ELSEVIER

Antiviral Research 24 (1994) 275–288

Antiviral Research

Bicyclic imidazo derivatives, a new class of highly selective inhibitors for the human immunodeficiency virus type 1

Christiane Moog[a,*], Alexander Wick [b,1], Pierre Le Ber[b], André Kirn [a], Anne-Marie Aubertin[a]

[a]*INSERM U74, Institut de Virologie, 3 rue Koeberlé, 67000 Strasbourg, France*
[b]*SYNTHELABO Recherche, Département de Chimie, 31 av. Paul Vaillant Couturier, 92225 Bagneux Cedex, France*

(Received 1 December 1993; accepted 10 March 1994)

Abstract

In the search for new antiviral agents against human immunodeficiency virus, different members of two imidazoheterocycle families (imidazothiazoles, imidazopyridines) have been found to display potent inhibitory effects on the replication of HIV-1. Three of these derivatives, which show significant anti-HIV-1 activity, have been chosen for further studies. The analysis of these compounds and their comparison to AZT and TIBO revealed that these bicyclic imidazo derivatives represent a class of highly specific inhibitors of HIV-1, but not of HIV-2 or simian immunodeficiency virus (SIV). Their inhibition of HIV-1 is mediated through interaction with the reverse transcriptase (RT). The mechanism of action of these bicyclic imidazo derivatives may be similar to that of the other non-nucleoside RT inhibitors (NNRTIs).

Key words: HIV type 1; Reverse transcriptase; NNRTI; Synergism

1. Introduction

Since the causative agent of the acquired immunodeficiency syndrome AIDS was identified in 1983, different inhibitors of the reverse transcriptase (RT) of HIV have been discovered. Two main classes of compounds emerged from the studies: (1) the

*Corresponding author.

[1]Present address: SYNTHELABO Pharmacie, 22 av. Galilée, 92352 Le Plessis-Robinson Cedex, France.

SSDI 0166-3542(94)00026-5

nucleoside RT inhibitors – AZT (Zidovudine), and various other nucleoside analogues such as ddI or ddC –; and (2) the non-nucleoside RT inhibitors (NNRTIs). Although AZT, the first approved drug for the treatment of HIV, does prolong survival in AIDS patients (Cooper et al., 1993; Vella et al., 1994), its use is compounded by serious side effects and the emergence of AZT-resistant HIV-1 mutants (Kellam et al., 1992; Mohri et al., 1993; Shirasaka et al., 1993). By contrast, the administration of AZT to asymptomatic patients seems not to be of much benefit for them (Aboulker, J.P. and Swart, A.M., 1993).

Concerning NNRTIs, disheartening results from the preliminary clinical trials of some derivatives show the rise of drug-resistant variants, only a few weeks after beginning the treatment (Saag et al., 1992). Under these circumstances, novel chemotypes of anti-HIV agents are of great interest. The screening strategy we have adopted led us to the discovery of a new class of anti-HIV-1 compounds: bicyclic imidazo derivatives (imidazothiazoles and imidazopyridines).

In this paper, in which the activity of various members of these imidazoheterocycle families on the replication of HIV-1 in vitro is examined, we demonstrate that three compounds show a potent anti-HIV-1 activity.

2. Materials and methods

2.1. Compounds

The bicyclic imidazo derivatives have been synthesized by Synthelabo Recherche. TIBO (R 82913) and AZT were purchased from JANSSEN and SIGMA, respectively. Compounds were dissolved in DMSO and then serially diluted in RPMI 1640 medium. The control cultures received the same volumes of DMSO, never exceeding 1%.

2.2. Cell culture and virus infections

The cell cultures were maintained at 37°C in 5% CO_2. The effects of the compounds on the replication of HIV in MT-4 cells, CEM-SS cells, human PBMC, human monocyte-macrophages and chronically-infected U1 cells, were studied. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: U1 from Dr. Thomas Folks, CEM-SS from Dr. Peter Nara and HIV-1_{BaL} from Dr. Suzanne Gartner.

In the first test, the activity of the compound against HIV-1_{IIIb} was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. Cells were infected with HIV-1_{IIIb} isolate at 5 times the tissue culture infective dose 50 ($TCID_{50}$), which was determined by evaluating the RT activity released from MT-4-infected cells after 5 days), a virus dose which decreases the number of viable cells by 90% within 5 days. After adsorption at 37°C for 30 min, infected cells were adjusted to 2×10^5 cells/ml in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and seeded

in 96-well flat-bottom tissue culture plates (COSTAR 3596) (100 μl/well) with 100 μl of the compound at various concentrations. On day 5, cell viability was measured with the colorimetric reaction (MTT) based on the capacity of mitochondrial dehydrogenase of living cells to reduce 3-(4,5-dimethylthiazol-2yl)-2.5 diphenyl tetrazolium bromide into formazan. The quantity of formazan produced and measured at an optical density (OD) of 540 nm was correlated with the number of living cells (Pauwels et al., 1988). The percentage of protection of infected cells treated with the compound was calculated according to the formula proposed by Pauwels et al. (1988):

$$\% \text{ Protection} = \frac{[OD_{540} \text{ of infected cells treated with the compound}] - [OD_{540} \text{ of control-infected cells}]}{[OD_{540} \text{ of control-uninfected cells}] - [OD_{540} \text{ of control-infected cells}]}$$

The 50% inhibitory concentration (IC_{50}) is the concentration of a compound conferring 50% protection.

