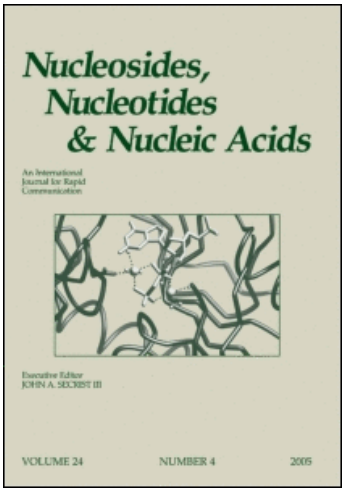

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Nucleoside Analogues Exerting Antiviral Activity Through a Non-nucleoside Mechanism†

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ABSTRACT

In analogy with maribavir [1-(β-L-ribofuranosyl)-isopropylamino-5,6-dichlorobenzimidazole], a nucleoside analogue that acts against human cytomegalovirus (HCMV) by a non-nucleoside mechanism, here I present three other examples of classes of nucleoside analogues (i.e. bicyclic furo[2,3-d]pyrimidine as well as HEPT and TSAO derivatives) that act against either HCMV or human immunodeficiency virus (HIV) through a non-nucleoside mode of action.

Key Words: Neucloside analogues; HCMV; HIV.

INTRODUCTION

Toyocamycin, tubercidin, sangivamycin and sugar-modified analogues thereof have been known for a long time as nucleoside analogues effective against a large variety of viruses, albeit endowed with a relatively small therapeutic index.[1,2] When the sugar moiety of these compounds was replaced by a benzyl moiety, these compounds, i.e. the pyrrolo[2,3-d]pyrimidine derivatives 951 and 1028 (Figure 1) were turned into specific

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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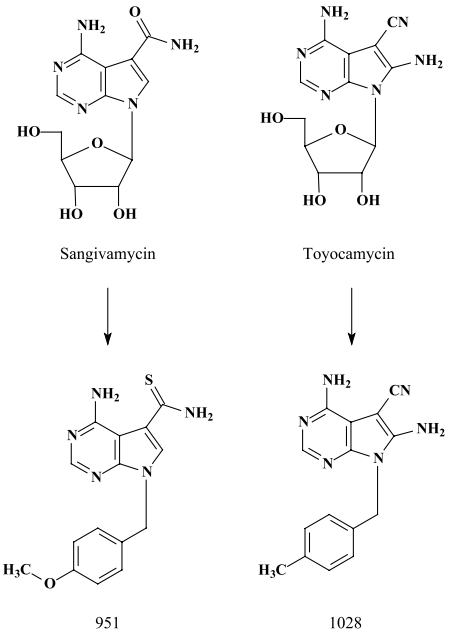

Figure 1. Pyrrolo[2,3-d]pyrimidine derivatives sangivamycin, toyocamycin and their derivatives 951 and 1028, respectively.

inhibitors of human cytomegalovirus (HCMV) replication;[3] they were found to be effective against HCMV strains that were resistant towards the acyclic nucleoside analogue ganciclovir and postulated to interfere with an early step of HCMV replicative cycle, presumably coinciding with immediate early antigen expression.[3]

While the compounds 951 and 1028 are, stricto sensu, non-nucleoside analogues, the benzimidazole derivatives TCRB [1-β-D-ribofuranosyl-2,5,6-trichloro-benzimidazole], BDCRB [1-(β-D-ribofuranosyl)-2-bromo-5,6-dichlorobenzimidazole] and its L-counterpart, 1263W94 (maribavir), in which, in addition to the D → L configuration switch, the bromine is replaced by an isopropylamine moiety, do qualify as nucleoside analogues (Figure 2). Yet, they do not interact as would be expected from nucleoside analogues that is with the RNA (or DNA) polymerisation reaction, but appear to be targeted at totally different processes. BDCRB is targeted at the cleavage of viral high-molecular weight DNA concatemers and packaging of monomeric genomes into procapsids (a process catalyzed by the so-called ''terminase'', which is composed of the UL89 and UL56 gene products).[4,5] Maribavir, on the other hand, appears to be targeted at the UL97 protein kinase,[6] an enzyme that has been known to phosphorylate ganciclovir to its monophosphate form, and has been recently held responsible for the nuclear egress, i.e. release, of HCMV nucleocapsids from the nucleus.[7]

