Synthesis and Antiviral Activity of New Benzothiadiazine Dioxide Derivatives

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A series of 2,1,3- and 1,2,4-benzothiadiazine derivatives were synthesized by alkylation *via* Mitsunobu reaction and evaluated for their antiviral activity against ADV, HHV-6, Cox-B5 and H-CMV. Most of them were active at micromolar level against one or more viral strains. All the molecules studied are poorly cytotoxic (maximum non toxic concentrations were >25μM), except one compound that presents a higher cytotoxicity (maximum non toxic concentration was 6 μM).

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Introduction.

During the last past decade, a greater understanding of viral life cycles has resulted in the discovery and validation of several targets for therapeutic intervention, and an increase in the number of licensed antiviral drugs from 5 in 1990 to over 30 in 2004. However, it is necessary to continue the studies in this field, as these compounds are not always efficacious (owing to virus resistance) or well tolerated [1].

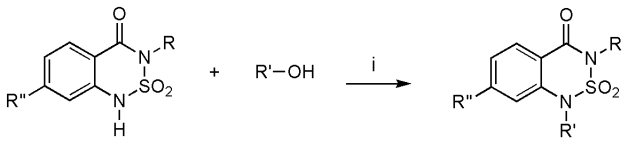
Martinez and co-worker have reported interesting antiviral properties for 2,1,3-benzo and imidazothiadiazine derivatives particularly against Human Cytomegalovirus and Varicella Zoster Virus infections [2-8]. Also the 1,2,4-benzothiadiazine and the 1,1,3-trioxo-2*H*, 4*H*thieno[3,4-*e*][1,2,4]thiadiazine rings were investigated as antiviral systems and found to effectively inhibit the replication of a variety of HIV-1 strains at the reverse transcription steps, including strains that are resistant to AZT but not against HIV-2 (ROD) [9-10]. The benzothiadiazine derivatives (BTDs) were described also as heterocyclic inhibitors of PDE7 with concurrent inhibitory activity at PDE4 and PDE3 [11-12].

As a part of an ongoing search in the field of benzothiadiazine molecules [13-16], we reported here the synthesis and the antiviral activities against Adenovirus (ADV), Human-Herpesvirus 6 (HHV-6), Coxsackievirus B5 (Cox-B5) and Human Cytomegalovirus (H-CMV) of new N-substituted 2,1,3- and 1,2,4-benzothiadiazine derivatives. The tested viruses widely diffused in the population, are etiological agents of important diseases and hardly tractable with the drugs actually available, especially in immuno-compromised subjects such as HIV and transplant patients. Moreover, in the absence of effective vaccines to control the mentioned viral infections new active compounds are greatly desired. It is worth mentioning that some of these compounds are substituted with the 2,6-di-*tert*-butylphenol group endowed with antioxidant and anti-inflammatory properties and were therefore tested as free radical scavengers by reaction with 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazil (DPPH) using UV spectrometry.

Chemistry.

The N-1-,N-3-disubstituted-2,1,3- (**1-9**) and the N-2-monosubstituted 1,2,4-benzothiadiazine (**10**) were obtained by alkylation *via* Mitsunobu reaction [17] with the appropriate alcohol from the *N*-3-aralkyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-ones 2,2-dioxide (**11-15**) (Scheme 1) and the 6-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (**16**) (Scheme 2). The site of alkylation of **16** was determined by means of NOESY experiments: thus aromatic H-5 shows NOE effect with N-H of **10** but not with CH_2 of 4-OH-benzyl moiety.

