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Synthesis and Biological Evaluation of Didemnin Photoaffinity Analogues

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Abstract—The synthesis of four benzophenone-containing analogues of the antiproliferative natural product didemnin B is presented. In vitro protein biosynthesis inhibition potency and antitumor activity were evaluated. The results indicate that all four analogues are biologically active and could serve as photoaffinity reagents for the study of receptor-binding interactions of didemnins. These analogues could also be useful in studying antitumor effects of didemnins. © 2001 Elsevier Science Ltd. All rights reserved.

The didemnin class of marine depsipeptides (**1** and **2**, Fig. 1) has attracted considerable interest resulting from the wide spectrum of biological activities exhibited by didemnin B, the initial lead congener.[1–3] Although didemnins have shown antiviral, immunosuppressive, and protein biosynthesis inhibition effects, the antiproliferative and cytotoxic actions have led to clinical trials of some congeners against cancer.[4] The clinical efficacy of didemnins remains uncertain. Moreover, little is understood about the molecular basis of their biological activities.

Two didemnin binding proteins, eukaryotic elongation factor 1α (EF-1α)[5] and palmitoyl protein thioesterase 1 (PPT1)[6] have been identified. While the mechanistic relevance of the interaction with PPT1 is unclear,[7] binding of didemnin B to EF-1α explains the protein biosynthesis inhibition effects.[8] Using a tritiated didemnin B radioligand, we recently reported evidence showing that a ternary complex consisting of ribosome, didemnin B, and EF-1α serves to inhibit the EF-2-mediated translocation step of eukaryotic peptide elongation in vitro.[9,10] Although mechanistically interesting, the inhibition of protein biosynthesis by didemnins is not sufficient to explain the cellular apoptosis induced by these natural products.[11] Moreover, the interaction between didemnin B and its two binding proteins is not understood at any level of structural detail.

In order to address this aspect of didemnin biochemistry, we now report the synthesis and biological evaluation of didemnin photoaffinity analogues. Before attempting to use any analogues for photoaffinity labeling, we decided to address two separate questions. First, we wished to establish whether the presence of a photophore in the side chain would be tolerated. Secondly, we wished to determine whether there is an optimal length of the linker between the photophore and the didemnin nucleus. Analogues **3**–**6** (Fig. 1) were designed to explore both issues.

Photoaffinity analogue candidates **3**–**6** are formally didemnin A derivatives containing the 4-benzoylbenzoic acid moiety. We chose this type of benzophenone derivative as the photoreactive group because of its multiple advantages in photoaffinity labeling.[12,13] Benzophenones are easily manipulated in ambient light, but are photoactivated at about 350 nm, a wavelength which will not damage the target protein. The activated diradical of benzophenone tends to avoid solvent molecules and nucleophiles, cross-linking preferentially at unreactive carbon centers. Because the photoexcitation of benzophenones is reversible, a given molecule of the analogue which is not proximal to the ligand binding site can undergo several excitation–relaxation cycles without suffering nonspecific side reactions. Thus,

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benzophenones tend to cross-link with greater site-specificity than photophores such as aryl azides, which undergo an irreversible photolysis reaction.

The side-chain portion of didemnins is easily modified by chemical synthesis. Based upon the SAR profile for protein biosynthesis inhibition by didemnins,[3,8] EF-1α was anticipated to be tolerant of a structural change such as a benzophenone moiety in the side chain. Likewise, PPT1 has been shown to be rather undiscriminating among side-chain variants.[7] The consequences of benzophenone incorporation for antitumor activity, however, were more difficult to predict.

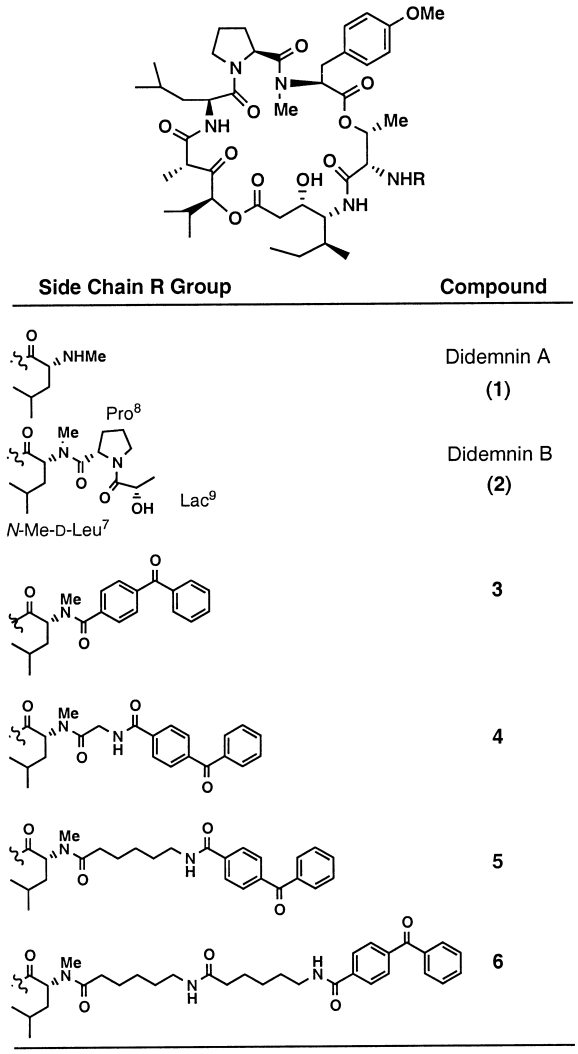
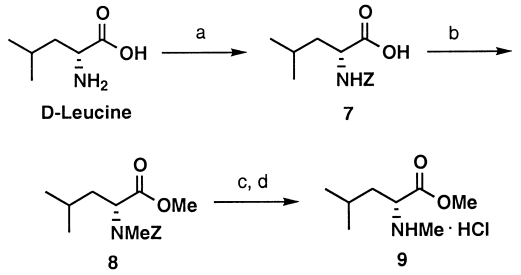


