

The Synthesis and Antifungal Activity of *N*-Alkylated Analogs of Echinocandin B

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The synthesis and biological activity of a series of *N*-alkylated derivatives of echinocandin B are described and compared with the *N*-acylated analogs. The linear, rigid geometry of the side chain that was essential to improve the antifungal potency of the *N*-acylated series gave similar *in vitro* results with the *N*-alkylated derivatives. However the slight structural variation forfeited all *in vivo* activity.

Echinocandin B (ECB) (Fig. 1) belongs to a family of lipopeptide natural products produced by *Aspergillus* spp.¹⁾ Recently, we reported the structure-activity relationship that focused on the enzymatic deacylation and semisynthetic reacylation of the linoleic side chain with polyaromatic groups²⁾ (Scheme 1). These new derivatives have received much attention due to their potent broad spectrum fungicidal activity against clinically important fungi pathogenic to humans such as *Candida* spp. (including *Candida albicans*), *Aspergillus fumigatus* and *Pneumocystis carinii*^{3,4)}. The shape of the side chains played a pivotal role in improving potency and spectrum of this chemical class. Although these extensive structure-activity relationships have focused on *N*-acylated analogs, we report here the effects of synthesizing a new series of *N*-alkylated echinocandins.

Chemistry

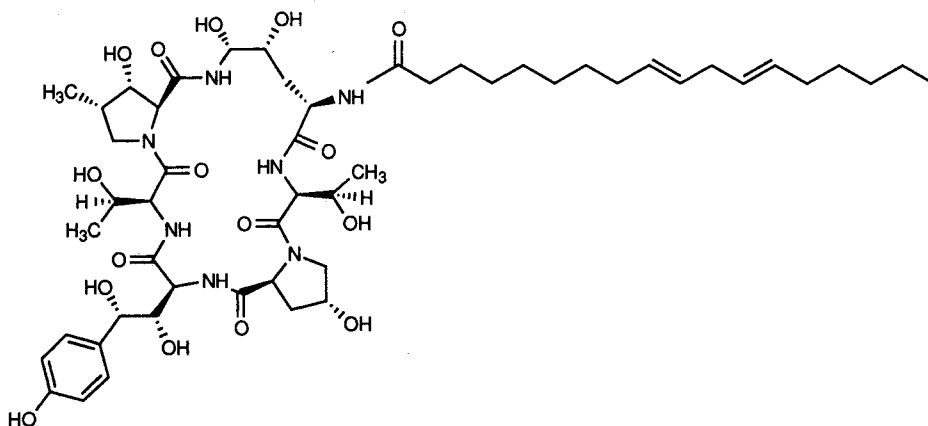
The echinocandin nucleus⁵⁾ (EN) was *N*-alkylated using reductive alkylation conditions whereby the EN

was treated with a slight excess of aldehyde in the presence of sodium cyanoborohydride in refluxing methanol/dimethylformamide for 24~48 hours. Unlike the reacylation methods, the reactivity of the hemiaminal hydroxy group toward the reaction conditions proved difficult to control^{6,7)}. Although the ring opened material was the major by-product, sufficient quantities of the desired *N*-alkylated analogs were isolated. The products were easily purified by preparative C18 reverse phase HPLC and analyzed by high resolution FAB-MS analysis. Mass spectroscopy was the primary physical-chemical method in confirming the structural assignment. Analysis of the fragmentation pattern shows the loss of the side chain cleaved at the benzylic methyl group (Fig. 2) is unique to the *N*-alkylated series.

