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Benzothiadiazine Dioxides (BTD) Derivatives as Non-nucleoside Human Cytomegalovirus (HCMV) Inhibitors. Study of Structural Requirements for Biological Activity†

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Abstract—Two new series of BTD derivatives have been synthesised allowing to explore the steric requirements for their biological activity. The *N*3-alkylBTD compounds have shown antiviral activity in the same order or lower than previously prepared compounds. However, the cytotoxicity values observed prevent this new series of BTD derivatives from its potential therapeutic application. Concerning BTD derivatives with the modified linker attached to *N*1 position, we have obtained new non-nucleoside anti-HCMV derivatives. The activity against HCMV is shown at concentrations that were 10-fold lower than the concentration that was toxic for the host cells, which confirm that these derivatives show a specific antiviral effect against HCMV. SAR conclusions derived from these last compounds have provided new knowledge about the structural requirements of BTD showing certain positions that could be modified for enhancing the anti-HCMV action.

Introduction

The importance and the interest of human cytomegalovirus HCMV as a pathogen has increased over the two past decades, with an increase in the number of patients undergoing immunosuppresive therapy following organ and bone marrow transplantation, as well as the rising amount of AIDS patients.[1,2] Despite new drugs and strategies for prophylaxis and treatment of HCMV disease, the virus is still today one of the leading causes of morbidity and mortality in immunocompromised patients.[3] In addition, a number of studies have demonstrated that HCMV may be involved in the development of vascular diseases such as atherosclerosis, restenosis after coronary angioplasty and transplant vascular sclerosis (chronic rejection).[4–6]

Current treatment options for HCMV disease are limited to ganciclovir, foscarnet, cidofovir and fomivirsen.[7] All of them achieve a selective inhibition of HCMV replication by inhibition of the viral DNA polymerase. While these drugs have made significant contributions to the treatment of HCMV disease, they all possess significant side effects and have poor oral bioavailability, requiring intravenous or intraocular administration. Moreover, the increased and prolonged use of these compounds in the clinical setting has led to the emergence of viral resistance against most of these drugs.[8,9] There is a clear, unmet medicinal need for a convenient therapy for HCMV which acts by new molecular mechanisms of action. This has renewed the current interest in the search for novel HCMV inhibitors.[10,11]

As a part of our ongoing search for new antiviral compounds[12,13] we have discovered the benzothiadiazine dioxide (BTD) modified acyclonucleosides which showed a marked activity against HCMV and Varicella–Zoster virus (VZV) infection.[14] The structures of these compounds are quite unique, not only because of the nature of the heterocyclic base, but also for the lack of the 5′-OH mimetic group present in ganciclovir and other current anti-HCMV drugs, which points to a different mechanism of action not involving obligate

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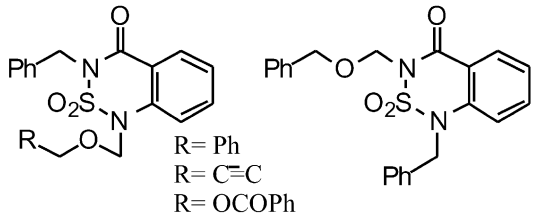
5′-phosphorylation (Chart 1). Preliminary structure–activity analysis showed the necessity of a double substitution in the heterocycle together with the lipophilicity in the acyclic side chain.[15] These factors were considered in the first optimisation step performed on this family of compounds leading to the BTD dibenzyl derivatives as potent non-nucleoside HCMV inhibitors.[16]

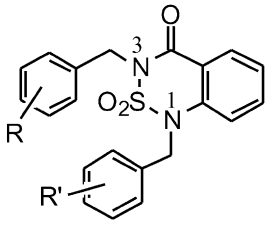
The BTD anti-HCMV drugs show comparable activity to ganciclovir on laboratory (AD-169 and Davis) and isolated viral strains and are active against some current drugs resistant strains. Pharmacological studies revealed that the selective biological action exerted by the BTD derivatives against HCMV is in the early stages of the viral replicative cycle.[17]

As the pharmacological viral receptor is yet unknown and with the aim of gaining further insights into the structural requirements for the biological activity of BTD, a CoMFA analysis was performed. It suggested that the steric component is a predominant factor in the antiviral activity of these analogues with electrostatic factors playing a smaller yet significant role.[18] From these results, here we propose two different series of new BTD derivatives (Fig. 1) which allow us to explore the structural BTD steric requirements in their interaction with the viral receptor.

According to the steric CoMFA contour plot (Fig. 1), in the first designed series, the benzyl moiety attached to the *N*3 of the BTD framework is changed by different alkyl chains with the aim of diminishing the steric volume in this region of the space. In the second one, the length and the nature of the linker between the BTD heterocycle and the phenyl ring attached to *N*1 as well as the size of the substituents in *para* position of the phenyl ring are varied. These last modifications would provide molecules that increase the steric bulk in this other region of the space. The synthesis, biological evaluation and further SAR conclusions of these new compounds are here described.



Benzothiadiazine Dioxides (BTD) Modified Acyclonucleosides



BTD Dibenzyl Derivatives

Chart 1.

