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Antiviral properties of simple difunctionalized enols targeted to the HIV-1 protease

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Abstract

The human immunodeficiency virus type 1 (HIV-1) protease catalyses the specific cleavage of the virion structural polyproteins p55gag and p160gag-pol and is, therefore, essential for viral maturation. We have previously reported a series of low molecular weight non-peptidic enol-based compounds that inhibit the HIV-1 protease activity in a competitive fashion (Vaillancourt et al., Bioorg. Med. Chem., 2 (1994) 343–355). Here we demonstrate that VS-215 and VS-261, two of these non-peptidic inhibitors, impair viral polyprotein maturation and exhibit antiviral activity in infected MT4 cells. The ID_{50} for these two compounds ranged between 24 and 50 μM whereas their TD_{50} ranged between 60 and 200 μM depending on the cell lines used. The calculated therapeutic index of these two inhibitors both had values of 2.5 even though they were shown to be non cytotoxic at their ID_{50}. Their calculated permeability index ranged between 0.09 and 0.79 suggesting that these enol-based inhibitors efficiently reach the site of protease activity. These results provide new information on the therapeutic potential of this new class of protease inhibitors and emphasize the usefulness of enol chemistry in the development of anti-HIV-1 protease inhibitors.

Keywords: HIV-1 protease; Antiviral activity; Non-peptidic inhibitors; Enols

1. Introduction

The Human Immunodeficiency Virus (HIV), a retrovirus of the lentivirus subgroup, is the etiological agent of AIDS (Montagnier, 1983). The spread of AIDS throughout the world represents one of the most important medical challenges that the scientific

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community has faced in the past two decades. The only drugs approved for treatment of HIV are aimed at interfering with the reverse transcriptase activity. AZT, ddI and ddC have all brought some improvement in the quality of life of AIDS patients. Treatment with AZT primarily generates an increase in $CD4^+$ T lymphocyte cell counts and a decrease in blood p24 antigen within the first 48 weeks of treatment (Volberding et al., 1990). However, the three years follow up reported by the Concorde Trials indicates that AZT does not slow the patients' progression from seropositivity to AIDS (Aboulker and Swart, 1993). The need to develop novel therapeutic agents directed against different viral targets becomes obvious and urgent.

The HIV life cycle provides many opportunities for therapeutic intervention. The antiviral strategies targeting pre-integrational events utilize agents which interfere with viral entry, uncoating, reverse transcription and integration (Tomasselli et al., 1991). Other inhibitors of viral replication are directed against post-transcriptional events. Among these post-transcriptional inhibitors are Tat inhibitors (benzodiazepine) (Hsu et al., 1991; Johnston and McGowan, 1992), glycosylase inhibitors (casternospermine and deoxynojirimycine) (Gruters et al., 1987; Sunkara et al., 1987; Tyms et al., 1987), *N*-myristoyltransferase inhibitors (myristic acid derivatives) (Bryant et al., 1989), α-interferon (Lane et al., 1990) and protease inhibitors (Craig et al., 1991; Kempf et al., 1991; Lambert et al., 1992; Meek et al., 1990; Tucker et al., 1992).

The retroviral protease is responsible for the specific processing of the gag and gag-pol polyproteins during viral budding (Darke et al., 1988). Impairment of its activity by mutagenesis (Guenet et al., 1989; Kohl et al., 1988; Loeb et al., 1989a,b) or by inhibitors (Ashorn et al., 1990; DeWit et al., 1988; Martin, 1992; Rich et al., 1992; Thaisrivong et al., 1991) has been demonstrated to lead to the production of non-infectious particles by infected cells. Our understanding of the HIV protease mode of action and substrate specificity has proven extremely useful in designing a variety of potent and highly specific transition state inhibitors. However, these inhibitors retain substantial peptide character, which make them biologically unstable, poorly absorbed and rapidly metabolized (Kempf et al., 1991; Martin, 1992). We have previously described enol-based molecular structures that may serve as the prototype for the design of non-peptide inhibitors of HIV protease (Vaillancourt et al., 1993,1994; Vaillancourt et al., in preparation). The complete characterization of the inhibitory properties of these enols (i.e. structure-activity relationship, molecular modeling and inhibitory behavior) led us to evaluate the antiviral activity of the two most efficient compounds. The results described herein reveal that these two enol-based protease inhibitors, VS-215 and VS-261 (Fig. 1), efficiently inhibit viral replication in various cell lines at concentrations below cytotoxic levels. In addition we show that these compounds inhibit viral replication by preventing the processing of gag and gag-pol precursor proteins. This study also demonstrates the usefulness of the enol chemistry in the development of anti-HIV-1 protease inhibitors.

