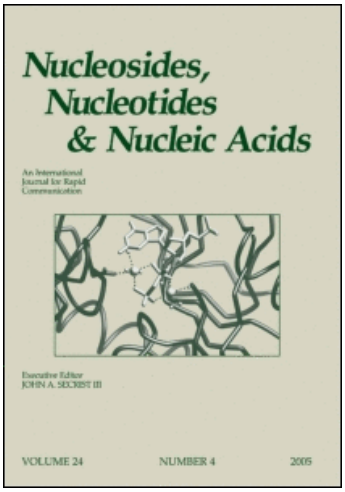

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Novel Non-Nucleoside Human Cytomegalovirus Inhibitors Based Upon Tsao Nucleoside Derivatives: Structure-Activity Relationships

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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

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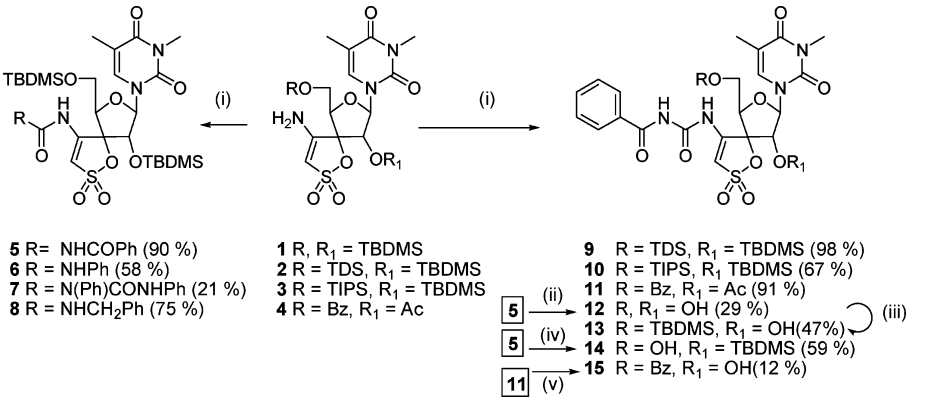

SCHEME 1 Reagents and conditions (i) RNCO, NaH, THF, 70°C (ii) (iii) TBDMSCI, Py, rt (iv) 1N HCI, methanol, 0°C (v) NH_3, methanol.

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″-Subtituted TSAO-m^3T 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-m^{3}T derivatives bearing other groups at 2′- and 5′-positions were prepared as follows. A reaction of **5** with tetrabutylamonium 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 NH_3 in methanol at 0°C gave the 5′-benzoyl-2′-*O*-deprotected derivative **15** in low yield.

Biological Evalutation

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 μM) at a concentration below their toxicity threshold. Compounds **9** and **10**, in which the 5′-*tert*-buthyldimenthylsilyl groups were replaced by other silyl groups, showed a similar activity to that of compound **5** (EC_{50} = 1.1 and 1.2 μM versus 0.8 μM), although they were more toxic (CC_{50} => 50 μM versus 200 μM). 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.

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