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# C32-O-PHENALKYL ETHER DERIVATIVES OF THE IMMUNOSUPPRESSANT ASCOMYCIN: A TETHER LENGTH STUDY

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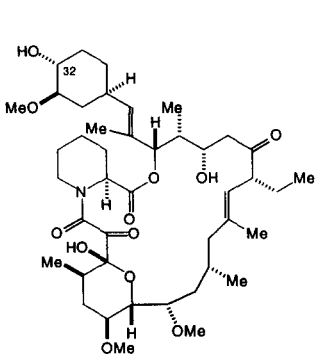
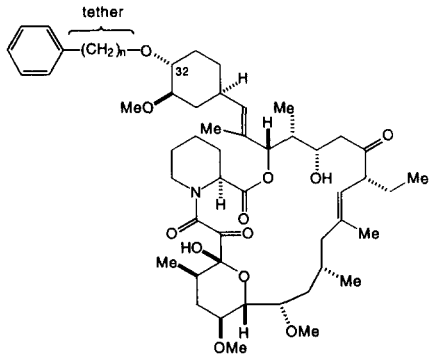
**Abstract:** A tether length study of C32-O-phenalkyl ether derivatives of ascomycin was conducted wherein it was determined that a 2-carbon tether provides optimum in vitro immunosuppressive activity. Oxygen-bearing substituents along the 2-carbon tether can further increase the potency of this design. 

As a part of our effort to develop an immunosuppressant in the FK-506 (tacrolimus)[1,2] class with an improved therapeutic profile we have investigated numerous C32-O-ether analogs of the related natural product ascomycin, **1**.[3] Derivatives of ascomycin containing C32-O-aryl,[4] -heteroaryl,[5,6] -aralkyl,[7] and -heteroaralkyl ethers[8] have in certain instances exhibited in vitro potency comparable to FK-506 and an improved therapeutic index in our animal models of neuro- and nephrotoxicity.[8,9] The C32-O-aralkyl ether class, in particular, has demonstrated exceptional safety in rodent-based models but has not undergone further development due to a lack of sufficient in vivo potency.[8] To improve the activity of this design, we sought first to gain a better understanding of the SAR about the alkyl tether region. The present study involved determining the dependence of in vitro activity on tether length within a class of phenalkyl ethers **2a–d**.



**1** ascomycin




**2a–d** (n = 0–3)

## Chemistry

The syntheses of C32-O-phenalkyl ether ascomycin derivatives with tethers of zero, one, and three methylene units (**2a,b,d**) were conducted by direct alkylation of the aryl or aralkyl components and have been described previously.[4,7] The C32-O-phenethyl ether analog **2c**, however, could not be formed by these methods and required attachment of the phenyl group to a pre-existing 2-carbon tether.[10] To achieve this goal, ascomycin was converted to the C24-OTBS, C32-O-acetaldehyde derivative **3** in a 5-step procedure.[7] Addition of phenylmagnesium bromide to **3** provided alcohol **4** as a 1:1 mixture of diastereomers.[11] Removal of the free

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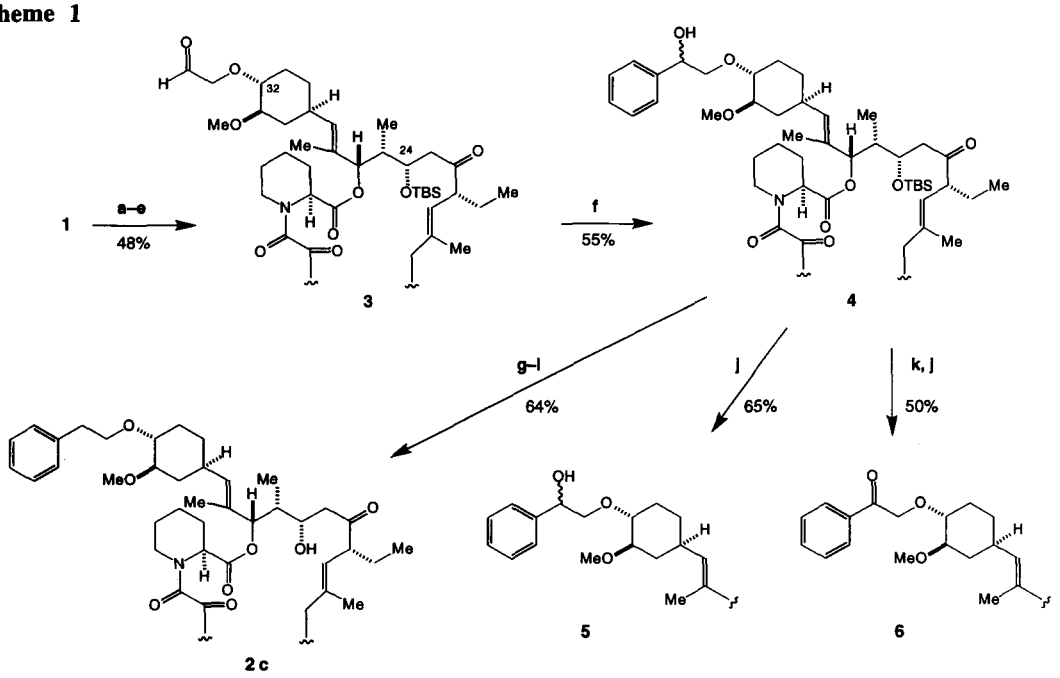
**Scheme 1**




