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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF NOVEL 3′-HTY-SUBSTITUTED PNEUMOCANDINS

Regina M. Black,*[a] James M. Balkovec,[a] Karl M. Nollstadt,[b]
Sarah Dreikorn,[c] Kenneth F. Bartizal,[d] and George K. Abruzzo[d]

*Departments of [a]Medicinal Chemistry, [b]Parasite Biochemistry and Cell Biology,
[c]Antibiotic Discovery and Development, and [d]Infectious Disease Research,
Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065-0900*

Abstract: A series of pneumocandin B_0 analogs substituted at the 3′-position of the homotyrosine (Hty) residue have been prepared and evaluated for their inhibition of 1,3-β-(D)-glucan synthesis and for their antifungal activity against *C. albicans*. Cationic analogs displayed enhanced antifungal properties. The phenolic hydroxyl is involved in a critical hydrogen-bond at the binding site of the enzyme. © 1997 Elsevier Science Ltd.

Introduction

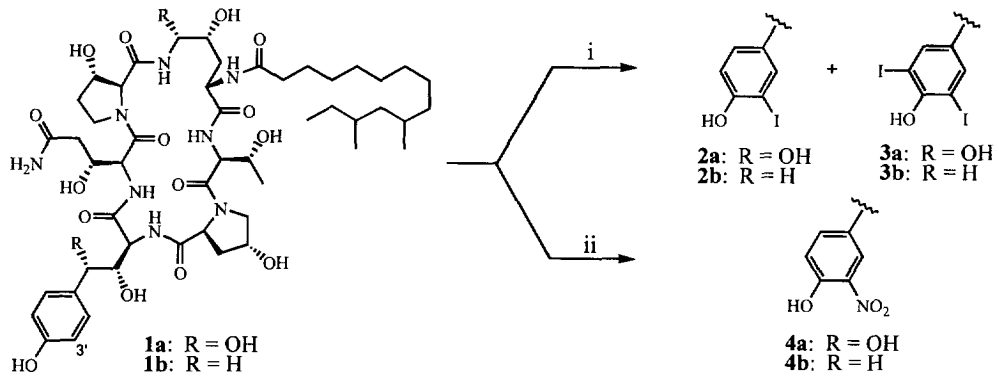
There is a critical need to develop new antifungal agents with novel modes of action due to an increase in the number of systemic fungal infections and more frequent accounts of resistance.[1] The pneumocandins inhibit the synthesis of 1,3-β-(D)-glucan, an important structural element of the cell wall of a number of pathogenic fungi such as *C. albicans*.[2] We have previously described modifications of the fungicidal lipopeptide, pneumocandin B_0 (**1a**). Studies on these cyclic hexapeptides have shown that several structural elements must be conserved in order to retain potent inhibitory activity, including the phenolic hydroxyl of the homotyrosine residue.[3] Removal of the phenolic hydroxyl of pneumocandin B_0 abolished glucan synthase inhibition and significantly reduced the antifungal activity against *Candida* spp. Because of the critical nature of this phenol, we investigated the effects of substitution at the 3′-position of the homotyrosine residue, the position *ortho* to the phenol of pneumocandin B0 (**1a**), and its dideoxy-analog (**1b**). The resulting structure–activity profile of this series of novel compounds is detailed herein.

Chemistry

Electrophilic aromatic substitution of the Hty moiety of either pneumocandin B_0 (**1a**) or its dideoxy analog (**1b**)[4] provided two key intermediates, which served as starting materials for a series of 3′-substituted derivatives. Syntheses of these compounds, the 3′-iodo derivatives (**2**) and the 3′-nitro derivatives (**4**), are shown in Scheme I.

Iodination was accomplished by two different routes. Treatment with one equivalent of iodine monochloride in DMF yielded a 1:1:1 mixture of starting material (**1a,b**), mono-iodinated (**2a,b**), and diiodinated (**3a,b**) products. Dideoxypneumocandin B_0 (**1b**) could be exclusively monoiodinated by treatment with one equivalent of sodium iodide and aqueous sodium hypochlorite in methanol. The presence of the base-sensitive hemiaminal moiety[3a] precluded the use of these conditions with pneumocandin B_0 (**1a**). Treatment of **1a** or **1b** with excess aqueous sodium nitrite in acetic acid gave the 3'-nitro derivatives **4a** and **4b**, respectively. While treatment of phenols with excess nitrous acid in acidic media has been reported to lead to the direct introduction of a diazonium group,[5] we only observed nitration. Presumably, the initially formed nitroso adduct is further oxidized by the nitrous acid.

