Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 935–938

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STUDIES ON AN IMMUNOSUPPRESSIVE MACROLACTAM, ASCOMYCIN: SYNTHESIS OF A *C*-33 HYDROXYL DERIVATIVE

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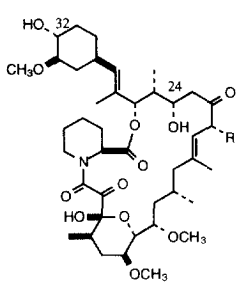
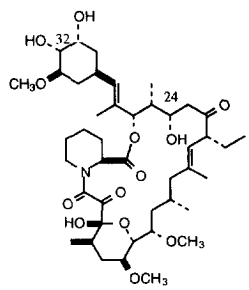
Received 26 January 1998; accepted 9 March 1998

Abstract : Ascomycin **2**, a close analogue of the immunosuppressant FK506 **1**, was modified to incorporate a hydroxyl group at the *C*-33 position. This increased the aqueous solubility of ascomycin by a hundred-fold at pH 7.4 and by approximately 300-fold at pH 6.5. Ascomycin **3** also exhibited an excellent immunosuppressive activity in vitro, as tested in a human mixed lymphocyte proliferation (HuMLR) assay, and in vivo using a rat popliteal lymph node (rPLN) hyperplasia assay.

The immunosuppressants FK506 **1**[1] and ascomycin **2**[2] are both fermentation products expressing a powerful antiproliferative activity. FK506 has been clinically successful in transplant patients.[3] It has also shown promise as a useful drug for the treatment of various autoimmune diseases, such as rheumatoid arthritis, atopic dermatitis as well as psoriasis.[4,5] Because of this potential utility, intensive studies have been initiated to overcome some of its undesired side effects.



(**1**) R = allyl
(**2**) R = ethyl

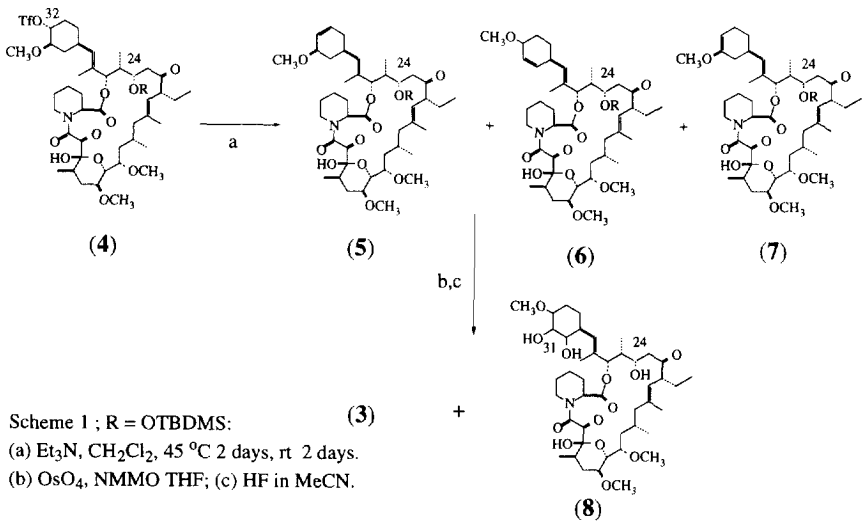


(**3**)

FK506 **1** and ascomycin **2** are hydrophobic macrolactams and are practically insoluble in aqueous solution. We began synthesis of novel ascomycin (**2**) analogues to elucidate the structural features governing this property. Increased water solubility may be a key to changing not only its physicochemical properties, but also its pharmacological behavior such as pharmacokinetics. The NMR and X-ray crystallographic analyses of the macrolactam–FKBP complex have shown that the *C*32,33 and *C*34 region do not interact directly with the FKBP molecule, rather being exposed to solvent.[6,7] Thus, this area can be used for chemical modifications without affecting FKBP binding affinity. This paper describes a synthetic route for the preparation of *C*33-[R]-hydroxyl-ascomycin **3** and biological data of some of the derivatives.

