Nonnucleoside Human Cytomegalovirus Inhibitors: Synthesis and Antiviral Evaluation of (Chlorophenylmethyl)benzothiadiazine Dioxide Derivatives

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A second generation of benzothiadiazine dioxide (BTD) derivatives was synthesized employing benzylation reactions mainly. The chlorophenylmethyl BTD derivatives showed activity against human cytomegalovirus (HCMV) with IC_{50} values ranging from 3 to 10 μM. Their 50% cytotoxic concentrations were often >200 μM to lung fibroblast HEL cell proliferation and between 20 and 35 μM for lymphocyte CME cell growth. When cytotoxicity for cell morphology was considered, the minimum cytotoxic concentration for the different BTD derivatives varied between 5 and 200 μM. Some of the anti-HCMV compounds also showed activity against HIV-1 and HIV-2. The chlorophenylmethyl derivative **21** was active against a variety of HCMV clinical isolates from patients with different clinical manifestations and fully maintained its activity against a ganciclovir-resistant HCMV strain. The dibenzyl BTD derivatives did not inhibit HCMV protease, and preliminary pharmacological experiments revealed that their anti-HCMV action stems from interference with an early stage of the viral replicative cycle.

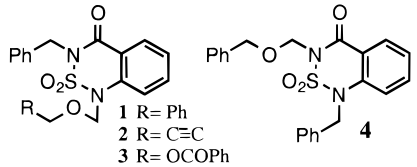
Introduction

Human cytomegalovirus (HCMV) is a highly prevalent member of the herpesvirus family infecting up to 80% of the general population.[1] This virus is responsible for opportunistic infections in immunocompromised individuals including organ transplant recipients and AIDS patients.[2] Clinical manifestations include disseminated disease, pneumonitis, retinitis, and gastrointestinal infections such as esophagitis and colitis. Of particular significance are HCMV infections of neonates. This disease is the most common congenitally acquired viral infection in the world.[3]

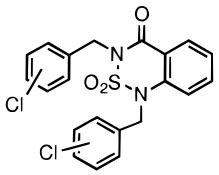
The treatment of HCMV infection is difficult because few therapeutic options are available. Ganciclovir,[4] foscarnet,[5] cidofovir,[6] and, recently, fomivirsen[7] have been approved by the FDA for the treatment of HCMV diseases. All of them achieve a selective inhibition of HCMV replication by inhibition of the viral DNA polymerase. Unfortunately, toxicity associated with these drugs, poor oral bioavailability, and high relapse rates have made their use suboptimal.[8] In addition, concomitant with the increased use of antiviral drugs, there has been an increased emergence of drug-resistant HCMV strains.[9] Therefore, there is still an urgent need for effective, nontoxic anti-HCMV drugs with good oral availability which act by new molecular mechanisms of action.[10,11]

In our search for new antiviral agents,[12−14] we have recently discovered the benzothiadiazine dioxide (BTD) modified acyclonucleosides which showed a marked activity against HCMV and varicella-zoster virus (VZV).[15]

Chart 1



Benzothiadiazine Dioxides (BTD) Modified Acyclonucleosides



Chlorophenylmethyl BTD

The structure of these compounds is quite unique, not only with respect to the nature of the heterocyclic base but also because of the lack of the 5′-OH mimetic group present in ganciclovir and other current anti-CMV drugs, which points to a different mechanism of action (Chart 1). First structure−activity (SAR) data showed the necessity of a double substitution in the heterocycle, while monosubstituted compounds were completely devoid of antiviral activity.[16] Simultaneously, lipophilicity in the acyclic side chain is essential to preserve the anti-HCMV activity. These two factors were considered when preparing these second-generation chlorophenylmethyl BTDs.[17,18] Their synthesis and antiviral evaluation are here described. To assess the influence of the acyclo chain and the benzyl moiety in the antiviral activity of these compounds, dibenzyloxy and dibenzyl BTD derivatives were also synthesized.

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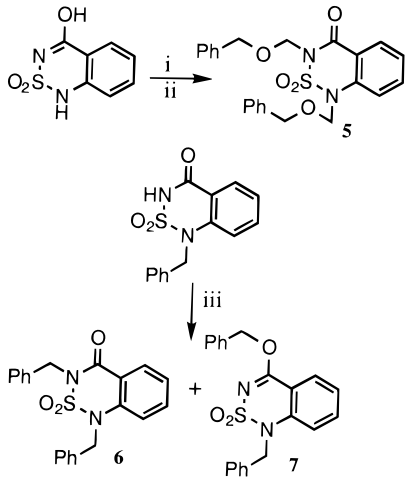
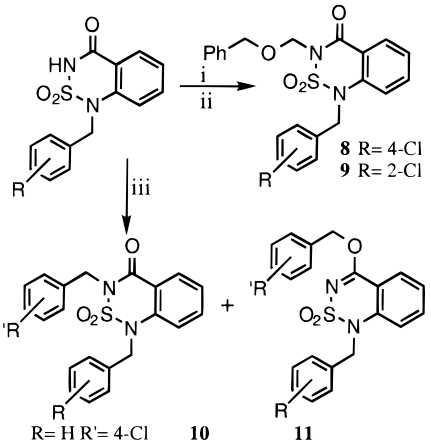
Scheme 1[a]



[a] Reagents: (i) HMDS/N_2; (ii) BF_3·Et_2O/CH_2Cl_2/$PhCH_2OCH_2OAc$; (iii) BrBn/DMF/NaH/Δ.

