Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 117–120

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antifungal Sordarins. Synthesis and Structure–Activity Relationships of 3′-*O*-Substituted Derivatives

Enrique M. Arribas, Julia Castro, Ian R. Clemens, Juan C. Cuevas,* Jesús Chicharro, Mª Teresa Fraile, Silvestre García-Ochoa, Federico Gómez de las Heras and José R. Ruiz

GlaxoSmithKline, Research Department, Parque Tecnológico de Madrid, Severo Ochoa, 2. 28760 Tres Cantos, Madrid, Spain

Received 1 August 2001; revised 26 September 2001; accepted 3 October 2001

Abstract—A number of novel 3′-*O*-acyl and alkyl sordarins were synthesised for structure–activity relationship studies. Many of these derivatives exhibit high activity against *Candida albicans*, *Candida pseudotropicalis*, *Candida tropicalis* and *Cryptococcus neoformans*. © 2002 Elsevier Science Ltd. All rights reserved.

Systemic fungal diseases arouse great concern due to the increasing number of immunosuppressed patients (cancer, transplants, premature infants, broad spectrum antibiotic or glucocorticoid therapy, peritoneal dialysis or hemodialysis, ITU's, AIDS, etc.). Treatments for these fungal infections are still far from satisfactory because of therapeutic limitations (azoles, polyenes, echinocandins). Limited spectrum of action, emergence of resistant strains, toxicity, and limited ways of administration decrease in variable degrees the broad use of current antifungal drugs.[1a,b] Therefore, the development of satisfactory drugs with novel modes of action continues to be a major challenge.

Sordarins[1c,d] are new potent and selective inhibitors of fungal protein synthesis, the mode of action of which involves binding to elongation factor-2 (EF-2) and P0 (a ribosomal stalk protein). Sordarin derivative **1a**[2] (GR 135402; Fig. 1), structurally related to the previously known antibiotics sordarin (**1b**)[3,4] and zofimarin (**1c**),[5] was discovered in a cell-free screening programme aimed to identify inhibitors of fungal protein synthesis.[6] Compound **1a**, obtained by bioproduction from *Graphium putredinis*,[2] is a potent and selective inhibitor of *Candida albicans* protein synthesis ($IC_{50}=0.03$ μg/mL), which also inhibits the growth of *C. albicans* whole cells (MIC = 0.03 μg/mL). However, 3′-*O*-acylated sordarin derivative **1a** is fungistatic and shows a limited spectrum of action. These values compare favourably with those of sordarin ($IC_{50}=0.036$ μg/mL; MIC = 31 μg/mL), which suggest that the 3′-substitution may have a role in binding to EF-2 and P0 and/or uptake in whole cells, as previously suggested.[2]

Due to the promising antifungal activity of these compounds and the novelty of their mode of action, a chemical programme was initiated in order to improve the activity of GR 135402 (**1a**). In this work, we report on the synthesis and biological properties of 3′-*O*-substituted sordarin derivatives.

Chemistry

The synthetic route used for the preparation of the 3′-*O*-acylated derivatives (esters **4**–**13**, carbonates **14** and **15**, and carbamate **16**; see Table 1) is shown in Scheme 1. Sordarin (**1b**) from large scale fermentation of *Sordaria araneosa* was used.[2] The aglycon of these molecules present a rather unusual tetracyclic diterpene structure bearing a formyl and a carboxylic acid group in a vicinal arrangement with a high enough dihedral angle to avoid internal hemi-acetalisation. However, this carboxylic acid may react with acylating reagents to give mixed anhydrides. To avoid these unwanted reactions,

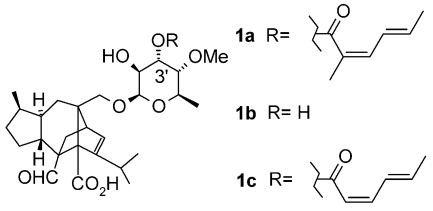


Figure 1. Natural GR 135402 (**1a**), sordarin (**1b**) and zofimarin (**1c**).

