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Articles

Synthesis and in Vitro Anti-HIV Activity in Human Monocyte-Derived Macrophages of 2-Oxothiazolidine-4(*R*)-carboxylic Acid Derivatives

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Oxidative stress and glutathione (GSH) deficit may play an important role in HIV infection pathogenesis, and oral administration of GSH-replenishing drugs such as *N*-acetylcysteine (NAC) and 2-oxothiazolidine-4(*R*)-carboxylic acid (OTC) may be associated with an increased survival rate of HIV-infected patients. Nevertheless, beneficial effects of these molecules are restricted in vivo by the high concentrations that are necessary to obtain biological effects, rapid extracellular metabolization, and low availability and plasma concentrations. We synthesized OTC derivatives that are more lipophilic than OTC and theoretically able to overcome these limitations and to generate, in addition to cysteine, other substrates of the γ-glutamyl cycle. Their antiviral effects were investigated in human HIV-1/Ba-L-infected monocyte-derived macrophages. In our experimental conditions, OTC exhibited anti-HIV-1 effects and little cytotoxicity at high doses. None of the nine tested derivatives showed higher cytotoxicity than OTC, nor anti-HIV-1/Ba-L activity.

Introduction

The production of highly reactive oxygen intermediates (ROI) is counter-balanced by antioxidants in physiological conditions, and oxidative stress results from an imbalance between pro- and anti-oxidant molecules. Glutathione (GSH) is a cysteine-containing tripeptide (γ-glutamylcysteinylglycine) found in all eukaryotic cells. It is synthesized and degraded intracellularly, mainly via its reduced form. GSH participates in several cell functions, such as synthesis of proteins and DNA precursors, amino acid transport, and maintenance of sulfhydryl redox status. It also plays a central role in the antioxidant defense system, protecting cells against free radicals and toxic compounds of endogenous and exogenous origin.[1]

HIV-infected individuals have decreased levels of acid-soluble thiols, particularly GSH, in tissue, e.g. lung and central nervous system (CNS), in plasma, and in CD4+ T lymphocytes and monocytes.[2−5] This GSH deficit and the oxidative stress that follows may participate in human immunodeficiency virus (HIV)[6] infection pathogenesis, by enhancing viral replication, CD4+ T lymphocyte deficiency,[7] inflammatory syndrome, apoptosis, chronic weight loss, and drug toxicities and by decreasing immune cell proliferative response and immune functions.[8,9] HIV replication is probably potenti-

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ated through NF-κB transcription factor activation[10] and subsequent induction of inflammatory cytokines, e.g. tumor necrosis factor α (TNF-α).[11]

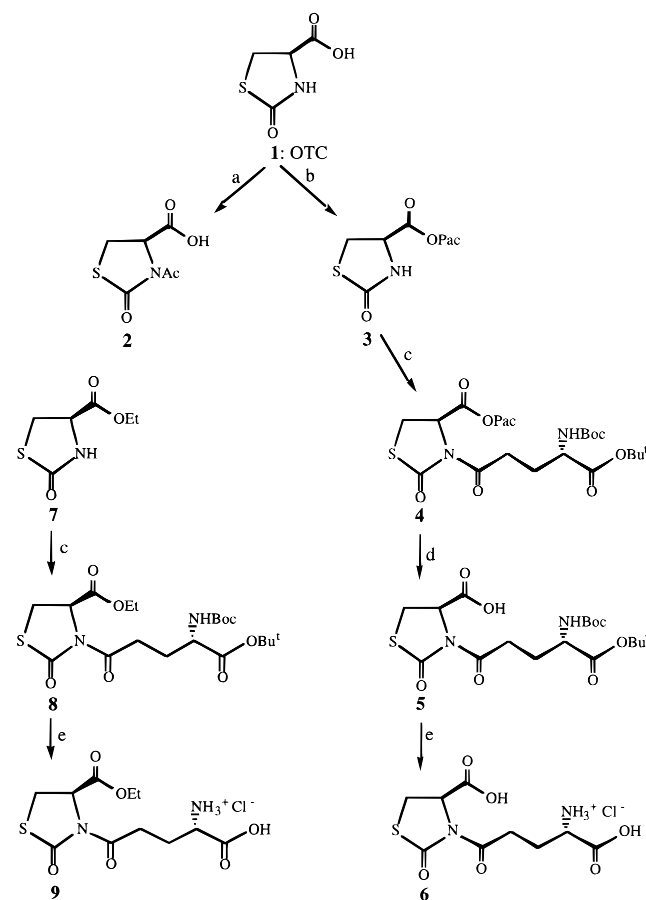
Administration of *N*-acetylcysteine (NAC) increases GSH levels and is associated with decreased mortality in HIV-infected patients.[12] In vitro, GSH and NAC inhibit viral replication in monocyte-derived macrophages (MDM) and/or lymphocytes.[13−15] These compounds suppress the activation of the transcription factor NF-κB. However, the direct inhibition of the reverse transcriptase (RT) activity may be an alternative mechanism.[16] As a consequence, beneficial effects may be expected by compensating GSH levels in HIV-infected patients. Nevertheless, the efficiency of GSH and NAC is restricted by the high concentrations that are necessary to obtain biological effects,[11] their rapid oxidation and hydrolysis by intestinal and hepatic γ-glutamyltransferase,[17] and their low oral bioavailability.[18,19] Moreover, the rate of intracellular GSH synthesis is also limited by the availability of cysteine. 2-Oxothiazolidine-4-carboxylic acid (OTC, procysteine) is converted to cysteine by the intracellular 5-oxoprolinase enzyme[20] and is able to overcome these limitations. Indeed, oral OTC administration results in effective intracellular delivery of cysteine and increased cellular GSH.[20−22] Nevertheless, in the light of the short half-life of OTC,[23] OTC derivatives were synthesized, and their in vitro antiretroviral activity was investigated in primary cultures of HIV-1/Ba-L-infected MDM. Theoretically, these derivatives are more lipophilic and therefore could be less susceptible than OTC to extracellular metabolization. They could generate, in addition to cysteine, one or two other substrates of the γ-glutamyl cycle, i.e. glycine and glutamic acid, and so could facilitate GSH synthesis. Antiviral effects on macrophages were evaluated because these cells or related cells such as microglia are the major source of ROI and have a powerful antioxidant system which is supported by efficient uptake of cysteine.[24] They are one of the main HIV targets involved in the inflammatory process and AIDS encephalopathy[25,26] and alter GSH level in response to HIV infection.[27]

