

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199943856 B2
(10) Patent No. 759480

(54) Title
Novel cyclosporin with improved activity profile

(51)⁶ International Patent Classification(s)
C07K 007/64 A61K 038/13

(21) Application No: **199943856** (22) Application Date: **1999.06.30**

(87) WIPO No: **W000/01715**

(30) Priority Data

(31) Number (32) Date (33) Country
1405/98 1998.07.01 CH

(43) Publication Date : **2000.01.24**

(43) Publication Journal Date : **2000.04.06**

(44) Accepted Journal Date : **2003.04.17**

(71) Applicant(s)
Debiopharm S.A.

(72) Inventor(s)
Roland M. Wenger; Manfred Mutter; Thomas Ruckle

(74) Agent/Attorney
A J PARK and SON, Level 11, 60 Marcus-Clarke Street, CANBERRA ACT 2601

43856/99



PCT

ORGANISATION MONDIALE DE LA PROPRIÉTÉ INTELLECTUELLE
Bureau international

DEMANDE INTERNATIONALE PUBLIÉE EN VERTU DU TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

(51) Classification internationale des brevets ⁷ : C07K 7/64, A61K 38/13	A1	(11) Numéro de publication internationale: WO 00/01715 (43) Date de publication internationale: 13 janvier 2000 (13.01.00)
(21) Numéro de la demande internationale: PCT/IB99/01232 (22) Date de dépôt international: 30 juin 1999 (30.06.99) (30) Données relatives à la priorité: 1405/98 1er juillet 1998 (01.07.98) CH (71) Déposant (pour tous les Etats désignés sauf US): DEBIO-PHARM S.A. [CH/CH]; 17, rue des Terreaux, CH-1000 Lausanne 9 (CH). (72) Inventeurs; et (75) Inventeurs/Déposants (US seulement): WENGER, Roland, M. [CH/CH]; Grenzacherweg 45, CH-4125 Riehen (CH). MUTTER, Manfred [DE/CH]; 9, chemin de la Venoge, CH-1028 Préverenges (CH). RUCKLE, Thomas [DE/CH]; Université de Lausanne, Institut de Chimie Organique BCH - DORIGNY, CH-1015 Lausanne (CH). (74) Mandataire: CURRAT, Vanessa; Debiopharm S.A., 17, rue des Terreaux, CH-1000 Lausanne 9 (CH).	(81) Etats désignés: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, brevet ARIPO (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), brevet eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), brevet européen (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	Publiée Avec rapport de recherche internationale.

(54) Title: NOVEL CYCLOSPORIN WITH IMPROVED ACTIVITY PROFILE

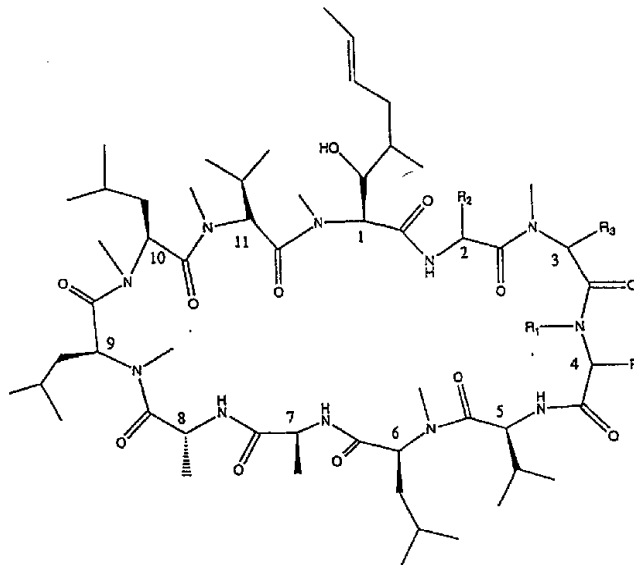
(54) Titre: NOUVELLE CYCLOSPORINE AYANT UN PROFIL D'ACTIVITÉ AMÉLIORÉ

(57) Abstract

The invention concerns a novel cyclosporin, its pharmaceutical use and a pharmaceutical composition containing it.

(57) Abrégé

La présente invention traite d'une nouvelle cyclosporine, de son utilisation pharmaceutique ainsi que d'une composition pharmaceutique la comprenant.



WO 00/01715

PCT/IB99/01232

2

Leu = L-leucine
MeLeu = N-methyl-L-leucine
MeVal = N-methyl-L-valine
Nva = L-norvaline
5 Sar = sarcosine
Thr = L-threonine
Val = L-valine

The amino acids described according to their
conventional abbreviation have the configuration L unless
10 otherwise specified.

Since this first cyclosporin was discovered, a large
number of other varieties have been isolated and identified,
as have non-natural varieties obtained by synthetic or semi-
synthetic methods, or even by the application of modified
15 culture techniques. The production of cyclosporin A is
described by [Kobel et al. European Journal of Applied
Microbiology and Biotechnology 14, 237-240 (1982)]. The
production of artificial cyclosporins produced by a purely
synthetic method developed by R. Wenger is also described -
20 see Traber et al. 1, Traber et al. 2 and Kobel et al., US
4,108,985; 4,210,581; 4,220,641; 4,288,431; 4,554,351 and
4,396,542; EP 34 567 and 54 782; WO 86/02080; Wenger 1,
Transpl. Proc. 15, Suppl. 1:2230 (1983); Wenger 2, Angew.
Chem.Int. Ed., 24,77 (1985); and Wenger 3, Progress in the
25 Chemistry of Organic Natural Products 50, 123 (1986).