Scheme 2[a]



R= H R'= 4-Cl	**10**	**11**
R=R'= 4-Cl	**12**	**13**
R=R'= 3,4-diCl	**14**	**15**
R= 3-Cl R'= H	**16**	**17**
R= 3,4-diCl R'= H	**18**	**19**
R= H R'= 2-Cl	**20**	
R= 4-Cl R'= H	**21**	
R= 2-Cl R'= H	**22**	
R=R'= 2-Cl	**23**	
R=R'= 3-Cl	**24**	

[a] Reagents: (i) HMDS/N_2; (ii) BF_3·Et_2O/CH_2Cl_2/$PhCH_2OCH_2OAc$; (iii) R′$PhCH_2X$/DMF/NaH/Δ.

Chemistry

Dibenzyloxy BTD derivative **5** was prepared from 4-hydroxy-2,1,3-benzothiadiazine dioxide[19] following the silylation procedure.[20] Thus, silylation of the heterocycle-like base was achieved with hexamethyldisilazane (HMDS) and acetonitrile as cosolvent under a nitrogen atmosphere. The benzyloxymethyl moiety was introduced in a second step using 2 equiv of acetoxymethyl benzyl ether[21] in dichloromethane using boron trifluoride as catalyst (Scheme 1). Following the same experimental conditions, it was also possible to obtain the modified acyclonucleosides **8** and **9** with chlorophenylmethyl fragments in their chemical structures (Scheme 2).

N,*N*-Dibenzyl BTD derivative **6** could be initially obtained by reaction of 1-benzyl-2,1,3-benzothiadiazin-4-one dioxide[22] with benzyl bromide in a polar, nonprotic solvent such as dimethylformamide (DMF), using sodium hydride (NaH) as base (Scheme 1). In these conditions, a mixture of disubstituted compounds was obtained, as previously observed for di(biphenylmethyl) derivatives.[23] *N*,*N*-Dialkylation predominated over the *N*,*O*-disubstitution in a 5:1 ratio. These two compounds could be separated and isolated by circular thin-layer chromatography.

These experimental conditions were used for the synthesis of a large series of chlorophenylmethyl BTD derivatives (Scheme 2) using different chloro-substituted benzyl halides. *N*,*O*-Disubstitution was also observed as side reaction in some cases. Circular thin-layer chromatography was employed in the isolation of all chlorophenylmethyl BTD compounds.

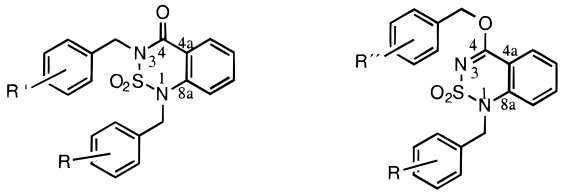
The structures of all new compounds were elucidated from their analytical and spectroscopic data (^1H and ^{13}C NMR) which are collected in Table 1 and in the Experimental Section. Unequivocal assignment of all chemical shifts (^1H and ^{13}C NMR) was done using bidimensional experiments such as COSY or HMQC for one-bond correlation. The site of alkylation was determined from the chemical shifts of benzylic CH_2 signals and by means of NOE experiments and sequences of HMBC for long-distance proton/carbon correlation. Thus, *N*1-CH_2 correlated exclusively with the quaternary carbon C-8a, while *N*3-CH_2 or *O*-CH_2 correlated with the heterocyclic carbon C-4. In the latter case, a deshielding in both proton and carbon signals was observed (Table 1), which additionally confirmed the *O*-substitution.

Biological Results

The new BTD derivatives here synthesized **(5−23)** were evaluated for their antiviral activity against HCMV, strains AD-169 and Davis, and VZV, strains OKA, YS, 07/1, and YS/R. The IC_{50} was determined by plaque reduction (VZV)[24] or CPE reduction (CMV)[25] assays in confluent human embryonic lung (HEL) fibroblasts. Cytotoxicity measurements were based on the inhibition of cell growth and alteration of cell morphology. Among the new series of BTD derivatives synthesized, compounds **10**, **12**, **18**, and **21** emerged as the most active HCMV inhibitors with IC_{50} values in the range of 3−4 μM, being 5−2-fold more potent than the previous BTD-modified acyclonucleosides lead compounds.[16] When the selectivity index (ratio of CC_{50} for cell growth to IC_{50} for virus replication) was calculated, the four compounds appered to have similar selectivity. However, if cytotoxicity for cell morphology was considered, compound **18** emerged as the most selective followed by compounds **12**, **10**, and **21**. Compounds **5**−**7**, **9**, and **22** showed slight activity against HCMV, while compounds **16**, **19**, **20**, and **23** were totally inactive. Only compounds **10**, **12**, and **21** were inhibitory to VZV replication.

