0026-895X/93/010109-06$03.00/0

MOLECULAR PHARMACOLOGY, 43:109–114

Human Immunodeficiency Virus Type 1-Specific [2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)-Purine Analogues Show a Resistance Spectrum that Is Different from that of the Human Immunodeficiency Virus Type 1-Specific Non-nucleoside Analogues

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Received July 29, 1992; Accepted October 16, 1992

SUMMARY

The [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (TSAO) derivatives of N^1-methylhypoxanthine with linkage to the TSAO moiety through the N^9 or N^7 atom of the hypoxanthine ring (designated TSAO-m^{1}Hx and 7-TSAO-m^{1}Hx, respectively) are potent and selective inhibitors of human immunodeficiency virus type 1 (HIV-1) but not HIV-2 or simian immunodeficiency virus. Their selectivity indices (ratio of cytotoxic concentration to antivirally active concentration) are >500. This is a >15-fold increase in therapeutic index, compared with TSAO-adenine. A HIV-1(III$_B$) variant selected for resistance to TSAO-m^{1}Hx (designated HIV-1/TSAO-m^{1}Hx) proved to be cross-resistant to the other TSAO-purine derivatives and to the TSAO-pyrimidine derivatives. However, HIV-1/TSAO-m^{1}Hx was highly sensitive to the HIV-1-specific non-nucleoside tetrahydroimidazobenzodiazepinone, nevirapine, pyridinone L697,661, and several HEPT derivatives. The reverse transcriptase (RT) of HIV-1/TSAO-m^{1}Hx shows a single amino acid change (138-Glu to Lys) that is identical to the amino acid change that has recently been observed in several HIV-1/TSAO-pyrimidine mutant strains. Our observations indicate that the TSAO-purines and TSAO-pyrimidines belong to one pharmacological class of HIV-1-specific RT inhibitors that are targeted at the same molecular site of the HIV-1 RT.

At least three different pharmacological classes of compounds, targeted at the viral RT, have been identified as potent and selective inhibitors of HIV-1 replication, i.e., (i) 2′,3′-dideoxynucleosides (AZT, DDC, DDI, and 2′,3′-didehydro-2′,3′-dideoxythymidine), which do not discriminate in their antiviral action between HIV-1 and HIV-2 and which need to be phosphorylated intracellularly to their triphosphate forms in three successive steps before they interfere with HIV RT (1–5); (ii) acyclic nucleoside phosphonates (PMEA and FPMPA), which also do not discriminate between HIV-1, HIV-2, and other retroviruses, i.e., SIV and feline immunodeficiency virus, and need to be converted to the diphosphate derivatives before they interact with RT (6–10); and (iii) non-nucleoside derivatives belonging to structurally unrelated compound classes, i.e.

This research was supported in part by the AIDS Basic Research Programme of the European Community, by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Krediet 3.0026.91 and 3.0097.87), the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek (Krediet 3.3010.91), and the Belgian Geconcerteerde Onderzoeksacties (Project 90/94-2), by NATO Collaborative Research Grant CRG 920777, and by the Spanish Plan Nacional de I+D Farmaceuticas (Project FAR 880160/1), the Minsterio de Educacion y Ciencia of Spain (grants to A.S.-F. and M.-J.P.-P.), the Medical Faculty of the Karolinska Institute, the Swedish Society of Medicine, and the Swedish Board of Technical Development (Project 623-90-02526).

ABBREVIATIONS: RT, reverse transcriptase; TSAO, [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide); $CCID_{50}$, 50% cell culture-infective dose; PCR, polymerase chain reaction; SIV, simian immunodeficiency virus; AZT, 3′-azido-2′,3′-dideoxythymidine; CC_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration; HIV, human immunodeficiency virus; BHAP, bis(heteroaryl)piperazine; α-APA, α-anilino-phenylacetamide; E-EPU-S, 1-ethoxymethyl-5-ethyl-6-phenylthio-2-thiouracil; E-BPU, 1-benzyloxymethyl-5-ethyl-6(phenylthio)-uracil; E-EPU, 1-ethoxymethyl-5-ethyl-6-(phenylthio)uracil; E-EBU-dM, 1-ethoxymethyl-5-ethyl-6-(3,5-dimethyl-benzyl)uracil; DDG, 2′,3′-dideoxyguanosine; DDC, 2′3′-dideoxycytidine; DD1, 2′3′-dideoxyinosine; HEPT, 1-[(2-hydroxyethoxy)-methyl)]-6phenylthiothymine; TIBO, tetrahydroimidazo(4,5,l-*jk*)(1,4-benzodiazepin-2(1*H*))-one; PHEA, 9-(2-phosphorylmethoxyethyl)adenine; FPMPA, g-(3-fluoro-2-phosphorylmethoxypropyl)adenine.

