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Prodrugs of 3-Amido Bearing Pseudomycin Analogues: Novel Antifungal Agents

Xicheng Sun,[†] Douglas J. Zeckner, Yan-zhi Zhang,[‡] Roberta K. Sachs, William L. Current, Michael Rodriguez and Shu-Hui Chen*

Lilly Research Laboratory, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

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Abstract—With the aim of identifying safer pseudomycin derivatives, we synthesized and evaluated a number of *N*-acyloxymethyl carbamate linked prodrugs of 3-amido pseudomycin analogues. To our satisfaction, all of the prodrug-amide combinations prepared exhibited good in vivo efficacy against murine *Candidiasis*. When evaluated in a dose elevation study, all of the newly synthesized combinations (e.g., **4A**, **6A**, **8A**, and **8B**) demonstrated improved toxicity profiles in comparison to their corresponding 3-amides as well as the parent pseudomycin B.

Introduction

The medical need for effective and safe antifungal agents has been intensified in recent years due to rapidly growing population of immunocompromised patients including those infected with AIDS and those receiving organ transplantation. Clinically, *Candida albicans, Cryptococcus neoformans*, and *Aspergillus fumigatus* are the most important fungi responsible for the majority of hospitalized patients suffering from systemic fungal infections (SFI).[1] Although amphotericin B (AMB) and fluconazole are the most widely used drugs for the treatment of SFI, the effectiveness of these drugs is limited by severe toxicity (e.g., AMB),[2] rapid development of resistance and narrow spectrum of activity (especially against *A. fumigatus* as observed with fluconazole).[3] In light of this urgent medical need, we became interested in the discovery and development of structurally unique novel agents for the treatment of systemic fungal infections.[4] Towards this end, we recently reported biological and toxicity profiles of a series of naturally occurring lipopeptides, pseudomycin A and B.[5,6] When evaluated against *C. albicans* and *C. neoformans*, both in vitro and in vivo, pseudomycin B (PSB) demonstrated superior activity to that achieved by amphotericin B.[6,7] Unfortunately, however, despite its promising antifungal activity, the development of pseudomycin B as a therapeutic agent was quickly discontinued due mainly to the irritation potential found at the injection site. With the aim to circumvent the toxicity issues associated with PSB, we synthesized and evaluated a series of *N*-acylated prodrugs (e.g., **2A** and **2B**)[8] and 3-amido pseudomycin B analogues (e.g., **4**, **6**, and **8** shown in Fig. 1).[9] When compared with the parent, these recently reported PSB prodrugs and 3-amido analogues exhibited comparable in vitro activity (for 3-amides) and in vivo efficacy. Some of these prodrugs and 3-amides were found to be free of tail vein irritation (e.g., **2A**, **2B**, and **4**).[8–10] Encouraged by these findings, we designed a series of *N*-acyloxymethyl carbamate linked prodrugs of 3-amides as novel antifungal agents. It is hoped that the amide-prodrug combinations described herein would possess even greater safety profiles than their corresponding 3-amides and the parent PSB. In this paper, we wish to disclose the synthesis and preliminary evaluation of these newly prepared compounds **3A**–**8B** as shown in Figure 1.

Chemical Synthesis

Following our recently developed regioselective method,[9] all of the combinations (**3A**–**8B**) (Table 1) were synthesized from PSB **1** via the following two-step sequence: (1) *N*-acylation of PSB with the requisite *iso*-propyl or *tert*-butyl prodrug linkers; (2) TBTU and $EtPr_2N$ mediated 3-amidation.[8,9] Whilst the isolated

*Corresponding author. Tel.: +1-317-276-2076; fax: +1-317-276-5431; e-mail: chen_shu-hui@lilly.com

[†]Current address: Array Biopharma Inc., 1885 33rd St., Boulder, CO 80301, USA.

[‡]Current address: Cubist Pharmaceuticals, Inc., 24 Emily St., Cambridge, MA 02139, USA.

