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Antiviral properties of new arylsulfone derivatives with activity against human betaherpesviruses

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Abstract

Based on our previous experience with arylsulfone derivatives displaying antiherpetic activity, we synthesized several analogues in which the sulfonyl group is part of a bicyclic structure. The benzene-fused derivative 2*H*-3-(4-chlorophenyl)-3,4-dihydro-1,4-benzo-thiazine-2-carbonitrile 1,1-dioxide and its thiophene-fused analogue were shown to have favorable activity and selectivity against the betaherpesviruses human cytomegalovirus (HCMV) and human herpesvirus 6 (HHV-6) and 7 (HHV-7). The benzene-fused derivative retained its anti-HCMV activity when evaluated against virus strains resistant to foscarnet, ganciclovir, and/or cidofovir. The compound conferred ≥95% inhibition of viral DNA synthesis in HHV-6-infected cells. RT-PCR analysis of immediate-early, early and late gene products revealed that this arylsulfone compound acts at a step preceding late gene expression, and coinciding with the inhibition exerted by foscarnet. No inhibitory effect was seen in an enzyme assay for DNA elongation catalyzed by the HCMV or HHV-6 DNA polymerase catalytic subunit. The arylsulfone derivatives had no effect on the functional interaction between the catalytic subunit of HCMV DNA polymerase and its accessory protein, nor did they disrupt the physical interaction between the two proteins. We conclude that these arylsulfone derivatives represent new betaherpesvirus inhibitors with a novel mode of action that results in indirect inhibition of viral DNA synthesis.

Keywords: Cytomegalovirus; Human herpesvirus 6; Betaherpesvirus; Antiviral; Arylsulfone derivative

1. Introduction

The availability of more than eight antiherpetic drugs allows control of human herpesvirus infections in various clinical settings (Coen and Schaffer, 2003). Considerable progress has been achieved in the therapy of life-threatening manifestations of herpesvirus reactivation in immunocompromised patients, such as transplant recipients undergoing immunosuppressive therapy. Ganciclovir (GCV) and, to a lesser extent, foscarnet, are the standard drugs for preemptive therapy of human cytomegalovirus (HCMV) infections in transplant recipients (Nichols and Boeckh, 2000). The value of ganciclovir or foscarnet treatment in transplant recipients showing clinical signs from human herpesvirus 6 (HHV-6) reactivation remains to be fully established (Zerr et al., 2002, 2005; De Bolle et al., 2005). Since HCMV reactivation in solid organ recipients can be enhanced by the other two betaherpesviruses HHV-6 and human herpesvirus 7 (HHV-7) (Mendez et al., 2001), antiviral drugs with activity against all three betaherpesviruses are to be preferred. Unfortunately, long-term administration of ganciclovir or foscarnet can lead to severe toxicity or emergence of drug-resistant virus strains (Nichols and Boeckh, 2000). Therefore, the need remains for new antiherpetic drugs (preferably with non-nucleoside structure) that combine efficacy and safety with a novel mechanism of action, thus excluding the possibility of cross-resistance with existing therapeutics (Wathen, 2002).

We previously reported on the synthesis and antiviral and antitumor activities of a series of new diarylsulfone derivatives (Stephens et al., 2001). While their anti-human immunodeficiency virus type 1 (HIV-1) activity may be based on inhibition of the HIV-1 reverse transcriptase (RT), in analogy to structurally

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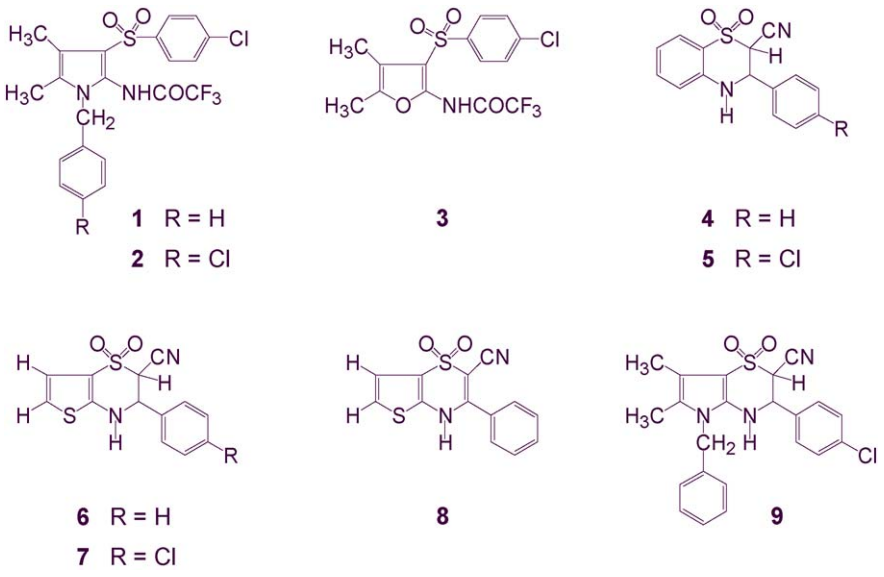

