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Structure–Activity Relationships of Side-Chain Modified Didemnins

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Abstract—The synthesis and antitumor activity of a novel didemnin B analogue containing a Ψ[CH_2NH] amide bond surrogate between *N*-Me-D-Leu[7] and Pro[8] are reported. The analogue shows activity (GI_{50} = 4 nM) comparable to that of didemnin B (GI_{50} = 13 nM) in the NCI-60 tumor cell screen. This result, along with new data from previously reported synthetic didemnin analogues, is discussed within the context of the side-chain SAR for didemnins. © 2001 Elsevier Science Ltd. All rights reserved.

The didemnin class of cyclic depsipeptides has attracted considerable interest because of its wide spectrum of biological activities. Didemnin B (Fig. 1), the initial lead congener, was isolated from the didemnidae family of tunicates.[1,2] It has shown potent antitumor, antiviral, and immunosuppressive activities, as well as the ability to inhibit in vitro protein biosynthesis[3] and to induce rapid apoptosis.[4] The antitumor action of these compounds has attracted substantial attention, with didemnin B entering Phase I and II clinical trials against cancer in the late 1980s.[5,6] Although didemnin B showed poor efficacy and high toxicity in the clinic, the molecular basis of its multifaceted activities is incompletely understood and still under investigation. In an attempt to improve the biological profile, we investigated the use of amide bond surrogates.

The amide bond of a peptide is a polar and enzymatically fragile moiety. These attributes cause poor rates of transfer across cell membranes and intestinal mucosa and result in rapid degradation by various proteases.[7] The use of amide surrogates is one approach to modulate the bioactivity of peptides. An appropriate amide surrogate may make a peptide analogue more resistant toward enzymatic degradation, more stable, and even orally active. The Ψ[CH_2NH] surrogate is one of the simplest isosteres of the amide bond. It has been used in the design of potent ACE inhibitors[8] and in the generation of antagonists against bombesin,[9] gastrin,[10] substance P,[11] and secretin[12] receptors. The reduced moiety Ψ[CH_2NH] acts as a nonhydrolyzable analogue of the tetrahedral transition state formed during hydrolysis of the peptide bond.[13] Although the reduced peptide bond is only one of many possible alternatives for the amide bond linkage, it has the advantage of being easily incorporated by reductive alkylation.

As a continuation of our investigation of the structure–activity relationships (SARs) of side-chain modified didemnin B analogues (**1**),[14] we have prepared a compound (**2**) containing a Ψ[CH_2NH] surrogate for the amide bond between the *N*-Me-D-leucine[7] and L-proline[8] residues. Analogue **2** contains a lactyl residue at the terminal position. Since, aplidine ([pyruvyl[9]]didemnin B), isolated from the polyclinidae family of tunicates,[15] is reported to be a highly potent cytotoxic agent,[16] we attempted to synthesize the Ψ[CH_2NH] surrogate analogue with an oxidized side chain (**3**). Unfortunately, compound **3** was not stable over extended periods of time.

The tolerance for changes in the residue beyond the *N*-Me-D-Leu[7] is precedented but limited. The introduction of unsaturation at positions 3 and 4 of the proline ring ([3,4-dehydroPro[8]]didemnin B) and the elimination of the lactyl group in the didemnin B side chain ([Pro[8]]didemnin A) were shown previously to produce two very potent analogues.[14] For the purposes of comparison to these previously tested analogues, we prepared the congener (**5**) that is truncated with 3,4-dehydroproline,[8] thus containing both modifications. To further probe the tolerance to side-chain modifications a fluorescent didemnin was prepared in order to study its cellular localization by fluorescence microscopy.[17]

The aforementioned analogues were prepared by coupling the modified side chains to the macrocycle. The

