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Synthesis and structure–activity relationships of bengazole A analogs

Roger J. Mulder [c,†], Cynthia M. Shafer [c,‡], Doralyn S. Dalisay [a], Tadeusz F. Molinski [a,b,c,*]

[a] Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[b] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[c] Department of Chemistry, University of California Davis, 1 Shields Avenue, Davis, CA 95616, USA

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ABSTRACT

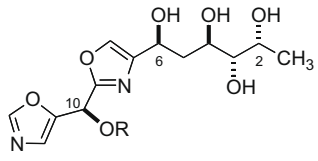
Analogs of the potent antifungal agent, bengazole A, were prepared and evaluated against *Candida* spp. in both microbroth dilution and disk diffusion assays.

Bengazoles A (**1**) and B (**2**) are homologous bis-oxazole natural products first reported by Crews and co-workers from the sponge *Jaspis* sp. with anthelmintic activity against the nematode *Nippostrongulus braziliensis*.[1]

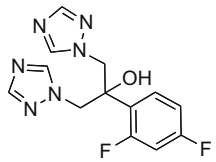
In 1990 we re-isolated **1** and **2**, together with five additional homologs, bengazoles C–G (**3a**–**e**), from *Jaspis* sp. collected on the Great Barrier Reef,[2] during a bioassay-guided screening of marine organisms for antifungal activity and found both compounds exhibited exceptionally potent in vitro activity against *Candida albicans* (e.g., MIC 1 μg/mL, *C. albicans* ATCC 14503),[3] a pathogenic fungus that is responsible for systemic infections in immunocompromised individuals, including AIDS patients. The absolute stereostructure of **1** was determined using a combination of chemical degradation, synthesis of model compounds and comparisons of the CD spectra of perbenzoyl derivatives of both models and the natural product.[2] Only total syntheses of bengazoles have been reported; bengazole A (**1**) by our group in 1999[4] and, more recently, **1** and **2** by Ley,[5] and 'bengazole Z' (**4**) by Shioiri and co-workers.[6] The bis-oxazole, digonazole from *Jaspis digonoxea* and its homologs,[1b] lack the C10 acyloxy group of **1**–**3**,[7] and have not been made or evaluated.

The mechanism of action of **1** is unknown and only limited of structure–activity relationships (SAR) are available.[7] The clinically important 'azole' antifungal drugs (e.g., fluconazole (**5**), miconazole and voriconazole) inhibit 14-α-demethylase in yeast by tight binding of one of the 1,2,4-triazole rings to the Fe-heme active site.[8] A cursory comparison of the structures of **1** and **4** suggested one of the oxazole rings in **1** might play a similar role, although this is not certain and other structural elements also appear to be important (Fig. 1).

Bengazole **1** exhibits ergosterol-dependent in vitro activity against *C. albicans* similar to amphotericin B (AmB). When grown on agar plates containing Sabouraud media and defined concentrations of ergosterol or cholesterol, *C. albicans* showed diminished susceptibility that was dose-dependent upon **1**, but inversely dependent upon the sterol concentration.[9] Conversely, the activity of miconazole, exhibited no ergosterol-dependence.[9b] Interestingly,



1: R = (C=O)$C_{10}H_{20}$-*n*-Pr
2: R = (C=O)$C_{10}H_{20}$-*i*-Bu, $C_{10}H_{22}$-*sec*-Bu (5:1)
3a: R = (C=O)$C_{10}H_{20}$-Et
3b: R = (C=O)$C_{10}H_{20}$-*i*-Pr
3c: R = (C=O)$C_{10}H_{20}$-*n*-Bu
3d: R = (C=O)$C_{11}H_{22}$-*sec*-Bu
3e: R = (C=O)$C_{11}H_{22}$-*n*-Bu
4: R = H



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6

Figure 1. Bengazoles A–G (**1**–**3**), –Z (**4**), fluconazole (**5**) and oxazole (**6**).

* Corresponding author. Tel.: +1 858 534 7115; fax: +1 858 822 0386.
E-mail address: tmolinski@ucsd.edu (T.F. Molinski).
† Present address: CSIRO Molecular and Health Technologies, Clayton, Vic 3169, Australia.
‡ Present address: Novartis Biosciences, Emeryville, CA, USA.

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incorporation of cholesterol or synthetic *ent*-cholesterol[9c] in the media elicited dose-dependent, differential suppression of susceptibility of *C. albicans* towards AmB, but no enantiospecific differences were observed with (–)- and (+)-cholesterol and **2**.[9a]

Here, we report the synthesis of new bengazole A analogs and an advanced SAR study of the new and known compounds that reveal the importance of structural features in **1** for anti-*Candida* activity; a long-hydrophobic side-chain, the polyol side chain, and at least one oxazole in the heterocyclic core.