Fig. 1. Chemical structures of arylsulfone derivatives.

related non-nucleoside RT inhibitors (Silvestri et al., 2003), their mode of action against herpesviruses such as HCMV, herpes simplex virus (HSV) and varicella zoster virus (VZV) has not yet been studied. In the present paper, we describe the anti-betaherpesvirus activity of some related heteroarylsulfone derivatives in which the sulfonyl group is part of a bicyclic structure. Our interest in such compounds was stimulated by a report on the potent anti-HIV-1 activity of some other bicyclic sulfones (Witvrouw et al., 1998). The present findings indicate that the mode of action of these new bicyclic derivatives against betaherpesviruses may be related to indirect inhibition of viral DNA synthesis.

2. Materials and methods

2.1. Chemical synthesis

The structures of the arylsulfone compounds are shown in Fig. 1. Sulfones **1–3** (Stephens et al., 2001) and **6–8** (Stephens and Sowell, 1998) and **9** (Stephens, 1998) were available as a result of previously described synthetic work. Sulfones **4** and **5** were prepared according to the literature (Baliah and Ananthapadmanabhan, 1972) starting from (2-nitrophenylsulfonyl)acetonitrile (Stephens, 1998).

2.2. Cells and viruses

The cell lines used were: human embryonic lung (HEL) fibroblasts, purchased from the American Type Culture Collection (ATCC; Manassas, VA), and the human T-lymphoblast cells HSB-2 (from ATCC), MOLT-3 (from Advanced Biotechnologies, Columbia, MD) and SupT-1 (from ATCC).

The reference human cytomegalovirus (HCMV) strains AD169 (ATCC VR538) and Davis (ATCC VR807) were obtained from ATCC. The HCMV clinical strains Der530 and Ly9990 were isolated in our laboratory; they were obtained from the lymphocytes of an AIDS patient and a transplant recipient, respectively, both undergoing GCV therapy.

Activity against HCMV was determined using the reference strains AD-169 and Davis, and drug-resistant strains: AD169 PFA^r (resistant to foscarnet); Der530 GCV^r/CDV^r (resistant to ganciclovir and cidofovir) and Ly9990 GCV^r (resistant to ganciclovir). For HHV-6, we used the reference strains GS (kindly provided by Dr. R. Gallo when at NIH), representing HHV-6 variant A, and Z29 (obtained from Advanced Biotechnologies), representing HHV-6 variant B. For HHV-7, the KHR strain (a kind gift from Dr. K. Yamanishi, Osaka, Japan) was used.

2.3. Antiviral assays

The detailed procedures for determining anti-betaherpesvirus activity can be found elsewhere (Snoeck et al., 2002; Neyts et al., 2001; Zhang et al., 1999). Briefly, HCMV was added at 100 PFU per well to 96-well plates containing confluent cultures of human embryonic lung (HEL) fibroblasts. Unadsorbed virus was removed after 2-h incubation, and replaced by serial dilutions of the test compounds. After 7 days incubation, the cytopathic effect (CPE) was scored by microscopic evaluation, from which the 50% antivirally effective concentration (EC_{50}) was calculated. HHV-6 assays were performed in fresh human cord blood mononuclear cells, or in human T-lymphoblast HSB-2 (for HHV-6A, strain GS) and MOLT-3 (for HHV-6B, strain Z29) cells. Virus stocks were added to concentrated cell suspensions at a multiplicity of infection of 100 $CCID_{50}$ (50% cell culture infective dose) per 10^6 cells. After 2 h, cells were centrifuged to remove unadsorbed virus, resuspended in medium containing serial dilutions of the compounds, and transferred to 48-well plates. After 10–12 days incubation, viral CPE was scored by microscopy, and total DNA was extracted from the cells for quantitation of the viral DNA by a non-radioactive slot-blot hybridization assay (Naesens et al., 2001). Anti-HHV-6 activity was expressed as EC_{50}, i.e., the compound concentration