The assay procedure for measuring the anti-HIV-1 activity of compounds in CEM-SS cells was based on a quantitative detection of reverse transcriptase (RT) activity in the culture supernatant. CEM-SS cells were infected with the HIV-1 LAI isolate at 20 $TCID_{50}$ (evaluated by using the same determination as described above). After adsorption for 30 min, the cells were washed twice to remove unadsorbed virus particles and cultured at a 10^5 cells/ml final concentration in the presence of various dilutions of the test compounds. On day 5 following virus infection, the RT activity in the supernatant was measured as described below.

For the detection of anti-HIV activity in PBMC, phytohemagglutinin-stimulated PBMC were infected with various doses of HIV-1_{IIIB} isolate or HIV-2_{ROD} (Clavel et al., 1986), HIV-2_{D194} (Kunel et al., 1989), HIV-2_{205} (Kunel et al., 1989) or SIV_{mac251} (Daniel et al., 1985). After virus adsorption for 30 min, the cells were washed and then cultured with the compounds at 0.4×10^6 cells/ml in RPMI 1640 with 10% FCS and 20 U/ml IL-2 (Boehringer-Mannheim). On day 5, half of the medium was removed and replaced by fresh medium containing the appropriate dilutions of the test compounds. After 7 days, the virus released from the cells was evaluated by the RT assay.

Cytotoxicity of the compounds was evaluated in parallel with their antiviral activity for all cell types used. It was based on the viability of mock-infected cells, as determined by the MTT method. The 50% cytotoxic dose (CC_{50}) is the concentration at which OD_{540} was reduced by half.

2.3. Chronically-infected U1 cells

The activation of HIV-1 expression in U1 cells may be induced by PMA, TNF-α or IL-6 (Kalebic et al., 1991). In order to study the effect of our bicyclic imidazo derivatives on PMA-, TNF-α-, or IL-6-mediated induction of HIV-1 expression, U1 cells were pretreated with various concentrations of the test compounds for 6 h at 37°C in 5% CO_2. Then, PMA (10 nM; Sigma), recombinant TNF-α (100 units/ml;

Genzyme, TEBU, France), or IL-6 (100 units/ml; Genzyme, TEBU, France) were added to the culture and further incubated for 3 days. To monitor HIV-1 production, the level of RT was measured in the supernatant as described below.

2.4. Human monocyte-macrophages

Monocytes were obtained from the blood of healthy HIV-1 seronegative adult donors. They were isolated after centrifugal elutriation of PBMC and plated on culture dishes. Non-adherent cells were removed after 2 h. The purified monocytes were cultured for 1 week in AIM V (GIBCO Laboratories, France), 2 mM glutamine and 100 U/ml GM-CSF (Genzyme, TEBU, France) until differentiation into macrophages was observed. The monocyte-macrophages (MM) obtained were removed from the dishes using a rubber-policeman (viable cells: 99%) and plated at a density of $0.35 \times 10^6/250$ μl/well in 48-well plates (COSTAR 3548). MM were infected with HIV-1_{BaL} (Gartner et al., 1986), at a volume of 0.1 ml/well with an RT activity of 1.5×10^4 cpm/well, for 2 h at 37°C. The inoculum was removed, the cells were washed and medium containing the appropriate dilutions of antivirals was added. The culture supernatants were replaced two times per week with fresh medium containing the compounds at various concentrations. Supernatants were assessed for their RT activities, and the results obtained 15 days after infection were used for calculating the IC_{50} values for each compound.

2.5. Reverse transcriptase (RT) assay

Virus particles (65 μl of culture supernatant) were dissociated within 10 min, at 4°C in the presence of lysis buffer (10 μl) containing 0.5% Triton X-100, 0.75 M KCl and 50 mM dithiothreitol (DTT). RT assays were then performed at 37°C for 60 min by adding 25 μl of reaction mixture containing 0.2 M Tris-HCl (pH 7.8), 20 mM $MgCl_2$, 4 mM EGTA, 3 μCi of [^{3}H]thymidine triphosphate (48 Ci/mmol, Amersham, France), 5 μg poly(rA)-oligo(dT) (Pharmacia France). The reaction was stopped with 20 μl of 120 mM tetra-sodium diphosphate decahydrate in 60% trichloroacetic acid (TCA). Precipitates were then collected on a filter with a micro cell harvester (Skatron, OSI, Paris) and the radioactivity measured with a Betaplate liquid scintillation counter (Pharmacia France). The assays were carried out in duplicate.

2.6. In vitro RT inhibition

HIV-1, HIV-2 or SIV_{MAC} virus particles were disrupted with the lysis buffer and then treated for 1 h at 37°C with the compounds. The RT activity was evaluated by performing the assay described above.