The pyrrolopyrimidine derivatives (951 and 1028) as well as the benzimidazole derivatives TCRB and BDCRB, which all originated from L.B. Townsend's Laboratory, have in common that they are specifically active against HCMV and act by a ''non-nucleoside mode of action''. Of these benzimidazole derivatives, synthesized through L.B. Townsend's collaboration with Burroughs Wellcome Co. (now

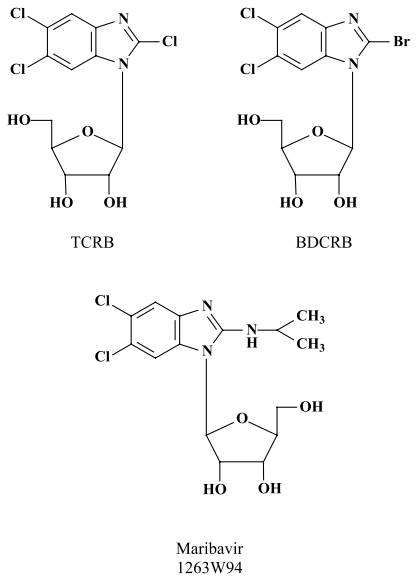

Figure 2. Benzimidazole derivatives TCRB, BDCRB and maribavir (1263W94).

GlaxoSmithKline), maribavir (1263W94) has proceeded to preclinical pharmacokinetic and toxicological studies in mice, rats and monkeys[8] and phase I/II dose escalation studies in HIV-infected men with asymptomatic HCMV shedding.[9] In the latter studies, maribavir demonstrated in vivo antiviral efficacy, as based on significant reductions in semen HCMV titers. Maribavir can be considered as the prototype of a nucleoside analogue, effecting in vivo and in vitro antiviral activity through a mode of action that is unusual and non-classical for the nucleoside type of compounds.[10] This article, in honor of L.B. Townsend, is meant to provide a few additional representative cases of nucleoside analogues exhibiting antiviral effects through a non-nucleoside mechanism: 1) HEPT [1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine] derivatives, 2) TSAO-T [(2′,5′-bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)thymine] derivatives and 3) bicyclic furo[2,3-d]pyrimidine nucleoside derivatives. Whereas the former 1) and 2) act as non-nucleoside reverse transcriptase inhibitors (NNRTIs) on the replication of human immunodeficiency virus type 1 (HIV-1), the latter 3) specifically interfere with HCMV replication at an early stage of viral life cycle.

HEPT [1-[(2-HYDROXYETHOXY)METHYL]-6-(PHENYLTHIO)THYMINE] DERIVATIVES

The starting point for the design and synthesis of the HEPT analogues (Figure 3) must have been the antiherpetic drug acyclovir [9-[(2-hydroxyethoxy)methyl]guanine], as they possess the same acyclic side chain. Yet, it had been shown earlier that acyclic nucleoside analogues of biologically important pyrimidine cyclic nucleosides (such as