**a.** TBSOTf (2.5 equiv), 2,6-lutidine (3 equiv), $CH_2Cl_2$; **b.** 10% pTsOH, $CH_3OH/CH_2Cl_2$ (1/1); **c.** allyl-2,2,2-trichloroacetimidate (2 equiv), TfOH (0.2 equiv), cyclohexane/$CH_2Cl_2$ (2/1); **d.** $OsO_4$ (0.2 equiv), 4-methylmorpholine *N*-oxide (6 equiv), aq THF; **e.** $NaIO_4$ (1.5 equiv), aq THF; **f.** phenylmagnesium bromide (3 equiv), THF, –78 °C; **g.** $(CF_3CO)_2O$ (2 equiv), $Et_3N$ (4 equiv), DMAP (cat), $CH_2Cl_2$; **h.** $H_2$ (1 atm), $Pd(OH)_2/C$, EtOH; **i.** 2% aq HF/$CH_3CN$; **j.** HF•pyridine, THF; **k.** TPAP (cat.), 4-methylmorpholine *N*-oxide (3 equiv), 4 Å sieves, $CH_2Cl_2$.

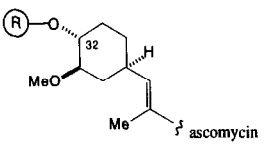
hydroxyl group was then accomplished by formation of the corresponding trifluoroacetate followed by hydrogenolysis of this group. Desilylation of the C24-OTBS protecting group with hydrogen fluoride in acetonitrile then gave **2c**. Alternatively, **4** could be desilyated under milder conditions (hydrogen fluoride•pyridine) to give benzyl alcohol **5**, or oxidized using tetrapropylammonium perruthenate (TPAP) and deprotected to provide the C32-O-acetophenone analog **6**.

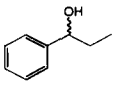
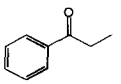
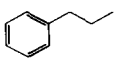
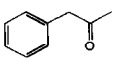
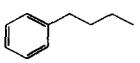
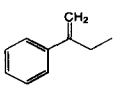
## Results and Discussion

The in vitro immunosuppressive activity and FKBP12 binding affinity of ethers **2a–d** were measured and the data compared with that of the parent natural product **1** (Table 1). In this study, the C32-O-phenalkyl ether analog in which the phenyl group is attached by a two-methylene tether, **2c**, was found to have immunosuppressive activity equivalent to ascomycin **1**. In contrast, the zero-, one-, and three-methylene tethered ethers (**2a,b,d**) were between four- and eightfold less active than **1**. The SAR of this tether region is also depicted graphically in Figure 1 where in vitro immunosuppressive activity as a percent of FK-506 activity[12] is plotted versus tether length. From this graph, the beneficial effect of a 2 atom tether is most evident.

Drugs in the FK-506 class suppress antigen induced T lymphocyte proliferation by their ability to bind the cytosolic protein FKBP12 and then, as a complex, bind the serine/threonine protein phosphatase calcineurin (CaN) and inhibit the activity of this enzyme.[13–15] To exert an effect, the drug must first enter the T lymphocyte

**Table 1.** Immunosuppressive activity of C32-O-alkyl ether derivatives of ascomycin



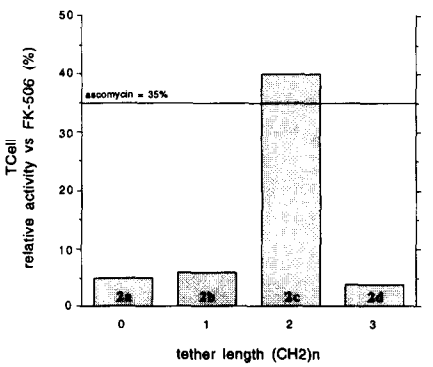

| Compound | R | TCell[a] $IC_{50}$ (nM) | FKBP12[b] $EC_{50}$ (nM) | Compound | R | TCell[a] $IC_{50}$ (nM) |
|---|---|---|---|---|---|---|
| **1** (ascomycin) | H | 0.69 | 1.6 | **5** |  | 0.18 |
| **2a**[c] |  | 5.4 | 12 | **7**[e] |  | 4.3 |
| **2b**[d] |  | 3.0 | 5.3 | **6** |  | 0.27 |
| **2c** |  | 0.61 | 9.2 | **8**[f] |  | 3.5 |
| **2d**[d] |  | 3.5 | 32 | **11**[g] |  | 1.0 |

[a]ref. 16; [b]ref. 17; [c]ref. 4; [d]ref. 7; [e]ref. 8; [f]ref. 18; [g]ref. 10.