Chemistry

The syntheses of compounds **2**, **3**, **5**−**7**, and **9** are outlined in Scheme 1. Compound **2** has been synthesized previously by heating of **1** with acetic anhydride in 52.1% yield, but two other byproducts [2-oxo-3-(2′-oxothiazolidin-4′(*R*)-ylcarbonyl)thiazolidine-4(*R*)-carboxylic acid and 1,4,5,8-tetraoxo-2,6-dithia-4a,8a-diazaperhydro-*s*-indacene] were also isolated by fractional recrystallizations.[28] To increase the yield of **2**, and also to simplify isolation, we introduced the *N*-acyl group by treatment of **1** in acetone, with acetyl chloride in the presence of TEA. Thus, **2** was easily obtained after flash chromatography and crystallization in 82% yield.

For the synthesis of **6**, we used the phenacyl ester as a temporary protecting carboxyl function of **1**, to avoid the side reactions during subsequent coupling. Thus, **3** was obtained by reaction of phenacyl bromide with **1** in the presence of TEA according to a classical method.[29−32] The preparation of **4** was accomplished in DMF for 2 days at 50 °C, by coupling of **3** with active ester Boc-L-Glu(OSu)-OBu^t in the presence of TEA and catalytic

Scheme 1[a]



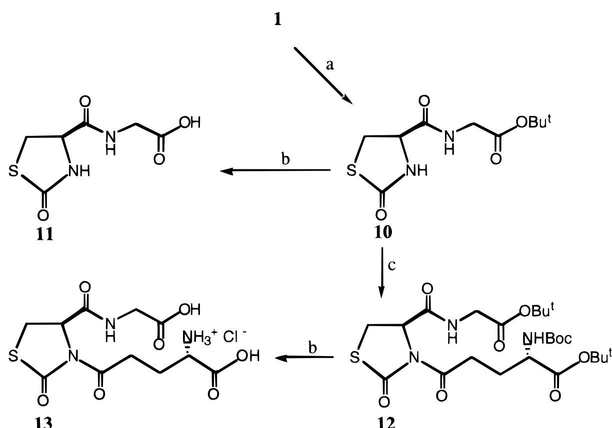
[a] Reagents: (a) TEA, acetone, then acetyl chloride, 0 °C to rt; (b) phenacyl bromide, TEA, DMF, rt; (c) Boc-Glu(OSu)-OBu^t, TEA, DMF and DMAP for 4, rt to 50 °C; (d) AcOH, H_2O, Zn, rt; (e) 4 M HCl/AcOH, 0 °C.

DMAP. It is noteworthy that the use of DMAP is essential to maximize the yield of this coupling. The phenacyl ester was removed, according to the previously described method,[33,34] by adding a severalfold molar excess of zinc powder at room temperature to a solution of **4** in dilute aqueous acetic acid. The resulting pseudo-dipeptide **5** was then deprotected at 0 °C by treatment with 4 M HCl in glacial acetic acid to give pure **6** as the hydrochloride salt.

In a similar sequence to that described above, **9** was prepared from OTC ethyl ester **7** which was obtained, according to a published procedure, [35] by one-pot reaction of L-cysteine ethyl ester with 1,1′-carbonyldiimidazole. Briefly, **7** was coupled with Boc-L-Glu(OSu)-OBu^t in the presence of TEA to give **8**. N,O-protecting groups of glutamyl residue were then removed to afford the expected compound as the hydrochloride salt.

The synthetic strategy for pseudo-dipeptides **10** and **11** and pseudo-tripeptide **13** is illustrated in Scheme 2. Condensation of **1** with Gly-OBu^t·HCl in DMF and TEA by the DCC/HOBt method[36] gave the desired derivative **10**. Removal of the *tert*-butyl protecting group with 4 M HCl in glacial acetic acid (30 min/0 °C, 2.5 h/room temperature) followed by lyophilization and trituration with ether afforded pure **11**. Through a reaction similar to that used for the preparation of **9**, **10** was coupled in acetonitrile with Boc-L-Glu(OSu)-OBu^t in the presence of TEA (1 h/0 °C, 12 h/room temperature) to give **12**.

Scheme 2[a]



[a] Reagents: (a) HOBt, DCC, DMF, 0 °C, then Gly-OBu^t·HCl, TEA/DMF, 0 °C to rt; (b) 4 M HCl/AcOH, 0 °C to rt; (c) Boc-Glu(OSu)-OBu^t, TEA, acetonitrile, 0 °C to rt.

Final compound **13** was isolated, as the hydrochloride salt, after removal of Boc and Bu^t protecting groups of glycyl and glutamyl residues, as already described.

Biological Results and Discussion

The redox imbalance or oxidative stress associated with HIV infection may be one of the mechanisms by which this retrovirus promotes its own replication. Moreover, this oxidative stress plays a part in pathogenesis and in survival impairment in HIV disease.[12] This supports the in vitro evaluation of effects on cell survival and viral replication of nine OTC derivatives. The anti-HIV effects of compounds **3**, **5**−**7**, **9**−**11**, and **13** were compared to those of OTC and those of compound **2** to those of OTC and NAC. This N-acetylated OTC derivative compound is able to generate NAC (Scheme 1).