groups of benzodiazepinones (11), acyclouridines (12–15), dipyridodiazepinones (16), pyridinones (17), BHAPs (18), and α-APAs (19). These compounds represent the most potent and selective agents that are specific for HIV-1 and thus not inhibitory to other retroviruses including HIV-2, SIV, and feline immunodeficiency virus. Representative congeners of these different classes of HIV-1-specific compounds are HEPT, TIBOs R82150 and R82913, nevirapine (BI-RG-587), pyridinones L697,639 and L697,661, BHAP 87201, and α-APA R89439. A common feature of all these compounds is that they do not require metabolic conversions before interacting with RT.

Recently, we identified a novel class of compounds, i.e. TSAO derivatives of pyrimidine nucleosides, that selectively inhibit the replication of HIV-1 but not HIV-2, SIV, or other retroviruses (20–23). The thymine derivative TSAO-T is antivirally active at 30 ng/ml and toxic for MT-4 cells at 7.7 μg/ml. It is a specific inhibitor of the HIV-1 RT (24, 25). Introduction of an alkyl function at the N^3-position of the thymine moiety markedly decreases cytotoxicity but does not affect antiviral activity. Consequently, the N^3-methyl- and N^3-ethyl-substituted TSAO derivatives (designated TSAO-m^3T and TSAO-e^3T, respectively) are more potent and selective inhibitors of HIV-1 replication than is the parent compound TSAO-T. Their selectivity index (ratio of the cytotoxic concentration to the antivirally effective concentration) exceeds 1000 in different cell systems (25). Only a few TSAO-purine derivatives have been synthesized so far, i.e., the adenine derivative TSAO-A, the hypoxanthine derivative TSAO-Hx, and the hypoxanthine derivative 7-TSAO-Hx, in which the sugar moiety is linked to N^7 instead of N^9 of hypoxanthine (25). In contrast to the N^3-substituted TSAO-pyrimidines, the TSAO-purine derivatives proved rather cytotoxic (CC_{50}, 7.3–8.7 μg/ml) and, consequently, exhibited relatively low selectivity indices (selectivity index, <100). We have now synthesized a series of TSAO-purine derivatives with markedly greater selectivity, due to higher antiviral activity and lower cytotoxicity. We also identified the molecular site of interaction of the TSAO-purine derivatives with the HIV-1 RT. An HIV-1 strain was isolated that proved to be resistant to the N^1-methyl-substituted TSAO-m^1Hx, cross-resistant to other TSAO-purine and TSAO-pyrimidines, but sensitive to the HIV-1-specific non-nucleoside analogues (i.e., TIBO, HEPT, nevirapine, and pyridinone L697,661).

Materials and Methods

Compounds. The thymine, N^3-methylthymine, N^3-ethylthymine, adenine, hypoxanthine, and N^7-linked hypoxanthine derivatives of TSAO (designated TSAO-T, TSAO-m^3T, TSAO-e^3T, TSAO-A, TSAO-Hx, and 7-TSAO-Hx, respectively) were synthesized according to previously published procedures (21–23). The synthesis of the 1-methylhypoxanthine (TSAO-m^1Hx), the N^7-linked 1-methylhypoxanthine (7-TSAO-m^1Hx) (Fig. 1), the N^7-linked 1-ethylhypoxanthine (7-TSAO-e^1Hx), the N^7-linked xanthine (7-TSAO-X), 6-methoxypurine (TSAO-m^6Hx), N^6-methyladenine (TSAO-m^6A), and N^6-dimethyladenine (TSAO-dm^6A) derivatives of TSAO will be reported elsewhere. Nevirapine was a gift from Dr. P. Ganong (Boehringer Ingelheim), TIBO R82150, TIBO R82913, and DDC were kindly provided by Dr. Z. Hao and Dr. D. G. Johns (National Institutes of Health), pyridinone L697,639 was supplied by Dr. M. Goldman (Merck, Sharp & Dohme), the HEPT derivatives E-EPU-S, E-BPU, E-EPU, and E-EBU-dM were provided by Dr. M. Baba (Fukushima, Japan), AZT was from Sigma Chemical Co. (St. Louis, MO), DDI was from Bristol-Myers Squibb (Wallingford, CT), DDG was from Calbiochem, and PMEA and FPMPA were kindly provided by Dr. A. Holy and Dr. J. Jindrich (Czechoslovak Academy of Sciences, Laboratory of Organic Chemistry and Biochemistry, Prague, Czechoslovakia).