0960-894X/98/$19.00
PII: S0960-894X(98)00145-0

In order to avoid synthetic complexity, *C*-24-OTBDMS-ascomycin[8] was employed, which was then converted to its *C*-32-trifluoromethanesulfonyl-*C*-24-OTBDMS-ascomycin (**4**) by treatment with triflic anhydride [$(TfO)_2O$] in methylene chloride at 0 °C for 20 min in the presence of pyridine. The elimination of **4** was conducted with triethylamine (2.5 equiv) in methylene chloride under a slightly elaborated temperature for 2 days and at room temperature for an additional day to yield mainly a mixture of three dehydro products (**5–7**). Two products (the ratio of **5** and **6** is approximately 3:1) were unseparable under the HPLC condition investigated, isolated in 30% yield, while the latter dehydro product (**7**) was isolated as a single isomer in 3% yield. We investigated several bases, triethylamine(Et_3N), diisopropylethylamine (DIPEA) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) for the dehydration reaction. More than 70% of ascomycin was destroyed when DBU was used in 30 min.



Scheme 1 ; R = OTBDMS:
(a) Et_3N, CH_2Cl_2, 45 °C 2 days, rt 2 days.
(b) OsO_4, NMMO THF; (c) HF in MeCN.

In contrast, Et_3N and DIPEA gave a mixture of three dehydro analogues in relatively satisfactory yield. The dehydro formations **5** and **6** preferably occurred in the presence of Et_3N rather than in the presence of DIPEA. When the latter base was employed, the dehydro **7** was preferably formed over **5** and **6**. There were also several additional products that have been isolated and characterized.[9,10]

Selective dihydroxylations of the *C*32,33 or *C*30,31 double bond were accomplished by reaction of a mixture of **6** and **7** with osmium tetroxide (OsO_4, 0.2 equiv) and *N*-methylmorpholine *N*-oxide (NMMO, 2 equiv) in tetrahydrofuran at room temperature for 2 days to yield the mixture of *C*-24-TBDMS-*C*-33-[R]-hydroxyl-ascomycin and *C*24-TBDMS-*C*30,31-dihydroxyl-*C*-32-methoxy-ascomycin, which were finally separated as a single isomer by HPLC. *C*24-TBDMS groups were selectively deprotected by 48%-HF in acetonitrile for 2 h at room temperature to obtain the desired products: *C*-33-[R]-hydroxyl-ascomycin **3** and *C*30,31-dihydroxyl-*C*-32-methoxy-ascomycin **8**, respectively.

The in vitro immunosuppressive activities of synthetic analogues were measured as inhibition of the human mixed lymphocyte reaction (MLR). Because it is known that binding to FKBP appears to be necessary for immunosuppressive activity,[11] we also investigated the ability of synthetic analogues to bind FKBP. As was expected, with the introduction of a hydroxyl functional group at *C*-33 position, **3** effects neither the inhibition of ascomycin binding with FKBP nor the immunosuppressive activity as illustrated by FKBP and human MLR assays (1.2 and 0.17 nM, respectively). On the other hand, **8** showed a 20-fold weaker FKBP binding than ascomycin **2** or **3**, but was almost equipotent in the human MLR assay.

Compd	HuFKBP IC_{50} (nM)	HuMLR IC_{50}(nM)	RtPLN i.p. ED_{50}mpk	Solubility(μg/mL) pH 6.5	Solubility(μg/mL) pH 7.4	Log D pH 6.5	Log D pH 7.4
(**1**)	2.54	0.37	0.15	1.0	5.2	4.36	4.38
(**2**)	2.06	0.25	0.31	1.6	4.2	3.98	4.22
(**3**)	1.21	0.17	0.72	464	455	3.6	3.4
(**8**)	39.2	0.72	36%@3	294	245	3.9	3.9

A previous NMR experiment has suggested that a methoxy at the *C*-31 position of FK506 also participates in a hydrophobic interaction with the FKBP molecule.[6] Therefore, a weak affinity of **8** is expected due to an unfavoured interaction, despite a good in vitro immunosuppressive activity. In vivo immunosuppressive activities were tested by measuring the ability to inhibit popliteal lymph node hyperplasia in response to injection of histoincompatible splenocytes in the foot, as has been described previously.[12] Derivative **3** was almost equipotent to the natural macrolides **1** and **2**, whereas the analogue **8** showed no or very little in vivo immunosuppressive potency.