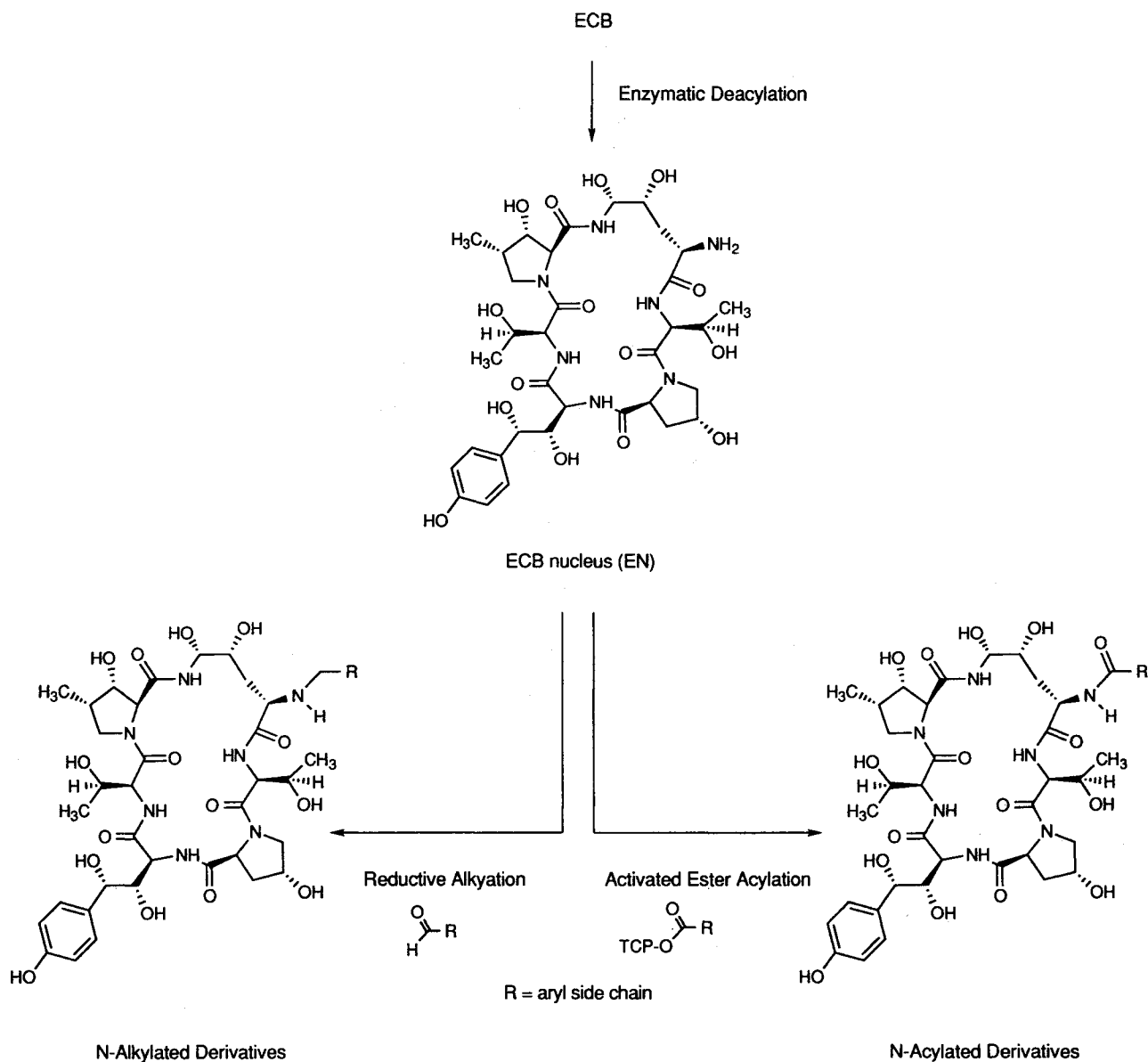
Biological Evaluation

The reintroduction of the side chain reestablished *in vitro* activity against *Candida albicans*, **1~5** (Table 1). The structure-activity relationship evaluated the effects

Fig. 1. Echinocandin B (ECB).



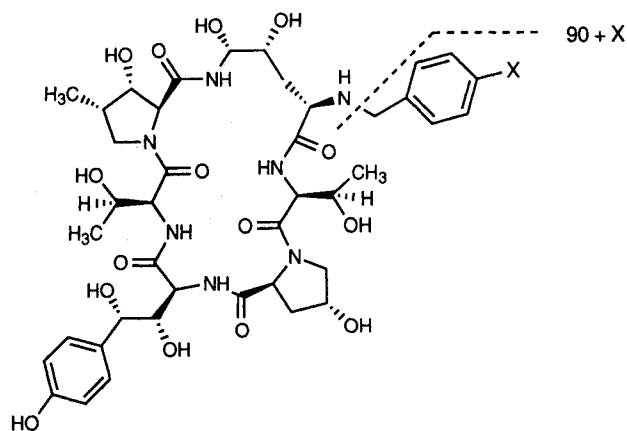
Scheme 1. ECB side chain derivatization.



of structurally modifying the side chain at the site of attachment. The lipophilicity of the side chain, as measured qualitatively by the CLOGP⁸⁾ value, causes a significant effect on *Candida* activity. A ten fold improvement over the natural product¹⁾ was observed with the terphenyl side chain, **5**. As observed with the *N*-acylated analogs, the lipophilicity of the side chain improves antifungal activity with the *N*-alkylated derivatives.

