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Synthesis and Antifungal Activity of (−)-(*Z*)-Dysidazirine

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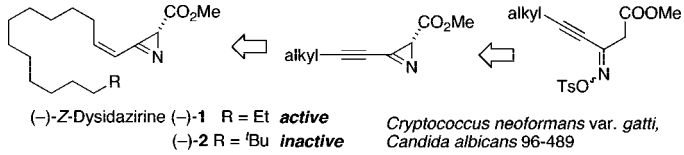
ABSTRACT



A short, flexible synthesis of the marine natural product (2*R*)-(*Z*)-dysidazirine (−)-1 has been completed. (−)-1 shows significant antifungal activity across a panel of seven human pathogens, whereas the structural analogue (−)-2, featuring a terminal *tert*-butyl group, is essentially inactive.

Invasive fungal infections, particularly those associated with *Candida* sp., pose a serious and increasing threat to human health. Widespread use of fluconazole since the mid-1990s has led to the rise of resistant *Candida* strains (e.g., *Candida glabrata* and *Candida albicans*) and attendant increased mortality among immunocompromised individuals.[1,2] New antifungal agents are urgently needed, and marine natural products represent an underexploited source of potential leads.[2]

(2*R*)-(*E*)-Dysidazirine, (−)-**3**, one of a few rare naturally occurring azacyclopropenes (2*H*-azirines), was isolated in 1987 from the marine sponge *Dysidea fragilis* (collected in Fiji).[3] Compound (−)-**3** exhibited potent antifungal activity against *C. albicans* and *Saccharomyces cerevisiae* (4 μg/disk, disk-diffusion assay). More recently, brominated analogues **4** and (+)-**6** (antazirines) were isolated along with (*Z*)-dysidazirine (**1**) and (2*S*)-(*E*)-dysidazirine [(+)-**3**] from the same sponge species collected in Micronesia.[4] Antifungal activity for **4** and (+)-**6** were not reported,[4] and they were found to be inactive against a panel of bacteria.

We recently isolated three new antazirine analogues [(+)-**5**, (+)-**7**, and (−)-**8**] from a sample of *D. fragilis* that also provided the two known antazirines [(+)-**4** and (+)-**6**].[5] All five antazirines proved to be inactive against a panel of *Candida* and *Cryptococcus* spp., in stark contrast with (−)-**3**. This surprising result led us to hypothesize that terminal substitution (e.g., Br, Cl, Me) attenuates antifungal activity in long-chain 2*H*-azirines. In order to explore this hypothesis, we have developed a short, flexible synthesis of (−)-**1** and structural analogue (−)-**2** that features a terminal *tert*-butyl group.

There is only one published synthesis of a long-chain 2*H*-azirine, that of *E*-dysidazirine, completed in 1995 by Davis and co-workers.[6] In this case, the key azirine ring was formed by treating an appropriately substituted *N*-(*p*-tolylsulfinyl)-

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(5) Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 2592–2597. All *naturally occurring* long-chain azirines from marine sponges appear to be heterochiral (non-racemic mixtures of enantiomers).

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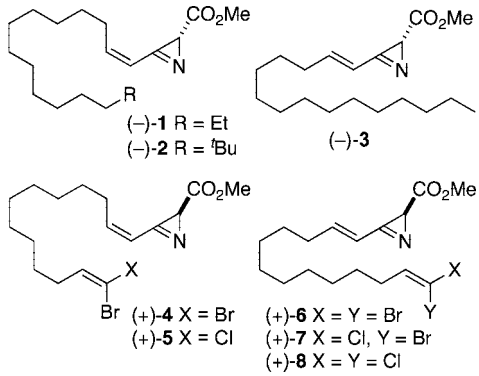


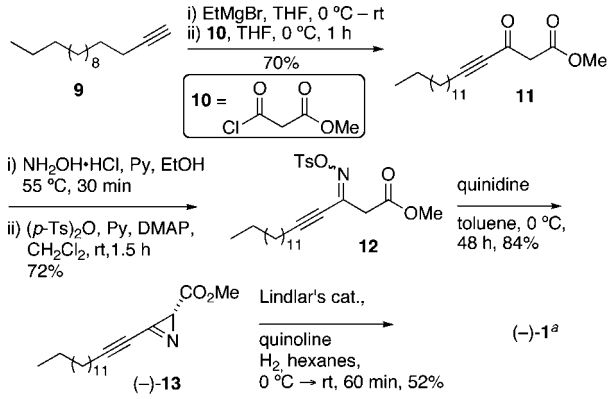
Figure 1. Structures of (*Z*)- and (*E*)-dysidazirine [(−)-**1** and (−)-**3**], antazirines [(+)-**4**−**8**], and synthetic (*Z*)-dysidazirine analogue [(−)-**2**].

