Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2733–2736

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of Novel Pyrrolidinyl Sordaricin Derivatives as Antifungal Agents

Masami Arai,[a] Tamako Harasaki,[b] Takashi Fukuoka,[b] Satoru Kaneko[a,*] and Toshiyuki Konosu[a]

[a]*Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*
[b]*Biological Research Laboratories, Sankyo Co., Ltd., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan*

Received 28 May 2002; accepted 22 June 2002

Abstract—*N*-Benzyl pyrrolidinyl sordaricin derivatives have been synthesized from *cis*-4-hydroxy-D-proline in a stereocontrolled manner. These compounds maintained moderate antifungal activity against several pathogenic fungal strains. Their MIC values against *Candida albicans* were in the range of 0.25–2 μg/mL.

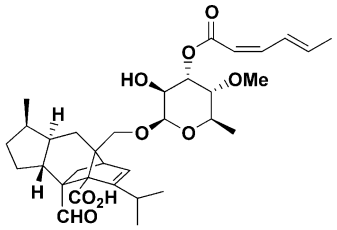
One of the most promising therapeutic approaches for the treatment of fungal infection involves inhibition of fungal protein synthesis by impairing the function of elongation factor 2 (EF-2) in the translation process.[1,2] In view of this point, zofimarin (**1**),[3] initially isolated from *Zopfiella marina* SANK21274, has interested us (Fig. 1) again as one of the most potent agents. This natural product showed moderate inhibitory activity against the growth of pathogenic fungi. Moreover, the sordarin family, which has the tetracyclic aglycon common to **1**, exhibited high selectivity for fungal EF-2 compared with a mammalian one, and a novel mode of action unlike known azole antifungal compounds.

Over the last several years, the selective inhibition of fungal EF-2 has been extensively investigated,[2] and many analogues of this class have been reported by us[4,5] and others.[6–9]

Recently, we have focused on azasordarins such as GW531920 (**2**)[2,10] and our 1,4-oxazepanyl sordaricins such as **3**[5] in view of the influence of serum on them.[11] Therefore, we were interested in a five-membered ring containing a nitrogen atom instead of a morpholine or an oxazepane. In addition, these compounds possess acetal moiety derived from the glycosyl bond of the parent natural product. Taking into account the chemical stability of linkage, we designed novel sordaricin analogues **4** bearing a pyrrolidine ring without an acetal linkage in place of the sugar. Herein, we report synthesis and in vitro antifungal activity of *N*-benzyl pyrrolidinyl sordaricin derivatives **4a**–**c** (Fig. 2).

Chemistry

The synthesis commenced with the preparation of the key intermediate **11**. As outlined in Scheme 1, alcohol **6** was synthesized from *cis*-4-hydroxy-D-proline (**5**) in 7 steps.[12] Sordaricin (**7**)[4,13] was converted to allyl ester **8**. Treatment of **8** with trifluoromethanesulfonic anhydride and pyridine gave unstable triflate **9**. Without further purification, the crude triflate **9** was subjected to the next displacement reaction. Treatment of **6** with sodium hydride followed by addition of **9** successfully intro-



1: zofimarin

Figure 1. Formula of zofimarin (**1**), an antifungal natural product.

*Corresponding author. Tel.: +81-3-3492-3131; fax: +81-3-5436-8563; e-mail: skanek@shina.sankyo.co.jp

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00534-6

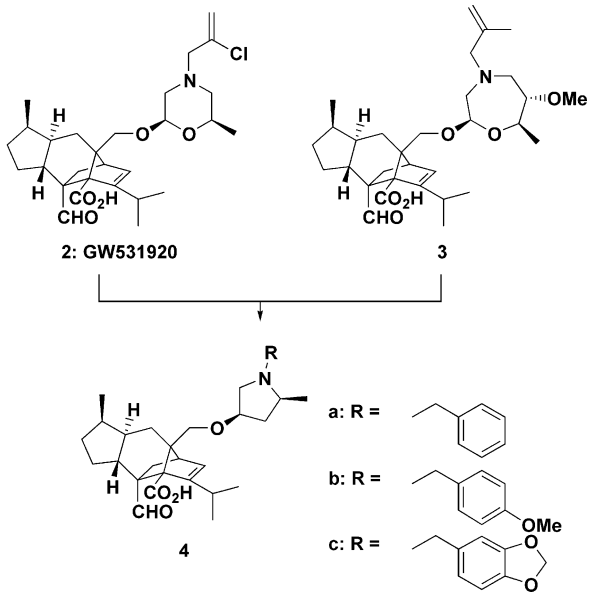
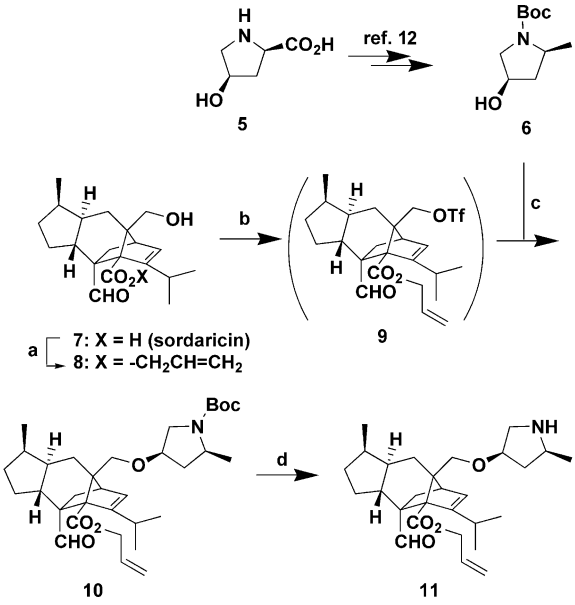