SCHEME I

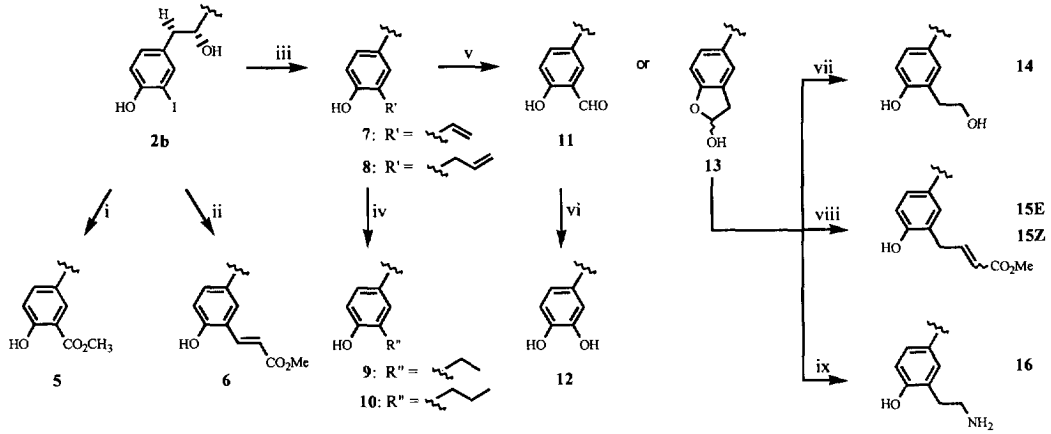


(i) See text; (ii) $NaNO_2$ (aq), HOAc (42–45%).

Ortho-iodo dideoxypneumocandin B_0 (**2b**) served as a substrate for various palladium(0)-catalyzed substitutions (Scheme II). These reactions were unsuccessful in the pneumocandin B_0 series, apparently due to the presence of the unstable hemiaminal functionality. Carboxymethylation of **2b** using $Pd(OAc)_2(dppp)_2$ under CO (1 atm) in methanol gave methyl ester (**5**). The vinylogous analog (**6**) was obtained by treating **2b** with methyl acrylate and triethylamine in the presence of 20 mol % $Pd(OAc)_2$, in acetonitrile/DMF at 70 °C for 3 h. The reaction proceeded to 80% completion and the product coeluted with starting material and therefore was resubmitted to the reaction conditions. After careful chromatography, **6** was obtained contaminated by ~2% of **2b** and 6% of the (*Z*)-isomer. Reaction of **2b** with vinyl- or allyltributylstannane in the presence of tetrakis(triphenylphosphine) palladium(0) smoothly gave the vinyl and allyl substituted analogs, **7** and **8**, respectively. Catalytic hydrogenation of the *o*-vinyl and *o*-allyl analogs gave the ethyl and propyl derivatives **9** and **10**, respectively. Ozonolysis of the *o*-vinyl and *o*-allyl analogs followed by workup with dimethyl sulfide gave the respective aldehydes, **11** and **13**. In the case of the allyl derivative, the resulting aldehyde existed as the lactol **13**. The *o*-formyl analog **11** was oxidized via a Baeyer–Villiger reaction followed by base hydrolysis to give the catechol **12**. Lactol **13** was also further derivatized. Reduction with sodium borohydride in methanol yielded the hydroxyethyl derivative **14**. Wittig reaction with methyl(triphenylphosphoranylidene) acetate afforded a mixture of the *trans*- and *cis*-isomers **15*E*** and **15Z** (*E*-isomer predominated), separable by preparative HPLC. Reductive amination with ammonium acetate/sodium cyanoborohydride gave the aminoethyl derivative **16**.

