SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A NOVEL ANTIFUNGAL AGENT, AZOXYBACILIN

Jun OHWADA, Isao UMEDA, Hitomi ONTSUKA, Yuhko AOKI, and Nobuo SHIMMA*

Nippon Roche Research Center, 200 Kajiwara, Kamakura 247, Japan

A new antifungal substance, azoxybacilin (an unusual amino acid with an azoxy moiety) and its derivatives have been synthesized from Boc-L-Asp-O^{t}Bu utilizing the Moss procedure for the preparation of the azoxy moiety. The ester derivative, Ro 09-1824, showed more potent antifungal activity and a broader antifungal spectrum than azoxybacilin did.

KEYWORDS azoxybacilin; antifungal; synthesis; structure-activity relationship

Azoxybacilin, a new antifungal substance having an azoxy moiety, was isolated from *Bacillus cereus* NR2991.[1] It inhibits methionine biosynthesis at the sulfur fixation step, and shows selective toxicity to fungi.[2] Azoxybacilin exhibits potent antifungal activity *in vitro* (in an amino acid free medium) especially against mycelial fungi such as *Aspergillus* spp. and *Trichophyton* spp., but not against most yeast type fungi such as *Cryptococcus neoformans*. It is only weakly active in the systemic infection model with *Asp. fumigatus* in mice. To develop a new type of antifungal agent, azoxybacilin was chemically modified.

In this communication, we wish to report the synthesis and the structure-activity relationships of azoxybacilin and its derivatives.

Synthesis of Azoxybacilin and Its Derivatives

The synthesis of azoxybacilin and its derivatives is outlined in Chart 1. The preparation of the azoxy moiety, a key step in this synthesis, was achieved according to the Moss procedure[3] which includes regio- and stereo-selective alkylation of the diazoate generated from N-alkyl-N-nitrosourethane. Iodide **3** was prepared from Boc-L-Asp-O^{t}Bu **1** in four steps according to the Olsen procedure[4] in 67% overall yield from **1**. Thus, N-nitroso-N-methylurethane **4** was treated with potassium tert-butoxide in ether at -78°C for 3 hours to generate diazoate anion **5**. Then, trapping intermediate **5** with iodide **3** in hexamethylphosphoric triamide (HMPA) gave the desired azoxy derivative **6** regio- and stereo-selectively. Finally, removal of the protecting groups of compound **6** with trifluoroacetic acid (TFA) gave azoxybacilin as a TFA salt in 74% yield from **4**. All spectral data of the synthetic azoxybacilin were identical with those of the natural product (TFA salt). The azoxybacilin congeners having different alkyl chain length such as **7** and **8**, and the desamino derivative **13**, were synthesized by a synthetic procedure similar to that described above with the use of Boc-L-Glu-O^{t}Bu, N-nitroso-N-n-propylurethane or ethyl 4-bromobutyrate as the starting material, respectively, instead of the starting material used in the synthesis of azoxybacilin.

