Novel Potential Agents for Human Cytomegalovirus Infection: Synthesis and Antiviral Activity Evaluation of Benzothiadiazine Dioxide Acyclonucleosides

Ana Martinez,*[,†] Ana I. Esteban,[†] Ana Castro,[†] Carmen Gil,[†] Santiago Conde,[†] Graciela Andrei,[‡] Robert Snoeck,[‡] Jan Balzarini,[‡] and Erik De Clercq[‡]

Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

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The first acyclonucleosides based on the benzothiadiazine dioxide system were synthesized following the silylation procedure. Several acyclic moieties, including acetoxyethoxymethyl, benzyloxymethyl, and propargyloxymethyl groups, were introduced. Two synthetic strategies were designed to selectively obtain the N-1 or N-3 derivatives. Lipase-mediated deacylation was used for the deprotection of the acyclonucleosides. Some of the benzothiadiazine dioxide acyclonucleosides, in particular **16**, proved active against human cytomegalovirus (CMV) at concentrations slightly higher than that found for ganciclovir [50% inhibitory concentration (IC_{50}) = 3.5−3.7 μg/mL, cytotoxicity (CC_{50}) ≥ 40 μg/mL, MCC = 20 μg/mL]. Additionally, compound **16** inhibited the replication of human immunodeficiency virus type 1 (HIV-1) and HIV-2 in CEM cells at concentrations that were 5-fold lower than its cytotoxic concentration.

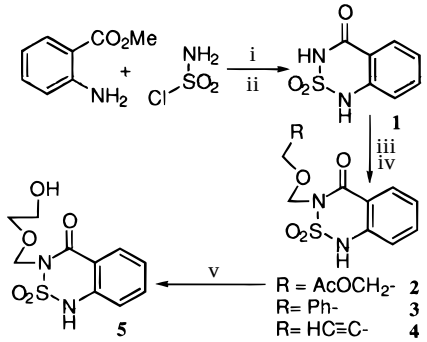
Introduction

Human cytomegalovirus (CMV) is a ubiquitous human pathogen that affects 50−90% of adults. In healthy individuals, CMV infections are usually asymptomatic. However, in immunocompromised individuals CMV is responsible for the most commom sight- and life-threatening opportunistic viral infection.[1] CMV can cause severe morbidity and mortality in congenitally infected newborns and immunocompromised patients. It is the primary cause of death in recipients of allogenic bone marrow transplants[2] and renal transplants.[3] In addition, patients suffering from the acquired immune deficiency syndrome (AIDS) are nearly always HCMV seropositive and often develop symptomatic reactivation disease as the immunodeficiency progresses, retinitis and colitis being the most frequent manifestations.[4]

Few therapeutic options are available for the treatment of CMV infections. Only ganciclovir,[5] foscarnet,[6] and cidofovir[7] have been approved by the FDA for the treatment of CMV diseases. Although treatment of CMV infections with these drugs has produced clinical improvement in the majority of the patients, the compounds suffer from poor oral bioavailability[8] and adverse side effects. Therefore, there still is an urgent need for effective, nontoxic anti-CMV drugs with good oral availability. Moreover, with the advent of virus strains there are resistant to current drugs,[9] new drugs that act by a new mechanism of action may be highly desirable for the treatment of CMV infections.

In our search for antivirally effective thiadiazine acyclonucleosides,[10,11] we discovered that benzothiadiazine dioxide acyclonucleosides could be considered as new lead compounds in the development of anti-CMV agents.[12] In the present report we describe the synthesis, molecular modeling, and further antiviral evaluation of these new compounds.

Scheme 1[a]



[a] Reagents: (i) C_6H_6; (ii) 6 N NaOH; (iii) HMDS/N_2; (iv) $BF_3{\cdot}Et_2O/CH_2Cl_2/RCH_2OCH_2OAc$; (v) CAL/*t*-BuOH/buffer pH = 7.

Chemistry

The benzothiadiazine dioxide ring was obtained in two steps following the Cohen and Klarberg procedure[13] starting from methyl anthranylate and sulfamoyl chloride.

