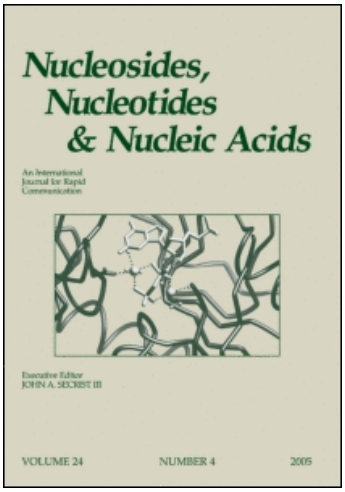


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

TSAO Derivatives: Highly Specific Inhibitors of Human Immunodeficiency Virus Type-1 (HIV-1) Replication

Maria J. Camarasa[a]; Maria J. Péarez-Péarez[a]; Sonsoles Velázquez[a]; Ana San-Féalix[a]; Rosa Alvarez[a]; Simon Ingate[a]; Maria Luisa Jimeno[a]; Anna Karlsson[b]; Erik De Clercq[c]; Jan Balzarini[c]
[a] Instituto de Quíamica Méadica (C. S. I. C.). Juan de la Cierva, Madrid, Spain [b] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium [c] Biochemical Laboratory I, Karolinska Institute, Stockholm, Sweden

To cite this Article Camarasa, Maria J. , Péarez-Péarez, Maria J. , Velázquez, Sonsoles , San-Féalix, Ana , Alvarez, Rosa , Ingate, Simon , Jimeno, Maria Luisa , Karlsson, Anna , De Clercq, Erik and Balzarini, Jan(1995) 'TSAO Derivatives: Highly Specific Inhibitors of Human Immunodeficiency Virus Type-1 (HIV-1) Replication', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 585 — 594
To link to this Article: DOI: 10.1080/15257779508012432
URL: http://dx.doi.org/10.1080/15257779508012432

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 585-594 (1995)

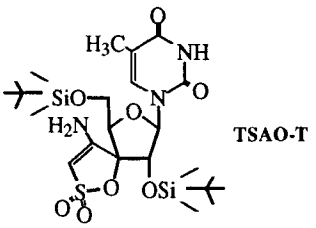
TSAO DERIVATIVES: HIGHLY SPECIFIC INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) REPLICATION

María José Camarasa*[§], María Jesús Pérez-Pérez[§], Sonsoles Velázquez[§], Ana San-Félix[§], Rosa Alvarez[§], Simon Ingate[§], María Luisa Jimeno[§], Anna Karlsson[¥], Erik De Clercq[+] and Jan Balzarini[+]

Instituto de Química Médica (C.S.I.C.). Juan de la Cierva, 3. 28006 Madrid, Spain[§]
Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium[+]
Karolinska Institute, Biochemical Laboratory I, S-10401, Stockholm, Sweden[¥]

Abstract: *TSAO derivatives represent a unique class of nucleosides that are specifically targeted at HIV-1 RT. This overview is focussed on the chemical synthesis, the conformational studies, the antiviral and metabolic properties of TSAO derivatives, as well as their mechanism of antiviral action and the molecular basis of the rapid selection of resistant HIV-1 strains that emerge in cell culture in the presence of TSAO derivatives.*

Several different kinds of nucleosides have proved to be effective anti-HIV agents. AZT (Azidothymidine), DDI (Dideoxyinosine), and DDC (Dideoxycytidine) and D4T (Didehydrodideoxythymidine) are, so far, the only drugs approved for the clinical treatment of acquired immunodeficiency syndrome (AIDS).[1,2] However, the clinical usefulness of these compounds is hampered by their severe toxic side effects.[2,3,4] This justifies the search for new compounds with potent and selective anti-HIV activity.