In addition, antiviral activity in a wide variety of assay systems,[26] including herpes simplex virus type 1 (strains KOS, F, McIntyre), herpes simplex virus type 2 (strains G, 196, Lyons), thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains B 2006, VMW 1837), vaccinia virus, and vesicular stomatitis virus in E_6SM cells; vesicular stomatitis virus, poliovirus type 1, and Coxsackie B4 virus in HeLa cells; parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie

Table 1. Some Representative ^{1}H and ^{13}C NMR Data (chemical shifts in $CDCl_3$) of Chlorophenylmethyl BTD Derivatives **5**−**24**



compd	R	R′	R″	C-4	C-4a	C-8a	*N*1-*C*H$_2$	*N*3-*C*H$_2$	*O*-*C*H$_2$	*N*1-C*H*$_2$	*N*3-C*H*$_2$	*O*-C*H*$_2$
6	H	H		162.09	122.81	139.79	56.21	46.54		4.83	4.93	
7	H		H	165.73	112.40	142.97	49.29		70.79	5.15		5.44
10	H	4-Cl		162.07	122.80	139.81	56.31	45.81		4.84	4.87	
11	H		4-Cl	165.59	112.33	143.07	49.39		69.84	5.20		5.46
12	4-Cl	4-Cl		161.98	122.75	139.68	55.59	45.76		4.79	4.88	
13	4-Cl		4-Cl	165.59	112.47	142.93	48.88		69.92	5.11		5.41
14	3,4-diCl	3,4-diCl		161.86	122.40	139.59	54.96	45.28		4.84	4.94	
15	3,4-diCl		3,4-diCl	165.48	112.31	142.74	48.36		69.18	5.08		5.39
16	3-Cl	H		161.95	122.58	139.71	55.36	46.57		4.85	5.03	
17	3-Cl		H	165.74	112.54	142.86	48.75		70.91	5.11		5.45
18	3,4-diCl	H		161.87	122.47	139.56	54.69	46.50		4.74	4.97	
19	3,4-diCl		H	165.71	112.47	142.55	48.17		70.97	5.08		5.45
20	H	2-Cl		162.23	122.85	140.06	56.35	44.51		5.02	5.16	
21	4-Cl	H		161.98	122.71	139.56	55.37	46.46		4.78	4.94	
22	2-Cl	H		162.14	121.75	139.82	52.66	45.56		5.03	5.14	
23	2-Cl	2-Cl		162.29	121.65	139.99	52.87	44.43		5.10	5.28	
24	3-Cl	3-Cl		161.97	122.58	139.76	55.52	45.90		4.86	4.96	

Table 2. Antiviral Activity and Cytotoxicity of BTD Derivatives against Human Cytomegalovirus and Varicella-Zoster Virus in HEL Cells

	antiviral activity IC_{50} (μM)[a]						cytotoxicity (μM)	
	HCMV		TK$^+$ VZV		TK$^-$ VZV		cell growth	cell morphology
compd	AD-169	Davis	OKA	YS	07/1	YS/R	CC_{50} [b]	MCC[c]
1	20	27	49	49	>12	>12	>122	122
2	34	34	59	67	31	36	>140	140
3	29	≥44	>44	>44	>44	26	37	110
4	8.5	9.0	>12	>12	>12	7.3	≥98	49
5	12	12	14	>50	>50	10	130	200
6	10	10	7.4	36	23	6.0	>200	≥50
7	7	7	>50	>50	>50	20	>200	200
9	10	10	>50	>50	>50	8	>50	200
10	3.2	3.5	2.6	8.2	10	2.6	121	20
12	3.2	3.2	3.6	3.8	11	2.4	>200	≥50
16	>5	>5	>50	>50	30	ND[d]	>200	≥5
18	3.3	3.5	>50	>50	27	4.5	>200	200
19	>50	>50	>50	>20	>50	>50	>200	200
20	>50	>50	>50	>20	>50	>50	>200	200
21	4	3	18	22	11	2.4	>200	≥5
22	15	12	>20	>20	>20	>20	88	200
23	>50	50	>50	>50	>50	5	>200	≥20
acyclovir			4.53	4.09	41	63	>400	>150
cidofovir	0.58	1.3					317	>159
ganciclovir	3.7	5.9					394	>150

[a] 50% Inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. Virus input was 100 PFU in CMV and 20 PFU in VZV. Assays were performed in duplicate. [b] 50% Cytotoxic concentration, or concentration required to reduce cell growth by 50%. Assays were performed in duplicate. [c] Minimun cytotoxic concentration that caused a microscopically detectable alteration of cell morphology. Assays were performed in duplicate. [d] Not determined.

