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[54] IMMUNOSUPPRESSIVE CYCLOSPORIN ANALOGS WITH MODIFIED AMINO ACIDS AT POSITION-8

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

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

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[57] **ABSTRACT**

New immunosuppressive cyclosporin analogs are disclosed consisting of [dehydro-Ala][8] cyclosporins and derived therefrom cyclosporins having a sulfur containing amino acid at position-8.

11 Claims, No Drawings

IMMUNOSUPPRESSIVE CYCLOSPORIN ANALOGS WITH MODIFIED AMINO ACIDS AT POSITION-8

BACKGROUND OF THE INVENTION

The cyclosporins are a family of, neutral, hydrophobic cyclic undecapeptides, containing a novel nine-carbon amino acid (MeBmt) at position 1 of the ring that exhibit potent immunosuppressive, antiparasitic, fungicidal, and chronic anti-inflammatory properties. The naturally occuring members of this family of structurally related compounds are produced by various fungi imperfecti. Cyclosporins A and C, are the major components. Cyclosporin A, which is discussed further below, is a particularly important member of the cyclosporin family of compounds. Twenty four minor metabolites, also oligopeptides, have been identified: Lawen et al, J. Antibiotics 42, 1283 (1989); Traber et al, Helv. Chim. Acta 70, 13 (1987); Von Wartburg and Traber Prog. Med. Chem., 25, 1 (1988).

Isolation of cyclosporins A and C, as well as the structure of A were reported by A. Ruegger et al., Helv. Chim. Acta 59, 1075(1976); M. Dreyfuss et al., J. Appl. Microbiol. 3, 125 (1976). Crystal and molecular structures of the iodo derivative of A have been reported by T. J. Petcher et al., Helv. Chim. Acta 59, 1480 (1976). The Structure of C was reported by R. Traber et al., ibid. 60, 1247 (1977). Production of A and C has been reported by E. Harri et al., U.S. Pat. No. 4,117,118 (1978 to Sandoz). Isolation, characterization and antifungal activity of B, D, E, as well as the structures of A through D have been reported by R. Traber et al., Helv. Chim. Acta 60, 1568(1977). Isolation and structures of E, F, G, H, I: eidem, ibid. 65, 1655 (1982). Preparation of [2-Deutero-3-fluoro-D-Ala]^8-CsA is disclosed by Patchett et al in GB 2,206,199A which was published on Dec. 29, 1988.

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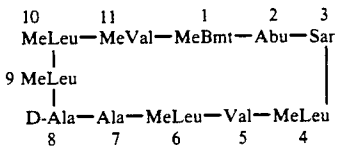
As exemplified by the ever expanding list of indications for which Cyclosporin A has been found useful, the cyclosporin family of compounds find utility in the prevention of rejection or organ and bone marrow transplants; and in the treatment of psoriasis, and a number of autoimmune disorders such as type 1 diabetes mellitus, multiple sclerosis, autoimmune uveitis, and rheumatoid arthritis. Additional indications are discussed infra.

As is generally accepted by those of skill in the art, inhibition of secretion of interleukin-2 (IL-2) and other lymphokines from lymphocytes, is a useful indicator of intrinsic immunosuppressive activity of a cyclosporin analog. For a recent review of cyclosporin uses and mechanisms of action see Wenger et al Cyclosporine: Chemistry, Structure-Activity Relationships and Mode of Action, Progress in Clinical Biochemistry and Medicine, vol. 2, 176 (1986).

Cyclosporin A is a cyclic peptide which contains several N-methyl amino acids and, at position-8, contains a D-alanine.

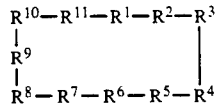
Structure of Cyclosporin A[a]



(I)

Abu = L-αAminobutyric acid
Ala = L-Alanine
MeBmt = N-Methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine
Leu = L-Leucine
MeLeu = N-Methyl-L-leucine
MeVal = N-Methyl-L-valine
Nva = L-Norvaline
Sar = Sarcosine
Thr = L-Threonine
Val = L-Valine
[a]Unless otherwise specified, each of the amino acids of the disclosed cyclosporin is of the L-configuration.