TSAO-T

Recently, we reported on an entirely novel class of functionalized nucleoside derivatives that are highly specific and potent inhibitors of human immunodeficiency virus type 1 (HIV-1) replication.[5-8] The prototype compound is [1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-

2",2"-dioxide) designated TSAO-T. TSAO derivatives inhibit the replication of human immunodeficiency virus type 1 (HIV-1) but not HIV-2 or other (retro)viruses. They are specifically targeted at the HIV-1-encoded reverse transcriptase (RT) with which they interact at a non-substrate binding site.[9] In this respect they behave like the non-nucleoside HIV-1-specific RT inhibitors (ie. HEPT, TIBO, Nevirapine, pyridinones, BHAP, α-APA, PETT derivatives ...).[10-12]

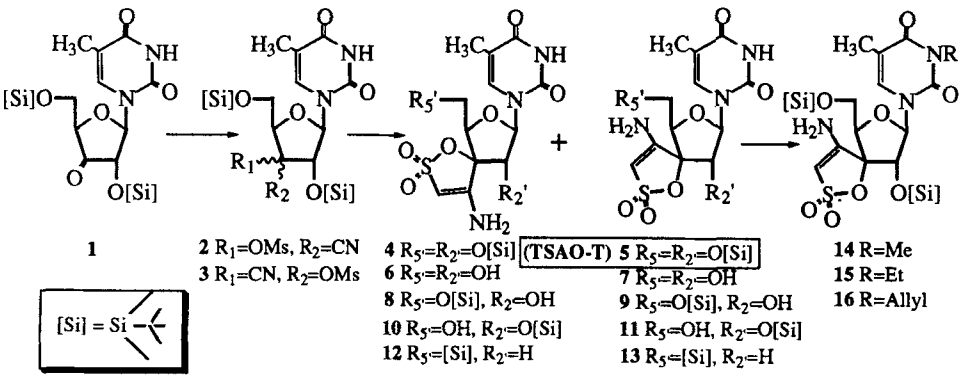
This overview is focussed on the chemical synthesis, the conformational studies, the antiviral and metabolic properties of TSAO derivatives, as well as their mechanism of antiviral action and the molecular basis of the rapid selection of resistant HIV-1 strains that emerge in cell culture in the presence of TSAO derivatives.

CHEMICAL SYNTHESIS OF TSAO DERIVATIVES

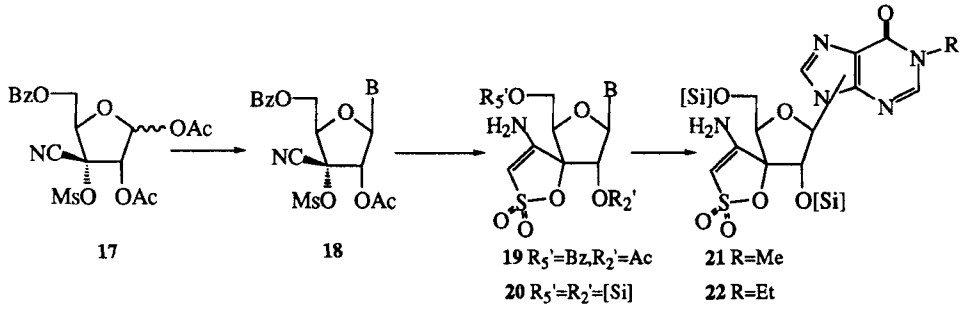
The synthesis of TSAO-T was carried out as outlined in Scheme 1. Reaction of the 3'-ketonucleoside **1**[7] with sodium cyanide followed by mesylation (mesyl chloride/pyridine) of the corresponding 3'-cyanohydrin epimers obtained, gave the respective 3'-*C*-cyano-3'-*O*-mesyl-β-D-xylo- and -ribofuranosyl thymine nucleosides **2** and **3**. The major diastereomer (**2**) resulted by the attack of the CN^- ion from the sterically less hindered α-face of the furanose ring.[13] Treatment of cyanomesylates **2** and **3** with Cs_2CO_3 gave the *xylo*- and *ribo*-spiro nucleosides **4** and **5**, respectively. Following standard procedures,[7] *xylo*- and *ribo*-spiro nucleosides afforded the fully deprotected compounds **6** and **7**, the 2'- or 5'-partially sylilated derivatives **8-11** and the 2' deoxy derivatives **12** and **13**. From *ribo* TSAO-T (**5**), 3-methyl-, 3-ethyl- or 3-allyl-TSAO-T derivatives **14** (TSAO-m^3T), **15** (TSAO-e^3T), and **16** (TSAO-a^3T), were prepared by selective N-3 alkylation.[8]