B4 virus, and Semliki forest virus in Vero cells, was determined. However, no antiviral activity was noted in any of these antiviral assay systems (at compound concentrations up to 400 μg/mL). These data point to the selective nature of the antiviral action of chlorophenylmethyl BTD derivatives against HCMV.

Further antiviral evaluation against HIV-1 (strain III_B) and HIV-2 (strain ROD) in human T-lymphocytes (CEM cells) was performed, and the results are shown in Table 3. The virus was exposed to the cells immediately prior to compound administration. Several compounds were found to inhibit the replication of HIV-1 and HIV-2 at concentrations that were only 2−5-fold below the cytotoxic concentration for the host cells, thus resulting in a low selective antiviral action.

Some SARs emerged concerning the potent anti-HCMV action found for these nonnucleoside inhibitors. Disubstitution of the BTD ring with a benzyl or chlorophenylmethyl moiety was more important maintaining the antiviral activity than when the benzyloxymethyl fragment was present. Additionally, the presence of a chlorine atom in the phenyl nucleus led to more potent HCMV inhibitors. This substitution is especially favorable in the *para* position of the aromatic ring. Thus *N*,*N*-dibenzyl BTD derivatives were 2−3-fold more potent against HCMV than the initial lead compound **4**.

The antiviral potential of the new compounds was also assessed by measuring their antiviral activity against seven HCMV clinical isolates. Chlorophenylmethyl BTD

Table 3. Activity and Cytotoxic Properties of BTD Derivatives against HIV Types 1 and 2 in Human T-Lymphocyte CEM Cells

compd	antiviral activity EC_{50} (μM)[a] HIV-1 (III_B)	HIV-2 (ROD)	cytotoxicity CC_{50} (μM)[b]
1	>8	>8	25 ± 1.4
2	>8	>8	23 ± 0.7
3	≥40	≥40	100
4	15	20	100
5	25.0 ± 7.1	25.0 ± 7.1	27.7 ± 4.3
6	25.0 ± 21.8	≥50	108 ± 51.5
7	>10	>10	22.2 ± 0.6
9	>10	≥10	22.2 ± 1.6
10	5.0 ± 2.6	≥10	27.8 ± 0.1
12	≥10	7.5 ± 3.5	35.8 ± 3.3
13	>50	>50	≥250
16	5.5 ± 2.1	>10	21.3 ± 1.4
18	≥2	>2	16.4 ± 3.4
19	>10	>10	30.5 ± 0.3
20	>250	>250	>250
21	6.5 ± 0.7	8.0 ± 2.8	19.7 ± 1.1
22	>10	>10	27.9 ± 1.7
23	15.0 ± 7.1	>10	40.4 ± 4.6

[a] 50% Effective concentration, or compound concentration required to protect CEM cells by 50% against the cytopathicity of HIV. Assays were performed in triplicate. [b] 50% Cytotoxic concentration, or compound concentration required to reduce CEM cell proliferation by 50%.

Table 4. Activity of BTD **21** Against Clinical Human Cytomegalovirus Isolates

HCMV strain	IC_{50} (μM)[a] ganciclovir	**21**	HCMV strain	IC_{50} (μM)[a] ganciclovir	**21**
A	6.2	2.4	E	4.1	1.2
B	6.2	2.4	F	6.2	3.6
C	6.2	2.4	G	8.2	2.4
D	4.1	2.4			

[a] 50% Inhibitory concentration, or compound concentration required to reduce virus plaque formation by 50%. Assays were performed in duplicate.

derivative **21** was selected for these determinations. The wild-type strains were isolated from patients with different clinical conditions: congenital infection (strains A−C), mononucleosis in an immunocompetent child (strain D), AIDS (strains E, F), and bone marrow transplant recipient (strain G). In all cases the BTD derivative showed an antiviral potency greater than that of ganciclovir (Table 4).

It is worth mentioning that the chemical structure of these active compounds is unique since they lack the 5′-OH mimetic group present in ganciclovir and other nucleoside analogues. This may point to a different molecular mode of action than inhibition of the viral DNA polymerase. Compound **21** showed an $IC_{50} = 3\ \mu M$ against HCMV AD-169 strain resistant to ganciclovir, while ganciclovir showed an $IC_{50} = 31.5\ \mu M$ for this strain. This antiviral activity being 10-fold more potent than that observed for ganciclovir confirms that this new family of HCMV inhibitors exerts antiviral action by a biological mechanism different from that of ganciclovir.