that produces 50% inhibition of virus replication, as estimated from the CPE score, or the viral DNA band intensity in the hybridization assay. In the case of HHV-7, human T-lymphoblast SupT-1 cells were mixed with cell lysate from HHV-7-infected cells, and serial dilutions of the test compounds (Zhang et al., 1999). After 10 days incubation, cells were fixed and stained with anti-CD4 antibody, and then subjected to FACS analysis. The antiviral EC_{50} was defined as the compound concentration that afforded 50% protection against HHV-7-induced CD4 receptor down-regulation.

2.4. Cytotoxicity assays

The minimum cytotoxic concentration (MCC) was estimated by microscopy, and defined as the concentration producing minimal changes in cell morphology. The CC_{50} or 50% cytostatic concentration was determined in growing cultures of HEL cells, which were incubated with the compounds during 3 days, and then counted using a Coulter counter.

2.5. RT-PCR assays

The effect of the test compounds on the transcription of selected HHV-6 immediate early (IE), early (E) and late (L) genes was determined in MOLT-3 cells at 16 and 48 h after HHV-6B infection. Actinomycin D, cycloheximide and foscarnet (inhibitors of IE, E and L transcription, respectively) were included as control compounds. The detailed procedure can be found elsewhere (De Bolle et al., 2004a). After RNA extraction, any contaminating DNA was removed by DNase treatment, as verified by PCR analysis on the RNA extracts. Then, cDNA was synthesized with RAV-2 reverse transcriptase and oligo-$dT_{(15)}$ primers. Then, PCR amplification was performed with primers for the HHV-6 U86, U12, U67 and U100 mRNAs. To normalize for total cDNA input, a PCR for human β-actin mRNA was run in parallel.

For time-of-addition experiments, the compounds were added to HHV-6B-infected MOLT-3 cells at different time points (range: 2–32 h) post infection (p.i.). At 48 h p.i., RNA extracts were prepared and processed as described above, after which RT-PCR analysis was performed for HHV-6 U67 and human β-actin mRNA.

2.6. Enzyme assays for HCMV and HHV-6 DNA polymerase

The detailed procedure to determine the inhibitory effect of the compounds on the activity of HCMV- or HHV-6-encoded DNA polymerase has been published elsewhere (De Bolle et al., 2004b). The catalytic subunit of the DNA polymerase (pUL54 for HCMV and pU38 for HHV-6) was prepared by in vitro transcription/translation from an expression plasmid containing the UL54 gene of HCMV (Cihlar et al., 1997) or the U38 gene of HHV-6. The enzyme mixture contained activated calf thymus DNA as the primer/template, 1 μM of [^{3}H]-dGTP and 100 μM of dATP, dTTP and dCTP, and serial dilutions of the test compounds. After 40 min at 37 °C, nucleic acids were precipitated with trichloroacetic acid and collected on filters, in which incorporated radioactivity was quantified by scintillation counting.

The compounds were also tested in an assay that measures stimulation of pUL54 activity by pUL44 as previously described (Loregian et al., 2003, 2004). Briefly, the incorporation of [^{3}H]-dTTP into a poly(dA)-oligo(dT) template was measured using 100 fmol of purified pUL54 and 200 fmol of purified pUL44 in the absence or presence of various amounts of each compound. The purified baculovirus-expressed HCMV pUL54 and pUL44 proteins used in these assays, prepared as described (Loregian et al., 2003), were kindly provided by H.S. Marsden (Institute of Virology, Glasgow, UK). As a positive control for inhibition, a peptide corresponding to the 22 C-terminal residues of HCMV pUL54 (kindly provided by H.S. Marsden) was included.

2.7. pUL54–pUL44 interaction ELISA

This assay was performed as described in Loregian et al. (2003, 2004), using baculovirus-expressed and purified HCMV pUL54 and pUL44 proteins, and with various concentrations of each compound. As a positive control for inhibition, a peptide corresponding to the 22 C-terminal residues of pUL54 was used.