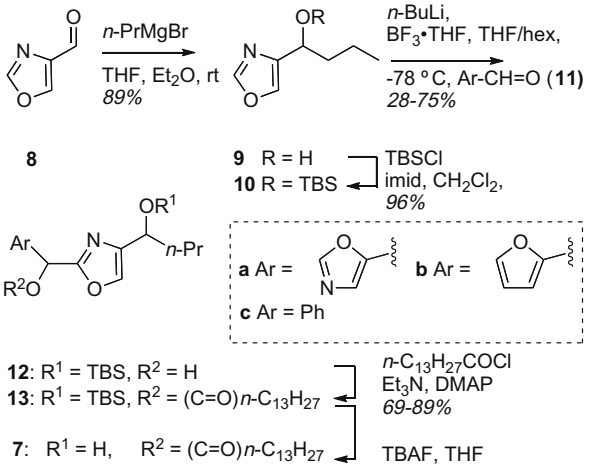
The fatty acyl side chain of **1** is important for antifungal activity. We observed that **1** was prone to spontaneous solvolysis in the presence of protic solvents, or more rapidly, by ammoniolysis (NH_3, MeOH).[2] The des-acyl product, 'bengazole Z' (**4**),[10] showed no anti-*Candida* activity in disk diffusion assays.

In order to examine the role of the heterocyclic core of **1**, analogs were prepared with replacement of the polyol side-chain with a simple 1-hydroxy-1-butyl group (the parent, 1,3-oxazole (**6**) is too volatile, bp 69–70 °C, for bioassay).

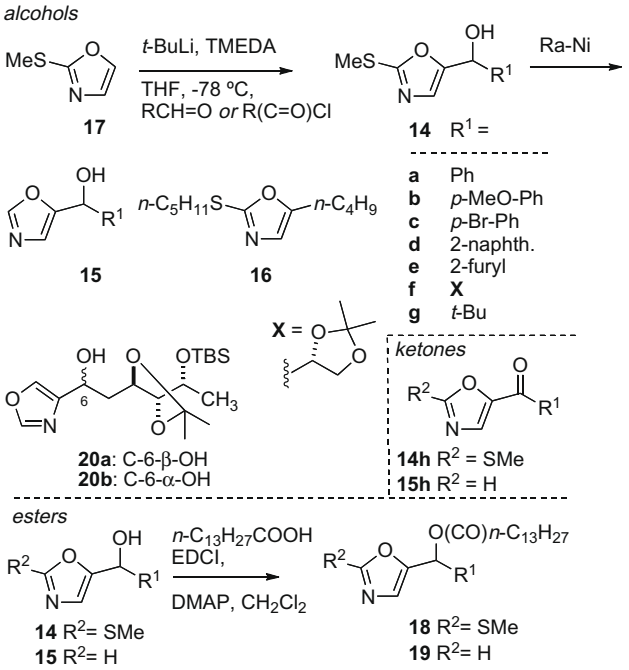
Compounds **7a–c** were prepared by a variant of the 'oxazole grafting' approach employed for the synthesis of **1** (Scheme 1).[4,11] Aldehyde **8** (prepared by DIBAL reduction of ethyl oxazole-4-carboxylate) was treated with *n*-PrMgBr and the product **9** was protected as the silyl ether **10** (TBSCl, imidazole, THF). Deprotonation of **10** using the Vedejs' protocol[12] (i: −78 °C, THF/hexanes, ii: BH_3·THF), followed by separate additions of three aldehydes (oxazole-5-carboxaldehyde, **11a**,[13] furfural, **11b** and benzaldehyde, **11c**), gave 2,4-substituted oxazole adducts **12a–c**.[14] Diastereomeric mixtures of the secondary carbinols **12a–c** were acylated in good yield (myristoyl chloride, Et_3N, DMAP) to give the long-chain esters **13a–c** that were deprotected (TBAF, THF) to afford bengazole A analogs **7a–c**. This small library, **7a–c**, **12a–c** and **13a–c**, was tested in paper disk diffusion assays against *Candida albicans* (96–489, a patient clinical isolate, and ATCC 14503), *C. glabrata* and *C. krusei*. To our surprise, none were active at doses of up to 300 μg/disk.

In order to determine the properties of mono-oxazole analogs of **1**, selected 5- and 2,5-substituted oxazoles (Scheme 2) were tested in disk diffusion assays against five species of *Candida* including fluconazole-resistant *C. glabrata*, *C. krusei* and *C. albicans* (Table 1).

