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Design and synthesis of simplified sordaricin derivatives as inhibitors of fungal protein synthesis

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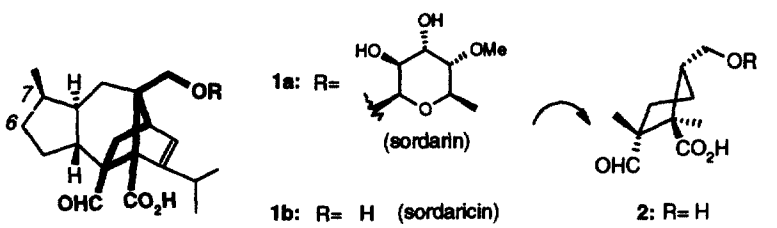
Abstract: A reduction of the tetracyclic skeleton of sordarins and sordaricins to a cyclopentane ring bearing the pharmacophore functional groups led to new derivatives retaining part of their *in vitro* and whole-cell activity.

Keywords: *antibiotics; enzyme inhibitors; fungi; molecular modeling/mechanics*

Sordarin (**1a**) is an antifungal mould metabolite isolated from *Sordaria araneosa*.[1] Derivatives of sordarin (**1a**) and its aglycone sordaricin (**1b**) are selective and potent fungal protein synthesis inhibitors.[2] In addition, these derivatives are endowed with a good whole-cell activity against the majority of fungal pathogens[3] and, therefore, are considered as promising antifungal agents. Sordaricin (**1b**) presents a rather unusual tetracyclic diterpene structure bearing what is supposed to be its pharmacophoric group: a formyl and a carboxylic acid group in a vicinal arrangement with a high enough dihedral angle to avoid internal hemiacetalisation, and a third appendage which not only might enhance binding but could function as cell uptake modulator as well. These fungal protein synthesis (FPS) inhibitors lose part of their potency in the presence of mouse serum and liver S-9 fraction which is likely due to serum binding and/or enzymatic degradation. On the basis of mass spectrometric studies of the metabolites coming from liver degradation, we have been able to determine that cyclopentane C-6 and C-7 positions suffer oxidative metabolism through cytochrome P-450 mediated hydroxylation.[4] In order to improve bioavailability of these antifungals and to ascertain whether a more reduced structure like **2** (Scheme 1) could retain inhibitory activity of the fungal protein synthesis we initiated a chemical program aimed to design and synthesize simpler sordarin related fungal protein synthesis inhibitors.



Scheme 1

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Molecular Modeling

One of the geometrical requirements for a reduced model like **2** was a dihedral angle OHC-C-C-COOH similar to that of natural sordaricin. Besides, the distances of the three main functionalities from each other should be close to those of **1b**. From chemical considerations the presence of a methyl group in each one of the corresponding α positions of the aldehyde and carboxylic acid groups was considered suitable in order to avoid possible epimerizations. To investigate the former arguments we have performed molecular modeling calculations of both structures. These were built using INSIGHT software[5] and energy minimized with cff95 force field using steepest-descent and convergent-gradients algorithms with a convergence criteria of 0.001 kcal/mol. In addition, we have carried out semiempirical molecular orbital calculations using the AM1 hamiltonian within MOPAC 6.0.[6]

Table 1. Geometrical constraints from energy minimisations.

compound	C-C distance (Å) (COOH,CH_2OH)	C-C distance (Å) (COOH,CHO)	dih. angle (C_{3a},C_{8a},CH_2OH) (C_1,C_5,CH_2OH)	dih. angle (C_4,C_{3a},COOH) (C_2,C_1,COOH)
sordaricin (**1b**)	3.1	3.2	60.3°	67.3°
simplified sordaricin (**2**)	3.3	3.4	85.5°	94.9°

From distances and dihedral angles obtained (Table 1) two points can be drawn: (a) simplified sordaricin **2** maintains the main structural features of **1b** regarding COOH to CH_2OH and COOH to CHO distances, and (b) the differences found for the key dihedral angles could be due to the lower strain energy for the simplified ring as compared with the tetracyclic structure. Figures 1 and 2 show minimised structures of **1b** and **2** and its molecular superimposition, respectively.

