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Synthesis and chain-dependent antifungal activity of long-chain 2*H*-azirine-carboxylate esters related to dysidazirine

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ABSTRACT

Analogues of the antifungal marine natural product (*E*)-dysidazirine were prepared and evaluated in broth ro-dilution assays against a panel of fungal pathogens. A simple structure–activity relationship was developed which provides insight into the mechanism of action of long-chain 2*H*-azirine carboxylates.

2*H*-Azirines are highly strained, weakly aromatic heterocycles rarely found in Nature. In fact, only nine natural products containing the 2*H*-azirine ring have been described to date. Azirinomycin[1] (**1**) was isolated from *Streptomyces aureus* while the other compounds constitute a small family of long-chain 2*H*-azirine carboxylates from marine sponges. The first of this family, (*E*)-dysidazirine (**2**), was isolated in 1988 from *Dysidea fragilis*, collected in Fiji, and shown to exhibit potent antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae* (4 μg/disk, disk diffusion assay).[2] Faulkner and co-workers later characterized (*Z*)-dysidazirine (**3**) and two ω-dibromovinylidene azirine-2-carboxylate methyl esters, **4** and **5** (*E*- and *Z*-antazirines) from *D. fragilis* and reported them as inactive 'against a standard panel of microorganisms.[3] Motualevic acid F, the parent carboxylic acid of *ent*-**4**, was reported by Bewley and co-workers along with related amides from *Siliquariaspongia* sp. with antibacterial activity against *Staphylococcus aureus* and MRSA.[4] In our search[5] for antifungal compounds from marine organisms with efficacy against Fluconazole-resistant strains of *Candida* and other pathogenic yeast,[6] we found three new antazirines (**6**–**8**) along with **4** and **5** from a sample of *D. fragilis* collected in Pohnpei, Micronesia that showed cytotoxicity in a crude screen.[7] Compounds **4**–**8** were moderately cytotoxic (HCT-116 cells), but to our surprise they were completely inactive against a panel of fungal pathogens including *C. albicans* (ATCC, UCD-FR1, and 96-489), less susceptible, non-*albicans* species such as *Candida glabrata* and *Candida krusei*, and two strains of *Cryptococcus neoformans*, var. *grubii* and *gatti* (Fig. 1).

In order to elucidate the relationship between structure and antifungal activity of long-chain 2*H*-azirine carboxylates we carried out the first synthesis of (*Z*)-dysidazirine[8] and a short series of structural analogues ((−)-**9**→**13**, Fig. 2), along with (*E*)-dysidazirine. We report here the structure-activity relationships for (−)-**2**, (−)-**3**, (±)-**3**, and (−)-**9**→**13** that reveal structural determinants for antifungal activity, including terminal branching [(−)-**9**], chain length [(−)-**10** and (−)-**11**], C4–C5 unsaturation [(−)-**12**, (−)-**13**] and C2 configuration [(±)-**3**].

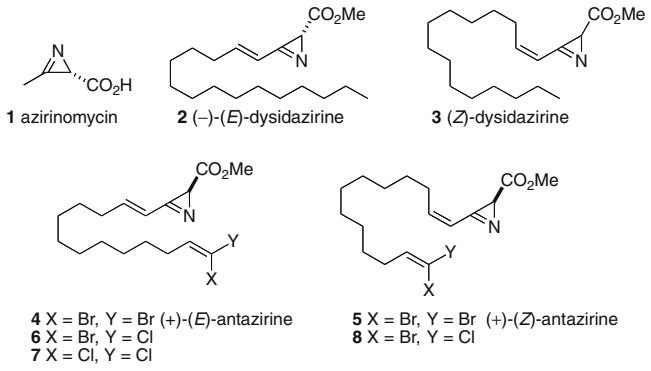

Figure 1. Naturally occurring 2*H*-azirines.

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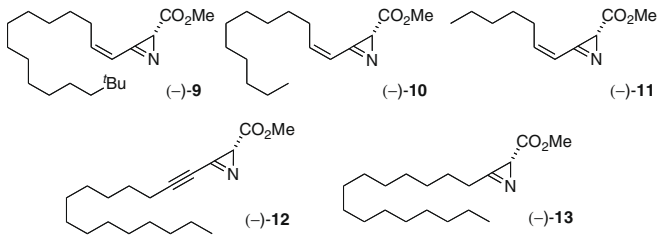
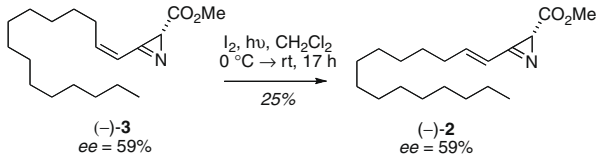