To be physiologically relevant, log D values were estimated at pH 6.5 (intestinal absorption) and 7.4 (blood). Additional hydroxyl incorporations indeed lowered the log D values at both pHs, from 4–4.4 down to 3.4–3.9. The observed lower log D values at pH 6.5 and 7.4 compared to those of **1** and **2** suggest that the analogue **3** may have an advantage for absorption.[13]

The determinations of solubility were also measured at specified pHs (6.5 and 7.4). As summarized in the Table, a hydroxylation at the *C*33 position (analogue **3**) increased the aqueous solubility of **2** by a 100-fold at pH 6.5 and 300-fold at pH 7.4, while preserving completely the in vitro and in vivo immunosuppressive activities. On the other hand, increasing water solubility did not always lead to a compound with good immunosuppressive activity. As pointed out earlier, **8** inhibited the HuMLR with good in vitro potency, but failed to show in vivo activity.

Analogue **3** may be one of the first synthetic derivatives that completely preserve in vitro as well as in vivo immunosuppressive activities while increasing water solubility more than 100-fold to 300-fold. Additional investigation is now in progress to study further whether analogue **3** may have an advantage over **1** or **2** in terms of a safety profile.

Acknowledgement: We thank Ms. Loan Miller for collecting biological data. We also special thank Mr. David Whittern who has done a complete structural determination of the compounds (**8**, **9** and **10**) by NMR spectroscopy, and Mr. Tony Borre and Drs. Dan Hoffman and Hugh Nellans who provided solubility and log D values at Abbott Laboratories.

References and Notes

1. Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1987**, *XL*, 1249.

2. Arai, T.; Koyama, Y.; Suenaga, T.; Honda, H. *J. Antibiot.* **1962**, *15*, 231.

3. Starzl, T.; Fung, J.; Jordan, M.; Shapiro, R.; Tzakis, A.; McCauley, J.; Johnston, J.; Iwaki, Y.; Jain, A.; Alessiani, M.; Todo, S. *J. Am. Med. Assoc.* **1990**, *264*, 63.

4. Thomson, A. W.; Starzl, T. E. *Autoimmunity* **1992**, *12*, 303.

5. Nakagawa, H.; Etoh, T.; Ishibashi, Y.; Higaki, Y.; Kawashima, M. *Lancet* **1994**, *344*, 883.

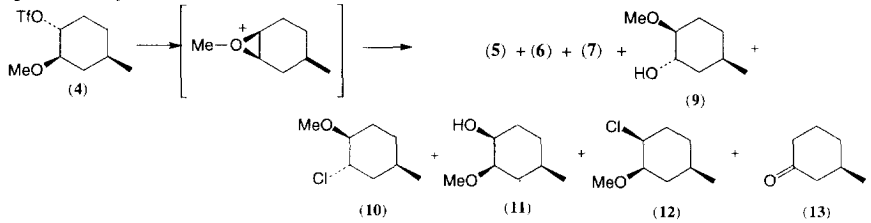
6. Petros, A. M.; Neri, P.; Fesik, S. W. *J. Biomolecular NMR* **1992**, *2*, 11.

7. Schreiber, S. L.; Liu, J.; Albers, M. W.; Rosen, M. K.; Standaert, R. F.; Wandless, T. J.; Somers, P. *Tetrahedron* **1992**,*48*, 2545.

8. Gunawardana, I. W. K.; Kawai, M.; Mollison, K. W.; Hsieh, G.; Lane, B. C.; Luly, J. R. submitted for publication to *J. Med. Chem.*

9. Deprotection of *C*-24–TBDMS from **9** has led to a compound having a keto at *C*-31 position; *C*-31keto-*C*32-deoxy-ascomycin in good yield.

10. Besides three dehydro products; **5**, **6** and **7**, several by-products (**9–13**) were isolated in 0.5–2.5% yield, respectively, presumably due to a formation of oxonium salt.



We were not able to explain the formations of **10** and **12** in the presence of Et_3N. Despite our careful experimental procedure, a very small percentage of chloro substitution always occurred. However, no chloro substitutions were observed when DIPEA was used.

11. Bierer, B. E., Somers, P. K., Wandless, T. J., Burakoff, S. J. and Schreiber, S. L. *Science*, **1990**, *250*, 556.

12. Kawai, M.; Lane, B. C.; Hsieh, G. C.; Mollison, K. W.; Carter, G. W.; Luly, J. R. *FEBS Lett.* **1993**, *316*, 107.

13. Hadgraft, J.; Guy, R. H. In *Transdermal Drug Delivery*; Marcel Dekker: NY, 1989; pp 59.