TSAO analogues were stereoselectively prepared (Scheme 2) by condensation[14] of trimethylsilylated pyrimidine[8] or purine[15] bases with the suitably functionalized and protected ribofuranosyl derivative **17**.Basic treatment (Cs_2CO_3) of the cyanomesyl nucleosides obtained (**18**) gave , exclusively, β-D-ribo-spiro nucleosides **19**.[8,15] The *ribo* configuration of the TSAO derivatives **18** and **19** was determined by the configuration of the starting cyanohydrin used in the preparation of the sugar intermediate **17**.[15,16] Subsequent deprotection and silylation of these derivatives (**19**) gave the TSAO-purine analogues **20.** By this method a variety of TSAO analogues of pyrimidines and purines were prepared.[8,15,17] Selective N-1-alkylation of the TSAO-purine derivatives **20** gave 1-alkyl-purine nucleosides **21** and **22.**[15]

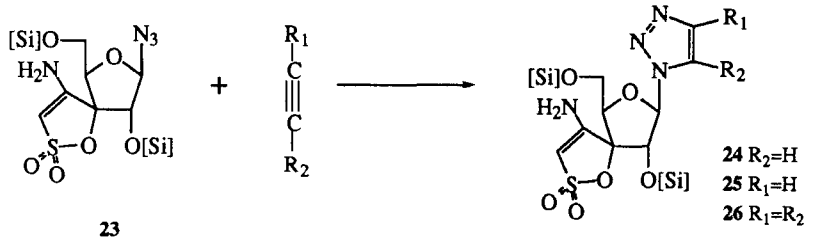
Finally, a series of 1,2,3-triazole-TSAO derivatives (Scheme 3) were stereoselectively prepared by 1,3-dipolar cycloaddition of a suitably functionalized and protected ribofuranosyl azide intermediate **23** to differently substituted acetylenes, to give β-D-ribo-spironucleosides.[18] Cycloaddition of azide **23** to unsymmetrical acetylenes gave



SCHEME 1



SCHEME 2



SCHEME 3

a mixture of the corresponding 4- and 5-substituted isomers **24** and **25**, whereas cycloaddition of azide **23** to symmetric acetylenes gave difunctional 1,2,3-triazole nucleosides **26**.

STRUCTURE-ACTIVITY RELATIONSHIP

TSAO-T is endowed with potent anti-HIV-1 activity (EC_{50} = 0.058 μM),[5] the antiviral activity of TSAO-T does not vary markedly from one cell line to another (EC_{50} range: 0.017-0.058μM).[5] TSAO derivatives are not inhibitory to HIV-2 strains, simian immunodeficiency virus (SIV), Moloney murine sarcoma virus and a broad range of DNA and RNA viruses at subtoxic concentrations.[5] TSAO-T does not act as a DNA chain terminator. Interaction of TSAO-T with the enzyme is non-competitive with respect to both the natural substrate (dGTP) and the template/primer [poly(rC).oligo(dG)]. TSAO-T, like the other HIV-1-specific inhibitors, interferes with a non-substrate binding site at HIV-1 RT that seems not to be present at other DNA polymerases including HIV-2 RT.[9]

A structure-activity relationship analysis including more than 100 different TSAO derivatives revealed the structural requirements that the TSAO molecule has to fulfil to inhibit HIV-1 replication. (*i*) The presence of *tert*-butyldimethylsilyl (TBDMS) groups at both C-2' and C-5' of the ribose is a prerequisite for anti-HIV-1 activity. Removal of these lipophylic groups, either at C-2', C-5' or both C-2' and C-5' positions, renders the TSAO derivatives completely inactive at subtoxic concentrations (EC_{50}: 30μM-1000 μM)[7]. The 5'-silyl protecting group seems to be more critical for activity than the 2'-silyl protecting group. Thus, replacement of the silyl moiety at the C-5' position by other groups that mimic either the lipophylic or the steric properties of TBDMS, results in antivirally inactive TSAO-T derivatives. However, a similar replacement of the silyl moiety at the C-2' position leads to compounds with only 2- to 10- fold reduced anti-HIV-1 activity[19] *(ii)* The presence of the unique 3'-spiro group [3'-spiro-5"-(4'-amino-1",2"-oxathiole-2",2"-dioxide)], in nucleosides having a *ribo* configuration, is also a prerequisite for antiviral activity. The *xylo* enantiomer renders the TSAO-T molecule completely inactive (EC_{50} >10 μM).[7] Replacement of the 3'-spiro group by other 3'-spiro moieties or change of this spiro group from position 3' to 2' of the sugar moiety in TSAO-T , results in annihilation of the antiviral activity of TSAO-T.[20] *(iii)* In contrast to the stringent structural requirements of the sugar part of the TSAO derivatives, the nature of the base part is less critical for activity. The thymine moiety of TSAO-T can be replaced by a number of other pyrimidines, purines and 1,2,3-triazoles without marked decrease of antiviral efficacy[7,8,15,17,18] (TABLE 1). The TSAO-purine derivatives are in general 3- to 5-fold less effective than the most active TSAO-pyrimidine derivatives. Among the TSAO-1,2,3-triazole compounds several derivatives with various substitutions at C-4 or C-5 of the triazole ring, show potent anti-HIV-1 activities. The 5-substituted amido, methylamido- and dimethylamido derivatives were the most potent inhibitors of HIV-1 replication with activities (EC_{50} = 0.056-0.52 μM) comparable to that of TSAO-T. Interestingly, the