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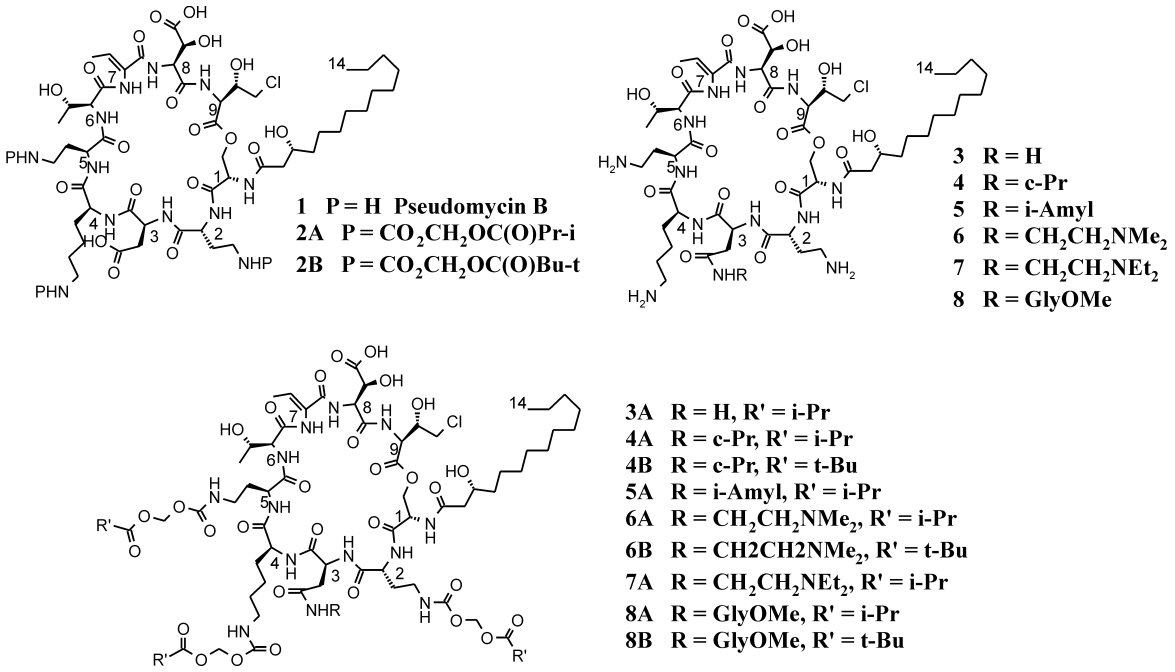

Figure 1. Structures of pseudomycin B prodrugs and analogues.

yields of the *N*-acylated products (**2A** and **2B**) ranged between 60 and 70%, the 3-amidation reactions generally afforded the desired products in low to modest yields along with various amounts of recovered **2A** or **2B**. It is important to mention that the desired *N*-acylated 3-amides (**3A**–**8B**) were obtained always as the predominant products. The selectivity of 3-amide[9]/8-amide[11] was usually greater than 20:1 when TBTU was used as the coupling reagent. In contrast, the use of PyBOP as the coupling reagent led to the production of 8-amido PSB derivatives as the major products.[11] Owing to their structural similarities, the composition of all combo prepared were confirmed by their mass spectra analyses (for all) and also by proton NMR analysis (for **4A**). The key chemical shifts of **4A** and PSB **1** are listed in Table 2. On the basis of the upfield shift ($\sim$0.20 ppm) of 3β and 3β′ protons found with **4A**, it is evident that the cyclopropyl amido moiety in **4A** was indeed attached to residue 3 of the depsipeptide core.[9]

Table 1. Yield and molecular weight of prodrugs 3-amido PSB analogues **3A**–**8B**