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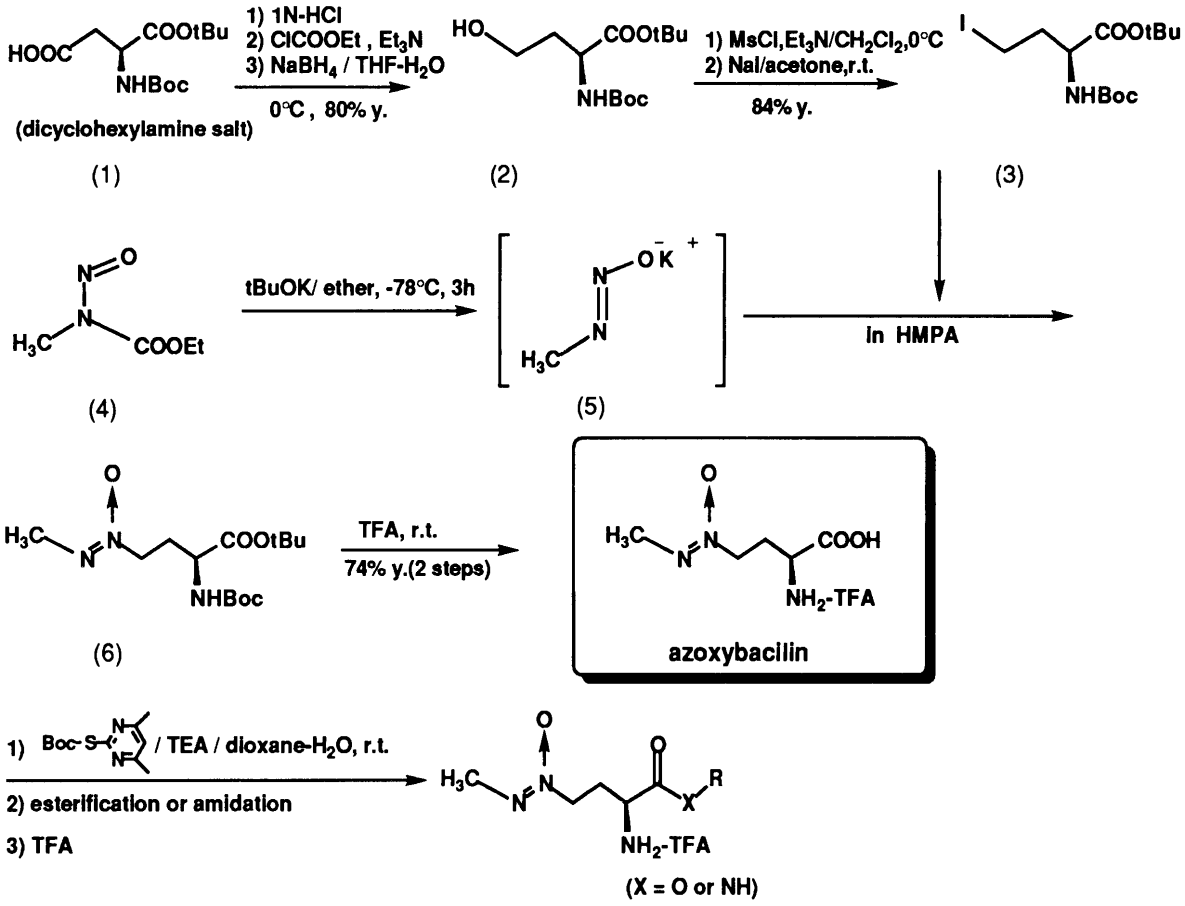

Chart 1

The ester and amide derivatives of azoxybacilin were prepared in 3 steps : (i) protection of the amino group of azoxybacilin with the Boc group, (ii) esterification of the resulting N-Boc-azoxybacilin with various alkyl or substituted benzyl halides, or amidation with various amines by active ester methods, and (iii) removal of the Boc group with TFA.

Structure-Activity Relationships

The *in vitro* antifungal activity of azoxybacilin was significantly affected by even minor structural modifications (Table I). The elongation of the alkyl chain (compound **7** and **8**) resulted in a complete loss of antifungal activity. The primary alcohol (**10**), des-amino (**13**), and amide derivatives (**11** and **12**) of azoxybacilin were also devoid of the activity. On the other hand, the esterification of azoxybacilin led to the significant improvement of antifungal activity against yeast-type fungi such as *C. albicans* and *Cry. neoformans*. Among them, the benzyl ester derivative, Ro 09-1824, showed well-balanced and improved antifungal activity against all three major systemic pathogens. More interestingly, the *in vitro* antifungal activity of azoxybacilin was significantly antagonized by the addition of a physiological concentration (6 μg/ml) of methionine in the assay media[2], while Ro 09-1824 was antagonized to a lesser extent: The (IC_{80} value for *C. albicans* CY1002 with methionine) / (IC_{80} without methionine) ratio is 54 for azoxybacilin and 3 for Ro 09-1824. The detailed biological studies on these derivatives will be reported elsewhere.