The synthesis of the acyclonucleosides was achieved following the silylation procedure.[14] Thus benzothiadiazine **1** was first silylated using hexamethyldisilazane (HMDS) and acetonitrile as cosolvent under nitrogen atmosphere. Several acyclic moieties, including acetoxyethoxymethyl, benzyloxymethyl, and propargyloxymethyl groups, were introduced in a second step using dichloromethane and boron trifluoride as catalyst (Scheme 1). In all conditions assayed, acycloglycosylation took place exclusively at the N-3 position yielding derivatives **2**−**4**. Deprotection of compound **2** was achieved quantitatively using a lipase-mediated deacylation procedure with *Candida antarctica* lipase (CAL) in a hydrolytic medium (*t*-BuOH/buffer pH = 7). This methodology has been previously described by our group[10] and has been applied efficiently to the regioselective deacylation of thiadiazine dioxide diacyclonucleosides.[15]

[†] Instituto de Química Médica.
[‡] Katholieke Universiteit Leuven.

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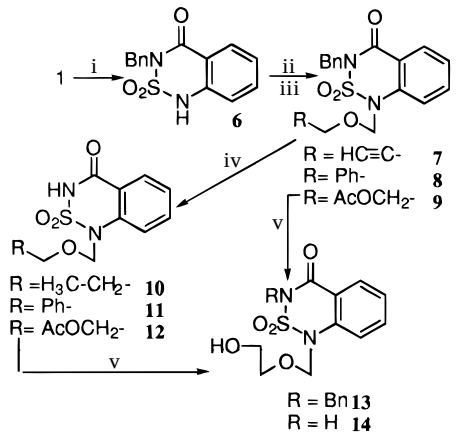
Scheme 2[a]



[a] Reagents: (i) BrBn, HCO_3Na; (ii) HMDS/N_2; (iii) $BF_3{\cdot}Et_2O$/CH_2Cl_2/RCH_2OCH_2OAc; (iv) [H_2]/Pd; (v) CAL/*t*-BuOH/buffer.

The N-1-substituted acyclic nucleosides were prepared using a protection−deprotection strategy from 3-benzylbenzothiadiazine **6**, which was previously obtained by reaction of **1** with benzyl bromide in aqueous bicarbonate. Silylation of **6** with HMDS and catalytic amounts of ammoniun sulfate, followed by reaction with several acyclic moieties in dichloromethane and boron trifluoride etherate as catalyst, yielded, after chromatographic purification, the N-3-protected, N-1 acyclic nucleosides **10**−**12** (Scheme 2). The *N*-benzyl group was eliminated by catalytic hydrogenolysis using Pd/C as catalyst. It is worth mentioning that in the case of compound **7**, simultaneously with the cleavage of the *N*-benzyl moiety, reduction of the acyclic moiety multiple bond was accomplished. The lipase-mediated deacylation procedure was employed to obtain the hydroxyethoxymethyl derivates **13** and **14** in quantitative yields.

The structures of all new compounds were elucidated according to analytical and spectroscopic data (see Experimental Section). Unequivocal assignment of all chemical shifts (^{1}H and ^{13}C NMR) was done using bidimensional experiments such as COSY or HMQC for one-bond correlation. The site of glycosylation was determined from sequences of HMBC for long distance proton/carbon correlation experiments.

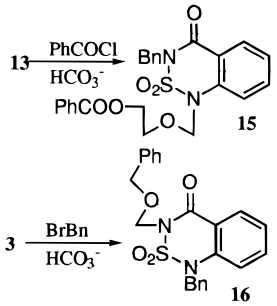
Biological Results and Discussion

All acyclonucleosides derived from benzothiadiazine dioxide system were evaluated for their antiviral activity in a wide variety of assay systems:[16] 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 B4 virus, and Semliki forest virus in Vero cells. However, no antiviral activity was noted in any of these antiviral assay systems (at compound concentrations up to 400 μg/mL).

In addition, antiviral activity against CMV, strains AD-169 and Davis, and varicella-zoster virus (VZV), strains OKA, YS, 07/1, and YS/R, was determined by plaque reduction (VZV)[17] or CPE reduction (CMV)[18] assays in confluent human embryonic lung (HEL) fibroblasts. Cytotoxicity measurements were based on the inhibition of cell growth. The results are presented in Table 1. Two of the test compounds (**7** and **8**) were active against CMV and VZV at concentrations that were only 2.5−5-fold below the cytotoxic concentration for the host cells, which resulted in a low selective antiviral action. Taking into account the chemical structure of compounds **7** and **8** that is unique because of the nature of the heterocyclic base and the lack of the hydroxyl group in the acyclic side chain, some preliminar structure−activity analysis of compounds **2**−**14** can be done. So, it pointed to the necessity of a double substitution in the benzothiadiazine moiety together with the lipophilicity in the acyclic side chain. These factors were considered when preparing the new acyclonucleosides **15** and **16**.