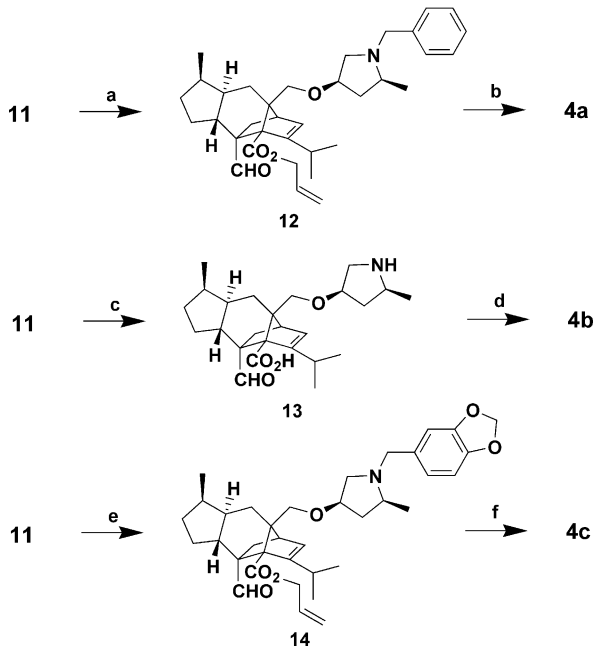
Figure 2. Molecular design of novel pyrrolidinyl sordaricis.



Scheme 1. Reagents and conditions: (a) allyl bromide, $NaHCO_3$, DMF, rt, 94%; (b) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (c) NaH, DMA, 0 °C, 66%; (2 steps from **8**); (d) TFA, CH_2Cl_2, rt, 83%.

duced the ether linkage in **10**. Subsequent removal of the Boc group of **10** afforded pyrrolidine **11**.

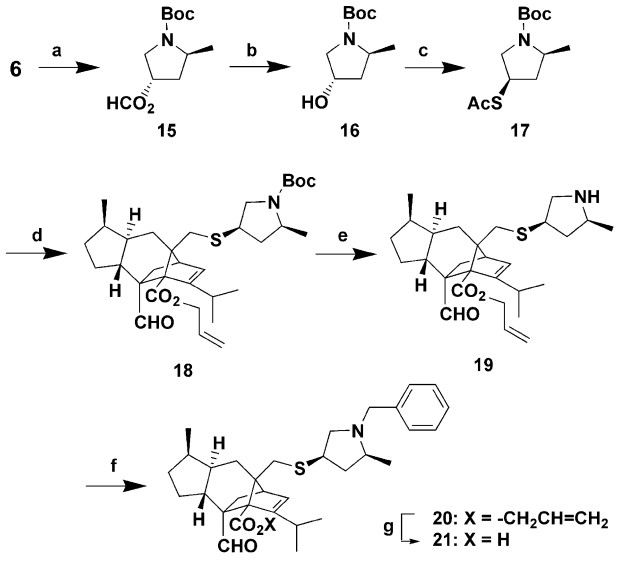
As shown in Scheme 2, the pyrrolidine **11** was converted to tertiary amine **12**. Finally, cleavage of the allyl group of **12** proceeded via π-allyl-palladium complex, to afford the desired product **4a**. For **4b**, removal of the allyl group of **11** was first performed, to afford liberated acid **13**. Subsequent selective alkylation of the nitrogen atom in **13** gave rise to the target compound **4b**. For **4c**, treatment of **11** with piperonyl chloride and triethylamine afforded tertiary amine **14**. At last, deprotection employing tetrakis(triphenyl phosphine)palladium as a catalyst furnished the desired product **4c**.[14]