Chemistry

The *N*3-alkyl BTD compounds were obtained by alkylation in basic medium of the monobenzyl derivatives **1** and **2** previously prepared.[19] The 4-chlorophenylmethyl and 3,4-dichlorophenylmethyl substituents attached to the *N*1 of BTD heterocycle were chosen because of their good anti-HCMV activity previously shown in other derivatives.[16,17] Thus, treatment of BTDs **1** and **2** with the corresponding alkyl halides or alkyl sulphates in the presence of sodium hydride and dimethylformamide as solvent, afforded mixtures of *N,N*- and *N,O*-disubstituted compounds **3–21** (Scheme 1). In general, the overall reaction yields were lower than those previously described for the benzylated compounds.[17–19]

Until now, with all the different benzyl halides previously used as alkylating agents,[17–19] the major product obtained was the *N,N*-disubstituted derivative and only traces of the *N,O*-derivative were isolated. Here, when alkyl halides or alkyl sulphates were used as reagents, the *N*/*O* alkylation ratio decreased with increasing hardness of the alkylating agent. Thus, an equimolecular mixture of both possible disubstituted compounds with a slight prevalence of the *N,O*-derivative were obtained in almost all cases assayed. Circular thin-layer chromatography was employed in the isolation of all these disubstituted compounds.

Different linkers between the BTD ring and the phenyl group attached to its *N*1 position were introduced on the *N*3-benzyl derivative **22**.[18] This starting material was unequivocally prepared in an efficient two-step synthesis following the Cohen and Klarberg procedure and could avoid the mixtures of final compounds.[20] Alkylation of compound **22** in basic medium (DMF/NaH) with the

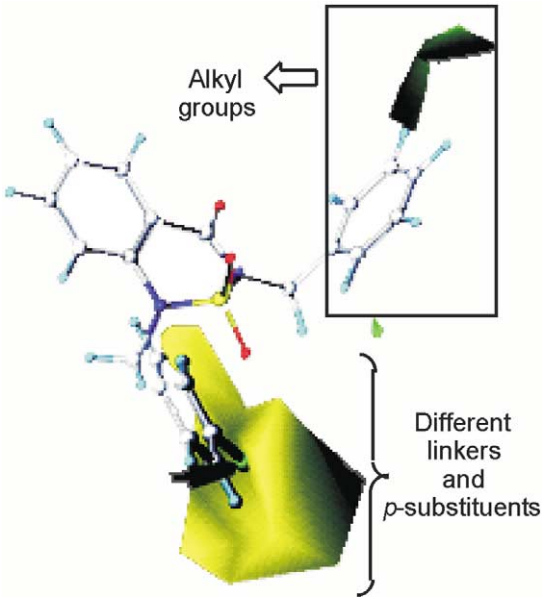
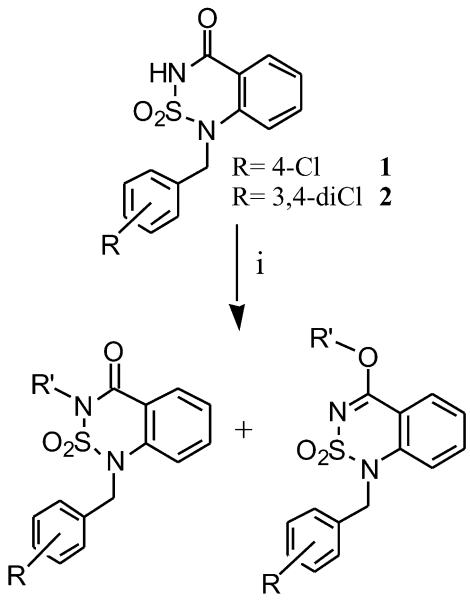


Figure 1. View of CoMFA steric contour plot.[18] Regions where increased steric bulk is associated with enhanced activity are indicated in yellow, while regions where increase in bulk is associated with diminishing activity are indicated in green. Structural modifications proposed are marked.



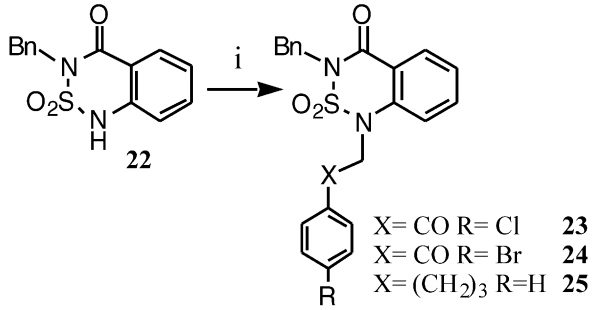
3	R= 4-Cl R'= Me	**4**
5	R= 4-Cl R'= Et	**6**
7	R= 4-Cl R'= Pr	**8**
9	R= 4-Cl R'= iPr	**10**
11	R= 4-Cl R'= iBu	**12**
13	R= 4-Cl R'= Chexmethyl	**14**
15	R= 4-Cl R'= Propargyl	-
16	R= 3,4-diCl R'= Et	**17**
-	R= 3,4-diCl R'= iPr	**18**
19	R= 3,4-diCl R'= iBu	**20**
-	R= 3,4-diCl R'=Chexmethyl	**21**

appropriate reagent afforded, in good yields, exclusively the *N,N*-disubstituted BTD derivatives **23–25** (Scheme 2).

