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A NOVEL NON NUCLEOSIDE COMPOUND WITH HIGH *IN VITRO* ANTI-HIV-1 ACTIVITY. ABSOLUTE STEREOCHEMISTRY DETERMINATION

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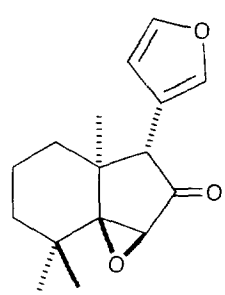
Abstract. Here we report a novel non-nucleoside compound, PM-92131(+), with *in vitro* anti-HIV-1 activity. This product was synthesized as a pure enantiomer *via* diastereomeric ester formation and selective crystallisation and its absolute stereochemistry was determined by X-ray diffraction analysis. Results from the activity assays suggest PM-92131(+) could be a promising new compound for further assessment as a potential anti-HIV-1 agent.

Since the discovery that HIV is the etiological agent of AIDS[1], great efforts have been made to discover and develop compounds to provide effective therapy for AIDS patients. Currently, active compounds used in the treatment of this syndrome all share a common target, the inhibition of the reverse transcriptase enzyme. The therapeutic benefit of this family of nucleoside analogs is limited by acute and cumulative toxicities, and the emergence of resistant viral strains[2].

The identification of new therapeutic entities with potential anti-HIV therapy is one of the most important priorities in new drug development. This paper reports on a structurally new HIV-1 *in vitro* inhibitor discovered from our screening program. It was first identified with interesting anti-HIV-1 activity as a racemic mixture, **PM-92131(±)** Figure I; its structure is related to degraded systems of the limonoid family[3]; compounds in this class are known to have insect antifeedant activity, but this is the first report of antiviral activity against HIV-1 for this kind of structure.

Figure I



PM92131(±)

The measurement of anti-HIV activity *in vitro* was done with two different assays: XTT cytoprotection assay[4] and syncytium-forming assay[5]. The virus used for testing was HIV-1 RF(HTLV-III_{RF}/H9) strain. HIV-1 stock virus was cultured in H9 cells, and both assays were carried out in CEM-SS cells. The results from both assays are shown in Table I.

Table I. Anti-HIV-1 *in vitro* activities (μM).

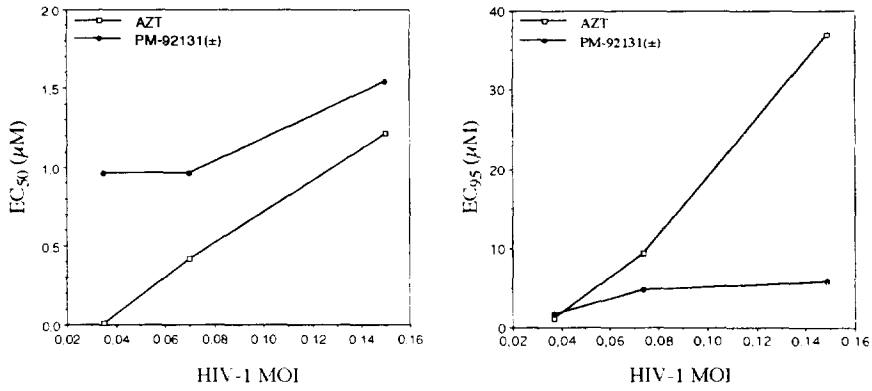
	Cytoprotection EC_{50}[a] (IC_{50})[b]	Syncytia EC_{50}[a] (IC_{50})[b]
PM-92131 (±)	1.3 (45.7)	1.7 (53.8)
PM-92131 (+)	0.8 (42.1)	0.6 (134.6)
PM-92131 (-)	42.1 (48.3)	15.4 (153.8)
AZT	0.3 (>1887)	0.02 (>1887)

[a] EC_{50} (effective drug concentration for 50% inhibition of viral replication).
[b] IC_{50} (drug concentration that inhibits cell growth by 50%).

High antiviral activity against the *in vitro* HIV-1 replication was observed in both assays.

The antiviral activity of **PM-92131(±)** was compared *in vitro* to AZT[6] in the cytoprotection assay with three different concentrations of HIV-1 virus (different multiplicities of infection) (Figure II). Results from this assay indicated that at low viral concentrations AZT appears to be a better inhibitor of viral replication. However, as the concentration of test virus increases, **PM-92131(±)** shows activity closer to that of AZT. When the multiplicity of infection (MOI) is higher than 0.04 and EC_{95} is determined, **PM-92131(±)** appears to have higher activity than AZT.

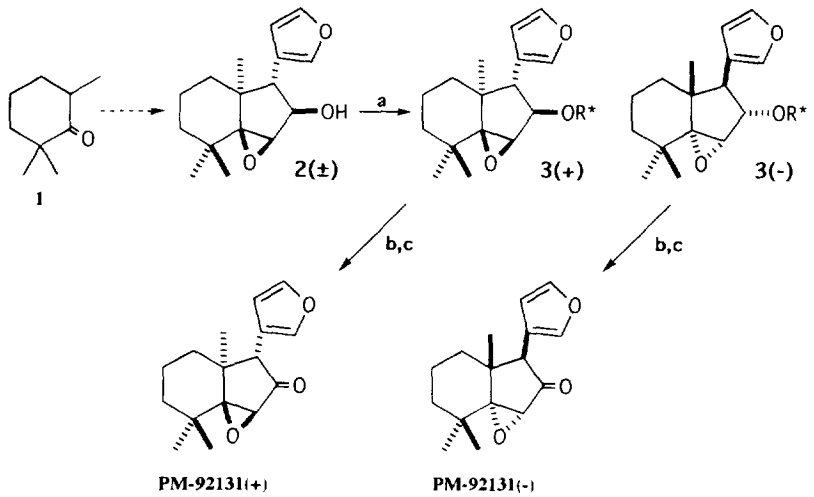
Figure II Anti-HIV-1 *in vitro* activity of PM-92131(±) vs. AZT at three different multiplicities of infection (MOI) of virus, determined by cytoprotection assay



The data from these *in vitro* assays indicate that **PM-92131(±)** could be a potential therapeutic agent.