The *N*-alkylated analogs, **1**~**2**, with lower CLOGP values gave the anticipated antifungal activities based on our earlier work with the *N*-acylated derivatives²⁾. Surprisingly, the best *N*-alkylated analogs of the series,

3~**5**, having potent *in vitro* antifungal activity lost all *in vivo* activity. Our investigations with *Candida* infected mice indicated that this slight structural variation resulted in an increase in i.p. ED₅₀ values. The relationship between biological activity and the type of linkage between the nucleus and side chain (*i.e.* amine or amide) is unclear. However, poor membrane permeability and/or the increase in basicity at the site of attachment may have affected an important receptor-drug interaction. Further studies are underway to provide additional insights to the side chain structural requirements necessary for maintaining antifungal activity for this class of antibiotics.

Fig. 2. FAB-MS fragmentation pattern for *N*-alkylated echinocandins.

Compound	FAB-MS	Fragment ^c	Side chain: 90 + X
1	970 ^b	191	90 + C ₈ H ₅
2	964 ^a	167	90 + C ₆ H ₅
3	998 ^b	219	90 + C ₈ H ₁₇ O
4	1046 ^b	267	90 + C ₁₄ H ₉
5	1040 ^a	243	90 + C ₁₂ H ₉

^a(M + H)^b(M + H - H₂O)^cSide chain fragmentation ion observed.Table 1. Antifungal activity against *C. albicans* of *N*-alkylated and *N*-acylated derivatives.

Compound	Side Chain, R	ClogP Side chain	MIC (μg/ml) ^a / ED ₅₀ (mg/kg) ^b	
			N-Alkylated	N-Acylated
ECB	Linoleoyl	7.15	-----	0.625/>20
1		4.63	20/>2.5	10/>20
2		4.68	20 />2.5	>20/>20
3		6.41	0.625/>20	0.312/ 7.0
4		6.52	0.039/>20	.0039/0.6
5		6.57	0.005/>20	0.078/1.3

^a MIC performed in a dilution assay in Antibiotic 3 broth (DIFCO), pH 7.0^b i.p. administration

Experimental

Compounds

EN derivatives were dissolved in methanol and diluted in aqueous broths for *in vitro* studies. Each compound was suspended in β -cyclodextrin (Sigma, St. Louis, MO) for intraperitoneally (i.p.) treated *in vivo* studies.

Animals

Outbred, male ICR mice (mean weight, 18~20 g; Harlan-Sprague Dawley, Indianapolis, IN) were used for systemic candidiasis survival studies.

Survival Studies

Systemic candidiasis mice were X-irradiated with a sublethal dose of 400 r 24 hours prior to infection with a Gammacell 40 (Atomic Energy of Canada Limited Commercial Products, Ottawa, Canada). The mice were then infected intravenously (i.v.) through the lateral tail vein with a 0.1 ml saline suspension of *Candida albicans* A26 (2×10^6 conidia/mouse). The mean day of death for untreated control mice was 3.0~3.5 days. Morbidity and mortality were recorded for 7 days. EN derivatives were administered i.p. at 0, 4, 24 and 48 hours post-infection. Compounds were tested at titrated concentrations using serial two-fold dilutions. Ten mice were used per drug level. Ten untreated infected control mice were administered only the vehicle.

In Vitro Susceptibility Studies

Antifungal susceptibility studies were conducted using a microdilution assay. Compounds were diluted serially using 2-fold dilutions equivalent to $2 \times$ the final concentration ($20 \sim 0.0006 \mu\text{g/ml}$) desired. Aliquots of 100 μl of the diluted compound were placed in the wells serially. The 12th well received 100 μl of broth only and served as a positive growth control. Broths were adjusted to contain 2.0×10^5 cells/ml with a spectrophotometer (optical density at 660 nm). Aliquots of 100 μl of the inoculated broth were added to each well. Plates were incubated at 35°C for 48 hours in ambient air. *Candida albicans* was tested in Antibiotic 3 broth (DIFCO Laboratories, Detroit, MI). MICs were interpreted as the lowest concentration of an antifungal which inhibited the growth of the organism detected visually with the naked eye.

General

FAB mass spectra were obtained using a V6 ZAB-2SE mass spectrometer. High-resolution mass spectra were

generated for all final products and were consistent with the theoretical empirical formulas. Analytical reversed-phase HPLC work was done using the Varian 2050 system with Waters $\mu\text{bondapak}^{\text{®}}$ (C18, 3.9×300 mm) column (40/60 water/acetonitrile isocratic solvent system) with a flow rate of 2 ml/minute and using UV detection 280 nm. Preparative HPLC work performed with a Waters Prep 2000 system using Waters 3X preppak Nova-pak[®] (C18, 40×100) column. All final products were >90% pure as determined by analytical HPLC. 4-Biphenylcarboxaldehyde was commercially available and used without further purification. All other aldehydes were prepared analogously to those described in reference 2.

