Mycopyranone: A 8,8′-binaphthopyranone with potent anti-MRSA activity from the fungus Phialemoniopsis sp.

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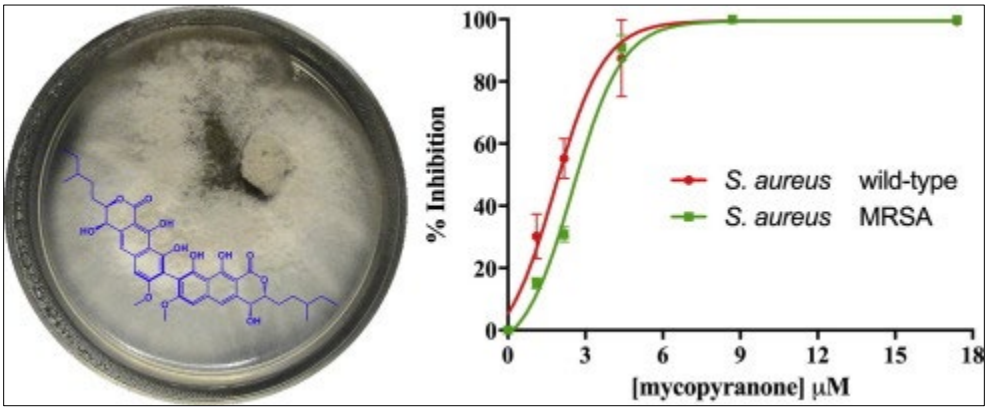
Abstract:

A new 8,8′-binaphthopyranone (mycopyranone, 1) was isolated from a solid fermentation of Phialemoniopsis sp. (fungal strain MSX61662), and the structure was elucidated via analysis of the NMR and HRESIMS data. The axial chirality of 1 was determined to be M by ECD. The central chirality at C-4/C-4′ was assigned through a modified Mosher's method, while the absolute configuration at C-3/C-3′ was deduced based on analysis of the 3JH-3-H-4 values and NOESY correlations. Compound 1 was evaluated for its antimicrobial properties against Staphylococcus aureus SA1199 and a clinically relevant methicillin-resistant S. aureus strain (MRSA USA300 LAC strain AH1263). Compound 1 inhibited the growth of both strains in a concentration dependent manner with IC50 values in the low μM range. Molecular docking indicated that compound 1 binds to the FtsZ (tubulin-like) protein in the same pocket as viriditoxin (2), suggesting that 1 targets bacterial cell division.



Keywords: Binaphthopyranone | Phialemoniopsis sp. | FtsZ protein | Viriditoxin | MRSA

Article:

Introduction

Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are a serious health problem [1], [2], [3]. In the United States alone, over 80,000 severe MRSA infections were documented in 2013, 11,000 of which were fatal [4]. Unfortunately, despite the imminent problem MRSA and similar drug resistant infections pose, most pharmaceutical companies do not focus on the discovery and development of new antimicrobial agents [5], [6]. In this context, the World Health Organization has started a cooperative program with Academia to search for new molecules and strategies to combat antibiotic resistance [7], [8].

One promising strategy to combat MRSA infections is targeting cell division via the inhibition of the proto ring protein FtsZ [2], [9], a vital and highly conserved protein involved in bacterial division and formation of new cells; it is a homolog of tubulin in eukaryotic cells [10], [11]. However, despite the importance of this protein as a molecular target to combat infections against MRSA, just a few examples of molecules have been described as potential inhibitors, including natural products [12], [13], [14], [15], [16] and synthetic probes [9], [17]. Among natural products, viriditoxin (**2**), a 6,6'-binaphthopyranone, inhibited the polymerization of FtsZ, resulting in the inhibition of bacterial cell division, and this compound has been proposed as a promising lead for the development of anti-MRSA drugs [16], [18].