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

WO 00/01715

PCT/IB99/01232

3

Cyclosporin A (CsA) isolated 20 years ago from *Tolypocladium inflatum* has considerable immunosuppressive activity. It has revolutionised organ transplantation and is commonly used in the treatment of autoimmune diseases. For a recent review of the use of CsA and its 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).

The therapeutic effect of CsA results mainly in the selective suppression of the activation of T lymphocytes. This immunosuppressive activity is explained by the fact that CsA binds to an intracellular proteic receptor, cyclophilin A (CyP) forming a CyP-CsA complex which interacts with calcineurin (CaN) and thus inhibits its phosphatase activity. Thus, the transcription of families of genes exhibiting precocious activation will be blocked (cf. O'Keefe, S.J; Tamura, J; Nature 1992, 357, 692-694).

The present invention provides the production of a novel cyclosporin with considerable HIV-1 (human immunodeficiency virus) inhibitory activity and not having the immunosuppressive activity of CsA.

The mode of infection of HIV type 1 is unique amongst the retroviruses because it requires the specific incorporation into its virion of the cellular protein CyP which interacts with the polyprotein Gag (cf. Eltaly Kara Franke, Bi-Xing Chem. Journal of Virology, Sept. 1995, vol. 69 no. 9). It is well known that CyP binds to CsA and CaN in



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

a ternary complex. However, the native function of CyP is to catalyse the isomerisation of peptidyl-prolyl bonds, a limiting and important step in the process allowing proteins to acquire a definitive three-dimensional structure. CyP
5 also protects cells from thermal shocks or acts as a chaperone protein. Unlike CsA, the product of the Gag gene of HIV-1 prohibits the formation of a ternary complex with CyP and CaN. In fact, the HIV virus needs CyP in order to bind to the product of the Gag gene so as to form its own
10 virions (cf. Franke, E.K; 1994 Nature 372, 359-362). In the presence of CsA, there is direct competition with the polyprotein derived from the Gag gene of HIV-1 to bind to CyP. This CsA acts at two levels on the replication of the viral cycle. Firstly, at the level of translocation towards
15 the core of the pre-integrated complex, then in the production of infectious viral particles.

US patent 4,814,323 already describes anti-HIV activity of CsA, but the latter also has considerable immunosuppressive activity which is undesirable for the
20 treatment of patients infected with the HIV virus. Recently, another type of cyclosporin has been developed, namely derivatives in position 4 such as MeIle¹Cs, MeVal⁴Cs, or (4-OH)MeLeu⁴-Cs to mention only the most anti-HIV and the least immunosuppressive substances. The derivative [(4-OH) MeLeu⁴-
25 Cs] is synthesised by oxidation of cyclosporin A using a microorganism. Another patent WO 97/04005 uses the method of preparation of the patent EP 484 281 and the method developed by Seebach EP 194972 in order to produce



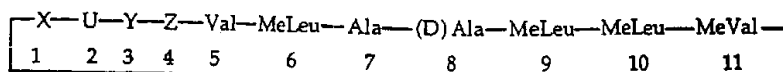
RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

derivatives in position 3 such as, for example, (D)-MeSer³-(4-OH)MeLeu⁴ cyclosporin. This substance has a better affinity for CyP but only has limited anti-HIV activity compared with the reference derivative MeIle⁴-Cs(NIM 811).

5 The more hydrophilic nature of this substance prevents it penetrating the cells and the organism. This is reflected directly in the reduced anti-HIV activity of this substance (cf. Christos Papageorgiou, J.J. Sanglier and René Traber - *Bioorganic & Medicinal Chemistry Letters*, Vol, 6, No. 1, pp. 10 23-26, 1996).

The substances described in this invention have the dual advantage of retaining the same affinity for CyP as that observed with [(4-OH)MeLeu⁴]-Cs or cyclosporin A whilst having anti-HIV activity which is identical to or greater than that of the reference derivatives (MeVal⁴-Cs or MeIle⁴-Cs) and appreciably greater than the anti-HIV activity of cyclosporin A or of (4-OH)MeLeu⁴-Cs. The object of the invention is to provide a novel cyclosporin which does not have the immunosuppressive activity of CsA and has an improved profile of activity. This new family of Cs is characterised by the formula (I):



25

(I)



WO 00/01715

PCT/IB99/01232

6

wherein:

X is -MeBmt or 6,7-dihydro-MeBmt-

U is -Abu, Nva, Val, Thr

Y is Sar or (D)-MeSer or (D)-MeAla or (D)-MeSer (OAcyl)

5 Z is (N-R)aa where aa={Val, Ile, Thr, Phe, Tyr, Thr (OAc),
Thr (OG₁), Phe (G₂), PheCH₂(G₃), Tyr (OG₃)} with R = {alkyl >
CH₃};

G₁ = {phenyl-COOH, phenyl-COOMe, phenyl-COOEt};

G₂ = {CH₂COOH, CH₂COOMe(Et), CH₂PO(OMe)₂, CH₂PO(OH)₂};

10 G₃ = {PO(OH)₂, PO(OCH₂CH=CH₂)₂, CH₂COOH, CH₂COOMe(Et)}

Thus, by replacing the natural MeLeu group in position
4 by an N-(alkyl)aa group (where alkyl > CH₃), the anti-HIV
1 activity of this derivative is improved.

15 The new cyclosporin molecule thus obtained offers the
unexpected and surprising advantage of having much better
stability towards metabolism than all the other
cyclosporins known hitherto.

20 This new molecule is much more resistant to the
phenomena of oxidation and degradation which take place in
the cell. Consequently, the "in vivo" life of this new N-
alkyl aa Cs is particularly prolonged.

