Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 18, pp. 2193-2196, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00398-8

PREPARATION AND IN VITRO ACTIVITIES OF NAPHTHYL AND INDOLYL ETHER DERIVATIVES OF THE FK-506 RELATED IMMUNOSUPPRESSIVE MACROLIDE ASCOMYCIN

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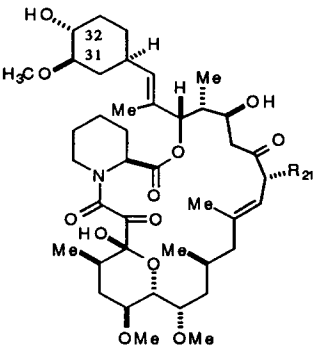
Abstract: The synthesis of naphthyl- and indolyl-ethers of the immunosuppressive macrolide ascomycin using pentavalent bismuth reagents is described. The in vitro activities of the aryl ether analogs are reported. The indole ether analogs show increased immunosuppressive activity in vitro relative to the parent macrolide.

The macrolide FK-506 (**1**) is a fungal metabolite first isolated and characterized in 1987.[1] It has been shown to be a powerful immunosuppressant in vitro and in vivo,[2] being 50 to 100 times more potent than cyclosporin A, the therapy of choice for prevention of graft rejection following organ transplantation. Although structurally dissimilar, FK-506 and cyclosporin elicit their immunosuppressive effects through a common mechanism.[3] Both bind with cognate receptors (FKBP and cyclophilin, respectively) and those drug-receptor complexes inhibit the Ca^{2+}-dependent protein phosphatase calcineurin. Inhibition of calcineurin disrupts early gene transcription following T-cell activation and results in suppression of lymphocyte proliferation. FK-506, like cyclosporin, is effective in the prevention of organ graft rejection in the clinic.[4] There are, however, side effects associated with the clinical use of FK-506: notably nephrotoxicity, neurotoxicity, diabetogenicity and gastrointestinal toxicity.[4,5] Research in these laboratories concentrated on derivatizing ascomycin (**2**) (L-683,590, FK-520) in an effort to identify a less toxic immunosuppressant. This communication is a preliminary account of the preparation and in vitro activity of 32-O-naphth-2-yl- and 32-O-indol-5-yl-ascomycin analogs. Significantly, the appendage of an indole group to the macrolide results in an unexpected and encouraging increase in activity in vitro as measured by the inhibition of T-cell proliferation.



compound		R_{21}
1	FK-506	allyl
2	Ascomycin	ethyl

A recent report from these laboratories described the synthesis of simple substituted phenyl ethers of ascomycin using pentavalent organobismuth reagents.[6] This etherification methodology proceeds under exceedingly mild conditions and works best on substrates containing a heteroatom proximal to the hydroxy group being arylated.[7] As such, this chemistry was exploited for the etherification at the 32-position of ascomycin without disrupting the highly functionalized macrocycle. The simple phenyl ethers of ascomycin showed a trend of increasing activity with increasing electron donating ability of the aromatic substituent. However, the most potent aryl ether made was only equipotent with ascomycin.[6] In an effort to advance the lead, the bismuthine arylation methodology was extended to naphthyl and indolyl ethers. Encouragingly, good in vitro activity was observed with the naphthyl ether and an unexpected boost in potency was seen in the case of the indolyl ethers.

In general, the bismuth(V) reagents are prepared by addition of 3 equivalents of an aryl Grignard or aryl lithium species to $BiCl_3$ followed by oxidation.[8] Tri(naphth-2-yl)bismuthine was prepared by addition of 2-naphthylmagnesium bromide to bismuth trichloride. The tri(indol-5-yl)bismuthines were prepared by lithium-halogen exchange on 5-bromoindole and N-methyl-5-bromoindole[9] followed by treatment with $BiCl_3$. The unsubstituted tri(indol-5-yl)bismuthine was unstable and thus prepared in lower yields. Arylation was achieved by preparation of the desired Bi(V) reagent in situ by addition of 1-1.2 equivalents of peracetic acid (32% solution in dilute acetic acid) to a solution of the triarylbismuthine in 3:1 CH_2Cl_2:THF. Ascomycin and copper(II)acetate were then added. The reactions were generally allowed to proceed overnight at room temperature or, if necessary, at reflux. While small amounts of the 24,32-diaryl compounds were isolated in some cases, arylation takes place preferentially at the 32-hydroxyl thus making protection of the 24-hydroxyl unnecessary. Arylation with the naphthyl or N-methylindolyl bismuthines proceeded as expected. The yields are not optimized.

