United States Patent [19]

Witzel

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[54] **CYCLOSPORIN ANALOGS WITH MODIFIED "C-9 AMINO ACIDS"**

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[73] Assignee: **Merck & Co., Inc.,** Rahway, N.J.

[*] Notice: The portion of the term of this patent subsequent to Jan. 17, 2006 has been disclaimed.

[21] Appl. No.: **261,868**

[22] Filed: **Oct. 24, 1988**

Related U.S. Application Data

[62] Division of Ser. No. 57,196, Jun. 3, 1987.

[51] **Int. Cl.**[4] **A61K 37/07;** C07K 5/12

[52] **U.S. Cl.** **514/11;** 530/317; 530/321

[58] **Field of Search** 530/317, 321; 514/11

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,396,542	8/1983	Wenger	530/321
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Sandorama (1984) 5–11 III Wenger.

Primary Examiner—Delbert R. Phillips
Attorney, Agent, or Firm—Robert J. North; Joseph F. DiPrima

[57] **ABSTRACT**

New cyclosporin analogs with modified "C-9 amino acids" have been made and are found to be effective immunosuppressive agents.

8 Claims, No Drawings

CYCLOSPORIN ANALOGS WITH MODIFIED "C-9 AMINO ACIDS"

CROSS-REFERENCE TO RELATED APPLICATION

This Application is a continuation of U.S. Ser. No. 057,196, filed by B.E. Witzel on June 3, 1987 (now allowed).

BACKGROUND OF THE INVENTION

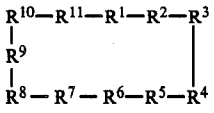
Immunoregulatory abnormalities have been shown to exist in a wide variety of "autoimmune" and chronic inflammatory diseases, including systemic lupus erythematosis, chronic rheumatoid arthritis, type 1 diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohns disease, ulcerative colitis, bullous pemphigoid, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they have in common the appearance of a variety of autoantibodies and self-reactive lymphocytes. Such self-reactivity may be due, in part, to a loss of the homeostatic controls under which the normal immune system operates.

Similarly, following a bone-marrow or an organ transplantation, the host lymphocytes recognize the foreign tissue antigens and begin to produce antibodies which lead to graft rejection.

One end result of an autoimmune or a rejection process is tissue destruction caused by inflammatory cells and the mediators they release. Antiinflammatory agents such as NSAID's and corticosteroids act principally by blocking the effect or secretion of these mediators but do nothing to modify the immunologic basis of the disease. On the other hand, cytotoxic agents such as cyclophosphamide, act in such a nonspecific fashion that both the normal and autoimmune responses are shut off. Indeed, patients treated with such nonspecific immunosuppressive agents are as likely to succoumb from infection as they are from their autoimmune disease.

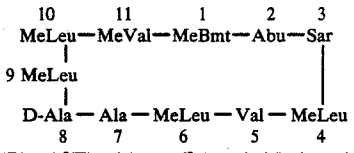
The cyclosporins are a family of immunospressive compounds isolated from fermentation broths of various fungal species including *Tolvoocladium inflatum* and *Cylindrocaroon lucidum.*

The generic structure of the class of cyclosporins has been established as a cyclic peptide of formula (I) which contains 11 amino acids.



(I)

For example, cyclosporin A of formula (II) contains several N-methylated amino acids and one novel amino acid "MeBMT" designated as the 1- "C-9 amino acid". This novel amino acid is located in position 1 and has been found to be important for the biological activity of cyclosporin. We have found that replacing the double bond of the "C-9 amino acid" (MeBMT) with a hetero atom such as S and O decreases the toxicity of the parent cyclosporin. Substantial activity in the various assays in which cyclosporin A expresses immunosuppressive activity is also exhibited.