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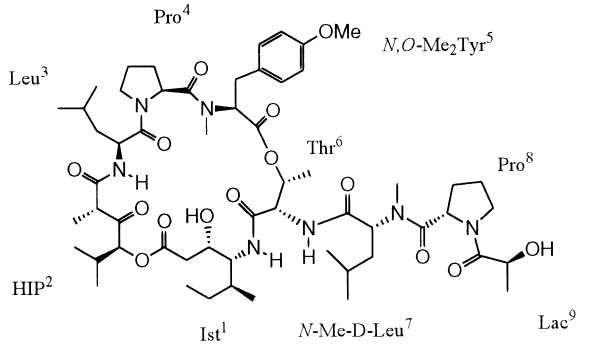
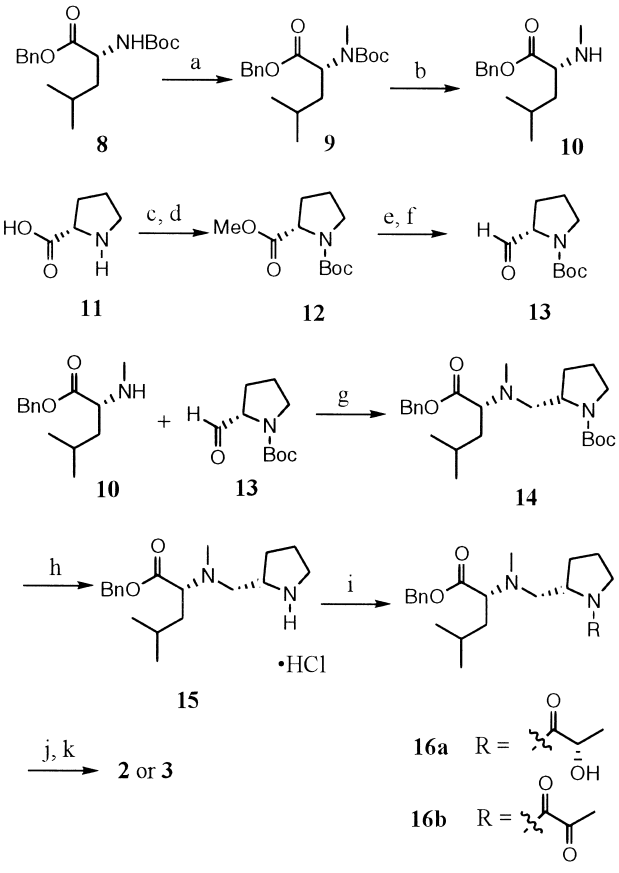


Figure 1. Didemnin B.

synthesis of the macrocycle was based on the approach previously developed in our group,[14,18] with some modifications including cyclization with HATU.[19]

The synthesis of the two Ψ[CH_2NH] surrogate side chains is described in Scheme 1. Although the Z-D-leucine methyl ester was initially used, its saponification at a later stage proved difficult and required large amounts of base. As the resultant acid was very hydrophilic, its purification was difficult. Therefore, the scheme was altered to employ a benzyl ester, which then could be removed under non-aqueous conditions. The Boc-protected D-leucine benzyl ester (**8**) was methylated to yield **9**. The Boc group was then removed with trifluoroacetic acid to afford **10**, which was subsequently condensed with protected prolinal **13** by reductive amination. The secondary amine was liberated by treatment with HCl in dioxane to produce **15**. Coupling of the amine hydrochloride salt with the appropriate acid (lactic or pyruvic acid) was accomplished with BOP and NMM in methylene chloride. Hydrogenolysis of the benzyl esters (**16a**,**b**) gave the desired unprotected side chains, and the final coupling to the didemnin macrocycle salt was performed in methylene chloride using HATU and DIEA.



Scheme 1. (a) MeI, NaHMDS, CH_2Cl_2 (78%); (b) TFA/CH_2Cl_2 (90%); (c) MeOH, $SOCl_2$ (95%); (d) Boc_2O, Et_3N, CH_2Cl_2 (85%); (e) $NaBH_4$, LiCl, THF/EtOH (85%); (f) SO_3·pyridine complex, DMSO, Et_3N, CH_2Cl_2 (81%); (g) $Na(AcO)_3BH$, AcOH, CH_2Cl_2 (88%); (h) HCl in dioxane (90%); (i) lactic acid or pyruvic acid, BOP, NMM, CH_2Cl_2 (70%); (j) H_2, Pd/C; (99%); (k) didemnin macrocycle salt, DIEA, HATU, CH_2Cl_2 (72%).