2. Materials and methods

Viral strains and cell lines

The UHC-1 viral strain was produced using a chronically infected U_{937} cell line obtained from Dr. M.A. Wainberg, Lady Davis Institute for Medical Research, Mon-

treal, Canada. SVC21 plasmid contains the DNA of the HXBc2 provirus, an infectious molecular clone derived from the HIV-IIIB isolate (Ratner et al., 1985). MT4, U_{937} and CEM cell lines were maintained in RPMI-1640 supplemented with 10% (MT4, U_{937}) or 20% (CEM) fetal calf serum (FCS) and 1% penicillin/streptomycin (pen/strep). COS cell line was maintained in DMEM supplemented with 10% FCS and 1% pen/strep.

Protease inhibitors

VS-215 and VS-261 were prepared as described previously (Vaillancourt et al., 1993,1994) by the activation of the *N*-protected amino acid by 1,1'carbonyldiimidazole in dry tetrahydrofurane at 0° C. Addition of the activated methylene treated with sodium hydride yielded the corresponding enol in a one step synthesis. Purification of the newly formed enol was performed by flash chromatography. $K_{i_{app}}$ values for VS-215 and VS-261 were evaluated with the Henderson progressive curve evaluation using high performance liquid chromatography as described previously (Vaillancourt et al., 1994).

Cytotoxicity and antiviral activity

The inhibitors were dissolved in 10% DMSO/90% FCS-complemented RPMI-1640 (10 mM solution) and were added to the culture media to achieve correct concentrations (0.1% final DMSO concentration). The compound SKF 108 842 (Smith Kline Beecham Pharmaceuticals) was used at 1% DMSO concentration. Cytotoxicity of VS-215 and VS-261 was evaluated by incubating infected and non-infected cells with increasing concentrations of the drugs. The total number of viable cells and the percentage of trypan blue-staining cells in the cultures were monitored by light microscopy. The growth efficiency experiment was performed as follow. 1000 MT4 cells were incubated for 9 days in 5 ml of RPMI-1640, 10% FCS, 1% pen/strep in 60-mm petri dish in presence of increasing concentrations of VS-215. Cells were then centrifuged and viable cells counted by trypan blue exclusion dye. The intra-variation for the cellular counts was evaluated at $11 \pm 6\%$ while the inter-variation was evaluated at $24 \pm 7\%$.

MT4 cells were infected at a multiplicity of infection (MOI) of 0.1 as measured by end-point dilution titration. Cells were infected at day 0 by adsorbing the virus onto cells for 2 h. Following two rounds of wash (RPMI-1640 medium), cells were incubated at 5×10^5 cells/ml in 24 or 96-wells plastic ware at 37° C in 5% CO_2. The antiviral potentials of VS-215 and VS-261 were evaluated at viral peak production by measurement of reverse transcriptase activity in the supernatant as described previously (Ratner et al., 1985). Antiviral activity is quantified by determining the inhibitor concentration required to reduce reverse transcriptase activity by 50% (ID_{50}). Results are expressed as percentage of RT activity obtained compared with that of infected controls without drug. Viral cytotoxicity in the presence of drugs was also evaluated by monitoring the formation of syncytia in the cultures using light microscopy as described previously (Yao et al., 1993).

