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Antifungal Sordarins. Part 3: Synthesis and Structure–Activity Relationships of 2′,3′-Fused Oxirane Derivatives

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Abstract—A number of new 2′,3′-fused oxirane derivatives were synthesized for structure–activity relationship study. Many of these derivatives exhibit high potency against *Candida* spp. In addition, sordarin *manno* epoxide derivative **6** presents in vivo therapeutic effect in mice and is considered a promising antifungal lead within this series. © 2002 Elsevier Science Ltd. All rights reserved.

The incidence of both nosocomial and community acquired fungal infections has increased over the past two decades with a consequent rise in mortality. A number of factors have contributed to this trend. Among them are: a growing population of immunocompromised patients, the use of broad spectrum antibiotics and immunosuppressive therapeutic regimes, and the use of new and agressive therapeutic strategies or life-support systems. Treatments for these fungal infections are unsatisfactory and restricted to few classes of agents like polyenes (e.g., amphotericin), azoles (e.g., fluconazole), candins (e.g., caspofungin) or amines (e.g., terbinafine).[1a,1b] The use of these drugs has some issues like short spectrum of action, limited ways of administration, and emergence of resistance and toxicity. Therefore, the development of new drugs with novel modes of action for treating fungal infections continues to be a major challenge.

Sordarins[1c,1d] are a new class of antifungal agents which are potent and selective inhibitors of fungal protein synthesis. Sordarins bind to EF-2. This binding is stronger when this elongation factor is complexed with the ribosome. A ribosomal stalk protein, P0, is also involved in binding since its mutations lead to resistance to sordarins. Natural acylated derivatives (e.g., GR 135402, **1a**) of sordarin (**1b**)[2,3] are potent antifungals with a moderate spectrum of action (Fig. 1).[4] Their chemical structures comprise a diterpene aglycon and a modified sugar moiety (sordarose). Sordarin aglycon is endowed with structural elements which seem to be essential for activity: the vicinal aldehyde and carboxylic acid groups and the rigid tetracyclic skeleton. Based on our discoveries in this area,[4–6] we have continued a chemical programme aimed at the preparation of sordarose modified derivatives. The main objective was to widen the spectrum of this family to cover some of the *Candida* spp. not initially included like *Candida glabrata*. Previous work in this direction has been centered on modifications around the 3′ and 4′ positions in the sordarose ring.[5,6]

In this communication, we describe the effects on antifungal activity caused by the arrangement of substituents at 2′ and 3′ positions as fused oxirane or thiirane ring. Although the compound presenting a 2′,3′-fused epoxide was initially considered as key intermediate to obtain other 3′-substituted derivatives, this unusual and simplified arrangement has led to very active compounds. In addition, other slight modifications around the pyranose ring have been done. In

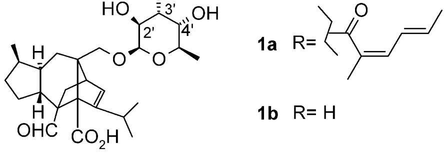

Figure 1. Natural GR 135402 (**1a**) and sordarin (**1b**).

