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Synthesis and Evaluation of Novel Pseudomycin Side-Chain Analogues. Part 3

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Abstract—To increase the therapeutic utility of C-18 side-chain bearing pseudomycin analogue **2**, we prepared additional analogues and prodrugs of **2** containing further modifications at various positions within its core structure. Each of the newly synthesized derivatives (**10**–**15**) exhibited reduced tail vein toxicity relative to the parent compound. Some of the new pseudomycin derivatives (e.g., **14**) also showed improved in vivo antifungal activity relative to its corresponding parent compound.

Introduction

The rising incidence of serious fungal infections (e.g., systemic fungal infection), especially in the immunocompromised patients, attests to the need for more effective therapies.[1] All current agents have some serious liabilities: inadequate spectrum of activity (e.g., fluconazole), limited dosage forms, narrow therapeutic window (e.g., amphotericin B), and rapid emergence of fungal resistance. Thus the search for new agents, particularly those with a novel mode of action, continues to attract more and more attention from the medical community. These efforts led to the discovery of several new triazole based antifungal agents (e.g., voriconazole,[2a] posaconazole,[2b] BMS-207147,[2c] etc.) as well as several cyclic peptide based novel agents such as aureobasidins,[2d] echinocandin,[2e] FR901469,[2f] and recently disclosed pseudomycins.[3] Compared with the commonly used antifungal drug amphotericin B, pseudomycin B **1** (the most potent member within pseudomycin family) demonstrated improved in vitro and in vivo activity against *Candida albicans* and *Cryptococcus neoformans*.[3c]

To further expand the therapeutic utility of pseudomycin B (e.g., activity against *Aspergillus fumigatus*), we carried out rather extensive SAR modifications at both the hydrophilic core structure[4a] and the lipophilic side-chain portion.[4b,4c] Towards the latter goal, we synthesized and evaluated many types of side-chain analogues of pseudomycin B.[4b,4c] Based on the in vitro antifungal activity data obtained with these newly prepared PSB side-chain analogues, two C-18 aliphatic side-chain bearing analogues (**2** and **3**) exhibited improved activity (up to 8-fold) against *A. fumigatus* relative to PSB **1** (see Fig. 1). Furthermore, compound **2** also showed impressive MIC towards *C. albicans* (MIC = 0.625 μg/mL) and *C. neoformans* (MIC = 0.01 ug/mL) without tail vein toxicity being observed at the dose of $\leq$10 mg/kg. Although slight discoloration and swelling in tail vein irritation assay were observed with **2** at the top dose (20 mg/kg), the level of tail vein irritation detected with **2** was better than that found with the parent PSB **1**. Despite its encouraging in vitro activity, compound **2** exhibited only marginal in vivo activity in the mouse disseminated *Candidiasis* model (@5 mg/kg dose). All animals receiving **2** at higher dose (10 or 20 mg/kg) died shortly after dosing. It is evident that in order to translate its promising in vitro activity into in vivo efficacy, the inherent irritation potential of **2** must be circumvented.

Two main approaches we used to improve the toxicity profile of **2** utilized both analoging and prodruging strategies. The rationales behind these approaches were based upon our recent success achieved through core modifications such as *N*-acylated prodrugs (e.g., **4**–**6**)[5,6] and 3-acid derivatizations (e.g., ester **7** and amides **8**

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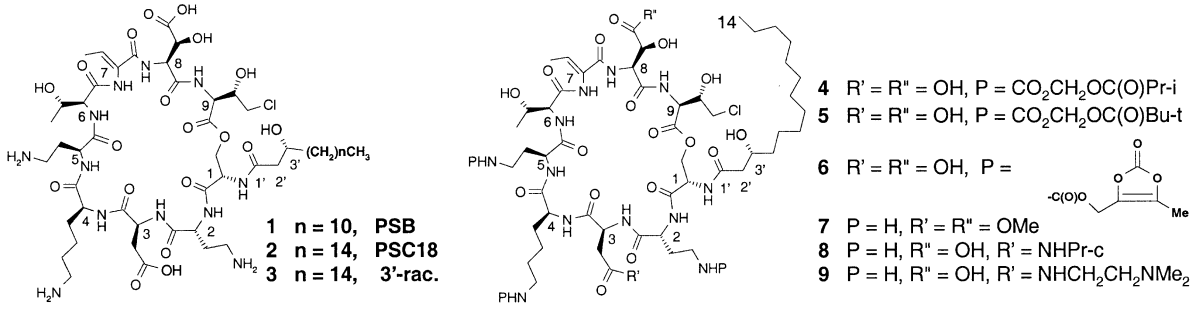