Transfection, metabolic labeling and electrophoretic analysis

COS cells grown to 50–60% confluence in 80-cm^2 tissue flasks were transfected with 20 μg of plasmid SVC21, using the calcium phosphate precipitation method (Cullen, 1987). The transfection solution was added dropwise to cells, and incubated

without mixing for 16–20 h at 37° C in 5% CO_2. At 20 h post-transfection, medium was replaced and the drug was added. At 26 h, transfected COS cells were washed once and resuspended in starvation media without cysteine for 30 min in presence of drugs. Cells were metabolically labelled with [^{35}S]cysteine (100 μCi/ml) for 6 h. After several washes with PBS, labelled cells were lysed by adding 1 × RIPA buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.25% deoxycholate, 0.2% phenylmethylsylfonyl fluoride). Supernatants were also collected and lysed by adding 10 × RIPA buffer. Labelled proteins were immunoprecipitated by incubating the cell lysate and supernatant samples with 6 μl of HIV-1-positive human serum. After a 5 h incubation at 4° C, protein A Sepharose was added and the suspensions were incubated overnight at 4° C. Following centrifugation, pellets were washed several times with RIPA and PBS, resuspended in SDS-PAGE sample buffer and then boiled for 4 min. Immunocomplexes were resolved in a 12.5% SDS polyacrylamide gel. Radioactive detection and analysis were performed with a Molecular Dynamics Phosphoimager and Image Quant software version 3.3.

p24 assay

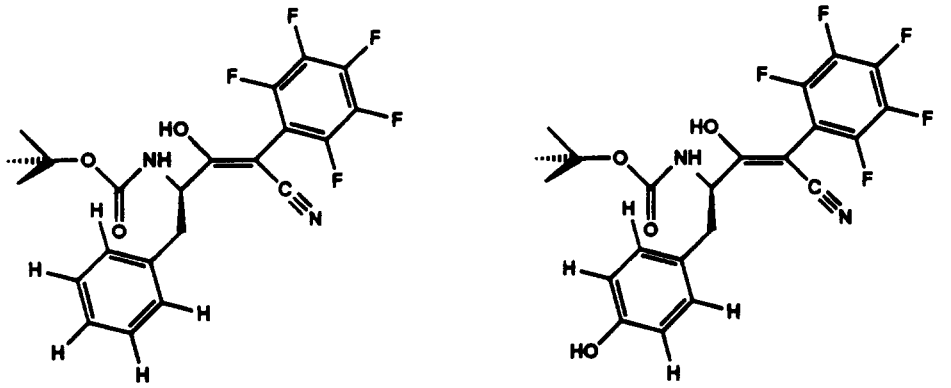
SVC21 transfected COS cell supernatants were collected 48 h post-transfection. HIV p24 was measured using the Coulter HIV-1 p24 ELISA kit according to the manufacturer's instructions.

3. Results and discussion

HIV-1 peptide-based protease inhibitors exhibit low oral absorption and poor stability in vivo (Tomasselli et al., 1991). Studies aimed at increasing their pharmacokinetic properties are currently underway. Recently, there has been a growing interest in protease inhibitors which are thought to be more bioavailable and more biostable than their peptide-based counterparts. To date, two non-peptidic retroviral protease inhibitors, cerulenin and haloperidol (Blumenstein et al., 1989; Desjarlais et al., 1990), have been described. They show high cytotoxicity and cannot be used as HIV-1 antiviral agents in vivo. Recently, non-peptidic cyclic ureas have been reported to inhibit the viral protease with K_i values in the nanomolar range (Lam et al., 1994). These compounds have peak plasma concentrations 15–20 times higher than their K_i values, but exhibit poor stability in rats when orally absorbed. These results emphasize the need to improve the oral stability of the non-peptide-based inhibitors.

3.1. Cytotoxicity studies

Structure-activity relationship of VS-215 and VS-261 (Fig. 1) was evaluated in vitro using recombinant HIV-1 protease as previously described (Vaillancourt et al., 1993,1994). The non-peptidic inhibitors possess an analog isostere of the tetrahedral transition state intermediate and share some inhibitory characteristics with the C_2 symmetric inhibitor class (Fig. 1). Their $K_{i_{app}}$ and IC_{50} values (in vitro evaluation) are listed in Table 1. Cytotoxicity evaluation of VS-215 and VS-261 was evaluated in three