2-carbomethoxyaziridine with LDA at −78 °C. While this approach allowed for synthesis of (−)-**3** with high optical purity, the necessity to preserve the *Z* configuration in (−)-**1** required an alternate approach. We chose a strategy based on a variant of the asymmetric cinchona base-catalyzed Neber reaction of *O*-tosyl-oximes first reported by Zwanenburg and co-workers.[7]

Synthesis of (2*R*)-(*Z*)-dysidazirine [(−)-**1**] began with addition of the lithio-anion of pentadecyne (**9**) to methyl malonyl chloride (**10**, Table 1). The addition gave poor, variable yields when the deprotonation of **9** was effected with *n*-BuLi; using EtMgBr, however, led to clean formation of **11** in a reproducible 70% yield.

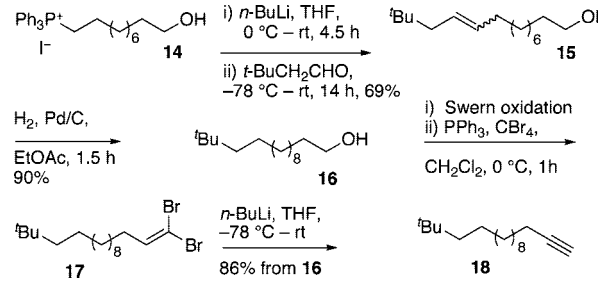
Keto-ester **11** was converted to the corresponding oxime (NH_2OH·HCl, pyridine, EtOH, 55 °C, 30 min), which was tosylated immediately (*p*-toluenesulfonic anhydride, pyridine, DMAP, 1.5 h) giving **12** in two steps (72% yield). Treatment of **12** with quinidine (0 °C, 48 h) lead to clean, albeit slow, formation of the desired 2*H*-azirine ring.[7] While the product azirine was formed with only modest enantioselectivity, this methodology is notable for its practical simplicity and high chemical yield.

Scheme 1. Synthesis of (−)-(*Z*)-Dysidazirine **1**



[a] % ee for (−)-**1** = 59% (determined by chiral HPLC, Chiralpak AD, 90:10 hexanes/*i*-PrOH).

Scheme 2. Synthesis of 14,14-Dimethylpentadecyne (**18**)

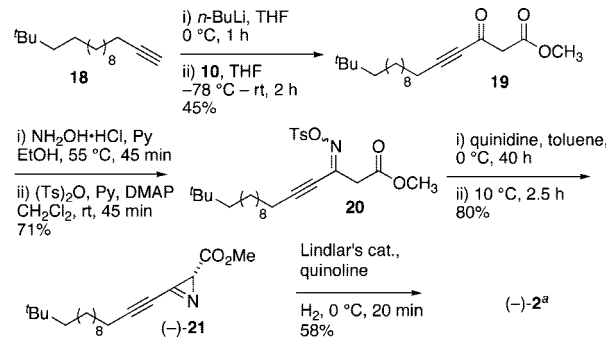


Partial hydrogenation of (−)-**13** using Lindlar's catalyst at ambient temperature in EtOH proved difficult due to facile reduction of the product alkene and the azirine ring within minutes. An improvement was found by lowering the temperature of hydrogenation (0 °C, hexanes) to give (*Z*)-dysidazirine [(−)-**1**] in 52% yield and optical purity (59% ee) comparable to the related natural products.[5]

In order to examine the effect of terminal substitution on antifungal activity, analogue (−)-**2** was synthesized starting with Wittig reaction between phosphonium bromide **14** and 3,3-dimethylbutanal, giving alkene **15** as a mixture of double bond isomers. Hydrogenation of **15** (H_2, Pd/C, EtOAc, 1.5 h) gave saturated alcohol **16**, which was oxidized under Swern conditions to give the corresponding aldehyde.[8] Treating the crude aldehyde immediately with PPh_3/CBr_4 (CH_2Cl_2, 0 °C, 1 h)[9] afforded dibromoalkene **17**, which was subsequently converted to terminal alkyne **18** (*n*-BuLi, THF, −78 °C → rt) in 86% overall yield from **16**. Addition of the lithiated acetylide ion of **18** to **10** proceeded reliably to give **19**, albeit in moderate yield (45%).

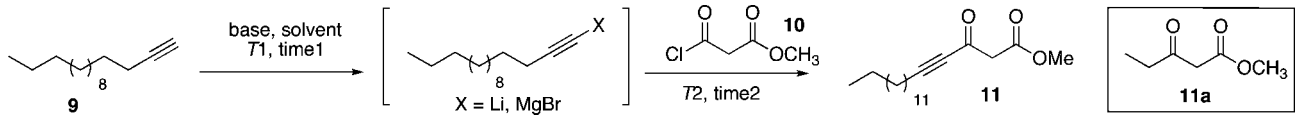
Keto-ester **19** was converted to the corresponding oxime-tosylate **20** (71%), which underwent quinidine-mediated cyclization to give the desired azirine (−)-**21** in 80% yield. Cyclization of **20** proceeded even more slowly than the corresponding reaction with **12** and required brief warming to 10 °C to ensure complete consumption of starting material. Lindlar reduction of (−)-**21** gave (−)-(*R*)-17,17-dimethyl-(*Z*)-dysidazirine [(−)-**2**] in 58% yield.