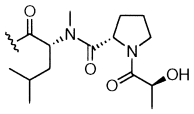
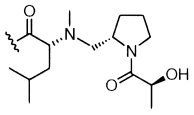
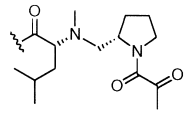
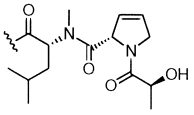
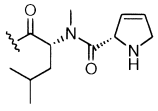
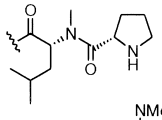
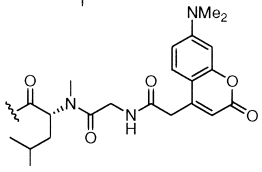
The 3,4-dehydroproline side chain was synthesized according to a previously reported procedure[14] and was attached to the macrocycle as described above. The synthesis of the fluorescent analogue **7** has been published.[17] All analogues were submitted to the NCI-60 tumor cell screen.[20] The results are collected in Table 1.

Results from several groups have demonstrated that in vitro antitumor activity is closely related to the structure of the side-chain peptide attached to the amino group of Thr.[6] As shown in Table 1, replacement of the amide bond with the Ψ[CH_2NH] surrogate led to a compound (**2**) having antitumor activity similar to that of didemnin B.

Antitumor activity is quite intolerant of modifications to the absolute configuration[21] of the *N*-Me-D-Leu.[7] However, *N*-acylation of this residue, whether by Pro^8Lac^9 or short (C2–C6) aliphatic residues, enhances cytotoxicity.[16] This effect has been attributed to improved cell permeability resulting from either increased lipophilicity or the elimination of basic nitrogen.[16] Since the Ψ[CH_2NH] surrogate (**2**)[24] exhibited activity comparable to that of didemnin B, the *N*-acylation of *N*-Me-D-Leu[7] is apparently not a strict requirement for intact biological activity, and a basic nitrogen is also tolerated.

Compounds **5** and **6** address the importance of the Lac^9 residue. The enhanced cytotoxicity of aplidine, having a ketone rather than a secondary hydroxyl at position 9, seems to imply a significant role for residue 9 as a determinant of cytotoxicity. However, our results demonstrate that the Lac^9 residue can be deleted without adverse effects on cytotoxicity. In fact, compound **6** exhibits modestly enhanced growth inhibition and cytotoxic activities.[14] Rinehart has independently demonstrated that several didemnins having *O*-acyl substituents on the Lac^9 hydroxyl group also show enhanced cytotoxicity.[16] These apparently conflicting results could be reconciled with the observation that analogues **5** and **6**, as well as aplidine and the *O*-acylated analogues, contain no hydrogen bond donor at position 9. Kessler has reported a solution NMR study of didemnin B showing evidence of a hydrogen bond between the Lac^9 hydroxyl group and the carbonyl of the macrocyclic Me_2Tyr^5 residue.[22] It is possible to speculate that the absence of this hydrogen bond may alter the conformation of the molecule in such a way as to enhance antitumor activity. The possibility that potency enhancements result from

Table 1. NCI-60 mean data from NCI-60 tumor cell screen

Compound	Side-chain structure	GI_{50} (nM)	TGI (nM)	LC_{50} (μM)	Reference
1		13	66	3.8	1,2
2		4	76	3.0	24
3					
4		2.8	20	0.083	14
5		3	39	7	14
6		0.62	12	0.21	14
7		11	20.5	7.2	17

increased lipophilicity cannot be ruled out. However, this hypothesis would be at odds with a result from Schmidt, showing that the more polar hydroxylactic acid at position 9 results in equal or enhanced growth inhibition potency.[23]

Compound **4** illustrates that installation of an unsaturation in the ring of Pro^8 leads to an incremental increase in growth inhibition potency and a larger increase in cytotoxicity. Comparison with **5** implies that the effects of the unsaturated proline and the deletion of Lac^9 do not appear to be additive.