A lipophilic moiety was introduced in the side chain of acyclonucleoside **13** by treatment with benzoyl chloride in refluxing pyridine yielding compound **15** (Scheme 3). Additionally, the positions of the substituents in derivative **8** were interchanged. Thus, acyclonucleoside **3** was benzylated by reaction with benzyl bromide in aqueous bicarbonate. Compound **16** was isolated from the reaction medium after column chromatography purification (Scheme 3).

Scheme 3



The new benzothiadizine acyclonucleosides thus prepared (**15** and **16**) were evaluated for their activity against CMV and VZV in HEL cells. Compound **16** was active against CMV at a concentration that was only slightly higher than for ganciclovir [50% inhibitory concentration (IC_{50}) 3.6 μg/mL for compound **16** compared to 1.2−1.9 μg/mL for ganciclovir]. Although compound **16** only inhibited cell growth after 3 days of incubation at a concentration of ≥40 μg/mL, cell morphology was altered at 20 μg/mL when cell cultures were exposed to the compound for 7 days.

Additionally, the benzothiadiazine derivatives **2**−**16** were also evaluated for their activity against HIV-1 (strain III_B) and HIV-2 (strain ROD) in CEM cells, and the results are shown in Table 2. Compound **16** was found to inhibit the replication of HIV-1 and HIV-2 in CEM cells at an IC_{50} of 15−20 μg/mL. The CC_{50} was around 100 μg/mL for **16** in CEM cells. All the other compounds were inactive against the replication of HIV-1 at subtoxic concentrations.

The conformation of **16** was studied with ab initio Hartree−Fock methods to asses its structural determinants. The geometry was optimized with a 3-21G* basic set to determine the optimum position of the two aromatic systems. Molecular modeling, using the Gaussian 94 program,[19] revealed that the benzothiadiazine

Table 1. Antiviral Activity and Cytotoxicity of Benzothiadiazine Dioxide Acyclonucleosides **2**−**16** against Human Cytomegalovirus (CMV) and Varicella-Zoster Virus (VZV) in Human Embryonic Lung (HEL) Cells

	antiviral activity IC_{50} (μg/mL)[a]						cytotoxicity (μg/mL)	
	CMV		TK^+ VZV		TK^- VZV			
compd no.	AD-169	Davis	OKA	YS	07/1	YS/R	cell growth CC_{50}[b]	cell morphology MCC[c]
2	>50	>50	>50	>50	>50	>50	>200	>50
3	>50	>50	34	46	38	31	148	>50
4	>50	>50	>50	>50	>50	>50	169	>50
5	>50	>50	>50	>50	>50	>50	160	>50
6	>20	>20	>50	>50	48	>50	>50	50
7	12	12	21	24	11	13	>50	50
8	8	11	20	20	>5	>5	>50	≥50
9	>20	>50	42	>50	44	>20	100	50
10	>50	>50	>50	>50	>50	>50	>50	>50
11	>50	>50	>50	>50	>50	>50	>50	>50
12	>50	>50	>50	>50	>50	>50	>200	>50
13	>20	>50	43	>50	55	>20	125	>50
14	>50	>50	>50	>50	>50	>50	112	>50
15	13	≥20	>20	>20	>20	12	17	50
16	3.5	3.7	>5	>5	>5	3.0	≥40	20
acyclovir			0.73	0.78	24	26	>200	>50
ganciclovir	1.2	1.9					>50	>50

[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] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. Assays were performed in duplicate.

Table 2. Anti-HIV-1 and Anti-HIV-2 Activity and Cytotoxic Properties of Benzothiadiazine Dioxide Acyclonucleosides **2**−**16** in Human T-Lymphocyte (CEM) Cells

compd no.	antiviral activity EC_{50} (μg/mL)[a]		cytotoxicity CC_{50} (μg/mL)[b]
	HIV-1(III_B)	HIV-2(ROD)	
2	>200	>200	>200
3	>40	>40	91 ± 0.71
4	>200	>200	>200
5	>40	>40	>200
6	>200	>200	>200
7	>8	>8	23 ± 0.71
8	>8	>8	25 ± 1.4
9	>40	>40	103 ± 3.5
10	>40	>40	120 ± 28
11	>40	>40	>200
12	>200	>200	>200
13	>40	>40	89 ± 1.4
14	>40	>40	15 ± 4.9
15	≥40	≥40	100
16	15	20	100

[a] 50% effective concentration, or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%. Assays were performed in triplicate. [b] 50% cytotoxic concentration, or concentration required to reduce CEM cell viability by 50%. Assays were performed in triplicate.

