Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2089–2094

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

POTENT IMMUNOSUPPRESSIVE C32-O-ARYLETHYL ETHER DERIVATIVES OF ASCOMYCIN WITH REDUCED TOXICITY.

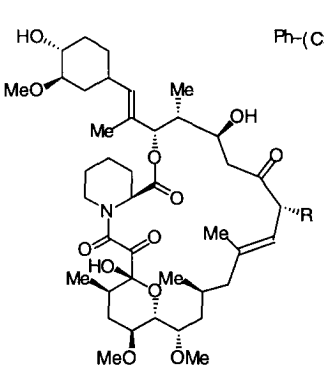
Helen M. Armstrong,*[a] Frederick Wong,[a] Mark A. Holmes,[a] Peter J. Sinclair,[a] Mark T. Goulet,[a] Francis J. Dumont,[b] MaryJo Staruch,[b] Samuel Koprak,[b] Laurence B. Peterson,[c] Raymond Rosa,[c] Mary Beth Wilusz,[c] Gregory J. Wiederrecht,[b] John G. Cryan,[b] Matthew J. Wyvratt,[a] and William H. Parsons.[a]

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

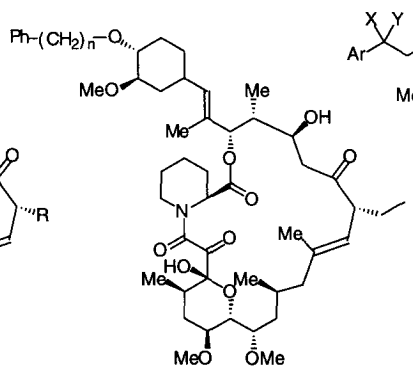
Received 19 March 1999; accepted 11 June 1999

Abstract: The synthesis of C32-O-arylethyl ether derivatives of ascomycin that possess equivalent immunosuppressant activity but reduced toxicity, compared to FK-506, is described .

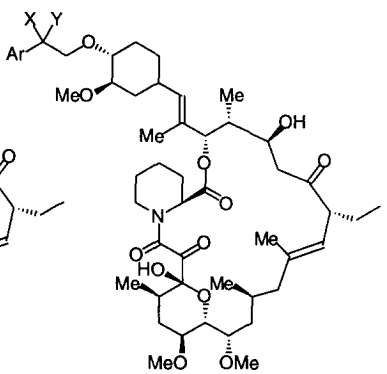
FK-506 (tacrolimus) (**1**)[1] is a potent agent for the suppression of T-cell proliferation in vitro and in vivo,[2] and has found broad application for the prevention of organ transplant rejection.[3] The toxicity of FK-506, however, appears to be largely related to its mechanism of action.[4,5] Our program has been focused upon finding chemical modifications of the FK-506 structure that will impart unique pharmacological properties such that toxicity and immunosuppressant activity can be separated.



(**1**) FK-506 R = $CH_2CH{=}CH_2$
(**2**) ascomycin R = CH_2CH_3



(**3**) n = 0 - 3



(**4**) X = Y = O
(**5**) X = OH, Y = H

Recent reports[6-10] have detailed our efforts to develop an immunosuppressant in the FK-506 class by the preparation of C32-O-ether analogs of the related natural product, ascomycin (**2**).[11] Certain members of this class of compounds have been of particular interest since they have demonstrated reduced toxicity in rodent models.[10,12] The study reported in the accompanying paper[13] shows that immunosuppressant activity in this class exhibits a marked dependence upon the tether length, a two atom tether being optimum (**3**, n = 2). It was

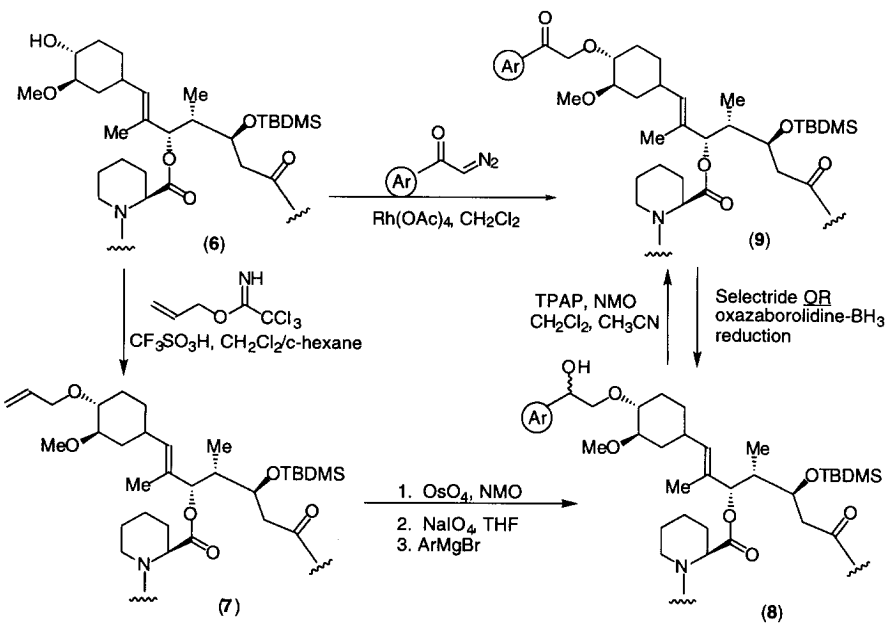
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00336-4