A generic structure, useful for describing cyclosprin A and analogs thereof is



wherein the superscript number defines the position of the amino acid. Because of our specific interest in the amino acid at position 8, we will hereinafter replace "R^8" with "Y", thereby emphasizing that amino acid.

As is the practice in the field, a particular cyclosporin analog may be named using a shorthand notation identifying how the analog differs from cyclosporin A. Thus, cyclosporin C which differs from cyclosporin A by the threonine at position-2 may be identified as [Thr]^2-cyclosporin or [Thr]^2-CsA. Similarly, cyclosporin B is [Ala]^2-CsA; cyclosporin D is [Val]^2-CsA; cyclosporin E is [Val]^{11}-CsA; cyclosporin F is [3-DesoxyMeBmt]^1-CsA; cyclosporin G is [NVa]^2-CsA; and cyclosporin H is [D-MeVal]^{11}-CsA.

D-Serine and D-Threonine have been introduced into the 8-position of cyclosporin A by biosynthesis resulting in active compounds. See R. Traber et al. *J. Antibiotics* 42, 591 (1989). D-Chloroalanine has also been introduced into position-8 of Cyclosporin A by biosynthesis. See A. Lawen et al *J. Antibiotics* 52, 1283 (1989).

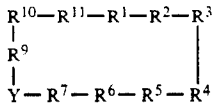
The present invention concerns new analogs of cyclosporin A and related cyclosporins for the care of immunoregulatory disorders and diseases, including the prevention, control and treatment thereof.

SUMMARY OF THE INVENTION

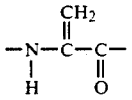
This invention relates to [dehydro-Ala]^8 cyclosporins and their preparation and conversion to novel cyclosporin analogs useful as alternatives to cyclosporin A. More specifically, the invention relates to [dehydro-Ala]^8 cyclosporins and derived therefrom cyclosporin analogs having a sulfur containing amino acid at position-8.

DETAILED DESCRIPTION OF THE INVENTION

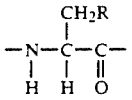
This invention relates to cyclosporin analogs of the formula



wherein the amino acid moiety at position-8 is Y, and Y is [dehydro-Ala], namely



or Michael thio adducts of [dehydro-Ala], namely



wherein

R is $CH_3(O—CH_2—CH_2)_n—S(O)_m—$, wherein m is 0 or 1 and n is 1,2,3, or 4; or

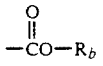
$R_aS(O)_m—$, wherein R_a is selected from the group consisting of

1) H, provided that m is 0;

2) C_{1-6} alkyl, such as methyl, ethyl, isopropyl or tert-butyl;

3) substituted C_{1-6} alkyl wherein the substitutent is selected from the group consisting of,

(a)



wherein R_b is C_{1-6} alkyl or hydrogen,

(b) $—NR_bR_c$ wherein R_c is C_{1-6} alkyl or hydrogen;

(c) C_{1-6} acylamino-;

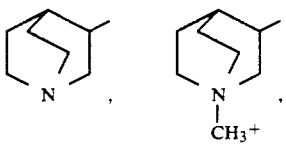
(d) -hydroxy; and

(e) C_{1-6} acyloxy-;

4) benzyl or phenyl;

5) substituted benzyl or phenyl wherein substitutents are selected from the group consisting of C_{1-4} alkyl, hydroxyl, C_{1-4} alkyloxy, and halo,

6)



wherein

R^1 may be, but is not limited to MeBmt, 3-desoxyMeBmt or dihydroMeBmt;

R^2 may be, but is not limited to Abu, Ala, Nva, SeR, Thr or Val;

R^3 may be, but is not limited to, Sar or N-methyl-D-alanyl;

R^4 may be, but is not limited to MeLeu or MeVal;

R^5 may be, but is not limited to Val or Nva;

R^6 may be, but is not limited to, MeLeu or MeVal;

R^7 may be, but is not limited to Ala, Abu, or L-phenylalanyl;

R^9 may be, but is not limited to MeLeu or MeVal;

R^{10} may be, but is not limited to MeLeu, or MeVal; and

R^{11} may be, but is not limited to MeVal, D-MeVal or MeNva.