The structure of all new compounds was elucidated from their analytical and spectroscopic data (^{1}H and ^{13}C NMR) which are collected in Table 1 for compound **3–22** and in the Experimental for compound **23–25**. 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 alkylation was determined from the chemical shifts of alkylic or 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*-susbtitution.

Biological Results and SAR

The new *N,N*- and *N,O*-disubstituted BTD derivatives **3–21** and **23–25** here described were evaluated for their activity against the laboratory strain of HCMV AD-169. Antiviral activity was determined by plaque reduction assay in confluent human embryonic lung MRC-5 fibroblasts. Cytotoxicity measurements were based on the inhibition of cell growth. Results are gathered in Table 2. For comparative purposes, some IC_{50} values of BTD derivatives (**26–31**) previously synthesised have also been also included.[18]

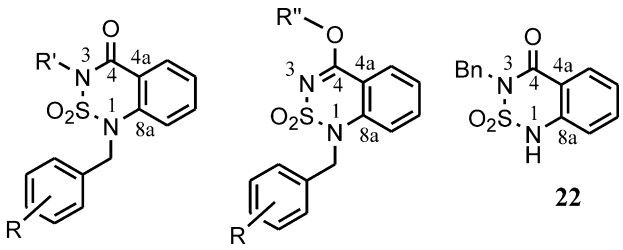


Scheme 2. Reagents: (i) NaH/DMF/R-C_6H_4-X-CH_2-Br/Δ.

Regarding the BTD derivatives **3–21**, we can observe that most of the compounds exhibit antiviral activity against HCMV, showing some of them IC_{50} values similar to those of the standard reference ganciclovir (Table 2). These data might validate the conclusions of our previous 3-D-QSAR study, which point to a decrease of the steric molecular volume in the substituent attached to *N*3 of BTD heterocycle for enhancing the antiviral inhibition. However, the introduction of an alkyl moiety in this position of the BTD heterocycle led to compounds toxic for the host cells. The high values of cytotoxicity found here prevent this new series of BTD derivatives from their potential therapeutic application.

Concerning BTD derivatives with the modified linker attached to *N*1 position, we have obtained non-nucleoside anti-HCMV inhibitors (Table 2). The activity against HCMV is shown at concentrations that were 10-fold lower than the concentration that was toxic for the host cells, which suggest that these derivatives show a specific antiviral effect against HCMV. Indeed, compounds **23–25** are the most actives ones obtained at the moment. Interesting SAR conclusions could be drawn from this set. First of all, the carbonylmethyl linker is well tolerated by the viral receptor leading to active compounds (see **23–24** and **26–28** in Table 2). Moreover, the HCMV-inhibition increases with the steric bulk of the phenyl *para*-substituent. The observed antiviral potency (Br > Cl > Me > H) might point to a gorge in the viral receptor that could be occupied by the inhibitor in the active conformation. Thus it is in agreement with the previous CoMFA results in which an increase in the volume of the *p*-substituent of the benzyl moiety attached to *N*1 of BTD enhance the antiviral activity. The same behaviour is observed when a dimethylene spacer is introduced between the BTD scaffold and the phenyl ring (compounds **29** and **30**). When the linker is elongated to a three-methylene chain (derivative **31**), the activity against HCMV is lost. This might suggest that the occupation limit receptor is reached. However, the inhibitory activity found in the longer BTD **25** is apparently in contrast with this last conclusion.

Table 1. Some representative ^{1}H and ^{13}C NMR data (chemical shifts in $CDCl_3$ of BTD derivatives **3–22**



