Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2253–2258

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

C32-O-IMIDAZOL-2-YL-METHYL ETHER DERIVATIVES OF THE IMMUNOSUPPRESSANT ASCOMYCIN WITH IMPROVED THERAPEUTIC POTENTIAL

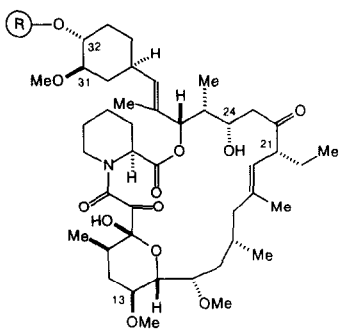
Mark T. Goulet,[a]* Shelli R. McAlpine,[a] Mary Jo Staruch,[b] Samuel Koprak,[b] Francis J. Dumont,[b] John G. Cryan,[b] Gregory J. Wiederrecht,[b] Raymond Rosa,[c] Mary Beth Wilusz,[c] Laurence B. Peterson,[c] Matthew J. Wyvratt,[a] William H. Parsons[a]

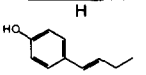
Departments of Medicinal Chemistry,[a] Immunology Research,[b] and Pharmacology[c]
Merck Research Laboratories
P.O. Box 2000, Rahway, NJ, 07065, U.S.A.

Received 30 March 1998; accepted 20 July 1998

Abstract: A series of C32-O-aralkyl ether derivatives of the FK-506 related macrolide ascomycin have been prepared based on an earlier reported C32-O-cinnamyl ether design. In the present study, the nature of the aryl tethering group was varied in an attempt to improve oral activity. An imidazol-2-yl-methyl tether was found to be superior among those investigated and has resulted in an ascomycin analog, L-733,725, with in vivo immunosuppressive activity comparable to FK-506 but with an improved therapeutic index.© 1998 Elsevier Science Ltd. All rights reserved.

Interruption of T lymphocyte receptor signaling by inhibition of the serine/threonine protein phosphatase calcineurin (CaN) is a therapeutically effective means of suppressing an immune response to antigen.[1-3] Two chemically distinct immunosuppressants that work by this mechanism, FK-506 (tacrolimus)[4] and cyclosporin A (cyclosporine)[5] are used clinically for the prevention of organ transplant rejection and have demonstrated utility in the treatment of other autoimmune diseases.[6] The goal of our research effort has been to develop an "FK-506-like" immunosuppressant that has an improved therapeutic index with respect to the neuro- and nephrotoxicity experienced by many patients receiving this therapy. To affect the therapeutic index of a drug whose toxicities are mechanism-based[7,8] we sought to prepare analogs of ascomycin that were distinct in some physical property (e.g., lipophilicity, FKBP12 binding affinity, etc.) that may alter the intra- or intercellular distribution of the drug while retaining the potent immunosuppressive activity of the parent natural product. We have previously reported on C32-derivatives of the related natural product ascomycin **1**,[9] such as the aralkyl ether **2**, that have good in vitro immunosuppressive potency.[10-14] Related aryl ether analogs have also demonstrated an improved therapeutic index over FK-506 in various animal models of efficacy and toxicity.[15,16] This report describes the synthesis and evaluation of C32-O-aralkyl ethers that incorporate heteroatoms in the tethering chain with an emphasis on the imidazol-2-yl-methyl group.



