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Zwittermicin A: Synthesis of Analogs and Structure-Activity Studies

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Abstract

Analogs and diastereomers of the natural product zwittermicin A were prepared. SAR studies of these compounds reveal the antifungal activity to be dependent singularly upon the natural constitution and configuration.

Keywords

antifungal; amino alcohol; synthesis

(+)-Zwittermicin A (**1**) (ZwA) is a highly polar aminopolyol antibiotic isolated from the soil-borne bacterium *Bacillus cereus*.¹ Compound **1** was first reported in 1994 and shows significant activity against human pathogenic yeast and phytopathogenic fungi.^{1,2} More importantly **1** has been shown to work synergistically with endo-toxin produced by *Bacillus thuringiensis* for control of gypsy moth.³ Research has shown strains of *B. cereus* producing **1** to be ubiquitous in the soil^{1c} and may be more benign to the environment than some synthetic pesticides.⁴ Biosynthesis of **1** arises from a non-ribosomal peptide synthetase/polyketide synthase pathway (NRPS/PKS) involving two new type 1 PKS extender units: hydroxymalonyl-acyl carrier protein (ACP) and aminomalonyl-ACP.⁵

(+)-Zwittermicin A (**1**)

Preliminary studies show that **1** appears to exhibit a unique mechanism of action.⁶ Investigations of ZwA-resistant mutants found that the resistance mapped to *rpoB* and *rpoC*,

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genes that encode subunits of RNA polymerase.⁶ However, **1** showed no effect on total RNA or DNA synthesis implying a mechanism of action that differs from that of other antibiotics that target RNA polymerase.⁶

Previously, we synthesized (–)-**1**, the enantiomer of ZwA, its diastereomer **2**, and analogs **5** through **10** (Figure 1).⁷ This report describes the synthesis of the additional diastereomers **3** and **4** as well as analogs *ent*-**6**, **11** and **12** together with a comprehensive SAR study of all compounds.

During the course of our synthesis of (–)-**1** a number of models were made to evaluate synthetic strategies and some of these were later used to make analogs **11** and **12** as shown in Scheme 1. Analog *ent*-**6** was synthesized by reduction of diazide **13** (Scheme 1).⁸ The known alcohol **14**⁹ was converted to aldehyde **15** in three high-yielding steps; MOM protection of the primary OH group,¹⁰ desilylation of the primary OTBS group,¹¹ followed by Swern¹² oxidation. Evan's aldol addition of the boron enolate of chiral glycolate equivalent **16**¹³ to **15** (*dr* 47:1) followed by separation and removal of the chiral auxiliary under standard conditions afforded carboxylic acid **17** (57% over two steps).¹⁴ Coupling of **17** to known *N*-ureido- L-1,3-diaminopropionamide (–)-**18**⁷ gave **19** in 83% yield; global deprotection of the latter provided the truncated ZwA analog **11** in 76% yield. Similarly, analog **12** was prepared from (+)-**18**.

Azidodiol **20**, prepared from L-serine as described earlier,⁷ was refunctionalized by TBDPS protection¹⁵ of the terminal alcohol, MOM protection of the secondary alcohol and removal of the TBDPS group to give **21** in high yield (86% three steps, Scheme 2). Transformation of the azido group in **21** to an *N,N*-dibenzyl group by hydrogenolysis (Lindlar's catalyst¹⁶) followed by *N*-benzylation¹⁷ gave a primary alcohol that was easily oxidized to the stable aldehyde **22** (74% three steps).

Aldol addition of the glycolate equivalent **23**¹⁸ to aldehyde **22** (9:1 *dr*) followed by hydrolysis with lithium hydroxide under standard conditions¹⁹ afforded carboxylic acid **24** in (41% over two steps).

EDCI coupling of **24** to (–)-**18** gave **25** (86% yield) and global deprotection provided zwittermicin A diastereomer **3** in 57% yield. In a similar manner, diastereomer **4** was synthesized from (+)-**18** and **24**.

Antifungal assay of natural (+)-**1** and the 13 synthetic compounds was conducted against the fungal strains *Candida albicans* 96-489, *C. glabrata*, *C. albicans* UCDFR1, *C. albicans* ATCC 144503, *C. krusei*, and the phytopathogenic bacteria *Erwinia carotovora*, and *E. amylovora* and oomycete *Phytophthora infestans* (Table 1). Natural zwittermicin A [(+)-**1**] showed activity against *C. albicans*, *C. glabrata*, *E. carotovora*, and *E. amylovora*. Despite structural similarities of the synthetic compounds to natural ZwA, particularly the enantiomer (–)-**1** and stereoisomeric **3** and **4**, only the natural product showed detectable activity.

