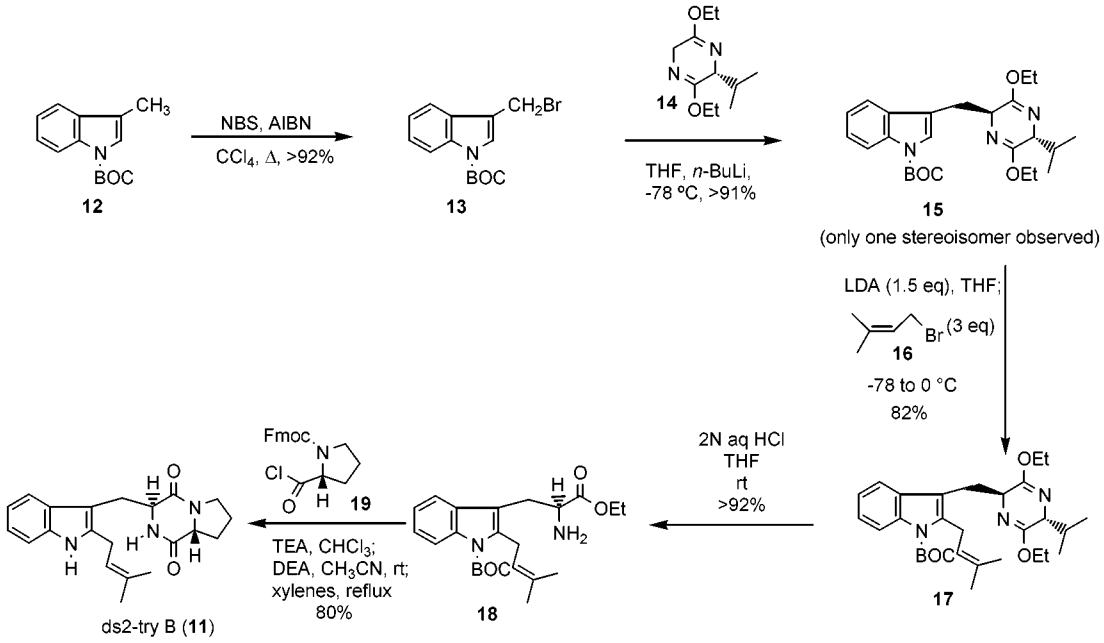


Table 1. Cell Growth Inhibition of Tryprostatins and Their Enantiomers and Diastereomers (at 10, 100 μM) on Human Lung, Breast, and Prostate Cancer Cell Lines

	percent cell survival[a]					
	H520		MCF7		PC-3	
compound	10 μM	100 μM	10 μM	100 μM	10 μM	100 μM
try A **1**	80.1 ± 4.1	79.4 ± 4.2	>100	95.0 ± 4.7	99.2 ± 4.2	95.6 ± 5.0
en-try A **6**	81.7 ± 3.9	75.2 ± 3.5	>100	>100	>100	83.7 ± 4.2
try B **2**	77.6 ± 3.6	60.5 ± 3.5	88.2 ± 5.8	66.7 ± 5.3	95.5 ± 2.8	68.9 ± 6.6
en-try B **7**	>100	99.8 ± 1.6	>100	>100	95.8 ± 1.3	78.9 ± 2.1
ds1-try A **8**	>100	>100	>100	>100	>100	>100
ds2-try A **10**	99.3 ± 1.8	98.5 ± 3.1	>100	99.0 ± 4.6	>100	>100
ds1-try B **9**	>100	76.5 ± 11.2	>100	>100	97.3 ± 5.9	68.5 ± 3.4
ds2-try B **11**	88.3 ± 8.4	0.1 ± 0.1	73.6 ± 5.3	0.0 ± 0.0	59.3 ± 3.9	0.2 ± 0.0

[a] CellTiter 96 AQ_{ueous} nonradioactive cell proliferation assay (Promega) was used to determine growth inhibition. Percent inhibition values were calculated versus control wells and were done in quadruplicate. Control wells contained 0.2% DMSO and the positive control was either etoposide or m-AMSA. Values are reported ± the standard deviation of the mean.

