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ANTI HIV-1 ACTIVITY OF A HYDROPHILIC CYCLOSPORIN DERIVATIVE WITH IMPROVED BINDING AFFINITY TO CYCLOPHILIN A

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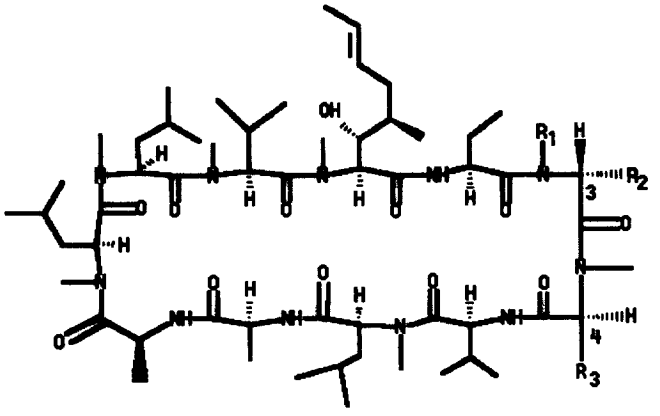
Abstract: Due to its conformation, the hydrophilic compound [(D)MeSer-3, (4-OH)MeLeu-4] cyclosporin, **3**, shows high affinity for cyclophilin A. This property, together with its lack of immunosuppression, confers **3** with very interesting anti HIV-1 activity.

Among the members of the cyclophilin (Cyp) family of proteins[1], cyclophilin A (CypA) is the cellular target of the immunosuppressant drug cyclosporin A **1** (CsA, *Sandimmun*^{R})[2] as well as the binding protein of the human immunodeficiency virus type 1 (HIV-1) related Gag polyprotein p55[3]. In the presence of CsA, CypA mediates immunosuppression through inhibition of calcineurin (CaN)[4] and in its absence, is specifically incorporated into HIV-1 virions through contacts with the Gag polyprotein[5,6]. The Gag-CypA interaction is efficiently and competitively disrupted by CsA at a 100-fold higher concentrations than those necessary for immunosuppression[3]. In addition, it was recently demonstrated that cyclosporin derivatives devoid of immunosuppressive activity but retaining binding capacity to CypA, exhibited potent and highly selective anti HIV-1 activity in various cell lines[7,8]. A representative example is (Me-Ile-4)cyclosporin **2** (NIM 811)[9]. It therefore appears that high affinity for CypA is a prerequisite for antiviral activity of cyclosporin derivatives.

To gain insight into this relationship, the hydrophilic derivative [(D)MeSer-3, (γ-OH)MeLeu-4] cyclosporin, **3**, was conceived as a cyclosporin with potentially increased CypA affinity and no immunosuppressive activity. Indeed, cyclosporin derivatives having (D)MeSer at position 3 instead of Sar show enhanced binding affinity for CypA. This affinity is not due to additional ligand/receptor interactions but solely to the increase in the binding conformer population[10]. Experimental evidence has been obtained by ^{1}H-NMR spectroscopy of triol **3** in polar solvents (D_2O or DMSO-d_6) where only one major conformation, which is very close to CsA's binding conformation, can be detected[11]. The presence of polar substituents at position 4 is known to be detrimental to the binding of the CypA/cyclosporin complex to CaN and therefore immunosuppression[12].



$R_1=R_2=H$ $R_3=CH_2CH(CH_3)_2$ CsA 1

$R_1=R_2=H$ $R_2=CH(CH_3)CH_2CH_3$ NIM 811 2

$R_1=CH_3$ $R_2=CH_2OH$ $R_3=CH_2C(OH)(CH_3)_2$ 3

$R_1=CH_3$ $R_2=CH_2OH$ $R_3=CH_2CH(CH_3)_2$ 4

Figure 1. Structures of compounds **1, 2 , 3, 4**

Compound **3** was synthesized in two steps starting from CsA by a combination of chemical and enzymatic methods. Treatment of **1** with an excess of n-butyllithium (-78°C, THF) and trapping of the resulting hexa-anion with solid paraformaldehyde afforded the hydroxymethylene derivative **4** in 42% yield[13]. The latter was subsequently enzymatically hydroxylated at MeLeu-4 with *Sebekia benihana*[14] to give **3** (20% yield). The ^{1}H-NMR spectrum of compound **3** in DMSO-d_6 clearly showed one predominant conformation (>90%) as judged by the NMe signals (Figure 2).