Moreover, this new N-alkyl aa⁴ cyclosporin has high
affinity for CyP and has anti-HIV activity which is equal to
or greater than the best existing cyclosporins.



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

Figure 1 represents the general structure of this novel cyclosporin. The groups R1, R2, R3 and R4 will be largely described in Table III. Thus, by transforming these 4 key positions, it was possible to retain a very good affinity
5 for cyclophilin and to prevent the formation of a ternary complex with CaN and, above all, to increase, in a particularly advantageous manner, its stability towards enzymatic oxidation and consequently its anti-HIV activity.

This novel cyclosporin is thus characterised
10 principally by the presence, in position 4, of a residue with $R > CH_3$ and $R < C_{10}H_{21}$. The substituent of nitrogen used will be, for example, ethyl, propyl, butyl or pentyl, but these examples are not limiting. This novel cyclosporin is particularly active if the residue in position 4 is an N-ethylated amino acid (see drawings 2 and 3).
15

The invention also claims the pharmaceutical composition of the substance as described by formula (I). This may be combined with a pharmaceutically acceptable solution. The pharmaceutical formulation thus produced makes
20 it possible to increase the solubility in water or to keep the composition in the form of microemulsions in suspension in water. The object of the present invention is also to provide a new medicinal product which may be used, for example, in the treatment and prevention of AIDS (acquired
25 immunodeficiency syndrome). The cyclosporin modified in position 4 by a residue Z, namely N-ethyl-valine will be used in particular for the production of a medicinal product



intended for the treatment and prevention of AIDS. The application for the prevention of AIDS is not limiting. This substance may also be used, for example, for its anti-inflammatory properties.

- 5 With regard to the process for the production of this novel cyclosporin, we used conventional techniques described in the literature and certain specific methods developed in the laboratory.

- The process for the synthesis of CsA is described in:
- 10 R. Wenger (Helv. Chim. Acta Vol. 67, p. 502-525 (1984)). The process for opening protected cyclosporin A (OAc) is described in Peptides 1996. The CsA molecule is treated with Meerwein's reagent $(CH_3)_3OBF_4$ then cleaved by treatment with acid in methanol or hydrolysed by water in order to convert
- 15 it to a linear peptide of 11 amino acid residues: H-MeLeu-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-OCH₃. This process was presented at the international conference of the European Society of Peptides (EPS-24) in Edinburgh 8-13 September 1996 and published in Peptides 1996
- 20 by R. Wenger. This linear peptide is then treated according to the conventional Edman process in order to cleave its last amino acid residue (MeLeu) and to provide our starting product: the decapeptide H-Val-MeLeu-Ala-(D)Ala-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-OMe. This product is then used in the
- 25 following steps:



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

Preparation of (1) (protection):

Boc-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeValMeBmt(OAc)-Abu-Sar-OMe (1)

0.72 ml (4.18 mmoles) of a solution of
5 diisopropylethylamine and 0.65 g (2.95 mmoles) of Boc
anhydride in 50 ml of dioxane are added to a solution of
2.83 g (2.46 mmoles) of the decapeptide H-Val MeLeu-Ala-
(D)Ala-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-OMe in 120 ml of
dioxane. 17 ml of water are added to the solution which is
10 mixed for 2 hours at ambient temperature. The solvent is
then evaporated and the resulting reaction mixture is
dissolved in 300 ml of ethyl acetate then washed 3 x with a
5% solution of citric acid, 3 x with a saturated solution of
NaHCO₃, and finally 3 x with a solution of NaCl. The organic
15 phases are dried with anhydrous Na₂SO₄, filtered, and the
solvent is finally evaporated under vacuum. 3 g (98%) of the
protected decapeptide (Boc-decapeptide methyl ester) are
thus obtained.

The product is then used for the following synthesis
20 routes without an additional purification step. This
substance is hydrolysed then activated and condensed with 1
corresponding amino acid in order to produce a new peptide
with 11 residues, the starting product for the cyclisation
and production of a novel cyclosporin with the desired
25 properties.



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

Preparation of (2) (hydrolysis of the ester):

Boc-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeValMeBmt(OAc)-Abu-Sar-OH (2)

192 mg (4.56 mmoles) of LiOH/H₂O dissolved in 36 ml of
5 water are added dropwise (at 15°C) to 4.08 g (3.26 mmoles)
of the previous compound (1) in 146 ml of tetrahydrofuran.
The whole mixture is stirred at 15°C. The reaction is
complete after 120 hours after the successive addition of 5
portions respectively of 1.4 equivalents of LiOH/H₂O each.
10 The solution obtained is neutralised with 0.1 N HCl and the
solvent is then evaporated. The solid product recovered is
then dissolved in 500 ml of ethyl acetate and washed 2 x
with a 5% solution of citric acid and 2 x with a brine
solution. The aqueous phases are extracted 4 x with 50 ml of
15 ethyl acetate and the combined organic phases are then dried
with anhydrous Na₂SO₄, filtered and evaporated. 3.84 g (95%)
of compound (2) are thus obtained. The product is then used
without additional purification.