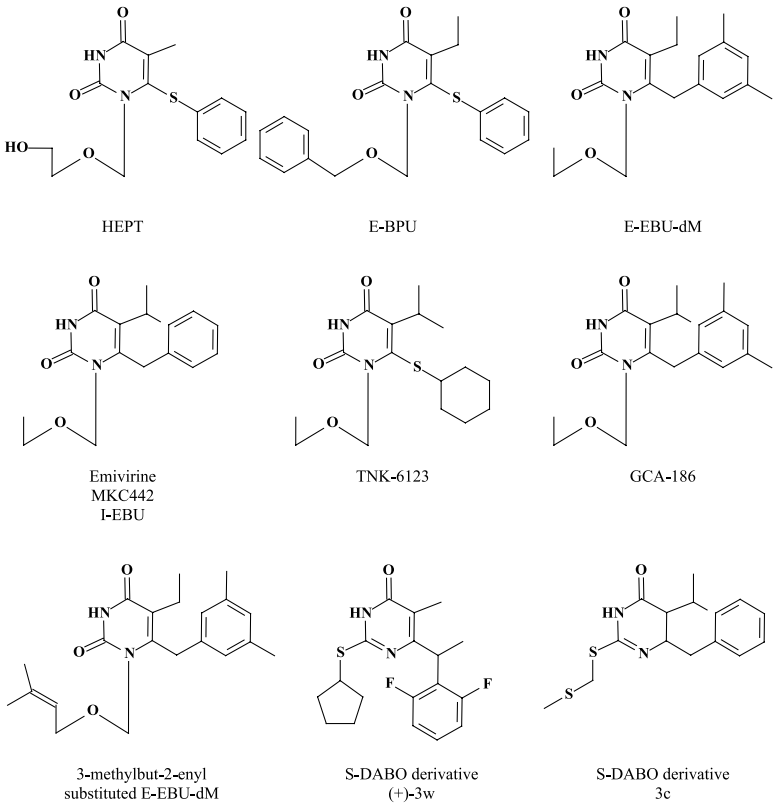

Figure 3. HEPT analogues (E-BPU, E-EBU-dM, I-EBU, TNK-6123, GCA-186, 3-methylbut-2-enyl substituted E-EBU-dM) and S-DABO [(+)-3w and 3c)] derivatives.

5-iodo-, 5-fluoro-, or 5-trifluoromethyl-2′-deoxyuridine) were inactive against herpes simplex virus (HSV) and several other DNA or RNA viruses.[11,12] In this perspective, it was not surprising that 1-[(2-hydroxyethoxy)methyl]-6-substituted thymine analogues had no activity against HSV. What was surprising, however, was that this 6-substituted acyclouridine had activity against HIV-1,[13,14] and thus HEPT was the first representative of a new class of HIV-1 inhibitors which, later on, would become known as NNRTIs (non-nucleoside reverse transcriptase inhibitors). Through lead optimization, several new HEPT congeners were developed, such as 5-ethyl-1-benzyloxymethyl-6-(phenylthio)uracil (E-BPU),[15] 5-ethyl-1-ethoxymethyl-6-(3,5-dimethylbenzyl)uracil (E-EBU-dM),[16] and, finally, the clinical drug candidate 5-isopropyl-1-ethoxymethyl-6-benzyluracil (I-EBU), MKC-442, emivirine.[17] Emivirine proved to be a potent inhibitor of the HIV-1 reverse transcriptase and HIV-1 replication, both in vitro and in vivo, with a favorable pharmacokinetic and toxicological profile (i.e. good oral bioavailability, and no mitochondrial, bone marrow or reproductive toxicity).[18] The compound was taken into the clinic, moved swiftly to phase III clinical trials, but then its further development was discontinued essentially because of economically competitive reasons.

The HEPT series of compounds snugly fit into a specific, allosteric ''pocket'' site (located at some 10 Å from the catalytic site) of the HIV-1 reverse transcriptase and thus disrupt its enzymatic activity. The interaction between the 6-benzyl ring of the inhibitors and residue Tyr181 of the protein would play a major role in the potency of the HEPT compounds.[19] The HEPT analogues, alike most other NNRTIs, are notorious for rapidly eliciting the emergence of resistance mutations in the HIV-1 reverse transcriptase, particularly at position 103 (K→N) and 181 (Y→C). To cope with this problem, several analogues of MKC-442 were synthesized, e.g. TNK-6123 and GCA-186.[20] Compound TNK-6123 has a C_6 thiocyclohexyl group designed to have more flexibility in adapting to the mutated drug-binding site. GCA-186 has additional 3′,5′-dimethyl substituents aimed at forming close contacts with the conserved residue Trp229. Both compounds showed a 30-fold increased inhibitory effect, relatively to MKC-442, towards the K103N and Y181C mutants.[20] Annulated analogues of HEPT, i.e. 2,3-dihydro-5-[(3,5-dimethylphenyl)methyl]-3-ethoxy-6-ethyl]-7*H*-thiazolo[3,2-a]pyrimidin-7-one[21] and MKC-442 analogues possessing an ω-functionalized acyclic structure[22] have also been synthesized, but none of these novel structures afforded antiviral activity superior to that of MKC-442. In contrast, N_1 allyloxymethyl and N_1 3-methylbut-2-enyloxymethyl substituted 5-ethyl-6-(3,5-dimethylbenzyl)uracils showed activity against wild-type HIV-1 in the picomolar range with selectivity indices greater than 5×10^6, and activity in the submicromolar range against the clinically important Y181C and K103N mutant strains.[23]

