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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF [*N*-MeLeu^5] DIDEMNIN B

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Abstract: Based on information from X-ray, NMR, and SAR data, the *N,O*-diMeTyr^5 unit of didemnin B was believed to interact with receptors. To ascertain the importance of this unit, an analog was synthesized in which the *N,O*-diMeTyr^5 moiety was replaced with *N*-MeLeu. Preliminary biological testing showed that the analog retained antitumor activity and the ability to inhibit protein biosynthesis.

Introduction and Background

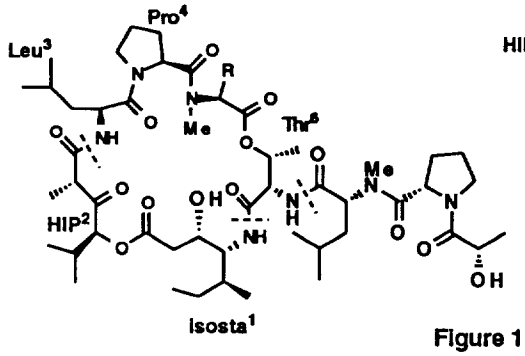
The didemnins, which were isolated in 1981 by Rinehart from a Caribbean tunicate of the family Didemnidae,[1] exhibit a wide variety of biological activities. Most didemnins share the same cyclic depsipeptide core, which contains a tetrapeptide portion and a nonpeptide unit, HIP-isostatine, and differ in the side chains attached to the threonine unit. The tetrapeptide and HIP-isostatine unit, drawn respectively as the northern and southern hemispheres of the macrocycle in Figure 1, are the key intermediates for our synthetic design.[2,3]

Didemnin B (**1**, Fig. 1), one of the most potent natural members of the didemnin family, has shown antiviral, antitumor, and immunosuppressant activities.[4-8] Unfortunately, it has also shown some side effects such as hepatotoxicity in clinical trials. Although a mechanistic model(s) of biological action is still evolving, some preliminary findings have appeared. Crews and Schreiber have identified eEF-1α as a binding protein for a didemnin A derivative in bovine brain.[9] SirDeshpande and Toogood have reported effects of didemnin A and B on protein biosynthesis in rabbit reticulocyte lysates.[10] Didemnin B has been recently reported to induce apoptosis in human HL-60 cells at the highest rate yet measured.[11] X-ray crystallographic studies[12] and solution NMR studies[13] suggest that the *N,O*-diMeTyr^5 unit and the isostatine hydroxyl group in the macrocycle, along with the lactylproline portion of the side chain (of didemnin B), are major contributors to an overall biologically important conformation. This conclusion is based on the observation that each of these groups is projected outward from the cyclic core and could describe a "pharmacophore". Support for this premise is drawn from the report that demethylation of the tyrosine methyl ether of didemnin B yields an analog with reduced ability to inhibit both protein and DNA synthesis in Nb2 node lymphoma cells.[14]

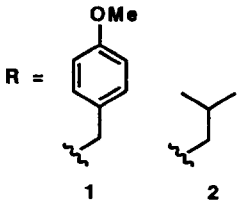
We have been involved in the synthesis of the didemnins in order to understand better the SAR.[15-17] In this context, we prepared the [*N*-MeLeu^5] analog and had it tested for both antitumor activity (at NCI) and its ability to inhibit protein biosynthesis (Dr. Peter Toogood; University of Michigan). Subsequently, in a review article on the SAR of the didemnins, Sakai and Rinehart have 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.[18] None of these changes resulted in significant loss of activity. It is this report that prompted us to communicate our recent synthetic efforts and the attendant biological results.

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 natural and unnatural amino acids. The first such analog to be completed is one in which *N*-MeLeu was substituted for *N,O*-diMeTyr^5 (**2**, Fig. 1). It was synthesized to explore the importance of potential *pi* stacking and steric effects at the side chain of *N,O*-diMeTyr^5.



HIP = α-(α-hydroxyisovaleryl)propionyl

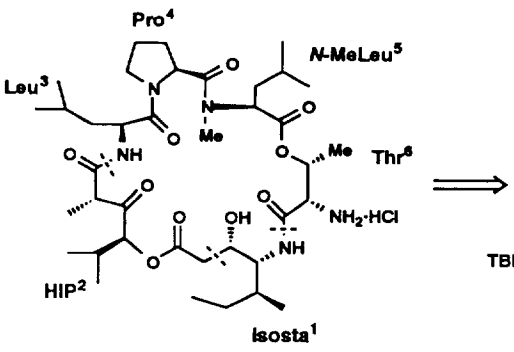


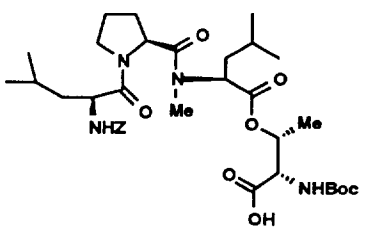
didemnin B

Figure 1

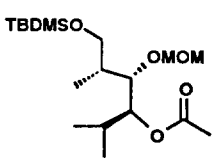
Synthesis

Our stereoselective synthesis of the didemnin macrocyclic core was reported previously[2,3] and was used here as the basis of the analog synthesis. For a retrosynthetic analysis (Fig. 2), the macrocycle can be broken into a tetrapeptide unit (**3**) containing Leu^3, Pro^4, diMeTyr^5 (or a variation), and Thr^6 as well as a nonpeptide portion, HIP-isostatine. The tetrapeptide portion was elaborated using standard solution-phase coupling reactions. The second portion, HIP-isostatine, is in turn derived from protected HIP-acetate (**4**) and Cbz-D-alloisoleucine (**5**).