2.7. Time of addition experiment

45 millions MT-4 cells were infected with 400 $TCID_{50}$ of HIV-1_{IIIb} in 7 ml

(determined by evaluating the RT activity released from MT-4-infected cells after 5 days). After adsorption for 30 min, the cells were thoroughly washed, plated at 10^5 cells/200 μl/well and 20 μl of the test compounds were added at different times after infection. Viral p24 antigen production was determined at 24 h post infection by a sandwich ELISA (Pasteur Diagnostic, France). The compounds were added at a standardized concentration, i.e., 100 times their IC_{50} value for MT4 cells as indicated in the legend of Fig. 3.

2.8. Drug combinations

The combined inhibitory effect of MS-888 and MS-1126 with AZT on HIV replication was examined by check-board combinations of various concentrations of the compounds. This was done on MT-4 cells and PBMC and the combined effect was analyzed using either the isobologram procedure (Baba et al., 1987) or the method developed by Chou and Talalay (Chou, T.C and Talalay, P., 1984).

For the isobologram procedure, the concentrations of compounds which inhibited viral cytopathogenicity by 50% in HIV-1-infected MT-4 cells were used to calculate the fractional inhibitory concentration (FIC) as follows: $FIC_{MS\text{-}1126}$ = (concentration of MS-1126 in the combination wells required for 50% inhibition)/(concentration of MS-1126 alone required for 50% inhibition). When the FIC index, which is the sum of FIC_{MS1126} and FIC_{AZT}, is equal to 1.0, the combination is additive. When it is below 1.0, the combination is synergistic, and when the minimum FIC index is higher than 1.0, the combination is judged as antagonistic.

With the method of Chou and Talalay (Chou, T.C. and Talalay, P., 1984), we have calculated the combination index (CI) giving 50% protection using the mutually non-exclusive equation for drugs having different sites/mechanisms as described elsewhere.

3. Results

3.1. Anti-HIV-1 assays for different bicyclic imidazo derivatives

In the course of screening for anti-HIV active compounds, a substituted imidazothiazole, MS-888 (Fig. 1) was found to display a marked antiviral effect (Table 1). Starting from this lead compound, a number of similar structures contained in our collection of synthetic chemicals were chosen for testing.

Table 1 gives the IC_{50} and the selective index (SI) of some of the analogues studied. It was observed that by changing the heterocycle from an imidazothiazole to an imidazopyridine (MS-1060) without changing the substituents, the good anti-HIV-1 activity as well as the favorable activity/toxicity ratio were preserved. A further change of the heterocycle to an imidazopyrimidine (MS-1256) led to a complete loss of activity.

In the imidazothiazole series, the change from a furan to a phenyl in the side-chain (MS-1010) reduced slightly the activity, while the substitution of this phenyl

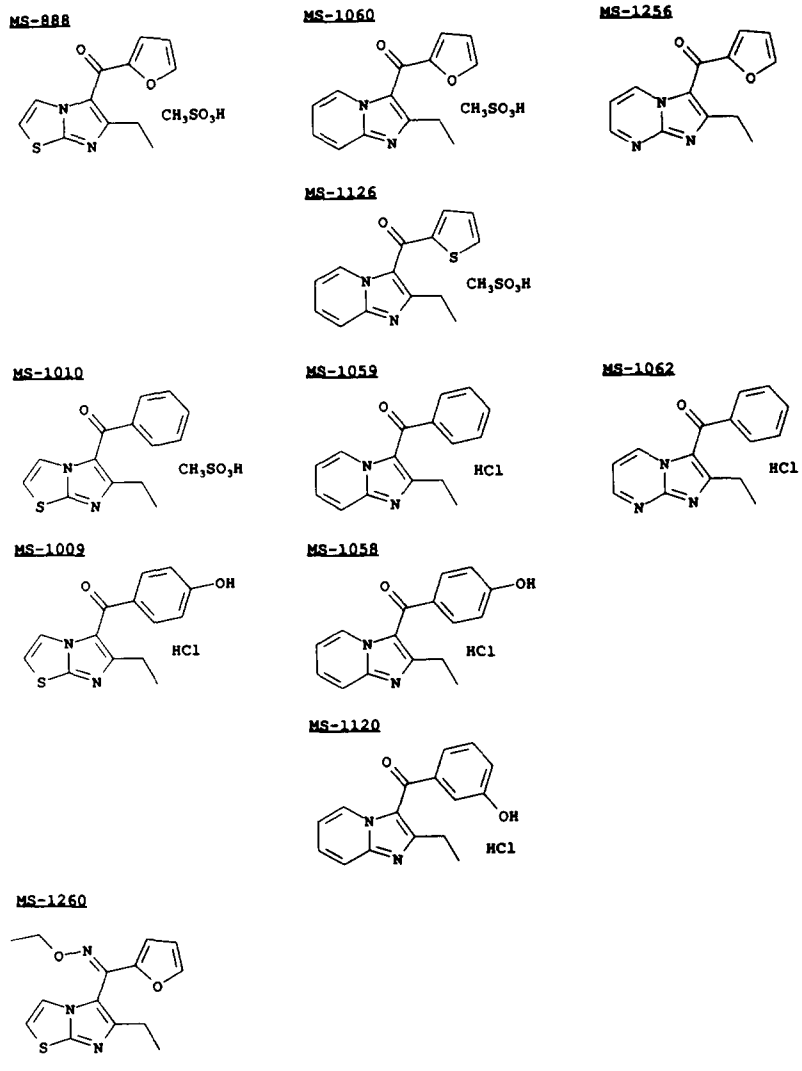

Fig. 1. Structure of bicyclic imidazo derivatives.

by a hydroxyl group in the 4 position (MS-1009) abolished all activity. The transformation of the keto function into an oxime-ether (MS-1260) was suggested by modelling studies; however, compound MS-1260 was clearly less active than MS-888.