Preparation of (3) (addition of a new amino acid):

20 **Boc-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-EtVal-OtBu (3)**

6.18 g (5 mmoles) of compound (2) are dissolved in 250
ml of anhydrous dichloromethane under argon. The solution is
then cooled and 3.9 ml of N-methylmorpholine (10 mmoles; pH
25 8.5) and 1.1 ml (10 mmoles) of isobutylchloroformate are
then added slowly under argon. The solution is stirred for



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

15 minutes at -15°C . A solution of 2.4 g (12 mmoles) of H-
NET Val-OtBu dissolved in 40 ml of anhydrous dichloromethane
is then added within a period of 20 minutes. The mixture is
then stirred for 1 hour at -15°C , then for 1 hour at 0°C and
5 finally overnight at ambient temperature. 400 ml of
dichloromethane are then added, then 3 extractions are
carried out with a 5% solution of citric acid followed by 3
extractions with a saturated solution of NaHCO_3 and finally
3 final extractions with a saturated solution of NaCl . The
10 organic phases are dried with anhydrous Na_2SO_4 then filtered
and finally the solvent is evaporated. After chromatography,
4.42 g (62%) of pure undecapeptide are recovered.

Preparation of (4) (deprotection):

15 H-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-
EtVal-OH (4)

830 mg (0.58 mmole) of protected undecapeptide (4) are
dissolved in 15 ml of pure dichloromethane. 3.2 ml of
trifluoroacetic acid are added to this solution within a
period of 3 minutes at ambient temperature. The reaction is
20 monitored by HPLC which proves to be complete after 1 h 30.
The solvent is evaporated and the remaining trifluoroacetic
acid is evaporated 2 x in the presence of ethyl acetate.

The crude product (900 mg) is purified by
chromatography [150 g of silica gel (0.4-0.63)], use of
25 dichloromethane/methanol/triethylamine (17:3:0.05) as



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:18

WO 00/01715

PCT/IB99/01232

12

eluants) to elute 700 mg (95%) of pure, deprotected undecapeptide (4).

Preparation of (5) (cyclisation):

MeBmt(OAc)¹-EtVal⁴-Cs (5)

5 275 mg of TFFH (1.04 mmole) are dissolved under argon in 3.45 l of anhydrous dichloromethane. The deprotected undecapeptide (4) [438 mg (0.347 mmole)] is then dissolved in 40 ml of anhydrous dichloromethane, and 0.52 ml (3.82 mmole) of collidine are added thereto. This slightly basic peptide solution is added dropwise to the solution of TFFH within a period of 20 minutes under argon and with vigorous stirring. After 1 h 30 all the starting material is cyclised. In order to trap the excess TFFH, 5 ml of water are added, then the solution is evaporated. 200 ml of 10 dichloromethane are added then the whole mixture is washed respectively 3x with a 0.1 N solution of aqueous HCl, 3 x with a brine solution then dried with Na₂SO₄, filtered, and the solvent is evaporated. 360 mg of a yellowish oil are obtained. The crude product is purified by chromatography on 15 silica gel using 100 g of silica gel (0.04-0.0063 mm) and 1% of methanol in ethyl acetate as eluant. 230 mg (54%) of the pure derivative (5) are thus produced. 20

Cleavage of the MeBmt (OAc)-EtVal⁴-Cs (5) acetate group and production of EtVal⁴-Cs (6):

25 1.44 ml of a 0.45 molar solution of NaOCH₃ in MeOH (0.647 mmole) are added dropwise, under argon, to a solution



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

WO 00/01715

PCT/IB99/01232

13

of 700 mg (0.562 mmole) of the derivative of Cs (5) in 28 ml of MeOH. [The solution of NaOCH₃ in methanol is prepared by adding sodium to pure MeOH.] The reaction is complete after 48 h with stirring at ambient temperature. The mixture is
5 adjusted to pH 5 by adding 50% acetic acid in water. The solvents are removed under vacuum. The crude product is dissolved in 200 ml of ethyl acetate and extracted 2 x with water. The aqueous phase is re-extracted with 50 ml of ethyl acetate then the combined organic phases are washed 2 x with
10 a brine solution, dried with Na₂SO₄, filtered and the solvent is evaporated.

The product obtained (750 g) is chromatographed on 180 g of silica gel (0.04-0.063 mm) using a solution of acetone/hexane 1:1 (20 ml fractions). 550 mg (82%) of
15 (EtVal⁴)Cs (6) are thus produced.

Preparation of H-EtVal-Ot-Bu:

4.1 ml (23.83 mmoles) of diisopropylethylamine are added, under argon, to a suspension of 5 g (23.8 mmoles) of H-ValOtBu x HCl in 1 l of trimethyloxoformate. At the end of
20 10 minutes the suspension becomes clear. 13.5 ml (0.24 mmole) of acetaldehyde dissolved in 30 ml of trimethyloxoformate are added dropwise to this solution under anhydrous conditions. The reaction mixture is stirred for 45 minutes under argon at ambient temperature.

25 Using a low vacuum, the excess acetaldehyde is removed by evaporation for 1 h 30. 25 g (0.112 mmole) of solid



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

WO 00/01715

PCT/IB99/01232

14

NaBH(OAc)₃ are added, under argon, to this solution. After 15 minutes, the solution is cooled to 0°C and 500 ml of a 2% aqueous solution of HCl are added slowly.

5 The trimethyloxofomate is evaporated under vacuum and the remainder of the aqueous solution is diluted in 300 ml of water. This solution is then extracted 2 x with 100 ml of diethylether. The organic phase is then re-extracted 3 x with a 0.1 N aqueous solution of HCl. The combined aqueous phases are cooled to 0°C then the pH is adjusted to 9 using
10 (2N)NaOH. The solution then becomes cloudy. The aqueous suspension is extracted 4 x with 100 ml of diethylether. The combined organic phases are then dried with Na₂SO₄, filtered and the solvent is finally evaporated.

