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8-Amido-Bearing Pseudomycin B (PSB) Analogue: Novel Antifungal Agents

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Abstract—During the course of a structure–activity relationship (SAR) study on novel depsinonapeptide pseudomycin B, we synthesized a total of 12 8-amidopseudomycin analogues via standard two-step sequence from either ZPSB **2** or AllocPSB **3**. A number of these amides exhibited good in vitro antifungal activities. © 2001 Published by Elsevier Science Ltd.

Introduction

The increasing incidence of systemic fungal infections in hospitalized patients, coupled with the shortage of effective and safe antifungal agents have stimulated renewed research interests in search for broad spectrum antifungal agents. Clinically, *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus* are the three major pathogens responsible for the life-threatening systemic fungal infections. Despite the severe side effects or narrow spectrum of activity, amphotericin B (AMB) and several triazole based drugs (e.g., fluconazole) still remain as the limited available drugs for the treatment of systemic fungal infections.[1] Clearly, the discovery and development of novel broad spectrum and safe antifungal agents constitutes a key step towards curing these fungal infections. Towards this end, we were inspired by several reports by Strobel et al.[2] detailing the preliminary biological profiles of pseudomycins, a novel class of depsinonapeptides produced by *Pseudomonas syringea* in plants. Within the pseudomycin family, pseudomycin B (PSB **1**) was identified as the most potent member, which showed excellent activity against all strains of *Candida* as well as *Cryptococcus*.[3,4] It also demonstrated weak activity against *Aspergillus fumigatus*.[3,4] In spite of its promising antifungal activity, the development of natural product pseudomycin B (PSB) as a therapeutic agent was complicated by its irritation potential at the injection site.[4] To circumvent this irritation potential found with PSB, we carried out rather extensive structure–activity relationship (SAR) investigations on both the pseudomycin side chain[5] and the cyclic lipopeptide core structure[6] in the hope of improving the toxicity profile of PSB by means of modification of the acid functions on residues 3 and 8. In a recent disclosure from this laboratory, we reported a 3-imido and an intramolecular lactone bearing (linking 8α-hydroxyl group and the acid function at residue 3) derivatives of PSB that were devoid of in vitro antifungal activity as well as tail vein toxicity.[6] To continue the core modification, we prepared a series of 8-amido containing pseudomycin B analogues. These new PSB analogues were designed based on the recent findings from Bruzzese et al., who reported that some amido-derivatives of amphotericin B exhibited reduced toxicity relative to the parent drug.[7] In this communication, we wish to report the synthesis and preliminary evaluation of these 8-amides **4**–**15** shown in Figure 1.

Chemical Synthesis

Attempted regioselective synthesis of 8-amides from either ZPSB **2** or AllocPSB **3** using HOBt/EDCI protocol was unsuccessful. In this case, almost equal amounts of 3-amides, 3,8-diamides, along with 8-amides were produced. Separation of the desired 8-amides from their respective 3-amides proved to be nontrivial. After careful survey of the peptide coupling reagents, we discovered that replacement of HOBt/EDCI with PyBOP

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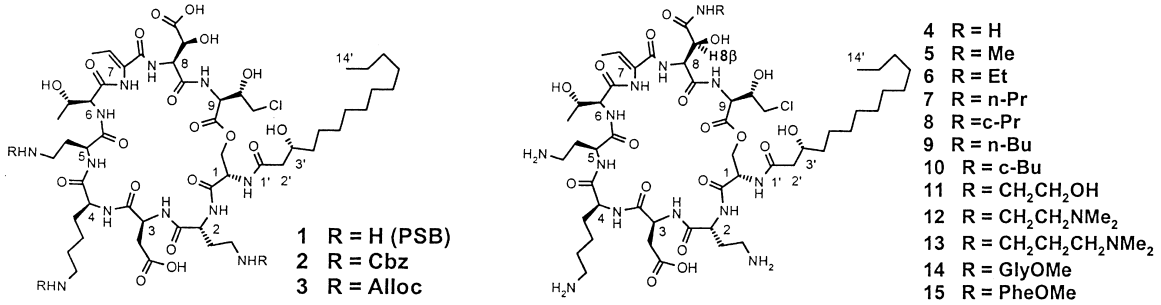

Figure 1. Structures of pseudomycin analogues.