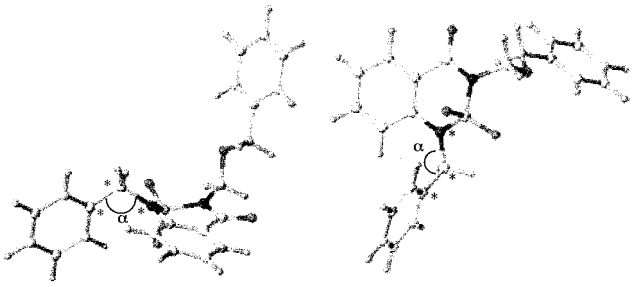


Figure 1. Two different views of the 3-21G*-optimized structure of benzothiadiazine **16**.

derivative **16** adopts a butterfly-like conformation, the valence angle α between the two rings being 114.10° (Figure 1).

In conclusion, the benzothiadiazine dioxide acyclonucleoside **16** can be considered as a new lead among the antiviral compounds, as it is the first to show activity against both CMV and HIV, at a yet to be identified target site.

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. ^{1}H 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. The 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. Elemental analyses were performed by the analytical departement at CNQO (CSIC), and the results obtained were within ±0.4% of the theoretical values. The *C. antarctica* lipase used was Novo Nordisk's immobilized preparation Novozym 435.

3-Benzyl-2,1,3-benzothiadiazin-4(1*H*)-one 2,2-Dioxide (6). Benzyl bromine (0.17 g, 1 mmol) was added to a solution of benzothiadiazine **1**[13] (0.198 g, 1 mmol) in a sodium bicarbonate aqueous solution (25 mL). The reaction mixture was refluxed for 1 h. After the mixture cooled to room temperature, the aqueous phase was extracted with AcOEt (5 × 10 mL). The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue was chromatographed on silica gel column using CH_2Cl_2:MeOH (50:3) as eluent. Compound **6** was obtained (0.10 g, 34%) as a white crystalline solid: mp 250−251 °C; ^{1}H NMR (DMSO-d_6) δ 4.93 (s, 2H, N−CH_2), 6.72 (d, 1H, J = 8.2 Hz, H-8), 6.85 (t, 1H, J = 6.7 Hz, H-6), 7.19−7.42 (m, 6H, Ar−H and H-7), 7.85 (d, 1H, J = 7.8 Hz, H-5); ^{13}C NMR (DMSO-d_6) δ 46.45 (CH_2), 113.66 (C-4a), 119.09 (C*p*), 119.73 (C-8), 126.59 (C*m*), 126.65 (C-7), 128.31 (C*o*), 128.75 (C*i*), 131.72 (C-5), 137.68 (C-6), 142.21 (C-8a), 165.67 (C-4). Anal. ($C_{14}H_{12}N_2O_3S$) C, H, N, S.

General Procedure for the Synthesis of Benzothiadiazine Acyclonucleosides. To a solution in CH_2Cl_2 (25 mL) of the silyl derivative of the benzothiadiazine dioxide (1 mmol)

prepared by refluxing the base in hexamethyldisilazane (3 mL) under nitrogen using suitable catalyst and cosolvents, the dissolved acyclic moiety precursor in CH_2Cl_2 (25 mL) was added. The mixture was cooled, and $BF_3{\cdot}Et_2O$ (1.40 mmol) was added under vigorous stirring and exclusion of moisture. The resulting mixture was stirred at room temperature for 1−3 h and was then shaken with saturated sodium hydrogencarbonate 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 as eluent mixtures of solvents in the proportions indicated for each particular case.

3-[(2-Acetoxyethoxy)methyl]-2,1,3-benzothiadiazin-4(1*H*)-one 2,2-Dioxide (2). Reagents: (1) benzothiadiazine dioxide **1** (0.39 g, 2 mmol), HMDS (6 mL), $SO_4(NH_4)_2$ (catalytic amounts), CH_3CN (1 mL); (2) acetoxyethyl acetoxymethyl ether[20] (0.35 g, 2 mmol), $BF_3{\cdot}Et_2O$ (2.8 mmol). Purification: compound **2** was obtained as white needles from the acidified aqueous phase (0.34 g, 54%); mp 122−123 °C; ^{1}H NMR (DMSO-d_6) δ 1.97 (s, 3H, CH_3CO), 3.77 (m, 2H, CH_2O), 4.12 (m, 2H, $AcOCH_2$), 5.35 (s, 2H, NCH_2O), 7.20 (d, 1H, J = 7.8 Hz, H-8), 7.33 (t, 1H, H-6), 7.71 (t, 1H, H-7), 8.04 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 20.63 (CH_3), 62.79 (CH_2), 67.03 (CH_2), 71.66 (N−CH_2−O), 117.64 (C-4a), 119.97 (C-8), 124.47 (C-7), 129.77 (C-5), 135.79 (C-6), 138.53 (C-8a), 162.40 (C-4), 170.74 (C═O). Anal. ($C_{12}H_{14}N_2O_6S$) C, H, N, S.