other analogues **8**−**10** in the series. In brief, the protected 3-methylindole **12**[15] was stirred with *N*-bromosuccinimide (NBS) in the presence of 2,2-azo-bisisobutyronitrile (AIBN) at reflux to provide the 3-(bromomethyl)indole **13**, as illustrated in Scheme 1. When bromide **13** was coupled with the anion of the Schöllkopf chiral auxiliary **14** (derived from D-valine), the desired trans diastereomer **15** was obtained with 100% trans diastereoselectivity. To introduce the isoprenyl group at the indole C(2) position of **15** and decrease the number of steps earlier reported by Gan et al.,[5] lithium diisopropylamide (LDA) was employed to form the anion at C(2)[7] (Scheme 1). The indole **15** was stirred with LDA at −78 °C, and this was followed by addition of dry, pure isoprenyl bromide **16** to furnish 2-isoprenyl-pyrazine **17** (82% yield). Since the Schöllkopf chiral auxiliary can tolerate strongly alkaline conditions, it served as an excellent protecting group for the amino acid functionality to prevent racemization. The pyrazine moiety was removed from **17** under acidic conditions (aqueous HCl, tetrahydrofuran) in greater than 92% yield to provide the 2-isoprenyltryptophan **18** and D-valine ethyl ester which could be recycled. With the key 2-isoprenyltryptophan derivative in hand, the diketopiperazine unit was now constructed. As illustrated in Scheme 1, 2-isoprenyl-tryptophan (represented by **18**) was stirred with *N*-Fmoc-D-prolyl chloride **19**[16] in the presence of triethylamine (in $CHCl_3$) at room temperature, and this was followed by removal of the solvent ($CHCl_3$). The Fmoc protecting group was then cleaved by addition of diethylamine (DEA) in acetonitrile. The acetonitrile and excess diethylamine were then removed under reduced pressure. Formation of the diketopiperazine unit in **11** as well as removal of the BOC protecting group from the indole N(H) function were achieved after each diastereomer was heated individually in refluxing xylenes (high dilution). Therefore, a stereospecific, enantiospecific total synthesis of the diastereomer of tryprostatin B (**11**), for example, was accomplished from **12** via alkylation of the corresponding 2-lithio-indole derivative.

Results and Discussion. The growth inhibition properties of all eight diastereomers were studied on three human cancer cell lines—MCF7 (breast), PC3 (prostate), and H520 (lung). Data from the assay results are illustrated in Table 1, and GI_{50} values are depicted in Figure 3. Outlined in Table 2 are the results obtained from the National Cancer Institute (NCI) on the same cancer cell lines which are in complete agreement with the present work.

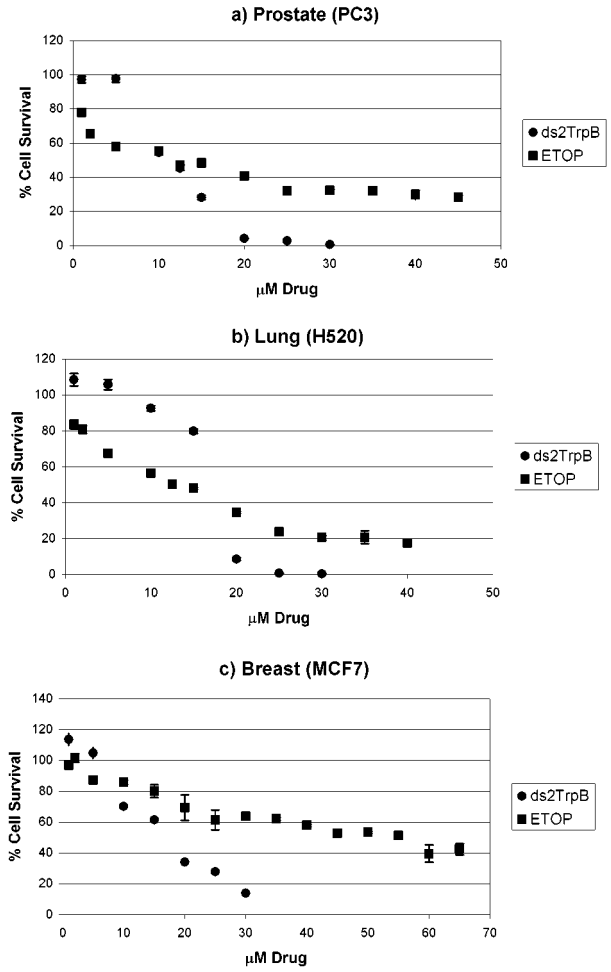


Figure 3. Comparison of GI_{50} determinations for ds2-try B **11** and etoposide **3** on selected human cancer cell lines. Concentrations were tested in quadruplicate or greater and are shown as ± the standard deviation of the mean.