4.2 g of a yellowish oil resulting from this step are
15 purified by chromatography using 900 g of silica gel (0.04-0.063 mm) and a mixture of hexane/ethyl acetate 8:2 as eluant. Finally, 3.13 g (65%) of pure H-EtLeu-OtBu are obtained.

The results of Table 1 show the affinity of the
20 derivatives of Cs for cyclophilin A in a competitive ELISA test described by Quesniaux in Eur. J. Immunology 1987, 17, 1359-1365. In this test, during incubation with cyclophilin, Cs bound to BSA (serum albumin) is added to the Cs to be tested. The concentration required to obtain 50% inhibition
25 (IC₅₀) of the reference reaction in the absence of competitor is then calculated. The results are expressed by the binding index BI which is the ratio of the IC₅₀ of the



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

03-JAN-2001 15:02

A J PARK

64 4 4723358 P.20/32

64 4 4723358

WO 00/01715

PCT/IB99/01232

15

derivative and the IC_{50} of CsA. A binding index (BI) of 1.0 indicates that the compound tested binds as well as CsA. A value lower than 1.0 indicates that the derivative binds better than CsA, and a value greater than 1.0 means that the
5 derivative binds less well to Cyp than CsA.



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

TABLE 1

Substance	Structure	BI	IR
UNIL 001	CsA	1.0	1.0
UNIL 002	MeVal ⁴ -Cs	0.6	>200
UNIL 004	EtVal ⁴ -Cs	1.0	>200
UNIL 007	MeIle ⁴ -Cs	0.5	>200
UNIL 013	EtIle ⁴ -Cs	1.3	>200
UNIL 014	EtPhe(4-CH ₂ PO(OMe) ₂)-Cs	0.5	>200

Cs is regarded as being immunosuppressive if its activity in the mixed lymphocyte reaction (MLR) is greater than 5%. The reaction (MLR) is described by T. Meo in
5 "Immunological Methods", L. Lefkovits and B. Devis, Eds, Académie Prev. N.Y. pp: 227-239 (1979).

Spleen cells ($0.5 \cdot 10^6$) originating from Balb/c mice (female, 8 to 10 weeks) are co-incubated for 5 days in the presence of treated spleen cells originating from CBA mice
10 (females, 8 to 10 weeks). These cells were treated with mitomycin C or were irradiated at 2000 rads. The non-irradiated allogenic spleen cells exhibit a proliferative response in Balb/c cells which can be measured by incorporating a labelled precursor in the DNA. If the
15 stimulator cells are irradiated (or treated with mitomycin C), the Balb/c cells no longer exhibit a proliferative



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

WO 00/01715

PCT/IB99/01232

17

response but retain their antigenicity. The IC_{50} calculated in the MLR test is compared with the IC_{50} corresponding to CsA in a parallel experiment. The IR index is thus found, this being the ratio of the IC_{50} of the MLR test of the derivatives over the IC_{50} of cyclosporin A.

As with the binding index (BI) above, a value of 1.0 for the IR means an activity similar to CsA. Similarly, a lower value means better activity and a value greater than 1.0 shows that the activity of the compound is lower than that of CsA.

An IR value of > 20 shows that the substance is not immunosuppressive. The immunosuppression values of the derivatives are given in Table I.

Table II describes the percentage protection during infection with HIV of a CEM-SS cell line. The protection of this line in the presence of a Cs derivative is compared with the infection of a line cultivated in the absence of Cs (reference control). A mean value is established at a concentration of the derivative of 2.10^{-6} molar. This anti-HIV activity was measured by the NCI (National Cancer Institute) in Washington in the USA.



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

Table II

Substance	Structure	Percentage HIV Protection
UNIL 002	MeVal ⁴ -Cs	66.4
UNIL 004	EtVal ⁴ -Cs	74.9
UNIL 007	MeIle ⁴ -Cs	68.5



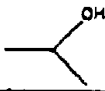
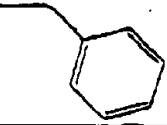

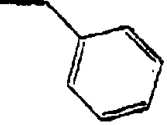



A better percentage of protection against HIV infection obtained with the compound EtVal⁴-Cs (compared with the two other references known to be 10 x better than CsA) shows the advantage of substitution by N-ethyl in position 4. This remark is even more pertinent if one compares the affinity for CyP of each substance. An affinity for CyP similar to that of CsA (BI = 1.0) is obtained for the EtVal⁴-Cs derivative, whereas the derivatives MeVal⁴-Cs and MeIle⁴-Cs exhibit greater affinity for CyP (BI = 0.6 and 0.5 respectively). A greater anti-HIV activity corresponds to a lower affinity for CyP of EtVal⁴-Cs. This clearly shows the value of this novel derivative.



RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

TABLE III

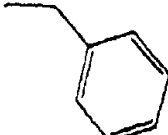
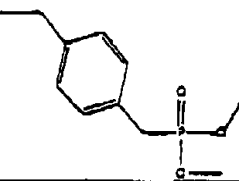
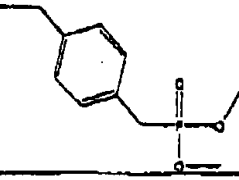
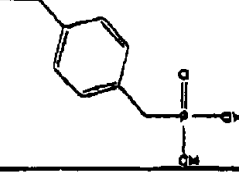
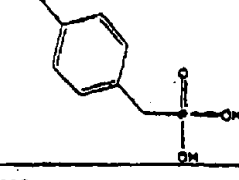
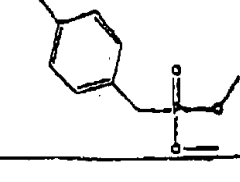
Substance	R ₁	R ₂	R ₃	R ₄	[α] _D ²⁰
EtV ₂ CS	-CH ₂ CH ₃	CH ₂ CH ₃	-H		ca. 0.07, MeOH -177
EtEtCS	-CH ₂ CH ₃	CH ₂ CH ₃	-H		ca. 0.05, MeOH -200
EtTHCS	-CH ₂ CH ₃	CH ₂ CH ₃	-H		
EtPhCS	-CH ₂ CH ₃	CH ₂ CH ₃	-H		ca. 0.14, MeOH -159
EtT ₃ CS	-CH ₂ CH ₃	CH ₂ CH ₃	-H		
MePhCS	-CH ₃	CH ₂ CH ₃	-H		ca. 0.06, MeOH -134
MeT ₃ CS	-CH ₃	CH ₂ CH ₃	-H		ca. 0.07, MeOH -95
D-MeAlEtV ₂ CS	-CH ₂ CH ₃	CH ₂ CH ₃	-CH ₃		ca. 0.12, MeOH -145
D-MeSeEtV ₂ CS	-CH ₂ CH ₃	CH ₂ CH ₃	-CH ₂ OH		



Replacement sheet (rule 26)

RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

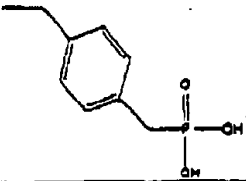
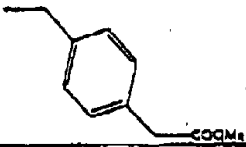
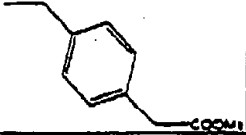
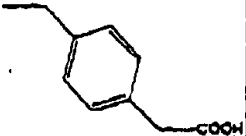
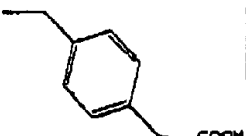
Substance	R ₁	R ₂	R ₃	R ₄	[α] _D ²⁰
D-MeAlEt ³ -EiPh ⁴ CS	CH ₂ CH ₃	CH ₂ CH ₃	CH ₃		c=0.06. MeOH -138
D-MeAlEt ³ -EiPh ⁴ (4-CH ₂ -PO(OMe) ₂)	CH ₂ CH ₃	CH ₂ CH ₃	CH ₃		
D-MeSeEt ³ -EiPh ⁴ (4-CH ₂ -PO(OMe) ₂)	CH ₂ CH ₃	CH ₂ CH ₃	CH ₂ OH		
D-MeAlEt ³ -EiPh ⁴ (4-CH ₂ -PO(OH) ₂)	CH ₂ CH ₃	CH ₂ CH ₃	CH ₃		
D-MeSeEt ³ -EiPh ⁴ (4-CH ₂ -PO(OH) ₂)	CH ₂ CH ₃	CH ₂ CH ₃	CH ₂ OH		
EiPh ⁴ (4-CH ₂ -PO(OMe) ₂)	CH ₂ CH ₃	CH ₂ CH ₃	-H		c=0.05. MeOH -134



Replacement sheet (rule 26)

RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

Substance	R ₁	R ₂	R ₃	R ₄	[α] _D ²⁰
EtPhe(4-CH ₂ -CO(OH)) ₂	CH ₂ CH ₃	CH ₂ CH ₃	-H		
EtPhe(4-CH ₂ -COOMe) ₂ CS	CH ₂ CH ₃	CH ₂ CH ₃	-H		α _D ²⁰ 0.17, MeOH -160
D-MeAl ² -EtPhe(4-CH ₂ -COOMe) ₂ CS	CH ₂ CH ₃	CH ₂ CH ₃	-CH ₃		
EtPhe(4-CH ₂ -COOH) ₂ CS	CH ₂ CH ₃	CH ₂ CH ₃	-H		
D-MeAl ² -EtPhe(4-CH ₂ -COOH) ₂ CS	CH ₂ CH ₃	CH ₂ CH ₃	-CH ₃		



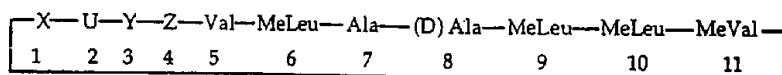
Replacement sheet (rule 26)

RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:17

Claims

1) Synthesised cyclosporin characterised by the formula:



wherein:

X is -MeBmt or 6,7-dihydro-MeBmt-;

U is -Abu, Nva, Val, Thr;

Y is Sar or (D)-MeSer or (D)-MeAla or (D)-MeSer (OAcyl); and

Z is (N-R)aa where aa={Val, Ile, Thr, Phe, Tyr, Thr (OAc), Thr (OG₁), Phe (G₂), PheCH₂(G₃), Tyr (OG₃)};

R = {alkyl > CH₃}:

G₁ = {phenyl-COOH, phenyl-COOMe, phenyl-COOEt};

G₂ = {CH₂COOH, CH₂COOMe(Et)₄; CH₂PO(OMe)₂, CH₂PO(OH)₂}; and

G₃ = {PO(OH)₂, PO(OCH₂CH=CH₂)₂, CH₂COOH, CH₂COOMe(Et)}.

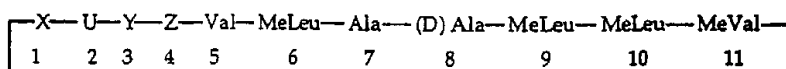
2) Cyclosporin according to claim 1, characterised in that the residue Z in position 4 is (R)Val where R > CH₃ and

R < C₁₀H₂₁.



3) Cyclosporin according to any one of the preceding claims, characterised in that the residue Z in position 4 is N-ethyl-valine.