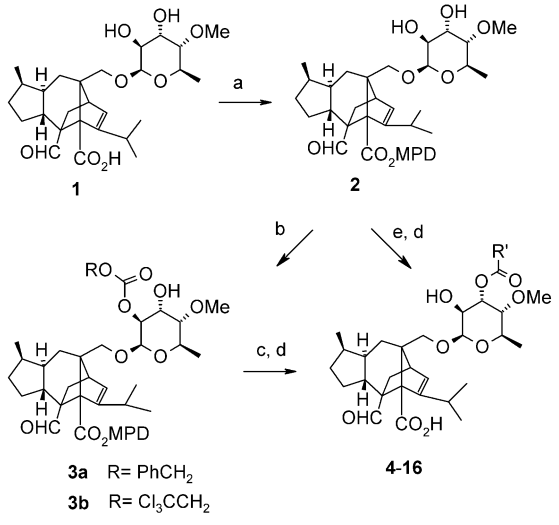
*Corresponding author. Fax: + 34-91-807-0550; e-mail: jccz9579@gsk.com

PII: S0960-894X(01)00729-6

Table 1. Antifungal activity (cell growth inhibitory activity) of 3′-*O*-acyl sordarins **4**–**16** [MIC (μg/mL)]

Compd	R′	Prep. method	*Candida albicans* 1208	*Candida albicans* 2005	*Candida albicans* 2402	*Candida pseudotropicalis* 2371	*Candida tropicalis* 2808	*Cryptococcus neoformans* 2867
4	$Me(CH_2)_2$	A	1	0.25	2	0.5	2	>125
5	$Me(CH_2)_4$	B	0.25	0.015	0.25	0.01	0.03	16
6	$Me(CH_2)_6$	A	0.06	<0.001	0.03	0.004	0.06	0.25
7	$Me(CH_2)_8$	B	1	0.25	1	0.25	8	1
8	$Cl(CH_2)_4$	A	2	0.12	2	0.25	1	16
9	$MeOCH_2$	A	>125	62	125	16	>125	125
10	$Me(CH_2)_3CHMe$	B	0.12	0.008	0.06	0.06	0.25	8
11	$Me(CH_2)_2CH=CMe$	B	1	0.015	0.5	0.12	0.5	62
12	Ph	A	1	0.12	1	0.25	0.5	>125
13	2-Furanyl	B	16	2	16	4	8	>125
14	$Me(CH_2)_7O$	A	1	0.25	0.5	0.12	8	8
15	$PhCH_2O$	A	8	2	8	2	8	>62
16	$Me(CH_2)_7NH$	C	4	0.06	2	1	2	31
1a	Me(E)CH=CH(Z)CH=CHMe		0.25	0.015	0.03	0.03	0.06	0.25
1b	Sordarin		31	8	31	16	16	>125

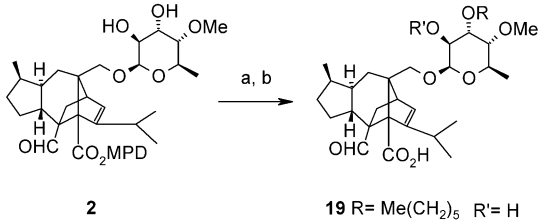
and for all the synthetic transformations, the sordarin carboxylic group was conveniently protected as diphenylmethyl (DPM) ester[7] to give compound **2**. Selective protection of the 2′-hydroxyl group was investigated in order to exclusively obtain 3′-*O*-acyl derivatives. The carbonate was found to be the most suitable protecting group. In this way, 3′-*O*-acyl and 3′-*O*-alkyloxycarbonyl derivatives **4**–**15** were prepared following a three-step sequence. Firstly, selective protection of the 2′-hydroxyl group as either benzyl or trichloroethyl carbonate[8] gave **3a** or **3b**. Then, esterification of the 3′-hydroxyl group of **3** was carried out either through reaction with an acid chloride (method A) or by coupling with a carboxylic acid in the presence of 2-chloro-1-methylpyridinium iodide (Mukaiyama's salt)[9] (method B).



Scheme 1. Reagents and conditions: (a) Ph_2CN_2, CH_2Cl_2 (90%); (b) ROCOCl, DMAP, MeCN (75%); (c) method A: R′COCl, DMAP, CH_2Cl_2 (75–90%); method B: R′CO_2H, Mukaiyama's salt, DMAP, CH_2Cl_2 (80–95%); (d) H_2, Pd/C, EtOAc (81–94%) [for **11** and **15**: (i) 5/2 TFA/H_2O (60–74%); (ii) Zn, aq 1 N KH_2PO_4, THF (75%)]; (e) method C: *n*-$Bu_2Sn(OAc)_2$, octyl isocyanate, toluene, reflux (50%).