HIV-1/Ba-L replicated efficiently in MDM (Figure 1) as previously described.[37] Cell death was observed in these HIV-1/Ba-L-infected MDM cultures when viral replication was maximal between 10 and 14 days after infection.[37] In these experimental conditions, OTC and NAC induced slight cytotoxicity in uninfected cells when used at high doses, 20 and 15 mM, respectively. These two molecules dose-dependently inhibited HIV-1 replication (Figures 1, 2) and favored cell survival of HIV-1/Ba-L-infected MDM. The ED_{50} was 7.5 and 2 mM for NAC and OTC, respectively. These results are in accordance with those of Ho et al.[38,39] and Lioy et al.[40] As also observed by Ho et al.,[38,39] the synthesis of HIV provirus was reduced by NAC (60% inhibition with 0.5 mM NAC compared with untreated control). On the other hand, no inhibition of RT activity was observed in an acellular system (data not shown). NAC significantly increased HIV replication in one experiment (Figure 3A,B). These deleterious effects may be related to an increased HIV particle production, i.e. 15 mM NAC dose (Figure 3A). Nottet et al.[41] associated these effects with hydroxyl radical overexpression and Chen et al.[42] with cell−cell interactions. In our experiment with this blood cell donor, contaminating lymphocytes persisted in MDM cultures and may have favored this increase in viral replication.

None of the new tested derivatives showed higher cytotoxicity nor higher anti-HIV-1/Ba-L activity than the reference compounds (Table 1). Neither the chemical modifications nor the delivery of additional substrates to the γ-glutamyl cycle increased cytotoxicity or antiviral activity. On the contrary, compound **13**, which theoretically released most γ-glutamyl cycle substrates by generating OTC, glycine, and glutamic acid, increased viral replication (cumulative RT activity: compound **13** 159 ± 45% of untreated control vs untreated control 100 ± 12%).

Taken together, these data do not support the use of these GSH-replenishing drugs as monotherapy in HIV infection. Nevertheless, these drugs are of value in adjuvant therapy of HAART,[43] because of their probable beneficial effects in restoration of the immune system and their ability to attenuate HIV-associated inflammatory disorders and to increase survival rate of HIV-infected patients.[12] Consequently, synthesis and antiviral evaluation of new cyclized derivatives with greater bioavailability are now in progress.

Experimental Section

Chemical Methods. Melting points were determined in a Büchi capillary melting point apparatus, and are uncorrected. IR spectra were recorded on a Beckman Acculab 4 spectrophotometer; absorbances are reported in ν (cm^{-1}). NMR spectra were recorded on a Varian EM 390 spectrometer unless otherwise stated, in which case a Bruker AC 250 instrument was used. Chemical shifts are reported in ppm and given in δ units relative to TMS as internal standard. Specific optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by the Service de Microanalyse de l'Ecole Nationale Supérieure de Chimie de Montpellier and were within ± 0.4% of calculated values. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates. Spots were visualized by ultraviolet light (254 nm), by iodine vapor, or by spraying with ninhydrin solution. Flash column chromatography was conducted with Merck silica gel 60 (230−400 mesh ASTM). All the solvents used were dried and purified in the usual manner. OTC was from Aldrich. Amino acid derivatives were from Bachem.

3-Acetyl-2-oxothiazolidine-4(*R*)-carboxylic Acid (2). A stirred solution of 2-oxothiazolidine-4(*R*)-carboxylic acid (OTC, **1**; 1.47 g, 10 mmol) and triethylamine (TEA; 3.63 mL, 26 mmol) in acetone (50 mL) was cooled to 0 °C and then treated dropwise with 0.782 mL of acetyl chloride (11 mmol). The reaction mixture was stirred for 1 h at 0 °C and at room temperature over 12 h. The alkaline solution was then reduced to an oil by evaporation in vacuo. This oil was taken up in water (10 mL), acidified to pH 3 with 2 M HCl, and extracted with ethyl acetate (5 × 30 mL). The combined extracts were dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The crude product was then purified by flash column chromatography (eluent: CH_2Cl_2/MeOH/AcOH, 9.5:0.4:0.08) and crystallized from ethyl acetate/petroleum ether to afford 1.56 g (82%) of **2** as white crystals: R_f 0.6 (CH_2Cl_2/MeOH/AcOH, 8:2:1); mp 153−154 °C; $[\alpha]_D^{20} = -140.5°$ (*c* 1.8 acetone/H_2O, 1/1) [lit.[28] mp 152−154 °C; $[\alpha]_D^{20} = -136.8°$ (*c* 1.8 acetone/H_2O, 1/1)]; ^{1}H NMR ($CDCl_3$) 2.52 (s, 3H, acetyl C*H*$_3$), 3.41 (dd, *J* = 1.9, 11.8 Hz, 1H, *H*5′), 3.68 (dd, *J* = 8.8, 11.8 Hz, 1H, *H*5), 5.23 (dd, *J* = 1.9, 8.8 Hz, 1H, *H*4), 9.03 (br, 1H, CO_2*H*).

