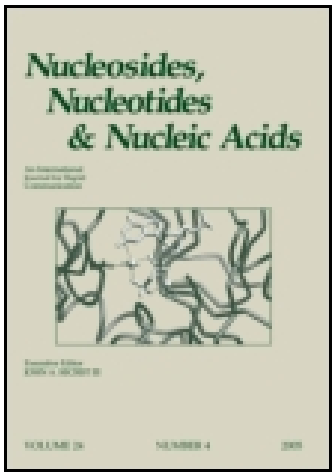

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"SECOND GENERATION" OF TSAO COMPOUNDS DIRECTED AGAINST HIV-1 TSAO-RESISTANT STRAINS

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"SECOND GENERATION" OF TSAO COMPOUNDS DIRECTED AGAINST HIV-1 TSAO-RESISTANT STRAINS

E. Lobatón,[1] S. Velázquez,[1] M. J. Pérez-Pérez,[1] M. L. Jimeno,[1] A. San-Félix,[1] E. De Clercq,[2] J. Balzarini,[2] and M. J. Camarasa[1]*

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ABSTRACT

A "second generation" of TSAO molecules directed against TSAO-resistant strains have been prepared. The presence of two neighboring carbonyl groups at the 4″ position of the 3′-spiro moiety seems to be important for the anti-HIV-1 activity against both wild type and TSAO-resistant strains. NMR conformational studies in solution and theoretical calculations of the novel compounds have also been carried out.

TSAO derivatives represent a particular and peculiar group of potent and highly specific inhibitors of the human immunodeficiency virus type 1 (HIV-1) replication that seem to interact at the interface between the p51 and p66 reverse transcriptase (RT) subunits (1,2). Well-defined aminoacids at both p51 and p66 RT subunits are needed for an optimal interaction of TSAO compounds with the HIV-1 RT (3). The prototype compound is the thymine derivative named TSAO-T (**1**). The most selective compound of this series is TSAO-m^3T (**2**) (Fig. 1). Selection of at least eight different TSAO-resistant strains in cell culture and molecular characterization of these strains revealed in all cases a single aminoacid mutation at position 138 (Glu138Lys). This mutation is important at the level of the p51 subunit of HIV-1 RT (4). Our experimental data strongly suggest a specific interaction of

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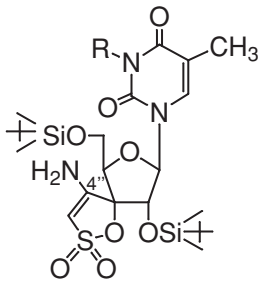


(**1**) R = H (TSAO-T)
(**2**) R = CH_3 (TSAO-m^3T)

Figure 1.

the 4″-amino group of the 3′-spiro moiety of TSAO molecules with the carboxylic group of the glutamic acid residue at position 138 (Glu-138) (2).

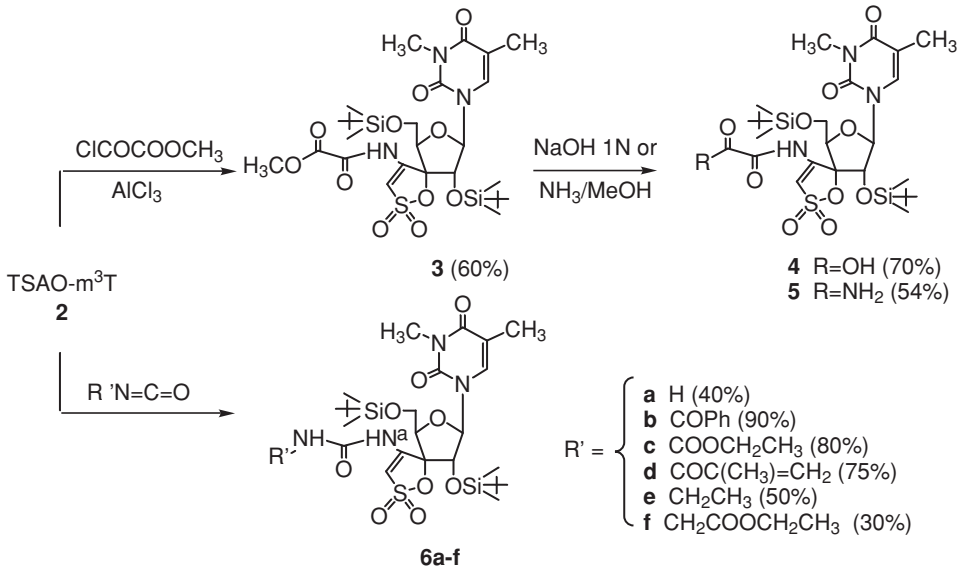
Based on this hypothesis we now focussed on modifications at the 4″-amino group of the spiro moiety of TSAO-m^3T in order to obtain a "second generation" of TSAO molecules directed against TSAO-resistant HIV-1 strains. These novel compounds contain at this 4″-position different carbonyl, carboxylic acid and ester groups that may form H-bonds with NH_2 of Lys-138 (TSAO-resistant strains).