Scheme 3. Synthesis of 17,17-Dimethyl-(*Z*)-dysidazirine



[a] % ee for (−)-**2** = 60% (HPLC, Chiralpak AD, 10:90, *i*-PrOH /hexanes).

Table 1. Optimization of Pentadecyne Addition to Methyl Malonyl Chloride



entry	base	solvent	$T1$ (°C)	time1 (min)	equiv **10**	$T2$ (°C)	time2 (min)	yield (%)
1	*n*-BuLi	THF	0	90	0.5	−78→0	150	0[a]
2	*n*-BuLi	Et_2O	−20	60	0.5	−60→0	120	14[a]
3	EtMgBr	Et_2O	0 → rt	80	0.5	0	40	0[b]
4	EtMgBr	THF	0 → rt	120	0.8	0	50	19
5	EtMgBr	THF	0 → rt	150	0.5	0	60	70

[a] Estimated from crude NMR. [b] Compound **11a** was isolated in 35% yield.

Both (−)-**1** and (−)-**2** were screened for antifungal activity against a panel of *Candida* and *Cryptococcus* spp. (Table 2). Synthetic (*Z*)-dysidazirine [(−)-**1**] showed strong activity against each fungal strain (MIC_{50} 2−8 μg/mL) with the exception of *C. krusei* (MIC_{50} 16 μg/mL). This appears to be consonant with the activity originally reported for isomeric (−)-*E*-**3** in 1988.[3] In contrast, compound (−)-**2** with the bulky *tert*-butyl chain terminus was effectively inactive across the entire panel.

Table 2. Antifungal activity of (−)-**1** and (−)-**2**[a]

	MIC_{50} (μg/mL)	
	(−)-**1**	(−)-**2**
C. albicans ATCC 14503	8	>64
C. albicans UCD-FR1[b]	8	>64
C. albicans 96−489[b]	4	>64
C. glabrata[b]	4	16
C. krusei	16	>64
Cryptococcus neoformans var. *grubii*	2	9
Cryptococcus neoformans var. *gatti*	2	>64

[a] The *in vitro* susceptibility of each compound was determined by the broth micro dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). [b] Fluconazole-resistant (MIC > 64 μg/mL).

This surprising result points to a subtle complexity in mechanism of action for (−)-**1**. The target of (−)-**1** in fungi is unknown; however, it is likely to involve essential metabolism of lipids.

The electrophilic properties of the strained α,β-unsaturated azacyclopropene suggests, at first glance, that the antifungal activity of (−)-**1** might arise from nonspecific Michael addition to nucleophilic amino acid residues (e.g., cysteine, lysine). The differential activity observed for (−)-**1** and (−)-**2** however, argues for a more specific effect that involves metering of the lipid chain.[10] We postulate that (−)-**1** engages its target protein in a two-point binding motif in which the lipid chain occupies a hydrophobic pocket with stringent steric requirements while the electrophilic α,β-unsaturated azirine carboxylate terminus binds at a distal site. The specificity of this interaction with respect to chain length remains to be investigated, however it is notable that dysidazirine shares a common chain length (C_{18}) with several sphingolipids important for fungal cell growth, such as phytosphingosine and ceramide.[2] This suggests dysidazirine may be an "antimetabolite" that interdicts fungal cell lipid metabolism, perhaps sphingolipid biosynthesis. A more extensive study of SAR and mechanism of action of (−)-**1** is underway, and our results will be reported in due course.

In summary, we have completed the first synthesis of the marine natural product (−)-(*Z*)-dysidazirine[4] and a structural analogue (−)-**2** that features a terminal *tert*-butyl group. Compound (−)-**1** shows significant antifungal activity toward a range of *Candida* and *Cryptococcus* species, but (−)-**2** is almost completely inactive. This surprising result suggests that the activity of (*Z*)-dysidazirine is modulated by two-point binding to an as-yet unidentified target protein in fungal cells. Interaction between (−)-**1** and its putative target likely involves sterically stringent interaction between the lipid side chain and a hydrophobic domain.

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Supporting Information Available: Full experimental details and characterization of synthetic intermediates; copies of ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) It is unlikely the effects are due to differential cell wall permeability since the *c* $\log_{10}$ *P* of (−)-**1** and (−)-**2** are similar (6.8 and 7.6, respectively, ChemDraw Ultra).