3-(Benzyloxymethyl)-2,1,3-benzothiadiazin-4(1*H*)-one 2,2-Dioxide (3). Reagents: (1) benzothiadiazine dioxide **1** (0.59 g, 3 mmol), HMDS (9 mL), $SO_4(NH_4)_2$ (catalytic amounts), CH_3CN (1 mL); (2) acetoxymethyl benzyl ether[21] (0.54 g, 3 mmol), $BF_3{\cdot}Et_2O$ (4.2 mmol). Purification: CH_2Cl_2:MeOH (50:1); yield 0.60 g (62%); ^{1}H NMR (DMSO-d_6) δ 4.59 (s, 2H, CH_2O), 5.25 (s, 2H, NCH_2O), 6.72 (t, 1H, H-7), 6.74 (d, 1H, J = 8.0 Hz, H-8), 7.25−7.34 (m, 6H, Ar−H, H-6), 7.78 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 69.80 (CH_2), 70.38 (N−CH_2−O), 115.45 (C-4a), 116.94 (C-8), 122.07 (C-7), 127.29 (C*p*), 127.55 (C*m*), 128.11 (C*o*), 128.60 (C-5), 133.51 (C-6), 138.41 (C*i*), 149.92 (C-8a), 164.89 (C-4). Anal. ($C_{15}H_{14}N_2O_4S$) C, H, N, S.

3-(Propargyloxymethyl)-2,1,3-benzothiadiazin-4(1*H*)-one 2,2-Dioxide (4). Reagents: (1) benzothiadiazine dioxide **1** (0.396 g, 2 mmol), HMDS (6 mL), $SO_4(NH_4)_2$ (catalytic amounts), CH_3CN (1 mL); (2) (propargyloxy)methyl chloride[22] (0.21 g, 2 mmol), $BF_3{\cdot}Et_2O$ (2.8 mmol). Purification: CH_2Cl_2:MeOH (50:1); yield 0.34 g (62%); ^{1}H NMR (DMSO-d_6) δ 3.47 (t, 1H, J = 1.5 Hz, HC≡), 4.25 (d, 2H, J = 1.5 Hz, ≡CCH), 5.36 (s, 2H, NCH_2O), 7.19 (d, 1H, J = 6.4 Hz, H-8), 7.34 (t, 1H, H-6), 7.71 (t, 1H, H-7), 8.04 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 56.60 (CHO), 70.72 (N−CH_2−O), 78.02 (CH≡), 79.88 (C≡), 118.14 (C-4a), 120.56 (C-8), 125.01 (C-7), 130.24 (C-5), 136.28 (C-6), 138.96 (C-8a), 162.78 (C-4). Anal. ($C_{11}H_{10}N_2O_4S$) C, H, N, S.

3-Benzyl-1-(propargyloxymethyl)-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (7). Reagents: (1) benzothiadiazine dioxide **6** (0.29 g, 1 mmol), HMDS (3 mL), $SO_4(NH_4)_2$ (catalytic amounts); (2) (propargyloxy)methyl chloride[22] (0.11 g, 1 mmol), $BF_3{\cdot}Et_2O$ (1.4 mmol). Purification: hexane:AcOEt (4:1); yield 0.27 g (77%); ^{1}H NMR (DMSO-d_6) δ 3.54 (t, 1H, J = 1.5 Hz, HC≡), 4.21 (d, 2H, J = 1.5 Hz, ≡CCH), 5.10 (s, 2H, CH_2Ph), 5.25 (s, 2H, NCH_2O), 7.33 (m, 5H, Ar−H), 7.49 (d, 1H, J = 7.8 Hz, H-6), 7.59 (d, 1H, H-8), 7.82 (t, 1H, H-7), 8.01 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 55.61 (CH_2), 56.57 (CHO), 71.44 (N−CH_2−O), 78.09 (CH≡), 79.58 (C≡), 122.10 (C-4a), 123.32 (C-8), 127.09 (C-7), 128.75 (C*o*, C*p*), 128.85 (C*m*), 130.12 (C-5), 134.23 (C*i*), 136.16 (C-6), 139.93 (C-8a), 162.04 (C-4). Anal. ($C_{18}H_{16}N_2O_4S$) C, H, N, S.