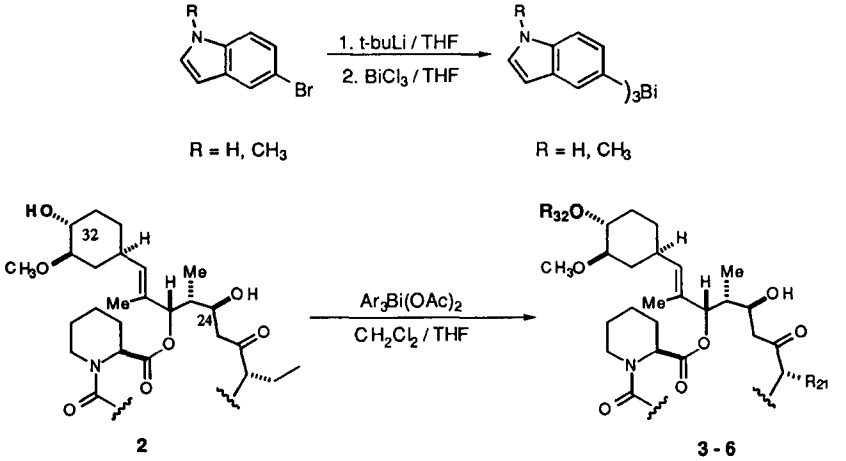


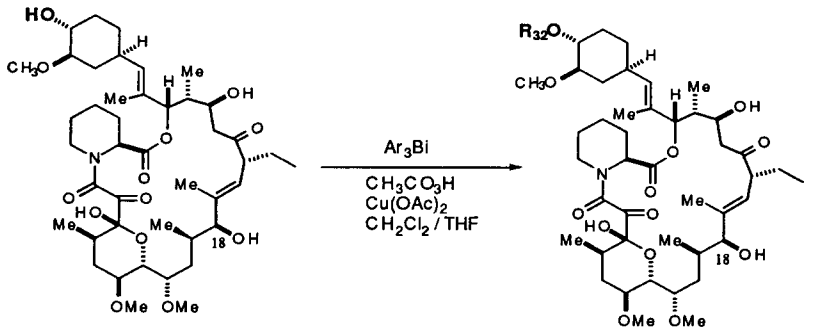
TABLE 1: IN VITRO ACTIVIY OF 32-O-ARYL ETHERS OF ASCOMYCIN

COMPOUND	R_{32}	R_{21}	Yield (%)[a]	T cell IC_{50} (nM)[b]	FKBP12 IC_{50}(nM)[c]
FK-506 **1**	H	allyl	--	**0.2**	0.8
ascomycin **2**	H	ethyl	--	0.7	1.6
3	phenyl	ethyl	42	5.4	10.0
4	2-naphthyl	ethyl	18	1.8	74.8
5	5-indolyl	ethyl	10	**0.2**	5.1
6	N-methyl-5-indolyl	ethyl	50	**0.2**	3.6

[a]Isolated yield of coupled product from arylation reaction.
[b]IC_{50} for inhibition of proliferation of PMA and Ionomycin stimulated murine T cells.[10]
[c]IC_{50} for competitive binding to FKBP12 versus tritiated FK-506.[11]

Table 1 shows the in vitro immunosuppressive activity of the phenyl, naphthyl, indolyl and N-methyl-indolyl ethers of ascomycin. The simple phenyl derivative **3** shows only one-seventh the activity of ascomycin. Substitution of a naphthyl group for the phenyl ring results in a modest increase in activity. Thus, naphthyl ether **4** is roughly three fold more active than phenyl ether **3**, but still only half as active as ascomycin. In contrast, substitution of an indole nucleus for the naphthyl system results in a 10-fold increase in activity. Moreover, the indole compound **5** is 3.5-fold more potent than the starting ascomycin and is now equipotent with FK-506. The N-methylindole ether **6** displays the same potent activity as unsubstituted indole **5**. Also shown in Table 1 are the affinities of the analogs for an FK-506 binding protein (FKBP12) as measured in a competitive binding assay against tritiated FK-506. The marked increase in potency for the indole ethers over ascomycin does not correlate with improved binding affinity for FKBP12.