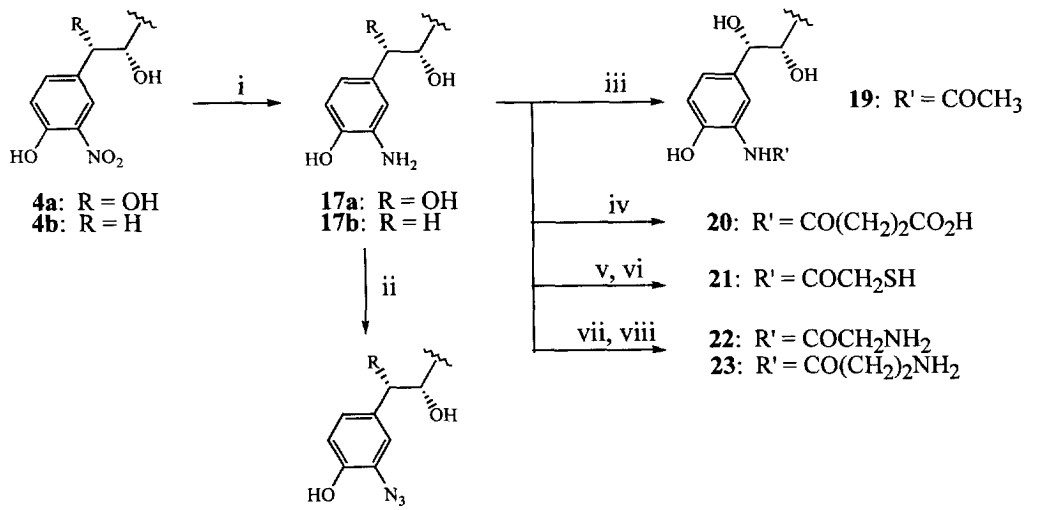
The 3′-nitro analogs of both pneumocandin B_0 and dideoxypneumocandin B_0 (**4a** and **4b**, respectively) served as starting materials for the preparation of various nitrogen-substituted derivatives (Scheme III). Reduction with sodium borohydride and palladium on carbon in aqueous methanol[6] afforded the 3′-amino analogs **17a,b**. Catalytic hydrogenation under similar conditions gave a poor yield of the 3′-amino analogs. Treatment of the amines with aqueous sodium nitrite in acetic acid followed by addition of sodium azide gave the 3′-azido derivatives **18a,b**. The 3′-amino analog of pneumocandin B_0 **17a** was further derivatized by a variety of acylating agents to give the 3′-amido analogs **19–23**. All final compounds were purified by preparative HPLC (>94% purity) and had satisfactory ^1H NMR and mass spectra.[7]

SCHEME II



(i) CO (1 atm), Et_3N, $Pd(OAc)_2$/dppp, MeOH, 60 °C (34%); (ii) CH_2=$CHCO_2Me$, 20 mol % $Pd(OAc)_2$, Et_3N, DMF, CH_3CN, 70 °C (20%); (iii) R'$SnBu_3$, $Pd(PPh_3)_4$, LiCl, NMP, 60 °C (36–58%); (iv) H_2, 10% Pd on C, MeOH (74–85%); (v) O_3, $S(CH_3)_2$, MeOH, 0 °C (53–67%); (vi) mCPBA, HOAc; $NaHCO_3$, MeOH (35%, 2 steps); (vii) $NaBH_4$, MeOH (53%); (viii) Ph_3P=$CHCO_2Me$, DMF (54%); (ix) NH_4OAc, $NaCNBH_3$, MeOH (10%).

SCHEME III



(i) $NaBH_4$, 10% Pd on C, MeOH, H_2O (42–45%); (ii) $NaNO_2$ (aq), HOAc; NaN_3 (aq) (43–59%); (iii) 1.0 equiv $(CH_3CO)_2O$, 2.5 equiv DIPEA, DMF (85%); (iv) succinic anhydride (42%); (v) S-acetylthioglycolic acid *N*-hydroxysuccinimide ester, DMF, 45 °C (50%); (vi) NH_2NH_2, MeOH (64%); (vii) N-CBZ-glycine or N-CBZ-β-alanine pentafluorophenyl ester, DMF (63 and 53%); (viii) H_2, 10% Pd on C, HOAc (32 and 81%).

Biology

The results of in vitro testing with the dideoxy-analogs are summarized in Table 1 and in vitro and in vivo testing results with pneumocandin B_0 analogs are summarized in Table 2. Inhibition (IC_{50}) of 1,3-β-(D)-glucan synthesis was determined in a *Candida albicans* membrane assay.[8] Minimum fungicidal concentrations (MFCs) were determined in a whole cell broth microdilution assay.[9] In vivo data were obtained in the target organ kidney assay (TOKA), a mouse model of disseminated candidiasis.[10] Activity is expressed as the minimum dose required for 99.9% reduction of colony forming units vs. sham treated controls ($ED_{99.9}$).