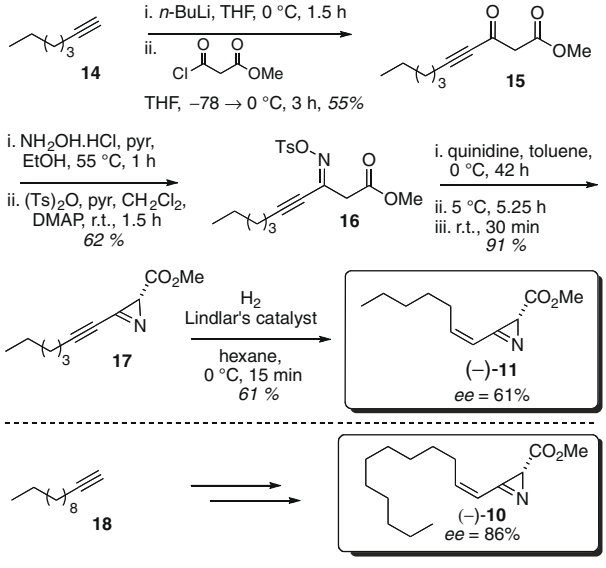
Figure 2. Targeted synthetic dysidazirine analogues.

The synthesis of (−)-(*Z*)-dysidazirine and analogues (−)-**9** and (−)-**12** has been described previously.[8] Photochemical isomerization (500 W sunlamp, Pyrex) of synthetic (−)-**3** (59% ee) provided (−)-**2** (Scheme 1) in low yield but without epimerization at C2. Natural dysidazirine and congeneric compounds have all been isolated as non-racemic mixtures of enantiomers. Neat, natural dysidazirine spontaneously epimerizes slowly in the dark ($t_{1/2}$ ~12y, −20 °C),[7] and the lack of racemization of **2** and **3** in the presence of light excludes a Photochemical mechanism.

The shorter chain analogues (−)-**10** and (−)-**11** were prepared in an analogous fashion to (−)-**3** (Scheme 2). Addition of the lithio-acetylide derived from alkyne **14** to methyl malonyl chloride gave -ketoester **15** in reasonable yield (55%). Treatment of **15** with $NH_2OH{\cdot}HCl$/pyridine led to the corresponding oxime which was converted to oxime tosylate **16** without purification. Cyclization in the presence of quinidine under Zwanenburg's conditions[9] provided 2*H*-azirine **17** in excellent yield. Partial hydrogenation with Lindlar's catalyst provided the truncated dysidazirine analogue (−)-**11**. The same sequence applied to alkyne **18** gave analogue (−)-**10**.



Scheme 1. Photochemical isomerization of (−)-(*Z*)-dysidazirine to (−)-(*E*)-dysidazirine.



Scheme 2. Synthesis of truncated analogues (−)-**10** and (−)-**11**.

Synthesis of (−)-**13**, the 4,5-dihydro analogue of **3**, began with generation of the enolate of dioxolane **19** (LDA, HMPA, −40 °C) followed by alkylation with 1-bromotetradecane (**20**) to provide **21** in low yield (18%) along with an equivalent amount of the product from α-alkylation (Scheme 3). Thermolysis of **21** (microwave) gave the incipient ketene that was captured with methanol to afford *O*-methyl β-ketoester **22**, and subsequently converted to oxime tosylate **23** in two steps as before. Treatment of **23** with quinidine led directly to (−)-**13** in 91% yield.

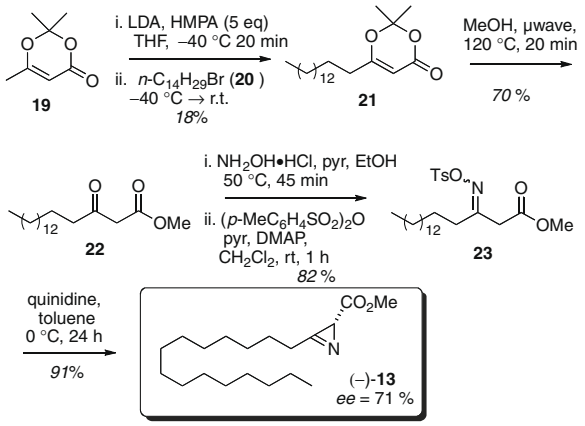
The antifungal MICs of the synthetic and natural azirines are compared in Figure 3.[10] Each yeast strain was susceptible to (−)-**3** and some synthetic analogues with the notable exception of Fluconazole-resistant *C. krusei*. The other Fluconazole-resistant strains (*C. glabrata*, *C. albicans* UCD-FR1 and 96-489) were all susceptible. Strains of *C. neoformans* (var. *grubii* and var. *gatti*) were the most susceptible overall.