(II)

Bmt = (4R)—4-[(E)—2-butenyl]-4-methyl-L-threonine
Me = Methyl
Abu = α-Aminobutyric acid
Val = Valine
Ala = Alanine
MeLeu = N—methyl Leucine
MeVal = N—Methyl valine
Sar = Sarcosine

Generally a cyclosporin such as cyclosporin A is not cytotoxic nor myelotoxic. It does not inhibit migration of monocytes nor does it inhibit granulocytes and macrophage action. Its action is specific and leaves most established immune responses intact. However, it is nephrotoxic and is known to cause the following undesirable side effects:

(1) abnormal liver function;
(2) hirsutism;
(3) gum hypertrophy;
(4) tremor;
(5) neurotoxicity;
(6) hyperaesthesia; and
(7) gastrointestinal discomfort.

Accordingly, an object of the present invention is to provide new cyclosporin analogs which will (1) restore the balance of the help-and-suppression mechanism of the immune system by acting at an earlier point than the anti-inflammatory agents and (2) induce specific long-term transplantation tolerance through a suppressor cell circuit without increasing the body's susceptibility to infection.

Another object of the present invention is to provide pharmaceutical compositions for administering to a patient in need of the treatment one or more of the active immunosuppressive agents of the present invention.

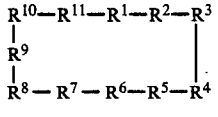
Still a further object of this invention is to provide a method of controlling graft rejection, autoimmune and chronic inflammatory diseases by administering a sufficient amount of one or more of the novel immunosuppressive agents in a mammalian species in need of such treatment.

Finally, it is the object of this invention to provide processes for the preparation of the active compounds of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A. Scope of the Invention

This invention relates to cyclosporins of formula (I)

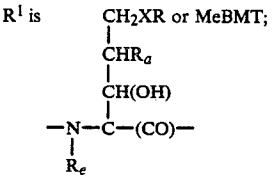


(I)

wherein

R^1 is

R^1 is



where X, R, R_a, and R_e are as defined below;

R^2 is L-2-aminobutyryl; norvalyl; L-threonyl; or R^1;

R^3 is sarcosyl or α-(methylmercapto)sarcosyl; N-methyl-D-alanyl or N-methyl-L-alanyl; or D-prolyl;

R^4 is N-methyl-L-leucyl;

R^5 is L-valyl; or norvalyl;

R^6 is N-methyl-L-leucyl;

R^7 is L-alanyl; L-2-aminobutyryl; or L-phenylalanyl;

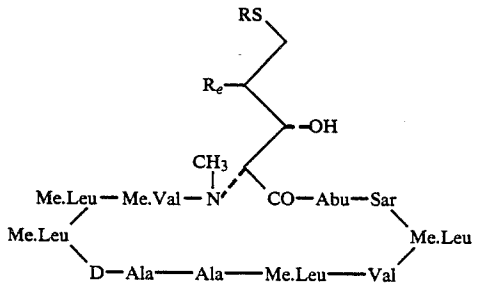
R^8 is D-alanyl or L-alanyl;

R^9 is N methyl-L-leucyl; or N-methyl-L-valyl;

R^{10} is N-methyl-L-leucyl; or L-leucyl;

R^{11} is N-methyl-L-valYl; L-valyl; or L-2-aminobutyryl;

Preferably, this invention relates to a cyclosporin A derivative having modified 1-"C-9 amino acid":



III

wherein

R is

(1) hydrogen; '(2) loweralkyl especially C_{1-6} alkyl such as methyl, ethyl, Propyl, isopropyl, t-butyl, pentyl, benzyl, cyclopropyl, cyclopentyl or cyclohexyl;

(3) loweralkenyl especially C_{2-6} alkenyl, for or example, vinyl, allyl, and buten-2-yl:

(4) haloloweralkyl especially C_{1-6} haloalkyl such as trifluoromethyl;

(5) aryl especially phenyl or substituted phenyl;

(6) oxyloweralkyl especially alkoxy C_{1-6} alkyl such as $—CH_2OR_b$ where R_b is H or C_{1-6} alkyl;