Compd	Linker	3-Amido R	Yield (%)	Molecular formula	M_r
3A	*i*-Pr	H	26	$C_{69}H_{112}ClN_{13}O_{30}$	1639
4A	*i*-Pr	c-Pr	40	$C_{72}H_{116}ClN_{13}O_{30}$	1679
4B	*t*-Bu	c-Pr	22	$C_{75}H_{122}ClN_{13}O_{30}$	1721
5A	*i*-Pr	*i*-Amyl	35	$C_{74}H_{112}ClN_{13}O_{30}$	1709
6A	*i*-Pr	$CH_2CH_2NMe_2$	39	$C_{73}H_{121}ClN_{14}O_{30}$	1710
6B	*t*-Bu	$CH_2CH_2NMe_2$	45	$C_{76}H_{127}ClN_{14}O_{30}$	1752
7A	*i*-Pr	$CH_2CH_2NEt_2$	50	$C_{75}H_{125}ClN_{14}O_{30}$	1738
8A	*i*-Pr	GlyOMe	50	$C_{72}H_{116}ClN_{13}O_{32}$	1711
8B	*t*-Bu	GlyOMe	$\sim$50	$C_{75}H_{122}ClN_{13}O_{32}$	1753

Table 2. Selected chemical shift assignments of **4A** and PSB (ppm)

Compd	3β, 3β′	8β	3-Cyclopropyl	Prodrug linker (*i*-Pr)
4A	**2.61, 2.70**	4.72	2.53, 0.39, 0.60	5.59, 2.48–2.57, 1.05–1.09
PSB	2.82, 2.87	4.75	None	none

Biological Evaluation

All of the prodrug-amide combinations (**3A**–**8B**) were evaluated in vitro and in vivo for assessments of their antifungal activities. *C. albicans*, *C. neoformans*, and *A. fumigatus* were included in the in vitro testing. The MIC values reported were defined as the lowest drug concentration required to inhibit 90–100% visible growth compared to controls. Following the previously established protocols,[8–11] all of the combo discussed herein were examined in the murine *Candidiasis* model (ip). A few selected compounds (**4A**, **6B**, and **8B**) were also evaluated in the murine *Cryptococosis* model (ip). The ED_{50} values reported were calculated using the method of Reed and Muench.[12] To evaluate the toxicity profiles of these newly prepared PSB combinations, we used the tail vein irritation assay and the dose elevation study, both in mice. The protocols used for these two assays are identical to those described in ref 9 of this manuscript.

The in vitro activity, in vivo efficacy, and tail vein toxicity profiles of three 3-amido PSB analogues (**3**, **5**, and **7**) and their corresponding *N*-acylated prodrugs (**3A**, **5A**, and **7A**) are listed in Table 3. As expected, all three prodrugs (**3A**, **5A**, and **7A**) showed markedly reduced in vitro activity in comparison to their parent amides. When compared with pseudomycin B, all 3-amides (**3**, **5**, and **7**) displayed essentially similar in vivo efficacy against *Candidiasis* with ED_{50} values ranging from <4.8 to 10.9 mg/kg. Unlike most of the 3-amido PSB analogues reported in our recent publication,[9] three 3-amides shown in Table 3 were capable of inducing tail vein irritation.[9] In contrast to the 3-amides discussed herein

Table 3. Biological and toxicity profiles of **3**, **5**, **7**, and their *N*-acylated prodrugs

Compd	Prodrug linker	3-Amido R	MICC (μg/mL)			*Candidiasis* ED_{50} (mg/kg)	Tail vein toxicity
			C. albicans	*C. neoformans*	*A. fumigatus*		
3A	*i*-Pr	H	20	20	20	<4.0	No
3	—	H	2.5	<0.01	10	<4.8	Yes
5A	*i*-Pr	*i*-Amyl	20	20	20	13.0	No
5	—	*i*-Amyl	0.312	<0.01	>20	10.9	Yes
7A	*i*-Pr	$CH_2CH_2NEt_2$	20	20	20	5.9	No
7	—	$CH_2CH_2NEt_2$	10	<0.01	10	4.9	Yes
PSB	—	—	0.625	0.01	20	2.8–8.4	Yes

Table 4. Biological and toxicity profiles of **4**, **6**, **8**, and their *N*-acylated prodrugs