Structurally related to the HEPT analogues are the 3,4-dihydro-2-alkoxy-6-benzyl-4-oxo pyrimidines (DABOs) and S-DABOs, i.e. 2-(cyclohexylthio)-3,4-dihydro-5-methyl-6-(3-methylbenzyl)-4-oxopyrimidine[24] and 5-alkyl-2-(alkylthio)-6-(2,6-dihalophenylmethyl)-3,4-dihydropyrimidin-4-(3*H*)-one.[25] Further lead optimization led to the identification of new S-DABOs (i.e. (+)-3w) with a potency and selectivity that were clearly higher than those of MKC-442.[26] Another DABO derivative, 5-isopropyl-2-[(methylthiomethyl)thio]-6-(benzyl)-pyrimidin-4-(1*H*)-one, also proved clearly more active than MKC-442 in abrogating HIV replication.[27] Other DABO (i.e. 2-alkylthio-3,4-dihydropyrimidin-4(3*H*)-one) derivatives have been prepared,[28,29] but did not supersede MKC-442 in either potency or selectivity. NNRTIs such as the HEPT and DABO derivatives should be envisaged as potential microbicides to prevent mucosal HIV transmission: under the experimental conditions where the nucleoside reverse transcriptase inhibitors (NRTIs) only delayed viral breakthrough, the NNRTIs MKC-442 and DABOs MC1047 and MC1220 were able to suppress HIV-1 replication for an entire period of 40 days.[30]

TSAO-T [(2′,5′-BIS-O-(*TERT*-BUTYLDIMETHYLSILYL)-β-D-RIBOFURANOSYL]-3′-SPIRO-5″-(4″-AMINO-1″,-2″-OXATHIOLE-2″,2″-DIOXIDE)THYMINE] DERIVATIVES

The TSAO-T derivatives (Figure 4), like the HEPT derivatives, selectively inhibit HIV-1, but not HIV-2, replication, due to a specific interaction with HIV-1 reverse transcriptase at a non-substrate binding site.[31–33] The TSAO-T derivatives have to fulfill stringent structural requirements, including the presence of *tert*-butyldimethylsilyl groups at both C-2′ and C-5′ of the ribose moiety and the presence of the unique 3′-spiro group [3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)] in the R (*ribo*)

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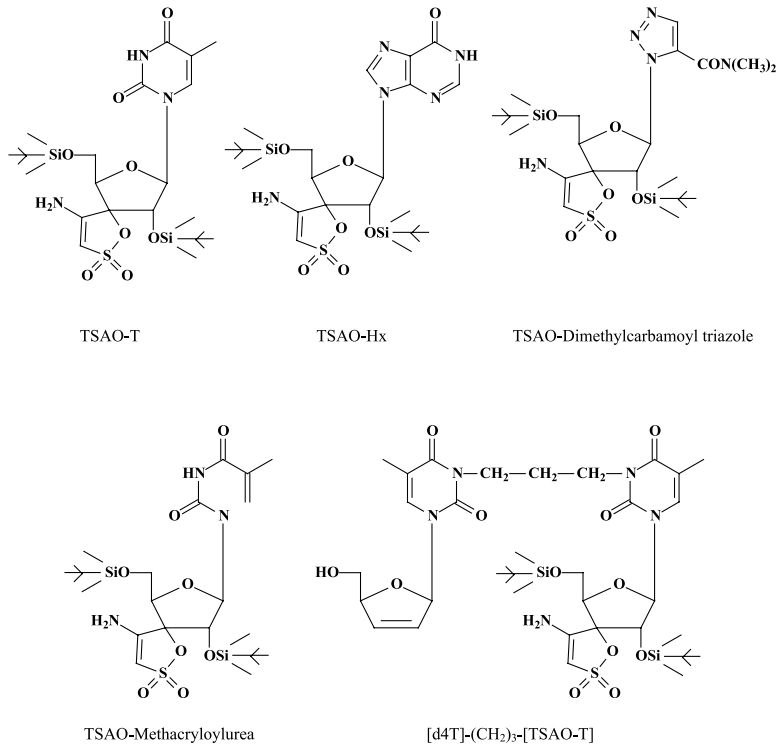

Figure 4. TSAO derivatives (TSAO-T, TSAO-Hx, TSAO-Dimethylcarbamoyltriazole, TSAO-Methacryloylurea and [d4T]-$(CH_2)_3$-[TSAO-T]) derivatives.