Table 1. Yields and molecular weight of the 8-amidopseudomycin analogues **4**–**15**

Product	Method of preparation	Yields (%)		Molecular formula	Molecular weight[a,b,c]
		Coupling	Deprotection		
4	A	10	68	$C_{51}H_{88}ClN_{13}O_{18}$	1207
5	C	91	94	$C_{52}H_{90}ClN_{13}O_{18}$	1221
6	C	78	75	$C_{53}H_{92}ClN_{13}O_{18}$	1235
7	B	34	91	$C_{54}H_{94}ClN_{13}O_{18}$	1249
8	C	49	62	$C_{54}H_{92}ClN_{13}O_{18}$	1247
9	B	28	86	$C_{55}H_{96}ClN_{13}O_{18}$	1263
10	C	44	58	$C_{55}H_{94}ClN_{13}O_{18}$	1261
11	C	71	46	$C_{53}H_{92}ClN_{13}O_{19}$	1251
12	C	95	74	$C_{55}H_{97}ClN_{14}O_{18}$	1278
13	C	39	73	$C_{56}H_{99}ClN_{14}O_{18}$	1292
14	B	40	83	$C_{54}H_{92}ClN_{13}O_{20}$	1278
15	B	12	61	$C_{61}H_{98}ClN_{13}O_{20}$	1369

[a]Method A: **2**/PyBOP/Rink resin; TFA; H_2/10%Pd/C in 1%HOAc/MeOH.
[b]Method B: **2**/PyBOP/DMF; H_2/10%Pd/C in 1%HOAc/MeOH.
[c]Method C: **3**/PyBOP/DMF; $Bu_3SnH/PdCl_2(PPh_3)_2$/HOAc/THF.

led to the expected regioselective 8-amidation. Following this new route, a total of 12 8-amides **4**–**15** were prepared from either **2** or **3**. In all 12 examples listed in Table 1, the desired 8-amides were isolated as the major products along with their corresponding 3-amides.[8] The regioselectivity for 8-amidation over 3-amidation was at least greater than 5:1. As indicated in Table 1, seven 8-amides (**5**, **6**, **8**, and **10**–**13**) were prepared from AllocPSB **3**, whereas the remaining five 8-amides (**4**, **7**, **9**, **14**, and **15**) were synthesized from ZPSB **2**. It should be mentioned that compound **4** was prepared using Rink resin via solid-phase chemistry. In most cases, the yields for coupling reactions ranged from 40 to 95%. The low coupling yields obtained with **4** and **15** were probably due to the bulky amines used in both cases. The final deprotection step was carried out using either hydrogenation (for Cbz-protected analogues)[9] or Bu_3SnH/Pd(0) mediated deallylation (for Alloc protected ones).[10] As also shown in Table 1, satisfactory mass spectra were obtained for all 8-amides prepared. Along with PSB, the detailed proton assignments for 8-methylamide **5** were listed in Table 2. The structural assignments of **5**, **8**, **12**, and **14** were secured based on the interactions between the 8-NHR protons with their corresponding H8β protons observed in the ROSEY experiments (see Fig. 1 for structures). The structures for the remaining 8-amido PSB derivatives were assigned in a similar fashion.

Table 2. Proton NMR assignments for **1**[a] and **5**[b]