and then participate constructively in both binding events. The close structural resemblance of ethers **2a–d** would allow one to assume similar cellular penetration within this series. Further, the FKBP12 binding affinities of **2a–d** are weaker than **1** and do not follow the same SAR pattern as that observed for in vitro immunosuppression (Table 1). Thus, the enhanced potency of **2c** is likely the result of a unique and favorable interaction of the C32-O-phenethyl appendage with CaN in the ternary complex (FKBP12•drug•CaN). Evidence for this type of interaction has been proposed based on data derived from other C32-derivatives of ascomycin.[6,8,19] Indeed, C-32-O-phenethyl ether **2c** had an $IC_{50}$ = 7.2 nM in a CaN inhibition assay,[6] which was two-fold more potent than FK-506 in the same experiment.[12] The $IC_{50}$ of homolog **2d** in this assay was 14 nM.

The immunosuppressive activity of C32-O-phenethyl ethers can be enhanced by substitution of certain functionality along the ethyl tether (Table 1). For example, addition of a hydroxyl group to **2c** increases in vitro potency three-fold (**5**). This effect is dependent on the presence of both the hydroxyl and phenyl groups, for the C-32-O-ethanol analog **7**[20] is much less efficacious (cf., **7** vs **5** and **1**). Addition of an oxo-group to the beta-carbon of the tether (**6**) likewise improves potency, while a similar substitution at the alpha-carbon (**8**) is detrimental. The oxygen atom in acetophone **6** appears to have a positive role in enhancing potency as the activity of the corresponding styrenyl analog (**11**, O → $CH_2$) is no better than **2c**.

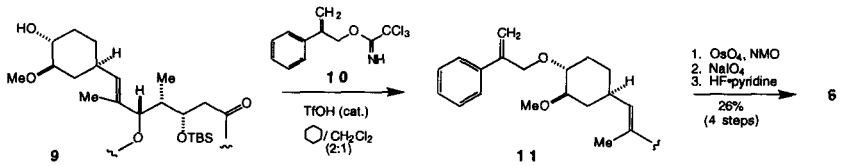
**Figure 1.** Tether length of ascomycin C32-O-phenalkyl ether derivatives vs relative immunosuppressive activity



In conclusion, a series of C32-O-phenalkyl ether derivatives of ascomycin was prepared and evaluated from which it was found that a 2-carbon tether analog, **2c**, provided maximal in vitro immunosuppression. Oxygen substitution along the ethyl tether can further increase the potency of these phenalkyl ethers. A more detailed examination of SAR within this class of ascomycin derivatives along with their in vivo properties is given in the accompanying report.[21]

## 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. First, M. R. *Am. J. Kidney Dis.* **1997**, *29*, 303.
3. 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.
4. Sinclair, P. J.; Wong, F.; Wyvratt, M.; Staruch, M. J.; Dumont, F. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1035.
5. Sinclair, P. J.; Wong, F.; Staruch, M. J.; Wiederrecht, G.; Parsons, W. H.; Dumont, F.; Wyvratt, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2193.
6. 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.
7. 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.
8. 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.
9. 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.
10. In subsequent studies it was found that alkylation of C24-OTBS ascomycin (**9**) with methylstyrenyl-2,2,2-trichloroacetimidate (**10**) followed by Johnson-Lemieux oxidation could provide **6** in good yield and without the requirement of carbon–carbon bond formation.



11. Satisfactory $^1$H NMR (400 MHz) and mass spectral data were obtained on all reaction products.
12. FK-506 was used as a positive control in each assay. Percent FK-506 activities were derived from the ratio of $IC_{50}$'s generated within a single experiment set (n = 3).
13. Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807.
14. Fruman, D. A.; Klee, C. B.; Bierer, B. E.; Burakoff, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3686.
15. O'Keefe, S. J.; Tamura, J.; Kincaid, R. L.; Tocci, M. J.; O'Neill, E. A. *Nature* **1992**, *357*, 692.
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 L.; 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.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature* **1989**, *341*, 755.
18. Preparation: 1. acylation of C24-OTBS ascomycin with phenylacetyl chloride; 2. desilylation (HF•pyr).
19. Becker, J. W.; Rotonda, J.; Cryan, J. G.; Martin, M.; Parsons, W. H.; Sinclair, P.J.; Wiederrecht, G.; Wong, F. *J. Med. Chem.* **1999**, *27*,in press.
20. see also: Mollison, K. W.; Fey, T. A.; Krause, R. A.; Andrews, J. M.; Bretheim, P. T.; Brandt, J. A.; Kawai, M.; Wagner, R.; Hsieh, G. C.; Luly, J. R. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1509.
21. Armstrong, H. M; Goulet, M. T.; Holmes, M. A.; Sinclair, P. J.; Wong, F.; Dumont, F. J.; Staruch, M. J.; Peterson, L. B.; Rosa, R.; Wilusz, M. B.; Wiederrecht, G. J.; Cryan, J. G.; Parsons, W. H.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2089.