Table 2. Growth Inhibition Data for ds2-try B **11** for NCI-H522 (Lung Cancer), MCF-7 (Breast Cancer), and PC-3 (Prostate Cancer) Cell Lines[a]

	NCI-H522	MCF-7	PC-3
GI_{50} (μM)	15.8	15.9	11.9

[a] Data were obtained from NCI.

One of the diastereomers of the tryprostatins, ds2-try B **11**, exhibited potent cytotoxic activity ($GI_{50} < 20$ μM, see Table 2) against all three cancer cell lines. This indicated that (1) the L-Try unit was required since none of the other tryprostatins (**6**, **7**, **8**, and **9**) which contained the D-Try unit exhibited activity; (2) the presence of the 6-methoxy group on ds2-try A **10** nearly eliminated the activity; (3) the stereochemistry of ds2-try B **11** is novel for the unnatural proline residue, may retard metabolism in vivo, and may provide an agent with a very long half-life. All eight of the diastereomers were examined for their ability to inhibit topoisomerase II-mediated DNA relaxation using an established protocol.[17,18] Analysis of the results for all eight diastereomers indicated there was no activity against topo II (data[19] not shown) for all eight compounds.

Analysis of the data for inhibition of tubulin polymerization of all eight compounds (microtubule assembly assay was prepared as described in the literature[20]) indicated that only tryprostatin A **1** was active against tubulin polymerization (~250 μM). This was in agreement with Osada et al.[21] who recently reported that tryprostatin A **1** was a novel inhibitor of MAP-dependent (MAP = microtubule-associated protein) microtubule assembly.

Interest in **11** and the development of new analogues based on the structure of ds2-try B **11** arises from examination of results of cell growth inhibition studies. In brief, ds2-try B **11** was more potent than etoposide **3** in the assay on the three human cancer cell lines described in this work.

Examination of the data in Tables 1 and 2 indicated the GI_{50} of ds2-try B **11** was 17.0 μM whereas the GI_{50} of etoposide **3** on MCF-7 cells was 55.6 μM. This clearly demonstrated that ds2-try B **11** was more potent at MCF-7 cells than etoposide **3**. Although the GI_{50} of ds2-try B **11** on H520 (GI_{50} = 11.9 μM) and PC-3 (GI_{50} = 12.3 μM) cell lines was similar to that of etoposide **3**, ds2-try B **11** was much more potent than etoposide **3** at higher concentrations (see Figure 3).

Further research is underway to determine the mechanism of action of the cytotoxic activity of ds2-try B **11** and the scope of its activity against other cancer cell lines (NCI data). In addition, results of this work provide the nature of the stereochemistry (L-Try-D-Pro) required for the synthesis of active analogues in the series related to **11**.

References

(1) Cui, C.; Kakeya, H.; Okada, G.; Onose, R.; Ubukata, M.; Takahashi, I.; Isono, K.; Osada, H. Tryprostatins A and B, Novel Mammalian Cell Cycle Inhibitors Produced by *Aspergillus Fumigatus*. *J. Antibiot.* **1995**, *48*, 1382−1384.
(2) Cui, C.; Kakeya, H.; Osada, H. Novel Mammalian Cell Cycle Inhibitors, Tryprostatins A, B and Other Diketopiperazines Produced by *Aspergillus Fumigatus* II. Physico-Chemical Properties and Structures. *J. Antibiot.* **1996**, *49*, 534−540.
(3) Cui, C.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. Novel Mammalian Cell Cycle Inhibitors, Tryprostatins A, B and Other Diketopiperazines Produced by *Aspergillus Fumigatus* I. Taxonomy, Fermentation, Isolation and Biological Properties. *J. Antibiot.* **1996**, *49*, 527−533.
(4) Depew, K. M.; Danishefsky, S. J.; Rosen, N.; Sepp-Lorenzino, L. Total Synthesis of Tryprostatin B: Generation of a Nucleophilic Prenylating Species from a Prenylstannane. *J. Am. Chem. Soc.* **1996**, *118*, 12463−12464.
(5) Gan, T.; Cook, J. M. Enantiospecific Total Synthesis of Tryprostatin A. *Tetrahedron Lett.* **1997**, *38*, 1301−1304.
(6) Gan, T.; Liu, R.; Yu, P.; Zhao, S.; Cook, J. M. Enantiospecific Synthesis of Optically Active 6-Methoxytryptophan Derivatives and Total Synthesis of Tryprostatin A. *J. Org. Chem.* **1997**, *62*, 9298−9304.
(7) Zhao, S.; Gan, T.; Yu, P.; Cook, J. M. Total Synthesis of Tryprostatin A and B As Well As Their Enantiomers. *Tetrahedron Lett.* **1998**, *39*, 7009−7012.
(8) Wang, H.; Usui, T.; Osada, H.; Ganesan, A. Synthesis and Evaluation of Tryprostatin B and Demethoxyfumitremorgin C Analogues. *J. Med. Chem.* **2000**, *43*, 1577−1585.
(9) van Loevezijn, A.; van Maarseveen, J. H.; Koomen, G.-J. Solid-Phase Synthesis of Fumitremorgin, Verruculogen and Tryprostatin Analogues based on a Cyclization/Cleavage Strategy. *Tetrahedron Lett.* **1998**, *39*, 4737−4740.
(10) Cardoso, A. S.; Lobo, A. M.; Prabhakar, S. Studies in the Aza-Cope Reaction: a Formal Highly Enantioselective Synthesis of Tryprostatin A. *Tetrahedron Lett.* **2000**, *41*, 3611−3613.
(11) Cline, S. D.; Macdonald, T. L.; Osheroff, N. Azatoxin is a Mechanistic Hybrid of the Topoisomerase II-Targeted Anticancer Drugs Etoposide and Ellipticine. *Biochemistry* **1997**, *36* (42), 13095−13101.
(12) Solary, E.; Leteurtre, F.; Paull, K. D.; Scudiero, D.; Hamel, E.; Pommier, Y. Dual Inhibition of Topoisomerase II and Tubulin Polymerization by Azatoxin, a Novel Cytotoxic Agent. *Biochem. Pharmacol.* **1993**, *45* (12), 2449−2456.