Initial pharmacological studies aimed at determining the mode of action of BTD derivatives have been performed. Potential inhibition of HCMV protease was evaluated for all the BTD compounds, but no inhibition of this viral enzyme could be demonstrated. In time of addition experiments, compounds **18** and **21** lost their capacity to inhibit HCMV replication, as measured by

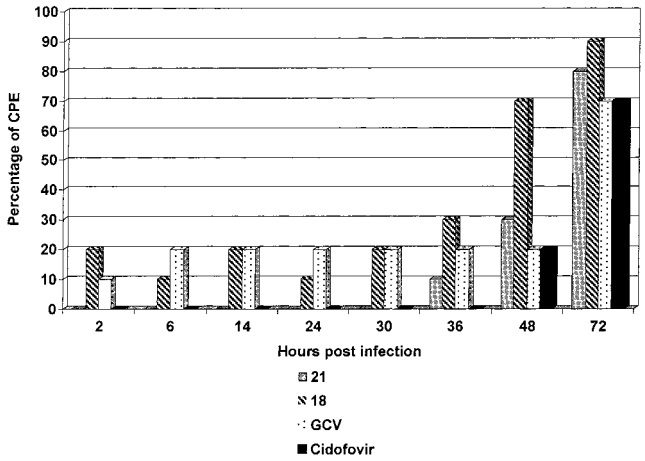


Figure 1. Effect of the time addition of chlorophenylmethyl BTD derivatives **18** and **21** on HCMV (Davis strain)-induced cytopathicity in HEL cultures.

CPE reduction assays when added after 48 h post-infection (Figure 1). Similarly, the control drugs cidofovir and ganciclovir, known to inhibit viral DNA synthesis, lost their capacity to inhibit HCMV replication between 48 and 72 h post-infection. Thus, time of addition experiments performed with compounds **18** and **21** revealed that the anti-HCMV action exerted by the BTD derivatives is in the early stages of the viral replicative cycle. Further experiments are in progress to warrant the mechanism of inhibition and molecular target of action of these novel compounds.

In conclusion, the chlorophenylmethyl BTD derivatives can be considered as potent nonnucleoside HCMV inhibitors with a new and interesting inhibition profile.

Experimental Section

Chemical Procedures. Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Flash column chomatography was carried out at medium pressure using silica gel (E. Merck, grade 60, particle size 0.040−0.063 mm, 230−240 mesh ASTM) with the indicated solvent as eluent. ^1H NMR spectra were obtained on Varian XL-300 and Gemini-200 spectrometers working at 300 and 200 MHz, respectively. Typical spectral parameters were: spectral width 10 ppm, pulse width 9 μs (57°), data size 32 K. NOE difference spectra were measured under the same conditions, using a presaturation time of 3 s. ^{13}C NMR experiments were carried out on the Varian Gemini-200 spectrometer operating at 50 MHz. Tha acquisiton parameters were: spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 μs (57°), data size 32 K. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si, and J values are reported in hertz (Hz). Elemental analyses were performed by the analytical departement at C.N.Q.O. (CSIC) and the results obtained were within ±0.4% of the theoretical values.

1,3-Di(benzyloxymethyl)-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (5). To a solution in CH_2Cl_2 (25 mL) of the silyl derivative of 4-hydroxy-2,1,3-benzothiadiazine dioxide[19] (0.30 g, 1.5 mmol), prepared by refluxing the base in HMDS (9 mL) under nitrogen using $SO_4(NH_4)_2$ (catalytic amounts) and CH_3CN (1 mL) as cosolvent, was added acetoxymethyl benzyl ether[21] (0.54 g, 3.0 mmol) dissolved in CH_2Cl_2 (25 mL). The mixture was cooled, and $BF_3{\cdot}Et_2O$ (0.5 mL) was added under vigorous stirring. The resulting mixture was stirred at room temperature for 17 h and then was shaken with saturated sodium hydrogen carbonate solution (50 mL). The organic phase was separated, dried over sodium sulfate, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column, using hexane:AcOEt (5:1) as

eluent. Compound **5** was obtained (0.12 g, 18%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 4.57 (s, 2H, $PhCH_2O$), 4.64 (s, 2H, $PhCH_2O$), 5.20 (s, 2H, N_1CH_2O), 5.43 (s, 2H, N_3CH_2O), 7.15−7.45 (m, 12H, Ar−H, H-6, H-8), 7.59 (t, 1H, J = 7.3 Hz, H-7), 8.16 (d, 1H, J = 7.9, H-5); ^{13}C NMR ($CDCl_3$) δ 71.24 ($PhCH_2O$), 71.52 ($PhCH_2O$), 72.28 (N_3CH_2O), 80.39 (N_1CH_2O), 120.97 (C-8), 121.59 (C-4a), 126.42 (C-6), 127.88, 128.00, 128.16, 128.36, 128.53, 136.34, 137.06 (Ar−C), 130.63 (C-5), 135.41 (C-7), 139.59 (C-8a), 162.55 (C-4). Anal. ($C_{23}H_{22}N_2O_5S$) C, H, N, S.