Figure 1. Structures of pseudomycin derivatives.

and **9**).[7,8] All six PSB prodrugs and analogues highlighted herein (**4**–**9**) exhibited impressive in vivo efficacy in the murine *Candidiasis* model without adverse tail vein irritation being detected (see Fig. 1). Thus, to take advantage of these exciting findings, we decided to prepare additional C-18 side-chain bearing derivatives (**10**–**15**) in hopes of discovering novel pseudomycin derivatives possessing improved overall biological and toxicology profiles in comparison to the parent pseudomycin B. Herein, we wish to report the synthesis and preliminary evaluation of these new pseudomycin derivatives (**10**–**15**) (Fig. 2).

Chemical Synthesis

The preparation of three C-18 side-chain bearing pseudomycin prodrugs **10**–**12** is outlined in Scheme 1. In this event, direct *N*-acylation of **2**[4] with the previously reported prodrug linkers[5,6] **17a**, **17b**, and **18** afforded the desired prodrugs **10** (45%), **11** (43%), and **12** (26%), respectively. All three products isolated via preparative HPLC showed proton NMR and mass spectra consistent with the structures reported.

Scheme 2 shows the synthetic sequence employed towards 3- and 8-bisester analogue **13**. Treatment of a methnol solution ZPSN **19** with catalytic amounts of HCl gas (dissolved in THF)[7] afforded the bis-OMe core **20** in high yield. Coupling of **20** with pre-activated HOBt C-18 side-chain ester **21** provided the corresponding adduct **22** (32%), which was further converted to the desired product **13** (52%) via standard hydrogenation.

As shown in Scheme 3, two 3-amido pseudomycin analogues **14** and **15** were synthesized from Cbz-protected intermediate **16** according to the previously established regioselective method.[8] Thus, with the aid of 5 equiv of $EtPr_2N$, TBTU mediated coupling reaction between **16** and *N,N*-dimethylethyldiamine provided the desired 3-amido intermediate **24** (60%). Compound **24** was then converted, upon removal of Cbz protecting groups, to the final product **15** in 87% yield. Following the same two-step sequence, the requisite 3-cyclopropyl derivative **14** was prepared, albeit in low overall yield. The low coupling yield obtained with **23** was in part due to the formation of the 3- and 8-bisamide side product. With the intention to prepare large amounts of **14** for in vivo evaluation, we devised a more efficient synthesis as also shown in Scheme 3. In this case, ZPSN **19** was first transformed into *N*-Boc protected derivative **25**, which was next converted to the 3-cyclopropyl amido nucleus **27** via *N*-Boc protected intermediate **26** in ~28% overall yield. It should be mentioned that the formation of unwanted bisamide was no longer an issue in this case. The 3-monoamide **27** was then converted smoothly to the desired product **14** via previously published side-chain *N*-acylation protocol.[9]

Biological and Tail Vein Toxicity Evaluation

All six newly synthesized C-18 side-chain bearing pseudomycin derivatives **10**–**15** were evaluated in the following three assays: (i) in vitro MIC against *C. albicans*, *C. neoformans* and *A. fumigatus*, the three major fungi responsible for systemic fungal infections; (ii) in vivo against murine disseminated *Candidiasis* mouse model

