Relationship between Structure and Biological Activity of Novel R106 Analogs

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The retro-aldol reaction at residue 8 of R106-1 produced a chemical handle, in the form of a sarcosine residue, that was amenable to classical aldol alkylation conditions. *In vitro* assay of several new hydroxylated analogs have shown that L isomers exhibit more potent antifungal activity than D isomers. However, all analogs exhibited a significant decrease in activity against *Cryptococcus neoformans*. By contrast, structural modifications to R106 were tolerated by some *Candida* spp., but the potency of activity was diminished as compared to that of the natural product R106-1. The full structure-activity relationship of the new R106 analogs has provided important information about the steric and electronic requirements of binding to target receptors. Furthermore, comparison of the structural differences between R106-1 and other derivatives, suggested that the potential for hydrogen bonding (at residue 8) was a key structural feature that was required to maintain activity against *Cryptococcus neoformans*.

R106-1 (LY295337), 1, a member of the aureobasidin class of antibiotics, is the major factor produced by fermentation of the microorganism *Aureobasidium pullulans* R106 (Fig. 1).[1] R106-1 is a broad spectrum fungicidal agent effective against several clinically important fungal pathogens such as *Candida* spp. and *Cryptococcus neoformans*.[2]

In the course of our studies on the relationship between structure and biological activity of R106-1 and other known factors, we recognized that the family of aureobasidins are divided into two categories: Those in which residue 8 is hydroxylated and those in which residue 8 is non-hydroxylated. Derivatives that are hydroxylated (*i.e.* the β-hydroxy *N*-methyl valine residue 8 of 1) are more active *in vitro* against *Candida* spp. and *Cryptococcus neoformans*.[3,4] It has been recognized by others,[5~10] that this residue is important for biological activity. We further suggest that the conformation of this residue and potential for hydrogen bonding to a receptor are essential for optimum activity against *Cryptococcus neoformans*.[11] In this article we expand our knowledge of this position and explore its importance in biological activity.

In contrast to other reported methods to acquire new derivatives,[5,6,12] recently we have reported a direct means of synthesizing new derivatives by selectively

modifying residue 8 of the natural product.[11] As shown in Scheme 1, new analogs were obtained in only two steps *via* a tandem retro-aldol, aldol reaction of R106-1. In this paper, we report the antifungal activities and structural relationships of several new derivatives.

Methods

Antifungal Susceptibility Testing Procedure

The microdilution microtiter testing procedure was used to determine MICs. Fungal isolates were grown in Sabouraud dextrose medium at 35°C. Spores or conidia were suspended in saline and adjusted to 1×10^5 cells/ml in an appropriate broth. Cell suspensions were counted using a hemocytometer. R106-1 was tested in Sabouraud

Fig. 1. The structure of R106-1 (LY295337), 1.
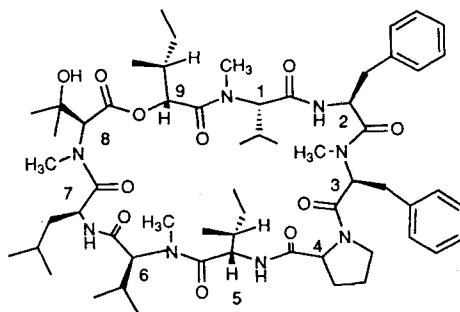
Scheme 1. The Retro-aldol, aldol reaction of R106-1.

R106-1 (LY295337), **1**

"Retro-Aldol"

acetone

R106-sarcpsome, **2**

"Aldol"