Cells. MT-4 cells were obtained from Dr. N. Yamamoto (Tokyo, Medical and Dental University School of Medicine, Tokyo, Japan), CEM cells were from the American Type Culture Collection (Rockville, MD), and C8166 cells were kindly provided by Dr. P. La Colla (Universitá degli Studi di Cagliari, Cagliari, Sardinia). Cells were grown in 75-cm^2 plastic culture bottles in the presence of RPMI 1640 culture medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 0.075% $NaHCO_3$.

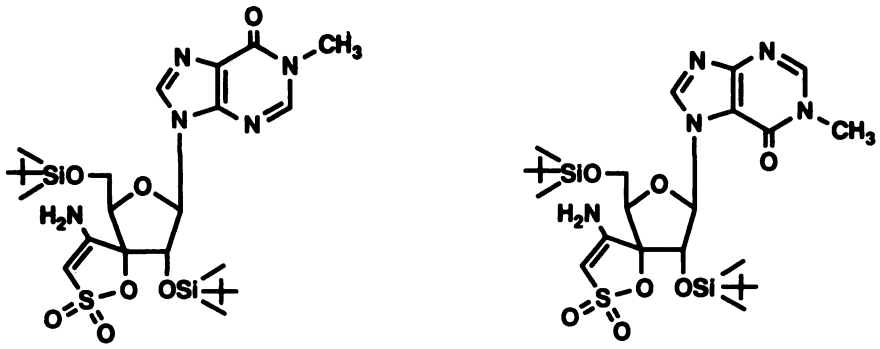
Viruses. HIV-1(III_B) was originally obtained from the culture supernatant of persistently HIV-infected H9 cells (26) and was kindly provided by Dr. R. C. Gallo and Dr. M. Popovic (National Institutes of Health). HIV-2(ROD) was a gift from Dr. L. Montagnier (Pasteur Institute, Paris, France) (27). SIV(MAC_{251}) was isolated by Daniel *et al.* (28) and obtained from Dr. C. Bruck (Smith Kline-Rit, Rixensart, Belgium).

Antiviral assays. MT-4, CEM, and C8166 cells (4.5×10^5 cells/ml) were suspended in fresh culture medium and infected with HIV-1, HIV-2, or SIV at 100 $CCID_{50}$/ml of cell suspension. Then, 100 μl of the infected cell suspension were transferred to microplate wells, mixed with 100 μl of the appropriate dilutions of the test compounds, and further incubated at 37°. After 5 days, the number of viable MT-4 cells was determined in a blood cell-counting chamber by trypan blue staining and was compared with the number of viable MT-4 cells in mock-infected cell cultures. After 4 days, syncytium formation in the CEM and C8166 cell cultures was recorded microscopically (29). The number of cells in mock-infected CEM cell cultures was determined in a Coulter counter (Coulter Electronics Ltd., Harpenden, Herts, England). The CC_{50} was defined as the concentration of compound that reduced the number of living cells by 50%.