One embodiment within the scope of the invention, is the cyclosporin analogs selected from the group consisting of:

(a) $[3\text{-DesoxyMeBmt}]^1[Y]^8$-CsA;

(b) $[Ala]^2[Y]^8$-CsA;

(c) $[Thr]^2[Y]^8$-CsA and dihydro $[Thr]^2[Y]^8$-CsA;

(d) $[Val]^2[Y]^8$-CsA and dihydro $[Val]^2[Y]^8$-CsA;

(e) $[Nva]^2[Y]^8$-CsA and dihydro and iso $[Nva]^2[Y]^8$-CsA;

(f) $[D\text{-MeVal}]^{11}[Y]^8$-CsA; and

(g) $[Val]^{11}[Y]^8$-CsA.

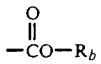
One class of compounds within the embodiment is the compounds wherein, R is $CH_3(OCH_2CH_2)_n—S(O)_m$ wherein m is 0 or 1 and n is 1,2,3 or 4 or $R_aS(O)m$; m is 0 is 1; and R_a is selected from the group consisting of

1) H unless m is 1,

2) C_{1-6} alkyl, such as methyl, ethyl, isopropyl or tert-butyl;

3)substituted C_{1-6} alkyl wherein the substitutent is selected from the group consisting of,

(a)



wherein R_b is C_{1-6} alkyl or hydrogen,

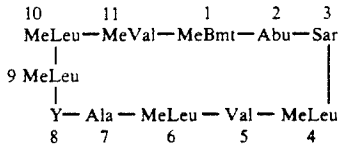
(b) C_{1-6} acylamino-,

(c) $—NR_bR_c$ wherein R_c is C_{1-6} alkyl or hydrogen;

(d) -hydroxy; and

(e) C_{1-6} acyloxy-.

A second embodiment within the scope of the invention, is the compounds of formula II



II

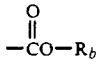
One class of compounds within this embodiment is the compounds wherein, R is $CH_3(OCH_2CH_2)_n—S(O)_m$ wherein m is 0 or 1 and n is 1,2,3 or 4 or $R_aS(O)m$; wherein m is 0 or 1; and R_a is selected from the group consisting of

1) H provided that m is 0,

2) C_{1-6} alkyl, such as methyl, ethyl, isopropyl or tert-butyl;

3) substituted C_{1-6} alkyl wherein the substitutent is selected from the group consisting of,

(a)



wherein R_b is C_{1-6} alkyl or hydrogen,

(b) C_{1-6} acylamino-,

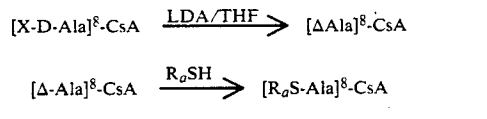
(c) —NR_bR_c wherein R_c is C_{1-6} alkyl or hydrogen;
(d) -hydroxy; and
(e) C_{1-6} acyloxy-.

Illustrating this class are:
D-[3-methylthio-Ala]^8-CsA;
D-[3-carbomethoxymethylthio-Ala]^8-CsA;
D-[3(2-hydroxyethylthio)Ala]^8-CsA; [dehydro-Ala]^8-CsA;
D-[3-benzylthio-Ala]^8-CsA;
D-[3-phenylthio-Ala]^8-CsA;
D-[3-methylthio-Ala]^8-CsA sulfoxide;
D-[3-(2-hydroxyethylthio)Ala]^8-CsA sulfoxide; and
D-[methoxyethoxy)ethoxyethylthio-Ala]^8-CsA.

Compounds of the present invention are conveniently prepared using the procedures described generally below and more explicitly in the Example Section thereafter.