TABLE 1. *Anti-HIV-1 activity of TSAO-T analogues*[a]

Compound[b]	EC_{50}[c] (μM)	CC_{50}[d] (μM)	SI[e]
TSAO-T	0.06	14	217
TSAO-m^3T	0.059	240	4088
TSAO-e^3T	0.123	123	1000
TSAO-a^3T	0.233	≥ 330	≥ 1418
TSAO-U	0.019	14	74
TSAO-C	0.76	≥ 360	460
TSAO-m^5C	0.127	30	250
TSAO-dm^4m^5C	0.16	62	388
TSAO-A	0.278	13	47
TSAO-m^6A	0.146	14	96
TSAO-Hx	0.158	14	89
TSAO-7Hx	0.173	15	86
TSAO-m^1Hx	0.514	> 150	> 292
TSAO-7m^1Hx	0.201	156	776
TSAO-7e^1Hx	0.604	> 150	> 242
TSAO-mes^4Tr	0.602	≥ 150	≥ 249
TSAO-mes^5Tr	0.90	128	142
TSAO-dmesTr	0.48	111	231
TSAO-mam^5Tr	0.16	28	175
TSAO-$dmam^5$Tr	0.056	20	357

[a] *Data taken from refs. 7, 8, 15, 17 and 18.* [b] *T=thymine; m^3T, e^3T, a^3T=3-methyl-, 3-ethyl-, 3-allyl-thymine; U=uracil; C=cytosine; m^5C=5-methylcytosine; dm^4m^5C=N^4-dimethyl-5-methylcytosine; A=adenine; m^6A=6-methyladenine; Hx=hypoxanthine; 7Hx=hypoxanthin-7-yl; m^1Hx=1-methylpoxanthine; ^{1}Hx, 7e^1Hx=1-methyl-, 1-ethyl-hypoxanthin-7-yl; mes^4Tr, mes^5, dmesTr=4-methylester-, 5-methylester-, 4,5-dimethylester-1,2,3-triazole; mam^5Tr, $dmam^5$Tr=methylamido-, 5-dimethylamido-1,2,3-triazole.* [c] *50% effective concentration.* [d] *50% cytotoxic concentration.* [e] *selectivity index, or ratio of CC_{50} to EC_{50}.*

cytotoxicity of the TSAO-pyrimidine or TSAO-purine analogues becomes significantly attenuated (10- to 20- fold), without affecting the anti-HIV-1 activity, by introduction of an alkyl or alkenyl group at N-3 (pyrimidines) or N-1 (purines) of the base moiety.[5,6,8,15]

CONFORMATIONAL STUDIES

The fact that the structural requirements for antiviral activity are more stringent with respect to the sugar part than to the base part, indicates that the sugar part must play a principal and crucial role in the interaction of the TSAO molecules with their antiviral target enzyme (reverse transcriptase). The conformation in solution (Figure 1) of TSAO-T was studied by NMR spectroscopy.[21] The conformation around the glycosidic bond (calculated from vicinal $^3J_{C6,H1'}$ and $^3J_{C2,H1'}$ coupling constants, using the Karplus equations) can be described as a *syn* =*anti* equilibrium shifted to the *anti* conformer ($X_{anti} \cong 0.7$). By using