Finally, and for deprotection purposes, two different procedures were followed depending on the protecting group used at 2′. When benzyl carbonate was used for hydroxyl protection at 2′, final release of both carboxylic acid and hydroxyl groups was carried out in one step via catalytic hydrogenation. When trichloroethyl carbonate was used, final deprotection was carried out in two steps: acidic hydrolysis to yield the free carboxyl group and then, reductive cleavage of trichloroethyl carbonate group to release the 2′-hydroxyl group. Trichloroethyl carbonate was used as protecting group in the preparation of **11** and **15**. In the other cases the benzyl carbonate **3a** was used. For the preparation of carbamate **16** tin catalysed (dibutyltin diacetate) regioselective addition of **2** to the corresponding isocyanate was followed (method C).

The synthetic route for the preparation of the 3′-*O*-ether derivative **19** is shown in Scheme 2. Direct alkylation of sordarin diphenylmethyl ester **2** with hexyl bromide by using sodium hydride, followed by acidic deprotection of the diphenylmethyl ester group, yielded 3′-*O*-alkylated derivative **19** along with varying amounts of the corresponding 2′,3′-dialkylated derivatives.

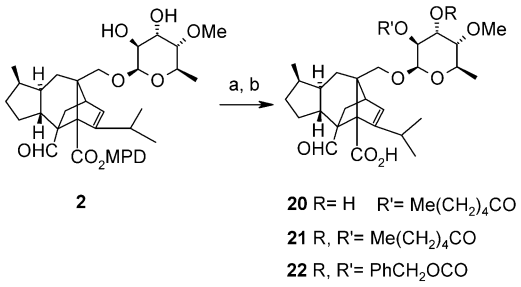


Scheme 2. Reagents and conditions: (a) NaH, R-Br, THF, reflux (55%); (b) 5/1 TFA/H_2O (60%).

In order to observe the effect of the position of sugar substitution, 2′-*O*-acylated compound **20** was prepared by reaction of **2** with one equiv of hexanoyl chloride in good yield. (Scheme 3).

Table 2. Antifungal activity (cell growth inhibitory activity) of 3′-*O*-alkyl sordarin **19**, 2′-*O*-acylated **20** and 2′,3′-di-*O*-substituted sordarins **21** and **22** [MIC (μg/mL)]

Compd	R, R′	*Candida albicans* 1208E	*Candida albicans* 2005E	*Candida albicans* 2402E	*Candida pseudotropicalis* 2371E	*Candida tropicalis* 2808E	*Cryptococcus neoformans* 2867E
19	$Me(CH_2)_5$ (R′ = H)	4	0.25	2	0.25	1	31
20	H (R′ = $Me(CH_2)_4CO$)	31	8	31	31	62	31
21	$Me(CH_2)_4CO$	8	4	8	>125	>125	>125
22	$PhCH_2OCO$	>62	62	>62	>62	>62	>62



2

20 R= H R'= $Me(CH_2)_4CO$

21 R, R'= $Me(CH_2)_4CO$

22 R, R'= $PhCH_2OCO$

Scheme 3. Reagents and conditions: (a) for **20**: hexanoyl chloride (1 equiv), DMAP, CH_2Cl_2 (49%); for **21** and **22**: RCl (excess), DMAP, CH_2Cl_2 (85%); (b) for **20** and **21**: H_2, Pd/C, MeOH (91–95%); for **22**: 5:2 TFA/H_2O (60%).

Finally, the 2′,3′-di-*O*-acylated derivatives **21** and **22** were prepared through acylation reaction of protected sordarin with excess of the corresponding acid chloride.

Biological Evaluation

Tables 1 and 2 show the in vitro antifungal activity (referred to as MIC, the minimum concentration inhibiting fungal cell growth)[10] of compounds synthesised. This assay was carried out in broth microdilution using RPMI + glucose as culture medium.[11] GR 135402 (**1a**) and sordarin (**1b**) were used as reference compounds.

Results and Discussion

Derivatives with structural similarity to natural GR 135402 (**1a**) were chosen. Several effects were tested: chain length, heteroatom substitution, α-substitution and unsaturations into the chain. Derivatives **4**–**11** are 3′-*O*-esters with chain lengths ranging from four to ten carbon atom members. The potency length of chain curve seems to have a Gauss bell shape, with a maximum for octanoate **6**. The substitution of the terminal methyl group of the hexanoate side chain by a chlorine atom (to see any lipophylicity effect) (**8**) seems to lead to lower potencies (up to 10-fold). Replacement of a lipophylic methylene by a more polar oxygen atom in the chain (such as in **9**) led to a general loss of antifungal activity. α-Methyl substitution in compounds **10** and **11** might add higher stability to the ester linkage. Methyl substitution in α-position (such as in **10**) does not alter antifungal potency much in comparison with the unsubstituted derivative **5**. However, α,β-unsaturation (see **11**) leads to lower potencies with respect to **10**. On the other hand, sordarin 3′-*O*-ester derivatives of aromatic or heteroaromatic carboxylic acids (**12**–**13**) show a noticeable decrease of antifungal potency. It is known that carbonate and carbamate functional groups (as in **14**–**16**) are more metabolically stable linkages than ester.[12] The replacement of the ester functionality by either carbonate (as in **14**–**15**) or carbamate (as in **16**) groups leads to compounds presenting similar activity levels. Thus, octyl carbonate **14** and octyl carbamate **16** show MIC values very close to those of their ester analogue **7** containing the same number of atoms. Carbonate **14** seems to be more potent than its corresponding carbamate counterpart **16** in some of the *Candida* strains.