		R	TCell (IC_{50}, nM)
1	ascomycin	H	0.69
2	L-702,992		0.80

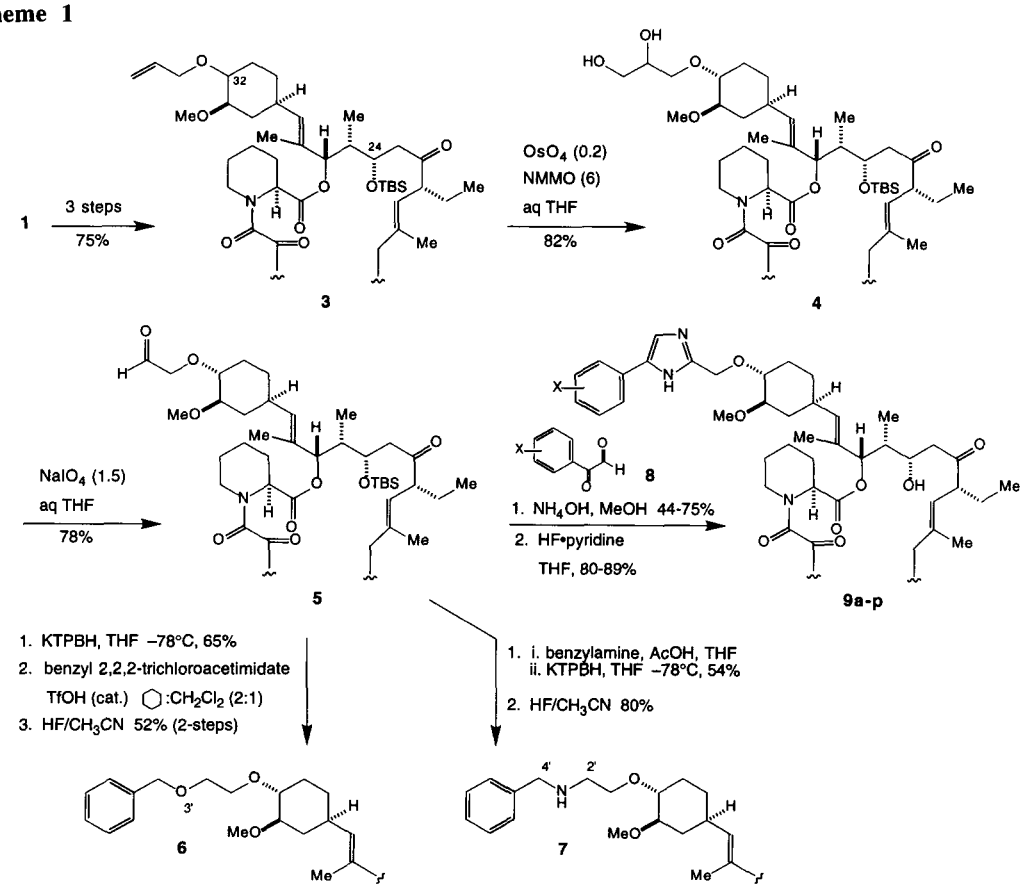
Chemistry

A variety of aralkyl ether derivatives of ascomycin bearing an oxygen or nitrogen in the tethering group were prepared using the synthetic routes depicted in Scheme 1.[17] The C32-O-allyl-C24-O-*tert*-butyldimethylsilyl

0960-894X/98/$ - see front matter © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(98)00397-7

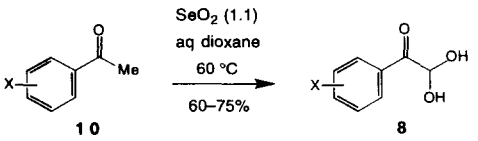
ether derivative **3** was prepared by a 3-step procedure described in a previous communication using allyl 2,2,2-trichloroacetimidate as the electrophilic coupling partner.[10] Oxidative cleavage of the terminal olefin in **3** was conducted in a 2-step manner to deliver the versatile intermediate aldehyde **5**. A four-atom tethered phenalkyl ether containing an oxygen at the 3' position, **6**, was prepared by reduction of aldehyde **5** with potassium triphenylborohydride (KTPBH) followed by benzylation of the resulting alcohol and removal of the silyl ether protecting group at C-24. The corresponding 3'-amino derivative, **7**, was obtained from **5** by reductive amination with benzyl amine followed by desilylation. An analog of **7** in which the 2' and 4' carbons are joined to form an imidazole ring is readily prepared from **5** using the Radziszewski synthesis.[18] Treatment of **5** with phenylglyoxal and ammonium hydroxide gave the corresponding 4(5)-phenylimidazol-2-yl-methyl ether derivative in 66% yield. Deprotection of the silyl ether using a solution of hydrogen fluoride•pyridine (40% in THF:pyridine, 2:1) provided the desired ascomycin analog **9a**.