3. Results

3.1. Anti-betaherpesvirus activity

The pyrrole derivatives **1** and **2** (already described in Stephens et al., 2001) were active against HCMV ($EC_{50} \leq 1.0$ μg/ml; Table 1), yet had little or no activity against HHV-6 (Table 2). The newly synthesized compounds **4**–**9**, in which the sulfonyl group is part of a bicyclic structure, showed some differences in their activity against HCMV, HHV-6 and HHV-7, depending on the fused aromatic ring system (Tables 1 and 2). The activity of the benzene-fused derivative **5** and the thiophene-fused derivative **7** was clearly greater against HHV-6 than HCMV. In contrast, no anti-betaherpesvirus activity was seen with the N-substituted

Table 1
Anti-HCMV activity of arylsulfone derivatives in human embryonic fibroblasts

Compound	EC_{50} based on CPE[a] (μg/ml)		MCC[b] (μg/ml)	CC_{50}[c] (μg/ml)
	AD169	Davis		
1	1.3	1.9	>20	28
2	0.5	0.6	20	50
3	>5	10	20	46
4	28	>20	50	>50
5	3.1	2.8	20	>50
6	50	50	>50	>50
7	7	9	50	35
8	>50	>50	>50	>50
9	>5	>5	20	>20
Ganciclovir	1.6	1.8	>50	>50

[a] Compound concentration that produces 50% inhibition of virus-induced cytopathic effect (CPE), as determined by microscopical examination.
[b] Minimum cytotoxic concentration, or concentration causing minimal changes in cell morphology, as determined by microscopical examination.
[c] Concentration that inhibits cell proliferation by 50%.

Table 2
Antiviral activity of arylsulfone derivatives in HHV-6- and HHV-7-infected human T-lymphoblasts

Compound	HHV-6A-infected HSB-2 cells			HHV-6B-infected MOLT-3 cells			HHV-7-infected SupT1 cells
	EC_{50} based on CPE[a] (μg/ml)	EC_{50} by DNA detection[b] (μg/ml)	MCC[c] (μg/ml)	EC_{50} based on CPE[a] (μg/ml)	EC_{50} by DNA detection[b] (μg/ml)	MCC[c] (μg/ml)	EC_{50} based on FACS[d] (μg/ml)
1	2.2	12 ± 5.8	20	>20	10	20	NA
2	5.0 ± 2.0	≥10	≥20	>20	>10	≥20	NA
3	>20	ND	20	>20	ND	≥20	ND
4	>20	ND	20	>20	ND	20	ND
5	0.4 ± 0.5	1.3 ± 1.0	20	1.5 ± 0.6	1.9 ± 1.1	20	5.7 ± 1.8
6	>20	ND	20	>20	ND	20	ND
7	1.8 ± 0.30	2.9 ± 0.86	50	2.1 ± 0.15	1.8 ± 1.1	50	ND
8	>20	ND	20	>20	ND	≥10	ND
9	>20	ND	20	>20	ND	≥20	ND
Foscarnet	2.8 ± 2.5	3.0 ± 3.3	500	6.6 ± 3.6	5.8 ± 4.3	500	2.9

Data are the mean ± S.D. from two to five independent experiments. ND, not determined; NA, not active at subtoxic concentrations.
[a] Compound concentration producing 50% inhibition of HHV-6-induced cytopathic effect (CPE), as determined by microscopical examination.
[b] Concentration producing 50% inhibition of HHV-6 replication, as estimated by hybridization assay for viral DNA.
[c] Minimum cytotoxic concentration, or concentration causing minimal changes in cell morphology, as determined by microscopical examination.
[d] Compound concentration producing 50% inhibition of HHV-7 replication, as estimated by FACS analysis of CD4 receptor down-regulation.

pyrrole derivative **9**. In addition, the *p*-chlorosubstituent on the phenyl ring of compounds **5** and **7** was prerequisite, since compounds **4** and **6** had very poor, if any, antiviral activity against HCMV and HHV-6. The antiviral activity of **5** and **7** against HSV-1 and VZV was inferior to their activity against HHV-6 and HCMV. For instance, the EC_{50} values for VZV were >50 and 13 μg/ml for **5** and **7**, respectively (data not shown).

Compound **5** retained its anti-HCMV activity when evaluated against HCMV isolates resistant to foscarnet, ganciclovir or cidofovir, and presumed to carry amino acid substitutions in the viral pUL38 DNA polymerase or pUL97 kinase (Table 3).