(7) thioloweralkyl especially alkylthio C_{1-6} alkyl such as $—CH_2SR_a$ wherein R_a is C_{1-6} alkyl; or mercapto C_{1-6} alkyl;

(8) heteroaryl especially pyridyl, pyrryl, furyl or thienyl;

the aryl or heteroaryl group above can be substituted with one or more functional groups e.g.,

(a) C_{1-6}alkyl,

(b) C_{1-6} alkanoyl;

(c) C_{1-6} haloalkyl;

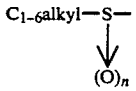
(d) halo;

(e) cyano;

(f) hydroxy C_{1-3} alkyl;

(g) C_{1-6} alkoxy;

(h)



where n is 0, 1 or 2;

(i) $—NR_bCOR_c$ R_b and R_c independently are H or C_{1-6} alkyl;

(j) $—NO_2$;

(k) $—NR_bR_d$;

(l) $—OR_b$;

(m) $—CONR_bR_c$;

(n) $—COR_b$;

(o) $—NR_bCONR_bR_c$;

(p) $—NR_bCOR_c$;

(q) $—OCOR_b$;

(r) $—SCOR_b$; or

(s) $—OCH_2O—$;

R_a is loweralkyl;

R_e is loweralkyl; loweralkylphenyl especially benzyl or aryl especially phenyl; and

X is S, SO, SO_2, O, or NR_b.

In a more preferred embodiment of this invention,

R is

(1) hydrogen;

(2) C_{1-6} alkyl;

(3) $—CF_3$;

(4) phenyl;

(5) CH_2OR_b; or

(6) CH_2SR_b;

R_a is CH_3;

X is S or O.

In a even more preferred embodiment of this invention,

R is C_{1-6} alkyl or phenyl;