The preparation of alcohols **14a–g**, **15a, d**, **g**, ketones **14h** and **15h**, and 2-methylthio-5-butyl-oxazole (**16**), starting with 2-methylthiooxazole (**17**),[16] have been reported previously[15] and summarized in Scheme 2. Selected alcohols **14** and **15** were O-acylated (EDCI, DMAP, *n*-$C_{13}H_{27}COOH$) group to give the new myristate esters **18** and **19**. When assayed against fungi (Table 1), all compounds were found to be less potent that **1** or AmB. The most active compounds were **14c** (9–17 mm zones of inhibition) and ketones **14h** (8–12 mm) and **15h** (9–12 mm). 2-Methylthio-5-oxazolyl analog **14a** appeared to be slightly less active than its 5-monosubstituted counterpart **15a**. Surprisingly, myristate esters **18a**,**c**,**g** and **19a**,**g** lost activity compared to their active parent alcohols, suggesting the activities of the compounds were limited by solubility. This contrasts with **1** where the fatty acyl chain is required for activity. Intermediates **20a** and **20b**, employed in the synthesis of **1**,[4] (Table 1) showed modest activity (9–10 mm)



Scheme 1. Synthesis of truncated 2,4-disubstituted oxazole analogs of **1**.



Scheme 2. 2-Methylthio-, 5- and 2,5-disubstituted oxazoles. See Ref. 15 for **14a–g**, and **15a**, **d**, **g**, Ref. 4 for **18a:18b**, and Ref. 15b for **16**.

Table 1
Anti-*Candida* activity of analogs of **1**: disk diffusion assays

	Zones of inhibition @ 300 μg/disk (mm)[a]				
Compd	*C. albicans*[c]	*C. glabrata*	*C. krusei*	*C. albicans*[d,e]	*C.albicans*[e]
1[b]	50	15	35	15	50
14a	10	0	9	9	9
14b	8	7.5	0	0	7.5
14c	13	10	9	12	17
14d	7.5	0	6.5	8	9
14e	8	7.5	7.5	0	8
14f	8	7	0	0	7
14g	0	0	0	0	0
14h	8	7	11	10	12
15a	10	0	9	12	12
15d	10	0	7.5	8	8
15g	0	0	0	0	0
15h	9	10	8	12	–
16	8	0	7	0	–
18a,**c**,**g**	0	0	0	0	0
19a,**g**	0	0	0	0	0
20a	10	0	9	0	0
20b	8	0	0	0	0
AmB[f]	22	19	15	12	23

[a] Sabouraud agar.'–', not tested.
[b] 10 μg/disk.
[c] ATCC 14503.
[d] 96–489, patient isolate, Ref. 9b.
[e] CDFR1, fluconazole-resistant (MIC >32 μg/mL), see Ref. 9b.
[f] AmB, amphotericin B (5 μg/disk).

Table 2
Anti-*Candida* activity of analogs of **1**: minimum inhibitory concentrations (MIC's)[a]

Compd	*C. albicans*[c]	*C. krusei*[b,d]	*C. albicans*[e]
1	1	—	1
14c	4	4	8
14d	8	16	8
15d	>64	>64	32
18c	>64	>64	>64
18d	>64	>64	>64
19d	>64	>64	>64
AmB	0.50	0.25	0.50

[a] RPMI media, 37 °C, 24 h incubation.
[b] 48 h incubation.
[c–e] See caption in Table 1 for strain identification.

although diastereomer **20a**, matching the configuration of **1**, was slightly more active with a susceptibility profile similar to that of **1**.

The most active compounds retained antifungal activity when tested in microbroth dilution assays (Table 2, for example **14c**, MIC = 4 μg/mL against *C. albicans* and *C. krusei*), but again, myristate esters **18c**, **d** and **19d** were inactive.

Clearly, the antifungal activity of **1** is not accounted for by simple analogs with one or two oxazole rings alone. At the very least, activity was correlated with the presence of a 5-monosubstituted oxazole (Scheme 2). 2,4-Disubstituted oxazolyl carbinol analogs, including their long-chain esters, were inactive. Interestingly, the closest heterocyclic analog, **7a**, to bengazole A (**1**), was inactive suggesting abrogration of biological activity upon replacement of the hydrophilic polyol side chain of **1** with a truncated *n*-propyl carbinol. The fatty acyloxy chain at C-10 potentiates activity *only* when the native polyol chain is present (e.g., **1**) since highly lipophilic **18a**,**c**,**g** and **19a**,**g** are inactive.

Although lipophilicity plays a role in activity of bengazole analogs, not all the effects can be explained by simple log*P* considerations, and subtle effects appear to be conferred by both the hydrophilic and fatty acyl chains.

In summary, several analogs of bengazole A (**1**) were prepared, containing one or two 1,3-oxazole rings, and modified side chains. Good activity was observed in some compounds but none were more potent than **1**.

Partial recovery of susceptibility of *Candida* strains was observed in some 5-monosubstituted oxazole analogs. These findings will guide further development of antifungal analogs of **1**, particularly modifications of the lipophilic acyl and hydrophilic polyol side-chains.[17]

Acknowledgements

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