The tolerance of side-chain modifications led us to attempt the incorporation of a glycine-linked coumarin fluorophore. The synthesis of this analogue (**7**), envisioned as a molecular probe to study cellular and tissue localization by fluorescence microscopy, was recently reported.[17] The data in Table 1 now establish **7** as essentially equipotent with didemnin B in the NCI-60 tumor cell screen. This result suggests that **7** could serve as a useful tool to study the fate of didemnins in cells. Work in this area is currently underway.

In summation, the acylation of *N*-Me-D-Leu^7 is not required for activity, and the presence of basic nitrogen in the side chain is tolerated in the case of compounds **2**, **5**, and **6**. However, the structure–activity relationship of the Pro^8Lac^9 is still unclear. The overall trends seem to indicate a quite stringent requirement for the intact *N*-Me-D-Leu^7 residue, preferably bearing an *N*-acyl group. Additionally, significant but ill-defined attenuation of cytotoxic activity results from modifications to the rest of the side chain. Nevertheless, there is notable tolerance to modifications at the side-chain terminus, as demonstrated by the successful replacement of Pro^8Lac^9 with the Gly-dimethylaminocoumarin moiety.

Acknowledgements

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24. Compound **2**: R_f 0.40 (10% $MeOH/CH_2Cl_2$); $[\alpha]_D^{25}$ +81.2 (*c* 0.15, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.85–0.97 (m, 24H), 1.17–1.27 (m, 2H), 1.28–1.39 (s, 3H), 1.40 (d, 3H), 1.43 (d, 3H), 1.46–1.50 (m, 1H), 1.61 (t, 1H), 1.68–1.73 (m, 1H), 1.74–1.79 (m, 1H), 1.82–1.88 (m, 2H), 2.02–2.08 (m, 1H) and 2.11–2.17 (m, 2H), 2.34–2.37 (m, 1H), 2.57 (s, 3H), 2.38 (d, 2H), 3.18 and 3.39 (dd, 1H), 2.83 (s, 3H), 3.37–3.57 (m, 2H), 3.60 (d, 1H), 3.70–3.73 (m, 1H), 3.80 (s, 3H), 4.05–4.14 (m, 3H) and 4.36–4.48 (m, 2H), 4.27 (q, 1H), 4.56 (dd, 1H), 4.65 (dd, 1H), 4.81 (t, 1H), 5.19 (d, 1H), 5.35 (dd, 1H), 5.42 (dd, 1H), 5.61 (dd, 1H), 5.73–5.75 (m, 1H), 6.11 (dd, 1H), 6.85 (d, 2H), 7.08 (d, 2H), 7.20 (d, 1H), 7.54 (d, 1H), 7.78 (d, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.7, 14.7, 15.2, 16.3, 16.9, 18.6, 20.1, 20.9, 23.3, 23.7, 24.8, 27.2, 24.9, 25.0, 33.9, 27.9, 31.2, 31.3, 33.9, 36.2, 38.7, 38.8, 41.3, 46.9, 49.46, 49.52, 53.0, 67.9, 55.3, 55.4, 55.5, 57.2, 57.6, 63.9, 65.9, 66.4, 66.5, 70.4, 81.5, 114.1, 124.0, 129.1, 130.0, 130.3, 158.6, 168.6, 169.3, 170.5, 170.6, 171.3, 171.5, 172.4, 173.9, 204.9; IR (neat) 3331 (br), 2956 (s), 2871 (m), 1732 (s), 1638.3 (br, overlap), 1543 (m), 1513 (s), 1448 (m), 1379 (m), 1247 (w), 1167 (w) cm^{-1}; HRMS *m*/*z* calcd for $C_{57}H_{91}N_7O_{14}$ (M + H) 1098.6702, found 1098.6726.