Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3653–3656

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL ACTIVITY OF [Tic^5] DIDEMNIN B

Amy J. Pfizenmayer, Matthew D. Vera, Xiaobin Ding, Dong Xiao, Wei-Chuan Chen, and Madeleine M. Joullié*

Department of Chemistry, University of Pennsylvania, 231 S. 34th St. Philadelphia, PA 19104-6323, U.S.A.

Deepika Tandon and Peter L. Toogood

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, U.S.A.

Received 25 August 1998; accepted 3 November 1998

Abstract: A didemnin B analog containing a Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) as a conformationally restrained replacement for tyrosine has been synthesized and shown to have comparable potency as a protein biosynthesis inhibitor. Synthetic highlights include an oxidation of an alcohol to an acid in the presence of the sensitive Tic heterocycle and a modified Schmidt-type one-pot macrocyclization.

Introduction and Background

The didemnins, which were isolated in 1981 by Rinehart from a Caribbean tunicate of the family Didemnidae,[1] exhibit a wide variety of biological activities. Most didemnins share the same 23-membered cyclic depsipeptide core and differ only in the side chains attached to the nitrogen of threonine.

Didemnin B (**1**, Figure 1), one of the most potent natural members of the didemnin family, has shown antiviral, antitumor, and immunosuppressant activities.[2–6] Although **1** is the first marine natural product to enter antitumor clinical trials, it was found to display some side effects such as hepatic toxicity[7] and neuromuscular toxicity.[8] Didemnin B has been reported to induce apoptosis in human HL-60 cells at an extremely high rate.[9] A mechanistic model
of biological activity is still evolving. To this end, Crews and Schreiber have identified eEF-1α[10] and palmitoyl thioesterase[11] as binding proteins for a didemnin A derivative.[10] Toogood has reported the actions of didemnins A and B on protein biosynthesis in rabbit reticulocyte lysates involving eEF-1α and EF-2.[12,13] X-ray crystallographic studies[14] and solution NMR studies[15] suggest that the *N,O*-diMeTyr^5 unit is one of three moieties (the other two being the isostatine hydroxyl group and the lactylproline portion of the side chain), which are important for biological activity since they project outward from the macrocycle and could be involved in receptor interactions. This notion was supported by the work of Shen in which *N,O*-diMeTyr^5 is replaced by *N*-MeTyr. This analog showed reduced ability to inhibit both protein and DNA synthesis in Nb2 node lymphoma cells.[16] However, changing the chirality of the tyrosine from L to D does not lower activity.[17]

With this information in mind, we initiated the syntheses of didemnin analogs with variations at this site to understand better the SAR. Our research group has also prepared a wide variety of different types of didemnin analogs.[18–20] Most recently, we have shown that replacing *N,O*-diMeTyr^5 with an aliphatic amino acid, *N*-MeLeu, provides an analog with activity comparable to the parent compound, **1**.[21] During the preparation of **2** (Figure 1), Sakai and Rinehart reported data on analogs in which the *N,O*-diMeTyr unit is replaced with H$_6$-*N*-MePhe, H$_6$-*N,O*-diMeTyr (stereochemistry undefined) and iodo-*N,O*-diMeTyr.[22] None of these changes resulted in significant loss of activity. In fact, [H$_6$-*N,O*-MeTyr^5] didemnin B was shown to be very cytotoxic to

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00662-3

lymphocytes. These data encouraged us to continue the preparation of didemnin B analogs which contain systematic variations at the tyrosine site.

Rationale

In order to understand better the importance of the *N,O*-diMeTyr^5 moiety for biological activity, we are synthesizing new analogs in which this unit is replaced with other coded and non-coded amino acids. To probe further the role of tyrosine, we prepared **2**. This analog, containing a Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) unit, retains the degree of substitution on the tyrosine nitrogen (tertiary), but restricts the rotation about the $\chi 1$ (α-β) and $\chi 2$ (β-γ) bonds of tyrosine.