The previous data relates to the antiviral activity of a racemic mixture; it was therefore important to test the activity of both enantiomers of **PM-92131** to identify if there was a difference between their individual activities. The synthesis of the two enantiomers is shown in Scheme I; the racemic epoxy alcohol **2**, an intermediate in the synthesis of **PM-92131(±)**[3a], was transformed by treatment with (-)-camphanic acid chloride in pyridine, in a diastereomeric ester mixture **3(+)** and **3(-)**. This mixture was partially separated by successive crystallisations from hexane/ether (7/3) to obtain **3(+)** as a white solid; **3(-)** was finally obtained by HPLC on silica gel as an oil[7]. Transformation of each diastereomer to the corresponding epoxy ketone **PM-92131(+)** and **PM-92131(-)**[8] was carried out by hydrolysis with KOH in ethanol and oxidation with Jones reagent with a resulting overall yield of 90%.

Scheme I



(**a**) (-)-Camphanic acid chloride,pyridine,0°C,1h (**b**) KOH,EtOH,rt,1h
(**c**) Jones reagent,acetone,0°C

Both pure enantiomers were tested *in vitro* against HIV-1, but only **PM-92131(+)** showed high antiviral activity (Table I); this product was also tested against the HIV reverse transcriptase and no inhibitory effect was found.

The absolute stereochemistry of **PM-92131(+)** was determined by single-crystal X-ray diffraction analysis[9]. A computer-generated perspective drawing of the final X-ray model is given in Figure III.

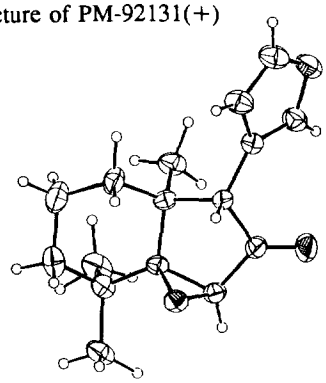
None of the intermediates in the synthesis of **PM-92131(+)** showed anti-HIV-1 activity.

Some other antifeedant compounds have been reported to have anti-HIV-1 activity that has been explained by inhibition of glycosidases[10]. However, inhibition of α and ß-mannosidase and α-glucosidase has not been observed for **PM-92131(+)**. This compound was also tested against herpes simplex virus type 1 and vesicular stomatitis virus and was found to have no activity.

A synthetic program is currently ongoing in our laboratory to determine structure-activity relationships. Biological assay systems are also being applied to investigate the mechanism of action.

In conclusion, a novel non-nucleoside compound **PM-92131(+)** with *in vitro* anti-HIV-1 activity, has been presented. The initial activity of this limonoid related compound suggets that further work must be carried out to fully assers the therapeutic potential of this agent and its related compouds.

Figure III. Solid-State molecular structure of PM-92131(+)



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References and Notes

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(4) (a) The HIV-1 RF(HTLV-III_{RF}/H9) strain, H-9 cells, and XTT cytoprotection assay protocol were provided to PharmaMar U.S.A. by Dr. Owen Weislow, Program Resources, Inc., Frederick, M.D. (b) Popovic, M.; Sarngadharan, M.C.; Read, E.; and Gallo, R.C. *Science*, **1984**, *224*, 597-598.

(5) (a) Cloned CEM-SS human lymphoid cells were provided by Dr. P.L. Nara for the protocol for the syncytium-forming assay. Both CEM-SS and H9 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2% L-glutamine (200mM), and 50μg/mL gentamicin. (b) Nara, P.L.; and Fischinger, P.J. *Nature*, **1988**, *332*, 469-470.

(6) AZT (3'-azido-3'-deoxythymidine) was purchased from Sigma Chemical Company, St. Louis, MO. **PM-92131(±)**, **PM-92131(+)** and **PM-92131(-)** were synthesized in our laboratory.

(7) HPLC was performed on a silica gel column (Spherisorb 10μm, 250x20 mm) in hexane/ethyl acetate 90/10, flow 9.9 mL/min, detection was carried out with a differential refractometer Waters-410. **3(+)** mp 183-184°C, $[\alpha]_D^{20}$= +67.9° (c=2.8, $CHCl_3$). **3(-)** $[\alpha]_D^{20}$= -67.7° (c=2.8, $CHCl_3$).

(8) **PM-92131(+)** $[\alpha]_D^{20}$= +28.7° (c=3.2, $CHCl_3$) and **PM-92131(-)** $[\alpha]_D^{20}$= -29.1° (c=2.8, $CHCl_3$).

(9) Crystal data: $C_{16}H_{20}O_3$, *Mr*=260.33, monoclinic space group $P2_1$, a=9.112(2)Å, b=7.387(2)Å, c=10.620(2)Å, β=97.88(2)°, V=708.1(3)Å^3, Z=2, Dx=1.221 Mg m^{-3}, Mo Kα radiation (graphite crystal monochromator, λ=0.71073Å), μ=0.776 cm^{-1}, *F(000)*=280, T=239K. Final conventional R=0.038 and *wR2*= 0.100 for 1869 'observed' reflections and 251 variables.

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