3-Benzyl-1-(benzyloxymethyl)-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (8). Reagents: (1) benzothiadiazine dioxide **6** (0.57 g, 2 mmol), HMDS (6 mL), $SO_4(NH_4)_2$ (catalytic amounts); (2) acetoxymethyl benzyl ether[21] (0.36 g, 2 mmol), $BF_3{\cdot}Et_2O$ (2.8 mmol). Purification: hexane:AcOEt (3:1); yield 0.56 g (70%); ^{1}H NMR (DMSO-d_6) δ 4.59 (s, 2H, CH_2O), 5.11 (s, 2H, CH_2Ph), 5.30 (s, 2H, NCH_2O), 7.58 (d, 1H, J = 6.4 Hz, H-8), 7.12−7.33 (m, 5H, Ar−H), 7.47 (t, 1H, H-6), 7.76 (t, 1H, H-7), 8.04 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 55.12 (CH_2Ph), 70.51 (CH_2), 72.19 (N−CH_2−O), 121.82 (C-4a), 122.91 (C-8), 126.70 (C-7), 127.39, 128.27 (C*p*), 128.33, 128.54 (C*m*), 128.80, 129.01 (C*o*), 129.87 (C-5), 134.01, 137.22 (C*i*), 135.81 (C-6), 139.67 (C-8a), 161.86 (C-4). Anal. ($C_{22}H_{20}N_2O_4S$) C, H, N, S.

1-[(2-Acetoxyethoxy)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (9). Reagents: (1) benzothiadiazine dioxide **6** (0.57 g, 2 mmol), HMDS (6 mL), $SO_4(NH_4)_2$ (catalytic amounts); (2) acetoxyethyl acetoxymethyl ether[20] (0.35 g, 2 mmol), $BF_3{\cdot}Et_2O$ (2.8 mmol). Purification: hexane:AcOEt (10:3); yield 0.39 g (50%); ^{1}H NMR (DMSO-d_6) δ 1.99 (s, 3H, CH_3CO), 3.71 (m, 2H, CH_2O), 4.12 (m, 2H, $AcOCH_2$), 5.10 (s, 2H, CH_2Ph), 5.23 (s, 2H, NCH_2O), 7.23−7.28 (m, 5H, Ar−H), 7.47 (t, 1H, H-6), 7.53 (d, 1H, H-8), 7.80 (t, 1H, J = 7.8 Hz, H-7), 8.00 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 20.63 (CH_3), 32.79 (CH_2), 67.03 (CH_2), 71.66 (N−CH_2−O), 122.02(C-4a), 123.17 (C-8), 126.93 (C-7), 130.04 (C-5), 136.05 (C-6), 139.91 (C-8a), 162.00 (C-4), 170.74 (C═O). Anal. ($C_{19}H_{20}N_2O_6S$) C, H, N, S.

General Procedure for the Enzymatic Cleavage of Acetyl Group. A solution of the acylated acyclonucleoside (2 mmol) in *t*-BuOH:buffer pH = 7 (90:10) was incubated with 10 mg·mL^{-1} *C. antarctica* lipase (CAL) at 45 °C and 250 rpm in an orbital shaker for 4 h. When all the starting material had disappeared, the enzyme was removed by filtration and washed with methanol. The filtrate was evaporated in vacuo, and the deprotected compound was obtained as a syrup. The compounds thus obtained were as follows.

3-[(2-Hydroxyethoxy)methyl]-2,1,3-benzothiadiazin-4(1*H*)-one 2,2-dioxide (5): yield 0.08 g (92%); ^{1}H NMR (DMSO-d_6) δ 3.54 (m, 4H, CH_2−CH_2O), 5.22 (s, 2H, NCH_2O), 6.83 (m, 2H, H-6 and H-8), 7.37 (t, 1H, J = 7.8 Hz, H-7), 7.82 (t, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 60.11 (CH_2), 70.53 (CH_2), 71.02 (N−CH_2−O), 115.87 (C-4a), 118.16 (C-8), 121.75 (C-7), 128.80 (C-5), 133.88 (C-6), 149.13 (C-8a), 164.48 (C-4). Anal. ($C_{10}H_{12}N_2O_5S$) C, H, N, S.