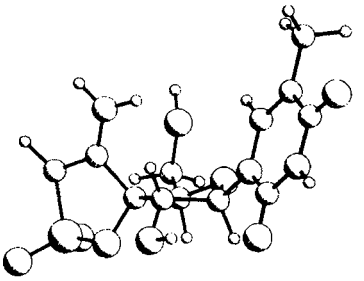


FIGURE 1. *Pluto representation of the major conformer of TSAO-T*

the concept of pseudorotation and by a modification of Pseurot method (to calculate the pseudorotational parameters), we could determine the ribose ring conformation from vicinal $^3J_{C,H}$ coupling constants. The pseudorotational parameters obtained by this method were compatible with a major conformation corresponding to an ^oE envelope ($P \cong 90°$ and $\tau \cong 50°$). Finally, the conformation around the exocyclic (C4'-C5') bond (determined from vicinal $^3J_{H,H}$ coupling constants, and by calculation of the relative distribution of g^+, g^t, and g^- populations of rotamers) showed preference for the g^+ rotamer ($n_{g+} = 0.66$).

METABOLISM AND PHARMACOKINETICS

Metabolic and pharmacokinetic studies performed[22] with radiolabelled [^{3}H]TSAO-m^{3}T , have shown that the drug tends to accumulate intracellularly within a short period of time (6 h), and 20-fold higher TSAO-m^{3}T levels are recovered intracellularly than initially added extracellularly. The high lipophilicity of TSAO-m^{3}T (Pa >> 10) is most likely responsible for the rapid uptake of the drug by the cells.

Upon intravenous bolus administration of TSAO-m^{3}T to mice at 0.75 mg/kg, the compound is rapidly cleared from the plasma in a mono-exponential manner (half-life of 22 min). Surprisingly, TSAO-m^{3}T preferentially accumulates in the lungs, far more than in the other organs *(i.e.,* heart, kidney, liver). TSAO-m^{3}T is detected at low but significant levels in the brain. These data clearly indicate that TSAO-m^{3}T is able to cross the blood-brain barrier, and thus may be able to inhibit virus replication in the brain compartment.[22]

TSAO-T is not metabolized *in vitro.* No intracellular conversion of the test compound was seen in CEM, MT-4 and Molt-4 (clone 8) cells, even when the cells were incubated as long as 72 h in the presence of the test compound. TSAO-m^{3}T was also stable in the presence of dThd phosphorylase, indicating that the test compound is not susceptible to hydrolytic attack by this enzyme.

In contrast with our *in vitro* findings, TSAO-m^{3}T is metabolized *in vivo* (mice). At least three metabolites have been unambigously characterized, *i.e.*, the TSAO-m^{3}T derivative lacking the silyl moiety at C-2', the TSAO-m^{3}T derivative lacking the silyl moiety at C-5' and the TSAO-m^{3}T derivative lacking the silyl groups at both the C-2' and C-5' positions of the sugar moiety. However, a fourth (eventual) metabolite tends to accumulate progressively with time in different tissues *(i.e,* liver, kidney, spleen). So far, the nature of this metabolite is unknown, but it is clearly different from the free base m ^{3}T and may be related to a TSAO-m^{3}T derivative with an opened 3'-spiro moiety.

VIRUS-DRUG RESISTANCE

Rapid emergence of TSAO-resistant HIV-1 strains occurred when HIV-1-infected cells were exposed to TSAO derivatives.[23,24] These TSAO-resistant HIV-1 strains proved cross-resistant to the other TSAO-pyrimidines, -purines and -triazole derivatives,but not cross-resistant to most if not all other classes of HIV-1 RT specific inhibitors, as well as, to the 2',3'-dideoxynucleosides (AZT, DDI, DDC,D4T etc.) and to the acyclic nucleoside phosphonates [PMEA, (*S*)FPMPA, (*R*)PMPDAP].[23-26]

Molecular characterization of the RT from at least 10 different TSAO-resistant HIV-1 strains revealed a single amino acid change at position 138. Invariably, glutamic acid was replaced by lysine at position 138 of all HIV-1 RT mutants. This amino acid change is exclusively associated with HIV-1 RT resistance to the TSAO derivatives[24] and must play a crucial role in the recognition of TSAO derivatives by the enzyme.