2-Oxothiazolidine-4(*R*)-carboxylic Acid Phenacyl Ester (3). To a stirred solution of **1** (1.61 g, 11 mmol) in DMF (30 mL) were added α-bromoacetophenone (phenacyl bromide; 3.28 g, 16.5 mmol) and TEA (1.53 mL, 11 mmol). A white precipitate formed within a few minutes and the suspension was stirred overnight at room temperature. The reaction mixture was filtered and the filtrate was reduced to an oil by evaporation in vacuo. This oil was diluted with ethyl acetate (200 mL) and washed with water (3 × 100 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The crude product was then crystallized from

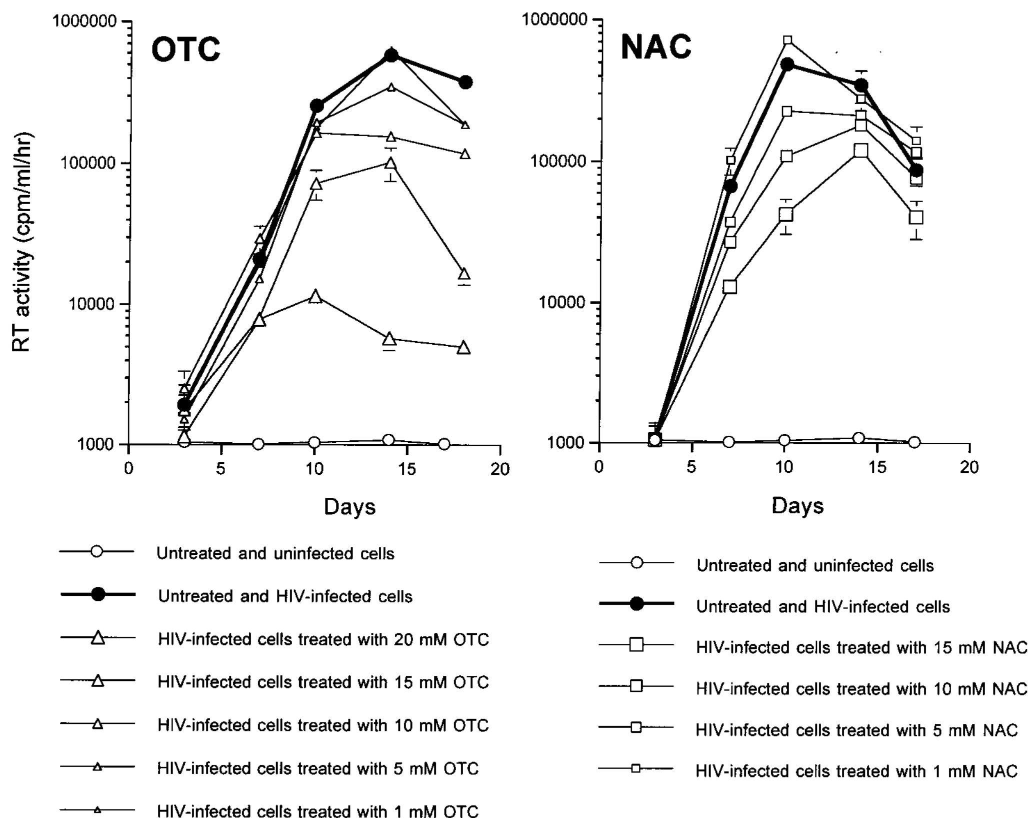


Figure 1. Antiviral activity of NAC and OTC in HIV-1/Ba-L-infected MDM. Results are expressed as mean ± SD and are representative of three (OTC) or four (NAC) blood donors.

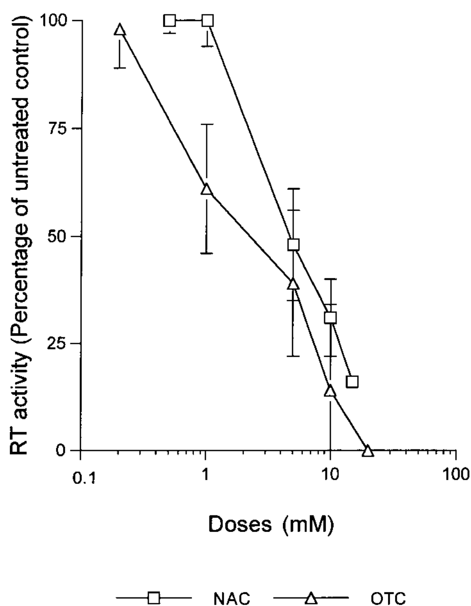


Figure 2. Dose−effect of antiviral activity of NAC and OTC in HIV-1/Ba-L-infected MDM. Cumulative RT activities were considered to calculate the percentages of untreated control. Results are expressed as mean ± SD and are representative of three (OTC) or four (NAC) blood donors.

ethyl acetate/petroleum ether to afford 2.62 g (90%) of **3** as white needles: R_f 0.6 (ethyl acetate/petroleum ether, 7:3); mp 124−125 °C; $[\alpha]_D^{20}$ = −29.9° (*c* 1.17 $CHCl_3$); IR (Nujol mull) 3200, 1755, 1675, 1655, 1445, 1205; ^{1}H NMR ($CDCl_3$) 3.78 (dd, *J* = 7.7, 11.4 Hz, 1H, *H*5), 3.83 (dd, *J* = 5.5, 11.4 Hz, 1H, *H*5′), 4.64 (ddd, *J* = 0.9, 5.5, 7.7 Hz, 1H, *H*4), 5.39 (d, *J* = 16.3 Hz, 1H, phenacyl C*H*a), 5.54 (d, *J* = 16.3 Hz, 1H, phenacyl C*H*b), 6.16 (br, 1H, N*H*), 7.46−7.53, 7.59−7.66 and 7.86−7.91 (3m, 5H, Ar*H*). Anal. ($C_{12}H_{11}NO_4S$) C, H, N.