No.	R	R′	R″	C-4	C-4a	C-8a	*N*1-$\underline{C}H_2$	*N*3-$\underline{C}H$	*O*-$\underline{C}H$	*N*1-C$\underline{H}_2$	*N*3-C$\underline{H}$	*O*-C$\underline{H}$
3	4-Cl	Me		162.08	122.66	139.61	55.77	28.50		4.91	3.31	
4	4-Cl		Me	166.47	112.46	142.68	48.64		55.94	5.15		4.12
5	4-Cl	Et		161.66	122.76	139.69	55.58	38.64		4.91	3.94	
6	4-Cl		Et	165.89	112.59	142.67	48.59		65.36	5.13		4.53
7	4-Cl	Pr		161.91	122.73	139.70	55.47	45.02		4.91	3.82	
8	4-Cl		Pr	165.99	112.63	142.68	48.60		70.82	5.14		4.44
9	4-Cl	^{i}Pr		162.14	123.19	134.49	55.40	49.38		4.88	4.93	
10	4-Cl		^{i}Pr	165.25	112.93	142.80	48.71		73.44	5.14		5.46
11	4-Cl	^{i}Bu		162.27	122.73	139.81	55.29	50.61		4.90	3.68	
12	4-Cl		^{i}Bu	165.97	112.69	142.76	48.65		75.17	5.15		4.27
13	4-Cl	ChM		162.32	122.73	139.77	55.21	49.56		4.90	3.68	
14	4-Cl		ChM	166.04	112.72	142.73	48.64		74.26	5.14		4.28
15	4-Cl	Pgyl		161.52	122.87	139.81	56.12	31.79		4.89	4.59	
16	3,4-diCl	Et		161.57	122.63	139.58	54.95	38.72		4.87	3.95	
17	3,4-diCl		Et	165.89	112.65	142.54	48.09		65.47	5.11		4.56
18	3,4-diCl		^{i}Pr	165.26	112.92	142.58	48.16		73.61	5.11		5.46
19	3,4-diCl	^{i}Bu		162.18	122.60	139.73	54.59	50.66		4.83	3.64	
20	3,4-diCl		^{i}Bu	165.96	112.62	142.46	48.02		75.21	5.06		4.21
21	3,4-diCl		ChM	166.03	110.08	140.35	48.70		74.36	5.19		4.29
22[a]	—	Bn	—	161.97	117.81	138.10		44.61			5.05	

ChM, cyclohexylmethyl; Pgyl, propargyl.
[a]Ref 18.

Preliminary pharmacological studies were performed in BTD derivatives[17] showing that the anti-HCMV action may be targeted at an early stage of the viral replicative cycle, probably during the translation or transcription process of the viral DNA.

Additionally, to understand this biological response, a molecular fitting of some of the linkers used was performed. Thus, Figure 2a depicts the molecular fitting of the benzyl moiety together with the phenylcarbonylmethyl and 4-methylphenylcarbonylmethyl ones, showing the space direction in which an increase in the volume is tolerated by the viral receptor. In Figure 2b, the phenylpropyl moiety is added. The different disposition of the aromatic groups might lead to the reduction in antiviral activity probably by steric interactions with the viral receptor. Finally, in Figure 2c the phenylbutyl fragment is added to the fitting. As can be seen, an increase of the steric volume in the favoured region is shown, which could explain the antiviral inhibition

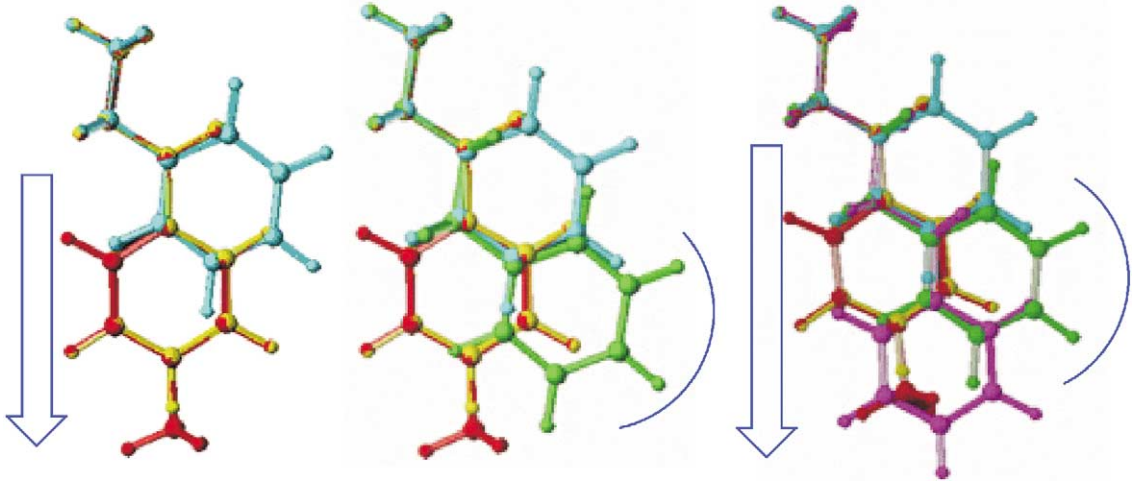
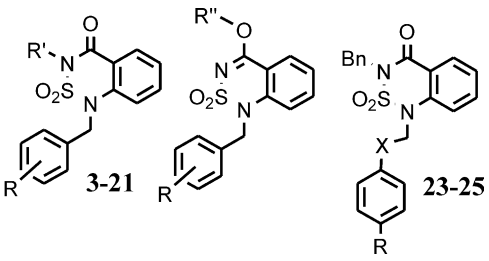


Figure 2. Molecular fitting of some BTD linkers: benzyl (cyan), phenylcarbonylmethyl (yellow), 4-methylphenylcarbonylmethyl (red), phenylpropyl (green), phenylbuthyl (magenta).