1-[(2-Hydroxyethoxy)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (13): yield 0.08 g (98%); ^{1}H NMR (DMSO-d_6) δ 3.52 (m, 4H, CH_2−CH_2O), 5.12 (s, 2H, CH_2Ph), 5.21 (s, 2H, NCH_2O), 7.13−7.27 (m, 5H, Ar−H), 7.57 (d, 1H, J = 7.8 Hz, H-8), 7.45 (t, 1H, H-6), 7.78 (t, 1H, H-7), 8.01 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 55.10 (CH_2Ph), 59.88 (CH_2), 71.21 (CH_2), 72.96 (N−CH_2−O), 121.87 (C-4a), 122.89 (C-8), 126.68 (C-7), 128.29 (C*p*), 128.31 (C*m*), 128.54 (C*o*), 129.77 (C-5), 134.01 (C*i*), 135.76 (C-6), 139.67 (C-8a), 161.81 (C-4). Anal. ($C_{17}H_{18}N_2O_5S$) C, H, N, S.

1-[(2-Hydroxyethoxy)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (14): yield 0.12 g (90%); ^{1}H NMR (DMSO-d_6) δ 3.59 (m, 4H, CH_2−CH_2O), 5.21 (s, 2H, NCH_2O), 6.78 (d, 1H, J = 8 Hz, H-8), 6.76 (t, 1H, H-6), 7.32 (t, 1H, H-7), 7.79 (t, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 60.49 (CH_2), 70.78 (CH_2), 71.30 (N−CH_2−O), 116.00 (C-4a), 117.95 (C-8), 122.35 (C-7), 129.80 (C-5), 134.14 (C-6), 149.55 (C-8a), 165.17 (C-4). Anal. ($C_{10}H_{12}N_2O_5S$) C, H, N, S.

1-(Propyloxymethyl)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (10). A solution of acyclonucleoside **7** (0.09 g, 0.3 mmol) in MeOH (15 mL) was hydrogenated with 10 psi of hydrogen in the presence of 20% palladium/charcoal catalyst (0.018 g) at room temperature. After 30 min, the catalyst was filtered off and the solvent removed in vacuo. The residue was dissolved in AcOEt and washed with water. The organic phase was dried over sodium sulfate and the solvent eliminated under reduced pressure yielding 0.06 g (90%) of derivative **10**: ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H, CH_3), 1.49 (q, 2H, CH_2), 3.50 (m, 2H, CH_2O), 5.30 (s, 2H, NCH_2O), 7.17 (d, 1H, J = 7.8 Hz, H-8), 7.29 (t, 1H, H-6), 7.68 (t, 1H, H-7), 8.02 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 10.68 (CH_3), 22.48 (CH_2), 70.71 (CH_2O), 71.89 (N−CH_2−O), 117.88 (C-4a), 120.32 (C-8), 124.29 (C-7), 129.95 (C-5), 135.84 (C-6), 139.44 (C-8a), 162.78 (C-4). Anal. ($C_{11}H_{14}N_2O_4S$) C, H, N, S.

1-(Benzyloxymethyl)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (11). Following the above procedure, benzyl acyclonucleoside **8** (0.11 g, 0.3 mmol) was hydrogenated in the presence of palladium/charcoal (0.02 g). After workup, compound **11** was obtained (0.08 g, 97%) as a syrup: ^{1}H NMR

(DMSO-d_6) δ 4.66 (s, 2H, CH_2O), 5.45 (s, 2H, NCH_2O), 7.20 (t, 1H, J = 8.0 Hz, H-6), 7.31−7.35 (m, 5H, Ar−H), 7.30 (d, 1H, H-8), 7.68 (t, 1H, H-7), 8.04 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 70.44 (CH_2), 71.32 (N−CH_2−O), 117.67 (C-4a), 120.04 (C-8), 124.36 (C-7), 127.71 (C*p*), 128.28 (C*m*), 128.39 (C*o*), 129.79 (C-5), 137.41 (C*i*), 135.72 (C-6), 138.76 (C-8a), 162.49 (C-4). Anal. ($C_{15}H_{14}N_2O_4S$) C, H, N, S.

1-[(2-Acetoxyethoxy)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (12). According to the method described for derivative **10**, the benzothiadiazine acyclonucleoside **9** (0.20 g, 0.4 mmol) was hydrogenated with 50 psi of hydrogen using 20% palladium/charcoal (0.04 g) as catalyst. After 2 h, the reaction mixture was worked up yielding 0.13 g (90%) of compound **12**: ^{1}H NMR (DMSO-d_6) δ 1.98 (s, 3H, CH_3CO), 3.76 (m, 2H, CH_2O), 4.13 (m, 2H, $AcOCH_2$), 5.35 (s, 2H, NCH_2O), 7.20 (d, 1H, J = 7.8 Hz, H-8), 7.32 (t, 1H, H-6), 7.70 (t, 1H, H-7), 8.04 (d, 1H, H-5); ^{13}C NMR (DMSO-d_6) δ 21.02 (CH_3), 63.19 (CH_2), 67.40 (CH_2), 72.06 (N−CH_2−O), 117.93 (C-4a), 120.45 (C-8), 124.56 (C-7), 130.01 (C-5), 136.08 (C-6), 139.42 (C-8a), 162.90 (C-4), 170.74 (C═O). Anal. ($C_{12}H_{14}N_2O_6S$) C, H, N, S.