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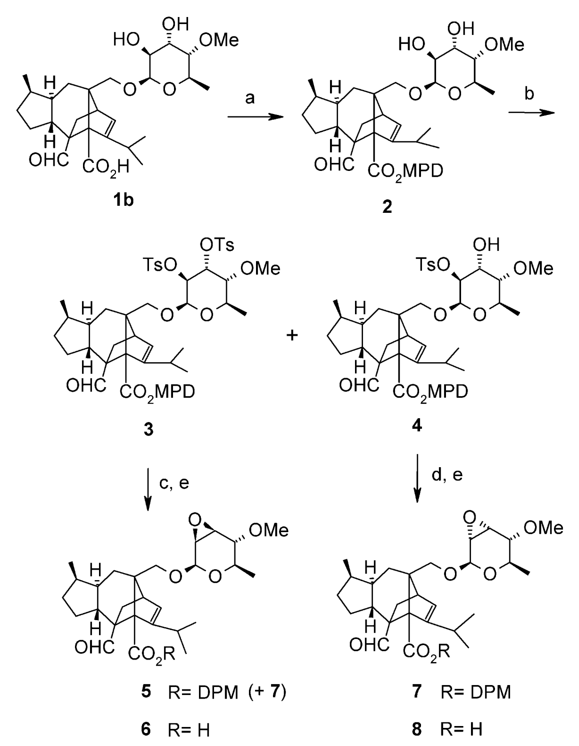
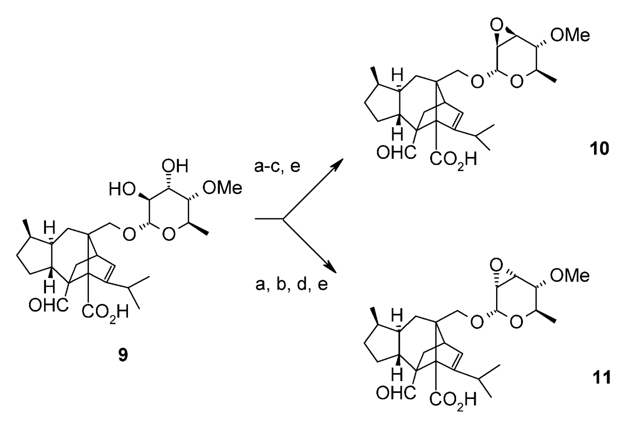
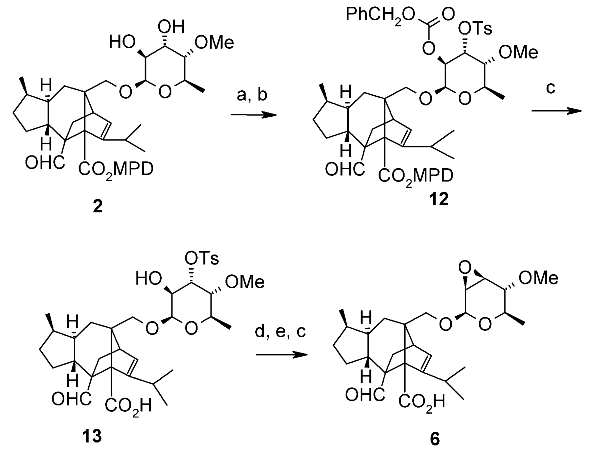


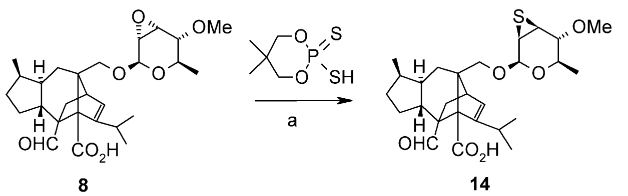
Scheme 1. (a) Ph_2CN_2, CH_2Cl_2, (90%); (b) TsCl, DMAP, CH_2Cl_2 (70% for the mixture **3**+**4**); (c) NaMeO, MeOH, (70%, for the mixture **5**+**7**); (d) NaH, DMF (86%); (e) H_2, Pd/C, EtOAc (97%).



Scheme 2. (a) Ph_2CN_2, CH_2Cl_2, (90%); (b) TsCl, DMAP, CH_2Cl_2 (35%); (c) NaMeO, MeOH (40–60%); (d) NaH, DMF (91%); (e) H_2, Pd/C, EtOAc (95%).



Scheme 3. (a) $PhCH_2OCOCl$, DMAP, CH_2Cl_2 (60%); (b) TsCl, DMAP, CH_2Cl_2 (89%); (c) H_2, Pd/C, EtOAc; (d) NaH, DMF (94%); (e) Ph_2CN_2, CH_2Cl_2 (87%, e+c).



Scheme 4. (a) Et_3N (52%).

this direction, we have also studied the importance of both the anomeric stereochemistry (C-1′) and the substitution in the position C-6′.

