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Synthesis of a Novel Bicyclic Scaffold Inspired by the Antifungal Natural Product Sordarin

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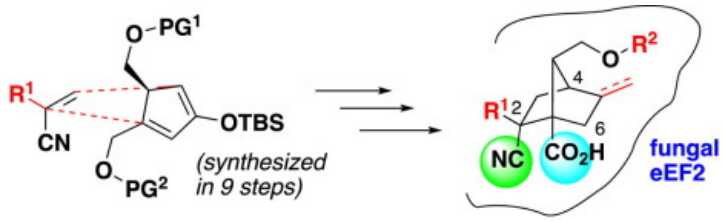
Abstract

A simplified bicyclic scaffold inspired by the antifungal natural product sordarin was designed and synthesized which maintains the carboxylic acid/aldehyde (or nitrile) pharmacophore. Docking studies with the target for sordarin, the fungal protein eukaryotic elongation factor 2 (eEF2), suggested that the novel scaffolds may bind productively. A densely functionalized chiral cyclopentadiene was constructed in 8 steps and utilized in a Diels-Alder reaction with acrylonitrile. The resulting [2.2.1] cycloheptene was transformed into a scaffold possessing vicinal carboxylic acid and nitrile groups, with orientations

predicted to provide high affinity for eEF2. The synthetic approach disclosed here sets the stage for a renewed medicinal chemistry campaign against eEF2.

Graphical abstract

A simplified bicyclic scaffold inspired by the natural product sordarin was designed and synthesized which will facilitate the preparation of novel antifungal agents.



An approach to novel scaffolds for use in eEF2 inhibitors is disclosed

Keywords Function-oriented synthesis; Antifungal agents; Sordarin; Diels-Alder reaction

An estimated 1.5 million people die each year from invasive fungal infections (IFIs) [1]. Clinical options for the treatment of IFIs are extremely limited and generally only include a small number of azole, echinocandin, and polyene (amphotericin B) antifungals. Of these treatments, only the azoles are orally available, but their value has been diminished by the increasing prevalence of resistant strains [2]. For these reasons, novel classes of antifungal drugs are urgently needed [3]. In the 1990s it was discovered that derivatives of the natural product sordarin (**1**), known since the 1960s as an antibacterial and antifungal agent [4], are highly active against pathogenic fungal species, particularly *C. albicans* (Fig. 1, e.g. **3** to **5**) [5], [6], [7]. A mode of action was deduced for sordarin that is unique for antifungals, and appears to be related to that of the antibacterial fusidic acid[8], [9]. Sordarin halts protein synthesis at fungal ribosomes by binding to eukaryotic Elongation Factor 2 (eEF2) and inhibiting the interaction of eEF2 with ribosomal stalk proteins [10], [11], [12]. Importantly, sordarin derivatives are able to selectively eradicate numerous fungal strains, including fluconazole-resistant *C. albicans*, without significant toxicity to mammalian cells [13], are orally available, and have shown promising results in animal models of invasive fungal infections [7], [14], [15], [16].

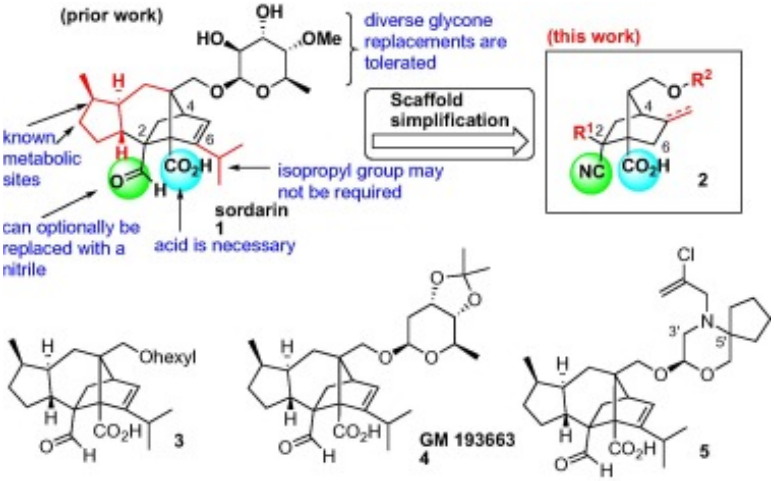


Figure 1. SAR of semisynthetic sordarin analogs and designed simplified bicyclic scaffold.