2-Oxo-3-[α-[*N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyl)]-L-γ-glutamyl]thiazolidine-4(*R*)-carboxylic Acid Phenacyl Ester (4). To a stirred solution of **3** (2.65 g, 10 mmol) in DMF (80 mL) were added *N*-(*tert*-butoxycarbonyl)-L-glutamic acid α-*tert*-butyl γ-*N*-succinimidyl ester (4 g, 10 mmol), TEA (1.39 mL, 10 mmol), and 4-(dimethylamino)pyridine (DMAP; 50 mg) at room temperature. The mixture was then heated for 2 days at 50 °C and concentrated to dryness in vacuo. After cooling, the resulting precipitate was filtered and the filtrate was then concentrated to dryness in vacuo. The residual paste was diluted with CH_2Cl_2 (300 mL) and washed consecutively with water, ice-cold saturated aqueous sodium bicarbonate, water, 1 N aqueous citric acid, and water (neutral pH). The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The crude product (oil) was then purified by flash column chromatography [eluent: stepwise gradient of ether (40−70%) in petroleum ether] and crystallized from ether/petroleum ether to afford 2.69 g (65%) of **4** as white needles: R_f 0.4 (ethyl acetate/petroleum ether, 3:7); mp 83−85 °C; $[\alpha]_D^{20}$ = −87.7° (*c* 1.14 $CHCl_3$); IR (Nujol mull) 3380, 1760, 1710, 1690, 1500, 1450, 1365, 1230, 1170; ^{1}H NMR ($CDCl_3$) 1.42, 1.45 (2s, 18H, t- butyl *H*), 1.76−1.93, 2.13−2.30 (2m, 2H, β-Glu C*H*a, C*H*b), 2.91−3.08 (m, 2H, γ-Glu CH_2), 3.72−3.80 (m, 2H, *H*5, 5′), 4.15−4.28 (m, 1H, α-Glu C*H*), 5.10 (d, *J* = 8 Hz, 1H, N*H*), 5.27 (d, *J* = 16.3 Hz, 1H, phenacyl C*H*a), 5.27−5.36 (m, 1H, *H*4 overlapping with the dd at 5.27), 5.65 (d, *J* = 16.3 Hz, 1H, phenacyl C*H*b), 7.43−7.53, 7.57−7.66, 7.84−7.92 (3m, 5H, Ar*H*). Anal. ($C_{26}H_{34}N_2O_9S$) C, H, N.

2-Oxo-3-[α-[*N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyl)]-L-γ-glutamyl]thiazolidine-4(*R*)-carboxylic Acid (5). A solution of **4** (1.5 g, 2.72 mmol) in a mixture of acetic acid (230 mL) and water (40 mL) was stirred at room temperature and Zn powder (22.1 g, 0.34 mol) was then added in one portion. The reaction, followed by TLC, was over in 2 h. The reaction mixture was filtered and the filtrate was evaporated to dryness in vacuo. The crude product was diluted with aqueous 0.1 M

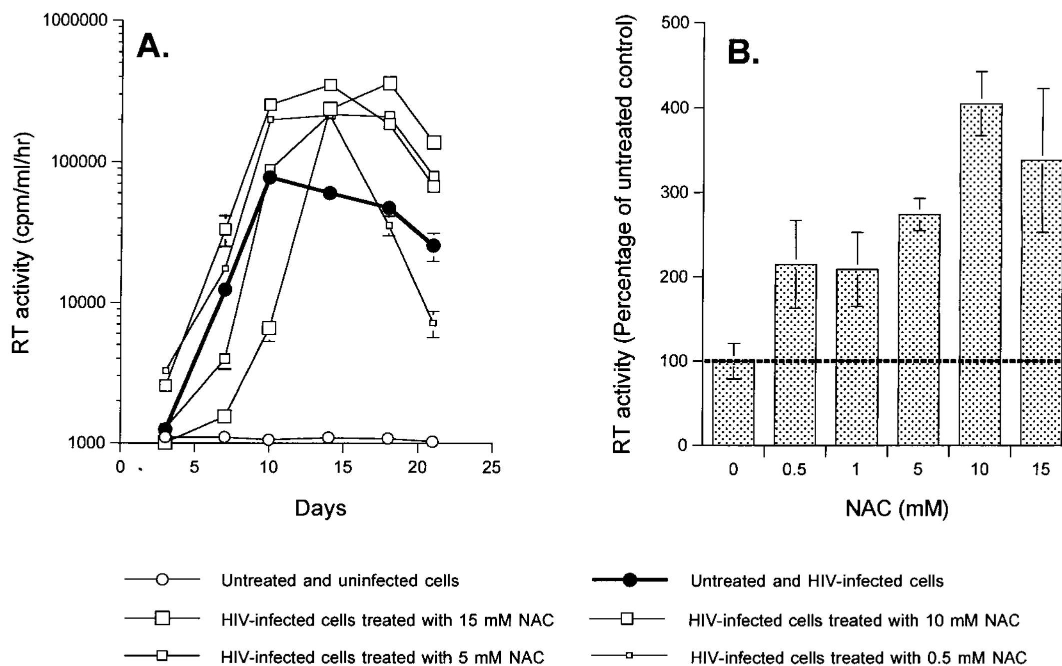


Figure 3. Deleterious effects of NAC on HIV-1/Ba-L replication in MDM (A, kinetic of viral replication; B, dose−effect). Results are expressed as mean ± SD. In panel B, cumulative RT activities were considered to calculate the percentages of untreated control.

Table 1. Antiviral Activity of OTC Derivatives in HIV-1/Ba-L-Infected MDM[a]

compd	percent of untreated control (%)	
	at 0.5 mM	at 2 mM
untreated control	100 ± 7	100 ± 12
NAC		88 ± 5
OTC, **1**	81 ± 16	37 ± 17
2		34 ± 21
3[b]	69 ± 12	
4[c]		
5		102 ± 26
6		83 ± 15
7		86 ± 39
8[c]		
9		37 ± 10
10		104 ± 15
11		45 ± 29
12[c]		
13		159 ± 45

[a] Cumulative RT activities were considered to calculate the percentages of untreated control. Results are expressed as mean ± SD and are representative of two blood donors. [b] Compound **3** was cytotoxic at 2 mM and was tested at 0.5 mM. [c] Compounds **4**, **8**, and **12** were insoluble in solvent concentrations compatible with cell culture.