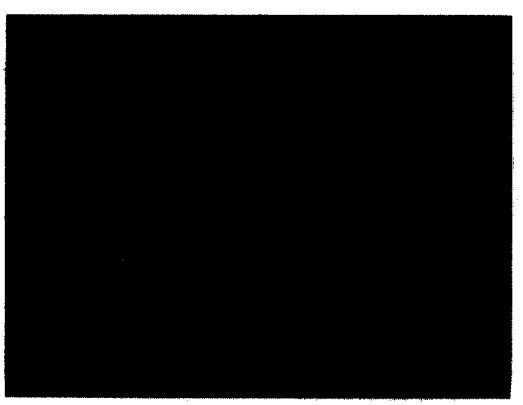


Figure 1. AM1 structure of **1b** and **2**

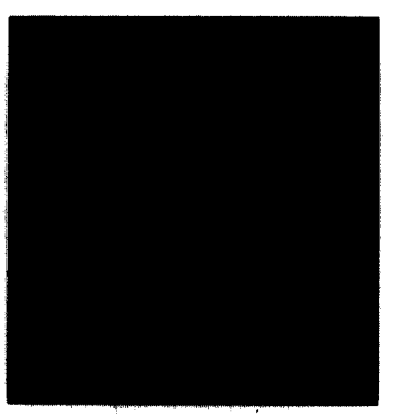
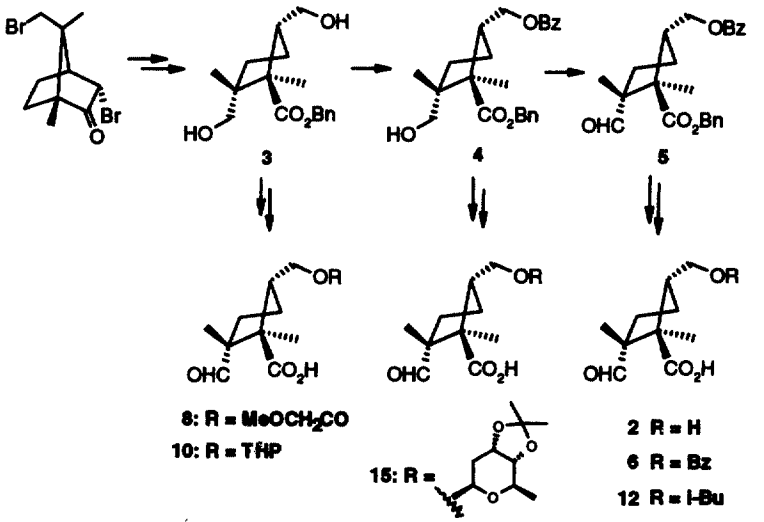


Figure 2. Fitting of **1b** and **2**

Chemistry

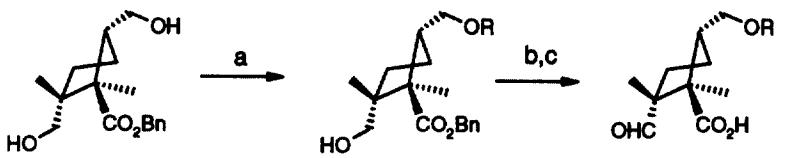
Apart from the simplified sordaricin analogue **2** we selected five representative simplified derivatives, **6**, **8**, **10**, **12**, and **15** (see scheme 2). We used different substituents to cover a range of lipophilicity (clogP from 0 to 4)[7] and several functional links to our simplified sordaricin moiety (ester, acetal, ether and glycoside). In addition, derivative **15** was selected because its sugar substructure resembles sordarin in the stereochemical arrangement of ring substituents and it is easily accessible from commercial (+)-D-digitoxose.