For most arylsulfone compounds, the selectivity was higher in the HCMV assay than in the HHV-6 assay. In slowly dividing HEL cells, the cytostatic effect of **5** and **7** was low (CC_{50} > 50 μg/ml; Table 1), whereas the same compounds produced minimal toxicity in HHV-6-infected HSB-2 or MOLT-3 cells at a concentration of 20 or 50 μg/μl, resulting in a selectivity index (ratio of MCC to EC_{50}) of 11–50 (Table 2). The selectivity index for HHV-6 may be biased by the fact that our HHV-6 experiments were performed in a 10-day assay in highly proliferating human T-lymphoblasts, which may overestimate the cytostatic activity of these arylsulfone compounds (Stephens et al., 2001).

3.2. Synergistic activity between compound 5 and foscarnet

A combination experiment was performed, in which different concentrations of compound **5** and foscarnet were combined in a checkerboard scheme, and evaluated for activity against HHV-6. The EC_{50} value of compound **5** was found to be markedly reduced upon combination with foscarnet. After isobologram analysis (Greco et al., 1995), the curve connecting the EC_{50} values of the combination fell between the line connecting the EC_{50} values of the compounds alone (Fig. 2; solid line) and the line connecting the $EC_{50}/2$ values (Fig. 2; dotted line), indicating that the combination of compound **5** with foscarnet is slightly synergistic.

3.3. Inhibitory effect on viral DNA synthesis in HHV-6-infected cells

The inhibitory effect of the arylsulfone compounds on viral DNA synthesis was determined using a hybridization assay for HHV-6 DNA. Overall, the EC_{50} values obtained by the CPE and DNA hybridization assay were very similar (Table 2). The highest activity was noted for compound **5**: at concentrations ≥4 μg/ml, **5** inhibited HHV-6 DNA synthesis by ≥95%, both

Table 3
Activity of compound **5** against drug-resistant HCMV strains

Compound	EC_{50} based on CPE (μg/ml)[a]			
	Wild-type HCMV (AD-169)	GCV^r HCMV (Ly9990)[b]	PFA^r HCMV (AD169 PFA^r)[b]	GCV^r/CDV^r HCMV (Der530)[b]
5	4.6 ± 1.9	2.7 ± 2.0	3.2 ± 0.3	5.6 ± 0.8
Foscarnet	19 ± 5.7	25 ± 0.7	94 ± 16	34 ± 3.5
Ganciclovir	1.2 ± 0.4	7.0 ± 3.5	1.7 ± 0.4	12 ± 5.2
Cidofovir	0.063 ± 0.021	0.090 ± 0.042	0.067 ± 0.035	0.52 ± 0.021

Data are the mean ± S.D. from two to four independent experiments.
[a] Compound concentration that produces 50% inhibition of virus-induced cytopathic effect (CPE), as determined by microscopical examination.
[b] See Section 2 for origin of HCMV mutants.

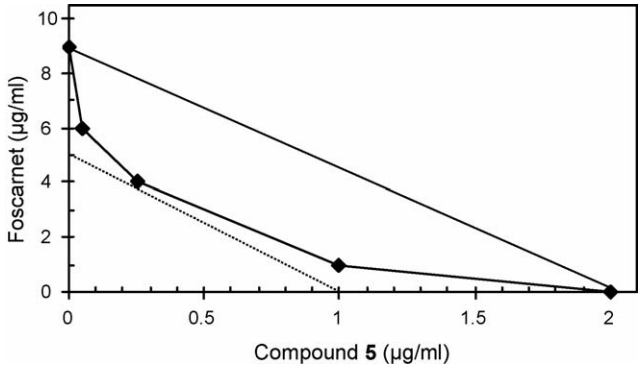

Fig. 2. Isobologram representation of the combined inhibitory effects of compound **5** and foscarnet on HHV-6B replication in MOLT-3 cells. The full line connects the EC_{50} values (determined by CPE assay) of both compounds in the absence of the combining compound, while the dotted line connects the $EC_{50}/2$ values. The EC_{50} values for the combination of **5** and foscarnet fall close to the dotted line, indicating slight synergism.

in T-lymphoblasts (Fig. 3A) and in fresh human cord blood mononuclear cells (Fig. 3B).