Selection of an HIV-1(III_B) strain resistant to TSAO-m^1Hx. CEM cell cultures at 4×10^5 cells/ml were infected with 200 $CCID_{50}$ of HIV-1(III_B) and were subjected to two subcultivations in the presence of TSAO-m^1Hx at 0.2 μg/ml (i.e., 2–3 times the EC_{50}). When abundant giant cell formation in the cell cultures appeared, 0.5 ml of the infected cultures was added to a fresh CEM cell culture volume of 5 ml (4×10^5 cells/ml) in the presence of a 5-fold increased concentration of the test compound (0.5 μg/ml). This procedure was repeated twice in the presence of 2.5 μg/ml TSAO-m^1Hx and 10 μg/ml TSAO-m^1Hx. After an additional subcultivation in the presence of the test compound (10 μg/ml), the cell cultures were frozen in aliquots at −70°.

Preparation of HIV-1 samples for PCR analysis and sequencing of the *pol* gene. MT-4 cells (3×10^5 cells/ml) were infected with different HIV-1 strains at 200 $CCID_{50}$ and were incubated in RPMI 1640 culture medium for 3 days at 37°. Then, cells were centrifuged and washed twice with phosphate-buffered saline in 1.5-ml Eppendorf tubes. To 10^6 MT-4 cells were added 100 μl containing 10 μl of PCR buffer (10 × concentrated: 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% (w/v) gelatin; Cetus-Vanderheyden, Brussels, Belgium), 8 μl of $MgCl_2$ (25 mM), 72 μl of Milli-Q water, 10 μl of proteinase K (10 μg) (Calbiochem), 0.5% Tween-20, and 0.5% Nonidet P-40 in H_2O. The cell suspension was then incubated at 56° for 1 hr and subsequently heated at 95° for 10 min. The samples were stored at −20° before PCR analysis.

Proviral DNA was amplified from total cellular DNA after extraction of the proteinase K lysates with phenol/chloroform. Amplification was performed with an extract from 1×10^5 cells by a Perkin Elmer Cetus GeneAmp PCR protocol, with 3 mM $MgCl_2$ and 0.15 μM levels of each primer. Oligonucleotides were chosen (sense primer, 5′-CCTGAAAATCCATACAATACTCCAGTATTTG-3′; reverse complement primer, 5′-AGTGCTTTGGTTCCTCTAAGGAGTTTAC-3′) to give a 727-base pair fragment covering amino acids 50–270. The PCR product was purified from a 1% low-melting agarose gel by MagicPCR Preps



TSAO-m^{1}Hx 7-TSAO-m^{1}Hx

Fig. 1. Structural formulae of TSAO-m^{1}Hx (*left*) and 7-TSAO-m^{1}Hx (*right*).

(Promega), directly sequenced with a Taq Dye Deoxy Terminator sequencing kit (Applied Biosystems), and analyzed on a model 373A DNA sequencer (Applied Biosystems).

Results

Antiretroviral activity of TSAO-purine derivatives. A series of novel TSAO-purine derivatives were evaluated for their inhibitory effect on HIV-1, HIV-2, and SIV replication in three different human T4 lymphocyte cell lines (MT-4, CEM, and C8166). None of the TSAO derivatives proved effective against HIV-2 or SIV at subtoxic concentrations (Table 1). HIV-1-induced cytopathicity in MT-4 cells was inhibited to a similar extent by all TSAO-purine derivatives evaluated. The EC_{50} values ranged from 0.09 μg/ml (7-TSAO-Hx) to 0.55 μg/ml (TSAO-A and 7-TSAO-e^{1}Hx). The antiviral activity of the TSAO-purine derivatives was 3–15-fold lower than that of the TSAO-pyrimidine derivatives TSAO-T, TSAO-m^{3}T, and TSAO-e^{3}T (20–23). However, striking differences were noted with regard to the cytotoxicity of the test compounds. The unsubstituted TSAO-purines TSAO-Hx, 7-TSAO-Hx, TSAO-A, and TSAO-X, the 6-methoxypurine derivative (TSAO-m^{6}Hx), and the N^6-methyl- (TSAO-m^{6}A) and N^6-dimethyl-substituted (TSAO-dm^{6}A) adenine derivatives were almost as toxic to MT-4 cells (CC_{50}, 7.7–13 μg/ml) as was TSAO-T (CC_{50}, 7.2 μg/ml). In contrast, derivatives with N^1-methyl- and N^1-ethyl-substituted hypoxanthine linked to the TSAO moiety through N^9 (TSAO-m^{1}Hx) or N^7 (7-TSAO-m^{1}Hx and 7-TSAO-e^{1}Hx) were nontoxic at concentrations of 89–100 μg/ml for MT-4 cells and 15–98 μg/ml for CEM cells. These concentrations are >200–800-fold higher than the antivirally effective concentrations of these compounds.