Scheme 1



A variety of substituted phenylimidazol-2-yl-methyl ether analogs of ascomycin were prepared by this procedure, **9b–p**, that often required the prior synthesis of phenylglyoxal component **8**. For this purpose, we have found the method of Riley et al. (Equation 1) to be a reliable means of preparing substituted phenylglyoxal hydrates from the corresponding acetophenones.[19,20]

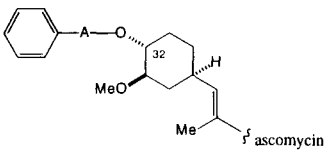
Equation 1

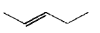
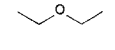
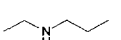
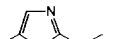


Results and Discussion

C32-O-Cinnamyl ether analogs, such as **2**, were found to be essentially equipotent to ascomycin in an in vitro assay of immunosuppression[10] but were less efficacious in vivo, especially upon oral administration (vida infra). In an attempt to improve the oral activity of this design we sought to increase the polarity of these analogs by incorporating heteroatoms into the alkyl tether. The effect of the tether on in vitro immunosuppressive activity and FKBP12 binding was investigated with a series of phenalkyl ethers shown in Table 1. Replacement of the allyl group in **11**[10] with a -CH_2OCH_2- linkage, **12**, had no effect on in vitro immunosuppressive activity with both analogs being approximately three-fold less potent than ascomycin. Lengthening the tether by one methylene unit as in analogs **6** and **7** results in further loss of activity. It is interesting that the uncharged ether tether in **6** and the amine tether in **7**, which would be positively charged as the protonated species both physiologically and under the present assay conditions, provide analogs with equivalent activities. This would suggest that the heteroatoms in these tethers are not making a close contact with protein in the cytosolic FKBP12•drug•CaN complex.[21,22] Incorporation of the 2' and 4' carbons of **7** into an imidazole ring, **9a**, imparts a severe rotational constraint in the tether and results in an analog with improved activity compared to the acyclic 4-atom tether analogs **6** and **7**. Analog **13** places fewer conformational restrictions in a 4-atom, imidazole-containing tether. Its immunosuppressive activity is reduced compared to **9a** and is similar to that of the unsubstituted C32-O-imidazol-2-yl-methyl ether analog **14**. Comparing the activity of **9a** to **14** gives a measure of the potency gained (three-fold) by substitution of a phenyl group to the 4(5)-position of an imidazol-2-yl-methyl tether. This gain in

Table 1.
Immunosuppressive activity and FKBP12 binding affinity of C32-O-phenalkyl ether derivatives of ascomycin

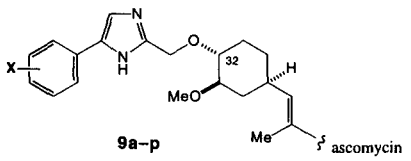


Compound	Tethering Group (A)	TCell[23] IC_{50} (nM)	FKBP12[24] EC_{50} (nM)
1 (ascomycin)	H (no phenyl)	0.69	1.6
11		1.9	30
12[25]		2.1	12
6		4.9	–
7		4.1	–
9a		0.80	22
13[26]		2.6	7.4
14[27]	(no phenyl)	2.7	–

potency is identical to that observed by substitution of a phenyl to the γ-position of an allyl tether (cf., **11** vs C32-O-allyl ascomycin: IC_{50} = 7.4 nM).[10] Consistent with previous observations, the affinity of these C32-O-alkyl ether ascomycin derivatives toward their major cytosolic binding protein, FKBP12, is reduced compared to parent and not correlative with in vitro activity. Immunosuppressive activity is more likely associated with cohesion in the FKBP12•drug•CaN complex which, in turn, can be profoundly affected by groups appended to C32.[15]