Table 2. Anti-HCMV (strain AD-169) activity of BTD derivatives



Compd	R	R′	R″	X	IC_{50} (μM)[a]	CC_{50} (μM)[b]
3	4-Cl	Me			14.8	2.9
4	4-Cl		Me		14.8	7.4
5	4-Cl	Et			2.8	7.1
6	4-Cl		Et		2.8	7.1
7	4-Cl	Pr			6.8	13.7
8	4-Cl		Pr		2.7	6.8
9	4-Cl	^{i}Pr			13.7	6.8
10	4-Cl		^{i}Pr		6.8	2.7
11	4-Cl	^{i}Bu			18.4	65.9
12	4-Cl		^{i}Bu		5.2	65.9
13	4-Cl	ChM			5.9	11.9
14	4-Cl		ChM		5.9	2.3
15	4-Cl	Pgyl			6.9	13.8
16	3,4-diCl	Et			12.9	6.4
17	3,4-diCl		Et		2.5	6.4
18	3,4-diCl		^{i}Pr		6.2	2.5
19	3,4-diCl	^{i}Bu			14.5	60.4
20	3,4-diCl		^{i}Bu		9.6	60.4
21	3,4-diCl		ChM		11.0	5.5
23	4-Cl			CO	2.2	56.7
24	4-Br			CO	2	51.5
25	H			$(CH_2)_3$	2.3	59.4
26[c]	H			CO	24.6	61.5
27[c]	4-OMe			CO	3.2	57.2
28[c]	4-Me			CO	2.8	59.4
29[c]	H			CH_2	7.6	63.7
30[c]	4-OMe			CH_2	4.7	59.1
31[c]	H			$(CH_2)_2$	19.1	57.4
Ganciclovir					5.9	98

ChM, cyclohexylmethyl; Pgyl, propargyl.
[a]50% inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. Assays were performed in duplicate.
[b]50% cytotoxic concentration, or concentration required to reduce cell growth by 50%. Assays were performed in triplicate.
[c]Ref 18.

found in this compound. Moreover, this preliminary study provides new knowledge about the structural requirements of BTD showing certain positions that could be modified for enhanced the anti-HCMV action.

Conclusions

The two new series of BTD derivatives synthesised have allowed us to explore the steric requirement for their biological activity. Thus, *N*3-alkylBTD compounds have shown antiviral activity in the same order or lower than previously prepared compounds. However, the cytotoxicity values observed prevent this new series of BTD derivatives from its potential therapeutic application. On the other hand, concerning BTD derivatives with the modified linker attached to *N*1 position, we have obtained non-nucleoside anti-HCMV inhibitors showing specific antiviral effects. SAR conclusions derived from these last compounds have provided new knowledge about the structural requirements of BTD showing certain positions that could be modified for enhancing the anti-HCMV action.

Experimental

Chemical procedures

Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Flash column chromatography was carried out at medium pressure using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–240 mesh ASTM) and preparative centrifugal circular thin layer chromatography (CCTLC) on a circular plate coated with a 1-mm layer of Kieselgel 60 PF254, Merk, by using a Chromatotron® with the indicated solvent as eluent. Compounds were detected with UV light (254 nm). ^{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. ^{13}C NMR experiments were carried out on the Varian Gemini-200 spectrometer operating at 50 MHz. The acquisition 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 department at C.N.Q.O. (CSIC) and the results obtained were within ±0.4% of the theoretical values.

General procedure for the synthesis of 3-alkyl BTD derivatives

To an equimolecular suspension of sodium hydride in DMF (25 mL), was added the corresponding monosubstituted BTD and alkylant agent (1.5 mmol). The reaction mixture was stirred in the indicated conditions in each case. 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 sulphate and the solvent evaporated under reduced pressure. The residue, was chromatographed on thin layer chromatography using as eluent mixtures of solvents in the portions indicated.

1-[(4-Chlorophenyl)methyl]-3-methyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (3) and 1-[(4-chlorophenyl)methyl]-4-(methyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (4). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.07 g, 0.2 mmol), dimethyl sulfate (0.04 g, 0.3 mmol). Conditions: 100 °C, 24 h. Purification: hexane/AcOEt (10:1). From the first fraction was isolated derivative **3**: yield 0.03 g (36%) as a white solid; mp 105–107 °C. Anal. calcd for $C_{15}H_{13}N_2O_3SCl$: C, 53.49; H, 3.89; N, 8.32; S, 9.52; found C, 53.81; H, 3.69; N, 7.98; S, 9.20.

From the second fraction was isolated derivative **4**: yield 0.004 g (5%) as a white solid; mp 115–117 °C. Anal.

calcd for $C_{15}H_{13}N_2O_3SCl$: C, 53.49; H, 3.89; N, 8.32; S, 9.52; found: C, 53.69; H, 4.00; N, 8.27; S, 9.43.

1-[(4-Chlorophenyl)methyl]-3-ethyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (5) and 1-[(4-chlorophenyl)methyl]-4-(ethyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (6). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), diethyl sulfate (0.07 g, 0.5 mmol). Conditions: 60 °C, 48 h. Purification: CH_2Cl_2/hexane (1:1). From the first fraction was isolated derivative **5**: yield 0.01 g (13%) as a white solid; mp 110–112 °C. Anal. calcd for $C_{16}H_{15}N_2O_3SCl$: C, 54.78; H, 4.31; N, 7.99; S, 9.14; found: C, 54.51; H, 4.60; N, 7.69; S, 8.79.