1-[(2-Benzoyloxyethoxy)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (15). A solution of acyclonucleoside **13** (0.08 g, 0.2 mmol) in CH_2Cl_2:pyridine (10:1) (10 mL) was cooled at −20 °C for 15 min, and benzoyl chloride (107 μL, 0.9 mmol) dissolved in CH_2Cl_2 (3 mL) was slowly added. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography eluting with hexane:AcOEt (1:3) and yielding 0.14 g (75%) of derivative **15** as a syrup: ^{1}H NMR ($CDCl_3$) δ 3.91 (m, 2H, CH_2O), 4.42 (m, 2H, $AcOCH_2$), 4.89 (s, 2H, CH_2Ph), 5.29 (s, 2H, NCH_2O), 7.04−8.10 (m, 14H, Ar−H, H-5, H-6, H-7, H-8); ^{13}C NMR ($CDCl_3$) δ 56.56 (CH_2Ph), 63.62 (CH_2), 67.69 (CH_2), 72.84 (N−CH_2−O), 122.71 (C-4a), 122.61 (C-8), 126.65 (C-7), 130.67, 130.17 (C*p*), 128.45, 128.27 (C*m*), 128.73, 129.70 (C*o*), 132.93 (C-5), 133.72, 133.56 (C*i*), 135.13 (C-6), 140.32 (C-8a), 162.47 (C-4), 166.48 (C═O). Anal. ($C_{24}H_{22}N_2O_6S$) C, H, N, S.

1-Benzyl-3-(benzyloxymethyl)-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (16). Benzyl bromine (0.8 μL, 0.8 mmol) was added to a solution of acyclonucleoside **3** (0.05 g, 0.2 mmol) in a sodium bicarbonate aqueous solution (10 mL). The reaction mixture was refluxed for 4 h. After cooling to room temperature, the aqueous phase was extracted with AcOEt (5 × 10 mL). The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue was chromatographed on silica gel column using hexane:AcOEt (4:1) as eluent. Compound **16** was obtained (0.07 g, 95%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 4.60 (s, 2H, CH_2O), 4.89 (s, 2H, CH_2Ph), 5.26 (s, 2H, NCH_2O), 7.06−7.36 (m, 12H, Ar−H, H-6, H-8), 7.54 (t, 1H, J = 7.3 Hz, H-7), 8.09 (d, 1H, H-5); ^{13}C NMR ($CDCl_3$) δ 56.44 (CH_2Ph), 71.44 (CH_2), 72.21 (N−CH_2−O), 122.49 (C-8), 122.68 (C-4a), 126.58 (C-7), 127.87, 128.64 (C*p*), 128.37, 128.75 (C*m*), 128.02, 128.23 (C*o*), 130.69 (C-5), 135.04 (C-6), 133.71, 137.14 (C*i*), 140.34 (C-8a), 162.48 (C-4). Anal. ($C_{22}H_{20}N_2O_4S$) C, H, N, S.

Antiviral Evaluation. The compounds were evaluated for antiviral activity following established procedures, as reviewed in ref 16.

Cells. Human embryonic lung (HEL) fibroblasts were propagated in Eagle's minimum essential medium (MEM) supplemented with 10% inactivated fetal calf serum, 1% 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 as infected 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 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, 1% L-glutamine, and 0.3% sodium bicarbonate containing serial dilutions of the test compounds (in duplicate). After 5 (VZV) or 7 (CMV) days of incubation at 37 °C in 5% CO_2 atmosphere, the cells were fixed with ethanol and strained with 2.5% Giemsa solution. Virus plaque formation (virus input: 20 PFU, VZV) or viral cytopathic 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.

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 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 toxic concentration (MTC) to cause a microscopically detectable change in morphology of normal cells treated with the compounds was evaluated.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 [HIV-1 (HTLV-IIIb)] was kindly provided by Dr. R. C. Gallo (when at the National Institutes of Health, Bethesda, 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. 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 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.

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