New R106 Derivatives
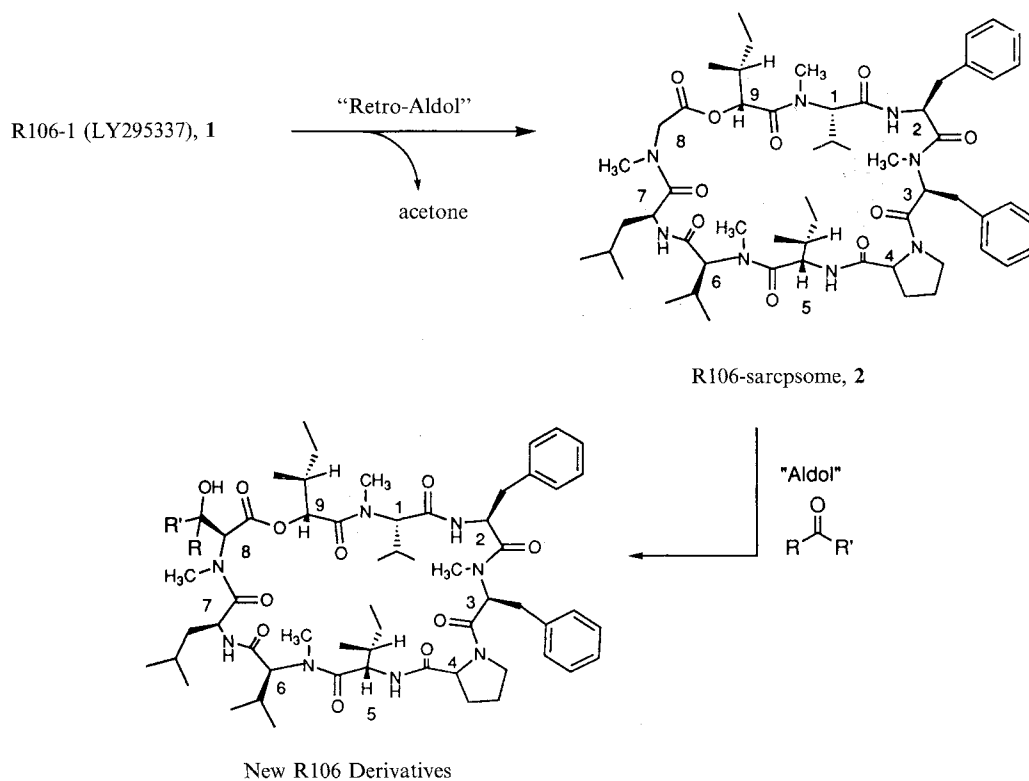
dextrose medium. Aliquots containing 100 μl of the above were added to each well of a 96 well microtiter plate. The test compound was solubilized at 1.0 mg/ml in methanol. The solution of test compound was diluted to yield 80 μg/ml in the first well when added in a 100 μl volume. Serial 2-fold dilutions were made in each well to the eleventh well. The twelfth well served as a positive growth control. All new derivatives were tested in duplicate. Plates were incubated 48 hours at 35°C. The MIC was defined as the lowest concentration of drug that inhibited 90~100% of visible growth of the fungal pathogen.

Results

The aldol condensation with acetone produced a mixture of two products identified as the D and L isomers of R106-1. We were not successful in separating the two products by C-18 reversed phase HPLC. However, by altering the alkylation conditions the product D/L ratio varied from 5:1 to 2:1.[11] In testing each mixture, we observed that the mixture with the highest D/L ratio was least active. This observation implied that the pure D isomer was considerably less active than the L isomer. Hence, as the D/L ratios decreased, the activity approached that of the natural product (Table 1).

When acetone was replaced with a more sterically

hindered electrophile, such as 2-butanone, 3-pentanone or cyclopentanone to give products **5**, **6**, and **7**, we observed an increase in the D/L ratio (2:1 to 12:1). The D/L mixtures were tested for antifungal activity and with the exception of cyclopentanone **7**, the MIC's against *Candida* spp. were at least 10-fold less active than that of the prototype R106-1.

The replacement of a methyl group in R106-1 with an electron withdrawing group, such as a trifluoromethyl group given by **4**, resulted in a sharp decrease in activity. Compounds **4A** and **B** (D isomers) were surprisingly inactive against all *Candida* spp. while **4C** and **D** (L isomers) retained modest activity against only *Candida albicans* and *Candida parapsilosis*. In all cases, these new trifluoromethyl derivatives lost activity against *Cryptococcus neoformans*.

The products derived from aldehydes gave a mixture of 4 diastereomers (**8**~**9** **A**~**D**) that were separated by C-18 reversed phase chromatography. According to ^{1}H NMR spectral interpretation, compounds **A** and **D** were assigned the L configuration; and compounds **B** and **C** were assigned the D configuration (the carbon configuration attached to the hydroxyl group is unknown).[11] Our stereochemical assignments were consistent with the observed antifungal activities, in that two of the four

Table 1. MIC's for tertiary alcohol derivatives of R106-1.

Electrophile	Product:[a] R, R'	Residue 8: D/L ratio	MIC (µg/ml)			
			Candida albicans	Candida parapsilosis	Candida glabrata	Cryptococcus neoformans
R106-1	Standard	L	0.097~0.156	0.097~0.312	0.097~0.156	0.39~1.25
Acetone	(3) CH₃, CH₃	2:1	0.156	0.312	0.312	2.5
		5:1	0.625	1.25	2.5	20
Trifluoroacetone	(4)[b] CH₃, CF₃	A: D	80	80	80	80
		B: D	80	80	80	80
		C: L	1.25	2.5	80	10
		D: L	0.625	1.25	1.25	80
2-Butanone	(5)[c] CH₃, CH₃CH₂	A: 3.6:1	0.625	2.5	2.5	10
		B: 4.0:1	0.625	2.5	2.5	80
3-Pentanone	(6) CH₃CH₂, CH₃CH₂	12:1	2.5	10	20	80
Cyclopentanone	(7) –CH₂(CH₂)₂CH₂–	3.5:1	0.195	0.097	0.195	80

[a] Products derived from the tandem retro-aldol, aldol reaction as shown in Scheme 1. The stereochemical assignment for the carbon attached to the hydroxy group is unknown for 4~5. Compounds 4~5 A~D are listed in order in which they are retained by C18 HPLC. [b] All four diastereomers (A~D) were separated by HPLC and activities of each product measured separately. [c] Products A and B are D/L mixtures of diastereomers.