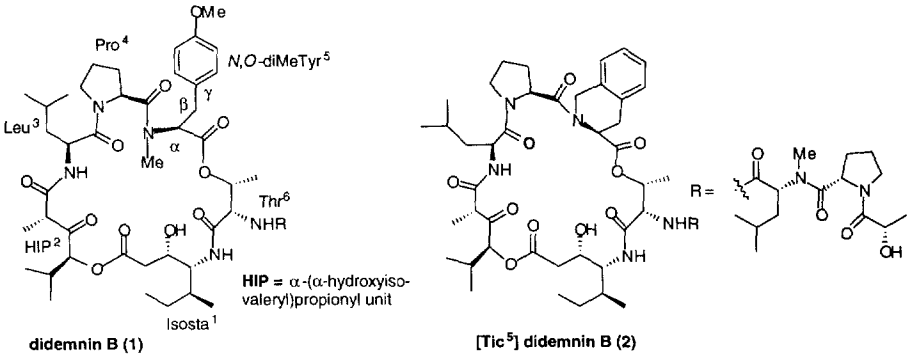


Figure 1. Didemnin B (**1**) and [Tic^5] didemnin B (**2**).

Synthesis

Our stereoselective synthesis of the didemnin macrocycle, reported previously,[24,25] was used as the basis for the synthesis of **2**. Retrosynthetically (Figure 2), the macrocycle can be broken into a tetrapeptide unit (**3**) containing Leu^3, Pro^4, Tic^5, and Thr^6 as well as a nonpeptide portion, HIP-isostatine. Tetrapeptide **3** was elaborated using standard solution-phase coupling reactions. Tic was prepared via a Pictet–Spengler reaction from Phe and formaldehyde.[23] HIP-isostatine (**4**),was derived from protected HIP-acetate (**5**) and Cbz-D-alloisoleucine.[24,25]

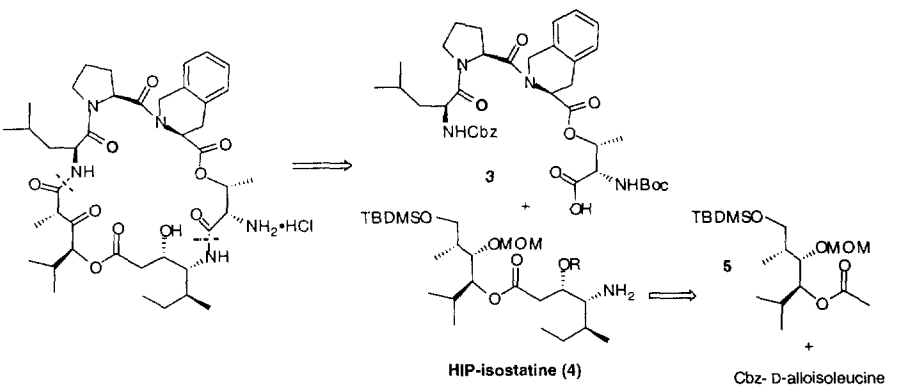
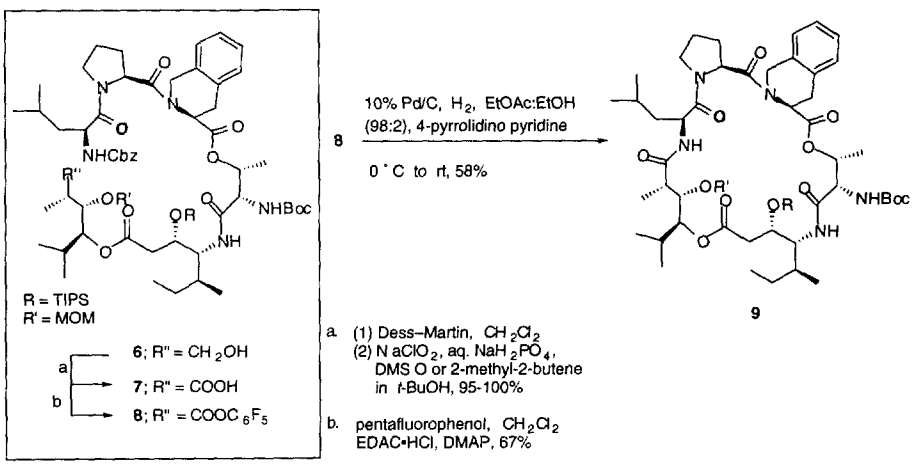


Figure 2. Retrosynthesis of **2**.