The effect of aromatic substitution in the 4(5)-phenylimidazol-2-yl-methyl ether design was explored as an attempt to improve immunosuppressive activity (Table 2). Many substituents at the 3-phenyl position result in analogs with improved activity relative to **9a**. This effect would appear to be independent of electronic factors as both the 3-CH_3 (**9b**, numbering refers to the terminal phenyl group) and 3-CF_3 (**9d**) analogs have equivalent in vitro activities. In two instances the 3-substituted and corresponding 3,5-disubstituted derivatives had similar activities irrespective of the ability of these substituents to donate (**9l,m**) or withdraw (**9f,g**) electron density from the phenyl ring. In contrast, the 3,5-di(CF_3) substituted **9e** is 20-fold weaker than mono-substituted **9d** which may be a result of increasing hydrophobicity. Substitution at the 4-position provides analogs that are either equivalent in activity to the corresponding 4-H parent (**9h,k** vs **9f,a**) or weaker (**9p** vs **9m**). In all cases, incorporation of fluorine improves activity (**9f–h**) whereas chlorine appears to have no beneficial effect over hydrogen (**9i** vs **9a**). Oxygen substituents also have a pronounced effect on in vitro activity and are superior to alkyl groups in this regard (cf., **9l** vs isosteric **9c**). The 3-methoxy and 3,5-dimethoxy derivatives (**9l,m**) are at least as active as FK-506 in vitro (IC_{50} = 0.29 nM) and three-fold more potent than the parent natural product ascomycin. It is interesting that hydroxyl-substitution does nothing to increase activity in this series (**9n**), whereas this group was potency enhancing in the C32-O-cinnamyl ethers. The divergence of SAR in these classes of ascomycin derivatives implies that the phenyl groups displayed by the two tethers adopt distinct binding interactions with CaN.

Table 2.
Immunosuppressive activity of substituted 4(5)-phenylimidazol-2-yl-methyl ether derivatives of ascomycin



9a–p

Compound	Substitution (X)	TCell[23] IC_{50} (nM)
9a	H	0.80
9b	3-CH_3	0.51
9c	3-CH_2CH_3	0.71
9d	3-CF_3	0.45
9e	3,5-di(CF_3)	9.1
9f	3-F	0.36
9g	3,5-di(F)	0.32
9h	3,4-di(F)	0.43
9i	3,5-di(Cl)	0.86
9j	2-OCH_3	0.34
9k	4-OCH_3	0.87
9l	3-OCH_3	0.18
9m	3,5-di(OCH_3)	0.20
9n	3-OH	1.1
9o	3-OCF_3	0.58
9p	3,4,5-tri(OCH_3)	1.2

The in vivo activities of selected analogs were evaluated in the mouse and compared to those of FK-506 (Table 3). Immunosuppression was measured in a murine ex vivo assay and ED_{50} values were derived for both intravenous (iv) and orally (po) administered drug.[15] The C32-O-cinammyl ether **2** and 4(5)-phenylimidazol-2-

yl-methyl ether **9a** are both approximately five-fold weaker than FK-506 when administered intravenously. This result is, in part, explained by their three-fold difference in intrinsic potency. When administered orally the ED_{50} of **2** is shifted 33-fold compared to the iv value, whereas the potency of FK-506 and **9a** are reduced only 10-fold. More potent arylimidazole analogs such as **9g** and **9m** have iv activities that are similar to FK-506. The 3,5-dimethoxyphenyl analog **9m** is also close to FK-506 in oral activity while the more lipophilic 3,5-difluorophenyl analog **9g** is less potent.

Table 3[a].
Evaluation of FK-506 and C32-O-aralkyl ether derivatives of ascomycin in murine-based models of immunosuppression and toxicity

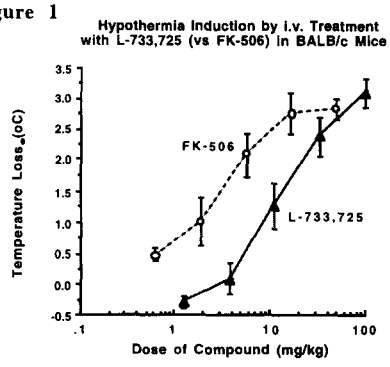
Compound	murine ex vivo, iv ED_{50} (mpk)	murine ex vivo, po ED_{50} (mpk)	mouse hypothermia, iv ED_{50} (mpk)	Therapeutic Index[b]
FK-506	0.24 ± 0.03	2.6 ± 0.18	3.5 ± 0.6	15
2	1.1	37	100	91
9a	1.4	15	11.8	8.4
9g	0.41	10	18.9	46
9m (L-733,725)	0.33 ± 0.07	4.4 ± 0.4	18.6 ± 5.6	56