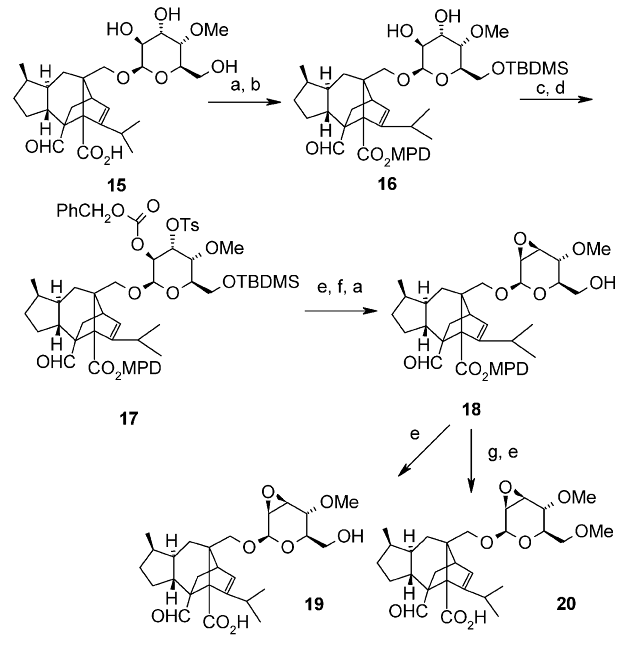
Chemistry

The synthetic route followed for the preparation of these derivatives is outlined in Scheme 1. Sordarin from large scale fermentation was used.[2] For all the synthetic transformations the sordarin carboxylic group was conveniently protected as diphenylmethyl (DPM) ester[7] to give compound **2**.

Treatment of **2** with tosyl chloride yielded a 1:1 mixture of mono (**4**) and di-tosyl derivatives (**3**). Reaction of the di-tosyl derivative **3** with sodium methoxide in methanol[8] afforded a 2:1 mixture of protected epoxides **5** (*manno*) and **7** (*allo*). The *allo* isomer was obtained unequivocally through internal nucleophilic substitution of the 2′-*O*-tosyl derivative **4** (sodium hydride/dimethyl formamide). Final catalytic hydrogenation afforded the deprotected compounds **6** and **8**. This same route was followed for the preparation of the α-glycosidic isomers **10** and **11** starting from α-sordarin **9** through the corresponding di- and mono-tosyl derivatives, respectively (Scheme 2).[9]

A preparative synthetic route (Scheme 3) which avoids the obtention of the *allo* epoxide derivative was developed. Thus, selective protection of the 2′ hydroxyl group of **2** as benzyl carbonate and subsequent reaction of the free 3′ hydroxyl group with tosyl chloride afforded **12**. Complete deprotection of the 2′-hydroxyl and carboxylic acid groups was achieved through catalytic hydrogenation to afford acid **13**. This compound cyclized to the *manno* epoxide **6** in basic media (sodium hydride/dimethylformamide). For handling purposes, the cyclized acid was converted into its diphenylmethyl ester, purified and then deprotected via catalytic hydrogenation. By this route, compound **6** was obtained in a 36% overall yield.

Thiirane ring was also considered as another modification within this series. No protection was required for the preparation of the 2′,3′-fused thiirane derivative. Single reaction of the *allo* 2′,3′ epoxide **8** with 2-mercapto-5,5-dimethyl-2-thioxo-1,3,2-dioxa phosphorinane[10] under reflux of triethylamine yielded the corresponding episulfide **14** (Scheme 4). This compound presents the



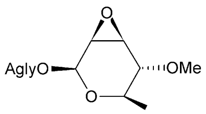
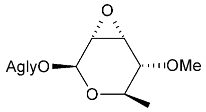
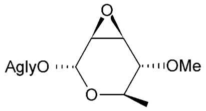
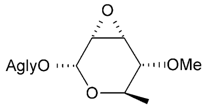
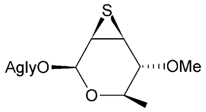
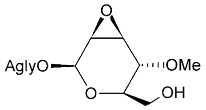
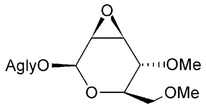
Scheme 5. (a) Ph_2CN_2, CH_2Cl_2 (94%); (b) TBDMSCl, imidazole, CH_2Cl_2 (86%); (c) $PhCH_2OCOCl$, DMAP, CH_2Cl_2 (53%); (d) TsCl, DMAP, CH_2Cl_2 (92%); (e) H_2, Pd/C, EtOAc (87–95%); (f) NaH, DMF (86%, f+a); (g) NaH, MeI, THF (85%).

opposite *manno* stereochemistry since the reaction takes place with inversion of both 2′ and 3′ chiral configurations.

Chemical modification of position 6′ was possible starting from bioproduced 6′-hydroxysordarin **15** which is obtained along with sordarin on fermentation of *Sordaria araneosa*.[11] The synthetic route described in Scheme 5 was followed. The carboxylic acid and the 6′-hydroxyl groups were protected as diphenylmethyl ester and *tert*-butyldimethylsilyl ether, respectively, to give **16**. The oxirane ring formation was accomplished using the above-mentioned procedure for **6** (Scheme 3).