From the second fraction was isolated derivative **6**: yield 0.02 g (15%) as a white solid; mp 135–137 °C. Anal. calcd for $C_{16}H_{15}N_2O_3SCl$: C, 54.78; H, 4.31; N, 7.99; S, 9.14; found: C,54.78; H, 4.60; N, 7.79; S, 8.85.

1-[(4-Chlorophenyl)methyl]-3-propyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (7) and 1-[(4-chlorophenyl)methyl]-4-(propyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (8). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), propyl iodide (0.08 g, 0.5 mmol). Conditions: 100 °C, 36 h. Purification: hexane/AcOEt (10:1). From the first fraction was isolated derivative **7**: yield 0.03 g (24%) as a white solid; mp 100–102 °C. Anal. calcd for $C_{17}H_{17}N_2O_3SCl$: C, 55.97; H, 4.70; N, 7.68; S, 8.79; found: C, 55.69; H, 4.84; N, 7.49; S, 8.68.

From the second fraction was isolated derivative **8**: yield 0.01 g (13%) as a white solid; mp 109–110 °C. Anal. calcd for $C_{17}H_{17}N_2O_3SCl$: C, 55.97; H, 4.70; N, 7.68; S, 8.79; found: C, 55.86; H, 4.86; N, 7.44; S, 8.57.

1-[(4-Chlorophenyl)methyl]-3-isopropyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (9) and 1-[(4-chlorophenyl)methyl]-4-(isopropyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (10). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), isopropyl iodide (0.08 g, 0.5 mmol). Conditions: 80 °C, 48 h. Purification: hexane/AcOEt (10:1). From the first fraction was isolated derivative **9**: yield 0.003 g (3%) as a syrup. Anal. calcd for $C_{17}H_{17}N_2O_3SCl$: C, 55.97; H, 4.70; N, 7.68; S, 8.79; found: C, 55.92; H, 4.97; N, 7.91; S, 8.77.

From the second fraction was isolated derivative **10**: yield 0.01 g (9%) as a syrup. Anal. calcd for $C_{17}H_{17}N_2O_3SCl$: C, 55.97; H, 4.70; N, 7.68; S, 8.79; found: C, 55.88; H, 4.50; N, 7.53; S, 8.99.

1-[(4-Chlorophenyl)methyl]-3-isobutyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (11) and 1-[(4-chlorophenyl)methyl]-4-(isobutyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (12). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.13 g, 0.4 mmol), isobutyl chloride (0.09 g, 0.6 mmol). Conditions: 100 °C, 12 h. Purification: CH_2Cl_2/hexane (2:1). From the first fraction was isolated derivative **11**: yield 0.01 g (8%) as a white solid; mp 55–56 °C. Anal. calcd for $C_{18}H_{19}N_2O_3SCl$: C, 57.06; H, 5.05; N, 7.39; S, 8.46; found: C, 56.85; H, 5.15; N, 7.25; S, 8.31.

From the second fraction was isolated derivative **12**: yield 0.03 g (21%) as a white solid; mp 54-55 °C. Anal. calcd for $C_{18}H_{19}N_2O_3SCl$: C, 57.06; H, 5.05; N, 7.39; S, 8.46; found: C, 56.78; H, 5.32; N, 7.31; S, 8.16.

1-[(4-Chlorophenyl)methyl]-3-cyclohexylmethyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (13) and 1-[(4-chlorophenyl)methyl]-4-(cyclohexylmethyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (14). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), cyclohexylmethyl bromide (0.08 g, 0.5 mmol). Conditions: 70 °C, 36 h. Purification: hexane/AcOEt (10:1). From the first fraction was isolated derivative **13**: yield 0.004 g (3%) as a syrup. Anal. calcd for $C_{21}H_{23}N_2O_3SCl$: C, 60.21; H, 5.53; N, 6.69; S, 7.65; found: C, 60.29; H, 5.81; N, 6.55; S, 7.58.

From the second fraction was isolated derivative **14**: yield 0.004 g (3%) as a white solid; mp 119–121 °C. Anal. calcd for $C_{21}H_{23}N_2O_3SCl$: C, 60.21; H, 5.53; N, 6.69; S, 7.65; found: C, 60.23; H, 5.82; N, 6.39; S, 7.49.

1-[(4-Chlorophenyl)methyl]-3-propargyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (15). Reagents: 1-[(4-chlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), propargyl bromide (0.05 g, 0.5 mmol). Conditions: 80 °C, 12 h. Purification: CH_2Cl_2/hexane (1:1). Yield 0.01 g (12%) as a white solid: mp 118–120 °C. Anal. calcd for $C_{17}H_{13}N_2O_3SCl$: C, 56.59; H, 3.63; N, 7.76; S, 8.89; found: C, 56.44; H, 3.90; N, 7.54; S, 8.72.