VS-215 **VS-261**

Fig. 1. Structure of VS-215 and VS-261. Simple *N*-protected amino acid enols achieved in a one step chemical synthesis generate potent inhibitory potential of the retroviral protease. VS-215: 4- (*tert*-butoxycarbonylamino)-3-hydroxy-5-(phenyl)-2-(pentafluorophenyl)-2-pentenenitrile. VS-261: 4- (*tert*-butoxycarbonylamino)-3-hydroxy-5-(4-hydroxy-phenyl)-2-(pentafluorophenyl)-2-pentenenitrile. The active moiety interacting with the two aspartic acids of the catalytic site is thought to be the hydroxyl of the enol.

different cell types: human CD4 + T lymphocytes (MT4 and CEM), human monocytic cells (U937) and African green monkey kidney cells (COS). We observed that regardless of the cell line, compounds VS-215 and VS-261 exhibited similar cytotoxicity levels at

Table 1
In vitro inhibitory potential, cytotoxicity potential and antiviral evaluation of VS-215 and VS-261. Data are expressed in μM. All the experiments were performed in duplicate. The intra-variation assay for the viable cellular count performed with the Trypan blue exclusion dye was calculated at $11 \pm 6\%$ while the inter-variation assay was calculated at $24 \pm 7\%$. $K_{i_{app}}$ and IC_{50} were evaluated using a synthetic peptide as described in Vaillancourt et al. (1994).

	VS-215	VS-261
HIV-1 protease inhibition		
$K_{i_{app}}$	1.49	0.64
IC_{50}	19	3.5
Cellular toxicity (TC_{50}) [a]		
MT4	61	73
CEM	77	77
U_{937}	100	67
COS	130	> 200
Antiviral activity		
ID_{50}	24	43
PI [b]	0.79	0.09
TI [c]	2.5	1.8

[a] TC_{50} were measured at day 4 except for COS cells where they were measured at day 2.
[b] Permeability index is calculated by the ratio IC_{50}/ID_{50}.
[c] Therapeutic index is calculated by the ratio TC_{50}/ID_{50}.

day 4 of treatment (Table 1). Their TC_{50} varied from 61 to 100 μM and 67 to 77 μM in MT4, CEM and U937 cell lines, respectively. These data are comparable to previously reported peptide-based HIV-1 protease inhibitors (Kageyama et al., 1992; Roberts et al., 1990; Sham et al., 1991). Cytotoxicity of VS-215 and VS-261 was evaluated at day two in COS cells and generated TC_{50} of 130–200 μM, respectively. The difference in TC_{50} observed in COS cells may reflect the shorter treatment time or the reduced susceptibility of these cells to the cytotoxic effect of these compounds.

3.2. Drug associated protection assays

The close relationship between the concentrations that inhibit enzyme activity in vitro and the cytotoxic concentrations led us to analyze the effect of the compounds on HIV-1 induced cytopathic effects. MT4 cells were infected for 2 h with the UHC-1 viral strain at a MOI of 0.1. After several washes, cells were incubated in presence of various concentrations of drugs. When the infected MT4 cells were incubated without drugs, large syncytia were observed (data not shown). However, examination of the cells at concentrations of 20 and 40 μM of VS-215 and VS-261 respectively indicated no detectable changes in the cells morphology as compared to the non-infected controls without drug. Also, a decreased in syncytium formation was seen for both compounds, suggesting that the drugs impair viral cytopathic effect without altering the cellular morphology. However drug-associated cytotoxicity was evident for both compounds at TD_{50} values (60 and 70 μM for VS-215 and VS-261 respectively) when compared to the non-infected control. Overall, the addition of increasing concentrations of VS-215 and VS-261 to infected MT4 cell cultures clearly showed a decrease of syncytium formation.