3.4. Effect on HHV-6 gene expression

In order to locate the stage in the viral replication cycle at which compound **5** exerts its effect, we performed RT-PCR studies to measure the immediate-early (IE), early (E) and late (L) transcripts at 16 hr or 48 h after HHV-6 infection. Whereas the IE and E mRNAs were found to be unaffected, there was a clear inhibition of L gene expression (Fig. 4). This pattern of inhibition was similar to that observed for the DNA polymerase inhibitor foscarnet. This analogy between compound **5** and foscarnet was also apparent from time-of-addition studies. Production of the late HHV-6 U67 transcript was measured by RT-PCR after one replication cycle (48 h) during which the compounds were added at increasing time points (ranging from 2 to 32 h p.i.). Both compound **5** and foscarnet retained their anti-HHV-6 activity when their addition was delayed until 2–12 h p.i., yet became inactive when added at 18 h p.i. or later (Fig. 5).

3.5. Direct effects on HCMV or HHV-6 DNA synthesis

Several experiments were performed to investigate whether compound **5** or related arylsulfone derivatives cause a direct inhibition of viral DNA synthesis. First, we determined the direct inhibitory effect of **5** on the DNA elongation process catalyzed by the catalytic subunit of HCMV or HHV-6 DNA polymerase (De Bolle et al., 2004b). Whereas the reference compounds foscarnet and ganciclovir triphosphate exerted a marked inhibitory effect on polymerase activity, we did not observe any inhibition with **5** at concentrations up to 50 µg/ml (data not shown). We also observed no inhibitory effect of the arylsulfone derivatives on viral DNA polymerization in an enzyme assay with the catalytic subunit of the HCMV DNA polymerase (pUL54) complexed with its accessory protein pUL44. Finally, the compounds did not disrupt the physical interaction between the pUL54 and pUL44 subunits, as determined in an ELISA interaction assay (data not shown).

4. Discussion

The diarylsulfone derivatives included in our previous investigations were compounds with a furan, thiophene, or N-

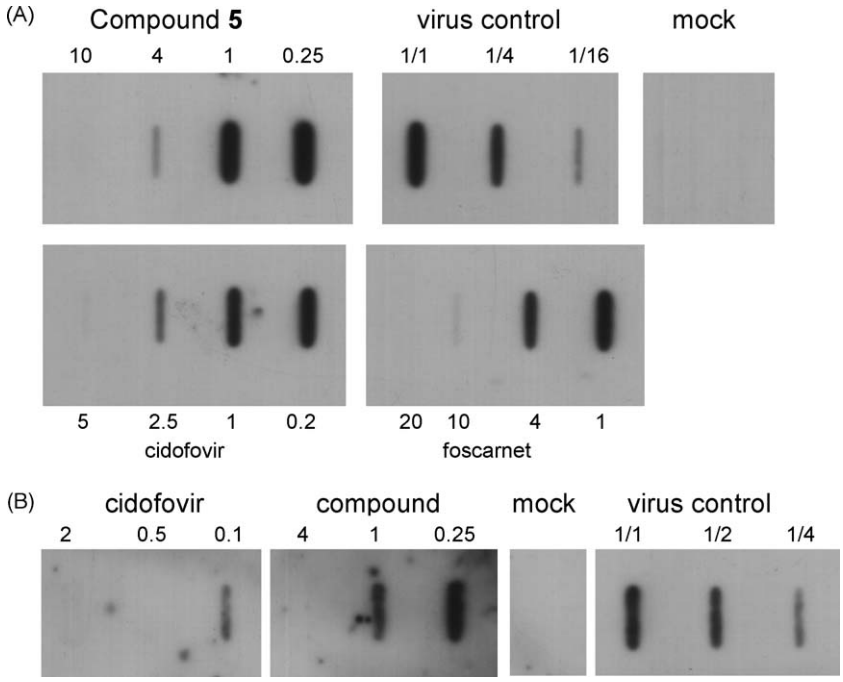

Fig. 3. Inhibitory effect of compound **5** on viral DNA synthesis, as determined by hybridization assay in HHV-6B-infected MOLT-3 cells (panel A) or HHV-6B-infected CBMCs (panel B). Equal amounts of total DNA (extracted at 10 days p.i.) were blotted; for the virus control, a dilution series was included to allow quantification of viral DNA band intensity. Compound concentrations are in µg/ml.