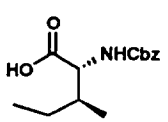




Leu-Pro-(NMe)Leu-Thr (3)



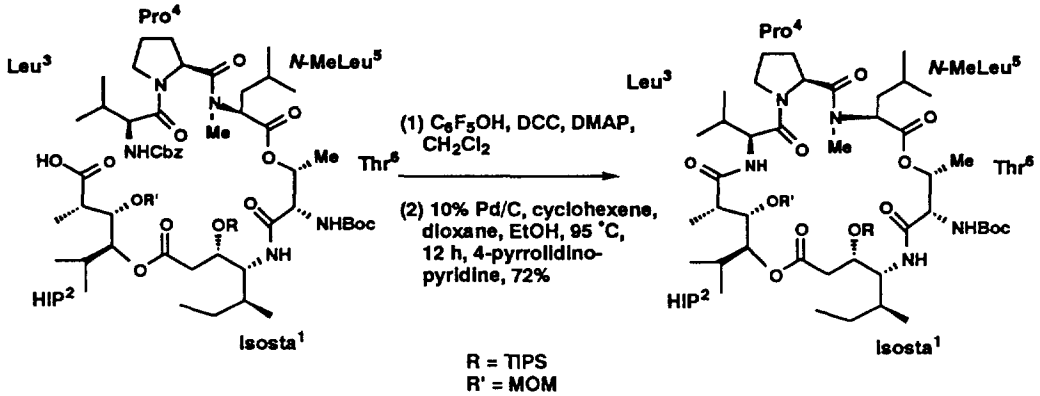
HIP-acetate (4)



D-*allo*-Isoleucine (5)

Figure 2

Once the tetrapeptide was in hand, the synthesis of the macrocycle followed that of the natural didemnin macrocycle with one exception. Normally, after coupling of the tetrapeptide to the HIP-isostatine at one point (the Thr^6-isostatine amide bond), the resulting linear precursor was cyclized to the macrocycle using FDPP[19] to form the final amide bond (between the amine of Leu^3 and and the carboxylic acid of HIP). However, this cyclization, as well as cyclizations using BOP and HBTU reagents, were unsuccessful. Instead, a modified version of Schmidt's protocol, in which in situ Cbz deprotection of an amine was followed by cyclization onto an activated ester was used.[20,21] Schmidt's method used hydrogen gas for Cbz deprotection. Heffner's modification[22] allowed a slow evolution of hydrogen via catalytic transfer hydrogenolysis over 10% palladium on carbon to remove the Cbz group on Leu^3. This safer, more convenient method produced the cyclized product in 72% yield (Scheme 1). After cyclization and a few deprotections, the didemnin B side chain was attached to the macrocycle to afford **2** (HRMS calcd: 1048.6545, obsd: 1048.6514).



Scheme 1

Biological Data

The title product (**2**) was tested by Dr. Toogood's group at the University of Michigan in a protein synthesis inhibition assay and by the National Cancer Institute against various cancer cell lines in vitro. A representative sample of cytotoxic activity data, derived from 48 h continuous drug exposure of at least five concentrations at 10-fold dilutions, is shown in the table. Growth inhibition concentrations (GI_{50}) were below 2.5 x 10^{-9} M for 49 of 52 NCI cell lines tested, but lower concentrations of drug will have to be tested to determine the exact values.

Table. Cytotoxic Activity of Didemnin B Analog

LC_{50} (μM)

Compound	Melanoma/ SK-MEL-5	CNS cancer/ SNB-75	Renal cancer RXF-393	Non-Small Cell Lung Cancer A549/ATCC	Colon Cancer HT29
didemnin B (1)	<0.0100	0.432	0.494	>100	38.2
2	0.0799	0.399	0.258	>25.0	0.445

Compound **2** was approximately 3-fold less active than didemnin B (**1**) as an inhibitor of in vitro protein synthesis in rabbit reticulocyte lysates.[10] The IC_{50} value for **1** was reported as 3 μM ± 2, while it is 9 ± 2 μM for **2**. Compound **2** was surprisingly active in these assays.

Conclusions

An analog of didemnin B in which the *N,O*-diMeTyr[5] unit was replaced with *N*-MeLeu was synthesized utilizing a cyclization step preceeded by an in situ deprotection. Biological testing showed that the analog was as active if not more active than didemnin B against many tumor cell lines. In order to evaluate further the importance of tyrosine, additional analogs are being synthesized and their preparations and bioactivities will be reported shortly.

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