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United States Patent [19]

Caufield

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[54] RAPAMYCIN ESTERS

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[73] Assignee: **American Home Products Corporation**, New York, N.Y.

[21] Appl. No.: **777,983**

[22] Filed: **Oct. 17, 1991**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 584,833, Sep. 19, 1990, abandoned.

[51] **Int. Cl.**[5] **C07D 487/06**; A61K 31/33

[52] **U.S. Cl.** **514/183**; 540/455

[58] **Field of Search** 540/455; 514/183

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,929,992 12/1975 Sehgal et al. 424/122

(List continued on next page.)

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Huffer et al "Introduction to Human Immunology" p.

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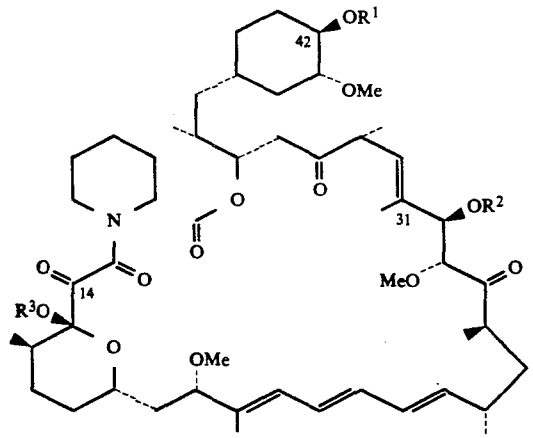
[57] **ABSTRACT**

A compound of the structure

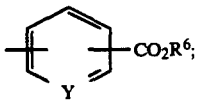


where

R^1, R^2, and R^3 are each, independently, hydrogen or

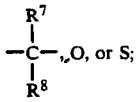
with the proviso that R^1, R^2, and R^3 are not all hydrogen;

R^4 is $-(CH_2)_mX(CH_2)_nCO_2R^5$ or



R^5 and R^6 are each, independently, alkyl, aralkyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl, alkoxy, hydroxy, cyano, halo, nitro, carbalkoxy, trifluoromethyl, amino, or a carboxylic acid;

X is



R^7 and R^8 are each, independently, hydrogen or alkyl;

Y is CH or N;

m is **0–4**; n is **0–4**;

with the proviso that m and n are not both **0** when X is O or S;

or a pharmaceutically acceptable salt thereof, which is by virtue of its immunosuppresive activity is useful in treating transplantation rejection, host vs. graft disease, autoimmune diseases, and diseases of inflammation, by virtue of its antitumor activity useful in treating tumors, and by virtue of its antifungal activity is useful in treating fungal infections.

19 Claims, No Drawings

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RAPAMYCIN ESTERS

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation in part of Ser. No. 584,833, filed Sep. 19, 1990 now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to novel esters of rapamycin and a method for using them in the treatment of transplantation rejection, host vs. graft disease, autoimmune diseases, diseases of inflammation, tumors, and fungal infections.

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antigugal activity, particularly against *Candida albicans*, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Seghal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31,539 (1978); U.S. Pat. No. 3,929,992; and U.S. Pat. No. 3993,749].

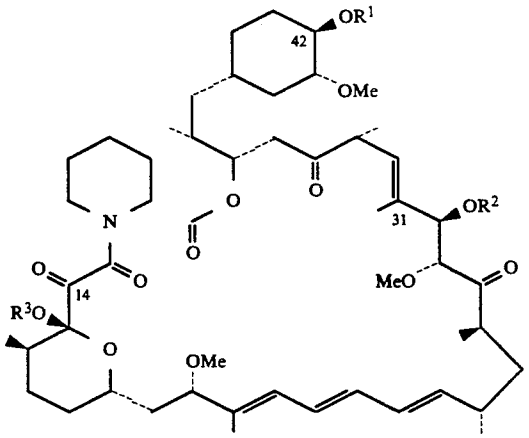
Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989), rapamycin has been shown to be effective in inhibiting transplant rejection (U.S. Pat. application Ser. No. 362,354 filed Jun. 6, 1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); and R. Y. Calne et al., Lancet 1183 (1978).

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions.

DESCRIPTION OF THE INVENTION

This invention provides derivatives of rapamycin which are useful as immunosuppressive, anti-inflammatory, antitùmor, and antifungal agents having the structure

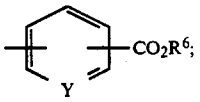


wherein

R^1, R^2, and R^3 are each, independently, hydrogen or

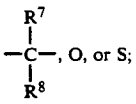
with the proviso that R^1, R^2, and R^3 are not all hydrogen;

R^4 is $-(CH_2)_mX(CH_2)_nCO_2R^5$ or



R^5 and R^6 are each, independently, alkyl of 1–6 carbon atoms, aralkyl of 7–10 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, or a carboxylic acid;

X is



R^7 and R^8 are each, independently, hydrogen or alkyl of 1–6 carbon atoms;

Y is CH or N;

m is 0–4;

n is 0–4;

with the proviso that m and n are not both 0 when X is O or S;

or a pharmaceutically acceptable salt thereof.