When evaluated for their inhibitory effect on HIV-1-induced syncytium formation in CEM and C8166 cell cultures, the TSAO-purine derivatives proved to be 2–5-fold more potent in CEM cells and equally potent to 4-fold less potent in C8166 cells, compared with MT-4 cells. Irrespective of the cell type (MT-4, CEM, or C8166), TSAO-m^{1}Hx, 7-TSAO-m^{1}Hx, and 7-TSAO-e^{1}Hx emerged as the most active and least toxic of all TSAO-purine derivatives that were evaluated.

Development of a TSAO-m^{1}Hx-resistant HIV-1(III$_B$) strain. When HIV-1(III$_B$)-infected CEM cells were incubated in the presence of TSAO-m^{1}Hx at about 3–4 times its EC_{50}

TABLE 1
Antiretroviral activity of TSAO derivatives *in vitro*

Compound	EC_{50}[a]					CC_{50}[b] (MT-4)	SI[c] (MT-4)	CC_{50} (CEM)	SI (CEM)
	MT-4			CEM, HIV-1(III$_B$)	C8166, HIV-1(III$_B$)				
	HIV-1(III$_B$)	HIV-2(ROD)	SIV-(MAC$_{251}$)						
	μg/ml					μg/ml		μg/ml	
TSAO-m^{1}Hx	0.18	>100	>100	0.06	0.10	>100	>555	19[d]	317
7-TSAO-m^{1}Hx	0.15	>100	>100	0.05	0.10	89	593	15	300
7-TSAO-e^{1}Hx	0.55	>100		0.12		>100	>180	98	817
TSAO-Hx	0.14	>4	>4	0.19	0.40	7.8	56	7.7	40
7-TSAO-Hx	0.09	>4	>4	0.07	0.40	8.5	94	8.3	118
7-TSAO-X	0.23	>4	>4	0.08	0.40	7.7	33	9.1	114
TSAO-m^{6}Hx	0.16	>4	>4	0.07	0.70	13	81	9.9	141
TSAO-A	0.27	>4	>4	0.05	0.40	8.3	31	5.5	110
TSAO-m^{6}A		>4		0.16		4–20		7.8	49
TSAO-dm^{6}A	0.27	>4	>4	0.15	0.80	10	37	6.1	41

[a] Compound concentration required to inhibit HIV-1-, HIV-2-, or SIV-induced cytopathicity (MT-4) or HIV-1-induced syncytium formation (CEM and C8166) by 50%.
[b] Compound concentration required to reduce MT-4 and CEM cell viability by 50%.
[c] Selectivity index or ratio of CC_{50}/EC_{50}.
[d] At 100 μg/ml TSAO-m^{1}Hx, CEM cell proliferation was inhibited by 69%.

(i.e., 0.2 μg/ml), giant cells appeared after the second passage and became abundant after the third passage in the continuous presence of the test compound. When the TSAO-m^{1}Hx concentration was then increased by 5-fold and the infected CEM cells were further subcultured in the presence of fresh CEM cells (ratio, 1:10), virus replication clearly proceeded, even if the concentration of TSAO-m^{1}Hx in the two subsequent passages was increased to 10 μg/ml (i.e., 200 times the EC_{50} of the test compound in CEM cells).