Table 1. Glucan Synthase IC_{50} and Anti-*Candida* Activity of Dideoxy-Analogs

Compound	3′-Substituent	GS IC_{50} (μM)	MFC *C. albicans* (μg/mL)[a]
1b	-H	0.07	0.25
2b	-I	0.1	0.5
4b	$-NO_2$	4.0	2
5	$-CO_2CH_3$	3.0	2
6	(*E*)-$CH{=}CHCO_2CH_3$	0.15	---
7	$-CH{=}CH_2$	0.08	1
8	$-CH_2CH{=}CH_2$	0.12	1
9	$-CH_2CH_3$	0.26	1
10	$-CH_2CH_2CH_3$	0.6	0.5
11	-CHO	>5	1
12	-OH	0.1	0.5
13	$-CH_2CHO$[b]	0.03	1
14	$-CH_2CH_2OH$	0.06	0.5
15 (*E*)	(*E*)-$CH_2CH{=}CHCO_2CH_3$	0.55	1
15 (*Z*)	(*Z*)-$CH_2CH{=}CHCO_2CH_3$	0.45	---
16b	$-CH_2CH_2NH_2$	0.2	---
17b	$-NH_2$	0.05	2
18b	$-N_3$	0.2	4

[a]In vitro activity against *C. albicans* (MY1055). [b]Compound exists in the lactol form.

Several interesting observations regarding the importance of the homotyrosine residue have been made. Introduction of a substituent such as NO_2 (**4a**,**b**), CO_2CH_3 (**5**), or CHO (**11**) at the 3′-position greatly reduced activity against the enzyme. These substituents can form a tight hydrogen bond to the phenolic hydroxyl group, reducing the hydrogen-donating ability of the phenol. The effect on pK_a by these groups cannot explain the reduced activity since *o*-iodophenol and salicaldehyde have similar pK_a's (8.4 and 8.5, respectively), yet **2b** was significantly more potent than **11**. The steric bulk of the substituents cannot account for the difference in activity since the vinyl and formyl analogs **7** and **11** have greatly different IC_{50}'s and yet are similar in size. Finally, the vinylogous analog **6**, which is expected to have a similar pK_a compared to **5** but cannot form a hydrogen bond to the phenol, retained considerable activity against the enzyme. *These results suggest that the phenolic hydroxyl functions as an important hydrogen bond donor at the target site of 1,3-β-(D)-glucan synthase.* However, lactol analog **13** provides an interesting observation. Although the phenolic hydroxyl is blocked, activity is retained suggesting that the lactol hydroxyl is suitably positioned as a hydrogen bond donor.

Severe steric bulk around the phenol is not well tolerated. An especially large effect is seen when both the 3′- and 5′-positions are substituted as in the diiodide **3a** (vs. monoiodide **2a**). In the mono-substituted analogs, it would be possible to rotate the substituent so that it would lie outside a potential binding pocket but di-substitution would preclude the aromatic group from fitting into such a pocket. A more subtle effect can be seen in the comparison of **7** vs. **9** and **8** vs. **10**. The analogs with bulkier saturated substituents have reduced activities. The effect of lipophilicity can be examined (**10** vs. **14** and **16**). Polar analogs (**14** and **16**) show enhanced activity relative to the carbon analog. Polar substituents as in compounds **12**, **17a**, **17b**, and **19** generally retain glucan synthesis inhibitory activity relative to the unsubstituted parent compounds **1a,b**.