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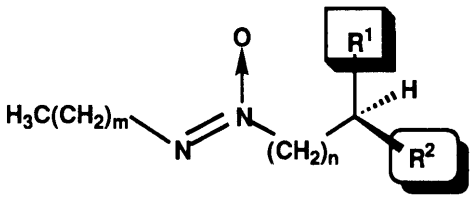

Table I *In Vitro* Antifungal Activity

Compound	Structure				IC_{80} (μg/ml)		
	m	n	R^1	R^2	*C. albicans**	*C. neoformans**	*Asp. fumigatus**
Azoxybacilin	**0**	**2**	**$-CO_2H$**	**NH_2**	**4.2 ~ 83.0**	**>200**	**0.71 ~ 0.78**
7	2	2	$-CO_2H$	NH_2	>200	>200	>200
8	0	3	$-CO_2H$	NH_2	>200	>200	>200
9	0	2	$-CO_2CH_3$	NH_2	4.5 ~ 15.0	55 ~ 180	1.80
Ro 09-1824	**0**	**2**	**$-CO_2CH_2$-Ph**	**NH_2**	**2.1 ~ 4.8**	**6.9 ~ 15**	**0.43 ~ 0.64**
10	0	2	$-CH_2OH$	NH_2	>200	>200	>200
11	0	2	$-CONH_2$	NH_2	>200	>200	>200
12	0	2	$-CONHCH_2Ph$	NH_2	>200	>200	>200
13	0	2	-COOH	H	>200	>200	>200

*3 strains; Medium: YNBPA (pH7.0), inoculum size: 1 x 10^4cfu/ml, incubation: 1 day at 27°C.

Spectral Data

Synthetic Azoxybacilin (TFA Salt): ^{1}H-NMR (400MHz, CD_3OD) δ: 4.47 (2H, br.t), 4.09 (1H, br.t), 3.19 (3H, s), 2.51 (2H, m). FAB-MS (glycerol): m/z 162 (MH^+). **7 (TFA Salt):** ^{1}H-NMR (400MHz, CD_3OD) δ: 4.47 (2H, t, J=6.4Hz), 4.09 (1H, t, J=6.8Hz), 3.39 (2H, t, J=7.3Hz), 2.60-2.42 (2H, m), 1.75 (2H, m), 1.00 (3H, t, J=7.3Hz). FAB-MS (m-nitrobenzyl alcohol): m/z 190 (MH^+). **8 (TFA Salt):** ^{1}H-NMR (400MHz, CD_3OD) δ: 4.28 (2H, br.t), 4.00 (1H, br.t,) 3.20 (3H, s), 2.17-1.89 (4H, m). FAB-MS (glycerol): m/z 176 (MH^+). **9 (TFA Salt):** ^{1}H-NMR (400MHz, $CDCl_3$) δ: 4.51-4.33 (3H, m), 3.84 (3H, s), 3.22 (3H, s), 2.71 (2H, m). FAB-MS (glycerol): m/z 176 (MH^+). **Ro 09-1824 (TFA Salt):** ^{1}H-NMR (400MHz, CD_3OD) δ: 7.40 (5H, m), 5.29 (2H, s), 4.41 (2H, t, J=6.4Hz), 4.24 (1H, t, J=6.8Hz), 3.18 (3H, s), 2.59-2.42 (2H, m). FAB-MS (m-nitrobenzyl alcohol): m/z 252 (MH^+). **10 (TFA Salt):** ^{1}H-NMR (400MHz, CD_3OD) δ: 4.38 (2H, m), 3.77 (1H, dd, J=11.6, 3.6Hz), 3.62 (1H, dd, J=11.6, 6.0Hz), 3.45 (1H, m), 3.18 (3H, s), 2.26 (2H, m). EI-MS: 148 (MH^+). **11 (TFA Salt):** ^{1}H-NMR (400MHz, CD_3OD) δ: 4.40 (2H, m), 4.03 (1H, m), 3.20 (3H, s), 2.46 (2H, m). FAB-MS (glycerol): m/z 161 (MH^+). **12 (TFA Salt):** ^{1}H-NMR (400MHz, CD_3OD) δ: 7.32 (5H, s), 4.43 (2H, s), 4.34 (2H, m), 4.01 (1H, t, J=6.8Hz), 3.18 (3H, s), 2.47 (2H, m). FAB-MS (m-nitrobenzyl alcohol)): m/z 251 (MH^+). **13:** ^{1}H-NMR (400MHz, $CDCl_3$) δ: 4.28 (2H, t, J=7.2Hz), 3.21 (3H, s), 2.49 (2H, t, J=7.2Hz), 2.28 (2H, m). EI-MS: 146 (M^+).

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