Effective compounds that inhibit viral replication are thought to increase cellular survivability of HIV-1 infected cells by preventing virus-induced cytopathic effects and cell death. In a second experiment, we analyzed the protective effect of VS-215 by monitoring cellular viability of infected MT4 cells 36 h after the appearance of syncytia. Cellular viability is expressed as percentage of the non-infected MT4 cells in the presence of increasing concentration of drugs. The data in Fig. 2 indicate that at low drug concentrations (< 10 μM), viral cytopathicity is predominant as VS-215 does not impair viral replication at such concentrations. At concentrations ranging from 20 to 40 μM, we observed an increase in the protective effect of the drug as cell viability was similar to the one obtained in the non-infected control. It is likely that this protective effect is the result of impairment of virus replication as VS-215 was not cytotoxic at these concentrations. At higher drug concentrations (> 50 μM), there was no protective effect as the cytotoxicity of VS-215 was predominant. The bell-shaped curve generated by VS-215 suggests that at non toxic concentrations VS-215 protects the cell culture from HIV-induced cytopathic effects. We have also assessed the cytotoxic effect on MT4 cells in stress conditions. 1000 cells were incubated for 9 days in 5 ml RPMI-1640, 10% FCS, 1% pen/strep in a 60-mm petri dish in presence of VS-215 (growth efficiency, Fig. 2). We observed no drug-associated cytotoxicity at concentrations lower than 30 μM. However, the cells were all dead at 50 μM. These data indicate no observed cytotoxicity at inhibitory concentrations (ID_{50} of 24 μM). Next, we assessed

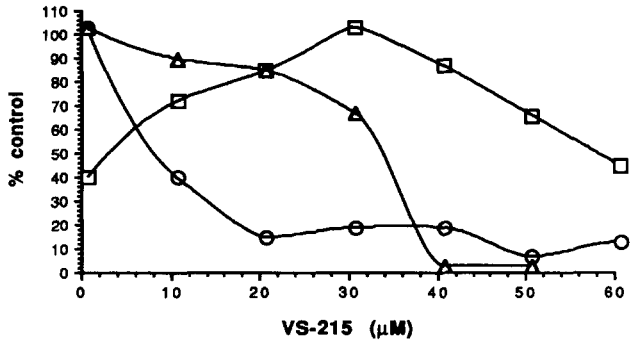


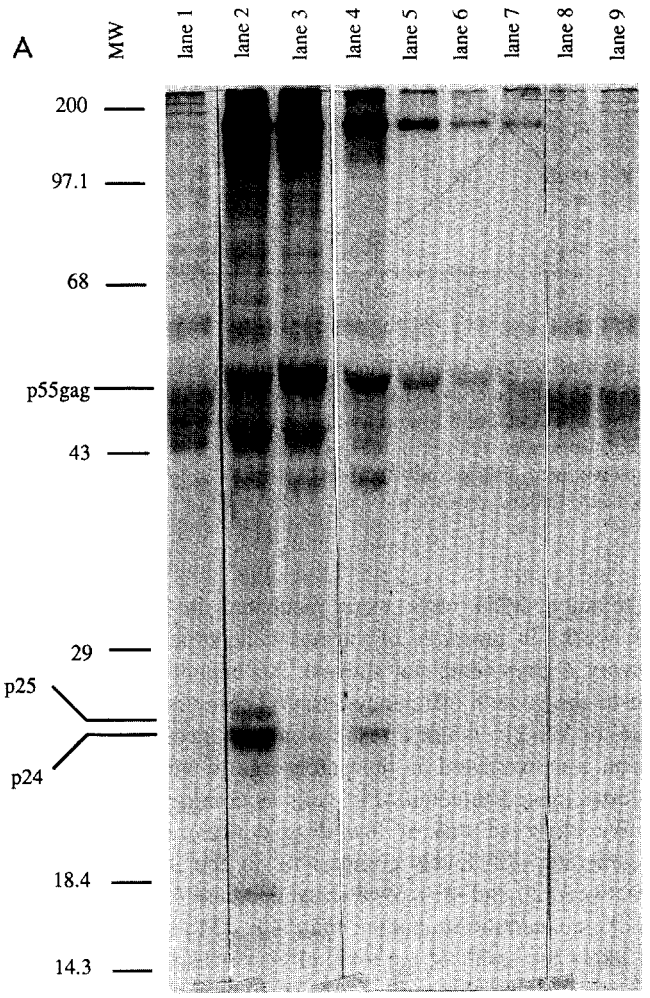
Fig. 2. Protective effect of VS-215 on HIV-1 induced syncytia formation: viable cell count (□) and RT activity (○) monitored 36 hours after syncytia formation in non-treated infected MT4 cells. MT4 cells were infected with HIV-1 virus at a MOI of 0.1 in presence of various concentrations of VS-215. Survivability was analysed by counting viable cells with trypan blue exclusion dye. Growth efficiency study (△): 1000 cells were incubated in 60-mm petri dish in 5 ml RPMI-1640, 10% FCS, 1% pen/strep containing the indicated concentrations of VS-215. Cellular viability was analyzed by counting viable cells at day 9 with trypan blue exclusion dye.