Despite significant efforts by the pharmaceutical industry in the 1990s and early 2000s to develop semisynthetic sordarin analogs via ready modification of the glycosyl portion of the molecule, no eEF2 inhibitors have advanced to clinical stages. The unmet potential of this class of molecules is amplified by findings that some derivatives also show broad spectrum activity, including against pathogenic *Aspergillus* species (**5**, Fig. 1) [7]. However, this potential is attenuated by the synthetic challenge of modifying the complex sordarin core, which is prone to *in vivo* oxidation of the cyclopentane ring to generate poorly active metabolites [17, 18]. Impressive total syntheses of sordarin or its aglyconesordaricin have been reported by Kato [19], Mander [20], and Narasaka [21], but the reported routes are lengthy and not amenable to convenient modifications of the sordarin core.

Our interest in function-oriented synthesis [22] as a strategy for simplifying and modifying natural products [23] led us to re-examine the complex diterpene core of sordarin, with the goal of generating novel scaffolds that could be more easily modified to improve properties such as metabolic stability and activity against resistant strains. An unsuccessful attempt at identifying a simplified sordarin scaffold with potent antifungal activity was reported by Cuevas in 1998, involving a monocyclic cyclopentane[17], but otherwise we are not aware of the de novo synthesis of sordarin-inspired scaffolds for antifungal applications. Novel scaffolds and synthetic approaches to this class of inhibitors could reignite the dormant interest in eEF2 as a target for potent and safe antifungal agents.

More recently, our interest in novel scaffolds is supported by the x-ray crystal structures of sordarin or related compounds with eEF2 that were reported subsequent to the majority of semisynthetic medicinal chemistry efforts [9, 24, 25]; these could enable the prioritization of novel compound designs with routine docking algorithms. Published patents and structure-activity relationship (SAR) studies, and inspection of the sordarin–eEF2 x-ray structure reported by Andersen [24], highlight the necessity of a carboxylic acid at C1 and an aldehydeor nitrile [5] at C2 of the bicyclic core of sordarin (Fig. 2). A carboxylic acid at the bridgehead position of the scaffold forms hydrogen bonds with a backbone amide (Glu524) of eEF2, as well as two bridging water molecules (Fig. 2, left). The acid moiety is essential for activity, and no alternative functional groups have been reported to be effective. The aldehyde of sordarin acts as a hydrogen bond acceptor for the backbone amide of Ala562; a nitrile was reported to be an effective replacement of this aldehyde moiety, and in some cases was more potent [5]. Interestingly, the glycosyl moiety is not critical for activity against specific strains, and highly potent analogs have been reported possessing aliphatic alkyl chains [5].

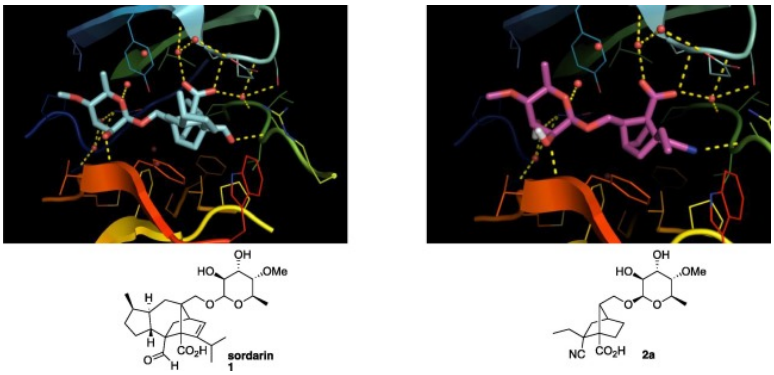


Figure 2. X-ray structure of sordarin with eEF2 (left); docked structure of designed analog **2a** (right).