1-[(4-Chlorophenyl)methyl]-3-(benzyloxymethyl)-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (8). To a solution in CH_2Cl_2 (25 mL) of the silyl derivative of 1-[(4-chlorophenyl)-methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.10 g, 0.3 mmol), prepared by refluxing the base in HMDS (5 mL) under nitrogen using $SO_4(NH_4)_2$ (catalytic amounts), was added acetoxymethyl benzyl ether[21] (0.05 g, 0.3 mmol) dissolved in CH_2Cl_2 (25 mL). The mixture was cooled, and BF_3·Et_2O (0.5 mL) was added under vigorous stirring. The resulting mixture was stirred at room temperature for 2 h and was then shaken with saturated sodium hydrogen carbonate solution (50 mL). The organic phase was separated, dried over sodium sulfate, and evaporated under reduced pressure. The residue was chromatographed on circular thin-layer chromatography, using CH_2Cl_2:hexane (1:1) as eluent. Compound **8** was obtained (0.03 g, 24%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 4.60 (s, 2H, $PhCH_2O$), 4.83 (s, 2H, $PhCH_2$), 5.30 (s, 2H, NCH_2O), 7.01−7.37 (m, 11H, Ar−H, H-6, H-8), 7.53 (t, 1H, J = 7.4 Hz, H-7), 8.10 (d, 1H, J = 7.8, H-5); ^{13}C NMR ($CDCl_3$) δ 55.55 ($PhCH_2$), 71.58 ($PhCH_2O$), 72.22 (NCH_2O), 122.20 (C-8), 122.58 (C-4a), 126.68 (C-6), 127.91, 127.96, 128.40, 129.03, 129.54, 132.47, 134.67, 137.11 (Ar−C), 130.88 (C-5), 135.14 (C-7), 140.20 (C-8a), 162.00 (C-4). Anal. ($C_{22}H_{19}N_2O_4SCl$) C, H, N, S.

1-[(2-Chlorophenyl)methyl]-3-(benzyloxymethyl)-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (9). To a solution in CH_2Cl_2 (25 mL) of the silyl derivative of 1-[(2-chlorophenyl)-methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.16 g, 0.5 mmol), prepared by refluxing the base in HMDS (5 mL) under nitrogen using $SO_4(NH_4)_2$ (catalytic amounts), was added acetoxymethyl benzyl ether[21] (0.09 g, 0.5 mmol) dissolved in CH_2Cl_2 (25 mL). The mixture was cooled, and BF_3·Et_2O (0.5 mL) was added under vigorous stirring. Following the workup described above, compound **9** was obtained (0.08 g, 36%) as a white solid: mp 80−83 °C; ^{1}H NMR ($CDCl_3$) 4.78 (s, 2H, $PhCH_2O$), 5.11 (s, 2H, $PhCH_2$), 5.56 (s, 2H, NCH_2O), 6.99 (d, 1H, J = 8.2 Hz, H-8), 7.24−7.50 (m, 10H, Ar−H, H-6), 7.55 (t, 1H, J = 7.4 Hz, H-7), 8.24 (d, 1H, J = 7.8, H-5); ^{13}C NMR ($CDCl_3$) δ 52.81 ($PhCH_2$), 71.56 ($PhCH_2O$), 72.24 (NCH_2O), 121.13 (C-8), 121.41 (C-4a), 126.20 (C-6), 127.37, 127.89, 127.98, 128.36, 129.23, 129.59, 129.80, 132.38, 132.86, 137.02 (Ar−C), 130.81 (C-5), 135.15 (C-7), 140.27 (C-8a), 162.54 (C-4). Anal. ($C_{22}H_{19}N_2O_4SCl$) C, H, N, S.

General Procedure for the Synthesis of Benzyl and Chlorophenylmethyl BTD Derivatives. To an equimolecular suspension of sodium hydride in DMF (25 mL) were added the corresponding monosubstitued benzothiadiazines and benzyl halide (1.5 mmol). The reaction mixture was refluxed for 2 h. After cooling the solvent was evaporated under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue was chromatographed on circular thin-layer chromatography using CH_2Cl_2: hexane (1:1) as eluent.

1,3-Dibenzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (6) and 1-Benzyl-4-(benzyloxy)-2,1,3-benzothiadiazine 2,2-Dioxide (7). Reagents: 1-benzyl-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.25 g, 0.8 mmol), benzyl bromide (0.22 g, 1.3 mmol). From the first fraction, derivative **6** was isolated as a white solid: yield 0.14 g (44%); mp 120−122 °C. Anal. ($C_{21}H_{18}N_2O_3S$) C, H, N, S.

From the second fraction, derivative **7** was isolated as a white solid: yield 0.03 g (8%); mp 110−111 °C. Anal. ($C_{21}H_{18}N_2O_3S$) C, H, N, S.