After coupling **3** to the amine of HIP-isostatine (**4**) the TBDMS ether of the resulting linear precursor was removed to yield alcohol **6** (Scheme 1). The two step oxidation procedure (Dess–Martin periodinane followed by $KMnO_4$) did not proceed as it had in the synthesis of the natural compound. After oxidizing **6** to the

corresponding aldehyde using the Dess–Martin reagent, we used $NaClO_2$[26] with a chlorine scavenger (DMSO[27–29] or 2-methyl-2-butene[30]) to convert the aldehyde to acid **7** in the presence of the oxidatively sensitive Tic residue. Removal of the Cbz carbamate of acid **7** resulted in the deprotected linear precursor. Attempts to cyclize this intermediate to form the final amide bond of the macrocycle (HIP/Leu connection) with TBTU and FDPP gave poor yields. We then returned to the Cbz protected acid **7** and converted it to its pentafluorophenol ester (**8**) in order to perform a one-pot Cbz deprotection/cyclization. This procedure, originally developed by Schmidt,[31,32] has been modified by Heffner and Joullié[33] and has been used in the syntheses of other didemnin analogs.[20,21] We used yet a different variation here: solvent (EtOAc/EtOH, 98/2), source of H_2 (external) and temperature (0 °C to rt). Cyclization of **8** proceeded in 58% yield to form **9**. This procedure was more convenient than previous ones but the yield was comparable. Lastly, after removing the MOM group, oxidizing the resulting alcohol to the ketone, and removing the Boc and TIPS protecting groups, the didemnin B side chain was attached to the macrocycle to afford **2** (HRMS calcd: 1102.6052, obsd: 1102.6046).

Scheme 1



Biological Data

The title product (**2**) was tested at the University of Michigan in a protein synthesis inhibition assay.[12] The IC_{50} values for rabbit reticulocyte lysates are as follows: **2** = 2.68 μM ± 0.17 μM; **1** = 4.4 μM ± 0.1 μM; **[*N*-MeLeu^5] didemnin B** = 9 μM ± 2 μM. It is currently being tested by the National Cancer Institute against various cancer cell lines.

Conclusions

An analog of didemnin B in which the *N,O*-diMeTyr^5 unit was replaced with Tic was synthesized. The synthesis utilized a $NaClO_2$ oxidation of an aldehyde to an acid in the presence of a Tic moiety and a one-pot Cbz deprotection/cyclization. Preliminary biological testing showed that **1** and **2** display comparable activity in a protein synthesis inhibition assay. These results suggest that the bioactive conformation of didemnin B around the tyrosine residue is similar to the crystal structure conformation.

Acknowledgement: We gratefully acknowledge the National Institutes of Health (CA 40081) and the University of Pennsylvania for financial support.