With this and other SAR data in mind, we designed novel scaffolds that maintain the pharmacophore of sordarin, but with removal of the fused cyclopentane ring, and replacement with

alternative metabolically stable groups (**2**, Fig. 1). We hypothesized that scaffolds with decreased complexity such as **2**could also facilitate SAR studies and the subsequent improvement of physico/physiochemical properties that are not feasible with the natural scaffold. A docking study was performed with compounds of type **2** and the sordarin–eEF2 x-ray structure (PDB 1N0U[24]) using FITTED® by Molecular Forecaster [26]. Our simplified sordarin analogs generally yielded similar docking poses to sordarin and comparable docking scores to compounds with simple alkyl glycosyl replacements such as **3**that have been reported to be potent antifungal agents against *S. cerevisiae*[5]. A representative docking pose is given in Fig. 2(right), in comparison to the x-ray structure in Fig. 2 (left) of sordarin with eEF2, which suggests that nitriles such as **2a** will indeed be able to effectively replace the aldehyde moiety of sordarin as an H-bond acceptor for the backbone amide of Ala 562.

A retrosynthesis of compounds of type **2** is depicted in Fig. 3. The Diels-Alder cycloaddition could permit the late stage introduction of a variety of substituents at C-2. We prioritized nitrile-containing compounds over aldehydes for their better stability and tolerance of a range of reaction conditions. For ease of synthesis, we also prioritized analogs alkylated at C-5 instead of C-6, especially since the x-ray structure of eEF2 suggests that various substituents could be tolerated in both positions. Cyclopentadienes of type **6** were selected as key synthetic targets, with the silylether substituent able to polarize the diene to provide the desired regioselectivity with the nitrile and latent carboxylic acid moieties on adjacent carbons, as well as increasing its reactivity. A related intermolecular Diels-Alder reaction was reported by Ciufolini [27]. One important disadvantage to substituted cyclopentadienes is that they are prone to 1,5-hydride or alkyl shifts [28], but we were inspired by the work of Gleason and coworkers disclosing that the silylether could greatly increase the stability of cyclopentadienes to undesired hydride shifts (isomerization) [29]. Cyclopentadienes of type **6** could be generated by enolization of an enone; enones of type **7** could be prepared via a carbonylation of triflate **8**, followed by an allylic oxidation reaction. Aldol reaction between cyclopentanone and formaldehyde, with a subsequent generation of the kinetic enolate and trapping with an appropriate electrophile, would generate enol triflate **8**.

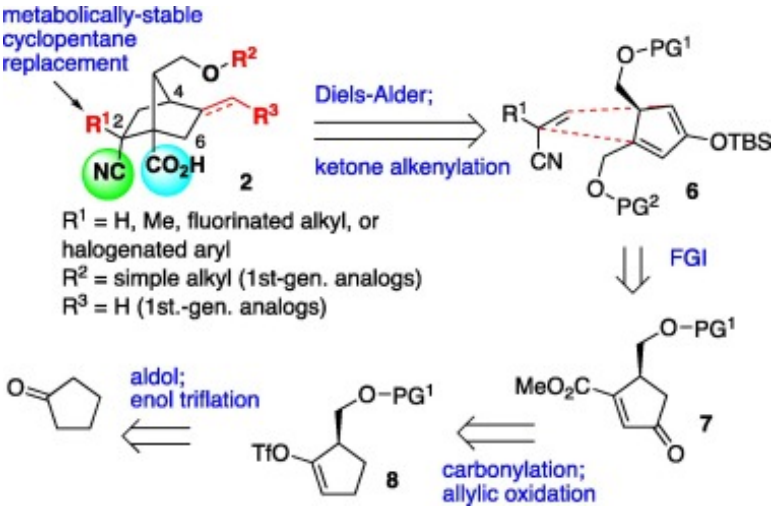
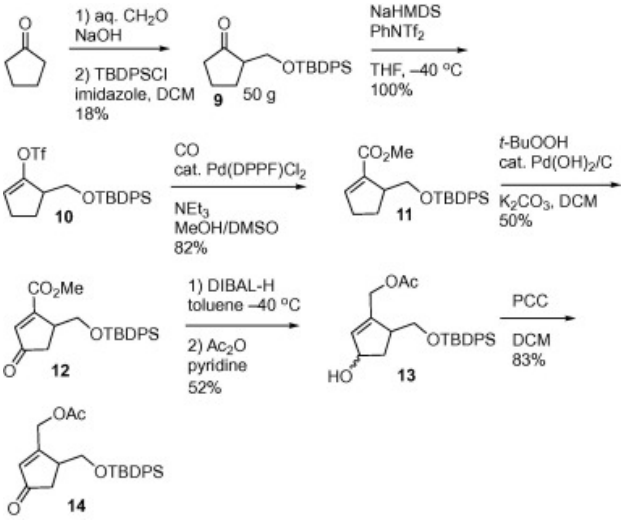