In the imidazopyridine family, it was shown that the furan moiety (MS 1060) could be replaced by a thiophene (MS 1126) with total retention of the antiviral activity and comparable toxicity. In contrast to the corresponding imidazothiazole MS-1010, MS-1059 was clearly less active than its parent compound. All substitutions on the phenyl ring gave either inactive or toxic products (e.g., MS-1120, MS-1058).

Table 1
Antiviral activity of different bicyclic imidazo derivatives evaluated on MT-4 cells and on CEM-SS cells

	MT4/IIIB			CEMSS/LAI		
	CC_{50} (μM) ± S.D.	IC_{50} (μM) ± S.D.	SI	CC_{50} (μM) ± S.D.	IC_{50} (μM) ± S.D.	SI
MS 888	358 ± 38	17 ± 5	21	473 ± 46	3 ± 1.5	158
MS 1060	280 ± 51	18 ± 6	16	377 ± 92	5 ± 0.5	75
MS 1126	240 ± 69	13 ± 5	18	320 ± 82	2 ± 1	160
MS 1010	223 ± 75	37 ± 9	6	410 ± 84	6 ± 2	68
MS 1059	180 ± 17	77 ± 25	2	255 ± 7	41 ± 14	6
MS 1062	620 ± 60	>100	–	723 ± 25	62 ± 2	12
MS 1256	>100	>100	–	>100	>100	–
MS 1009	42 ± 21	–	–	64 ± 36	–	–
MS 1058	80 ± 10	>100	–	>100	>100	–
MS 1120	5 ± 2	–	–	5 ± 2	4 ± 1	1.3
MS 1260	160 ±	82 ± 17	2	195 ± 21	16 ± 5	12
R 82913	67 ± 6	0.33 ± 0.16	203	54 ± 5	0.12 ± 0.08	450

IC_{50} (expressed in μM) is based on the inhibition of HIV-1-induced cytopathogenicity in MT-4 cells or the inhibition of RT activity in the supernatant of infected CEM-SS cells. CC_{50} (expressed in μM) is based on the drop in viability of mock-infected MT-4 or CEM-SS cells. All data represent mean values ± S.D. of 2 to 5 separate experiments.

As expected, all members of the imidazopyrimidine family (e.g., MS 1256 and MS-1062) were devoid of any antiviral activity.

Thus, MS-888, MS-1060 and MS-1126 display a marked anti-HIV-1 activity in our screening tests, especially on CEM-SS cells, where their selectivity indexes are only 3-fold lower than that of the TIBO derivative (R 82913). These three active compounds are all methane sulfonate salts. We demonstrated that this anion does not directly interfere with their antiviral activity. Indeed, MS-888 as the free base displays a similar antiviral effect. Moreover, anti-HIV activity could not be induced by adding methanesulfonic acid to less active (MS 1059, MS 1062) or inactive (MS 1009) analogues (data not shown).

In conclusion, we have demonstrated, with this limited number of compounds available from our collection, certain structure-activity relations which can serve as the basis for the search of optimized inhibitors of HIV-1 replication.

3.2. Studies of the antiviral activity of bicyclic imidazo derivatives on different cell types and with various virus strains

Compounds MS-888, MS-1060 and MS-1126, which display potent anti-HIV-1 activity in our screening tests, were examined for their inhibitory effects on HIV replication in a variety of cell systems including PBMCs. These compounds are highly inhibitory to HIV-1_{IIIb} replication in human PBMC (Table 2). The 50% inhibitory concentrations (IC_{50}) were, respectively, 2 and 0.7 μM for compounds MS-888 and MS-1126. In contrast, the replication of three HIV-2 strains (ROD, D194 and 205) as well as of SIV_{mac251} strain was not inhibited by non-toxic concen-

Table 2
IC_{50} and CC_{50}, expressed in μM, of MS-888, MS-1060 and MS-1126 for various cell types infected with different virus strains

Cell type	Virus	MS 888			MS 1060			MS 1126		
		IC_{50} (μM) ± S.D.	CC_{50} (μM) ± S.D.	SI	IC_{50} (μM) ± S.D.	CC_{50} (μM) ± S.D.	SI	IC_{50} (μM) ± S.D.	CC_{50} (μM) ± S.D.	SI
PBMC	HIV-1_{IIIb}	1.5±1	110±10	73	–	–	–	0.7±0.3	120±13	171
MM*	HIV-1_{BAL}	1	100	100	1	100	100	0.5	100	200
PBMC	HIV-2_{ROD}	>100	>100	–	>100	>100	–	>100	>100	–
PBMC	HIV-2_{194}	>100	>100	–	>100	>100	–	>100	>100	–
PBMC	SIV_{mac251}	>100	>100	–	>100	>100	–	>100	>100	–
U1	Activation PHA	>100	>100	–	>100	>100	–	>100	>100	–
U1	Activation IL-6	>100	>100	–	>100	>100	–	>100	>100	–

*MM, Monocyte-macrophages.
All data represent mean values ± S.D. for 2 to 5 independent experiments. For M/M, the RT activities in the supernatants were measured twice a week. The IC_{50} values were calculated from the results obtained 15 days after infection.

trations of the bicyclic imidazo derivatives (Table 2).