4) Pharmaceutical composition containing the compound characterised by the formula:



wherein:

X is -MeBmt or 6,7-dihydro-MeBmt-;

U is -Abu, Nva, Val, Thr;

Y is Sar or (D)-MeSer or (D)-MeAla or (D)-MeSer (OAcyl); and

Z is (N-R)aa where aa={Val, Ile, Thr, Phe, Tyr, Thr (OAc), Thr (OG₁), Phe (G₂), PheCH₂(G₃), Tyr (OG₃)};

R={alkyl>CH₃};

G₁ = {phenyl-COOH, phenyl-COOMe, phenyl-COOEt};

G₂ = {CH₂COOH, CH₂COOMe(Et), CH₂PO(OMe)₂, CH₂PO(OH)₂}; and

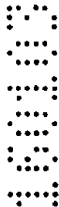
G₃ = {PO(OH)₂, PO(OCH₂CH=CH₂)₂, CH₂COOH, CH₂COOMe(Et)}.



5) Pharmaceutical composition according to claim 4, characterised in that it is combined with a pharmaceutically acceptable solution.

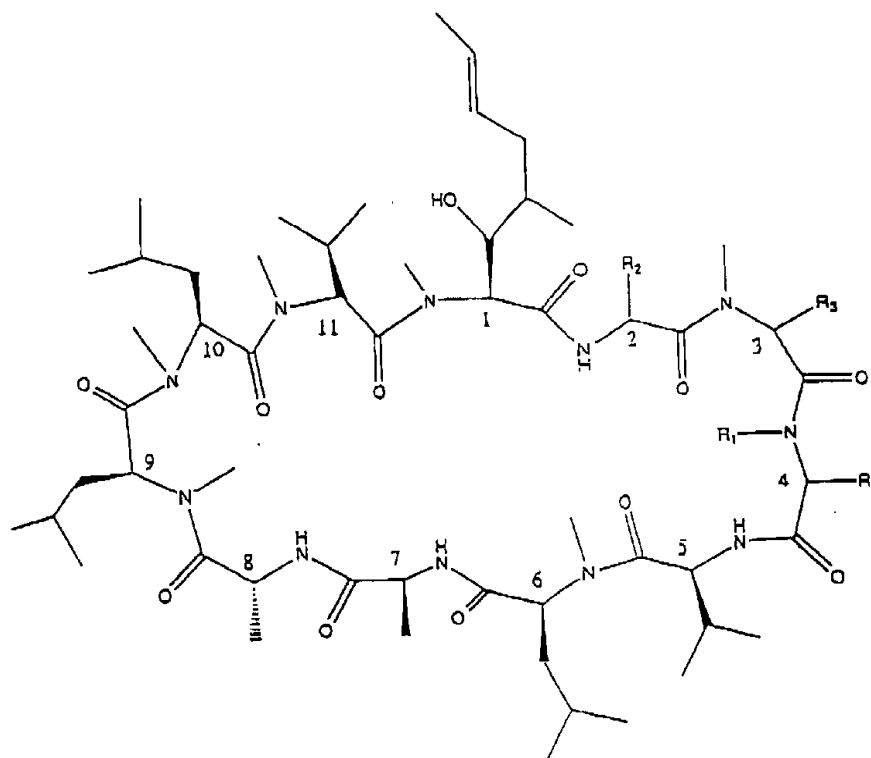
6) Use of the cyclosporin according to any one of the preceding claims for the production of a medicinal product intended for the treatment and prevention of AIDS.

7) Use of the cyclosporin according to claim 3 for the production of a medicinal product intended for the treatment and prevention of AIDS.