also noted that addition of a carbonyl or hydroxyl group at the β-carbon of such a tether further enhances immunosuppressant activity. This report details the investigation of a series of C32-O-acetophenone (**4**) and C32-O-arylhydroxyethyl (**5**) derivatives of ascomycin.

Chemistry

Acetophenone derivatives (**4**) were prepared by one of two routes. The initial route involved the direct attachment of the acetophenone moiety onto the protected ascomycin nucleus (**6**) by rhodium catalyzed insertion of an α-diazoketone into the C32 hydroxyl group to give protected acetophenone (**9**) (Scheme 1). While this provided an efficient way to generate simple mono- and disubstituted aromatic derivatives it was not successful in generating heteroaromatic and bicyclic aromatic derivatives due to low yields and complicated product separations. To generate these derivatives the more circuitous, but higher yielding, route through intermediates (**7**) and (**8**) was taken and has been previously described.[10]

Scheme 1



Arylhydroxyethyl ether products (**8**) were obtained by reduction of protected acetophenones (**9**), derived from the rhodium insertion procedure, using L-Selectride® to give a ~9:1 diastereomeric mixture of benzylic alcohols (as determined by HPLC analysis). This procedure was eventually supplanted by a chiral reduction using the oxazaborolidine-borane reagent,[14] which gave a much more desirable ~40:1 ratio of diastereomers. For derivatives prepared by the alternative procedure, the arylhydroxyethyl products (**8**) were obtained directly by HPLC separation of the diastereomers formed in the Grignard addition. In all cases the *t*-butyldimethylsilyl (TBDMS) protecting group was removed by treatment with hydrogen fluoride in pyridine. The absolute configuration at the benzylic center was assigned by X-ray crystallographic analysis of the C32-O-3,5-

dimethylphenylhydroxyethyl ether derivative (compound **(15)**, Table 1) bound to FK-506 binding protein (FKBP-12),[15] and for other derivatives by detailed comparison of NMR spectra with **(15)**.

Results and Discussion

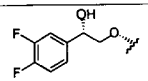
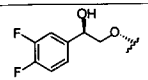
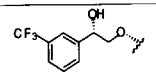
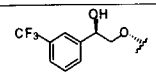
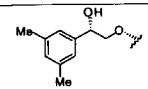
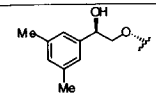
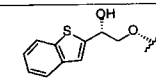
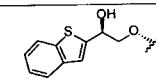
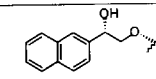
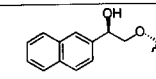
While the acetophenone derivatives **(4)** showed efficacy in our models of immunosuppression (data not shown) it was found that they were rapidly converted to the arylhydroxyethyl ether products **(5)** when incubated in rat blood at 37°C, consequently attention was focused on the latter series.

An extensive series of substituted arylhydroxyethyl ethers **(5)** was prepared of which a representative selection is shown in Table 1. Compounds **10–19** were initially evaluated for their in vitro[16] immunosuppressive potential. The data show that the compounds are potent inhibitors of in vitro T-cell proliferation and are of comparable activity to FK-506. Further, there does not appear to be a particular trend in activity with either electronic or steric factors since electron withdrawing groups (as in **10** and **11**) and electron donating groups (as in **14** and **15**) do not result in a profound difference in in vitro immunosuppressant potency. The larger bicyclic aromatic groups, as in **16–19**, also do not appear to have a profound effect upon immunosuppressant potential. However, all the compounds shown exhibit significantly weaker binding to FKBP-12 than does FK-506.[17] Although binding to FKBP-12, a major cytosolic protein, is a prerequisite for immunosuppressant activity it is not solely sufficient. After entering the cell and binding to FKBP-12 the resulting complex binds to and inhibits the activity of the serine/threonine phosphatase calcineurin (CaN).[18–20] Since the phosphatase activity of CaN is an integral part of the T-cell signal transduction cascade, the ability of the [drug•FKBP-12] complex to inhibit the phosphatase activity of CaN, as well as the stability of the complex itself, contribute to the level of immunosuppression.[8] These compounds are all significantly more potent inhibitors of CaN phosphatase activity than is FK-506, even though they do not bind as well to FKBP-12; these two factors combine to yield compounds of comparable immunosuppressant activity to FK-506.