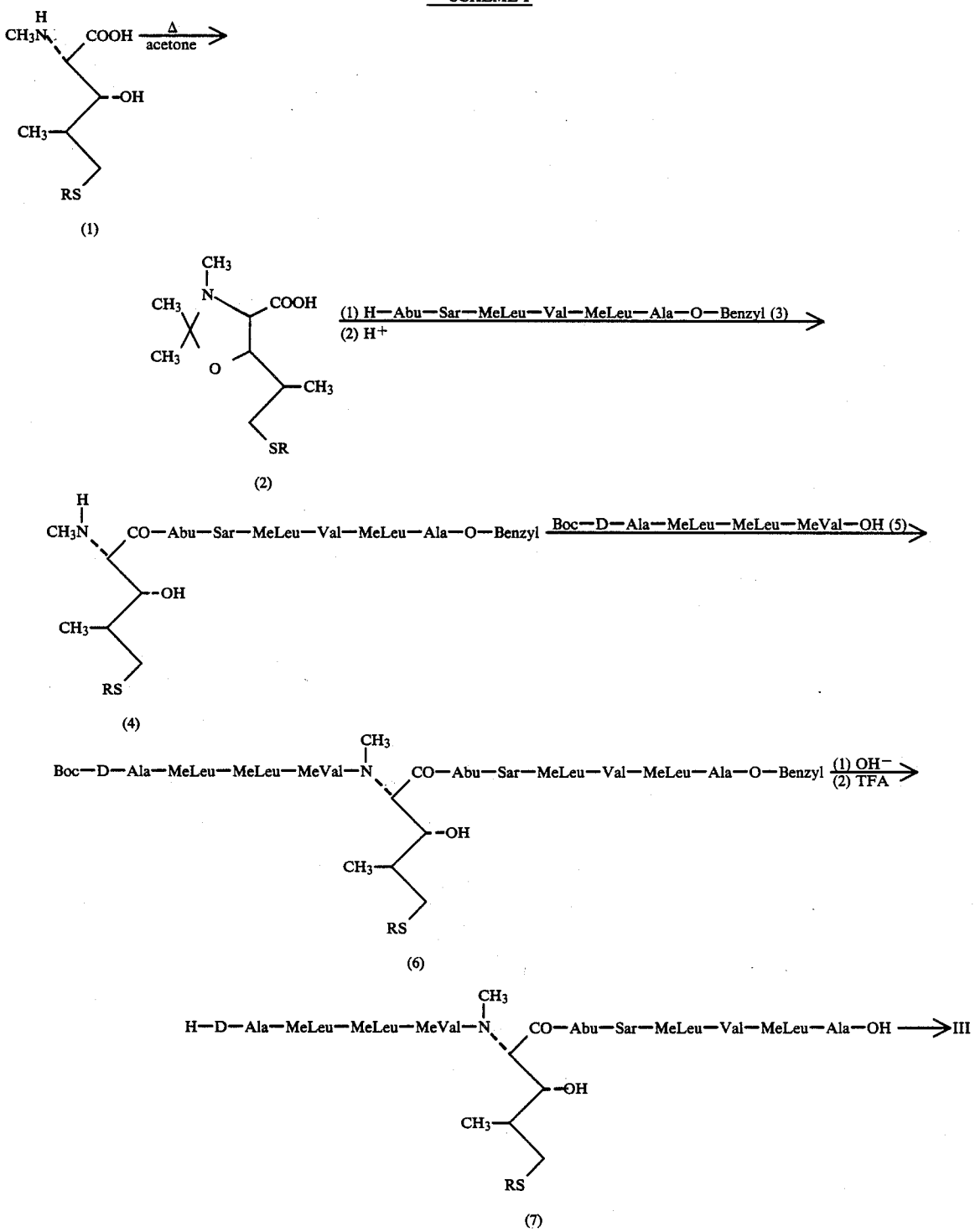
R_a is CH_3; and

X is S.

B. Preparation of the compounds within the scope of the present invention

The cyclosporins of this invention are prepared via cyclization of appropriate linear undecapeptide following well-established procedures which were slightly modified for better results. The procedure most used is published by R.W. Wenger et al. in *Helv. Chim. Acta,* 67. 502(1984). The following scheme illustrates the application of this procedure to the cyclosporins of this invention.

SCHEME I



According to the scheme, the threonine derivatives of formula (1) were converted to various cyclic undecapeptides of formula (8) utilizing, for the most part, published procedures. Notably, the methods described in *Helv. Chim. Acta.* 67, 502 (1984) are the preferred procedures.

Generally, the key starting material, (1) was heated in acetone to form the intermediate oxazolidinecarboxylic acid (2). Condensation of compound (2) with the hexapeptide ester (3) in the Presence of DCC, N-hydroybenzotriazole and N-methylmorpholine yielded an intermediate which upon treatment with an acid, for example, HCl in methanol afforded the heptapeptide (4). Further condensation with the tetrapeptide (5) followed by treatment with a strong base (e.g. NaOH or KOH) and then an acid such as TFA yielded the linear undecapeptide (7). Cyclization of compound (7) at high

dilution and in the presence of a condensation agent such as 1-proPanephosphonic acid cyclic anhydride and dimethylaminopyridine led to the cyclosporin derivative, (8).

Table I below lists the representative compounds prepared by following essentially the same procedures described in Scheme I.

Alternatively, cyclosporins of this invention may be made from existing analogs. For example, treatment of cyclo(2S,3R,4S)-N,4-dimethyl-4-(methylthiomethyl)-threonyl)-L-2-aminobutyrylsarcosyl-N-methyl-L-level-L-valyl-N-methyl-L-leucy-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-) with excess lithium diisopropylamide followed by excess methyl iodide via published procedures yields cyclo-((2S,3R,4R)-N,4-dimethyl-4-(methYlthiomethyl)-threonyl)-L-2-aminobutyryl-N-methyl-D-alanyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L- alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl) .

Also, treatment of the same substrate with a variety of oxidants, e.g., sodium metaperiodate or m-chloroperbenzoic acid produces the corresponding sulfoxide or sulfone.

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