1-Benzyl-3-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (10) and 1-Benzyl-4-[(4-chlorophenyl)methyloxy]-2,1,3-benzothiadiazine 2,2-Dioxide (11). Reagents: 1-benzyl-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.10 g, 0.3 mmol), 4-chlorophenylmethyl chloride (0.07 g, 0.4 mmol). From the first fraction, derivative **10** was isolated as a white solid: yield 0.03 g (27%); mp 94−96 °C. Anal. ($C_{21}H_{17}N_2O_3SCl$) C, H, N, S.

From the second fraction, derivative **11** was isolated as a white solid: yield 0.003 g (2%); mp 146−147 °C. Anal. ($C_{21}H_{17}N_2O_3SCl$) C, H, N, S.

1,3-Di[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (12) and 1-[(4-Chlorophenyl)methyl]-4-[(4-chlorophenyl)methyloxy]-2,1,3-benzothiadiazine 2,2-Dioxide (13). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.10 g, 0.3 mmol), 4-chlorophenylmethyl chloride (0.07 g, 0.4 mmol). From the first fraction, derivative **12** was isolated as a white solid: yield 0.03 g (23%); mp 108−110 °C. Anal. ($C_{21}H_{16}N_2O_3SCl_2$) C, H, N, S.

From the second fraction, derivative **13** was isolated as a syrup: yield 0.005 g (3%). Anal. ($C_{21}H_{16}N_2O_3SCl_2$) C, H, N, S.

1,3-Di[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (14) and 1-[(3,4-Dichlorophenyl)-methyl]-4-[(3,4-chlorophenyl)methyloxy]-2,1,3-benzothiadiazine 2,2-Dioxide (15). Reagents: 1-[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.1 g, 0.3 mmol), 3,4-chlorophenylmethyl chloride (0.08 g, 0.4 mmol). From the first fraction, derivative **14** was isolated as a syrup: yield 0.02 g (16%). Anal. ($C_{21}H_{14}N_2O_3SCl_4$) C, H, N, S.

From the second fraction, derivative **15** was isolated as a white solid: yield 0.004 g (2%); mp 180−182 °C. Anal. ($C_{21}H_{14}N_2O_3SCl_4$) C, H, N, S.

1-[(3-Chlorophenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (16) and 1-[(3-Chlorophenyl)-methyl]-4-(benzyloxy)-2,1,3-benzothiadiazine 2,2-Dioxide (17). Reagents: 1-[(3-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.10 g, 0.3 mmol), benzyl bromide (0.08 g, 0.4 mmol). From the first fraction derivative **16** was isolated as a syrup: yield 0.06 g (44%). Anal. ($C_{21}H_{17}N_2O_3SCl$) C, H, N, S.

From the second fraction, derivative **17** was isolated as a white solid: yield 0.004 g (3%); mp 135−136 °C. Anal. ($C_{21}H_{17}N_2O_3SCl$) C, H, N, S.

1-[(3,4-Dichlorophenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (18) and 1-[(3,4-Dichlorophenyl)methyl]-4-(benzyloxy)-2,1,3-benzothiadiazine 2,2-Dioxide (19). Reagents: 1-[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.15 g, 0.4 mmol), benzyl bromide (0.10 g, 0.6 mmol). From the first fraction, derivative **18** was isolated as a white solid: yield 0.08 g (45%); mp 138−140 °C. Anal. ($C_{21}H_{16}N_2O_3SCl_2$) C, H, N, S.

From the second fraction, derivative **19** was isolated as a white solid: yield 0.01 g (6%); mp 148−150 °C. Anal. ($C_{21}H_{16}N_2O_3SCl_2$) C, H, N, S.

1-Benzyl-3-[(2-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (20). Reagents: 1-benzyl-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.20 g, 0.7 mmol), 2-chlorophenylmethyl chloride (0.17 g, 1.0 mmol); yield 0.03 g (13%) as a white solid; mp 180−182 °C. Anal. ($C_{21}H_{17}N_2O_3$-SCl) C, H, N, S.

1-[(4-Chlorophenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (21). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.10 g, 0.3 mmol), benzyl bromide (0.08 g, 0.4 mmol); yield 0.03 g (29%) as a syrup. Anal. ($C_{21}H_{17}N_2O_3SCl$) C, H, N, S.

1-[(2-Chlorophenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (22). Reagents: 1-[(2-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.20 g, 0.6 mmol), benzyl bromide (0.15 g, 0.9 mmol); yield 0.06 g (25%) as a white solid; mp 68−70 °C. Anal. ($C_{21}H_{17}N_2O_3SCl$) C, H, N, S.

1,3-Di[(2-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (23). Reagents: 1-[(2-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.38 g, 1.0 mmol), 2-chlorophenylmethyl chloride (0.24 g, 1.5 mmol); yield 0.05 g (10%) as a white solid; mp 125−128 °C. Anal. ($C_{21}H_{16}N_2O_3SCl_2$) C, H, N, S.