1-[(3,4-Dichlorophenyl)methyl]-3-ethyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (16) and 1-[(3,4-dichlorophenyl)-methyl]-4-(ethyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (17). Reagents: 1-[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), diethyl sulfate (0.06 g, 0.4 mmol). Conditions: 60 °C, 24 h. Purification: hexane/AcOEt (10:1). From the first fraction was isolated derivative **16**: yield 0.008 g (7%) as a syrup. Anal. calcd for $C_{16}H_{14}N_2O_3SCl_2$: C, 49.88; H, 3.66; N, 7.27; S, 8.32; found: C, 50.08; H, 3.41; N, 7.47; S, 8.26.

From the second fraction was isolated derivative **17**: yield 0.01 g (12%) as a syrup. Anal. calcd for $C_{16}H_{14}N_2O_3SCl_2$: C, 49.88; H, 3.66; N, 7.27; S, 8.32; found: C, 50.00, H, 3.57; N, 7.41; S, 8.20.

1-[(3,4-Dichlorophenyl)methyl]-4-(isopropyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (18). Reagents: 1-[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), isopropyl iodide (0.07 g, 0.5 mmol). Conditions: 80 °C, 48 h. Purification: hexane:AcOEt (10:1). Yield 0.003 g (6%) as a white solid: mp 98–100 °C. Anal. calcd for $C_{17}H_{16}N_2O_3SCl_2$: C, 51.14; H, 4.04; N, 7.02; S, 8.03; found: C, 51.00; H, 4.25; N, 7.07; S, 8.13.

1-[(3,4-Dichlorophenyl)methyl]-3-isobutyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (19) and 1-[(3,4-dichlorophenyl)methyl]-4-(isobutyloxy)-2,1,3-benzothiadiazine 2,2-

dioxide (20). Reagents: 1-[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), isobutyl chloride (0.06 g, 0.5 mmol). Conditions: 100 °C, 12 h. Purification: CH_2Cl_2/hexane (1:1). From the first fraction was isolated derivative **19**: yield 0.02 g (18%) as a syrup. Anal. calcd for $C_{18}H_{18}N_2O_3SCl_2$: C, 52.31; H, 4.39; N, 6.78; S, 7.76; found: C, 52.40; H, 4.28; N, 6.93; S, 7.65.

From the second fraction was isolated derivative **20**: yield 0.03 g (21%) as a white solid; mp 63–65 °C. Anal. calcd for $C_{18}H_{18}N_2O_3SCl_2$: C, 52.31; H, 4.39; N, 6.78; S, 7.76; found: C, 52.21; H, 4.53; N, 6.69; S, 7.52.

1-[(3,4-Dichlorophenyl)methyl]-4-(cyclohexylmethyloxy)-2,1,3-benzothiadiazine 2,2-dioxide (21). Reagents: 1-[(3,4-dichlorophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide[19] (0.10 g, 0.3 mmol), cyclohexylmethyl bromide (0.07 g, 0.5 mmol). Conditions: 70 °C, 48 h. Purification: hexane/AcOEt (10:1). Yield 0.002 g (2%) as a syrup. Anal. calcd for $C_{21}H_{22}N_2O_3SCl_2$: C, 55.63; H, 4.89; N, 6.18; S, 7.07; found: C, 55.43; H, 4.81; N, 6.36; S, 7.28.

1-[(4-Chlorophenyl)carbonylmethyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (23). To an equimolecular suspension of sodium hydride in DMF (25 mL) were added N_3-benzyl benzothiadiazine **22**[18] (0.10 g, 0.3 mmol) and 2-bromo-4′-chloroacetophenone (0.09 g, 0.4 mmol). The reaction mixture was refluxed for 12 h. 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 sulphate 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. Compound **23** was obtained (0.07 g, 46%) as a white solid: mp 122–124 °C; ^{1}H NMR ($CDCl_3$) δ 5.11 (s, 2H, N_3-CH_2), 5.22 (s, 2H, N_1-CH_2), 7.09 (d, 1H, $J_{H_7H_8}$ = 8.1, H-8), 8.18 (d, 1H, $J_{H_5H_6}$ = 7.9, H-5), 7.22–7.78 (m, 11H, Ar–H, H-6, H-7); ^{13}C NMR ($CDCl_3$) δ 46.92 (N_3-CH_2), 56.51 (N_1-CH_2), 120.18 (C-8), 122.60 (C-4a), 126.07 (C-6), 127.91, 128.50, 128.67, 129.32, 129.38, 132.12, 135.71, 140.91 (Ar–C), 130.65 (C-5), 134.74 (C-7), 139.59 (C-8a), 162.24 (C-4), 190.61 (Ph<u>C</u>O). Anal. ($C_{22}H_{17}N_2O_4SCl$) C, H, N, S. Anal. calcd for $C_{22}H_{17}N_2O_4SCl$: C, 59.93; H, 3.89; N, 6.35; S, 7.27; found: C, 60.21; H, 3.90; N, 6.07; S, 6.98.