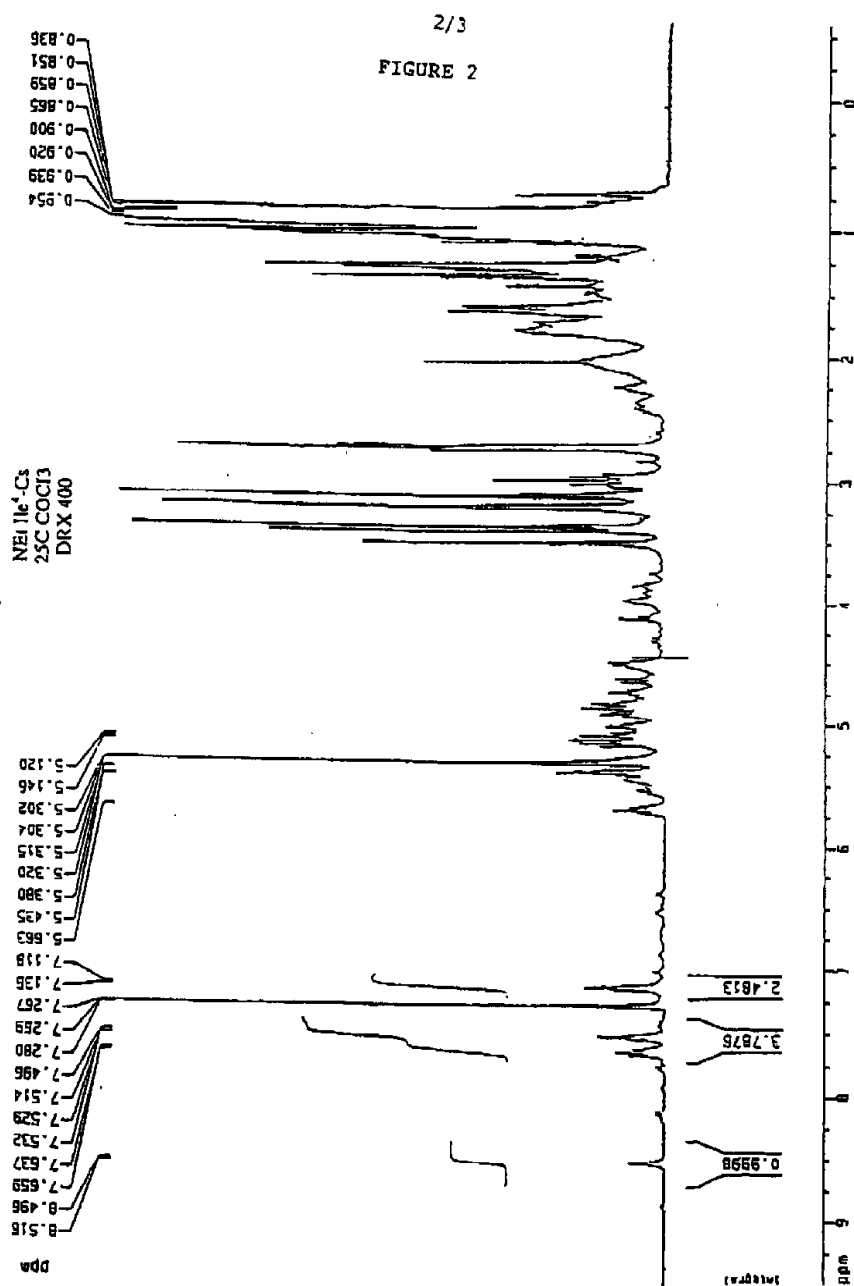
1/3

FIGURE 1



SUBSTITUTE SHEET (RULE 26)

64 4 4723358



SUBSTITUTE SHEET (RULE 26)

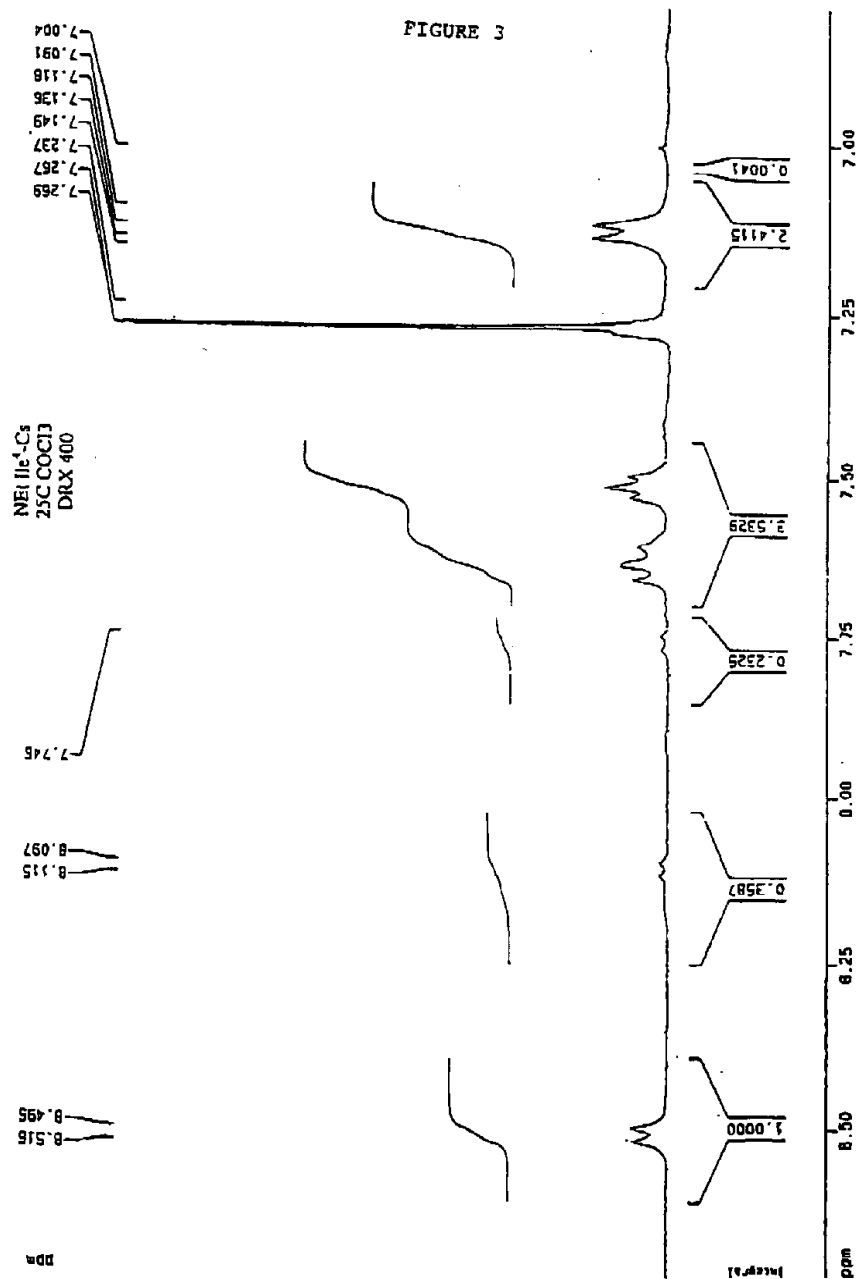
RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:16

64 4 4723358

3/3

FIGURE 3



SUBSTITUTE SHEET (RULE 26)

RECEIVED TIME 3. JAN. 13:37

PRINT TIME 3. JAN. 14:16

TOTAL P.32