The biological data indicate that the mechanism of action is highly stereospecific. The enantiomer (–)-**1** and all of the diastereomers were inactive.⁷ Changing the configuration of **1** at only two stereocenters (C-13 and C-14 in **3**) resulted in complete loss of activity, even in the most susceptible strain, *E. amylovora*. None of the truncated analogs (Figure 1) showed activity. In fact, the activity of (+)-**1** is quite singular; it not mimicked by simple synthetic stereoisomers or analogs. Antifungal activity in simple vicinal aminoalkanols has been observed for other natural products, such as oceanapiside,²⁰ oceanalin,²¹ even sphingosine and its synthetic short-chain analogs (C₆), irrespective of the relative configurations.²² Consequently, the stringent stereochemical requirements observed for antifungal activity properties in **1** were unexpected and surprising. The mechanism of action of **1** is presently

unknown, but clearly differs from that of the former compounds which appear to interdict sphingolipid metabolism.²³

In summary, two zwittermicin A diastereomers and three analogs were synthesized and compared with natural zwittermicin A and eight other previously synthesized analogs and stereoisomers in antifungal assays. The microbial susceptibility profile of **1–12** indicates that the mechanism of action is highly stereospecific, and the complete complement of functionality found in the natural enantiomer zwittermicin A [(+)-**1**] is required for efficacy. Further investigations to identify the mechanism of action may benefit from *selective* affinity tagging of **1** and deployment in cell-free pull-down experiments to identify the cellular target.²⁴

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Figure 1.
ZwA diastereomers and analogs for SAR studies.

Scheme 1.

Synthesis of *ent*-**6**, **11**, and **12**. Reagents and conditions: (a) H₂O, H₂ (1 atm), Pd/C, 2 h, 100%; (b) MeOCH₂Cl, EtN(*i*-Pr)₂, CH₂Cl₂, 0 °C-rt, 14 h, 91%; (c) TBAF, THF, -10 °C, 16 h, 93%; (d) (i) (COCl)₂, DMSO, CH₂Cl₂, -78 °C, (ii) Et₃N, 94%; (e) (i) **16**, *n*-Bu₂BOTf, Et₃N, CH₂Cl₂, -78 to 0 °C, 3 h, (ii) **15**, -78 to 0 °C, 2.5 h, 85%, dr 47:1; (f) H₂O₂, LiOH, 0 °C, 30 min, 67%; (g) (i) **17**, EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (-)-**18**, Et₃N, 0 °C-rt, 2.5 h, 83%; (h) (i) HCl, MeOH, H₂ (5 atm), Pd/C, 1 h, (ii) HCl, H₂O, H₂ (5 atm), Pd/C, 1 h, 76%; (i) (i) **17**, EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (+)-**18**, Et₃N, 0 °C-rt, 1.5 h, 67%; (j) (i) HCl, MeOH, H₂ (5 atm), Pd/C, 1 h, (ii) HCl, H₂O, H₂ (5 atm), Pd/C, 1 h, 73%. EDCI= 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBT= 1-hydroxybenzotriazole.

Scheme 2.

Synthesis of ZwA diastereomers **3** and **4**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, 0 °C-rt, 3.5 h, 91%; (b) MeOCH₂Cl, Hünig's base, CH₂Cl₂, 0 °C-rt, 48 h, 96%; (c) TBAF, THF, -10 °C, 4 h, 98%; (d) Lindlar's cat., H₂, (1 atm), EtOH, 15 h, 89%; (e) BnBr, K₂CO₃, CH₃CN, 83 h, 92%; (f) (i) (COCl)₂, DMSO, CH₂Cl₂, -78 °C, (ii) Et₃N, 90%; (g) (i) **23**, *n*-Bu₂BOTf, Hünig's base, Et₂O, -78 °C, 1.5 h, (ii) **22**, -78 to 0 °C, 2 h, 49%; (h) LiOH, H₂O:MeOH:THF, 0 °C, 4 h, 84%; (i) (i) EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (-)-**18**, Et₃N, 0 °C-rt, 2.5 h, 86%; (j) (i) HCl, MeOH, H₂ (5 atm), Pd/C, 1 h, (ii) HCl, H₂O, H₂ (5 atm), Pd/C, 1.3 h, 57%; (k) (i) **24**, EDCI, HOBT, DMF, 0 °C, 10 min, (ii) (+)-**18**, Et₃N, 0 °C-rt, 1.5 h, 86%; (l) (i) HCl, MeOH, H₂ (5 atm), Pd/C, 1 h, (ii) HCl, H₂O, H₂ (5 atm), Pd/C, 1 h, 73%. TBDPSCl = *tert*-butyldiphenylsilyl chloride.

Table 1

Biological testing of zwittermicin A and synthetic compounds.

Pathogenic microbes	(+)-1 MIC ^{a,b} (µg/mL)	(-)-1, 2–10, <i>ent</i> -6, 11 and 12 MIC ^{a,b} (µg/mL)
<i>Candida albicans</i> 96-489	55.7	>128
<i>C. glabrata</i>	59.5	>128
<i>C. albicans</i> UCDFR1	>128	>128
<i>C. albicans</i> ATCC 144503	>128	>128
<i>C. krusei</i>	>128	>128
<i>Erwinia carotovora</i>	22.2	>128 ^c
<i>E. amylovora</i>	18.8	>128 ^c
<i>Phytophthora infestans</i> ^d	>32	>32 ^c

^aThe MIC endpoint is defined as the lowest concentration (µg/mL) with 90% growth inhibition.

^b(+)-1, (-)-1, 2–4, 11 and 12 were converted to the free amine before testing. Compounds 5–10 were used as their HCl salts.

^c5–10 not tested.

^dThe maximum concentration used was 32 µg/mL due to limitations of the nutrient agar well diffusion assay.