1-[(4-Bromophenyl)carbonylmethyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (24). To an equimolecular suspension of sodium hydride in DMF (25 mL) were added N_3-benzyl benzothiadiazine **22**[18] (0.10 g, 0.3 mmol) and 2,4′-dibromoacetophenone (0.11 g, 0.4 mmol). The reaction mixture was refluxed for 12 h. 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 sulphate 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. Compound **24** was obtained (0.09 g, 54%) as a white solid: mp 140–142 °C; ^{1}H NMR ($CDCl_3$) δ 5.12 (s, 2H, N_3-CH_2), 5.22 (s, 2H, N_1-CH_2), 7.10 (d, 1H, $J_{H_7H_8}$ = 8.1, H-8), 8.18 (d, 1H, $J_{H_5H_6}$ = 7.9, H-5), 7.22–7.69 (m, 11H, Ar-H, H-6, H-7); ^{13}C NMR ($CDCl_3$) δ 46.91 (N_3-CH_2), 56.47 (N_1-CH_2), 120.17 (C-8), 122.57 (C-4a), 126.05 (C-6), 127.90, 128.50, 128.65, 129.42, 129.67, 132.30, 132.50, 135.71 (Ar–C), 130.63 (C-5), 134.74 (C-7), 139.57 (C-8a), 162.23 (C-4), 190.85 (Ph<u>C</u>O). Anal. ($C_{22}H_{17}N_2O_4SBr$) C, H, N, S. Anal. calcd for $C_{22}H_{17}N_2O_4SBr$: C, 54.44; H, 3.53; N, 5.77; S, 6.61; found: C, 54.42; H, 3.58; H, 5.88; S, 6.21.

1-(Phenylbuthyl)-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide (25). To an equimolecular suspension of sodium hydride in DMF (25 mL) were added N_3-benzyl benzothiadiazine **22**[18] (0.10 g, 0.3 mmol) and phenylbuthyl chloride (0.07 g, 0.4 mmol). The reaction mixture was refluxed for 12 h. 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 sulphate 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. Compound **25** was obtained (0.01 g, 7%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.53 (q, J = 7.9 Hz, J = 6.8 Hz, $PhCH_2C\underline{H}_2C\underline{H}_2$), 2.47 (t, J = 7.9 Hz, $PhC\underline{H}_2CH_2CH_2$), 3.74 (t, 2H, N_1-CH_2), 5.07 (s, 2H, N_3-CH_2), 7.20 (dd, 1H, $J_{H_7H_8}$ = 7.8, $J_{H_6H_8}$ = 0.7, H-8), 8.16 (dd, 1H, $J_{H_5H_6}$ = 7.9, $J_{H_5H_7}$ = 1.5, H-5), 7.58 (t, 1H, $J_{H_7H_6}$ = 7.6, H-7), 7.37 (t, 1H, H-6), 7.06–7.37 (m, 10H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 27.13, 28.15. ($PhCH_2\underline{C}H_2\underline{C}H_2$), 35.12 ($PhCH_2\underline{C}H_2\underline{C}H_2$), 46.40($N_3$-$CH_2$), 52.37($N_1$-$CH_2$), 122.65 (C-4a), 124.54 (C-8), 126.18 (C-6), 125.98, 128.05, 128.32, 128.41, 128.54, 129.03, 135.88, 141.50 (Ar–C), 130.64 (C-5), 134.74 (C-7), 140.13 (C-8a), 162.27 (C-4). Anal. ($C_{24}H_{24}N_2O_3S$) C, H, N, S. Anal. calcd for $C_{24}H_{24}N_2O_3S$: C, 68.55; H, 5.75; N, 6.66; S, 7.62; found: C, 68.61; H, 5.60; N, 6.75; S, 7.40.

Antiviral evaluation

Cells. Human embryonic lung MRC-5 fibroblasts were propagated in Hepes modified medium 199 supplemented with 10% inactivated foetal calf serum and 1% l-glutamine.

Viruses. AD-169 strain of human cytomegalovirus was used. Virus stocks consisted of cell-free virus obtained from the supernatant of infected cell cultures that have been sonicated and clarified by low speed centrifugation. The virus stocks were stored at −80 °C.

Antiviral assays. Confluent MRC-5 cells grown in 24-well plates were infected with the AD-169 strain at 50 (CMV) plaque forming units (PFU/well). After a 1.5-h incubation period, residual virus was removed and the infected cells were further incubated with Hepes modified medium 199 supplemented with 2% inactivated FCS and 1% l-glutamine containing serial dilutions of the test compounds (in duplicate). After 8 days of incubation at 37 °C in 5% CO_2 atmosphere, the cells were stained with 0.2% crystal violet in ethanol/water

(20:80). PFU (virus input: 50 PFU/well) was monitored microscopically. The antiviral activity is expressed as IC_{50} that represents the compound concentration required to reduce virus plaque formation by 50%. IC_{50}'s were estimated from graphic plots of the number of plaques (percentage of control) as a function of the concentration of the test compounds.

Cytotoxicity assays. Cytotoxicity measurements were based on the inhibition of MRC-5 cell growth. MRC-5 fibroblasts were seeded at a rate of 5×10^3 cells/well microtitre plates and were allowed to prolifarate 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%.

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