N-[4-(Phenylethynyl)phenylmethyl]EN (1)

A dry 250 ml, single-necked, round bottom flask was charged with EN (395.3 mg, 0.4958 mmol), 4-(phenylethynyl)benzaldehyde (158.3 mg, 0.7682 mmol), NaBH_3CN (47.25 mg, 0.7497 mmol) and crushed 4A molecular sieves. A mixture of anhydrous MeOH (30 ml) and DMF (10 ml) was then added. The resulting homogeneous reaction mixture was then heated to reflux overnight. The reaction mixture was allowed to cool to room temperature and excess ether was then added to precipitate the product. Filtration of the reaction mixture yielded a brown solid which contained the crude product. This crude product was then taken up in MeOH and filtered again to remove the molecular sieves. Preparatory HPLC of this methanolic solution, eluting with 40/60 $\text{H}_2\text{O}/\text{ACN}$ under isocratic conditions, yielded 23.0 mg (2.33%) of a white amorphous powder. Analytical HPLC retention time, 3.48 minutes; HRMS (FAB) calcd for $\text{C}_{49}\text{H}_{60}\text{N}_7\text{O}_{14}$ ($\text{M} + \text{H} - \text{H}_2\text{O}$)⁺ 970.4198, found 970.4222.

N-(4-Biphenylmethyl)EN (2)

According to a procedure similar to that described for 1; To EN (1.50 g, 1.88 mmol), 4-biphenylcarboxaldehyde (376.8 mg, 2.069 mmol), NaBH_3CN (130.0 mg, 2.063 mmol) and crushed 4A molecular sieves was added a mixture of anhydrous MeOH (75 ml) and DMF (25 ml). Preparatory HPLC of this methanolic solution yielded 68.0 mg (7.05%) of a white amorphous powder. Analytical HPLC retention time, 2.57 minutes; HRMS (FAB) calcd for $\text{C}_{47}\text{H}_{62}\text{N}_7\text{O}_{15}$ ($\text{M} + \text{H}$)⁺ 964.4304, found 964.4348.

N-[4-(Octyloxy)phenylmethyl]EN (3)

According to a procedure similar to that described for

1; To EN (1.50 g, 1.88 mmol), 4-octyloxybenzaldehyde (697.5 mg, 2.987 mmol), NaBH₃CN (142.0 mg, 2.253 mmol) and crushed 4A molecular sieves was added anhydrous MeOH (75 ml) and DMF (25 ml). Preparatory HPLC of this methanolic solution yielded 41.7 mg (1.67%) of a white amorphous powder. Analytical HPLC retention time, 2.65 minutes; HRMS (FAB) calcd for C₄₉H₇₂N₇O₁₅ (M+H-H₂O)⁺ 998.5086, found 998.5076.

N-[4'-(Phenylethynyl)-4-biphenylmethyl]EN (4)

According to a procedure similar to that described for **1**; To EN (1.50 g, 1.88 mmol), 4'-(phenylethynyl)-4-biphenylcarboxaldehyde (583.1 mg, 2.067 mmol), NaBH₃CN (125.7 mg, 1.994 mmol) and crushed 4A molecular sieves was added a mixture of anhydrous MeOH (75 ml) and DMF (25 ml). Preparatory HPLC of this methanolic solution yielded 98.0 mg (9.22%) of a white amorphous powder. Analytical HPLC retention time, 3.79 minutes; HRMS (FAB) calcd for C₅₅H₆₄N₇O₁₄ (M+H-H₂O)⁺ 1046.4511, found 1046.4530.

N-(4-*p*-Terphenylmethyl)EN (5)

According to a procedure similar to that described for **1**; To EN (1.50 g, 1.88 mmol), 4-*p*-terphenylcarboxaldehyde (540.2 mg, 2.093 mmol), NaBH₃CN (135.1 mg, 2.144 mmol) and crushed 4A molecular sieves was added a mixture of anhydrous MeOH (75 ml) and DMF (25 ml). Preparatory HPLC of this methanolic solution yielded 37.9 mg (3.65%) of a white amorphous powder. Analytical HPLC retention time, 3.59 minutes; HRMS

(FAB) calcd for C₅₃H₆₆N₇O₁₅ (M+H)⁺ 1040.4617, found 1040.4636.

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