configuration. However, the nature of the heterocycle (pyrimidine or purine) seems to be less important, as a multitude of antivirally active TSAO analogues have been described with either (modified) pyrimidines or purines.[34,35] TSAO-purines and TSAO-pyrimidines must be targeted a similar, unique molecular site of HIV-1 reverse transcriptase, as they lead to the emergence of the same unique resistance mutation (E138K).[36,37] This E138K mutation is located on the p51 subunit,[38] in a region that must be in close contact with the NNRTI-binding pocket site of the HIV-1 RT p66 subunit. Apparently, the TSAO analogues interact, through the 4″-amino group of the 3′-spiro-substituted ribose moiety, with the carboxylic acid group of glutamic acid at position 138 of the HIV-1 reverse transcriptase (p51 subunit).[39]

During the past decade several new TSAO-T derivatives have been synthesized, i.e. 4-, 5-, and 6-substituted pyrimidine analogues, among which some derivatives were found to be superior to their unsubstituted TSAO counterparts with regard to antiviral potency and/or selectivity.[40] Among the 4- or 5-substituted 1,2,3-triazole-TSAO analogues, the 5-substituted amido-, (methyl)amido- and (dimethyl)amido-1,2,3-triazole derivatives proved to be potent HIV-1 inhibitors with a resistance profile similar to that of TSAO-T.[41] The L-conformer of TSAO-T, however, did not show antiviral activity.[42] Likewise, L-*lyxo*-TSAO-T analogues with an inverted configuration at the

C-4′ position of the sugar moiety and 5′-deoxy-5′-modified TSAO-T analogues were not antivirally active.[43] 4″-H-TSAO-T, a TSAO-T derivative lacking the 4″-amino group at the 3′-spiro moiety, retained antiviral activity and failed to select for the E138K mutation,[44] thus corroborating the notion that the 4″-amino function is directly involved in an interaction with glutamic acid at position 138 (of the HIV-1 RT p51 subunit).

Also, abasic TSAO-T analogues with substituted urea moieties, mimicking both the shape and the electrostatic potential of the thymine ring, retained marked antiviral activity.[45] However, replacement of the 5′-*tert*-butyldimethylsilyl (TBDMS) group by alkyl-, alkenyl-, or aromatic ether groups, substituted amines, carbamoyl or (thio)acyl groups markedly diminished or even annihilated anti-HIV activity.[46] Heterodimers of TSAO-T with azidothymidine, i.e. [TSAO-T]-$(CH_2)_n$-[AZT] proved markedly inhibitory to HIV-1, although they were less potent than the parent compounds from which they were derived.[47] However, the [TSAO-T]-$(CH_2)_3$-[d4T] heterodimer derivative containing a propyl linker between the N-3 positions of TSAO-T and d4T (2′,3′-dideoxy-2′,3′-didehydrothymidine) was at least as active as TSAO-T and significantly more inhibitory to HIV-1 than the corresponding AZT heterodimer.[48]

Recently, 3″-substituted TSAO derivatives have been prepared.[49] Introduction of a bromine and particularly iodine at the 3″-position conferred considerable anti-HIV-1 activity; the presence at the 3″-position of vinyl, alkynyl, phenyl or thienyl groups significantly diminished the anti-HIV-1 activity; and, surprisingly, several 3″-alkenyl-substituted TSAO derivatives acquired anti-HIV-2 activity while retaining (albeit diminished) anti-HIV-1 activity.[49] The latter data are compatible with a recently proposed hypothesis[50] that the TSAO derivatives may interact with the interphase between the two subunits (p51 and p66) of the HIV reverse transcriptase.

BICYCLIC FURO[2,3-d]PYRIMIDINE NUCLEOSIDE DERIVATIVES

The furo[2,3-d]pyrimidine nucleoside analogues (Figure 5) were originally obtained as by-products in Pd-catalyzed coupling of terminal alkynes with 5-iodonucleoside analogues.[51] The 6-octyl substituted derivative Cf1368 was found to inhibit the replication of varicella-zoster virus (VZV), but not of any other virus, at nanomolar concentrations, with a selectivity index in excess of 5000.[52] Further lead optimization resulted in the synthesis of 6-(*p*-alkylphenyl)-substituted furopyrimidine nucleoside analogues, among which the 6-(*p*-pentylphenyl)- and 6-(*p*-hexylphenyl)-substituted derivatives (Cf1743 and Cf1742, respectively) emerged as the most potent and selective anti-VZV compounds ever reported[52] (activity at subnanomolar concentrations; selectivity index higher than 100,000). While the precise mechanism of anti-VZV action of these compounds still remains to be determined,[53] it has been ascertained that to exert their antiviral activity, the furo[2,3-d]pyrimidine nucleoside analogues need to be phosphorylated by the VZV-encoded thymidine kinase.[54] In their anti-VZV activity, the bicyclic furo[2,3-d]pyrimidine derivatives thus follow the activation pathway characteristic of thymidine analogues. In this sense, the compounds behave as genuine nucleosides; whether they also interact by a nucleotide mechanism with their final target (enzyme) is still unclear.