Both (−)-**2** and (−)-**3** were consistently among the most active compounds against all strains, with MIC values in the range of 2–8 μg/mL. For *Candida* spp., (−)-**3** was notably more active than (−)-**2** (Fig. 3a). Shorter chain analogues (−)-**10** and (−)-**11** were comparable in activity to (−)-**3**, although Fluconazole-resistant *C. albicans* UCD-FR1 was susceptible to (−)-**10**, but not to (−)-**11**.

Alkynyl analogue (−)-**12** retains most of the activity of the natural products (−)-**2** and (−)-**3**, however the 4,5-dihydro analogue (−)-**13** lost activity across the entire panel of fungal cells. The C2 configuration appears to be less important. Race *Z*-dysidazirine [(±)-**3**] shows good activity against all strains, comparable to or slightly better than the optically enriched homologue (−)-**3**.

As previously observed,[8] the *tert*-butyl terminus analogue (−)-**9** is essentially inactive. Clearly terminal substitution or branching abrogates antifungal activity, consistent with the lack of activity for ω-halo-alkenyl antazirines (**4**–**8**).[3,7]

The foregoing results suggest specific structural attributes determine antifungal activity rather than non-specific toxicity. Our original hypothesis regarding a putative sub-cellular target of dysidazirine, based on data obtained from (−)-**3** and (−)-**9**, is supported by the wider range of analogues presented here.[8] We favor a model for activity of long-chain azirines involving interaction with a putative protein target through a 2-point binding motif and propose the following. We envision the lipid chain occupies a tightly constrained hydrophobic pocket that is intolerant of bulky ω-substitution, which helps to explain the loss of activity seen for **4**–**8** and (−)-**9** despite log *P*s and molecular size that are comparable to (−)-**3**. The polar, electrophilic azirine terminus—a potent Michael acceptor capable of covalent modification of nucleophilic sites (e.g., cysteine, lysine)—binds at a distal site, possibly interacting with one or more nucleophilic amino acid residues. The absolute requirement of C4–C5 unsaturation for high antifungal



Scheme 3. Synthesis of 4,5-dihydrodysidazirine [(−)-**13**].