As monocyte-macrophages play an important role in the pathogenesis of AIDS, the three compounds were examined for their inhibitory effect on the replication of HIV-1_{BaL} in human macrophage cultures. They all proved to be markedly inhibitory to the replication of the HIV-1 monotropic strain (Table 2). Thus, our new class of compounds seems to be specific for HIV type 1.

Studies in chronically-infected U1 cells revealed that the compounds are not able to decrease the virus replication induced, in this cell type, by various factors (TPA, TNF-α, IL-6) (Table 2).

3.3. Mechanism of action of bicyclic imidazo derivatives: comparative studies with AZT and TIBO (R 82913)

To pinpoint which stage of the HIV replicative cycle is impaired by our bicyclic imidazo derivatives, a time-of-addition experiment was carried out. This experiment was aimed at identifying the drug-sensitive phase under single growth cycle conditions. In practice, we measured how long the addition of compounds could be delayed without loss of activity, depending on the stage at which they interact and on their need for intracellular metabolism. Dextran sulfate for example, which acts at the virus adsorption step, (McClure et al., 1992), must be added together with the virus in order to be active (Fig. 2). For AZT or TIBO, which act at the reverse transcription step (Pauwels et al., 1990), addition to the cells could be delayed until 5 h after infection. From the time-of-addition experiment, it appeared that the bicyclic imidazo derivatives interact with a process that is early in the life cycle (Fig. 2).

In order to demonstrate whether the inhibition of HIV-1 replication by bicyclic imidazo derivatives is mediated through interaction with the RT, MS-888 was di-

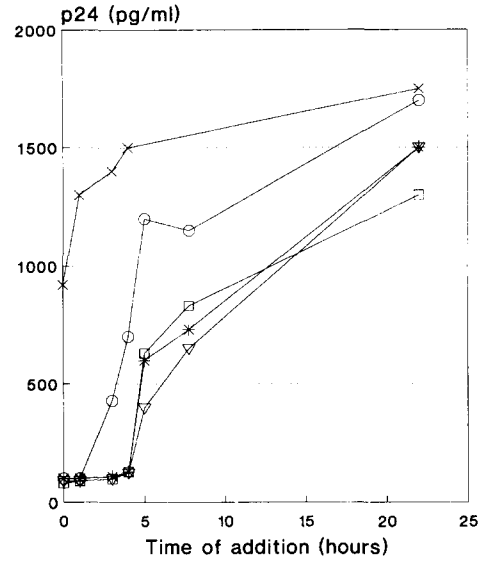

Fig. 2. Time-of-addition experiment. MT-4 cells were infected with HIV-1_{IIIB} and the compounds AZT (10^{-6} M, circle), dextran sulfate (50 μg/ml, cross), TIBO (10^{-5} M, triangle), MS-888 (10^{-4} M, asterisk) and MS-1126 (5×10^{-5} M, square) were added at different times after virus adsorption. Viral p24 antigen production was determined 24 h after infection by ELISA.

rectly added to RT released by the lysis of virions. It was found that MS-888, and MS-1060 and MS-1126 as well (data not shown), inhibited the activity of HIV-1 RT in vitro with an IC_{50} of 200 μM, 86% inhibition being measured at 1 mM (the highest concentration used) (Fig. 3). As observed for TIBO derivatives, the inhibitory concentration is 50- to 100-fold higher than the antiviral concentration measured in infected cells. As also noted with the TIBO compounds, the enzymatic activity of HIV-2 RT and SIV RT was not modified by the presence of bicyclic imidazo derivatives.

3.4. Drug combination experiment

Because the mode of action of bicyclic imidazo derivatives differs from that of nucleoside analogues, we were prompted to determine the potential for interactions between compounds of these two classes. Combinations of MS-1126 and AZT were prepared and the antiviral potencies of these mixtures were determined in MT-4 cell cultures infected with HIV-1_{IIIb}. These analyses indicated substantial synergism in the combined use of MS-1126 and AZT (Fig. 4). The fractional inhibitory concentration indexes were about 0.5. The association of other drugs, for example the combination of TIBO (R 82913) and AZT, gave less effective synergism (Fig. 4). Similar results were obtained for HIV-1_{IIIb}-infected PBMC by using the median effect principle and calculating the combination index (CI) of Chou and Talalay (data not shown).