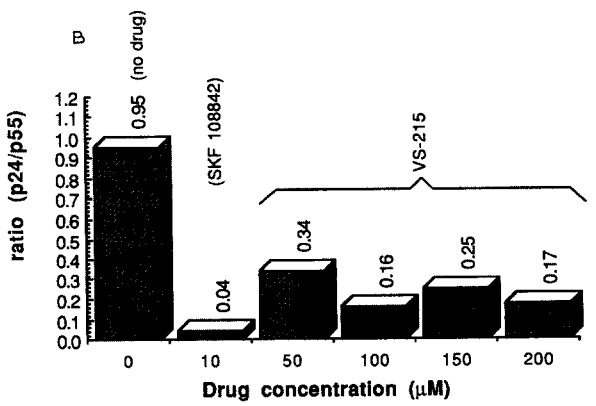
the long-term toxicity. MT4 cells were incubated in RPMI-1640, 10% FCS, 1% pen/strep for 6 weeks in presence of various concentrations of VS-215. Viable cells were counted every 4 days (data not shown). No enhanced cytotoxicity was observed when compared to the growth efficiency assay. These results strongly suggest that the antiviral potential of VS-215 is not a result of a non-specific cytotoxic artifact.

Viral inhibition was evaluated as the percentage of virion-associated RT activity in supernatants of drug treated infected MT4 cells as compared to the non-treated infected cells (Fig. 2). We observed that at low drug concentrations (0–10 μM), the virus replicates and generates RT levels comparable to the untreated infected control. At concentrations between 20 and 40 μM, a sharp decrease of reverse transcriptase activity was measured. At concentration higher than 40 μM the sustained decrease of RT activity is most likely the result of the toxic effect of the drug. Overall the result of this experiment indicates that at non toxic concentrations the enol-based inhibitors have a protective effect on the infected MT4 culture by altering viral replication and spread.

3.3. Analysis of viral-associated polyprotein precursors in transfected COS cells

In order to determine whether the antiviral effects of VS-215 was the result of inhibition of the viral protease, we have analyzed viral-associated polyprotein maturation in COS cells transfected with the HXB_c2 infectious molecular clone of HIV-1 (SVC21) (Fig. 3). Even though VS-261 exhibit a higher anti-protease activity in vitro when compared to VS-215, we have chosen the latter one for this study. Indeed, VS-261 contains a tyrosine residue at position P1 which has been shown in peptidic HIV-1 protease inhibitors to result in a significant decrease of antiviral activity even though K_i values indicated an increased inhibition potential (Thompson et al., 1992). Our preliminary data seemed to suggest a similar phenomenon.


A
MW
lane 1
lane 2
lane 3
lane 4
lane 5
lane 6
lane 7
lane 8
lane 9
200
97.1
68
p55gag
43
29
p25
p24
18.4
14.3


B
ratio (p24/p55)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.95
(no drug)
0.04
(SKF 108842)
0.34
0.16
0.25
0.17
VS-215
0
10
50
100
150
200
Drug concentration (μM)