Scheme 2. Reagents and conditions: (a) BnBr, K_2CO_3, MeCN, rt, 38%; (b) $Pd(PPh_3)_4$, morpholine, THF, rt, 42%; (c) $Pd(PPh_3)_4$, morpholine, THF, rt, 76%; (d) *p*-methoxybenzyl bromide, $NaHCO_3$, DMF, 70 °C, 12%; (e) piperonyl chloride, Et_3N, CH_2Cl_2, rt, 37%; (f) $Pd(PPh_3)_4$, morpholine, THF, rt, 53%.

In order to judge the role of the ether linkage, the thioether analogue of **4a** was also synthesized as shown in Scheme 3. In this route, the Mitsunobu reaction was applied twice to induce a sulfur atom with retention of configuration. Treatment of **6** with formic acid under Mitsunobu reaction conditions afforded formate **15**.

Hydrolysis of the formate **15** furnished alcohol **16**. The alcohol **16** was subjected again to the Mitsunobu protocol



Scheme 3. Reagents and conditions: (a) Ph_3P, DEAD, HCO_2H, THF, rt, 83%; (b) aq NaOH, THF, rt, 90%; (c) Ph_3P, DEAD, AcSH, THF, rt, 66%; (d) NaOMe, DMF, 0 °C; then **9**, rt, 47%; (e) TFA, CH_2Cl_2, rt, 81%; (f) BnBr, K_2CO_3, KI, MeCN, rt, 21%; (g) $Pd(PPh_3)_4$, morpholine, THF, rt, 52%.

Table 1. In vitro antifungal activity of pyrrolidinyl sordaricins

Organism	MIC (μg/mL)						
	FCZ[a]	**1**	**3**	**4a**	**4b**	**4c**	**21**
Candida albicans ATCC24433	0.5	0.5	0.03	1	0.5	0.25	0.5
Candida albicans SANK51486	0.25	0.25	0.02	0.5	0.25	0.13	0.25
Candida albicans TIMM3164[b]	>4	0.5	0.03	2	0.5	0.5	1
Candida albicans ATCC64550[b]	>4	0.5	0.03	2	0.5	0.5	1
Candida parapsilosis ATCC90018	0.5	>4	>16	>16	>16	>16	>16
Candida glabrata ATCC90030	>4	>4	0.25	>16	16	>16	>16
Candida tropicalis ATCC750	2	0.5	0.13	8	4	4	4
Cryptococcus neoformans TIMM1855	>4	0.25	0.25	>16	>16	>16	>16
Aspergillus fumigatus ATCC26430	>4	>4	>16	>16	>16	>16	>16
Candida albicans ATCC24433[c]	NT[d]	4	0.5	4	1	1	8
Candida albicans SANK51486[c]	NT[d]	NT[d]	0.25	1	0.5	0.5	4

[a]Fluconazole.
[b]Low susceptibility to fluconazole (MIC >4).
[c]In the presence of horse serum (20%).
[d]Not tested.

using thioacetic acid, and was transformed to thioacetate **17**. Treatment of **17** with sodium methoxide generated sodium thiolate in situ. Sequential treatment of the triflate **9** constructed the thioether linkage in **18**. Removal of the Boc group in **18** proceeded smoothly, to give pyrrolidine **19**. Benzylation of the secondary amine **19** gave rise to tertiary amine **20**. Finally, the carboxyl group of **20** was deprotected to afford the desired thioether analogue **21**.

Antifungal activity

With compounds **4a–c** and **21** in hand, in vitro antifungal activity was examined.[15] The results are summarized in Table 1.

These synthesized compounds exhibited good activity (MICs: 0.13–2 μg/mL) against *Candida albicans* including strains with decreased susceptibility to fluconazole. However, they did not have as much activity as the oxazepane derivative **3**. Against *Candida tropicalis*, they showed only moderate activity (MICs: 4–8 μg/mL). Against *Candida palapsilosis*, *Candida glabrata*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, they showed almost no activity.

Among this series, **4c** was the most effective compound, and exhibited MICs of 0.13–0.5 μg/mL against *C. albicans*. Moreover, it maintained certain activity even in the medium supplemented with 20% horse serum; the MICs determined in the presence of serum were fourfold higher than the corresponding MICs under serum-free conditions.