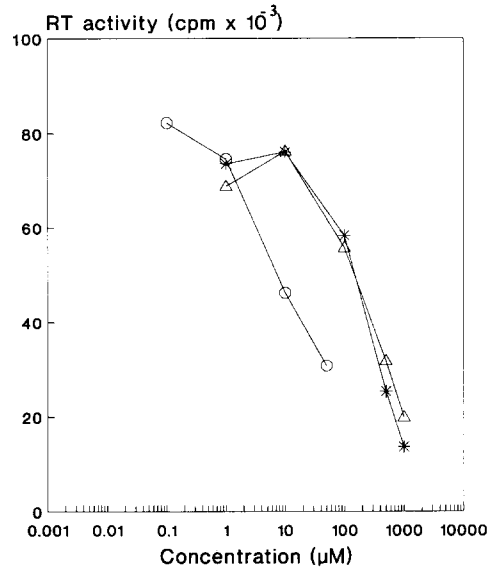

Fig. 3. Inhibitory effect of bicyclic imidazo derivatives on the activity of HIV-1 RT in vitro. HIV-1 RT activity was measured as described in Section 2, after a 1 h incubation of the enzyme at 37°C with various concentrations of MS-888 (triangle), MS-1060 (asterisk) or TIBO (circle). The experiment is representative of three of similar design.

From this combination analysis, we demonstrate a potent synergism between MS-1126 and AZT in HIV-1-infected cells.

4. Discussion

Given the necessity of long term chemotherapy for HIV-infected patients, more effective and less toxic treatment modalities are required. In this study, we found that some bicyclic imidazo derivatives have a significant inhibitory effect on HIV-1 replication in vitro. Chemical modifications of the MS-888 structure show relatively stringent structure-activity requirements as to the nature of the heterobicycle and the aryl moieties. Minor variations reduce the anti-HIV-1 activity, but the replacement of the imidazothiazole by an imidazopyridine and the replacement of the furane by a thiophene slightly enhance the antiviral effect. Synthesis of other analogues should permit an optimization of the anti-HIV-1 activity and a better understanding of the structure-activity relationship.

By analyzing the antiviral profile of the bicyclic imidazo derivatives, we found that these compounds are highly specific for HIV-1 replication. They do not inhibit HIV-2 or SIV. They interact with the HIV-1 reverse transcription process but not with HIV-2 or SIV reverse transcriptase. This antiviral activity spectrum clearly correlates with that of NNRTIs. Indeed, the NNRTIs including HEPT (Tanaka et al., 1991), TIBO (Pauwels et al., 1990), nevirapine (Merluzzi et al., 1990), L-697,639

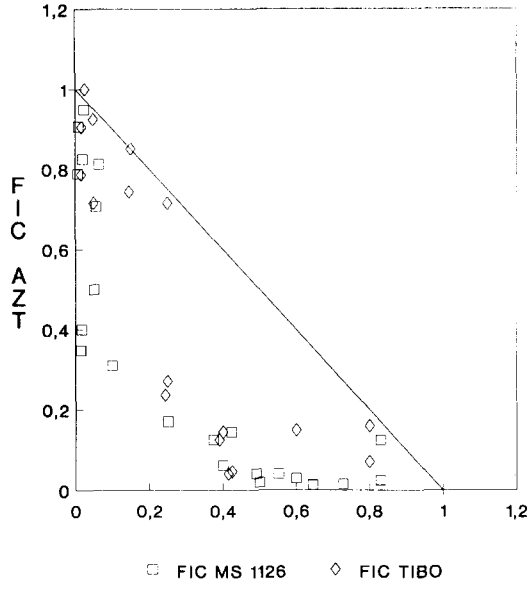

Fig. 4. Synergism in the combined use of MS-1126 and AZT, or TIBO and AZT. Fractional inhibitory concentrations (FICs) combining MS-1126 and AZT (square) or TIBO and AZT (rhombus) are shown. The diagonal line represents additivity.

(Goldman et al., 1991) and BHAP (Romero et al., 1991), which show little structural relationship with each other, also discriminate between HIV-1 and HIV-2 in terms of antiviral activity. Besides their antiviral properties, these five classes of compounds share another analogy, that is the emergence of HIV-1-resistant strains that are often cross-resistant. This multi drug resistance implicates that the inhibitors interact via a common binding site and constitute a single pharmacological class of RT inhibitors (Nunberg et al., 1991). Boyer et al. (1993) have shown that for some NNRTIs the binding sites overlap but are not exactly identical. These classes of HIV-1 specific RT inhibitors select for different amino acid mutations during the drug-resistant virus selection procedure (Balzarini et al., 1993a). The TSAO derivatives (Pérez-Pérez et al., 1992), which have been recently described as a new class of HIV-1-specific inhibitors (Balzarini et al., 1992), select for a single mutation at position 138 that is different from the mutations generally occurring with the NNRTIs (Balzarini et al., 1993a). However, NNRTI-resistant variants containing the Tyr-181→Cys mutation are also resistant to TSAO derivatives (Balzarini et al., 1993b).

The emergence of variants resistant to bicyclic imidazo derivatives is currently investigated. Studies comparing the different reference strains resistant to NNRTI (Richman et al., 1991) or AZT (Larder et al., 1989) and the imidazoheterocycle-resistant strains will demonstrate whether bicyclic imidazo derivatives are new members of the NNRTI family with or without a distinct binding site. In any case, although the emergence of multidrug resistance have been well demonstrated (Larder et al., 1993; Emini et al., 1993; Balzarini et al., 1993b), this class of HIV-1

inhibitors targeted to the RT should permit various drug combinations in the hope of achieving a prolonged suppression of HIV-1 replication.