Positions	PSB 1	5	Positions	PSB 1	5
Residue 1			Residue 5		
α	4.59	4.54	α	4.29	4.24
β1	4.38	4.40	β1	1.99	1.99
β2	4.53	4.51	β2	2.14	2.13
Residue 2			γ1	2.89	2.91
α	4.13	4.16	γ2	2.91	2.91
β1	2.01	2.00	Residue 6		
β2	2.07	2.07	α	4.27	4.32
γ1	2.90	2.91	β	3.92	3.91
γ2	2.97	2.97	γ	1.18	1.16
Residue 3			Residue 7		
α	4.55	4.63	β	6.53	6.36
β1	2.82	2.85	γ	1.70	1.70
β2	2.87	2.85	Residue 8		
Residue 4			*NHC(O)**Me***	—	2.66
α	4.13	4.20	α	4.96	4.86
β1	1.75	1.72	β	4.75	4.60
β2	1.78	1.78	Residue 9		
γ1	1.26	1.30	α	4.87	4.86
γ2	1.32	1.35	β	4.31	4.32
δ1	1.53	1.55	γ1	3.44	3.45
δ2	1.56	1.56	γ2	3.50	3.52
ε	2.84	2.85			
Side chain			Side chain		
2′α	2.25	2.26	4′	1.38	1.40
2′β	2.36	2.40	5′-13′	~1.22	~1.23
3′	3.86	3.90	14′	0.83	0.83

[a] ^1H NMR of **1** was recorded in acetonitrile-d_3/D_2O/CF_3CO_2D.
[b] ^1H NMR of **5** was recorded in acetonitrile-d_3/D_2O.

Table 3. In vitro and in vivo antifungal activity of novel 8-amidopseudomycin derivatives

Compd.	R	MIC[a] (μg/mL)			ED_{50}[b] (ip) (mg/kg × 4)	Tail vein (iv) assay
		C. albicans	*C. neoform.*	*A. fumigatus*		
4	H	0.312	< 1	>20	>20	Clean
5	Me	1.25	0.156	>20	>20	Clean
6	Et	10	1.25	>20	—	—
7	*n*-Pr	20	5.0	>20	14	Clean
8	*c*-Pr	5.0	0.312	>20	>20	Clean
9	*n*-Bu	20	1.25	>20	>20	Clean
10	*c*-Bu	5.0	1.25	>20	>20	Clean
11	CH_2CH_2OH	5.0	0.625	>20	—	Clean
12	$CH_2CH_2NMe_2$	5.0	1.25	>20	>20	Clean
13	$(CH_2)_3NMe_2$	20	1.25	>20	—	—
14	GlyOMe	2.5	0.08	>20	>20	Clean
15	PheOMe	10	1.25	>20	>20	Clean
PSB	—	0.625	0.078	>20	2.4–7.2	Positive

[a]MIC: Lowest drug concentration required to inhibit 90–100% of visible fungal growth compared to controls.
[b]ED_{50}: Drug concentration required to cure 50% of fungal infection compared with untreated animals.

Biological evaluation

In vitro assays

All 12 8-amides were tested against *C. albicans* (*A26*), *C. neoformans* (*M1 106*), and *A. fumigatus* (*WM1*), three major fungi responsible for systemic fungal infections. As shown in Table 3, all 8-amides showed excellent activity towards *C. neoformans,* with MIC values in the range of <0.01 to 1.25 μg/mL. However, none of these analogues exhibited better activity against this pathogen than that achieved by PSB. Based on the results shown in Table 3, it is also evident that all 12 8-amides exhibited only weak activity against *A. fumigatus*. When these newly synthesized 8-amides were assayed against *C. albicans*, several trends can be gleaned from the data accumulated so far: (1) The in vitro potency decreases according to the following order: **4** (R = H) > **5** (R = Me) > **6** (R = Et) > **7** (R = *n*-Pr) = **9** (R = *n*-Bu); **12** > **13**; **14** (GlyOMe) > **15** (PheOMe). Evidently, better activities were found with those bearing smaller alkyl groups. In fact, compound **4** (R = H) was identified as the most potent analogue within this series, being 2-fold more potent than PSB **1**. (2) *c*-Alkyl amides **8** and **10** displayed better activity than their corresponding *n*-alkyl bearing counterparts **7** and **9**, respectively. (3) Polar termini bearing analogues **11**–**13** were found to be at least 8-fold less active than the parent.