Molecular dynamics performed on TSAO-T derivatives[21] showed that the 4"-amino group on the spiro moiety of the antivirally active *ribo*-TSAO-T derivative is more accessible to interactions with other molecular entities (ie. the carboxylic acid group of glutamic acid at position 138 of HIV-1 RT), than the antivirally inactive *xylo* isomer.[21] Based on these observations together with our SAR studies on TSAO derivatives and site-directed mutagenesis studies on HIV-1 RT containing amino acids other than glutamic acid at position 138 (i.e. Arg, Lys),[24,27] we postulate that the 4"-amino group of the 3'-spiro moiety is most likely responsible for the specific interaction of the TSAO molecule with the carboxylic acid group of 138-Glu. Further evidence for our postulate was that when the 4"-amino group of TSAO-T was blocked by introduction of acetyl or benzyl groups, or removed, antivirally inactive TSAO derivatives were obtained.[19,20] The fact that the glutamic acid-138 is not present in HIV-2 and SIV, further corroborates this hyphothesis and may explain why HIV-2 or SIV are not sensitive to the antiviral action of the TSAO derivatives.

Recently, determination of the three-dimensional structure of HIV-1 RT has shown a totally different folding for the p66 and p51 subunits.[28] Most amino acid substitutions so far reported to confer resistance to the HIV-1-specific RT inhibitors are clustered in the palm domain of the p66 subunit. In contrast the amino acid 138, that is involved in the sensitivity and/or resistance to TSAO derivatives, is located at the top of the p66 finger domain of RT and distant from the binding site of the HIV-1-specific inhibitors. We initially thought that this peculiar characteristic may explain why TSAO-resistant HIV

strains retain sensitivity to the other NNRTIs. However, the crystallographic structure of HIV-1 RT revealed that the amino acid at position 138 of the p51 subunit closely approaches the hydrophobic pocket of p66 at which the HIV-1-specific RT inhibitors seem to bind.[28] Moreover, when the TSAO-specific resistance mutation 138 Glu → Lys was introduced in the p66, but not p51, subunit, the enzyme remained equally sensitive to several HIV-1 specific RT inhibitors including TSAO derivatives. On the other hand, when the mutation was introduced only in the p51 subunit of the p66/p51 RT heterodimer, the enzyme proved highly resistant to TSAO derivatives, but retained full sensitivity to other HIV-1-specific RT inhibitors and to ddGTP.[29] These data clearly indicate that among the HIV-1-specific RT inhibitors the TSAO molecules are, at present, the only example of compounds which seem to interact directly with the p51 subunit of the RT heterodimer, thus determining the sensitivity/resistance pattern of HIV-1 RT to the TSAO derivatives.[29]

The fact that other mutations in the RT at amino acid positions 181 (Tyr → Cys), 188 (Tyr → Cys) and 106 (Val → Ala) also lead to markedly reduced sensitivity of HIV-1 RT to TSAO derivatives strongly suggest the presence of additional interaction points that may serve either as direct attachment sites for the TSAO compounds or at least help in maintaining the desirable conformation of the TSAO binding sites in the RT heterodimer.[30]

CONCLUSIONS

TSAO derivatives represent a unique class of nucleosides that are specifically targeted at HIV-1 RT. The TSAO compounds are the first HIV-1-specific RT inhibitors for which a well defined part of the molecule (i.e. the 4"-amino group of the 3'-spiro moiety) has been identified as an essential pharmacophore interacting with a well-defined moiety (the -COOH group of Glu-138) of the RT target enzyme. They are, so far, the only compounds for which the p51 subunit of RT plays an important role in the sensitivity/resistance of HIV-1 strains to TSAO derivatives.

Acknowledgements. We thank the Spanish CICYT, the Plan Regional de Investigación de la Comunidad de Madrid, the NATO (Collaborative Research Programme) and the Biomedical Research Programme of the European Community for financial support.

REFERENCES

1 (a) Fischl, M.A.; Richman, D.D.; Grieco, M.H. and the AZT Collaborative Working Group. *New Engl. J. Med.* **1987**, *317*, 185-191. (b) De Clercq, E. *J. Acquir. Immun. Defic. Syndrom.* **1991**, *4*, 207-218.

2 (a) Yarchoan, R.; Broder, S. *Pharm. Ther.* **1989**, *40*, 329-384. (b) Yarchoan, R.; Mitsuya, H.; Thomas, R.V.; Pluda, J.M.; Hartman, N.R.; Perno, C.F.; Marczyk, K.S.; Allain, J.P.; Johns, D.G.; Broder, S. *Science* **1989**, *245*, 412-415.

3 (a) Richman, D.D.; Fischl, M.A.; Grieco, M.H. and the Collaborative Working Group *New Engl. J. Med.* **1987**, *317*, 192-197. (b) Larder, B.AQ.; Kemp, S.D. *Science*, **1989**, *246*, 1155.