Table 2. Glucan Synthase IC_{50} and in vitro and in vivo Anti-*Candida* Activity of Pneumocandin B_0 Analogs

Compound	3'-Substituent	GS IC_{50} (μM)	MFC *C. albicans* (μg/mL)[a]	TOKA $ED_{99.9}$ (mg/kg)[b]
1a	-H	0.07	0.25	6 (20%)[c]
2a	-I	0.1	4	---
3a	3',5'-diiodo	>10	8	---
4a	$-NO_2$	2.9	8	---
17a	-NH2	0.04	2	3 (10%)
18a	$-N_3$	0.2	0.5	---
19	$-NHCOCH_3$	0.09	1	---
20	$-NHCOCH_2CH_2CO_2H$	0.15	0.25	6 (0%)
21	$-NHCOCH_2SH$	0.8	2	---
22	$-NHCOCH_2NH_2$	0.035	0.25	0.75 (40%)
23	$-NHCOCH_2CH_2NH_2$	0.07	0.25	0.78 (0%)

[a]In vitro activity against *C. albicans* (MY1055). [b]In vivo activity against *C. albicans* (MY1055). [c]Numbers in parentheses represent percentage of animals in test group with kidneys that were completely sterilized of *C. albicans* at the ED99.9 dose.

The ionic nature of a 3′-substituent greatly influences activity. In the amido series of analogs, cationic groups (e.g., amine, **22** and **23**) are more favorable than neutral (**18**, **19**, and **21**) or anionic (**20**) substituents. In fact, among the *ortho*-substituted analogs, the glycine (**22**) and β-alanine (**23**) conjugates possessed the best in vivo activity in the TOKA with an eightfold enhancement over the parent compound, pneumocandin B_0. Enhanced activity through amine substitution has been observed elsewhere in the molecule,[11] namely, at the Orn and Gln residues and may be due to a more favorable interaction with the plasma membrane and hence the glucan synthase enzyme. It has since been shown that, in the case of L-733,560, a bis-amine analog of pneumocandin B_0, the improved activity is due to enhanced inhibition of the enzyme and is not due to membrane perturbation.[12]

The importance of the phenolic hydroxyl of the pneumocandins in the inhibition of 1,3-β-(D)-glucan synthesis is evident from the results of this study. Substitution at the position *ortho* to the phenol with bulky or electron-withdrawing substituents that interfere with the hydrogen-bond donating ability of the phenolic hydroxyl decreases activity against the enzyme. A significant enhancement in activity is seen with the introduction of a cationic substituent at the *ortho* position. Enhanced activity has also been seen with the introduction of cationic groups at the C5-Orn and/or Gln residues. Potency of the cationic analogs appears to be due to a change in the overall physical properties of the molecule rather than a specific interaction of these groups at the target site of the enzyme.

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7. The following are representative examples of the iodination and nitration procedures:
   Iodination, Method 1: Compound **1b** (2.00 g, 1.94 mmol) was dissolved in 50 mL of methanol and cooled to 0 °C. Sodium iodide (300 mg, 2.0 mmol) was added followed by aqueous sodium hypocholorite (5.25%, 2.8 mL, 2.0 mmol). The resulting yellow solution was stirred at 0 °C for 2 h. After quenching with aqueous sodium thiosulfate, the solution was concentrated in vacuo to give a dark orange residue. Purification by preparative HPLC (DeltaPak C18, 50/50 water/acetonitrile, 60 mL/min, 230 nm detection) gave 0.85 g (38%) of **2b** as a white solid after lyophilization. **Method 2**: Compound **1a** (0.25 g, 0.24 mmol) was dissolved in 2.5 mL of dimethylformamide and cooled to 0 °C. Iodine monochloride (1 M in CH_2Cl_2, 0.24 mL, 0.24 mmol) was added dropwise to the DMF solution. After 30 min, analytical HPLC (Zorbax C8, 50/50 water acetonitrile, 2 mL/min, 210 nm detection) showed no change in the product ratios (1:1:1 **1a**: mono-iodinated **2a**: diiodinated **3a**). Neutralization with sodium acetate (1 equiv) followed by purification via preparative HPLC (Zorbax C8, 50/50 water/acetonitrile, 10 mL/min, 230 nm detection) gave 30 mg (11%) of monoiodinated product **2a** and 60 mg (19%) of diiodinated product **3a**.
   Nitration: Compound **1a** (2.00 g, 1.88 mmol) was dissolved in 50 mL of glacial acetic acid. An aqueous solution of NaNO2 (1.0 N, 4.0 mL, 4.0 mmol) was added and the mixture was stirred at room temperature for 20 h. The resultant dark yellow solution was concentrated in vacuo to give a dark orange solid. Purification by preparative HPLC (DeltaPak C18, 55/45 to 45/55 water/acetonitrile gradient elution, 230 and 277 nm detection) gave 1.34 g (64%) of **4a** as a yellow solid after lyophilization.
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