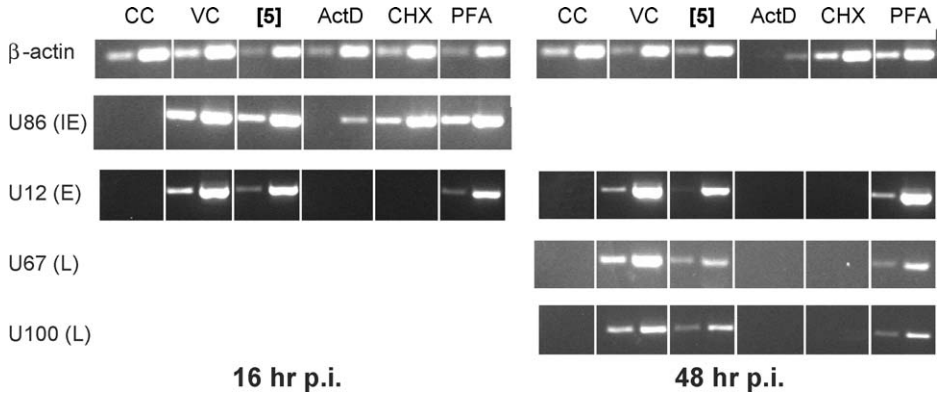

Fig. 4. Effect of compound **5** on HHV-6 gene expression. RT-PCR assay for HHV-6 immediate early (IE), early (E) and late (L) transcription at 16 or 48 h after infection of HHV-6B-infected MOLT-3 cells, treated with compound **5** (50 μg/ml); actinomycin D (ActD; 10 μg/ml); cycloheximide (CHX; 75 μg/ml) or foscarnet (PFA; 200 μg/ml). CC, uninfected control; VC, virus control. For each condition, the left and right band represent the PCR product formed after 34 and 40 thermal cycles, or 25 and 30 cycles for the PCR detecting β-actin mRNA.

substituted pyrrole (Stephens et al., 2001). Among these, several derivatives displayed selective activity against HIV-1, HCMV or VZV with EC_{50} values below 1 μg/ml. For the three viruses, the order of antiviral activity among the compounds was different; most notably, there was a clear dissection in their anti-HIV-1 and anti-HCMV activity. The structure–activity relationship obtained in the previous study provided the basis for a more specific molecular design to target compounds towards HCMV. In order to determine whether the antiviral activity was common to all human betaherpesviruses, we now included HHV-6 and HHV-7 in our experiments. Two compounds, i.e., the benzene-fused derivative **5** and its thiophene-fused analogue **7** emerged with broad-spectrum activity against the three human betaherpesviruses, although the activity against HHV-6 and HHV-7 was superior to that observed for HCMV. This can be explained by the fact that the overall genetic similarity between HCMV and HHV-6 is lower than among HHV-6 and HHV-7 (Dominguez et al., 1996).

Based on its superior activity against HCMV, HHV-6 and HHV-7, the benzene-fused derivative **5** was selected for further studies. The observation that **5** is not cross-resistant with foscarnet, ganciclovir or cidofovir, is an important advantage in the eventual clinical use of these arylsulfone compounds, since drug-resistant HCMV strains may emerge after long-term treatment with antiherpetic drugs (Emery, 2001). In addition, our observation that the anti-HHV-6 activity of compound **5** shows slight synergism upon combination with foscarnet, opens the possibility for drug combinations, although this is not routine practice in herpesvirus therapy.

The close correlation between the antiviral EC_{50} values obtained by the CPE assay and by measuring HHV-6 DNA synthesis after several rounds of replication, resembles our previous findings with antiherpetic drugs that target HHV-6 DNA polymerase (De Bolle et al., 2002). In contrast, no inhibition of HCMV DNA synthesis was seen for inhibitors of virus maturation such as the benzimidazole riboside BDCRB, which acts at a late stage in the HCMV replication cycle following viral DNA synthesis (Underwood et al., 1998). In addition, the observation that **5** exerts a complete shut-off of HHV-6 DNA synthesis, with high reproducibility regardless the virus strain or cell system, suggests that its target is a crucial viral factor that is equally involved in our different antiviral test systems. In contrast, we have observed that non-nucleoside antiherpetic compounds that interact with a cellular target (such as a cellular protein tyrosine kinase) show much higher variability in their antiviral activity depending on viral load and cell system (De Bolle et al., 2004a).