EDTA disodium salt dihydrate (150 mL) and extracted with ethyl acetate (3 × 80 mL). The organic phases were pooled, dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The crude product was then crystallized from ethyl acetate/petroleum ether to afford 970 mg (82%) of **5** as white crystals: R_f 0.5 (CH_2Cl_2/MeOH/AcOH, 8.8:0.7:0.5); mp 172−174 °C dec; $[\alpha]_D^{20}$ = −61.4° (*c* 1.14 $CHCl_3$); IR (Nujol mull) 3350, 3330−2480, 1755, 1740, 1690, 1660, 1535, 1390, 1220, 1150; ^{1}H NMR (DMSO-d_6 + D_2O) 1.33, 1.35 (2s, 18H, *tert*-butyl *H*), 1.65−2.0 (m, 2H, β-Glu CH_2), 2.70−2.97 (m, 2H, γ-Glu CH_2), 3.32 (dd, *J* = 1.0, 12 Hz, 1H, *H*5′), 3.62−3.86 (m, 2H, *H*5, α-Glu C*H*), 5.05 (dd, *J* = 1.0, 4.7 Hz, 1H, *H*4). Anal. ($C_{18}H_{28}N_2O_8S$) C, H, N.

2-Oxo-3-(L-γ-glutamyl)thiazolidine-4(*R*)-carboxylic Acid Hydrochloride (6). A solution of **5** (970 mg, 2.24 mmol) in 7 mL of 4 M HCl in glacial acetic acid was stirred at 0 °C under nitrogen. The reaction, followed by TLC, was over in 3 h. The hydrochloride was precipitated from the mixture by adding ice-cold anhydrous ether (100 mL), and the ether phase was then decanted. This procedure of washing with ether was performed three times. The crude product was then dried in a vacuum desiccator containing KOH pellets and P_2O_5 to afford 639 mg (91%) of **6** as an analytically pure white powder: R_f 0.4 (acetonitrile/water, 7:3); mp 184−186 °C; $[\alpha]_D^{20}$ = −77.1° (*c* 0.7 MeOH); IR (Nujol mull) 3230−2460, 1725, 1715, 1690, 1585, 1515, 1390, 1230; ^{1}H NMR (D_2O) 2.24−2.48 (m, 2H, β-Glu CH_2), 3.14−3.41 (m, 2H, γ-Glu CH_2), 3.58 (dd, *J* = 2.0, 11.9 Hz, 1H, *H*5′), 3.92 (dd, *J* = 9.0, 11.9 Hz, 1H, *H*5), 4.19 (t, *J* = 6.7 Hz, 1H, α-Glu C*H*), 5.34 (dd, *J* = 2.0, 9.0 Hz, 1H, *H*4). Anal. ($C_9H_{12}N_2O_6S$·HCl) C, H, N, Cl.

2-Oxothiazolidine-4(*R*)-carboxylic Acid Ethyl Ester (7). The synthesis of **7** was carried out as described by D'Ischia et al:[35] yield 90% (oil); R_f 0.4 (ether/petroleum ether, 8:2); $[\alpha]_D^{20}$ = −52.8° (*c* 0.89 $CHCl_3$) [lit.[35] $[\alpha]_D^{20}$ = −55.9° (*c* 2.1 $CHCl_3$)]; ^{1}H NMR ($CDCl_3$) 1.31 (t, *J* = 7.1 Hz, 3H, ethyl CH_3), 3.62 (dd, *J* = 5.5, 11.3 Hz, 1H, *H*5′), 3.71 (dd, *J* = 7.8, 11.3 Hz, 1H, *H*5), 4.27 (q, *J* = 7.1 Hz, 2H, ethyl CH_2), 4.42 (ddd, *J* = 0.8, 5.5, 8.6 Hz, 1H, *H*4), 6.03 (br, 1H, N*H*).

2-Oxo-3-[α-[*N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyl)]-L-γ-glutamyl]thiazolidine-4(*R*)-carboxylic Acid Ethyl Ester (8). This compound was prepared from **7** (2.3 g, 13.1 mmol) in DMF (60 mL), *N*-(*tert*-butoxycarbonyl)-L-glutamic acid α-*tert*-butyl γ-*N*-succinimidyl ester (4.6 g, 11.5 mmol), and TEA (1.83 mL, 13.1 mmol), following the same procedure as described for **4**. The pure product was obtained as a colorless paste (2.7 g, 51%): R_f 0.69 (ether/petroleum ether, 7:3); $[\alpha]_D^{20}$ = −61.2° (*c* 0.98 $CHCl_3$); IR (film) 3380, 1740, 1720, 1705, 1690, 1500, 1370, 1220, 1150; ^{1}H NMR ($CDCl_3$) 1.29 (t, *J* = 7.1 Hz, 3H, ethyl CH_3), 1.42, 1.45 (2s, 18H, *tert*-butyl *H*), 1.75−1.92, 2.13−2.31 (2m, 2H, β-Glu C*H*a, C*H*b), 2.89−3.06 (m, 2H, γ-Glu CH_2), 3.35 (dd, *J* = 1.7, 11.7 Hz, 1H, *H*5′), 3.67 (dd, *J* = 8.6, 11.7 Hz, 1H, *H*5), 4.16−4.36 (m, 3H, ethyl CH_2, α-Glu C*H*), 5.10 (d, *J* = 8.1 Hz, 1H, Glu N*H*), 5.19 (dd, *J* = 1.7, 8.6 Hz, 1H, *H*4). Anal. ($C_{20}H_{32}N_2O_8S$) C, H, N.