In comparison with **4c**, thioether **21** exhibited slightly less antifungal activity. However, the MICs determined in the presence of serum were 16-fold higher than the corresponding MICs under serum-free conditions. The finding suggests that the hydrophobicity of the thioether linkage in **21** may be concerned with the influence of serum on antifungal activity.

In conclusion, we synthesized a novel series of sordaricin derivatives possessing a pyrrolidine ring instead of the sugar. These compounds exhibited good antifungal activity. Introduction of the pyrrolidine moiety proved to be an efficient strategy to reduce the influence of serum. In particular, the *N*-piperonyl pyrrolidine derivative **4c** can be considered as a promising lead compound. These findings encourage us to continue studying further sordaricin analogues.

Acknowledgements

The authors thank Dr. T. Uchida of Sankyo Co., Ltd. for helpful discussion.

References and Notes

1. Gargallo-Viola, D. *Curr. Opin. AntiInfect. Invest. Drugs* **1999**, *1*, 297.
2. Odds, F. C. *Exp. Opin. Ther. Patents* **2001**, *11*, 283 and references therein.
3. Ogita, T.; Hayashi, T.; Sato, A.; Furutani, W. JP Patent 62,040,292, 1987.
4. Kaneko, S.; Uchida, T.; Shibuya, S.; Honda, T.; Kawamoto, I.; Harasaki, T.; Fukuoka, T.; Konosu, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 803.
5. Kaneko, S.; Arai, M.; Uchida, T.; Harasaki, T.; Fukuoka, T.; Konosu, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1705.
6. Arribas, E. M.; Castro, J.; Clemens, I. R.; Cuevas, J. C.; Chicharro, J.; Fraile, M. T.; García-Ochoa, S.; Heras, F. G.; Ruiz, J. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 117.
7. Bueno, J. M.; Cuevas, J. C.; Fiandor, J. M.; García-Ochoa, S.; Heras, F. G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 121.
8. Castro, J.; Cuevas, J. C.; Fiandor, J. M.; Fraile, M. T.; Heras, F. G.; Ruiz, J. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1371.
9. Serrano-Wu, M. H.; Laurent, D. R.; Mazzucco, C. E.; Stickle, T. M.; Barrett, J. F.; Vyas, D. M.; Balasubramanian, B. N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 943.
10. Herreros, E.; Almela, M. J.; Lozano, S.; Heras, F. G.; Gargallo-Viola, D. *Antimicrob. Agents Chemother.* **2001**, *45*, 3132.

11. It has been stated that the sordarin family has a higher affinity for serum protein. See: Aviles, P.; Falcoz, C.; Roman, R. S.; Gargallo-Viola, D. *Antimicrob. Agents Chemother.* **2000**, *44*, 2333.
12. Rosen, T.; Chu, D. T. W.; Lico, I. M.; Fernandes, P. B.; Marsh, K.; Shen, L.; Cepa, V. G.; Pernet, A. G. *J. Med. Chem.* **1988**, *31*, 1598.
13. Hauser, D.; Sigg, H. P. *Helv. Chim. Acta* **1971**, *54*, 1178.
14. Synthesized compounds showed satisfactory spectroscopic and analytical data. The data of **4c** is shown as follows. **4c**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.91 (1H, s), 6.88 (1H, s), 6.78 (1H, d, $J=8.1$ Hz), 6.73 (1H, d, $J=8.1$ Hz), 6.00 (1H, d, $J=3.7$ Hz), 5.93 (2H, s), 3.96 (1H, d, $J=13.2$ Hz), 3.88 (1H, m), 3.87 (1H, d, $J=9.5$ Hz), 3.17 (1H, d, $J=8.8$ Hz), 3.05 (1H, d, $J=13.2$ Hz), 2.92 (1H, d, $J=11.0$ Hz), 2.46 (1H, m), 2.38–1.20 (m), 1.19 (3H, d, $J=5.9$ Hz), 1.02 (3H, d, $J=6.6$ Hz), 0.95 (3H, d, $J=6.6$ Hz), 0.84 (3H, d, $J=6.6$ Hz); FABHRMS (m/z): calcd for $C_{33}H_{43}NO_6$ ($[M+H]^+$): 550.3169. Found: 550.3166.
15. In vitro antifungal activity was determined in RPMI1640 medium (for *Cr. neoformans*: yeast nitrogen base) buffered at pH 7.0. Microplates were incubated at 35 °C (for *A. fumigatus*: 30 °C). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound that inhibited the growth of the fungi by 80%. For experiments in the presence of horse serum, the medium was supplemented with 20% horse serum during incubation.