Now turning to Scheme 1, in one embodiment the cyclosporin analogs of this invention are conveniently prepared via conversion of [X-D-Ala]^8-CsA to [Δ-Ala]^8-CsA.

Scheme 1



wherein X is fluoro, chloro, methanesulfonyloxy, toluenesulfonyloxy and R_a is the broadest definition of R_a provided above.

According to Scheme 1 [2-deutero-3-fluoro-D-Ala]^8-CsA (abbreviated as [F-D-Ala]^8-CsA), in an aprotic solvent, is reacted with an aprotic base to yield [ΔAla]^8-CsA.

[F-D-Ala]^8-CsA possesses 17 active hydrogens (12-αCH, 4-NH AND 1-OH). Accordingly, a large excess of aprotic base is required to generate the polyanion. The molar ratio of aprotic base to [F-D-Ala]^8-CsA may range from 17 to 35 of which 20–25 is preferred. Suitable aprotic bases include, but are not limited to, mono or diC_{1-6}alkylamido derivatives such as lithium diethylamide, lithium diisopropylamide, sodium bis (trimethylsilyl)amide, lithium bis (trimethylsilyl)amide of which lithium diisopropylamide is preferred. Suitable aprotic solvents include, but are not limited to, diC_{1-4}alkoxy C_{1-4}alkane derivatives such as 1,2-dimethoxyethane; ethers such as diethyl ether di-n-butyl and diisopentyl ethers, cyclic ethers such as tetrahydropyran, dihydropyran, tetrahydrofurfuryl methyl ether, furan, tetrahydrofuran and 2-ethoxytetrahydrofuran, and mono or diC_{1-4}alkyl carbonyl amines such as dimethylformamide. Tetrahydrofuran is preferred.

The reaction may be conveniently conducted in a temperature range of −100° to −10° C., of which −70° to −30° C. is preferred. The reaction is allowed to proceed to completion in 1 to 24 hours, of which a 4 to 5 hour reaction time is preferred.

The [Δ-Ala]^8-CsA product can be isolated by standard chromatography, HPCL or TLC on silica gel plates as is known in the art.

[Δ-Ala]^8-CsA is then converted into the thio compound [R^aS-Ala]-CsA by reaction in a second solvent with a sulfur nucleophile in the presence of a second base.

The second base includes, but is not limited to, the alkali metal C_{1-6}alkoxides and hydrides, such as sodium, lithium or potassium methoxide or hydride, of which sodium methoxide is preferred.

The second solvent includes, but is not limited to C_{1-8}alkanols corresponding to the selected second base, such as methanol and ethers (as defined above) such as 1,2-dimethoxyethane, or tetrahydrofuran, of which methanol and tetrahydrofuran are preferred. One example of corresponding second solvent and base is methanol and sodium methoxide.

The sulfur nucleophile includes, but is not limited to RSH wherein R is $CH_3(O—CH_2—CH_2)_n—S(O)_m$, and R_aSH wherein R_a is given its broadest definition provided above.

The reaction can be conveniently conducted in a temperature range of 0° to 50° C., of which 15° to 30° C. is preferred. The reaction is allowed to proceed to completion in 1 to 36 hours, of which 15 to 18 hours is preferred.

In Scheme 1 the Δ-Ala moiety of [Δ-Ala]^8-CsA serves as a Michael acceptor to various nucleophiles.