Recently, we have reported an enantiospecific synthesis of the simplified aglycon **2** and its benzoylated derivative **6** starting from (+)-3,9-dibromocamphor (Scheme 2).[8] We have taken advantage of some of the intermediates used in this synthetic route to prepare the other simplified derivatives described in this work.



Scheme 2

In this way, methoxy acetate **8** and tetrahydropyranyl ether **10** were obtained by regioselective derivatization of the least hindered alcohol in diol **3**. The neopentylic primary alcohols **7** and **9** were then oxidized and deprotected to the corresponding aldehydes **8** and **10** (Scheme 3).



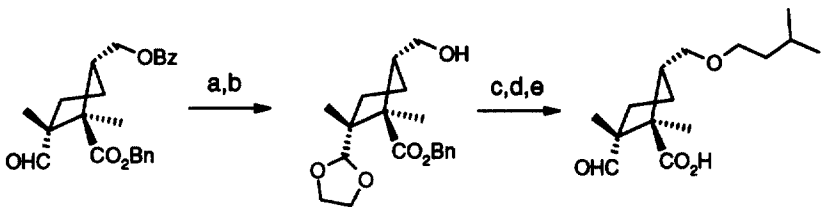
3 **7 R = MeOCH2CO** **8 R = MeOCH2CO**

9 R = THP (1:1 diast. mixt.) **10 R = THP** (1:1 diast. mixt.)

a: $MeOCH_2COCl$, CH_2Cl_2, -40°C, 30% (for **7**) and DHP, PPTS, CH_2Cl_2, 60% (for **9**);
b: PCC, CH_2Cl_2, r.t., 90%; **c:** H_2, Pd/C, EtOAc, 82%

Scheme 3

The benzoate **5** was the starting material for the preparation of the *i*-pentyl ether **12**. This preparation was carried out in five steps: aldehyde protection, benzoate hydrolysis, alkylation, hydrogenation and acidic acetal hydrolysis. (Scheme 4)

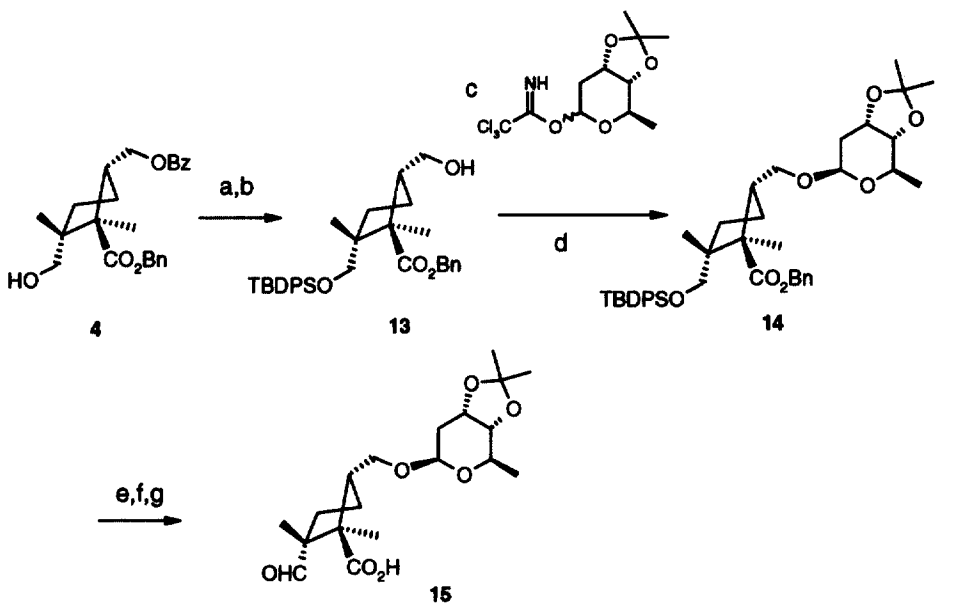


5 **11** **12**

a: $(CH_2OH)_2$, PPTS, toluene, refl., 87%; **b:** LiOH, THF/H_2O/MeOH, 90%; **c:** NaH, $Me_2C{=}CHCH_2Br$, THF, refl., 82%; **d:** H_2, Pd/C, EtOAc, 93%; **e:** 1N HCl, MeOH, r.t., 81%