As part of ongoing studies to identify structurally diverse and bioactive metabolites from fungal cultures, a new 8,8'-binaphthopyranone derivative (mycopyranone, **1**) was isolated from *Phialemoniopsis* sp. (strain MSX61662), which was identified phylogenetically and morphologically using methods that were detailed previously [19], [20]. The structure of **1** was established using a set of spectroscopic (1D and 2D NMR), spectrometric (HRESIMS), and chiroptic (ECD and OR) methods. The antimicrobial activity of **1** was evaluated against *Staphylococcus aureus* SA1199 [21] and a clinically relevant methicillin-resistant *S. aureus* strain (MRSA USA300 LAC strain AH1263) [22]. Growth inhibition by **1** was noted in both strains in a concentration dependent manner with IC_{50} values in the low μM range. We suggest that **1** targets bacterial cell division, as molecular docking studies predicted that viriditoxin (**2**) and mycopyranone (**1**) bind to the protein FtsZ in the same pocket.

Compound **1** [23] was isolated as an optically active ([a]D23=-229]) yellow amorphous powder, and its molecular formula was established as $C_{40}H_{46}O_{12}$ via HRESIMS [24]. Analysis of the ^{1}H, ^{13}C and HSQC NMR data indicated the presence of a chelated hydroxy group (δ_H 13.79), one phenolic proton (δ_H 9.67), two aromatic protons (δ_H 7.20, 6.79), two oxymethines (δ_H 4.48, 4.73), one aliphatic methine (δ_H1.42), three methylene (δ_H 1.98, 1.29/1.65, 1.22/1.44), one methoxy (δ_H 3.86) and two methyl groups (δ_H 0.91, 0.94), as well as nine fully substituted carbons, including three oxygenated and a carbonyl (lactone) moiety (Table 1 and Figures S3, S4, and S6).

Table 1. ^{1}H and ^{13}C NMR data for compound **1** recorded in $CDCl_3$ at 700 MHz.

Position	δ_C	**type**	δ_H, **multiplicity (*J* = Hz)**
1	171.0	C	

3	83.1	CH	4.48, *brt* (7.0)
4	66.9	CH	4.73, *brs*
4a	135.0	C	
5	117.3	CH	7.20, *s*
5a	140.1	C	
6	99.1	CH	6.79, *s*
7	161.7	C	
8	109.4	C	
9	155.6	C	
9a	109.2	C	
10	163.1	C	
10a	97.8	C	
11	28.0	CH_2	1.98, *m*
12a	32.0	CH_2	1.29, *m*
12b			1.65, *m*
13	34.5	CH	1.42, *m*
14	29.5	CH_2	1.22, *m*
			1.44, *m*
15	11.5	CH_3	0.91, *t* (7.3)
16	19.2	CH_3	0.94, *d* (6.5)
17	56.2	CH_3	3.86, *s*
4-OH			1.86, *brs*
9-OH			9.67, *s*
10-OH			13.79, *s*

These data accounted for a molecular formula of $C_{20}H_{23}O_6$, indicating that **1** was a symmetric dimer. In general, the ^{1}H and ^{13}C NMR data of **1** resembled those reported for viriditoxin (**2**) [25], pigmentosin [26], talaroderxines A and B [27], vioxanthin [28], and cladiosporinone [29], with minor differences attributed to the aliphatic chain at C-3/C-3′.

Analysis of the 2D NMR data, in particular COSY and HMBC experiments (Figures S5 and S7), allowed the identification of key fragments of the molecule (Figure 1).