Since it is our ultimate aim to produce a drug candidate that is not only a potent immunosuppressant, but also shows reduced toxicity relative to FK-506 in vivo, compounds **10–19** were also evaluated in murine models of immunosuppression and toxicity. Immunosuppression was measured, by intravenous and oral routes of administration, in an in vivo assay of splenic T-cell proliferation and ED_{50} values derived.[8] Toxicity was measured by the ability of the compounds to induce hypothermia in BALB/c mice.[12] This assay is considered to be a predictor of neurotoxicity since the regulation of body temperature is controlled by the CNS. Further, the compounds were evaluated for acute nephrotoxicity in the rat whereby male Sprague–Dawley rats were dosed daily intravenously for four days after which urine was collected and decreases in urinary sodium concentration measured.[12]

Ascomycin analogs **10–19** are all potent inhibitors of T-cell proliferation in vivo, whether administered intravenously or orally. As in the in vitro assays, there does not appear to be a correlation of in vivo immunosuppressant activity with steric or electronic features. In terms of toxicity however, this series has significantly less propensity to induce hypothermia than does FK-506, requiring two- to sixfold higher doses than FK-506 to produce the same effect. It has been postulated that this is due to their reduced binding affinity

Table 1. Evaluation of C32-O-arylhydroxyethyl ether derivatives in models of immunosuppression and toxicity.

#	C32 ether	T-cell proliferation IC_{50} (nM)[16]	FKBP-12 binding EC_{50} (nM)[17]	CaN phosphatase inhibition IC_{50} (nM)[8]	murine in vivo iv ED_{50} (mg/kg)[8]	murine in vivo po ED_{50} (mg/kg)[8]	Hypothermia induction[12] iv ED_{50}(mg/kg)	Therapeutic index [hypothermia ED_{50}]÷ [murine in vivo ED_{50}]	Rat nephrotox. 4 day iv % reduction. in [Na+] @ mg/kg*
(1)	FK-506	0.20	0.9	14.80	0.22	2.6	3.7	17	45% @ 1.7
(10)		0.14	7.5	7.25	0.19	2.8	10.0	52	44% @ 10
(11)		0.11	13.6	8.65	0.19	3.9	13.7	72	54% @ 5
(12)		0.38	45.0	4.93	0.31	3.7	12.8	41	NE @ 20
(13)		0.38	45.0	5.85	0.29	5.0	13.7	47	51% @ 5
(14)		0.23	7.5	7.91	0.45	4.5	10.3	23	NE @ 20
(15)		0.34	9.0	8.70	0.49	4.8	7.25	15	48% @ 10
(16)		0.39	11.25	3.96	0.75	6.4	14.8	20	32% @ 5
(17)		0.47	12.0	4.17	0.53	5.6	ND	-	ND
(18)		0.33	21.1	2.80	0.38	3.7	21.6	57	41% @ 20
(19)		0.34	18.0	7.32	0.38	3.4	14.2	37	40% @ 20

* dose (mg/kg) where a significant effect was observed, or the highest dose given where no effect (NE) was observed.

for FKBP-12 such that compounds of this type never become as concentrated in cells as does FK-506.[8] Thus for a given dose the concentration of intracellular [drug•FKBP-12] complex would be less than that of [FK-506•FKBP]. Since lymphocytes contain only low levels of CaN[21] there would be sufficient [drug•FKBP-12] available to inhibit the small amount of CaN present and produce immunosuppression, but in the brain, which contains much higher levels of CaN, there may not be sufficient [drug•FKBP-12] complex available to inhibit a significant amount of CaN present, perhaps resulting in the reduced neurotoxicity seen with this class of compounds.

Comparing the compounds on the basis of their therapeutic indices (defined as the ratio of the hypothermia assay ED_{50} to the murine in vivo ED_{50}), compounds **10**, **11** and **18** would seem to be the most promising potential drug candidates. In the acute 4-day iv model of nephrotoxicity in the rat, FK-506 produced a 45% reduction in urinary sodium concentration at a dose of only 1.7 mg/kg. Compounds **10**, **11** and **18** produced similar reductions in urinary sodium concentration but not until doses of 10, 5, and 20 mg/kg, respectively. On the basis of these results compound **18** was examined in a chronic three week nephrotoxicity model whereby **18** and FK-506 were administered by gavage to male Sprague–Dawley rats daily for three weeks.[12] Blood urea nitrogen (BUN) levels and plasma drug levels were recorded at the end of the study (Table 2). The data show that in order for FK-506 to produce a BUN level of 26 mg/dL, a plasma level of 611 ng/mL is required, and this is achieved at the 10 mg/kg dose. For **18** to produce a comparable BUN level of 24.7 mg/dL however, a plasma level of 1897 ng/mL is required and is not reached until the 20 mg/kg dose. This would indicate that the threshold at which significant alterations in kidney function are seen is at least threefold higher for compound **18** than it is for FK-506.