Thus, although their antiviral effects are comparable to that of NNRTIs our bicyclic imidazo derivatives should be considered as leads for a new class of potential drug candidates in the treatment of HIV-1 infections.

Acknowledgments

We are indebted to Sylvie Schmidt and Jacqueline Richert for their technical assistance and to Tania Kirn for correction of the English in the manuscript. The authors thank Dr. A. Saarmets (Synthelabo Research) for his modelling studies and Drs. G. Defosse and J. Frost (Synthelabo Research) for their skilled contribution in synthesis. This work has been carried out under the project "Action Coordonnée 3" of l'Agence Nationale de Recherches sur le Sida.

References

Aboulker, J.-P. and Swart, A.M. (1993) Preliminary analysis of the Concorde trial. Lancet 341, 889.

Baba, M., Pauwels, R., Balzarini, J., Herdewijn, P., De Clercq, E. and Desmyter, J. (1987) Ribavirin antagonizes inhibitory effects of pyrimidine 2′,3′-dideoxynucleosides but enhances inhibitory effects of purine 2′3′-dideoxynucleosides on the replication of human immunodeficiency virus in vitro. Antimicrob. Agents Chemother. 31, 1613–1617.

Balzarini, J., Karlsson, A., Pérez-Pérez, M.-J., Vrang, L., Walbers, J., Zhang, H., Oberg, B., Vandamme, A.-M., Camarasa, M.-J. and De Clercq, E. (1993a) HIV-1-specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acid substitutions in the reverse transcriptase. Virology 192, 246–253.

Balzarini, J., Karlsson, A., Pérez-Pérez, M.-J., Camarasa, M.-J., Tarpley, W.G. and De Clercq, E. (1993b) Treatment of human immunodeficiency virus type 1 (HIV-1)-infected cells with combinations of HIV-1-specific inhibitors results in a different resistance pattern than does treatment with single-drug therary. J. Virol. 67, 5353–5359.

Balzarini, J., Pérez-Pérez, M.-J., San Félix, A., Schols, D., Perno, C.-F., Vandamme, A.-M., Camarasa, M.-J. and De Clercq, E. (1992) [2′5′-bis-*O*-(tert-butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2 ″,2″-dioxide)pyrimidine (TSAO) nucleoside analogues: Highly selective inhibitors of human immunodeficiency virus type 1 that are targeted at the viral reverse transcriptase. Proc. Natl. Acad. Sci. USA 89, 4392–4396.

Boyer, P.L., Currens, M.J., McMahon, J.B., Boyd, M.R. and Hugues, S.H. (1993) Analysis of nonnucleoside drug-resistant variants of human immunodeficiency virus type 1 reverse transcriptase. J. Virol. 67, 2412–2420.

Cooper, D.A., Gastell, J.M., Kroom, S., Clumeck, N., Millard, J., Goebel, F-D., Bruun, J.N., Stingl, G., Melville, R.L., Gonzalez-Lahoz, J., Stevens, J.W., Fiddian, A.P. and the European-Australian collaborative group (1993) Ziduvudine in persons with asymptomatic HIV infection and CD4+ cell counts greater than 400 per cubic millimeter. N. Eng. J. Med. 329, 297–303.

Chou, T.C. and Talalay, P. (1984) Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs enzyme inhibitors. Adv. Enzyme Regul. 22, 27–55.

Clavel, F., Guyader, M., Guétard, D., Sallé, M., Montagnier, L. and Alizon, M. (1986) Molecular cloning and polymorphism of the human immune deficiency virus type 2. Nature 324, 691–695.

McClure, M.O., Moore, J.P., Blanc, D.F., Scotting, P., Cook, G.M.W., Keynes, R.J., Weber, J.N., Davies, D. and Weiss, R.A. (1992) Investigations into the mechanism by which sulfated

polysaccharides inhibit HIV infection in vitro. AIDS Res. Human Retoviruses 8, 19–26.

Daniel, M.D., Letvin, N.L., King, N.W., Kannagi, M., Sehgal, P.K., Hunt, R.T., Kanki, P.J., Essex, M. and Desrosiers, R.C. (1985) Isolation of T cell tropic HTLV-III like retrovirus from macaques. Science 228, 1201–1204.

Emini, E.A., Graham, D.J., Gotlib, L., Condra, J.H., Byrnes, V.W. and Schleif, W.A. (1993) HIV and multidrug resistance. Nature 364, 679.

Gartner, S., Markovits, P., Markovitz, D.M., Kaplan, M.H., Gallo, R.C. and Popovic, M. (1986) The role of mononuclear phagocytes in HTLV-III/LAV infection. Science 233, 215–219.

Goldman, M.E., Nunberg, J.H., O'Brien, J.A., Quintero, J.C. and Schleif, W.A., Freund, K.F., Gaul, S.L., Saari, W.S., Wai, J.S., Hoffman, J.M., Anderson, P.S., Hupe, D.J., Emini, E.A. and Stern, A.M. (1991) Pyridinone derivatives: specific human immunodeficiency virus type I reverse transcriptase inhibitors with an antiviral activity. Proc. Natl. Acad. Sci. USA 88, 6863–6867.