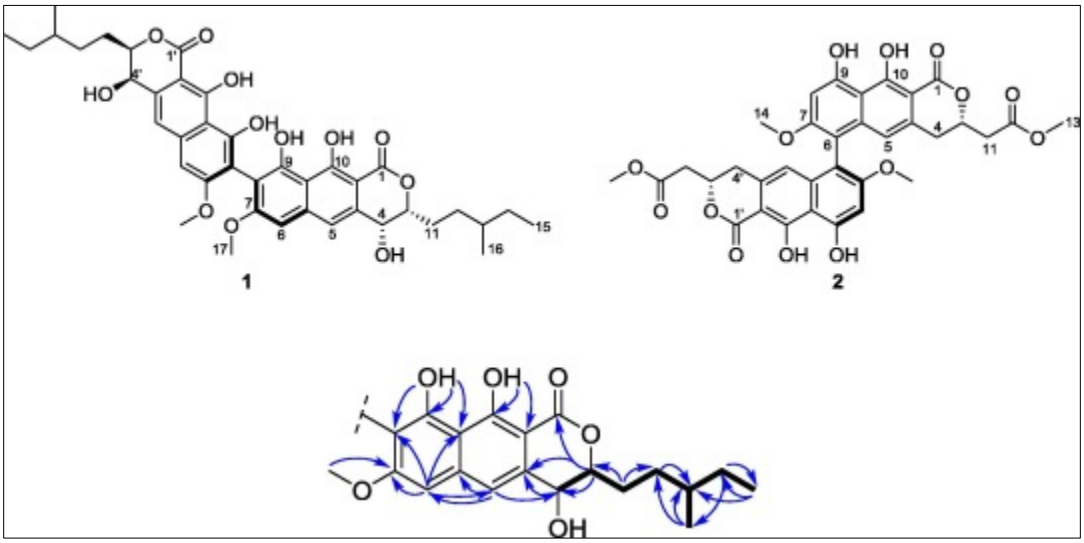


Figure 1. Structures of mycopyranone (**1**) and viriditoxin (**2**) and key COSY (bold bonds) and HMBC correlations (arrows) for compound **1**.

For example, the COSY data permitted the assignment of the spin system shown in black (Figure 1), which was confirmed by the HMBC correlations observed between H_3-15 to C-13 and C-14, H_3-16 to C-12, C-13 and C-14, H_2-14 to C-16, C-15 and C-13, H-13 to C-12, H_2-12 to C-14, C-13 and C-11, and H_2-11 to C-13, among others. The 9,10-dihydroxy-7-methoxy-naphthopyranone fragment was confirmed via HMBC correlations between H-3 to C-4a, C-4 and C-1, H-4 to C-4a, H-5 to C-6, C-5a and C-4, H-6 to C-5, C-7, C-8 and C-9a, 9-OH to C-9a, C-9 and C-8, 10-OH to C-10a, and C-10, and H_3-17 to C-7 (Figure 1). The connection between the 3-methylpentenyl moiety to the naphthopyranone fragment was established based on the HMBC correlations observed among H_2-11 and C-3, and H-3 to C-11 and C-12 (Figure 1). Finally, the 8, 8′ linkage between the homodimers was established based on the HMBC mutual correlation amongst H-6/H-6′ and C-8 and C-8′.

The axial chirality of the molecule was determined by ECD (Figure 2). Briefly, the spectrum of **1** in MeOH showed both positive and negative Cotton effects at 254 and 274 nm, respectively, similar to those of viriditoxin (**2**) [25], talaroderxine B [27], and *M*−vioxanthin [28]. The negative Cotton effect at 274 nm was attributed to the transitions of the naphthalene chromophores, indicating that the 8,8′ axis in **1** was twisted in a counter-clockwise manner, characteristic for *M* axial chirality [25], [26], [27].

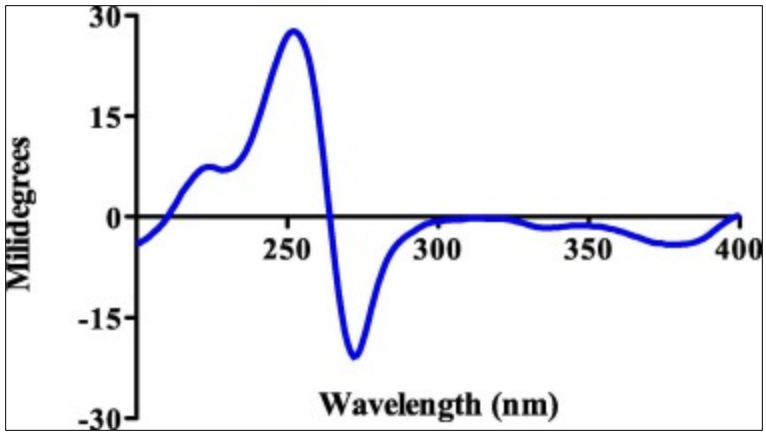


Figure 2. ECD spectrum of **1** recorded in MeOH (13 µM).