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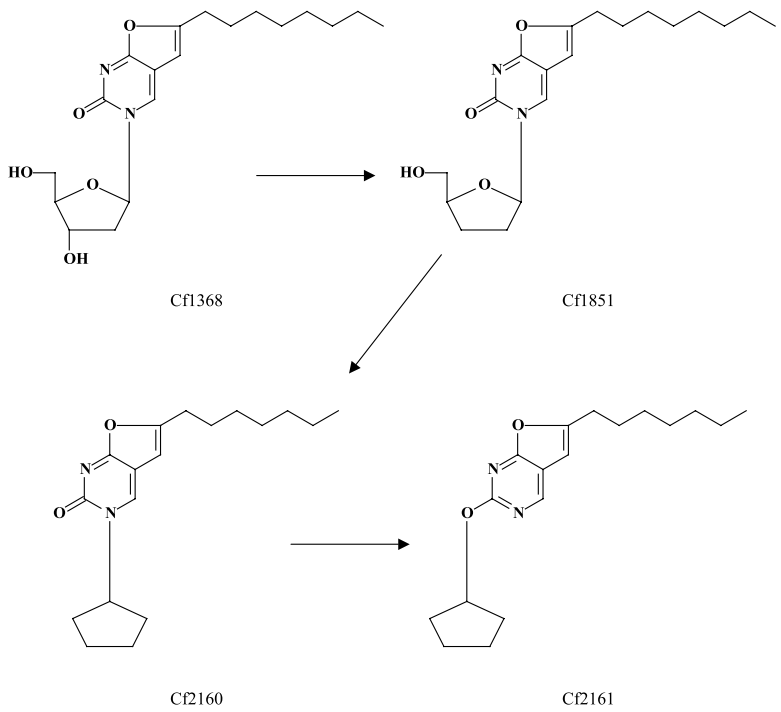

Figure 5. Bicyclic furo[2,3-d]pyrimidine nucleoside analogues Cf1368, Cf1851, Cf2160 and Cf2161.

If in Cf1368 the 2-deoxyribose moiety was replaced by 2,3-dideoxyribose, thus yielding Cf1851, the activity against VZV was abolished, and, instead, activity against HCMV was noted. If the ''sugar'' moiety was further modified to a cyclopentyl ring as in Cf2160, the activity against HCMV was retained, and if this cyclopentyl ring was moved from the N-1 position to C-2 and linked with the C-2 oxygen of the pyrimidine ring as in Cf2161, again selective activity was observed against HCMV.[55,56] The bicyclic furo[2,3-d]pyrimidine nucleoside analogues that inhibit HCMV replication clearly do so by a non-nucleoside mechanism. They are active against HCMV strains that are resistant to the nucleoside/nucleotide analogues ganciclovir and cidofovir, and, as revealed by time-of-addition experiments, they inhibit an early event in the viral replicative cycle, which may well correspond to viral entry into the cells.[56] Like the pyrrolo[2,3-d]pyrimidine derivatives 951 and 1028, the bicyclic furo[2,3-d]pyrimidine nucleoside analogues Cf1851, Cf2160 and Cf2161 were found to inhibit immediate early antigen expression.[56]

CONCLUSION

Maribavir (1263W94) is an example of a nucleoside analogue that exerts its antiviral activity (against HCMV) through a non-nucleoside mechanism. Other

examples of nucleoside analogues acting through a non-nucleoside mechanism are HEPT, TSAO and the recently discovered bicyclic furo[2,3d]pyrimidine nucleoside analogues. The HEPT and TSAO derivatives are active against the replication of HIV-1 by a specific interaction with a non-substrate (dNTP) binding site of the HIV-1 reverse transcriptase. The novel bicyclic nucleoside analogues inhibit HCMV replication by interfering with an early event in the viral life cycle.

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