A comparison of the 3′-*O*-hexylsordarin (**19**; Table 2) with their corresponding ester analogue **5** shows that it is less active. Esterification position is very important for activity as can be observed by comparing both hexanoate derivatives at 2′ (**20**, Table 2) and 3′ (**5**; Table 1). The 3′-derivative **5** is over a 100-fold more potent indicating the relevance of the attachment site. Finally, the doubly substituted sordarins at 2′ and 3′ (diester **21** and dicarbonate **22**) only maintain residual activities indicating the adverse effect of a large substituent at the 2′-hydroxyl group for activity.

From these results, we can draw some preliminary conclusions: the derivatisation of the 3′-hydroxyl group of sordarin to produce aliphatic esters with a variable degree of chain length or branching affords very potent antifungal compounds. Besides, from all four linkage types the ester seems to give the best results in terms of potency and spectrum. Due to the novel structure and mode of action of these molecules they will be the subject of further chemical modifications and substitutions in the search for spanning the spectrum of action.

Acknowledgements

The authors would like to thank the staff of the Bioprocessing Pilot Plant (GlaxoSmithKline, UK) for performing the large scale bioproduction of sordarin as starting material. We also thank our colleagues at the Mycology laboratory (GlaxoSmithKline, Spain) for running the antifungal assays.

References and Notes

1. (a) Halls, G. In *The Complete Guide to Anti-infectives*; Scrip Reports; PJB Publications and G. Halls: UK 1999, 111. (b) Di Dominico, B. *Curr. Opin. Microbiol.* **1999**, *2*, 509. (c) Gargallo-Viola, D. *Curr. Opin. Antiinfect. Invest. Drugs* **1999**, *1*, 297. (d) Odds, F. C. *Exp. Opin. Ther. Pat.* **2001**, *11*, 283.

2. Kennedy, T. C.; Webb, G.; Cannell, R. J. P.; Kinsmann, O. S.; Middleton, R. F.; Sidebottom, P. J.; Taylor, N. L.; Dawson, M. J.; Buss, A. D. *J. Antibiot*. **1998**, *51*, 1012.
3. Sigg, H. P.; Stoll, C. UK Patent 1,162,027, 1969.
4. Hauser, D.; Sigg, H. P. *Helv. Chim. Acta* **1971**, *54*, 1178.
5. Ogita, T.; Hayashi, A.; Sato, S.; Furutani, W. JP Patent 62-40292, 1987*; Chem. Abstr*. **1987**, *107*, 5745.
6. Kinsman, O. S.; Chalk, P. A.; Jackson, H. C.; Middleton, R. F.; Shuttleworth, A.; Rudd, A. M.; Jones, C. A.; Noble, H. M.; Wildman, H. G.; Dawson, M. J.; Stylli, C.; Sidebottom, P. J.; Lamont, B.; Lynn, S.; Hayes, M. V. *J. Antibiot*. **1998**, *51*, 41.
7. Kametani, T.; Sekine, H.; Hondo, T. *Chem. Pharm. Bull*. **1982**, *30*, 4545.
8. Windholz, T. B.; Johnston, D. B. R.. *Tetrahedron Lett*. **1967**, *8*, 2555.
9. Bald, E.; Saigo, K.; Mukaiyama, T. *Chemistry Lett*. **1975**, 1163.
10. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts, 1997. Approved Standard. Document M27-A. N.C.C.L.S. Villanova, Palo Alto, USA.
11. Herreros, E.; Martínez, C. M.; Almela, M. J.; Marriott, M. S.; Gómez de las Heras, F.; Gargallo-Viola, D. *Antimicrob. Agents Chemother*. **1998**, *42*, 2863.
12. Bundgaard, H., In *Design of Prodrugs*; Bundgaard, H. Ed., Elsevier Science: Publishers: Amsterdam, 1985; Chapter 1, p 1.