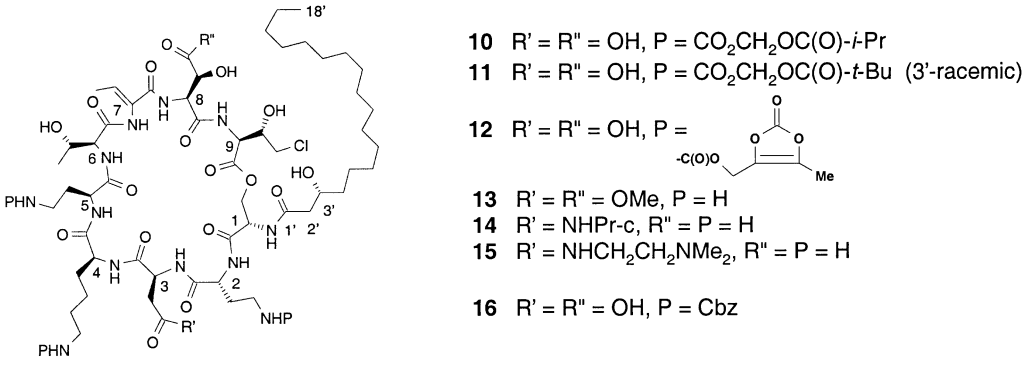
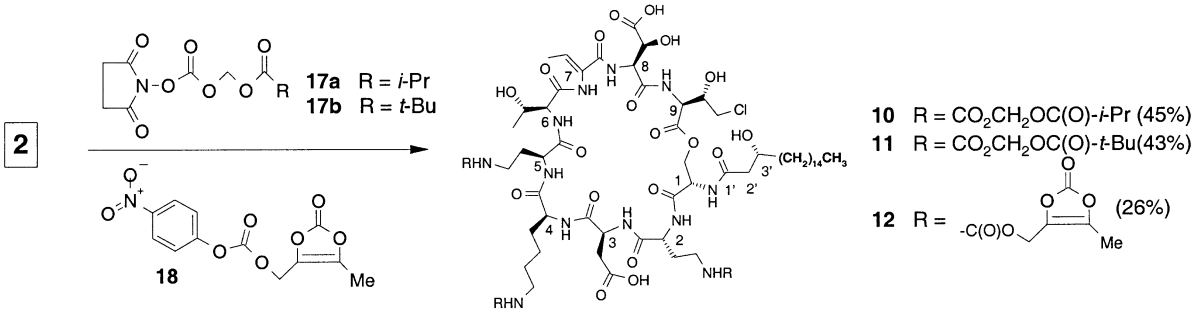
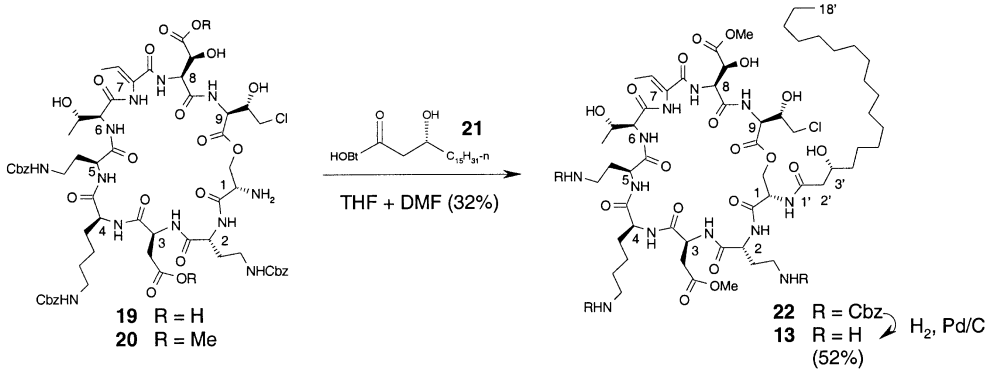