The effect of appending a 32-O-naphthyl- and 32-O-indolyl- group on the bioactivity of a known antagonist of FK506 was explored. The 18-hydroxy derivative of ascomycin (**7**, L-685,818), prepared by the action of SeO_2 on the macrolide, is a potent antagonist of FK-506. L-685,818 binds as well to FKBP12 as does FK-506, yet it antagonizes the effect of FK-506 both in vitro and in vivo.[12] Etherification of **7** was carried out to give the 32-O-(naphth-2-yl) derivative **8** and 32-O-(1-methylindol-5-yl) derivative **9** in 44% and 37% yield, respectively. As can be seen from the data in Table 2, etherification of L-685,818 resulted in moderately immunosuppressive macrolides. Assay of the indole analog **9** shows that the macrolide binds less well to FKBP12 than does L-685,818.



7 L-685,818 **8-9**

TABLE 2: IN VITRO ACTIVITY OF L-685,818 AND ITS 32-O-INDOLYL ETHER

COMPOUND	R_{32}	T Cell IC_{50} (nM)	FKBP12 IC_{50} (nM)
ascomycin **2**	H	0.7	1.6
L-685,818 **7**	H	**antagonist**	0.7
8	2-Naphthyl	**10.2**	--
9	N-methyl-5-indolyl	**5.5**	2.8

[a]Isolated yield of coupled product from arylation reaction.
[b]IC_{50} for inhibition of proliferation of PMA and Ionomycin stimulated murine T cells.[10]
[c]IC_{50} for competitive binding to FKBP12 versus tritiated FK-506.[11]

The underlying reason for the observed activities of these ascomycin derivatives can only be speculated upon at this time. The SAR cannot be explained by simply considering the analogs' binding affinities for FKBP. For instance, while ascomycin is approximately three-fold less potent in the T cell proliferation assay and two-fold less potent in the binding assay relative to FK-506, indole ether **6** is equipotent to FK-506 in the immunosuppression assay but is six-fold less potent than FK-506 in the FKBP12 binding assay. Additionally, L-685,818 (**7**) is equipotent to FK-506 in the binding assay but antagonizes the immunosuppressive effects of FK-506 while the 32-O-indole analog **8** binds less well to FKBP12 but is moderately immunosuppressive. One hypothesis is that the indole group enhances the binding interaction of the macrolide/FKBP complex with its target, the protein phosphatase calcineurin, thereby accounting for the observed increase in potency. L-685,818 (**7**) may be an antagonist due to unfavorable interactions of the 18-hydroxy group with calcineurin and it may be that these unfavorable interactions are overcome upon addition of an indolyl-, and to a lesser extent, naphthyl- group to the 32-position of the macrolide resulting in modestly immunosuppressive compounds. Further investigation is needed in order to determine the cause of the potency enhancing effect of the indole group.

In summary, this communication reports the preparation of 32-O-naphthyl- and 32-O-indolyl ethers of the immunosuppressive macrolide ascomycin. This chemistry is an extension of the utilization of triarylbismuth diacetates for the preparation of aryl ethers of complex molecules. Moreover, appendage of the indole group to ascomycin results in an exciting increase in potency as measured by the ability of the derivatives to inhibit the proliferation of T cells in vitro. The potency enhancing effect of the indole group was extended to a known antagonist of FK506, giving a moderately immunosuppressive compound. A more detailed study of the structure-activity relationship of indolyl ethers of ascomycin will be forthcoming.

Acknowledgement: The authors would like to thank Dr. G. Salituro and Mr. F. P. Gailliot for providing the ascomycin used in these studies, and Dr. L. Colwell and Ms. A. Bernick for mass spectral analyses.

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(*Received in USA* 22 *January* 1996; *accepted* 16 *August* 1996)