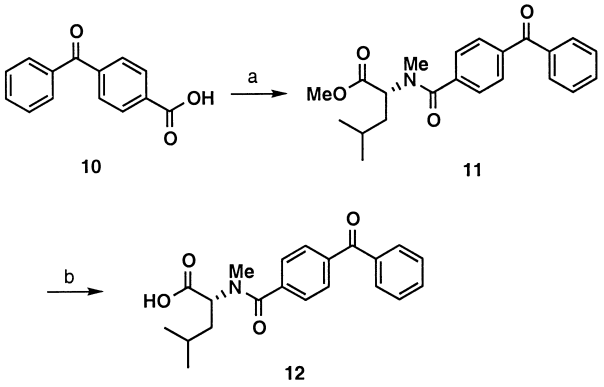
Figure 1. Structures of didemnins A and B and photoaffinity analogues **3**–**6**.



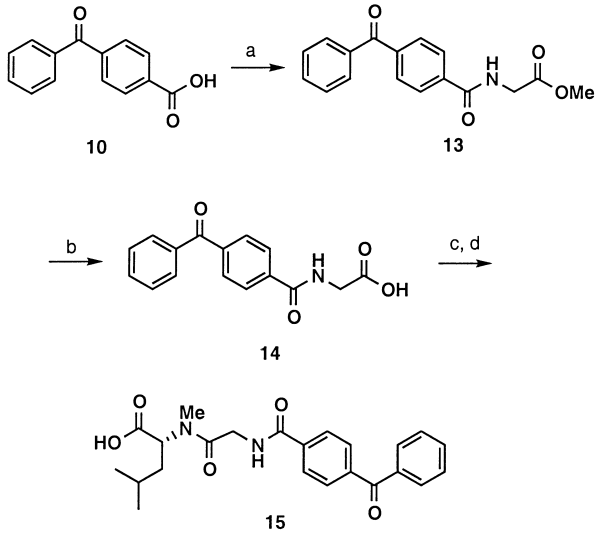
Scheme 1. (a) Benzyl chloroformate, $NaHCO_3$, H_2O, 87%; (b) Me_2SO_4, powdered KOH, THF, $Bu_4N^+HSO_4$ (cat.) 86%; (c) H_2, 10% Pd/C, EtOAc/MeOH (1:1); (d) HCl/MeOH, 96% (two steps).

Following our approach to the synthesis of didemnin congeners and analogues,[14,15] the side-chain portions of **3**–**6** were synthesized and then coupled to an advanced macrolide intermediate as a final step. Scheme 1 shows the synthesis of the D-leucine derivative which is common to all four analogues. D-Leucine was converted to its benzyl carbamate (**7**) and then dialkylated with dimethyl sulfate. Catalytic hydrogenolysis of the carbamate followed by trapping with HCl provided amine hydrochloride salt **9**. As shown in Scheme 2, **9** was coupled to 4-benzoylbenzoic acid using BOP-Cl, a reagent which is generally useful in coupling to *N*-methyl amines. Ester saponification delivered side chain **12**.

Benzophenone derivative **10** (Scheme 3) was elongated by BOP-mediated coupling with a glycine methyl ester linker. Saponification of ester **13** followed by coupling with amine salt **9** and saponification afforded side chain **15**.



Scheme 2. (a) BOP-Cl, NMM, DMF, 0 °C, 30 min, then amine hydrochloride **9**, 58%; (b) LiOH, H_2O, THF 85%.