2-Oxo-3-(L-γ-glutamyl)thiazolidine-4(*R*)-carboxylic Acid Ethyl Ester Hydrochloride (9). This compound was prepared from **8** (1.62 g, 3.52 mmol) in 19 mL of 4 M HCl in glacial acetic acid, following the same procedure as described for **6**, to afford 1 g (83%) of **9** as analytically pure white crystals: R_f 0.6 (CH_2Cl_2/MeOH/AcOH, 8:2:1); mp 150−152 °C; $[\alpha]_D^{20}$ =

−80.6° (c 0.98 MeOH); IR (Nujol mull) 3400−2450, 1735, 1715, 1700, 1690, 1500, 1370, 1220; ^{1}H NMR (D_2O) 1.30 (t, J = 7.1 Hz, 3H, ethyl CH_3), 2.16−2.37 (m, 2H, β-Glu CH_2), 3.05−3.34 (m, 2H, γ-Glu CH_2), 3.53 (dd, J = 2.0, 12.0 Hz, 1H, $H5'$), 3.85 (dd, J = 8.9, 12.0 Hz, 1H, $H5$), 4.06 (t, J = 6.7 Hz, 1H, α-Glu CH), 4.32 (q, J = 7.1 Hz, 3H, ethyl CH_2), 5.34 (dd, J = 2.0, 8.9 Hz, 1H, $H4$). Anal. ($C_{11}H_{16}N_2O_6S$·HCl) C, H, N, Cl.

***N*-(2-Oxothiazolidin-4(*R*)-ylcarbonyl)glycine *tert*-Butyl Ester (10).** To a cold (0 °C) stirred solution of **1** (3 g, 34 mmol) in DMF (200 mL) were added 1-hydroxybenzotriazole (HOBt; 4.8 g, 35.5 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC; 7 g, 34 mmol). After 30 min of stirring at 0 °C, glycine *tert*-butyl ester hydrochloride (7 g, 41,8 mmol) was added to the mixture, followed by the dropwise addition of TEA (8 mL, 57 mmol) in DMF (10 mL). Stirring was continued at 0 °C for 2 h and at 60 °C for 48 h. After cooling, the mixture was worked up as described for **4**. The crude product was purified by flash column chromatography [eluent: stepwise gradient of MeOH (0−2%) in CH_2Cl_2] to afford 4.1 g (46%) of **10** as a white solid. TLC [R_f 0.4 (CH_2Cl_2/MeOH, 9.5:0.5)] and NMR analysis indicated that the product was over 95% pure and can be used in the next step without further purification.

An analytical sample was obtained, by recrystallization from ethyl acetate and petroleum ether, as white crystals: mp 106−107 °C; $[\alpha]_D^{20}$ = −84.9° (c 0.73 $CHCl_3$); IR (Nujol mull) 3280, 1730, 1680, 1660, 1540, 1360, 1220, 1150; ^{1}H NMR ($CDCl_3$) 1.45 (s, 9H, *t*- butyl H), 3.60 (dd, J = 4.8, 11.4 Hz, 1H, $H5'$), 3.76 (dd, J = 8.7, 11.4 Hz, 1H, $H5$), 3.89 (dd, J = 5.2, 18.0 Hz, 1H, Gly CHa), 4.0 (dd, J = 5.7, 18.0 Hz, 1H, Gly CHb), 4.38 (dd, J = 4.8, 8.7 Hz, 1H, $H4$), 6.84 (br, 1H, NH), 7.03 (br, 1H, Gly NH). Anal. ($C_{10}H_{16}N_2O_4S$) C, H, N.

***N*-(2-Oxothiazolidin-4(*R*)-ylcarbonyl)glycine (11).** A solution of **10** (2.5 g, 9.61 mmol) in 13 mL of 4 M HCl in glacial acetic acid was stirred at 0 °C under nitrogen for 30 min and at room temperature. The reaction, followed by TLC, was over in 3 h. The mixture was then lyophilized. The residual paste was dissolved in distilled water (30 mL), washed with CH_2Cl_2 (2 × 20 mL), and again lyophilized to afford a gum. This was triturated with anhydrous ether to yield pure **11** as an analytically pure white powder (1.89 g, 96%): R_f 0.6 (2-propanol/ammonium hydroxide 30%, 7.5:2.5), 0.45 (acetonitrile/H_2O, 8:2); mp 165−167 °C; $[\alpha]_D^{20}$ = −87.4° (c 0.87 MeOH); IR (Nujol mull) 3450−2450, 1715, 1660, 1640, 1550, 1535, 1400, 1210; ^{1}H NMR (D_2O) 3.66 (dd, J = 4.8, 11.6 Hz, 1H, $H5'$), 4.01 (dd, J = 9.1, 11.5 Hz, 1H, $H5$), 4.16 (s, 2H, Gly CH_2), 4.74 (dd, J = 4.8, 9.1 Hz, 1H, $H4$). Anal. ($C_6H_8N_2O_4S$) C, H, N.

***N*-[2-Oxo-3-[α-[*N*-(*tert*-butoxycarbonyl)-*O*-(*tert*-butyl)]-L-γ-glutamyl]thiazolidin-4(*R*)-ylcarbonyl]glycine *tert*-Butyl Ester (12).** To a cold (0 °C) stirred solution of **10** (3.36 g, 12.9 mmol) in acetonitrile (150 mL) were added *N*-(*tert*-butoxycarbonyl)-L-glutamic acid α-*tert*-butyl γ-*N*-succinimidyl ester (5.2 g, 13 mmol) and TEA (1.8 mL, 12.9 mmol). The solution was stirred for 1 h at 0 °C, and at room temperature for 12 h. The mixture was then worked up as described for **4.** The crude product was purified by flash column chromatography [eluent: stepwise gradient of ether (0−6%) in CH_2Cl_2] to afford 3.52 g (50%) of **12** as a colorless paste. TLC [R_f 0.65 (CH_2Cl_2/MeOH, 9.65:0.35)] and NMR analysis indicated that the product was over 95% pure and can be used in the next step without further purification.