Table 2. MIC's for secondary alcohol derivatives of R106-1.

Electrophile	Product:[a] R, R'	Residue 8: D,L assignment	MIC (µg/ml)			
			Candida albicans	Candida parapsilosis	Candida glabrata	Cryptococcus neoformans
Acetaldehyde	(8) CH₃, H	A: L	0.156	0.625	10	80
		B: D	2.5	1.25	80	80
		C: D	2.5	1.25	80	80
		D: L	0.078	0.625	10	80
Butyraldehyde	(9) CH₃CH₂CH₂, H	A: L	0.312	0.312	5.0	80
		B: D	10	80	80	80
		C: D	10	2.5	80	80
		D: L	0.625	1.25	2.5	80

[a] Each of the Four possible diastereomers for 8~9 were assigned the amino acid configuration at residue 8. Compounds 8, 9 A~D are listed in order in which they are retained by C18 HPLC. The stereochemical assignment for the carbon attached to the hydroxy group is unknown for 8~9.

diastereomers were substantially more active (Table 2). Moreover, the higher *in vitro* activity of A and D over the other two isomers was consistent with our previous observation with R106-1 D/L mixtures; that the L isomer is more active.

Discussion

In order to learn more about the requirements for antifungal activity of the aureobasidins, we investigated compounds that differ from the prototype R106-1 by both steric and electronic features. The natural product R106-1, which possesses a propanol side chain at residue 8, provided the best activity and spectrum. Each methyl group of the propanol side chain was extended by a single methyl group as illustrated by compounds 5 and 6. As demonstrated by 5, the butanol side chain reduced the spectrum of the antibiotic to only *Candida abicans*. The

loss of activity was more dramatic with the extension of both methyl groups as in 6. Here, only very modest activity is seen against *Candida abicans*. Surprisingly, the cyclic analog 7 showed excellent *Candida* activity comparable to that of R106-1, however all activity against *Cryptococcus neoformans* was lost. The cyclopentanol group should be sterically more similar to the propanol side chain of 3, than to the 3-pentanol side chain of 6, since the bridging tether is essentially two connected methylenes rather than two methyl groups. The rigid cyclopentanol analog 7 apparently fits the steric requirements for the *Candida* receptors, while the freely rotating 3-pentanol analog 6 does not. Since all activity is lost against *Cryptococcus neoformans* it appears that this receptor is much less accomodating to steric changes of the aureobasidin structural motif.

The replacement of a methyl group of R106-1 with a trifluoromethyl group as in 4 was carried out to investigate the electronic effect at residue 8. The decrease

in activity across the spectrum seems to indicate that electron withdrawing groups are not tolerated. However, more studies are needed to confirm this result.

The activity results of the secondary alcohol analogs, 8 and 9, were surprising. The change from the tertiary analogs of Table 1 to these secondary alcohol analogs (Table 2) provided no antifungal advantage. The activities against Candida were generally diminished. Furthermore, as with the ketone SAR, all derivatives derived from aldehydes lost activity against Cryptococcus neoformans.

In our earlier work,[11] we carried out computational studies to understand the preferred conformation of R106-1 and new R106 derivatives. We found differences between the hydrogen bonding potential of the tertiary hydroxyl group of R106-1 and the secondary hydroxyl group of new analogs. Surprisingly, a dramatic difference between a conformer of the L configurational isomer was noted with tertiary and secondary alcohols of residue 8. In the case of R106-1 the hydroxyl group is available for possible interaction with a target receptor. By contrast, analysis of the same conformer of secondary alcohols indicates that the hydroxyl group is internally hydrogen bonded to the Leu-7 carbonyl.

The loss of Cryptococcus activity with the secondary alcohols was most interesting. In particular, the residue 8 derivative 7 should meet the steric requirements that are dictated by the prototype natural product, 1. The only difference between 1 and 7 that we have illustrated is the nature of the potential to hydrogen bond to a target receptor (in an L conformer of 1).

Evaluation of the new aureobasidin analogs described here have illustrated that slight structural alterations of the tertiary alcohol have a pronounced effect on biological activity. The chemical alterations we performed on residue 8 to explore steric and electronic requirements of the key β-turn region[9,10] of the aurebasidins revealed that activity against Candida was less sensitive as compared to activity against Cryptococcus neoformans. However, in all cases the spectrum of activity against these fungal pathogens was diminished as compared to that of the prototype natural product R106-1.

In summary, at this point it is still speculative as to specific interactions that occur between the target receptor of Cryptococcus neoformans and the hydroxyl group of residue 8 that potentially influence in vitro activity. However, the results described herein strongly suggest that the tertiary hydroxyl group is critical for maintaining overall activity. The influence of the tertiary alcohol over Cryptococcus activity was highlighted by

the SAR and our findings are consistent with known R106 factors isolated from fermentation in that alteration of the hydroxyl group is not tolerated and that non hydroxylated derivatives show no activity against Cryptococcus.[7,8]

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