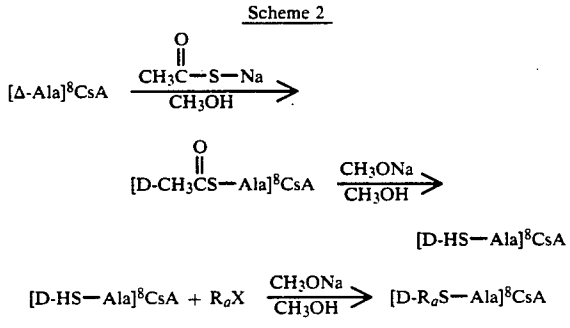
As an alternative to the above, [Δ-Ala]-CsA can be produced from [D-Ser]^8-CsA. In this procedure [D-Ser]^8-CsA is treated with a slight excess of methanesulfonyl chloride or toluenesulfonyl chloride in methylene chloride in the presence of 4-dimethylaminopyridine to yield, after chromatography, [methane or toluene substituted sulfonyloxy-D-Ser]^8-CsA. These compounds can be treated with excess LDA in THF at low temperatures to yield [Δ-Ala]^8-CsA. Similarly [D-Chloro-Ala]^8-CsA can be treated with excess LDA in THF at low temperatures to yield [Δ-Ala]^8-CsA.

Compounds [D-RS-Ala]^8-CsA (wherein R is $CH_3(O—CH_2—CH_2)$n-S(O)m) and [D-R_aS-Ala]^8-CsA may also be produced by an alternate route from [D-Cys]^8-CsA (which is the same as [D-HS-Ala]^{8}CsA) by reaction with RX and R_aX (wherein R_a is not phenyl or substituted phenyl and X is chlorine, bromine or a sulfonyloxy aryl or alkyl group such as mesyloxy or tosyloxy) as indicated in Scheme 2 which also shows production of [D-HS-Ala]^8-CsA by reaction of [Δ-Ala]^8-CsA with the sodium salt of thiolacetic acid followed by hydrolysis.

As appreciated by those of skill in the art, the remaining compounds within the scope of the invention can be produced in an analogous manner.

The SR_a groups in the disclosed compounds can be oxidized to the corresponding sulfoxides. A convenient route to sulfoxides is by periodate oxidation as described below.

Scheme 2

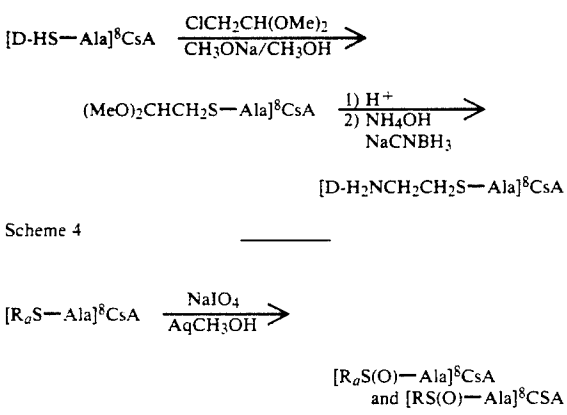


As shown in Scheme 2 treatment of [Δ-Ala]^{8}CsA with 5–10 equivalents of $CH_3COS—Na$ (generated in situ from equivalent quantities of CH_3COSH and CH_3ONa) in CH_3OH for 15–18 hr at 20–25° C. pro-

duces [D-CH_3COS-Ala]^8-CsA which is partly deacetylated. Deacetylation to [D-HS-ala]^{8}CsA is completed by reaction with CH_3ONa (1–5 equivalents) in a C_{1-8}alkanol such as methanol for 3–18 hr at 20–25° C. Reaction of [D-HS-Ala]^8-CsA with R^1X (5–10 equivalents) in the presence of CH_3ONa (1–2 equivalents) in an C_{1-8}alkanol such as CH_3OH for 15–18 hr at 20–25° C. yields [D R_aS-Ala]^{8}CsA. In this procedure R_a is not phenyl or substituted phenyl.

For example a compound accessible by this route is [D-3-thia-Lys]^8-CsA, ([D-$H_2NCH_2CH_2S$-Ala]^8-CsA). This compound is useful in preparing affinity chromatography columns for cyclosporin receptor isolation and to prepare cyclosporin antibodies.) [D-3-Thia-Lys]^{8}CsA may be prepared as indicated in Scheme 3.

Scheme 3



Scheme 4

As shown in Scheme 4, the [R_aS-Ala]^8 cyclosporins are converted into the corresponding sulfoxides by treatment with sodium periodate in aqueous alcohol with methanol-water in the ratio 3:1 as the preferred solvent. The time may range from 3 to 36 hours with 15–18 hours preferred. The preferred temperature range is 20–25° C.