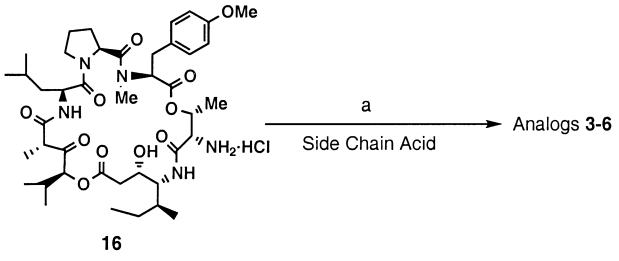
Scheme 3. (a) BOP, NMM, DMF, glycine methyl ester hydrochloride salt, 82%; (b) LiOH, H_2O, THF, MeOH, 83%; (c) BOP-Cl, NMM, DMF, amine salt **9**, 30%; (d) LiOH, H_2O, THF, MeOH, 93%.

The side chains leading to analogues **5** and **6** were elaborated using the strategy described above, incorporating either one or two 6-aminohexanoic acid moieties as linkers. The four benzophenone-containing side-chain acids were then coupled to the previously reported[15,16] macrocycle salt (**16**) using the BOP coupling reagent to obtain analogues **3**–**6** in 68–81% yield (Scheme 4, Table 1).

In order to evaluate their suitability for photoaffinity labeling of the EF-1α binding protein, analogues **3**–**6** were assayed for protein biosynthesis inhibition potency (Table 1) in a cell-free translation system as described previously.[8,9] In this assay, didemnin B (**2**) exhibited an IC_{50} of 3 μM. The results in Table 1 show that protein biosynthesis inhibition potency remains intact in spite of the incorporation of the large benzophenone moiety. The length of the linker seems to exert a marginal effect, with the two shortest side chains (**3** and **4**) being nearly equipotent with didemnin B. Increasing side-chain length correlates with modest reductions in inhibitor potency. These results are consistent with others[3,8] showing that protein biosynthesis inhibition is broadly tolerant of side-chain modification.

Table 2 shows preliminary results from the NCI-60 tumor cell screen.[17] The data shown indicate that the benzophenone photoaffinity analogues may also be useful in studying antitumor effects of didemnins. Examination of the 50% growth inhibition parameter (GI_{50}) shows that all four analogues retain potency comparable to didemnin B. In contrast to the result for in vitro protein biosynthesis, there does not appear to be any clear-cut relationship between linker length and activity. However, total growth inhibition (TGI) by the four analogues requires significantly higher concentrations than are required by didemnin B. Interestingly, all four of the analogues exhibit three to 10-fold lower toxicity as measured by the LC_{50}.

In conclusion, benzophenone-containing analogues of didemnins are accessible by total synthesis. Biological evaluation of the analogues indicates that incorporation of benzophenone in the side-chain peptide could be a feasible strategy for photoaffinity labeling of the molecular targets of didemnins.



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Scheme 4. (a) BOP, NMM, CH_2Cl_2.

Table 1. Coupling yields and protein biosynthesis inhibition activity for compounds **2**–**6**

Compound	Coupling yield (%)	IC_{50} (μM)	Ref
2		3.0	
3	68	4.0	18a
4	78	4.5	18b
5	74	24	18c
6	81	19	18d

Table 2. Mean-graph results from the NCI-60 tumor cell screen

Compound	GI_{50} (nM)	TGI (μM)	LC_{50} (μM)
2	13	0.066	3.8
3	3.0	0.35	15
4	13	2.0	23
5	4.3	21	19
6	17	5.0	48

Acknowledgements

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18. Compounds **3**–**6** gave satisfactory ^{1}H and ^{13}C NMR spectra. (a) Compound **3**: R_f 0.41 (5% MeOH/$CHCl_3$); $[\alpha]_D^{25}$ −43.8° (*c* 0.445, $CHCl_3$); HRMS *m*/*z* calcd for $C_{63}H_{86}N_6O_{14}+H$ (M + H) 1151.6280, found 1151.6261. (b) Compound **4**: R_f 0.29 (5% MeOH/$CHCl_3$); $[\alpha]_D^{25}$ −33.4° (*c* 0.445, $CHCl_3$); HRMS *m*/*z* calcd for $C_{65}H_{89}N_7O_{15}+H$ (M + H) 1208.6494, found 1208.6479. (c) Compound **5**: R_f 0.25 (5% MeOH/$CHCl_3$); $[\alpha]_D^{25}$ −60.9° (*c* 0.525, $CHCl_3$); HRMS *m*/*z* calcd for $C_{69}H_{97}N_7O_{15}+H$ (M + H) 1264.7120, found 1264.7153. (d) Compound **6**: R_f 0.21 (5% MeOH/$CHCl_3$); $[\alpha]_D^{25}$ −46.4° (*c* 0.835, $CHCl_3$); HRMS *m*/*z* calcd for $C_{75}H_{108}N_8O_{16}+Na$ (M + Na) 1399.7780, found 1399.7775.