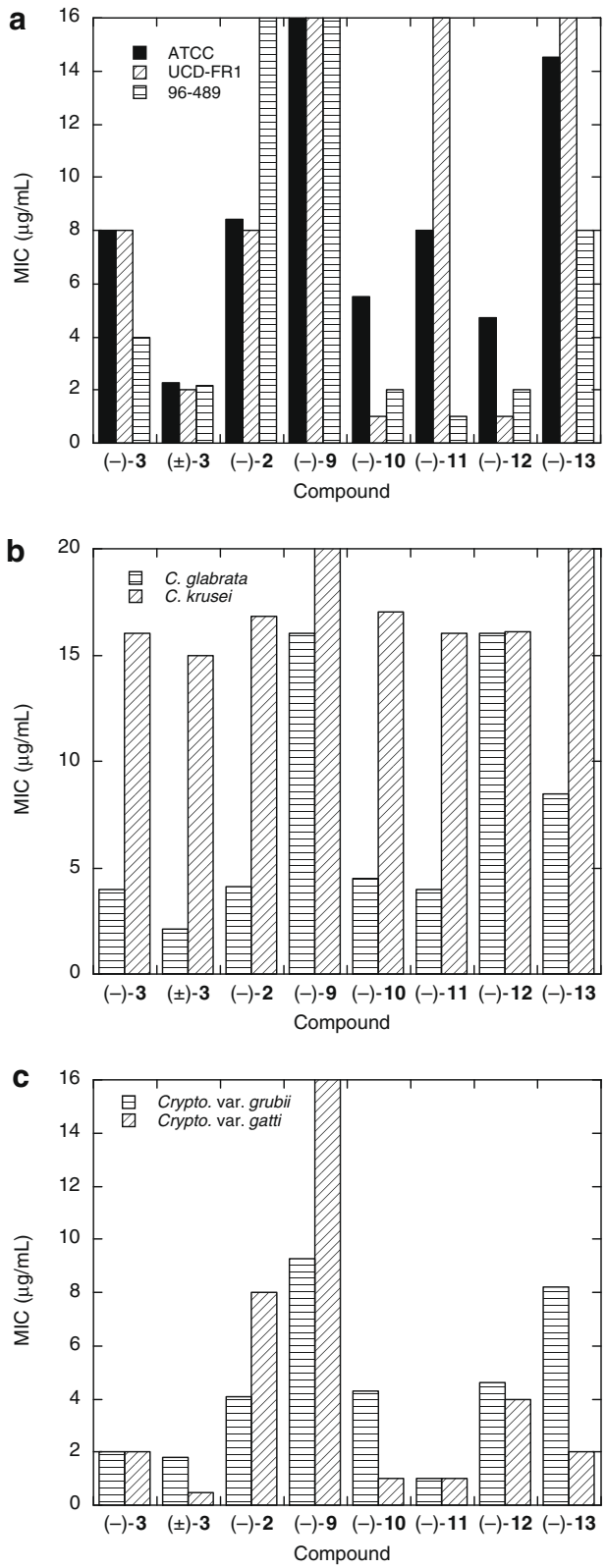


Figure 3. Antifungal activity of (−)-**2**, (−)-**3**, (±)-**3**, and (−)-**9**→**13** against: (a) *Candida albicans*, three strains: ATCC 14503, UCD-FR1, and 96-489 (see Ref. 18); (b) *Candida glabrata* and *Candida krusei*; (c) *Cryptococcus neoformans* var. *grubii* and *gatti*. Compounds with off-scale MICs were inactive up to 64 μg/mL.

activity supports the latter hypothesis. (*Z*)-Dysidazirine [(−)-**3**)], which is expected to be a superior Michael acceptor to (−)-**2**, shows enhanced antifungal activity against *C. albicans* strains.

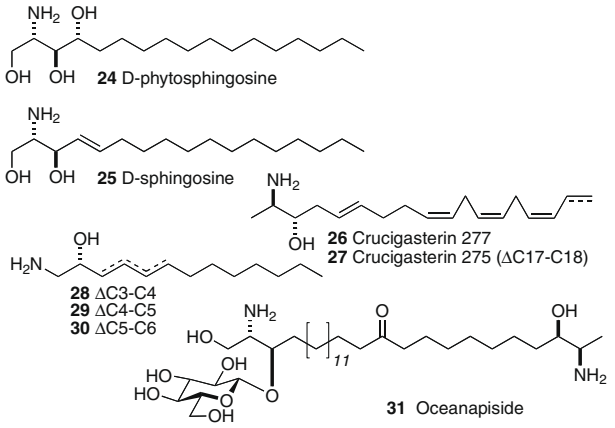


Figure 4. Structures of sphingoid bases and antifungal long-chain aminoalcohol marine natural products.

The structure of dysidazirine bears a resemblance to phytosphingosine (**24**) and sphingosine (**25**) (Fig. 4)—both **24** and **25** share the same carbon chain length (C_{18}) as dysidazirine. The long-chain base **24** occurs in plant and fungal cells and acts in multiple capacities, both as a cellular structural component and a signaling molecule.[11] Sphingosine (**25**) is the primary long-chain base of mammalian cells; it also plays roles in cell structure and signaling, and exhibits modest antifungal activity (30 μg/mL, *C. glabrata*).[12]

Several long-chain vicinal aminoalcohol marine natural products (Fig. 4) also exhibit antifungal activity,[5] including crucigasterins 275 (**26**) and 277 (**27**),[13] (*R*)-1-aminotridecen-2-ol[14] (**28**–**30**), and oceanapiside (**31**),[15] an unusual 'two-headed' sphingoid base.[16]

Natural products dysidazirine (−)-**3**, other long-chain azirines and **31** resemble sphingosine or its immediate precursor sphinganine (4,5-dihydrosphingosine) and may act as antimetabolites that competitively inhibit sphingolipid metabolism.

The biosynthesis of **24** and **25** in yeast follows the canonical sphingolipid pathway that begins with pyridoxal phosphate-dependent condensation of palmitoyl CoA thioester with serine. Long-chain azirines may compete for natural substrate in the condensation reaction or downstream reactions that tailor **24** and **25**. It is notable that the antifungal properties of **3** and other sphingoid base analogues are not solely determined by the presence of a long hydrophobic tail since simple lipids are inactive and other antifungal long-chain fatty acid conjugates appear to act by distinctly different mechanisms.[17]

In summary, we have completed the synthesis of a series of dysidazirine analogues and evaluated each for antifungal activity against a panel of clinically-relevant yeasts. Several generalizations can be made: (1) C4–C5 unsaturation is required for high activity. (2) Activity is not strongly dependent on minor changes in chain length or the configuration at C2. (3) Terminal branching of the lipid chain abolishes activity. Further investigations are underway in our laboratories to identify high-affinity binding targets of (−)-**3**.

Acknowledgments

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