Sensitivity of the TSAO-m^{1}Hx-resistant HIV-1 strain to various HIV-1-specific inhibitors. The HIV-1(III_B) strain that was selected after passage in the presence of a 100-fold higher concentration of TSAO-m^{1}Hx (and was designated HIV-1/TSAO-m^{1}Hx) was examined for its sensitivity to other TSAO-purine and TSAO-pyrimidine derivatives, HIV-1-specific non-nucleoside analogues, and various 2′,3′-dideoxynucleosides and acyclic nucleoside phosphonates. The HIV-1/TSAO-m^{1}Hx strain proved to be fully cross-resistant to all other TSAO-purine derivatives, as well as the TSAO-pyrimidine derivatives TSAO-m^{3}T and TSAO-e^{3}T. Its sensitivity to TSAO-T was decreased ~100-fold. However, HIV-1/TSAO-m^{1}Hx retained partial to full sensitivity to the dipyridodiazepinone BI-RG-587 (nevirapine), the benzodiazepinones TIBO R82150 and TIBO R82913, the pyridinone L697,661, and the HEPT derivatives E-EPU-S, E-EBU, E-EPU, and E-EBU-dM. Nevirapine was equally, if not more, inhibitory to HIV-1/TSAO-m^{1}Hx than to wild-type HIV-1(III_B), and the TIBO derivatives and pyridinone L697,661 were only 3.5–6.6-fold less active against HIV-1/TSAO-m^{1}Hx, whereas the HEPT derivatives were 5–14-fold less active. HIV-1/TSAO-m^{1}Hx replication was inhibited by all non-nucleoside derivatives at concentrations that were equal to or lower than 0.1 μg/ml. Also, the 2′,3′-dideoxynucleoside analogues AZT, DDC, DDI, and DDG proved to be equally inhibitory to HIV-1/TSAO-m^{1}Hx and to the wild-type HIV-1(III_B); the acyclic nucleoside phosphonate derivatives PMEA and FPMPA also were equally active against HIV-1/TSAO-m^{1}Hx and HIV-1(III_B) (Table 2).

Determination of the nucleotide sequence of the RT gene of the TSAO-m^{1}Hx-resistant HIV-1 strain. The specific nucleotide sequences of the first part of the RT gene (nucleotides 150–810) of HIV-1(III_B) and HIV-1/TSAO-m^{1}Hx were determined. A single transition mutation of the first base (guanine to adenine) of codon 138 was observed. No other mutations were found in the sequenced domain of the RT gene of HIV-1/TSAO-m^{1}Hx. Thus, resistance of the HIV-1/TSAO-m^{1}Hx strain to the TSAO derivatives must have resulted from a single amino acid change [glutamate (GAG) to lysine (AAG)] at position 138 of the RT. This particular substitution should be considered as relevant because of the change of a negatively charged to a positively charged amino acid residue. Interestingly, glutamic acid at position 138 in HIV-1 RT is conserved among all 15 HIV-1 strains with known amino acid sequences for RT (i.e., strains LAI, HXB2R, MN, RF, NDK, and MAL), whereas alanine is present at position 138 of the RT of all eight HIV-2 strains that have been sequenced so far (i.e., strains ROD, NIH2, ST, GHI, and D205) (except for HIV-2/BEN, where position 138 is occupied by methionine instead of alanine) (Table 3) (30).

Discussion

It has been shown previously that the structural features required for the potent anti-HIV-1 activity of the TSAO derivatives involve the presence of silyl groups at positions C-2′ and C-5′ and a spiro substituent in the *R*-configuration at position C-3′ of the ribose moiety. Also, introduction of an alkyl or alkenyl moiety at position N^3 of thymine results in markedly decreased cytotoxicity of the particular test compounds (i.e., compare TSAO-T with TSAO-m^{3}T and TSAO-e^{3}T) (Table 1). Introduction of a methyl or ethyl group at position N^1 of the TSAO-purine derivatives TSAO-Hx and 7-TSAO-Hx also resulted in a markedly increased selectivity index, mainly due to decreased cytotoxicity. However, additional derivatives have to be synthesized to obtain further insight into the structural requirements that TSAO-purine analogues must meet to be devoid of toxicity.