Figure 3. Retrosynthesis of simplified sordarin analogs.

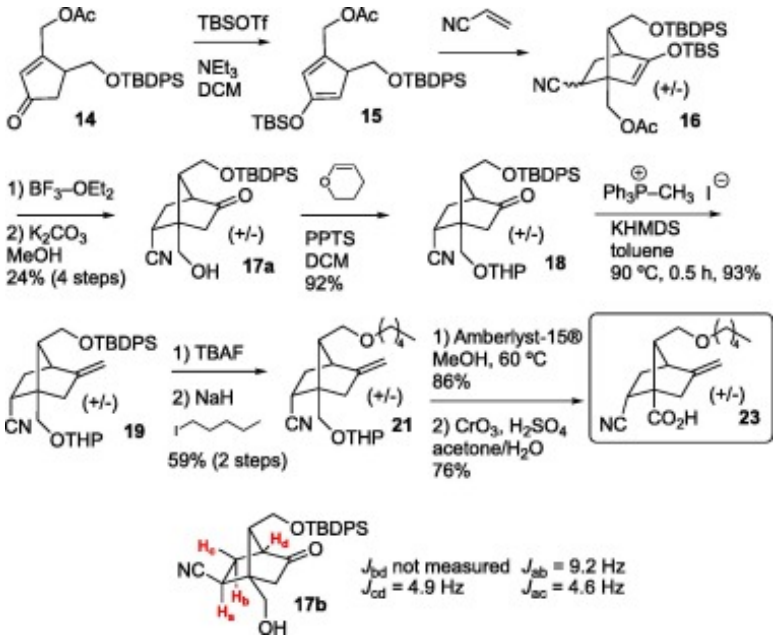
The synthesis of the desired cyclopentadienes proceeded broadly according to plan, with racemic materials generated in our first-generation synthesis disclosed here (Scheme 1). An excess of cyclopentanone was reacted with formaldehyde in an aldol reaction [30], followed by distillation and

protection of the alcohol with TBDPSCl to generate large quantities of silylether **9**, after recrystallization. After screening several bases and electrophiles, the kinetic enol triflate **10** was obtained in quantitative yield using NaHMDS and $PhNTf_2$ at –40 °C. Palladium-catalyzed carbonylation and trapping with methanolproceeded smoothly to yield enoate **11**. Allylic oxidation using Corey's reported protocol (*t*-BuOOH, cat. $Pd(OH)_2/C$) yielded enone **12**[31]. Reduction of both the ketone and ester moieties with DIBAL-H generated a diol intermediate as an inconsequential mixture of diastereomers, which was acetylated selectively at the primary alcohol to give **13**, then the secondary alcohol was oxidized with PCC to yield the enone **14**.



Scheme 1. Synthesis of cyclopentenone **14.**

Enone **14** was treated with TBSOTf and base to generate cyclopentadiene **15**, which was subjected to a variety of Diels-Alder reactions with different aldehyde, ester, and nitrile-containing dienophiles (Scheme 2). The most useful product was obtained from reaction with excess acrylonitrile; even though a 1:1 mixture of endo/exo diastereomers was obtained, these were separable by chromatography at a later stage. Diels-Alder reactions with carboxyl-substituted cyclopentadienes (instead of hydroxymethyl-substituted systems such as **15**), were unsuccessful, likely due to poor matching of HOMO/LUMO levels.