(13) Marchetti, F.; Bishop, J. B.; Lowe, X.; Generoso, W. M.; Hozier, J.; Wyrobek, A. J. Etoposide Induces Heritable Chromosomal Aberrations and Aneuploidy During Male Meiosis in the Mouse. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98* (7), 3952−3957.
(14) Behravan, G.; Leijon, M.; Sehlstedt, U.; Norden, B.; Vallberg, H.; Bergman, J.; Graslund, A. The Interaction of Ellipticine Derivatives with Nucleic Acids Studied by Optical and ^{1}H NMR Spectroscopy: Effect of Size of the Heterocyclic Ring System. *Biopolymers* **1994**, *34* (5), 599−609.
(15) Liu, R.; Zhang, P.; Gan, T.; Cook, J. M. Regiospecific Bromination of 3-Methylindoles with NBS and Its Application to the Concise Synthesis of Optically Active Unusual Tryptophans Present in Marine Cyclic Peptides. *J. Org. Chem.* **1997**, *62* (21), 7447−7456.
(16) Beyermann, M.; Bienert, M.; Niedrich, H. Rapid Continuous Peptide Synthesis via FMOC Amino Acid Chloride Coupling and 4-(Aminomethyl)piperidine Deblocking. *J. Org. Chem.* **1990**, *55*, 721−728.
(17) Spitzner, J. R.; Muller, M. T. A Consensus Sequence for Cleavage by Vertebrate DNA Topoisomerase II. *Nucleic Acid Res.* **1988**, *16*, 5533−5556.
(18) Muller, M. T.; Spitzner, J. R.; Didonato, J. A.; Metha, V. B.; Tsutsui, K. Single-strand DNA Cleavages by Eukaryotic Topoisomerase II. *Biochemistry* **1988**, *27*, 8369−8379.
(19) Zhao, S. Part I. The Enantiospecific Total Synthesis of Tryprostatin A and B As Well As Their Enantiomers and Mismatched Pairs. Part II. The Enantiospecific Total Synthesis of the Ring-A Oxygenated Sarpagine Indole Alkaloids (+)-Majvinine, (+)-10-Methoxyaffinisine and (+)-N_a-Methylsarpagine As Well As the First Total Synthesis of the *Alstonia* Bisindole Alkaloid Macralstonidine. Ph.D. Thesis, University of Wisconsin−Milwaukee, Milwaukee, WI, 2001.
(20) Bulman, A. L. Kinases and Drugs Targeting the Microtuble System. Ph.D. Thesis, University of Virginia, Charlottesville, VA, 1998.
(21) Usui, T.; Kondoh, M.; Cui, C.; Mayumi, T.; Osada, H. Tryprostatin A, a Specific and Novel Inhibitor of Microtubule Assembly. *Biochemistry J.* **1998**, *333*, 543−548.

JM0155953