Scheme 4

The key step for the attachment of the 2'-deoxyisopropylidene hexopyranose moiety was the glycosylation reaction[9] of the alcohol derivative **13**, obtained through silylation of **4**. Treatment of **13** with the protected trichloroacetimidate of D-(+)-digitoxose afforded a 2:3 mixture of α:β anomers from which the β–stereoisomer **14** could be separated by column chromatography. Final deprotection and re-oxidation steps led to inhibitor **15**. (Scheme 5)



4 **13** **14** **15**

a: TBDPSCl, imidazole, DMF, r.t., 94%; **b:** LiOH, MeOH/H_2O/THF, 83%; **c:** BF_3-Et_2O, CH_2Cl_2, 51%(β:α=3:2); **d:**chromatographic separation (hexane/ethyl acetate 5/1); **e:** TBAF, THF, r.t., 78%; **f:** PCC, CH_2Cl_2, r.t., 84%; **g:** H_2, Pd/C, EtOAc, 90%

Scheme 5

Biological results

It is known that all the sordarin derivatives act by inhibiting the translocational step of the elongation cycle. Recently, the complex of ribosome and EF-2 factor has been ascertained as the target for these types of antifungals.[10, 11, 12] The inhibitors prepared in this work were assayed for their inhibitory activity in a cell free *C. albicans* protein synthesis assay. This cell free translational system uses poly-U as synthetic messenger RNA template. The labeled amino acid, Phe-^{14}C, along with other energetic additives like ATP and GTP are added to these lysates. Upon incubation with the inhibitor, activity can be measured by scintillation counting of radiolabelled poly-Phenylalanine.

All the simplified inhibitors show inhibitory potency in this *C. albicans* protein synthesis assay[13] though at least twenty eight-fold lower than sordaricin. All of them, except **2**, also inhibit *C. albicans* cell growth with MIC values between 56 and 462 μM, which could account for the cell uptake modulator role of the attached substructures by increasing molecule lipophilicity. (Table 2)

Table 2. Antifungal activity of simplified derivatives: fungal protein synthesis (IC50) and cell growth (MIC) inhibition.

compound	clogP	IC50(*C.alb.2005E*) (μM)	MIC(*C.alb.2005E*)* (μM)
sordaricin (**1b**)	2.3	0.5	>376
2	-0.2	45	>624
6	2.7	32	102
8	0.4	81	459
10	1.1	14	56
12	2.4	185	462
15	0.9	162	84

*Broth microdilution assay. Medium: RPMIG

The molecular volume difference between **1b** and **2** might play a key role in enzyme interaction. In this way, sordaricin (**1b**), with the largest volume, shows the highest inhibition potency (lower IC_{50} value). On the other hand, lipophilicity, which could correlate with cell uptake, could be modulated by different substitution at the hydroxyl position as may be concluded from the MIC values for derivatives **6**, **8**, **10**, **12**, and **15**.

Even though the simplification of the molecules described here is too high, they maintain part of the *in vitro* and whole-cell activities of sordaricin (**1b**). In conclusion, these results show that it is possible to design and build simpler antifungals of this type. Further optimisation of all the parameters contributing to antifungal activity will lead to a more potent target molecule.

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13. The target of these new inhibitors was confirmed by searching for their inhibitory potency in cell free translational systems from *S. cerevisiae* mutants resistant to sordarin derivatives (*S.cerv.* SC373cpr5). These mutants also remain resistant to two of these simplified inhibitors suggesting that their fungal target is common to both families: IC_{50} (*S.cerv.*SC373, wild type)=184 μM (for **8**) and 70 μM (for **10**); IC_{50} (*S.cerv.*SC373cpr5, resistant type)>350 μM (for both **8** and **10**).