Scheme 2. Synthesis of first-generation antifungal scaffold: bicyclic nitrile acid **23.**

The racemic mixture of cycloadducts **16** underwent selective removal of the silylenol ether using BF_3 etherate [32]. The remaining acetate protecting group proved to be problematic for several transformations, so it was removed under basic conditions, and the *endo*/*exo* diastereomeric alcohols were separated by flash chromatography; the isolated yield is not reflective of mixed fractions that were omitted. The desired *endo*product **17a** and exo diastereomer **17b** were isolated and assigned via COSY and NOESY NMR, inspection of the ^{1}H NMR coupling constants, and comparison to literature coupling constants. Protons b and c (Scheme 2, bottom) of the exo isomer **17b** were differentiated by the negligible coupling of H_b with the bridgehead H_d, due to a dihedral angle approaching 90° [33]. $^3J_{ab}$(9.2 Hz) is consistent with the *cis* coupling reported by Williamson for a nitrile-substituted bicyclo[2.2.1]heptene (9.3 Hz) [34], therefore our data are consistent with H_a of **17b** residing on the *endo* face of the bicycle (see Supporting Information for spectra).

Initial efforts at protection of **17** with PMB or Bn were unsuccessful, so a THP protecting group was utilized to cleanly give **18**. Several functional group transformations of the C-5 ketone are presently being explored, but to maintain lipophilicity on the eastern face of the bicycle we elected to methenylate the ketone with a Wittig reaction. Elevated temperatures were required (90 °C), but the alkene **19** was cleanly obtained without epimerization of the α-nitrile carbon. Removal of the TBDPS protecting group with TBAF and alkylation of the resulting alcohol with *n*-pentyl iodide generated the ether **21**, containing a simple alkyl chain analogous to those on sordarin analogs reported to be highly potent against *S. cerevisiae*[5]. Analogs with extended alkyl chains are not expected to be metabolically stable (due to their likely accessibility to the active site of cytochromes P450), but for ease of synthesis we elected to build such an analog first to validate the scaffold synthesis prior to attaching more complex glycosyl groups presumably required for high potency against species such as *C. albicans*. The THP group of **21** was removed under acidic conditions, then subjected to a Jones oxidation to generate the desired carboxylic acid **23**, which represents our first simplified sordarin analog.

Though it was inactive against several strains of *C. albicans* at concentrations up to 8 μg/mL using the CLSI M27-A3 broth microdilution method (RPMI + MOPS, pH 7.0 as the liquid medium), the preparation of **23** validates our intermolecular Diels-Alder strategy towards the preparation of functionalized bicyclic scaffolds with the requisite positioning of carboxylic acid and aldehyde/nitrile moieties for inhibition of fungal eEF2. Our present efforts are directed towards the addition of alkyl and aryl substituents at C-2, the incorporation of validated glycosylgroups, and the development of an asymmetric synthesis of the desired bicyclic scaffolds. Our novel synthetic strategy facilitates the exploration of unaddressed structure-activity relationships of sordarin-type eEF2 inhibitors, and may lead to the identification of antifungal agents with improved properties.

Associated content

Supporting Information includes synthetic procedures, characterization data, NMR spectra, and select LC-MS traces.

Author contributions

Conceived the project: C.D. Designed compounds and synthetic routes: C.D., Y.W. Performed docking studies: C.D. Tested reactions, synthesized compounds, characterized products: Y.W. Wrote the manuscript: C.D. Wrote the Supporting Info: Y.W., C.D.

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Notes

A patent application including this work has been submitted. A version of this manuscript has been submitted to the preprint server ChemRxiv.

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Appendix A. Supplementary data

The following are the Supplementary data to this article:

https://ars.els-cdn.com/content/image/1-s2.0-S0040403918309572-mmc1.pdf

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