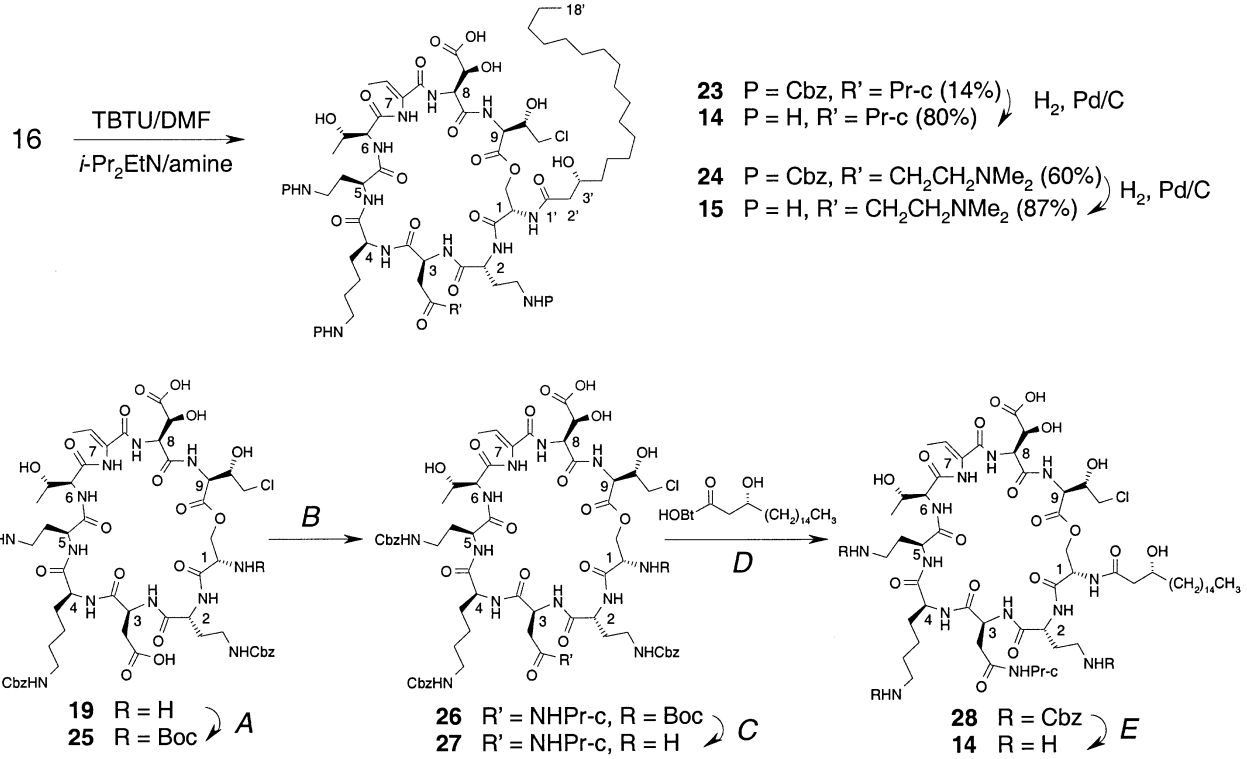
Figure 2. Novel core analogues and prodrugs of compound **2**.



Scheme 1. Synthesis of *N*-acylated prodrugs **10**–**12**.



Scheme 2. Synthesis of ester derivative **13**.



Scheme 3. Synthesis of 3-amido derivatives **14**–**15**. Conditions: (A) Boc_2O/TEA/DMF, 65%; (B) TBTU/c-PrNH_2/*i*-Pr_2EtN/DMF, 28%; (C) TFA/CH_2Cl_2, 95%; (D) **21**/DMF + THF, 35%; (E) H_2/Pd/C/1% HOAc/MeOH, 70%.

Table 1. In vitro and in vivo evaluation of pseudomycin derivatives **1–15**

Entry	Compd	MIC (μg/mL)			In Vivo (mg/Kg×4)	Tail vein toxicity (20 mg/kg)
		C. albicans	*C. neoformans*	*A. fumigatus*	ED_{50} (anal.)/ED_{50} (PSB)	
A	**1**	0.625	0.039	20	2.0–7.0	Positive
B	**2**	0.625	0.01	2.5-5.0	>20[a]	Positive
C	**3**	1.25	0.02	5.0	>20	Positive
D	**4**	>20	>20	>20	11.4 (7.0)	Negative
E	**10**	>20	>20	>20	>14	Negative
F	**5**	>20	>20	>20	6.4 (2.8)	Negative
G	**11**	>20	>20	>20	>20	Negative
H	**6**	>20	>20	>20	7.8 (3.8)	Negative
I	**12**	>20	>20	>20	>19	Negative
J	**7**	2.5	0.01	10-20	7.8 (2.8)	Negative
K	**13**	5.0	1.25	20	>20	Negative
L	**8**	0.156	<0.01	>20	<5.0 (6.6)	Negative
M	**14**	2.5-5.0	0.039	5.0	67% survival extension @ 10 mg/kg dose[b]	
N	**9**	0.156	<0.01	5.0	<5.0 (4.5)	Negative
O	**15**	5.0	0.156	20	>20	Negative