Radiolabelled viral proteins extracted and immunoprecipitated from HXBc2 transfected COS cells treated with 50, 100, 150 and 200 μM of VS-215 were analyzed on SDS-PAGE. Compound SKF 108 842, a competitive peptide-based HIV-1 protease inhibitor from Smith Kline Beecham, was reported to completely inhibit gag and gag-pol polyprotein processing in cell cultures at a concentration of 10 μM (Dreyer et al., 1992). The latter compound was used at 10 μM as an inhibitory control of the processing. The effect of VS-215 on the processing of the gag and gag-pol polyproteins is shown in Fig. 3A. Efficient processing of p55gag can be observed in non-treated infected cell lysates (lane 2) while addition of SKF 108 842 completely inhibited cleavage of the gag precursor (lane 3). Treatment of transfected cells with VS-215 with increasing concentrations of VS-215 (lanes 4, 5, 6 and 7: 50, 100, 150 and 200 μM respectively) resulted in a decrease of the total level of immunoprecipitable viral protein, most likely as a consequence of the drug cytotoxicity. However, polyprotein processing was still evident as p55gag and its cleavage products (p24, p17) could be detected. At 50 μM of VS-215 (lane 4), unprocessed p55gag and concomitant reduction of p24 resulting from protease activity impairment was detected. This effect was more evident at 100 μM (lane 5) and at higher concentrations (150 and 200 μM, lanes 6 and 7, respectively). Quantitative determination of p24 and p55gag levels was performed using a Molecular Dynamic and the Image Quant software version 3.3. Retroviral protease inhibition is expressed by calculating p24/p55gag ratios for each concentrations of VS-215. These results are shown in Fig. 3B. The ratio of the positive control is evaluated at 0.95, indicating that approximately half of the p55gag is cleaved to P24 in the cell lysate. SKF 108 842 which generated a ratio of 0.04 completely inhibit the polyprotein processing of the p55 precursor. In the case of VS-215, quantification of the p24/p55 ratios generated values of 0.34 at 50 μM and a mean value of 0.20 at higher concentrations. These data suggest that at high concentrations (> 100 μM), polyprotein processing inhibition can hardly be achieved as cytotoxicity is predominant. Nevertheless, the data indicates that VS-215 is able to interact with the retroviral enzyme.

3.4. Antiviral evaluation

The direct effect of the enol-based compounds on viral replication led us to evaluate their antiviral potential at non-toxic concentrations in MT4 cells. Infected cells in absence of drugs and non-infected MT4 cells were used as positive and negative controls. MT4 cells were infected with the UHC-1 viral strain at a M.O.I. of 0.1 in the

Fig. 3. Inhibition of polyprotein processing by VS-215. (A) Immunoprecipitation of HXBc2-transfected COS cell. 24 h post-transfection, transfected COS cells under treatment with various concentrations of VS-215 were metabolically radiolabelled with [^{35}S]cysteine after 6 h exposure to the drug prior to labelling. Then the cells were lysed and immunoprecipitated using an HIV-1-positive human polyclonal antibody 162 (Lavallée et al., 1994). Proteins were separated through a 12.5% SDS-PAGE gel. The standard molecular weights are shown on the left side. Lane 1, non-transfected COS cells without drug; lane 2, transfected COS cells without drugs, lane 3, transfected COS cells treated with SKF 108 842 at 10 μM; lane 4, 5, 6 and 7, transfected COS cells treated with 50, 100, 150 and 200 μM of VS-215, respectively; lane 8 and 9, non-transfected cells in presence of VS-215 and SKF 108 842 at 200 and 10 μM, respectively. (B) Quantification of p24 and p55. Bands were integrated by computer assisted analysis using a Molecular Dynamic phosphoimager apparatus. Ratios were calculated as p24/p55gag.

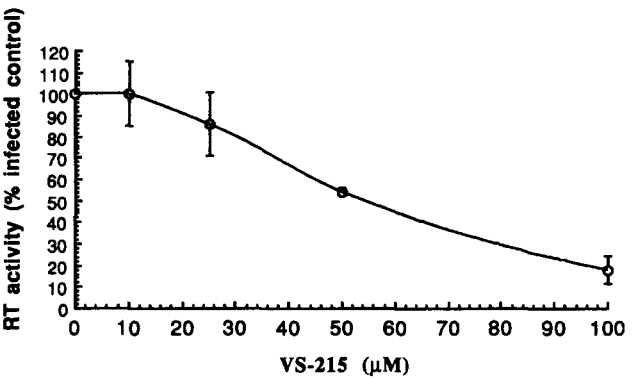


Fig. 4. RT activity monitored in MT4 cell supernatants in reinfection assays. Supernatants from HXBc2 transfected COS cells treated with VS-215 at 10, 25, 50 and 100 were collected 72 h post-transfection and used as infection materials in MT4 cells. MT4 cells were infected with an equivalent amount of p24 as measured by ELISA and incubated without drug (5500 ng/ml). RT activity was monitored in supernatants from infected MT4 cells 96 h post-infection. Results are expressed as percent inhibition compared to the no drug controls (transfected COS cells without drug).