The absolute configuration at C-4/C-4ˈ was determined as *R* based on the results of the modified Mosheŕs ester method (Figure 3) [30]. The absolute configuration at C-3/C-3ˈ was also determined as *R* by analysis of the NOESY experiment and analysis of the $^{3}J_{\text{H-3-H-4}}$, value (0.8 Hz) (Figures 3 and S8). Unfortunately, the absolute configuration at C-13 was not determined due to the high flexibility of the chain. Thus, the structure of compound **1** was elucidated as depicted, and assigned the trivial name mycopyranone.

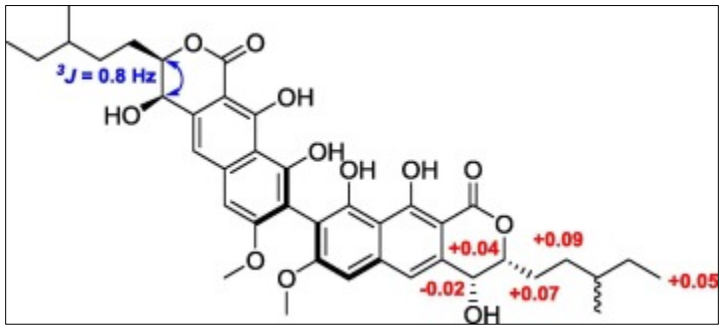


Figure 3. $\Delta\delta_H$ values [$\Delta\delta$ (in ppm) = $\delta S - \delta R$] (red) from the Mosher's esters experiment and key NOESY correlation (blue arrow) and coupling constant, all observed for compound **1**.

The activity of mycopyranone (**1**) was evaluated against *Staphylococcus aureus* (strain SA1199) [21] and a clinically relevant methicillin-resistant strain (MRSA USA300 LAC, AH1263) [22]. Compound **1** displayed promising antibacterial activity against both strains. Due to structural similarity to **2**, compound **1** likely functions as an FtsZ inhibitor [18]. Since FtsZ is highly conserved across many Gram-positive organisms [18], the ability of pathogens to develop resistance to 6,6ˈ- or 8,8ˈ-binaphthopyranone derivatives (including both compounds **1** and **2**) may be limited. The minimum inhibitory concentration (MIC) of compound **1** was ≤ 8.7 µM against both strains, and IC_{50} values were in the lower µM range (2.0 µM and 2.7 µM against SA1199 and AH1263, respectively; Figure 4).

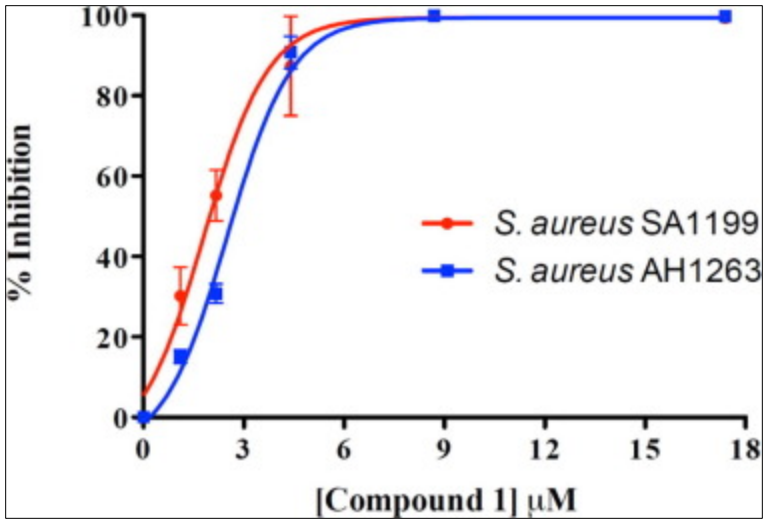


Figure 4. Concentration-response curves of compound **1** against *S. aureus* SA1199 (red circles) and methicillin-resistant *S. aureus* AH1263 (blue squares). The minimum inhibitory concentration (MIC) of compound **1** was ≤8.7 µM against both *S. aureus*strains. The MIC of the positive control, berberine, was 223 µM against both *S. aureus*strains.