Our hypothesis that compound **5** acts by either direct or indirect inhibition of viral DNA synthesis was based on time-of-addition studies and RT-PCR analysis of HHV-6 gene expression, showing that **5** inhibits HHV-6 late gene expression, in analogy to foscarnet. However, using different enzyme assays, we were able to demonstrate that the inhibition of HCMV or HHV-6 DNA synthesis is not caused by a direct effect of **5** at the level of the viral DNA polymerase or its interaction with accessory proteins. Herpesvirus DNA synthesis is a complex process involving DNA elongation and 3′-5′ exonuclease activity by the catalytic subunit of the DNA polymerase in association with the accessory protein that binds to the growing DNA chain (Cihlar

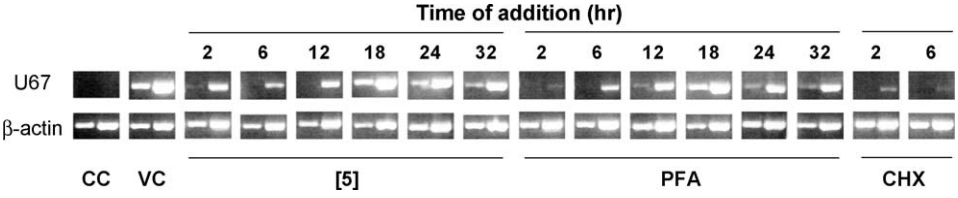

Fig. 5. Effect of delayed time of addition on the HHV-6 effect of compound **5**. MOLT-3 cells were infected with HHV-6B, and at the indicated time points (relative to virus infection), the compounds were added: compound **5** (5 μg/ml), foscarnet (PFA; 200 μg/ml) or cycloheximide (CHX; 75 μg/ml). HHV-6 replication was measured at 48 h p.i. by RT-PCR analysis of HHV-6 U67 mRNA. For each condition, the left and right band represent the PCR product formed after 30 or 35 cycles for HHV-6 U67 mRNA, or 25 and 30 cycles for human β-actin mRNA.

et al., 1997; Monahan et al., 1998). Compound **5** did not affect the DNA chain elongation by the catalytic subunit of HCMV or HHV-6 DNA polymerase. We can thus exclude that compound **5** would have a similar mode of action as the structurally related diarylsulfonyl derivatives which were found to directly inhibit HIV reverse transcriptase (Silvestri et al., 2003), or the 4-oxo-dihydroquinolines, the only class of non-nucleoside analogue inhibitors of herpesvirus DNA polymerases that is currently known (Brideau et al., 2002). Compound **5** also had no effect on viral DNA polymerization in an enzyme assay with the catalytic subunit of the HCMV DNA polymerase (pUL54) complexed with its accessory protein pUL44 (Loregian et al., 2003, 2004). Finally, the arylsulfone compounds did not disrupt the physical interaction between the HCMV DNA polymerase pUL54 and its accessory protein in an interaction ELISA assay (Loregian et al., 2003). Thus, we can exclude a mechanism of action as that described by Pilger et al. (2004), who described a new inhibitor of HSV replication that disrupts the protein–protein interaction between the catalytic subunit of HSV-1 DNA polymerase and its processivity factor. From these collective data, we conclude that the inhibition of betaherpesvirus DNA synthesis exerted by the arylsulfone derivatives in cell culture is not related to direct inhibition of the DNA polymerization process catalyzed by the viral DNA polymerase complex. Our data may seem reminiscent of what has been reported for the benzimidazole riboside maribavir (1263W94) that suppresses HCMV DNA synthesis, presumably by inhibition of the HCMV pUL97 kinase. In an enzymatic assay, maribavir was found to have no direct inhibitory effect on the HCMV DNA polymerase (Biron et al., 2002) although, in time-of-addition studies, its time point of action was shown to coincide with that of ganciclovir (Evers et al., 2004).

In conclusion, the promising anti-betaherpesvirus activity of the bicyclic heteroarylsulfone derivatives **5** and **7** makes them interesting lead compounds for further synthesis and development of novel antiherpetic drugs with a novel mode of action. Although the exact target for their anti-betaherpesvirus activity remains to be identified, our mechanistic data suggest that these non-nucleoside compounds indirectly interact with a process that is associated with viral DNA synthesis.

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