[a] All in vivo data represents an average of at least two experiments ($n \geq 2$), standard error is given in cases where $n \geq 4$.
[b] [mouse hypothermia (iv) ED_{50}] + [murine ex vivo (iv) ED_{50}].

A model of neurotoxicity used in this program measures drug-induced hypothermia in BALB/c mice.[16] Comparing ascomycin analogs **9a,g,m** and **2** to FK-506 in this model, it is apparent that they are all less effective inducers of hypothermia than FK-506 when administered intravenously (Table 3). One measure of therapeutic index is to ratio this activity with that from the murine ex vivo assay. When comparing the data in this manner, the unsubstituted phenylimidazole **9a** has a lower therapeutic index than FK-506, whereas the substituted analogs **9g,m** and the cinnamyl ether **2** are significantly (three- to six-fold) safer than FK-506 in this mouse model. The dose-response curves for hypothermia induction with **9m** (L-733,725) and FK-506 are shown in Figure 1.

To measure the potential for nephrotoxicity, **9m** and FK-506 were administered to spontaneously hypertensive rats for 5 days (ip) and blood urea nitrogen (BUN) levels were measured 2 hours after the final injection.[16] As shown in Table 4, FK-506 had a profound effect on BUN levels at both the 20 and 40 mg/kg/day doses whereas no significant effects were observed with **9m** at doses up to 80 mg/kg/day.

Figure 1



In summary, a series of C32-O-4(5)-arylimidazol-2-yl-methyl ether derivatives of ascomycin were prepared and evaluated for immunosuppressive activity and both neuro- and nephrotoxicity. The potent oral activity of L-733,725, **9m**, combined with a nearly four-fold improvement in therapeutic index over FK-506 makes this ascomycin analog an attractive candidate for further study as an immunosuppressant with improved therapeutic potential. Results from this work will be the subject of future reports.

Table 4.
Evaluation of FK-506 and **9m** in a 5-day model of rat nephrotoxicity.

	BUN (mg/dL)				
dose (mg/kg/day, ip)	0	10	20	40	80
FK-506	20 ± 0.8	22 ± 1.9	43 ± 12	76 ± 21	N.D.
9m	20 ± 0.8	N.D.	21 ± 0.8	22 ± 0.8	27 ± 2.2