Tail vein irritation

Selective members of the novel 8-amides were evaluated in the tail vein toxicity assay in vivo. In this experiment, the testing compounds (formulated in 5% dextrose and sterile water) were given to mice (two per compound) with a single intravenous injection at 75, 50, and 25 mg/kg. Following dosing, mice were observed closely for clinical signs of histamine induced pathology. As briefly outlined in Table 3, in sharp contrast to the parent, all 10 8-amides evaluated (**4**, **5**, **7**, **8**, **9**, **10**, **11**, **12**, **14**, and **15**) failed to induce tail vein irritation. It is particularly interesting to note that compound **4**, the bioisostere of the parent, was devoid of tail vein irritation while retaining excellent in vitro antifungal activity.

In vivo efficacy study

Most of the newly synthesized 8-amides were evaluated for their in vivo efficacy against murine systemic *Candidiasis*. Experimentally, mice were first infected by an intravenous injection in the lateral tail vein. The testing compounds (formulated in 4% hydroxypropyl cyclodextrin, sodium acetate pH 7 buffer and 1.75% dextrose) were given to mice (six per group) four times at 0, 4, 24, and 48 h post-infection. Infected sham-treated mice (10 animals) were dosed with the vehicle alone. Untreated controls were moribund within 3–4 days post-infection. The 50% effective dose (ED_{50}) was determined using the method of Reed and Muench.[11] Judging from the data listed in Table 3, it is rather disappointing to see that none of the 8-amides tested showed comparable in vivo efficacy to that achieved by pseudomycin B **1**. The reason for the lack of correlation between in vitro potency and in vivo efficacy is currently unclear.

In summary, we completed the synthesis of a novel series of 8-amido-bearing pseudomycin B analogues. These analogues displayed very different in vitro activity, ranging from 2-fold more potent to 30-fold less potent than the parent compound. It is encouraging to note that the 8-NH_2 containing analogue **4**, the bioisostere of PSB **1**, was found to be more potent than the parent yet devoid of tail vein irritation. On the basis of our testing results, it is also evident that the formation of a new amide bond at the hydroxylated aspartic acid (residue 8) was beneficial for improving toxicity profile, yet detrimental to in vivo efficacy.

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References and Notes

1. Kerridge, D. *Antifungal Therapy: Advances and Opportunities*; Connect Pharma: Oxford, 1992; pp 1–96.
2. (a) Harrison, L.; Teplow, D. B.; Rinald, M.; Strobel, G. A. *J. Gen. Microbiol.* **1991**, *137*, 2857. (b) Ballio, A.; Bossa, F.; Giorgio, D. D.; Ferranti, P.; Paci, M.; Pucci, P.; Scaloni, A.; Serge, A.; Strobel, G. A. *FEBS Lett.* **1994**, *355*, 96.
3. (a) Strobel, G. A.; Harrison, L. A.; Teplow, D. B. US Patent No. 5,576,298, 1996. (b) Strobel, G. A.; Harrison, L. A.; Teplow, D. B. US Patent No. 5,837,685, 1997.
4. Rodriguez, M. J.; Current, W. Presented at 14th Congress of the International Society for Human and Animal Mycology (ISHAM), Buenos Aires, Argentina, 8–11 May, 2000, Abstract No. 0270.
5. Jamison, J.; Levy, S.; Sun, X.; Zeckner, D.; Current, W.; Zweifel, M.; Rodriguez, M.; Turner, W.; Chen, S. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2101.
6. Zhang, Y.; Boyer, R.; Sun, X.; Paschal, J.; Chen, S. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 775.
7. Bruzzese, T.; Rimaroli, C.; Bonabello, A.; Ferrari, E.; Signorini, M. *Eur. J. Med. Chem.* **1996**, *31*, 965.
8. In contrast to the 8-amide series, most of the 3-amido-pseudomycin B analogues synthesized exhibited excellent in vivo efficacy. The detailed biological profiles of these 3-amides will be published elsewhere.
9. Deprotection of Cbz group(s) was conducted in 1–10% HOAc/MeOH using 10% Pd/C as catalyst.
10. Guibe, O. D. F.; Balavoine, G. *J. Org. Chem.* **1987**, *52*, 4984.
11. Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493.