Recently, several different classes of compounds (i.e., TIBO, HEPT, nevirapine, pyridinone, BHAP, TSAO, and α-APA) have been described that are endowed with potent and selective anti-HIV-1 activity. Although the compounds are structurally unrelated, it has been suggested that they belong to a single pharmacological class of compounds, for several reasons. (i) These compounds are highly specific for HIV-1 but not HIV-2 or SIV or other retroviruses. (ii) They are targeted at HIV-1 RT and show a preference for poly(C)·oligo(dG) as the homopolymeric template-primer. (iii) They inhibit HIV-1 RT noncompetitively with respect to [^{3}H]dGTP and noncompetitively or uncompetitively with respect to poly(C)·oligo(dG); this suggests that the compounds bind to a non-substrate binding site at HIV-1 RT and thus differ in this respect from the 2′,3′-dideoxynucleotide and acyclic nucleoside phosphonate analogues. (iv) HIV-1 strains selected for resistance against the pyridinones or nevirapine are cross-resistant to nevirapine, pyridinones, TIBO R82150, TIBO R82913, and the HEPT derivatives E-EPU and E-EBU. These findings indicate that all the HIV-1-specific RT inhibitors may share a common binding site on the HIV-1 RT. However, the finding that the TSAO-m^{1}Hx-resistant HIV-1 strain is cross-resistant to the other TSAO-purine and TSAO-pyrimidine derivatives but remains sensitive to the other HIV-1-specific non-nucleoside analogues at a concentration of 0.1 μg/ml or less strongly suggests that the mode of interaction of the TSAO derivatives with HIV-1 RT differs from that of the other HIV-1-specific RT inhibitors. The identification of a single mutation at position 138 in the RT of the TSAO-m^{1}Hx-resistant HIV-1 strain is unprecedented and indicates that this amino acid residue is involved in the interaction of the TSAO derivatives, but not that of the other HIV-1-specific compounds, with HIV-1 RT. When the TSAO-resistant HIV-1 strains were further passaged in the presence or absence of the particular TSAO derivative for at least 10 additional subcultivations, the degree of resistance to the TSAO derivatives and sensitivity to the other HIV-1-specific non-nucleoside analogues remained essentially constant (data not shown). In contrast to the TSAO derivatives, pyridinones and nevirapine seem to select for amino acid changes at positions 181 (181-Tyr to Cys), 100 (100-Leu to Ile), 103 (103-Lys to Asn), and 108 (108-Val to Ile) of HIV-1 RT (31–34).

Recently, the crystal structure (at 3.5-Å resolution) of HIV-1 RT complexes with nevirapine has been reported (35). The polymerase domain of p66 has an anatomical analogy to a right hand, containing a finger domain, a palm domain, a thumb domain, and a connection domain. All amino acid mutations that have been reported so far to be responsible for HIV-1

TABLE 2

Antiviral activity of test compounds against HIV-1/III_B and HIV-1/TSAO-m^1Hx in CEM cells

Compound	EC_{50}[a]		Ratio of EC_{50} for HIV-1/TSAO-m^1Hx/EC_{50} for HIV-1(III_B)
	HIV-1(III_B)	HIV-1/TSAO-m^1Hx	
	μg/ml		
TSAO-m^1Hx	0.063 ± 0.012	>50	>833
7-TSAO-m^1Hx	0.051 ± 0.027	>20	>400
TSAO-Hx	0.19 ± 0.16		
7-TSAO-Hx	0.07 ± 0.01		
7-TSAO-X	0.077 ± 0.025	>4	>50
TSAO-m^6Hx	0.07 ± 0.01	>4	>57
TSAO-A	0.05 ± 0.04		
TSAO-dm^6A	0.15 ± 0.07	>4	>20
TSAO-T	0.030 ± 0.005	2 ± 0.7	66
TSAO-m^3T	0.030 ± 0.005	≥50	≥1666
TSAO-e^3T	0.061 ± 0.057	>50	>820
Nevirapine	0.032 ± 0.029	0.01 ± 0.01	0.3
TIBO R82150	0.026 ± 0.006	0.07 ± 0.04	3.5
TIBO R82913	0.015 ± 0.009	0.10 ± 0.09	6.6
Pyridinone L697, 661	0.007 ± 0.003	0.035 ± 0.02	5
E-EPU-S	0.004 ± 0.0	0.055 ± 0.01	14
E-EBU	0.0026 ± 0.002	0.018 ± 0.02	6.9
E-EPU	0.005 ± 0.002	0.05 ± 0.03	10
E-EBU-dM	0.003 ± 0.001	0.015 ± 0.01	5.0
AZT[b]	0.003 ± 0.001	0.003 ± 0.002	1
DDC[b]	0.035 ± 0.007	0.02 ± 0.01	0.57
DDI[b]	4.6 ± 2.6	4.3 ± 2.9	0.9
DDG[b]	1.1 ± 0.28	4.0 ± 1.7	3.6
PMEA[b]	8.5 ± 2.1	8.0 ± 2.8	0.9
FPMPA	3.0 ± 0.0	1.5 ± 0.7	0.5

[a] Data are the mean of two or three independent experiments.
[b] Data expressed in μM.