presence of VS-215 and VS-261 concentrations varying from 10 to 75 μM. The replication of the virus was monitored by measuring RT activity every 24 hours for four days. Results of the antiviral activity of both compounds at day four are listed in Table 1. VS-215 and VS-261 inhibited viral replication in infected MT4 cells by 50% (ID_{50}) at concentrations of 24 and 40 μM respectively.

Since the concentrations required to completely inhibit polyprotein processing were not achievable in cell systems, we asked whether COS cells treated with non-toxic drug concentrations could produce non-infectious viruses. Indeed, Kaplan and co-workers (1993) have previously reported that partial inhibition of the retroviral protease result in a total block of the spread of infection in vitro. COS cells were transfected with SVC21 in the presence of VS-215 at concentrations below TD_{50} (10, 25 and 50 μM and 100 μM). 48 h post-transfection, cell-free supernatants were collected and level of p24 antigens was determined as described in Materials and Methods. MT4 cells were then infected with equivalent amounts of virions produced by drug-treated SVC21-transfected COS cells as measured by p24 assay (5500 pg/ml). Viral replication was allowed as infection and replication of MT4 cells was performed in absence of drug. Four days after the infection, RT activity was measured in the MT4 cells supernatant as shown in Fig. 4. Results are expressed as percentage of RT activity compared to the non drug-treated transfected COS cell supernatant that infected MT4 cells. The results indicate that as VS-215 concentration increased, infectivity of the virus produced by the COS cells decreases. At 50 μM of VS-215, a diminution of 54% of RT activity was measured in the MT4 target cells. A similar value was obtained when transfected COS cells were treated with 1 μM of SKF 108 842. At 100 μM of VS-215, RT activity reached its lower level at 15% and is thought to be representative of maximum inhibition. These results indicate that partial inhibition of HIV protease results in the formation of immature viral particles that are non-infectious.

These results led us to determine the properties of our enol-based compounds. The

permeability index (PI) is an indication of the potency of these drugs to penetrate cellular membranes and to act directly on its target. The PI are determined by calculating the ratio IC_{50}/ID_{50} from the results presented in Table 1. Indexes of 0.79 and 0.09 were obtained for VS-215 and VS-261 respectively. The VS-215 PI value suggests that this compound has the capacity to cross cell membranes with almost a one to one inhibition ratio. Even though VS-261 has a much lower PI value, it is comparable to or higher than the PI value of reported peptide-based protease inhibitors' PI (Martin, 1991). The decrease in the measured PI of VS-261 is also in agreement with results previously reported with peptide-based inhibitors containing a tyrosine at position P1 (Thompson et al., 1992).

Another important parameter is the therapeutic index (TI, Table 1). This parameter is calculated by the ratio TC_{50}/ID_{50}. In the case of our inhibitors, VS-215 and VS-261 generated therapeutic indexes of 2.5 and 1.8 respectively. These very low indexes are indicative of the necessity to improve the inhibitory potential of these compounds. Synthesis of novel drugs are currently underway to increase the inhibitory potential of this new class of HIV protease inhibitors.

4. Conclusions

In this paper we have described the usefulness of enol-based compounds in biological systems. VS-215 was able to impair the viral p55gag maturation in transfected COS cells. It was able to protect infected MT4 cells from the cytopathic effect of HIV-1 and was also able to block viral spread at concentrations lower than its TD_{50}. These results are important in the design of future compounds. By ruling out the possibility of a generalized cytotoxicity artefact, we clearly demonstrate that new modifications to increase the inhibitory potential of the enol-based compounds will generate promising future drugs.

Acknowledgements

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