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

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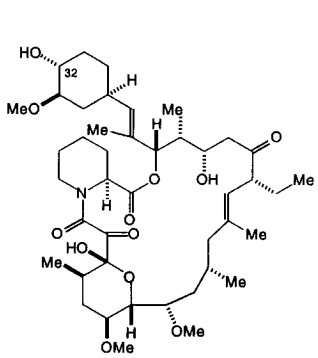
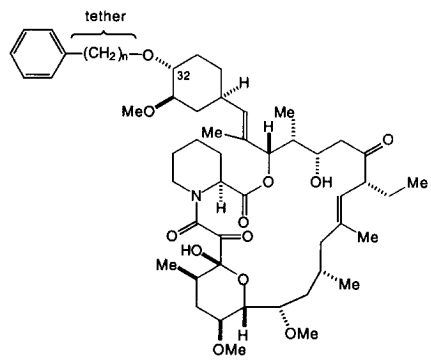
Abstract: A tether length study of C32-O-phenalkyl ether derivatives of ascomycin was conducted wherein it was determined that a 2-carbon tether provides optimum in vitro immunosuppressive activity. Oxygen-bearing substituents along the 2-carbon tether can further increase the potency of this design.

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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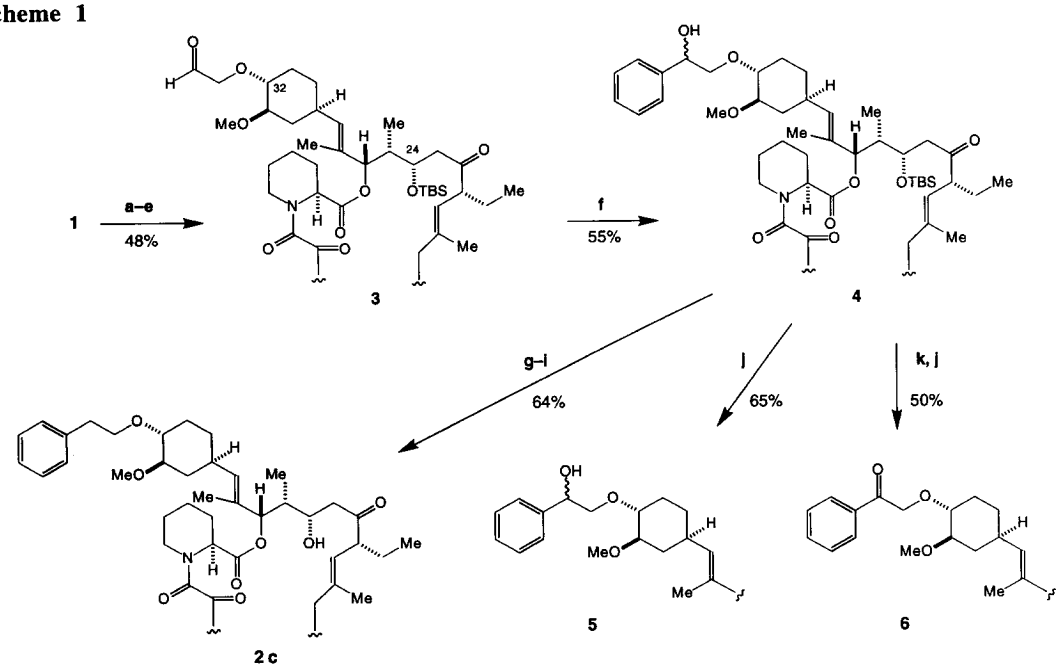
Scheme 1



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.

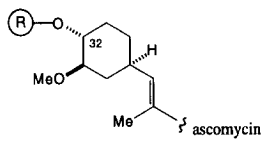
hydroxyl group was then accomplished by formation of the corresponding trifluoroacetate followed by hydrogenolysis of this group. Desilylation of the C24-OTBS protecting group with hydrogen fluoride in acetonitrile then gave **2c**. Alternatively, **4** could be desilyated under milder conditions (hydrogen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

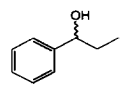
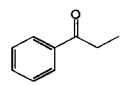
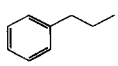
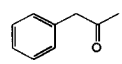
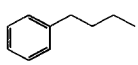
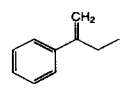
Results and Discussion

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



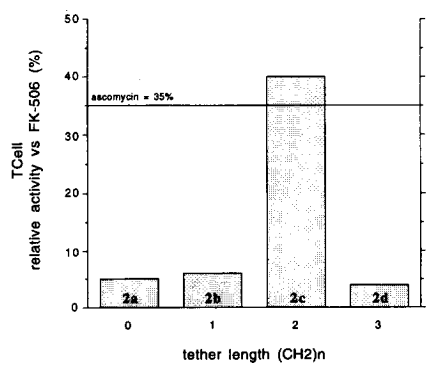
Compound	R	TCell[a] IC_{50} (nM)	FKBP12[b] EC_{50} (nM)	Compound	R	TCell[a] IC_{50} (nM)
1 (ascomycin)	H	0.69	1.6	**5**		0.18
2a[c]		5.4	12	**7**[e]		4.3
2b[d]		3.0	5.3	**6**		0.27
2c		0.61	9.2	**8**[f]		3.5
2d[d]		3.5	32	**11**[g]		1.0

[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]

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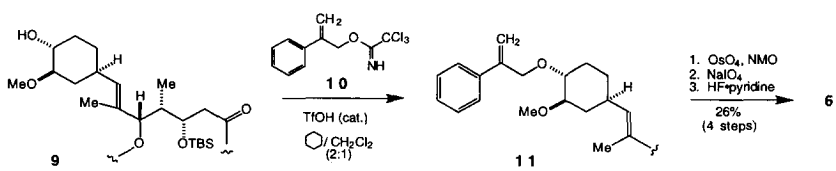
10. In subsequent studies it was found that alkylation of C24-OTBS ascomycin (**9**) with methylstyrenyl-2,2,2-trichloroacetimidate (**10**) followed by Johnson-Lemieux oxidation could provide **6** in good yield and without the requirement of carbon–carbon bond formation.



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).
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