Berberine, which was previously shown to target FtsZ in *Escherichia coli* [13], was used as a positive control. Berberine's antimicrobial activity (IC_{50} = 69.5 µM, MIC223 µM; Supporting Information, Figure S10) was consistent with previous reports [13], [31], [32]. In addition, the cytotoxic potential of compound **1** was evaluated against a panel of cancer cell lines, as described recently [33]. It did not show potent activity (IC_{50} value > 50 µg/mL), suggesting that **1** lacks toxicity against mammalian cells.

Based upon these results, and data from previous reports, we hypothesized that compound **1** binds to protein FtsZ, similar to viriditoxin (**2**), thereby inhibiting bacterial cell division [18]. To gain additional information about the putative binding site of mycopyranone (**1**) and viriditoxin (**2**) in FtsZ, molecular docking studies were carried out. Briefly, the results indicated that **1** and **2** bind to FtsZ (-5.09. and −6.60 kcal/mol, respectively) in a hydrophobic pocket conformed by the amino acids Arg-134, Pro-135, Gln-144, Pro-165, Asp-167, Arg-168, Asp-171, Ser-223, Ser-247, Pro-248, Leu-250, Glu-251, Ser-253, Val-255, Phe-315 and Asp-317 (Figures 5 and S11).

The forces that govern the interactions are mainly hydrogen bonds and van der Waals interactions (Figure 5). This pocket differs from those reported previously for the synthetic compounds PC190723 [9], TXA709 [34], [35] and TXA6101 [17], revealing a potential new druggable site in the FtsZ protein of *S. aureus*.

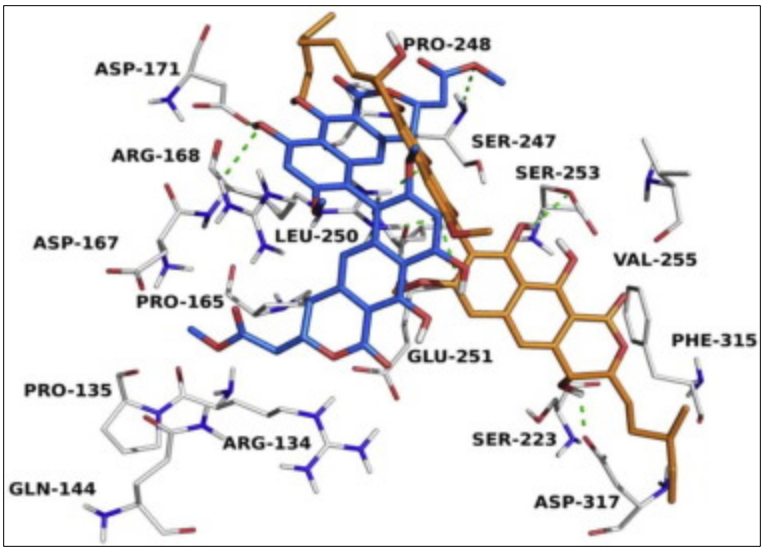


Figure 5. Structural model of the predicted binding pocket for viriditoxin (**2**) (blue) and mycopyranone **1** (orange) with FtsZ (PDB 4DXD).

Acknowledgements

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Appendix A. Supplementary data

The following are the Supplementary data to this article:

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Supplementary data 2.

Research data for this article

Data not available / Data will be made available on request

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[23] Mycopyranone (1): yellow amorphous powder: 1H and 13C-NMR see Table 1, and Figures S3 and S4 (Supporting Information); HRESIMS, [M+H]+= 719.3052 (calcd. for C40H47O12, 719.3062)(Figure S9).

[24] For details about the extraction and isolation of compound 1 see the Supporting Information file.

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