TABLE 3

Mutation of HIV-1 RT amino acid residue 138 in a TSAO-m^1Hx-resistant HIV-1 strain

Virus strain	Amino acid						
	135	136	137	138	139	140	141
HIV-1(III_B)	I	N	N	E	T	P	G
HIV-1/TSAO-m^1Hx	I	N	N	K	T	P	G
HIV-1 consensus	I	N	N	E	T	P	G
HIV-2/SIV consensus	V	N	N	A	E	P	G

resistance to the non-nucleoside inhibitors of HIV-1 (i.e., amino acids 100, 103, 108, 181, and 188) (31–34) are clustered in the palm domain. In striking contrast, the amino acid change (138-Gly to Lys) that confers resistance to TSAO is situated at the top of the finger domain, which is quite distant from the palm domain. These findings are in complete agreement with the observed lack of cross-resistance of the HIV-1/TSAO mutants to HIV-1-specific RT inhibitors other than TSAO. They may indicate that the TSAO nucleoside analogues bind to the HIV-1 RT enzyme at a different site than do the other non-nucleoside HIV-1-specific inhibitors. In fact, when the RT derived from a HIV-1/TSAO-resistant mutant strain (containing the 138-Glu to Lys change) was examined for its sensitivity to the prototype compound TSAO-T and several HIV-1-specific non-nucleoside analogues, it proved to be fully resistant to the inhibitory effect of TSAO-T (IC_{50} > 100 μg/ml) but still sensitive to nevirapine and TIBO R82913 (0.2 and 1.7 μg/ml, respectively).

We have recently observed the same mutation (GAG to AAG) at position 138 of the RT of three different TSAO-pyrimidine-resistant HIV-1 strains (HIV-1/TSAO-T, HIV-1/TSAO-m^3T, and HIV-1/TSAO-e^3T) (36).[1] These findings, together with the cross-resistance of TSAO-resistant HIV-1 strains to all other TSAO-purine and TSAO-pyrimidine derivatives, clearly indicate that (i) both TSAO-pyrimidine and TSAO-purine derivatives belong to the same pharmacological class of compounds, (ii) the single amino acid mutation at position 138 should be considered as relevant for all TSAO derivatives, irrespective of the nature of the base moiety of these molecules, (iii) the sugar part containing the silyl moieties at positions C-2′ and C-5′ and the spiro moiety at position C-3′ may determine the specific selection of HIV-1 strains containing the 138-Glu to Lys mutation, and (iv) the amino group of the C-3′ spiro substituent (in the *R*-configuration) may be directly involved in an interaction with the carboxyl group of the glutamic acid residue at position 138 of the wild-type HIV-1 RT. The nature of the interaction between glutamic acid and the amino group of the TSAO derivatives (i.e., ionic, covalent) needs further clarification. However, because we previously found that TSAO-T is a reversible inhibitor of HIV-1 RT (24) it is unlikely that covalent binding occurs between the TSAO derivatives and the RT.

Our findings that HIV-1 mutant strains that are resistant to one particular class of HIV-1-specific inhibitors (i.e., TSAO) are not markedly cross-resistant to other classes of HIV-1-specific inhibitors (i.e., TIBO, nevirapine, pyridinone, and HEPT) but retain sensitivity to the drugs at concentrations that are equal to or lower than 0.1 μg/ml are important not only for delineating the molecular determinants involved in the interaction of these compounds with their target enzyme but also for establishing therapeutic modalities for the clinical use of these compounds in the treatment of HIV-1 infections.

[1] J. Balzarini, A. Karlsson, A. Vandamme, and E. DeClerq, unpublished observations.

Acknowledgments

We thank Ann Absillis and Lizette van Berckelaer for their excellent technical assistance and Christiane Callebaut for her dedicated editorial help.

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