References and Notes

1. Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807.
2. Fruman, D. A.; Klee, C. B.; Bierer, B. E.; Burakoff, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3686.
3. O'Keefe, S. J.; Tamura, J.; Kincaid, R. L.; Tocci, M. J.; O'Neill, E. A. *Nature* **1992**, *357*, 692.
4. Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031.
5. Wenger, R. M.; *Angew. Chem. Int. Ed. Eng.* **1985**, *24*, 77.
6. Dumont, F. J. In *Drug Development in Transplantation and Autoimmunity*; Liebermann, R.; Muhkherjee, A.; Eds. R.G. Landes:Austin,TX, 1996; pp 175-205.
7. Dumont, F. J.; Staruch, M. J.; Koprak, S. L.; Siekierka, J. J.; Lin, C. S.; Harrison, R.; Sewell, T.; Kindt, V. M.; Beattie, T. R.; Wyvratt, M.; Sigal, N. H. *J. Exp. Med.* **1992**, *176*, 751.
8. Lyson, T.; Ermel, L. D.; Belshaw, P. J.; Alberg, D. G.; Schreiber, S. L.; Victor, R. G. *Circ. Res.* **1993**, *73*, 596.
9. Byrne, K. M.; Shafiee, A.; Nielsen, J. B.; Arison, B.; Monaghan, R. L.; Kaplan, L. In *Microbial Metabolites*; Nash, C., Ed.; Developments in Industrial Microbiology Series, Vol. 32; Wm. C. Brown: Dubuque, 1992; pp 29-45.
10. Goulet, M. T.; Hodkey, D. W.; Staruch, M. J.; Dumont, F. J.; Cryan, J. G.; Parsons, W. H.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 921.
11. Goulet, M. T.; Hodkey, D. W.; Staruch, M. J.; Dumont, F. J.; Lin, S.; Hung, S. H. Y.; Siekierka, J. J.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 927.
12. Sinclair, P. J.; Wong, F.; Wyvratt, M.; Staruch, M. J.; Dumont, F. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1035.
13. Sinclair, P. J.; Wong, F.; Staruch, M. J.; Wiederrecht, G.; Parsons, W. H.; Dumont, F.; Wyvratt, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2193.
14. Ok, H. O.; Szumiloski, J. L.; Beattie, T. R.; Goulet, M. T.; Staruch, M. J.; Dumont, F. J.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2199.
15. Peterson, L. B.; Cryan, J. G.; Rosa, R.; Martin, M. M.; Wilusz, M. B.; Sinclair, P. J.; Wong, F.; Parsons, J. N.; O'Keefe, S. J.; Parsons, W. H.; Wyvratt, M.; Sigal, N. H.; Williamson, A. R.; Wiederrecht, G. J. *Transplantation* **1998**, *65*, 10.
16. Dumont, F. J.; Koprak, S.; Staruch, M. J.; Talento, A.; Koo, G.; DaSilva, C.; Sinclair, P. J.; Wong, F.; Woods, J.; Barker, J.; Pivnichny, J.; Singer, I.; Sigal, N. H.; Williamson, A. R.; Parsons, W. H.; Wyvratt, M. *Transplantation* **1998**, *65*, 18.
17. Satisfactory ^{1}H NMR (400 MHz) and mass spectral data were obtained on all reaction products.
18. Weidenhagen, R.; Herrmann, R. *Ber.* **1935**, *68*, 1953.
19. Riley, H. L.; Morley, J. F.; Friend, N. A. C. *J. Chem. Soc.* **1932**, 1875.
20. Riley, H. A.; Gray, A. R.; In *Organic Syntheses, Col. Vol. 2.*; Blatt, A. H., Ed.; Wiley:New York, 1943; pp 509-511.
21. Griffith, J. P.; Kim, J. L.; Kim, E. E.; Sintchak, M. D.; Thomson, J. A.; Fitzgibbon, M. J.; Fleming, M. A.; Caron, P. R.; Hsiao, K.; Navia, M. A. *Cell* **1995**, *82*, 507.
22. Kissinger, C. R.; Parge, H. E.; Knighton, D. R.; Lewis, C. T.; Pelletier, L. A.; Tempczyk, A.; Kalish, V. J.; Tucker, K. D.; Showalter, R. E.; Moomaw, E. W.; Gastinel, L. N.; Habuka, N.; Chen, X.; Maldonado, F.; Barker, J. E.; Bacquet, R.; Villafranca, J. E.; *Nature* **1995**, *378*, 641.
23. Assay conducted using murine splenic T cells activated with PMA and ionomycin. In all cases the inhibition observed was reversed by the addition of exogenous IL-2, see: Dumont, F. J.; Staruch, M. J.; Koprak, S L.; Melino, M. R.; Sigal, N. H. *J. Immunol.* **1990**, *144*, 251.
24. Competitive binding assay using [^{3}H]-dihydro FK-506, see: Siekierka, J. J.; Hung, S. H.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature* **1989**, *341*, 755.
25. Prepared from **1**: benzyl chloromethyl ether, *N,N*-diisopropylethylamine, CH_2Cl_2.
26. Prepared from **5**: 1. 40% glyoxal, NH_4OH, MeOH; 2. benzylbromide, Ag_2O, CH_3CN; 3. HF•pyr., THF.
27. Prepared from **5**: 1. 40% glyoxal, NH_4OH, MeOH; 2. HF•pyridine, THF.