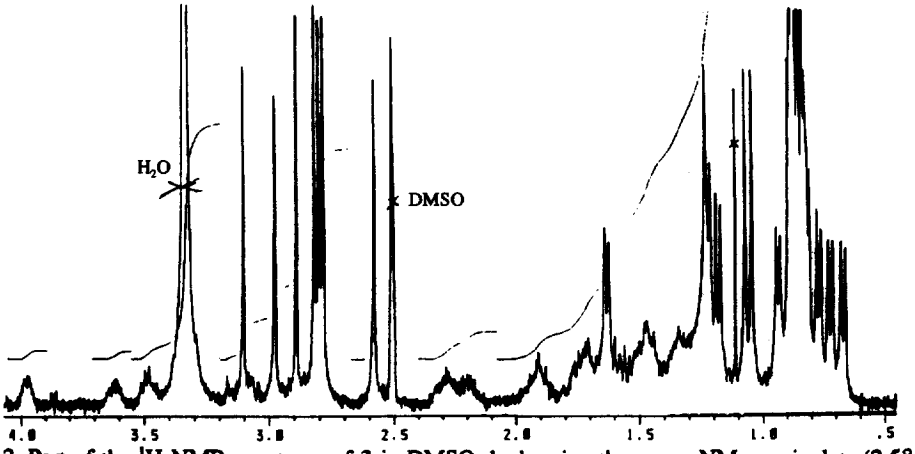


Figure 2. Part of the ^{1}H-NMR spectrum of 3 in DMSO-d_6 showing the seven NMe as singlets (2.58-3.11ppm) and the Me groups of the $C(OH)(CH_3)_2$ moiety (1.05ppm).

The *in vitro* profile of compounds **1**, **2** and **3** concerning their CypA binding affinity, their immunosuppression (IL2_RG and MLR_M) and their anti HIV-1 activity is summarized in the Table.

TABLE. *In vitro* biological activities of compounds

Compound	CypA[a,c]	IL2_RG[a,d]	MLR_M[a,e]	HIV-1[b,f]
CsA **1**	1	1	1	0.41
NIM 811 **2**	0.59	>1700	>100	0.08
3	0.16	>770	>3333	0.12

a)Mean relative IC_{50} values are shown (rel.IC_{50} represents the ratio : IC_{50}compound / IC_{50}CsA). Experiments were repeated at least three times.

b)Mean IC_{50} values in µg/ml. Experiments were repeated four times.

c)Binding of the derivative to CypA was determined by a solid phase enzyme immunoassay [15].

d)Immunosuppressive activity assessed with the interleukin-2 reporter gene assay (IL2_RG)in which IL-2 promoter activation upon T-cell stimulation was determined in a Jurkat cell line containing the β-galactosidase gene[16].

e)Immunosuppressive activity assessed with the mouse mixed lymphocyte reaction[17].

f)The human T-cell leukemia virus transformed T4 cell line was used and the inhibition of the cytopathic effect induced by HIV-1(IIIB) was determined[18].

The results of the biological evaluation clearly show that compound **3** fulfills the requirements set. It is devoid of immunosuppression and has 6-fold increased affinity for CypA as compared to CsA. In fact, the cyclosporin derivative **3** is one of the most potent cyclophilin binders ever reported. Nevertheless, **3** does not have increased anti-HIV activity as compared to NIM 811, **2**, which has lower binding affinity to CypA. The cell permeation properties of the two compounds could provide a possible explanation for this observation. It could well be that the polar character of **3** (three hydroxy functionalities as against one for **2**) prevents the compound from efficiently entering the cell in which case the potential advantage from the increased affinity would not be reflected in the cellular anti-HIV assay. Probing the physicochemical parameters governing cell penetration can be reached through the straightforward substitution of CsA **1** at position 3 (methylation, thioalkylation, acylation)[12]. The ensemble of these results indicates that cyclosporin "antagonists" exhibiting optimal physicochemical properties could be of great value in the anti-AIDS field.

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