Compd	Prodrug linker	3-Amido R	*Candidiasis* ED_{50} (mg/kg)	*Cryptococosis* ED_{50} (mg/kg)	Tail vein toxicity	Dose elevation (mg/kg)
4A	*i*-Pr	c-Pr	<5.0	0.56	No	125
4B	*t*-Bu	c-Pr	<5.0	N.T.	No	N.T.
4	—	c-Pr	<5.0	2.9	No	50
6A	*i*-Pr	$CH_2CH_2NMe_2$	6.6	N.T.	No	125
6B	*t*-Bu	$CH_2CH_2NMe_2$	<5.0	0.44	No	N.T.
6	—	$CH_2CH_2NMe_2$	<5.0	2.5	No	50
8A	*i*-Pr	GlyOMe	<5.0	N.T.	No	125
8B	*t*-Bu	GlyOMe	<5.0	>5.0	No	125
8	—	GlyOMe	<5.0	2.9	No	<50
PSB	—	—	2.8–8.4	1.4–1.8	Yes	20

(**3**, **5**, and **7**), all three respective combinations (**3A**, **5A**, and **7A**) exhibited identical in vivo efficacy to their parent analogues, yet without inherent tail vein irritation being observed. Thus, the results outlined in Table 3 clearly demonstrate the improved toxicity profiles of the prodrug-amide combinations (**3A**, **5A**, and **7A**) over their corresponding 3-amido PSB analogues as well as the parent drug PSB itself.

The in vivo efficacy and toxicity profiles of 3-cyclopropylamide **4**, 3-dimethylaminoethylamide **6**, 3-GlyOMe **8** and their respective *N*-acylated prodrugs are summarized in Table 4. As reported in our previous communication,[9] compounds **4**, **6**, and **8** displayed excellent in vitro activity against *Candida* and *Cryptococcus*. Basic-termini-bearing 3-amide **6** was also found to be 4-fold more potent toward *Aspergillus* than PSB in vitro. When tested against murine *Candidiasis* and *Cryptococosis* in vivo, all three amides (**4**, **6**, and **8**) demonstrated impressive efficacy comparable to that achieved by the parent PSB. Unlike the parent compound, all 3-amides listed in Table 4 were devoid of tail vein irritation.[9] Judging from their maximum tolerated dose found in the dose elevation study (~50 mg/kg), it is clear that amides **4**, **6**, and **8** were about 2.5-fold safer than PSB. Encouraged by the overall balanced biological and toxicity profiles obtained with **4**, **6**, and **8**, we further evaluated six of their prodrug combinations shown in Table 4. As predicted, all combinations tested displayed relatively poor in vitro potencies (data not shown). When evaluated in vivo, all six combinations (**4A/B**, **6A/B**, **8A/B**) exhibited excellent efficacy against murine *Candidiasis* with ED_{50} values ranging between <5.0 and 6.6 mg/kg. Three of such combinations (**4A**, **6B**, and **8B**) were further tested in the murine *Cryptococosis* model. As outlined in Table 4, the ED_{50} values for **4A** and **6B** were found to be about 5-fold lower than that achieved by their corresponding 3-amides **4** and **6**, respectively. In view of the exciting antifungal activity observed with these combinations, we selected four such compounds (**4A**, **6A**, **8A**, and **8B**) for further evaluation in the dose elevation study in mice (iv) at the following four doses: 50, 75, 100, and 125 mg/kg. To our satisfaction, the testing results showed that compounds **4A**, **6A**, **8A**, and **8B** were well tolerated at the top dose tested (125 mg/kg). Evidently, the newly synthesized prodrug-amide combinations exhibited even greater safety profiles than their corresponding parent 3-amides.

In conclusion, we succeeded in the preparation of novel *N*-acylated prodrugs of 3-amido pseudomycin B via a concise two-step sequence. All of the prodrugs evaluated showed very comparable in vivo activities against murine *Candidiasis* and *Cryptococosis* (for **4A**, **6B**, and **8B**) to that achieved by their parents, yet without inherent tail vein irritation. Furthermore, better therapeutic indices were obtained with such prodrug-amide combinations (e.g., **4A**, **6A**, **8A**, and **8B**) than that of their correponding 3-amides. Thus, considering all of the data presented herein together, we are convinced that it is possible to further improve the toxicity profiles of PSB analogues via prodrug approaches.

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