References and Notes

1. Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.
2. Montgomery, D. W.; Celniker, A.; Zukoski, C. F. *Transplantation* **1987**, *43*, 133.
3. Maldonado, E.; Lavergne, J. A.; Kraiselburd, E. *P. R. Health Sci. J.* **1982**, *1*, 22.
4. Fimiani, V. *Oncology* **1987**, *44*, 42.
5. Rinehart, K. L., Jr.; Cook, J. C., Jr.; Pandey, R. C.; Gaudioso, L. A.; Meng, H.; Moore, M. L.; Gloer, J. B.; Wilson, G. R.; Gutowsky, R. E.; Zierath, P. D.; Shield, L. S.; Li, L. H.; Renis, H. E.;McGovren, J. P.; Canonico, P. G. *Pure Appl. Chem.* **1982**, *54*, 2409.
6. Legrue, S. J.; Sheu, T.-L.; Carson, D. D.; Laidlaw, J. L.; Sanduja, S. K. *Lymphokine Res.* **1988**, *7*, 21.
7. Dorr, F. A.; Kuhn, J. G.; Phillips, J.; Von Hoff, D. D. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 1699.
8. Shin, D.; Holoye, P. Y.; Murphy, W. K.; Forman, A.; Papasozomenos, S. C.; Hong, W. K.; Raber, M. *Cancer Chemother. Pharmacol.* **1991**, *29*, 145.
9. Grubb, D. R.; Wolvetang, E. J.; Lawen, A. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 1130.
10. Crews, C. M.; Collins, J. L.; Lane, W. S.; Snapper, M. L.; Schreiber, S. L. *J. Biol. Chem.* **1994**, *269*, 15411.
11. Crews, C. M.; Lane, W. S.; Schreiber, S. L. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 4316.
12. SirDeshpande, B. V.; Toogood, P. L. *Biochemistry* **1995**, *34*, 9177.
13. Toogood, P. L. In *Molecular Design and Bioorganic Catalysis*; Wilcox, C. S. and Hamilton, A. D., Eds.; Kluwer Academic: Netherlands, 1996; p 243.
14. Hossain, M. B.; van der Helm, D.; Antel, J.; Sheldrick, G. M.; Sanduja, S. K.; Weinheimer, A. J. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 4118.
15. Kessler, H.; Will, M.; Antel, J.; Beck, H.; Sheldrick, G. M. *Helv. Chim. Acta* **1989**, *72*, 530.
16. Shen, G. K.; Zukoski, C. F.; Montgomery, D. W. *Int. J. Immunopharmac.* **1992**, *14*, 63.
17. Riedl, B. Dr. rer. nat Thesis, Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart, 1991.
18. Mayer, S. C.; Pfizenmayer, A. J.; Joullié, M. M. *J. Org. Chem.* **1996**, *61*, 1655.
19. Ramanjulu, J. M.; Joullié, M. M. *Tetrahedron Lett.* **1996**, *37*, 311.
20. Ramanjulu, J. R.; Ding, X.; Li, W.-R.; Joullié, M. M. *J. Org. Chem.* **1997**, *62*, 4961.
21. Pfizenmayer, A. J.; Ramanjulu, J.; Vera, M. D.; Ding, X.; Xiao, D.; Chen, W.-C.; Joullié, M. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2713.
22. Sakai, R.; Rinehart, K. L.; Kishore, V.; Kundu, B.; Faircloth, G.; Gloer, J. B.; Carney, J. R.; Namikoshi, M.; Sun, F.; Hughes, J., R.; Grávalos, D. G.; de Quesada, T. G.; Wilson, G. R.; Heid, R. M. *J. Med. Chem.* **1996**, *39*, 2819.
23. Majer, P.; Slaninová, I.; Lebl, M. *Int. J. Peptide Protein Res.* **1994**, *43*, 62.
24. Mayer, S. C.; Ramanjulu, J.; Vera, M. D.; Pfizenmayer, A. J.; Joullié, M. M. *J. Org. Chem.* **1994**, *59*, 5192.
25. Li, W.-R.; Ewing, W. R.; Harris, B. D.; Joullié, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7659.
26. Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888.
27. Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567.
28. Rich, R. H.; Bartlett, P. A. *J. Org. Chem.* **1996**, *61*, 3916.
29. Wood, J. L.; Porco, J. A.; Tuanton, J.; Lee, A. Y.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 5898.
30. Kraus, G. A.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825.
31. Schmidt, U.; Griesser, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 280.
32. Schmidt, U.; Lieberknecht, A. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 281.
33. Heffner, R. J.; Jiang, J.; Joullié, M. M. *J. Am. Chem. Soc.* **1992**, *114*, 10181.