1,3-Di[(3-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (24). Reagents: 1-[(3-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[22] (0.1 g, 0.3 mmol), 3-chlorophenylmethyl chloride (0.07 g, 0.4 mmol); yield 0.02 g (16%) as a syrup. Anal. ($C_{21}H_{16}N_2O_3SCl_2$) C, H, N, S.

Antiviral Evaluation. The compounds were evaluated for antiviral activity following established procedures, as reviewed in ref 26.

Cells. Human embryonic lung (HEL) fibroblasts were propagated in Eagle's minimal essential medium (MEM) supplemented with 10% inactivated fetal calf serum (FCS), 2 mM L-glutamine, and 0.3% sodium bicarbonate.

Viruses. Two reference strains of VZV expressing viral thymidine kinase (TK^+) (YS and Oka) and two reference strains of VZV lacking the viral thymidine kinase (TK^-) (07/1 and YS/R) were included in the study. Virus stocks were prepared in HEL cells. When 70% cytopathic effect was obtained, the cells were trypsinized, resuspended in medium containing 10% DMSO and stored in aliquots at −80 °C. The Davis and AD-169 strains of human cytomegalovirus were used. Virus stocks consisted of cell-free virus obtained from the supernatant of infected cell cultures that had been clarified by low-speed centrifugation. The virus stocks were stored at −80 °C.

Antiviral Assays. Confluent HEL cells grown in 96-well microtiter plates were infected with the different virus strains at 20 (VZV) or 100 (CMV) plaque-forming units (PFU). After a 2-h incubation period, residual virus was removed and the infected cells were further incubated with MEM supplemented with 2% inactivated FCS, 2 mM L-glutamine, and 0.3% sodium bicarbonate containing serial dilutions of the test compounds (in duplicate). After 5 days (VZV) or 7 days (CMV) of incubation at 37 °C in a 5% CO_2 atmosphere, the cells were fixed with ethanol and stained with 2.5% Giemsa solution. Virus plaque formation [virus input: 20 PFU (VZV)] or viral cytophatic effect [virus input: 100 PFU (CMV)] was monitored microscopically. The antiviral activity is expressed as IC_{50}, which represents the compound concentration required to reduce virus plaque formation or cytopathicity by 50%. IC_{50} values were estimated from graphic plots of the number of plaques (percentage of control) or percentage of cytopathicity as a function of the concentration of the test compounds. A variety of test compound concentrations were used and differed 5-fold from each other. The IC_{50} was calculated from the graphic plots using the compound concentrations that were just above and just below the IC_{50} value. Data are the mean of two independent experiments.

Confluent HEL fibroblasts, grown in 96-well microtiter plates, were infected with HCMV Davis strain at 100 PFU/well. After a 2-h adsorption period, residual virus was removed and the infected cells were further incubated with medium. A concentration of the test compounds at 5 μg/mL was added at different time points after infection: 2 h (control) or 6, 14, 24, 30, 36, 48, or 72 h. The cultures were further incubated at 37 °C in 5% CO_2 atmosphere and fixed at day 7. The percentage of CPE was then calculated for each point. The last time point for which an activity comparable to that of the control was recorded corresponds to the stage of the virus cycle at which the test compound interacts.

Cytotoxicity Assays. Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL fibroblasts were seeded at a rate of 5×10^3 cells/well in 96-well microtiter plates and allowed to proliferate for 24 h. Different concentrations of the test compounds were then added (in duplicate), and after 3 days of incubation at 37 °C in 5% CO_2 atmosphere, the cell number was determined with a Coulter counter. Cytotoxicity is expressed as CC_{50}, which represents the compound concentration required to reduce cell growth by 50%. As a second parameter of cytotoxicity, the minimum cytotoxic concentration (MCC) to cause a microscopically detectable change in morphology of normal cells treated with the compounds was determined.

Antiretroviral Evaluation. Human inmunodeficiency virus type 1 [HIV-1 (HTLV-IIIb)] was kindly provided by Dr. R. C. Gallo (when at the National Institutes of Health, Betheseda, MD). Virus stocks were prepared from supernatants of HIV-1-infected MT-4 cells. HIV-2 (strain ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France), and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells. Human lymphocyte (CEM) cells were obtained from the American Tissue Culture Collection (Rockville, MD). CEM cells were infected as follows: 4×10^5 cells/mL were infected with HIV-1 or HIV-2 at ∼100 $CCID_{50}$ (50% cell culture infective dose)/mL of cell suspension. Then 100 μL of the infected cell suspension was immediately transferred to 96-well microtiter plate wells and mixed with 100 μL of the appropiate dilutions of the test compounds. After 4 days giant cell formation was recorded microscopically in the HIV-infected cell cultures. The EC_{50} values were defined as the compound concentration required to inhibit HIV-induced giant cell formation in CEM cell cultures by 50%. The greater than sign (>) means that the compounds were unable to inhibit the virus-induced cytopathicity at subtoxic concentrations (as defined by the CC_{50} values).

Protease Assay. It was performed following the methodology described in ref 27.

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