

NOVEL NON-NUCLEOSIDE HUMAN CYTOMEGALOVIRUS INHIBITORS BASED UPON TSAO NUCLEOSIDE DERIVATIVES: STRUCTURE-ACTIVITY RELATIONSHIPS

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□ *TSAO derivatives are a unique group of potent and highly specific inhibitors of HIV-1 replication. We have recently reported 4''-ureido TSAO derivatives that are devoid of anti-HIV-1 activity, but inhibit human cytomegalovirus with an activity comparable to that of Ganciclovir. We herein report the synthesis and biological evaluation of novel 4''-ureido TSAO derivatives in order to evaluate the structural features required for anti-HCMV activity. Interestingly, these studies revealed that the compounds may inhibit HCMV at the DNA polymerase step via a non-nucleoside mechanism.*

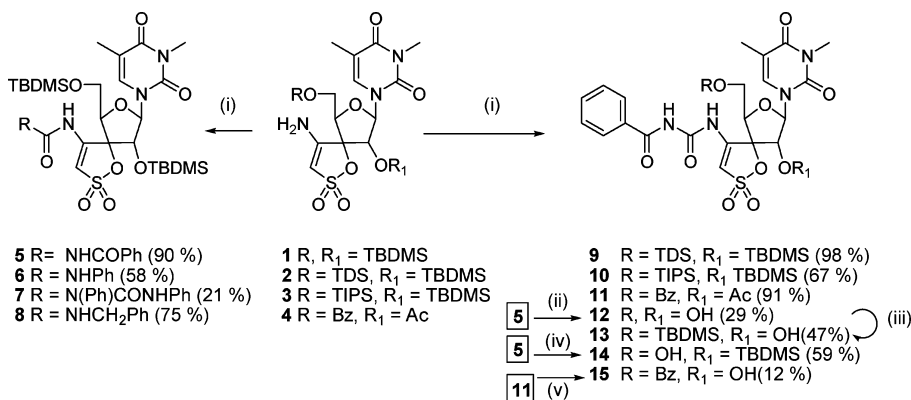
Keywords TSAO nucleosides; cytomegalovirus; ureidonucleosides; HCMV inhibitors

INTRODUCTION

Human cytomegalovirus (HCMV) is an ubiquitous member of the herpesvirus family. Although it rarely causes symptomatic disease in immunocompetent individuals, it is responsible for a variety of severe, often life-threatening diseases (i.e., pneumonia, retinitis) in immunocompromized or immunosuppressed individuals (transplant recipients and in AIDS patients).^[1] HCMV also is a major cause of congenital malformation in newborn children.^[2] Currently approved anti-HCMV agents (ganciclovir, foscarnet, valganciclovir, cidofovir, and formivisen) inhibit the viral DNA polymerase.^[3] However, these drugs suffer from a number of drawbacks such as dose-limiting bone marrow and kidney toxicity, unfavorable pharmacokinetic properties, as well as the emergence of single and multiple drug

We thank Susana Ruiz for excellent technical assistance. We also thank the Spanish MEC (project SAF 2006-12713-C02-01) for financial support.

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SCHEME 1 Reagents and conditions (i) RNCO, NaH, THF, 70°C (ii) (iii) TBDMSCl, Py, rt (iv) 1N HCl, methanol, 0°C (v) NH₃, methanol.

resistance.^[4] Therefore, there is a need for new anti-HCMV compounds, potent, safe, orally bioavailable, and with novel mechanisms of action.

We have recently reported^[5] that 4''-ureido-TSAO derivatives (TSAO compounds are a particular and peculiar group of potent and highly specific inhibitors of HIV-1 replication^[6]) are devoid of anti-HIV-1 activity, but are potent and selective inhibitors of HCMV in cell culture. The prototype compound (**5**) inhibit HCMV replication with an activity comparable to that of Ganciclovir.^[5] However, the highly modified structures of these TSAO nucleosides suggest a different mechanism of inhibition of the viral replication.

In order to evaluate the structural features required for anti-HCMV activity, modifications at the 4''-ureido moiety, and replacement of the 2' and/or 5' *tert*-butyldimethylsilyl groups by other groups, have been performed. In this communication we report on the synthesis, biological evaluation, and preliminary time of addition experiments of these novel 4''-ureido TSAO derivatives.