Table 2. Evaluation of (**18**) and FK-506 in a chronic model of nephrotoxicity.

Parameter	vehicle	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
FK-506 BUN (mg/dl)[a]	17.7 ± 0.5	18.1 ± 1.06	21.0 ± 1.2	26.0 ± 0.9	ND	ND
FK-506 plasma[b] drug level (ng/ml)	0	13 ± 5	45 ± 18	611 ± 177	ND	ND
(**18**) BUN (mg/dl)[a]	15.7 ± 0.5	ND	16.3 ± 0.6	17.7 ± 0.7	24.7 ± 1.0	39.3 ± 6.6
(**18**) plasma drug level (ng/ml)[b]	0	ND	239 ± 9	478 ± 83	1897 ± 308	3120 ± 308

[a] Each value is a mean ± SEM of 8–22 animals. [b] Each value is a mean ± SEM of 4–8 animals.

In conclusion, a series of C32-O-arylhydroxyethyl ether derivatives have been prepared from ascomycin (**2**) and evaluated for their immunosuppressant potential as well as their neuro- and nephrotoxicity. All of the compounds prepared were potent immunosuppressants in vitro and in vivo, showing good oral activity and reduced toxicity compared to FK-506. Compound (**18**) in particular has an improved therapeutic index compared to FK-506 and in acute and chronic nephrotoxicity models showed that it is at least three-fold safer than FK-506 and therefore is an attractive candidate for further study.

References and Notes

1. Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031.
2. Parsons, W. H.; Sigal, N. H.; Wyvratt, M. J. *Ann. New York Acad. of Sci.* **1993**, *685*, 22.
3. Dumont, F. J. In *Drug Development in Transplantation and Autoimmunity*; Leibermann, R. Muhkherjee, A. Eds.; R. G. Landes: Austin, TX, 1996; pp 175-205.
4. Dumont, F. J.; Staruch, M. J.; Koprak, S. L.; Siekeirka, J. J.; Lin, C. S.; Harrison, R.; Sewell, T.; Kindt, V. M.; Beattie, T. R.; Wyvratt, M. J.; Sigal, N. H. *J. Exp. Med.* **1992**, *176*, 751.
5. Lyson, T.; Ermel, L. D.; Belshaw, P. J.; Alberg, D. G.; Schreiber, S. L.; Victor, R. G. *Circ. Res.* **1993**, *73*, 596.
6. Sinclair, P. J.; Wong, F.; Wyvratt, M. J.; Staruch, M. J.; Dumont, F. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1035.
7. Sinclair, P. J.; Wong, F.; Staruch, M. J.; Wiederrecht, G.; Parsons, W. H.; Dumont, F.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2193.
8. 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. J.; Sigal, N. H.; Williamson, A. R.; Wiederrecht, G. J. *Transplantation* **1998**, *65*, 10.
9. 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.
10. Goulet, M. T.; McAlpine, S. R.; Staruch, M. J.; Koprak, S.; Dumont, F. J.; Cryan, J. G.; Wiederrecht, G. J.; Rosa, R.; Wilusz, M. B.; Peterson, L. B.; Wyvratt, M. J.; Parsons, W. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2253.
11. 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; W.C. Brown: Dubuque, 1992; pp 29-45.
12. 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. J. *Transplantation* **1998**, *65*, 18.
13. Goulet, M. T.; Sinclair, P. J.; Wong, F.; Staruch, M. J.; Dumont, F. J.; Cryan, J. G.; Wiederrecht, G. J.; Wyvratt, M. J.; Parsons, W. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2085.
14. Mathre, D. J.; Thompson, A. S.; Douglas, A. W.; Hoogsteen, K.; Carroll, J. D.; Corley, E. G.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 2880.
15. Becker, J.; Rotonda, J. unpublished results.
16. 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.; Melino, M. R.; Sigal, N. H. *J. Immunol.* **1990**, *144*, 251.
17. Competitive binding assay using [^{3}H]-dihydro FK-506, see: Siekierka, J. J.; Hung, S. H.; Pie, M.; Lin, C. S.; Sigal, N. H.; *Nature* **1989**, *341*, 755.
18. Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Wiessman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807.
19. Fruman, D. A.; Klee, C. B.; Bierer, B. E.; Burakoff, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3686.
20. O'Keefe, S. J.; Ramura, J.; Kincaid, R. L.; Tocci, M. J.; O'Neill, E. A. *Nature* **1992**, *357*, 692.
21. Asami, M.; Kuno, T.; Mukai, H.; Tanaka, C. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 1388.