Firstly, 2′ and 3′ hydroxyl groups were selectively derivatized to give **17**. This compound was transformed into 2′,3′-fused epoxide derivatives **19** and **20** in a three-step sequence: hydrogenation in order to release the 2′-hydroxy group, intramolecular epoxide ring formation (sodium hydride/dimethylformamide) and carboxylic protection as diphenylmethyl ester for ease of handling. During the cyclization reaction step the O–Si bond was also cleaved and **18** was obtained. Hydrogenolysis (H_2, Pd/C, EtOAc) of **18** gave 6′-hydroxy acid **19**. On the other hand, the 6′-methoxy acid **20** was obtained after previous methylation reaction.

Table 1. Antifungal activity as minimum cell growth inhibition [MIC (μg/mL)]

Compd	R	logP[a]	*Candida albicans* 4711E	*Candida albicans* 2005	*Candida glabrata* 2375E	*Candida pseudotrop.* 2371	*Candida tropicalis* 2808	*Cryptococcus neoformans* 2867
6		2.24	0.25	0.06	16	0.03	0.25	8
8		2.24	—	0.25	125	0.12	4	>125
10		2.24	8	4	31	1	31	>125
11		2.24	16	4	31	2	>125	125
14		2.84	0.12	0.03	16	0.008	0.5	>125
19		1.05	16	4	>125	16	31	>125
20		1.67	0.5	0.06	62	0.25	4	16
1a[4]	Me(E)CH=CH(Z)CH=CHMe	—	0.25	0.015	>125	0.03	0.06	0.25

[a]Calculated with MedChem v 3.53 interface with REACCs v 8.3. Molecular Design Limited, 2132 Farallon Drive, San Leandro, CA 94577, USA.

Biological Evaluation

Table 1 shows the in vitro antibacterial activity (referred to minimum concentration inhibiting fungal cell growth, MIC)[12] of compounds synthesized. This assay was carried out in broth microdilution using RPMI + glucose as culture medium. MIC values for GR 135402 (**1a**) are included as reference.

Results and Discussion

Among the 2′,3′ epoxides derived from sordarin, the *manno* isomer with β configuration at the anomeric carbon center (**6**) shows the best in vitro biological profile. Its antifungal spectrum includes *C. albicans*, *C. pseudotropicalis* and *C. tropicalis* ($\text{MIC} \leq 0.25$ μg/mL) as well as *C. glabrata* ($\text{MIC} = 16$ μg/mL) and *C. neoformans* ($\text{MIC} = 8$ μg/mL). Due to this fact, the structural arrangement present in **6** was chosen for additional modifications at the 6′ position. Change of epoxide stereochemistry to the *allo* configuration (**8**) led to a decrease in potency and a narrower spectrum as compared to the compound with the *manno* configuration. When stereochemistry change is produced at C-1′, as in compounds **10** and **11** (derived from the α-anomer of sordarin), a great decrease in potency can be observed. A similar result was obtained for other types of sordarin derivatives with 3′,4′-fused dioxolane rings.[6] This effect points out that β-stereochemistry at the glycosidic center may be essential for activity in the whole class of sordarin derivatives. Compounds with low lipophylicity ($\log P \leq 1$) like **19** show poorer antifungal activity. In contrast, the biological results obtained for the 6′-methoxylated **20** ($\log P = 1.67$) are close to those of unsubstituted epoxide **6**, though MIC values are still higher. On the other hand, the replacement of oxygen by sulfur in the three-membered ring (**14**) does lead to a slight improvement in activity against *Candida* spp, although activity against *C. neoformans* is missed. Further in vitro evaluation[13] for compound **6** was carried out against 54 clinical isolates of *C. albicans* (including some fluconazole resistant strains) giving a $\text{MIC}_{90} = 0.25$ μg/mL. MIC range for fourteen fluconazole resistant isolates is 0.03–0.004 μg/mL. Preliminary biological tests have pointed out that the *manno* epoxide **6** shows a significant in vivo protection for treatment of mice infected with *C. albicans* 4711E (84 and 93% survival after 7 days under sc and po administration, respectively, with respect to fluconazole treatment).[14] In conclusion, modification of the sordarin pyranose ring by fusing an oxirane ring to its C2′–C3′ bond has led to novel antifungals extending the spectrum of action. In particular, the 2′,3′-epoxide derivative **6** can be considered as a promising antifungal lead.

Acknowledgements

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14. Assay was carried out in male mice 8 weeks old. Infection was done by injecting 200 μL of *C. albicans* 4711E suspension (1.7×10^7 cfu/mL) in a lateral tail vein. Compound administration was done sc or po (50 mg/kg) at 0, 4, 24 and 28 h post-infection.