The synthesis of the target molecules was carried out by acylation of TSAO-m^3T (**2**) (Scheme 1), acylation that occurs exclusively on the 4″-NH_2 group in contrast to the acylation of "classical" enamines where *N*- and *C*-acylation has been reported (5). Thus, reaction of **2** with methyl oxalyl chloride in the presence of $AlCl_3$ gave the 4″-N-oxalyl derivative **3** in good yields. The methyl ester group of **3** was further transformed either into the free acid **4** or the amide **5** by treatment with NaOH 1N or NH_3/MeOH, respectively. Treatment of **2** with conveniently functionalized isocyanates gave the corresponding 4″-N-alkyl and acyl urea derivatives **6a**-**f** in moderate to good yields.

The novel TSAO derivatives **3**-**5** and **6a**-**f** were evaluated against HIV-1 replication in cell culture. The N-acyl derivatives **3**-**5**, bearing oxalyl substituents at the 4″ position of the spiro moiety, show similar anti-HIV-1 activity than the parent TSAO-m^3T. Moreover, compound **4** also show moderate activity against HIV-1 TSAO-resistant strains. In contrast, introduction of an ureido moiety (**6a**-**f**) annihilates activity.

Additionally, a comparative NMR conformational analysis in solution (acetone-d_6) between the 4″-N-oxalyl derivative **3** (active) and the 4″-N-urea compound **6e** (inactive) has been performed. The possibility of formation of intramolecular hydrogen bonds of the NH protons of the 4″ position of the spiro moiety, that may stabilize some conformations, has also been studied.

The conformation around the glycosidic bond in compounds **3** and **6e** was obtained from the vicinal carbon-proton couplings $^3J_{C2,H1'}/^3J_{C6,H1'}$ and the corresponding dihedral angles $\theta_{C2,H1'}/\theta_{C6,H1'}$, obtained by modeling studies, using the



Scheme 1.

modified Karplus relations (6). Although both compounds showed a *syn*/*anti* equilibrium, for compound **6e** the equilibrium is mainly shifted to the *syn* conformer ($P_{syn} = 83\%$, $P_{anti} = 17\%$) while for compound **3** a more equal distribution of *syn*/*anti* forms is observed ($P_{syn} = 56\%$, $P_{anti} = 44\%$). The *syn* conformer of **6e** can be stabilized by an intramolecular hydrogen bond between the amido group (NHa) directly attached to the spiro moiety (see Scheme 1) and the C-2 carbonyl of the thymine. The *syn*/*anti* populations of compounds **3** and **6e** were confirmed by n.O.e experiments.

Hydrogen bonds were studied by temperature dependent chemical shift NMR experiments (7) using acetone-d_6 as solvent. The temperature coefficients $\Delta\delta/\Delta T$ (ppb/K) values indicate that only the NHa of compound **6e** is involved in intramolecular hydrogen bonds, which is in agreement with the conformational analysis results described above.

Finally, semiempirical quantum mechanical AM1 calculations for *syn* and *anti* conformers of nucleosides **3** and **6e** were carried out. These studies indicate that the NHa...CO-2 bond distance in the *syn* conformer of compound **6e** is suitable for hydrogen bond formation which is in agreement with the results obtained by NMR studies in solution.

In conclusion, the presence of two neighboring carbonyl groups at the 4″ position of the spiro moiety is important for the activity. Compound **4** is the first example of a "second generation" of TSAO derivatives that is active against TSAO-resistant strains. NMR and theoretical studies show significant differences between 4″-N-oxalyl and 4″-N-urea TSAO derivatives **3** and **6e** in their ability of formation of intramolecular H-bonds that may compromise the interaction of these compounds with HIV-1 RT and therefore the observed activity.

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