Kalebic, T., Kinter, A., Poli, G., Anderson, M.E., Meister, A. and Fauci, A. (1991) Suppression of human immunodeficiency virus expression in chronically-infected monocytic cells by glutathione, glutathione ester and *N*-acetylcysteine. Proc. Natl. Acad. Sci. USA 88, 986–990.

Kellam, P., Boucher, C.A.B. and Larder, B.A. (1992) Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to Zidovudine. Proc. Natl. Acad. Sci. USA 89, 1934–1938.

Kunel, H., Von Briesen, H., Deitrich, U., Adamski, M., Mix, D., Biesert, L., Kreutz, R., Immelmann, A., Henco, K., Meichsner, C., Andreesen, R., Gelderblom, H. and Rubsamen-Waigmann, H. (1989) Molecular cloning of two West African human immunodeficiency virus type 2 isolates that replicate well in macrophages: a Gambian isolate, from a patient with neurologic acquired immunodeficiency syndrome, and a highly divergent Ghanian isolate. Proc. Natl. Acad. Sci. USA 86, 2383–2387.

Larder, B.A., Darby, G. and Richman, D.D. (1989) HIV with reduced sensitivity to Zidovudine (AZT) isolated during prolonged therapy. Science 243, 1731–1734.

Larder, B.A., Kellam, P. and Kemp, S.D. (1993) Convergent combination therapy can select viable multidrug-resistant HIV-1 in vitro. Nature, 365, 451–453.

Merluzzi, V.J., Hargrave, K.D., Labadia, M., Grozinger, K., Skoog, M., Wu, J.C., Shih, C.K., Eckner, R.K., Hattox, S., Adams, J., Rosenthal, A.S., Faanes, R., Eckner, R.J., Koup, R.A. and Sullivan, J.L. (1990) Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. Science 250, 1411–1413.

Mohri, H., Singh, M.K., Ching, W.T.W. and Ho, D.D. (1993) Quantitation of zidovudine-resistant human immunodeficiency virus type 1 in the blood of treated and untreated patients. Proc. Natl. Acad. Sci. USA 90, 25–29.

Nunberg, J.H., Schleif, W.A., Boots, E.J., O'Brien, J.A., Quintero, J.C., Hoffman, J.M., Emini, E.A. and Goldman, M.E. (1991) Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. J. Virol. 65, 4887–4892.

Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaeckers, A., Van Gelder, J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E. and Janssen, P.A.J. (1990) Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343, 470–474.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J. and De Clercq, E. (1988) Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20, 309–321.

Pérez-Pérez, M.-J., San-Félix, A., Balzarini, J., De Clercq, E. and Camarasa, M.-J. (1992) TSAO Analogues Stereospecific synthesis and anti-HIV-1 activity of 1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)pyrimidine and pyrimidine-modified nucleosides. J. Med. Chem. 35, 2988–2995.

Richman, D., Shih, C.-K., Lowy, I., Rose, J., Prodanovich, P., Goff, S. and Griffin, J. (1991) Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. Proc. Natl. Acad. Sci. USA 88, 11241–11245.

Romero, D.L., Busso, M., Tan, C.K., Reusser, F., Palmer, J.R., Poppe, S.M., Aristoff, P.A., Downey, K.M., So, A.G., Resnick, L. and Tarpley, W.G. (1991) Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type-1 replication. Proc. Natl.

Acad. Sci. USA 88, 8806–8810.

Saag, M.S., Douglas, J., Lapidus, W., DeLoach, L.J., Maples, V., Laskin, O., Massari, F., Whitley, R., Kappes, J., Shaw, G. and Emini, E. (1992) Safety and relative antiretroviral activity of L697, L661 versus zidovudine in HIV-1-infected patients, VIII International Conference on AIDS, Amsterdam, The Netherlands, 19–24 July 1992, WeB 1013 (Abstract).

Shirasaka, T., Yarchoan, R., O'Brien, M.C., Husson, R.N., Anderson, B.D., Kojima, E., Shimada, T., Broder, S. and Mitsuya, H. (1993) Changes in drug sensitivity of human immunodeficiency virus type 1 during therapy with azidothymidine, dideoxycytidine, and dideoxyinosine: an in vitro comparative study. Proc. Natl. Acad. Sci. USA 90, 562–566.

Tanaka, H., Baba, M., Hayakawa, H., Sakamaki, T., Miyasaka, T., Ubasawa, M., Takashima, H., Sekiya, K., Nitta, I., Shigeta, S., Walker, R.T., Balzarini, J. and De Clercq, E. (1991) A new class of HIV-1-specific 6-substituted acyclouridine derivatives: Synthesis and anti-HIV-1 activity of 5- or 6-substituted analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). J. Med. Chem. 34, 49–357.

Vella, S., Giuliano, M., Dally, L.G., Agresti, M.G., Tomino, C., Floridia, M., Chiesi, A., Fragola, V., Moroni, M. and Piazza, M. (1994) Long-term follow-up of Zidovudine therapy in asymptomatic HIV-1 infection: Results of a multicenter cohort study. J. Acq. Immun. Defic. Syndrome 7, 31–38.