4 (a) Yarchoan, R.; Pluda, J.M.; Thomas, R.V.;Mitsuya, H.; Browers, P.; Wyvill, K.M.; Hartman, N.R.; Johns, D.G.; Broder, S. *Lancet* **1990**, *336*, 526-529. (b) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. Usa* **1986**, *83*, 1911-1915.

5 Balzarini, J.; Pérez-Pérez, M.J.; San-Félix, A.; Schols, D.; Perno, C.F.; Vandamme, A.M.; Camarasa, M.J.; De Clercq, E. *Proc. Natl. Acad. Sci.* USA, **1992**, *89*, 4392-4396.
6 Balzarini, J.; Pérez-Pérez, M.J.; San-Félix, A.; Velázquez, S.: Camarasa, M.J.; De Clercq, E. *Antimicrob. Agents Chemother.* **1992**, *36*, 1073-1080.
7 Camarasa, M.J.; Pérez-Pérez, M.J.; San-Félix, A.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 2721-2727.
8 Pérez-Pérez, M.J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M.J. *J. Med. Chem.* **1992**, *35*, 2988-2995.
9 Balzarini, J.; Pérez-Pérez, M.J.; San-Félix, A.; Camarasa, M.J.; Bathurst, I.C.; Barr, P.J.; De Clercq, E. *J. Biol. Chem.* **1992**, *267*, 11831-11838.
10 (a) Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J.; Schols, D.; Nakashima, H.; Perno, C.F.; Walker, R.T.; Miyasaka, T. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1375-1381.(b) Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R.T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 2507-2509. (c) 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.; Janssen, P.A.J. *Nature* **1990**, *343*, 470-474. (d) Kukla, M.J.; Breslin, H.J.; Pauwels, R.; Fedde, C.L.; Miranda, M.; Scott, M.K.; Sherrill, R.G.; Raeymaeckers, A.; Van Gelder, J.; Andries, K.; Janssen, M.A.C.; De Clercq, E.; Janssen, P.A.J. *J. Med. Chem.* **1991**, *34*, 746-751. .
11 ((a) Merluzzi, V.J.; Hargrave, K.D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J.C.; Shih, C.K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A.S.; Faanes, R.; Eckner, R.J.; Koup, R.A.; Sullivan, J.L. *Science* **1990**, *250*, 1411-1413. (b) Koup, R.A.; Merluzzi, V.J.; Hargrave, K.D.; Adams, J.; Grozinger, K.; Eckner, R.J.; Sullivan, J.L. *J. Infect. Dis* **1991**, *163*, 966-970. (c) Goldman, M.E.; Nunberg, J.H.; O'Brien, J.A.; Quintero, J.C.; 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.; Stern, A.M. *Proc. Natl. Acad. Sci.* USA **1991**, *88*, 6863-6867. (d) 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.; Tarpley, W.G. *Proc. Natl. Acad. Sci.* USA **1991**, *88*, 8806-8810.
12 (a) Pauwels, R.; Andries, K.; Debyser, Z.; Van Daele, P.; Schols, D.; Stoffels, P.; De Vreese, K.; Woestenborghs, R.; Vandamme, A.-M.; Janssen, M.G.C.; Anné, J.; Cauwenbergh, G.; Desmyter, J.; Heykants, J.; Janssen, M.A.C.; De Clercq, E.; Janssen, P.A.J. *Proc. Natl Acad. Sci.* USA **1993**, *90*, 1711-1715. (b) Zhang, H.; Vrang, L.; Bäckbro, K.; Unge, T.; Engelhardt, P.; Högberg, M.; Kangasmetsä, J.; Lind, P.; Noréen, R.; Sahlberg, C.; Zhou, X.-X.; Johansson, N.G.; Cantrell,, A.S.; Jaskunas, R.; Morin, J.M. Jr.; Ternansky, R.J.; Bell, F.W.; Jordan, C.L.; Kinnick, M.D.; Palkowitz, J.A.; Parrish, C.A.; Pranc, P.; Vasileff, R.T.; West, S.J.; Öberg. Abstacts of the Second International workshop on HIV Drug-Resistance, Noordwijk, The Netherlands,3-5 June, **1993**, p 30.
13 Hayakawa, H.; Tanaka, H.; Itoh, N; Nakajina, M.; Miyasaka, T.; Yamaguchi, K and Iitaka, Y.; *Chem. Pharm. Bull*. **1987**, *35*, 2605-2608.
14 Vorbrüggen, H.; Kolikiewicz, K.; Bennua, B. *Chem.Ber.* **1981**, *114*, 1234-1256.
15 Velázquez, S.; San-Félix, A.; Pérez-Pérez, M.J.; Balzarini, J.; De Clercq, E.: Camarasa, M.J. *J. Med. Chem.* **1993**, *36*, 3230-3239.
16 Calvo-Mateo, A.; Camarasa, M.-J.; Díaz-Ortíz, A.; de las Heras, F G. *J .Chem. Soc., Chem. Commun.* **1988**, *11*, 14-15.
17 San-Félix, A.; Velázquez, S.; Pérez-Pérez, M.J.; Balzarini, J.; De Clercq, E.; Camarasa, M.J. *J. Med. Chem.* **1994**, *37*, 453-460.
18 Alvarez, A.; Velázquez, S.; San-Félix, A.; Aquaro, S.; De Clercq, E.; Perno, C.F.; Balzarini, J.; Camarasa, M.J. *J. Med. Chem.* **1994**, in press.
19 Ingate, S.; Pérez-Pérez, M.J.; De Clercq, E.; Balzarini, J.; Camarasa, M.J. **1994**, unpublished results.
20 Velázquez, S.; Jimeno, M.L.; Balzarini. J.; Camarasa, M.J. *Tetrahedron*, **1994**, in press.
21 Jimeno, M.L.; Pérez-Pérez, M.J.; Rozas, I.; San-Félix, A.; De Clercq, E.; Balzarini, J.; Camarasa, M.J. **1994**, unpublished results.
22 Balzarini, J.; Naesens, L.; Bohman, C.; Pérez-Pérez, M.J.; San-Félix, A.; Camarasa, M.J.; De Clercq, E. *Biochem Pharmacol* **1993**, *46*, 69-77.
23 Balzarini, J.; Velázquez, S.; San-Félix, A.; Karlsson, A.; Pérez-Pérez, M.J.; Camarasa, M.J.; De Clercq, E. *Mol Pharmacol* **1993**, *43*; 109-114.