Of the compounds, preferred members are those in which R^4 is $-(CH_2)_mX(CH_2)_nCO_2R^5$.

Aryl is defined as an organic radical derived from an aromatic hydrocarbon by the removal of one atom; e.g., phenyl from benzene. Aralkyl is defined as an arylated alkyl radical; a radical in which an alkyl H atom is substituted by an aryl group. The definition of aryl and aralkyl are also intended to encompass compounds in which the phenyl groups of such moieties are optionally

mono-, di-, or tri-substituted with a substituent selected from alkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, a carboxylic acid, or the like.

The pharmaceutically acceptable salts may be formed from inorganic cations such as sodium, potassium, and the like; mono-, di-, and trialkyl amines of 1–6 carbon atoms, per alkyl group and mono-, di-, and trihydroxyalkyl amines of 1–6 carbon atoms per alkyl group. Preferred salts are formed from sodium cations and tris(hydroxymethyl)methylamine.

The compounds of this invention can be prepared by acylating rapamycin with an acylating agent having the general structure

where X is OH, in the presence of a coupling reagent, such as dicyclohexylcarbodimide. The compounds of this invention also can be prepared using an anhydride of the above described carboxylic acid as the acylating species. Alternatively, the acylating species can be an acid halide, where X can be Cl, Br, or I. The acylating groups used to prepare the compounds of this invention are commercially available or can be prepared by methods that are disclosed in the literature.

Immunosuppressive activity was evaluated in an in vitro standard pharmacological test procedure to measure lymphocyte proliferation (LAF) and in two in vivo standard pharmacological test procedures. The first in vivo procedure was a popliteal lymph node (PLN) test procedure which measured the effect of compounds of this invention on a mixed lymphocyte reaction and the second in vivo procedure evaluated the survival time of a pinch skin graft.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated; radioactivity is determined. Inhibition of lymphoproliferation is assessed in percent change in counts per minute from non-drug treated controls. The results are expressed by the following ratio:

$$\frac{^3\text{H-control thymus cells} - \text{H}^3\text{-rapamycin-treated thymus cells}}{^3\text{H-control thymus cells} - \text{H}^3\text{-test compound-treated cells}}$$

A mixed lymphocyte reaction (MLR) occurs when lymphoid cells from genetically distinct animals are combined in tissue culture. Each stimulates the other to undergo blast transformation which results in increased DNA synthesis that can be quantified by the incorporation of tritiated thymidine. Since stimulating a MLR is a function of disparity at Major Histocompatibility antigens, an in vivo popliteal lymph node (PLN) test procedure closely correlates to host vs. graft disease. Briefly, irradiated spleen cells from BALB/c donors are injected into the right hind foot pad of recipient C3H mice. The drug is given daily, p.o. from Day 0 to 4. On Day 3 and Day 4, tritiated thymidine is given i.p., b.i.d. On Day 5, the hind popliteal lymph nodes are removed and dissolved, and radioactivity counted. The corresponding left PLN serves as the control for the PLN from the injected hind foot. Percent suppression is calculated using the non-drug treated animals as allogenic control. Rapamycin at a dose of 6 mg/kg, p.o. gave 86% suppression, whereas cyclosporin A at the same dose gave 43% suppression. Compounds evaluated in the PLN test procedure were administered orally, unless otherwise indicated, as being administered intraperitoneally. Carboxymethyl cellulose was used as the vehicle for administration, unless otherwise indicated. Results are expressed by the following ratio, unless otherwise indicated:

$$\frac{^3\text{H-PLN cells control C3H mouse} - ^3\text{H-PLN cells rapamycin-treated C3H mouse}}{^3\text{H-PLN cells control C3H mouse} - ^3\text{H-PLN cells test compound-treated C3H mouse}}$$

The second in vivo test procedure is designated to determine the survival time of pinch skin graft from male DBA/2 donors transplanted to male BALB/c recipients. The method is adapted from Billingham R. E. and Medawar P. B., J. Exp. Biol. 28:385–402, (1951). Briefly, a pinch skin graft from the donor is grafted on the dorsum of the recipient as a homograft, and an autograft is used as control in the same region. The recipients are treated with either varying concentrations of cyclosporin A as test control or the test compound, intraperitoneally. Untreated recipients serve as rejection control. The graft is monitored daily and observations are recorded until the graft becomes dry and forms a blackened scab. This is considered as the rejection day. The mean graft survival time (number of days±S.D.) of the drug treatment group is compared with the control group.

The following table summarizes the results of representative compounds of this invention in these three standard test procedures.