RESULTS AND DISCUSSION

Synthesis

4''-Substituted TSAO-m³T derivatives, bearing different ureido groups at the 4''-position (**6–8**), were prepared, in moderate to good yields, by reaction of **1** (Scheme 1) with an excess of conveniently functionalized isocyanates in the presence of NaH in dry acetonitrile at 70°C. On the other hand, compounds substituted with other silyl groups at the 5' position, such as 5'-*O*-*tert*-hexyldimethylsilyl (TDS) (**9**) or 5'-*O*-triisopropylsilyl (TIPS) (**10**), were prepared in good yields by reaction of the corresponding 5'-*O*-protected TSAO derivatives **2** or **3** with benzoyl isocyanate, in the presence of NaH at

70°C.^[7] Next, 4''-benzoylureido-TSAO-m³T derivatives bearing other groups at 2'- and 5'-positions were prepared as follows. A reaction of **5** with tetrabutylammonium fluoride in dry THF gave the 2',5'-*O*-deprotected 4''-benzoyl ureido derivative **12** that was silylated (TBDMSCl/pyridine) to give compound **13**. Similarly, treatment of **5** with a 0.1N solution of HCl in methanol at 0°C gave **14** in a 59% yield. Finally, reaction of **4**^[9] with benzoyl isocyanate in dry THF at room temperature gave the 2'-acetyl-5'-benzoyl-4''-benzoyl derivative **11** (91% yield). Treatment of **11** with NH₃ in methanol at 0°C gave the 5'-benzoyl-2'-*O*-deprotected derivative **15** in low yield.

Biological Evaluation

The novel TSAO derivatives (**6–10**, **11–15**) were evaluated against replication of human CMV in cell culture. Several compounds showed pronounced antiviral activity at concentrations in the lower micromolar range (i.e., 0.12–1.5 μ M) at a concentration below their toxicity threshold. Compounds **9** and **10**, in which the 5'-*tert*-buthyldimethylsilyl groups were replaced by other silyl groups, showed a similar activity to that of compound **5** (EC₅₀ = 1.1 and 1.2 μ M versus 0.8 μ M), although they were more toxic (CC₅₀ => 50 μ M versus 200 μ M). In contrast, 2'-acetyl-5'-benzoyl-4''-benzoyl-ureido derivative **11** was devoid of antiviral activity at subtoxic concentrations. Also, compounds containing a free OH at 2'- and/or 5'-position(s) (**13–15**) were devoid of antiviral activity at subtoxic concentrations. Given the nucleoside type structure of these compounds, one would expect that these new anti-HCMV compounds may act via a "conventional" nucleoside mechanism involving 5'-phosphorylation. However, the biological results point to a different mechanism of action since both 2' and 5'-OH groups need to be "blocked" with silyl groups to show anti-HCMV activity. Indeed, this new family of agents seem to act via a novel non-nucleosidic mechanism. Thus, the preliminary time of addition experiments, carried out with the prototype compound **5** suggests that this compound acts before GCV and HPMPC (compounds known to affect a late phase of HCMV replicative cycle). Interestingly, these studies revealed that the compounds may inhibit HCMV at the DNA polymerase step via a non-nucleoside mechanism. A more detailed SAR study of these nucleosides and of the mechanism of action is now in progress.

REFERENCES

1. Pass, R.F. Cytomegalovirus. In *Fields Virology*. Lippincott, Williams & Wilkins, Philadelphia, **2001**, Vol 2 . 4th ed., pp. 2675–2705.
2. Trincado, D.E.; Scott, G.M.; White, P.A.; Hunt, C.; Rasmussen, L.; Rawlinson, W.D. Human cytomegalovirus strains associated with congenital and perinatal infections. *J. Medical Virology* **2000**, 61, 481–487.

3. Yeh, R. and Cohen, D.M. Pharmacology of viral replication. In *Principles of Pharmacology: The Pathophysiologic Basis Of Drug Therapy*. Lippincott, Williams & Wilkins, Baltimore, **2004**, pp. 545–564.
4. Villareal, E.C. Current and potential therapies for the treatment of herpes-virus infections. *Progress in Drug Research* **2003**, 60, 263–307.
5. De Castro, S.; Lobatón, E.; Pérez-Pérez, M.J.; San-Félix, A.; Cordeiro, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Camarasa, M.J.; Velázquez, S. Novel [2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-ribofuranosyl]-3''-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide) derivatives with anti-HIV-1 and anti human cytomegalovirus activity. *J. Medicinal Chemistry* **2005**, 48, 1158–1168.
6. Camarasa, M.J.; San-Félix, A.; Velázquez, A.; Pérez-Pérez, M.J.; Gago, F.; Balzarini, J. TSAO compounds: The comprehensive store of a unique family of HIV-1 specific inhibitors of reverse transcriptase. *Current Topics Medicinal Chem.* **2004**, 4, 945–963.
7. Ingate, S.; Pérez-Pérez, M.J.; De Clercq, E.; Balzarni, J.; Camarasa, M.J. Synthesis and anti-HIV-1 activity of novel TSAO-T derivatives modified at the 2'- and 5'-position of the sugar moiety. *Antiviral Research* **1995**, 27, 281–299.
8. Pérez-Pérez, M.J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M.J. TSAO analogues. Stereospecific and anti-HIV-1 activity of 1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-ribofuranoxyl]-3-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide) pyrimidine and pyrimidine-modified nucleosides. *J. Medicinal Chem.* **1992**, 35, 2988–2995.

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