MIC: Lowest drug concentration required to inhibit 90–100% of visible fungal growth compared to controls. ED_{50}: Drug concentration required to achieve 50% survival of fungal infection compared with untreated animals.
[a]Extension of survival was recorded at low dose [5 (~20%) mg/kg].
[b]Extension of survival was observed at all doses [5 (37%), 10 (67%) and 20 (22%) mg/kg].

(ip); and (iii) in vivo tail vein toxicity assay in healthy mice. The detailed testing protocols for these assays are described in refs 6 and 8. The antifungal activity and tail vein toxicity profiles of the reference compounds (**1–9**) are included in Table 1 for comparison. The ED_{50} values reported were calculated using the method of Reed and Muench.[10]

As documented in Table 1, compound **2** showed similar MIC against *C. albicans* and *C. neoformans* to that found with PSB **1**. In addition, compound **2** exhibited improved in vitro activity against *A. fumigatus* relative to its parent compound. As also shown in Table 1, three *N*-acylated pseudomycin B prodrugs **4–6** displayed slightly reduced in vivo efficacy relative to the parent PSB. However, unlike PSB **1**, prodrugs **4–6** were found to be free of tail vein irritation. As expected, all three C-18 side-chain bearing prodrugs **10**, **11**, and **12** exhibited reduced in vitro activity relative to analogue **2**. In contrast to their corresponding parent **2**, compounds **10–12** did not induce tail vein irritation. Disappointingly however, none of the newly synthesized prodrugs **10–12** demonstrated significant in vivo efficacy.

As listed in entries J and K, both 3,8-bisester derivatives **7** and **13** displayed excellent safety profile in the tail vein irritation assay. When evaluated in the in vivo, unlike its C-14 side-chain bearing counterpart **7** (ED_{50} < 5.0 mg/kg), the newly prepared analogue **13** failed to show significant in vivo efficacy.

As shown in entries L and N, two 3-amido PSB analogues **8** and **9** exhibited comparable in vitro and in vivo activity to that observed with PSB, without adverse tail vein irritation. More interestingly, 3-dimethylaminoethyl-amdo bearing analogue **9** displayed improved in vitro activity against *Aspergillus* than the parent. The 3-cyclopropylamido analogues **14** showed not only impressive balanced in vitro activity against all three major fungi responsible for systemic fungal infections but also a clean result in tail vein toxicity assay. When evaluated against murine disseminated *Candidiasis* model, 67 and 37% extension of survival was obtained with mice receiving **14** at the dose of 10 and 5 mg/kg, respectively. This level of in vivo activity observed with **14** is in fact better than that obtained with its parent analogue **2**. Careful comparison of animal death pattern observed with **2** and **14** revealed that 3-amido analogue **14** was less toxic than **2**. On the other hand, 3-dimethylaminoethyl amide **15** possessed weaker in vitro potency in comparison to **2**. When tested in vivo, compound **15** was devoid of both tail vein irritation as well as antifungal efficacy.

In summary, we have synthesized six C-18 side-chain bearing pseudomycin derivatives. These include four prodrugs (**10–13**) and two analogues (**14** and **15**). Unlike parent compound **2**, all six newly synthesized derivatives were free of tail vein irritation (at 20 mg/kg dose). In contrast to the pseudomycin B prodrugs (**4–6**),[5,6] three *N*-acylated prodrugs of **2** (**10–13**) failed to demonstrate significant antifungal activity in vivo. Consistent with our previously finding with PSB,[8] 3-amidation on compound **2** led to the 3-cyclopropyl amido analogue **14** with improved in vivo efficacy as well as a reduction in tail vein toxicity in comparison to its parent **2**.

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