TABLE 1

Compound	LAF* (ratio)	PLN* (ratio)	Skin Graft (days + SD)
Example 1	0.37	++	8.2 ± 1.2
Example 2	0.9	0.69**	10.7 ± 1.2
Example 3	3.27	1.04**	12.7 ± 0.9
		0.20	
		2.08	
Example 4	0.56	1.68	10.2 ± 1.7
		0.42	
Example 5	0.02	1.11	8.0 ± 1.7
Example 6	0.01	0.48	8.0 ± 0.9
Example 7	0.97	0.70**	12.0 ± 1.0
Example 8	0.22	−1.93	9.3 ± 1.6
		0.37**,+	
Example 9	0.22	0.41	10.2 ± 1.2
Example 10	0.18	0.39	10.8 ± 0.8
Example 11	0.00	0.09	7.8 ± 1.7
Example 12	97%+++	1.04	10.8 ± 0.4
Example 13	2.11	1.02	10.6 ± 0.9
		0.40	
Rapamycin	1.0	1.0	12.0 ± 1.7

*Calculation of ratios was described supra.
**Administered using cremophore/ethanol as the vehicle.
+Administered intraperitoneally.
++Not evaluated.
+++Result expressed as percent inhibition at 100 nM.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this

invention. Positive ratios in the LAF and PLN test procedures indicate suppression of T cell proliferation. As a transplanted pinch skin grafts are typically rejected within 6–7 days without the use of an immunosuppressive agent, the increased survival time of the skin graft when treated with the compounds of this invention further demonstrates their utility as immunosuppressive agents. While it appears that the compound disclosed by Example 8 may cause T cell proliferation in the PLN test procedures because of the −1.93 ratio obtained, it is believed that this result is merely an anomaly in light of the other data obtained. Spurious results have been obtained in the PLN test procedure using compounds that have low bioavailability. Low bioavailability can be due to the compound itself, the dose used, the vehicle, the route of administration, or a combination of any of the above factors. When the negative ratio was obtained for the compound of Example 8, it was administered orally in carboxymethylcellulose. A negative ratio in the PLN test procedure was not observed for the compounds of Examples 9 and 10, which are pharmaceutical salts of the compound of Example 8. When the compound of Example 8 was administered i.p. in a mixture of cremophore and ethanol as the vehicle, a positive ratio was obtained indicating the compound had immunosuppressive activity. The positive ratio obtained in the LAF test procedure coupled with the increased survival time observed in the skin graft test procedure confirm the immunosuppressive activity of the compound of Example 8. The negative ratio obtained when the compound of Example 8 was administered orally in carboxymethyl cellulose is therefore beleived to be attributed to low bioavailability, and not a function of its immunosuppressive activity.

Antifungal activity of the compounds of this invention was measured against 5 strains of *Candida albicans* using a plate test procedure for measurement of inhibition. The following represents the typical procedure used. Compound to be tested was placed on sterile dried ¼" plate disks, and allowed to dry. Agar plates were seeded with fungi and allowed to solidify. The impregnated disks were placed on the seeded Agar surface and incubated for the time required for the particular culture. Results are expressed in MIC (μg/ml) to inhibit growth. The results of this test procedure showed that the compounds of this invention have antifungal activity; however, it was surprising that the compounds of this invention were less active that the parent compound, rapamycin. The compounds of Examples 12 and 13 were not evaluated for antifungal activity, but because of the structural similarity to the ones that were evaluated, they too are considered to have antifungal activity.

TABLE 2*

	Strain of *Candida albicans*				
Compound	ATCC 10231	ATCC 38246	ATCC 38247	ATCC 38248	3669
Example 1	>0.4	>0.4	>0.4	>0.4	>0.4
Example 2	>0.4	0.4	>0.4	0.4	0.4
Example 3	0.2	0.1	0.4	0.1	0.1
Example 4	>0.4	0.2	>0.4	0.2	0.4
Example 5	0.4	>0.4	>0.4	>0.4	>0.4
Example 6	0.4	>0.4	0.4	>0.4	>0.4
Example 7	0.1	0.4	0.1	0.1	0.2
Example 8	0.4	>0.4	0.4	>0.4	>0.4
Example 9	0.2	>0.4	0.2	0.4	>0.4
Example 10	0.1	>0.4	0.2	0.4	>0.4
Example 11	>0.4	>0.4	>0.4	>0.4	>0.4

TABLE 2*-continued

	Strain of *Candida albicans*				
Compound	ATCC 10231	ATCC 38246	ATCC 38247	ATCC 38248	3669
Rapamycin	0.003	0.025	0.003	0.006	0.025

*expressed as MIC (μg/ml)

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment of transplantation rejection such as, heart, kidney, liver, bone marrow, and skin transplant; autoimmune diseases such as, lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; diseases of inflammation such as, psoriasis, dermatitis, eczema, seborrhea, and inflammatory bowel disease; and fungal infections. As the compounds of this invention are structurally related to rapamycin, which has antitumor activity, they too are considered to be useful as antitumor agents.

The compounds may be administered neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intrave-

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