24 Balzarini, J.; Karlsson, A.; Vandamme, A.-M.; Pérez-Pérez, M.J.; Vrang, L.; Oberg, B.; San-Félix, A.; Velázquez, S.; Camarasa, M.J.; De Clercq, E. *Proc Natl Acad Sci USA* **1993**, *90*; 6952-6956.
25 Balzarini, J.; Karlsson, A.; Pérez-Pérez. M.-J.;Vrang, L.; Oberg, B.; Vandamme, A.M.; Camarasa, M.J.; De Clercq, E. *Virology* **1993**, *192*; 246-253.
26 Balzarini, J.; Karlsson, A.; Pérez-Pérez. M.-J.; Camarasa, M.J.; Tarpley, W.G.; De Clercq, E. *J Virol* **1993**, *67* , 5353-5359.
27 Balarini, J.; Kleim, J.-P.; Riess, G.; Camarasa, M.J.; De Clercq, E.; Karlsson, A. *Biochem. Biophys. Res. Commun.* **1994**.
28 (a) Kohlstaedt, L.A.; Wang, J.; Friedman, J.M.; Rice, PA.; Steitz, TA. *Science* **1992**, *256*, 1783-1790. (b) Jacobo-Molina, A.; Ding, J.; Nanni, R.G.; et al. *Proc Natl Acad Sci USA* **1993**, *90*, 6320-6324. (c) Nanni, R.G.; Ding, J.; Jacobo-Molina, A.; Hughes, S.H.; Arnold, E. *Perspect. Drug. Discov. Design.* **1993**, *1*, 129-150. (d) Smerdon, S.J.; Jàger, J.; Wang, J.; Kohlstaedt, T.L.A.; Chirino, A.J.; Friedman, J.M.; Rice, P.A.; Steitz, T.A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3911-3915.
29 Jonckheere, H.; Taymans, J.-M.; Balzarini, J.; Velázquez, S.; Camarasa, M.J.; Desmyter, J.; De Clercq, E.; Anné, J. *J. Biol. Chem.* **1994**, in press.
30 Balzarini, J.; Camarasa, M.J.; Karlsson, A. *Drugs of the Future*, **1993**, *18*, 1043-1055.