In view of their immunosuppressive activity, end product cyclosporins e.g. of formula II, are useful for the prophylaxis and treatment of diseases and conditions requiring a reduction of the immune response. Thus they may be used to suppress the proliferation of lymphocytes and immunocytes, e.g. in treatment of autoimmune diseases or in preventing the rejection of transplants e.g. skin, lung, heart, heart-lung, bone-marrow, kidney, spleen and corneal transplants.

Specific auto-immune diseases for which the cyclosporins of formula II are useful include all of those for which treatment with cyclosporine has been proposed or used, for example, aplastic anaemia, pure red cell anaemia, idopathic thrombocytopaenia, systemic lupus erythematodes, polychondritis, sclerodoma, Wegener granulomatosis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, Crohn's diseases, Graves opthalmopathy, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, primary juvenile diabetes, uveitis posterior, interstital lung fibrosis and psoriatic arthritis as well as insulin-dependent diabetes mellitus, nephrotic, syndrome and AIDS.

For all these uses the dosage will, of course, vary depending on the compound employed, mode of administration and treatment desired. However, in general, satisfactory results are obtained when administered at a daily dosage of from about 1 mg to about 200 mg per kg animal body weight, conveniently given in divided doses 2 to 4 times a day or in sustained release form. For the larger mammals, the total daily dosage is in the range from about 50 to about 5000 mg, and dosage forms suitable for oral administration comprise from about 15 mg to about 500 mg (e.g. 25–300 mg) of the compounds admixed with a solid or liquid pharmaceutical carrier or diluent.

The present invention also provides a pharmaceutical composition comprising a compound of formula II in association with a pharmaceutical carrier or diluent.

Such compositions may be in the form of, for example, a solution, a tablet or a capsule and in ointments especially for the treatment of psoriasis.

The cyclosporins of formula II may be administered by any conventional route, in particular in accordance with means currently practiced in relation to administration of cyclosporine, in particular via intravenous infusion, e.g. in the case or organ transplant, pre- and immediately post-transplant, as well as during episodes of gastrointestinal disturbance which might otherwise impair absorption, or orally, e.g. in the form of an oral solution.

Biological activity can be measured in terms of binding affinity for cyclophilin, the cytosolic receptor for cyclosporin (R. Handschumacher et al., Science, 226 (1984) 544), inhibition of interleukin-2 production, and inhibition of T-cell proliferation. Table 2 illustrates the pharmacological activity of representative compounds of the present invention.

T-cell proliferation was measured in mouse T-cell cultures stimulated with ionomycin plus phorbol myristate acetate (PMA). Spleen cell suspensions from C57B1/6 mice were prepared and separated on nylon wool columns. The recovered T-cells were suspended at 10^6 cells/ml in complete culture medium with addition of ionomycin (250 ng/ml) and PMA (10 ng/ml). The cell suspension was immediately distributed in 96 well-flat bottom microculture plates at 100 µl/well. Control medium or various concentrations of test compound were added in triplicate wells at 10 µl/well. The plates were incubated at 37° C. in a humidified atmosphere of 5% CO_2-95% air for 44 hours. At 44 hours of culture, the plates received 20 µl/well of a solution of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MMT) in PBS (10 mg/ml). To dissolve the purple crystals of MTT formazan produced by metabolically active cells, 100 µl of a 10% SDS-0.01 N hydrochloric acid solution was added to each well. The culture plates were incubated at 37° C. in a 5% CO_2 incubator. The plates were read at 570–600 nm in a multiwell scanning spectrophotometer. The absorbance (specific OD) of experimental wells was corrected for that of wells with unstimulated cells or no cells. The percent inhibition of proliferation was calculated according to the formula:

$$\% \text{ Inhib.} = 100 - \frac{\text{Specific } OD \text{ experimental}}{\text{Specific } OD \text{ control medium}} \times 100$$

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