An analytical sample was obtained, by crystallization from CH_2Cl_2 and hexane, as white crystals: mp 67−69 °C; $[\alpha]_D^{20}$ = −73.7° (c 1.14 $CHCl_3$); IR (Nujol mull) 3290, 3265, 1730, 1710, 1700, 1680, 1540, 1500, 1360, 1230, 1150; ^{1}H NMR ($CDCl_3$) 1.42, 1.45 (2s, 27H, t- butyl H), 1.76−2.0, 2.20−2.37 (2m, 2H, β-Glu CHa, CHb), 2.90−3.04 (m, 2H, γ-Glu CH_2), 3.60 (dd, J = 7.6, 11.1 Hz, 1H, $H5$), 3.66 (dd, J = 1.7, 11.1 Hz, 1H, $H5'$), 3.89 (dd, J = 4.8, 18.3 Hz, 1H, Gly CHa), 4.0 (dd, J = 5.3, 18.3 Hz, 1H, Gly CHb), 4.16−4.29 (m, 1H, α-Glu CH), 5.08−5.16 (m, 1H, Glu NH overlapping with the dd at 5.18), 5.18 (dd, J = 1.7, 7.6 Hz, 1H, $H4$), 6.76−6.83 (m, 1H, Gly NH). Anal. ($C_{24}H_{39}N_3O_9S$) C, H, N.

***N*-[2-Oxo-3-(L-γ-glutamyl)thiazolidin-4(*R*)-ylcarbonyl]-glycine Hydrochloride (13).** A solution of **12** (2.26 g, 4.14 mmol) in 15 mL of 4 M HCl in glacial acetic acid was stirred at 0 °C under nitrogen for 30 min and at room temperature. The reaction, followed by TLC, was over in 2 h. The mixture was then worked up as described for **6**. After drying, the crude hydrochloride was recrystallized from methanol and ether to afford 1.22 g (80%) of **13** as white flakes: R_f 0.6 (2-propanol/ammonium hydroxide 30%, 7:3); mp 175−178 °C; $[\alpha]_D^{20}$ = −104.3° (c 0.92 MeOH); IR (Nujol mull) 3330−2470, 1725, 1715, 1690, 1660, 1550, 1515, 1390, 1220; ^{1}H NMR (D_2O) 2.25−2.44 (m, 2H, β-Glu CH_2), 3.17−3.31 (m, 2H, γ-Glu CH_2), 3.52 (dd, J = 2.4, 11.9 Hz, 1H, $H5'$), 3.94 (dd, J = 9.2, 11.9 Hz, 1H, $H5$), 4.07−4.24 (m, 3H, Gly CH_2, α-Glu CH), 5.37 (dd, J = 2.4, 9.2 Hz, 1H, $H4$). Anal. ($C_{11}H_{15}N_3O_7S$·HCl) C, H, N, Cl.

Biological Methods. 1. Monocyte-Derived Macrophage Isolation. Human peripheral blood mononuclear cells (PBMC) were obtained from healthy HIV-, HCV-, and HBV-seronegative donors by Ficoll-Hypaque density gradient centrifugation (MSL 2000, Eurobio, Les Ulis, France). Monocytes were isolated from PBMC by countercurrent centrifugal elutriation as previously described with an enrichment degree ≥ 95%.[37,44] Freshly isolated human monocytes were resuspended in medium A: RPMI 1640 medium (Roche Diagnostics, Mannheim, Germany) supplemented with 10% heat-inactivated (56 °C for 30 min) fetal calf serum (FCS) (Roche Diagnostics), 2 mM L-glutamine (Roche Diagnostics), and 1% triantibiotic mixture (penicillin, streptomycin, neomycin, PSN; Life Technologies, Grand Island, NY). Cells were distributed (1 million/well) into 48-well plates (Becton Dickinson Labware, Lincoln Park, NJ) and maintained for 7 days in a humidified 5% CO_2 atmosphere to allow their differentiation in MDM.

2. Drugs. NAC and OTC (**1**) from Sigma (Saint Quentin-Fallavier, France) were solubilized in RPMI 1640 or dimethyl sulfoxide (DMSO; Sigma). The OTC derivatives were solubilized in RPMI 1640 (**5**, **7**, **10**, **13**) or DMSO (**2**, **6**, **9**, **11**, **13**) and stored at −80 °C. Solubilization of several molecules was difficult (**3**) or impossible (**4**, **8**, **12**). The pH of drug solutions was adjusted to 7.0 using 3 M NaOH when necessary.

3. Virus. MDM were infected with reference macrophage-tropic HIV-1/Ba-L strain.[45] This virus was propagated in vitro using human phytohemagglutinin (PHA)-P-activated umbilical blood mononuclear cells (UBMC). To eliminate soluble factors such as cytokines, the cell-free UMBC culture supernatant was ultracentrifuged at 360000*g* for 5 min and resuspended in RPMI 1640. This viral preparation was titrated using PHA-P-activated PBMC; 50% tissue culture infectious doses ($TCID_{50}$) and multiplicity of infection (moi) were calculated using Kärber's formula [46] and Poisson's law, respectively.

4. Antiviral Assay. One million MDM were pretreated for 1 h with different concentrations of compounds and infected with 10 000 $TCID_{50}$ (0.01 moi) of the HIV-1/Ba-L strain; 24 h later, MDM were washed once to eliminate excess virus and fed with fresh medium A. Twice a week, supernatants were removed and stored at −20 °C in order to measure viral replication. Cell culture medium and drugs were then renewed, and cells were microscopically observed to assess possible drug-induced cytotoxicity.

5. Assay of Viral Replication. HIV replication was assessed by the assay of RT activity in cell culture supernatants, as described elsewhere.[47]

6. Data Analysis. All experiments were repeated with cells isolated from a second blood donor. Results are expressed as the mean of RT activity ± standard deviation (SD). Cumulative RT activities were used to calculate the percentages of untreated control and 50% effective doses (ED_{50}). To calculate the percentages of untreated control, results obtained in cultures with treated MDM were normalized to the untreated control value. The ED_{50} was calculated using cumulative RT activity and microcomputer software (J. and T. C. Chou, Biosoft, Cambridge, U.K.).

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