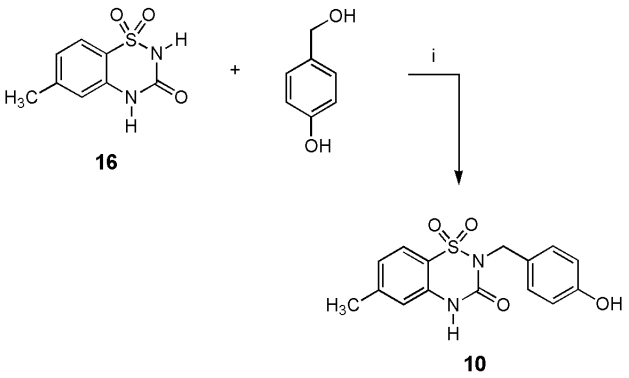
Scheme 1



11 - 15 **1 - 9**

(i) TPP, DIAD, THF, N_2, r.t., overnight.

Scheme 2

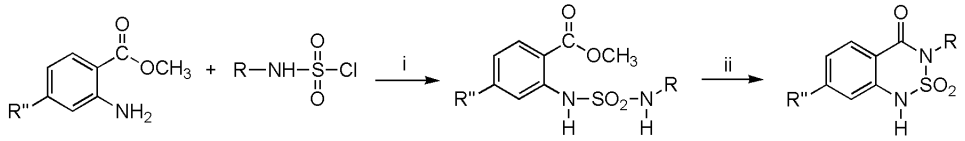


16 **10**

(i) TPP, DIAD, THF, N_2, r.t., overnight.

The N-3-monosubstituted 2,1,3-benzothiadiazines (**11-15**) were obtained following the Cohen and Klarberg procedure [18] starting from the appropriate methyl 2-aminobenzoate and sulfamoyl chloride. The use of sodium methoxide instead of 6 *N* NaOH for the cyclization of the intermediate *N*-sulfamoylaminobenzoates improves yields (Scheme 3).

Scheme 3



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(i) Toluene, Et_3N, 80°C, 1 hour; (ii) $NaOCH_3$, CH_3OH, 40 °C, 2 hours

The structures of new compounds were elucidated from their analytical and spectroscopic data (^1H and ^{13}C nmr). In the case of compound **1** unequivocal assignment of all chemical shifts was accomplished using bi-dimensional experiments such as COSY and Heteronuclear Multiple Quantum Coherence (HMQC) for one bond correlation and Heteronuclear Multiple Bond Correlation (HMBC) for long distance proton/carbon correlations. The nmr data are in agreement with values reported by A. Castro *et al.* [19] for closely related benzothiadiazine dioxide derivatives.

Radical Scavenging Effect on DPPH Radical.

UV measurement of free radical scavenging activity (S.A.%) for $1x10^{-4}$ *M* solutions, after storage for 6 hours at 37 °C show that compounds **4**, **6** and **7** scavenge the DPPH radical. The radical scavenging activity of each compound was expressed by the ratio of lowering of the absorption of DPPH relative to the absorption of DPPH solution in the absence of compound (control) (Table 1).

Table 1

Free Radical Scavenging Activity

Compd.	ΔA [a]	S.A.% [b]
4	0.1306 ± 0.0242	27.77
6	0.2012 ± 0.0122	42.78
7	0.2015 ± 0.0164	42.84

[a] Absorbance decrease at 514 nm against control for 1.0×10^{-4}M solutions after 6 hours; [b] Scavenging activity % calculated according to the equation $S.A.\% = 100 \times \Delta A/A_t$ where A_t is the control absorbance.

It is noteworthy that N-3 and also bz-substitution on heterocyclic system play a significant role on the parameter under investigation. *N*-3-(2-Chlorobenzyl)substitution (compound **4**) reduces scavenging activity, while 7-chloro-substitution (compound **7**) improves it.

Biological Results.

The antiviral activity of the synthesized compounds was evaluated on 5 different viruses, including DNA and RNA viruses: Adenovirus (ADV), Herpes simplex virus 1 (HSV-1), Human Cytomegalovirus (H-CMV), Human Herpesvirus 6 (HHV-6), and Coxsackievirus B5 (Cox-B5) (Table 2). Cytotoxicity measurements, based on the inhibition of cell growth, reveal all compounds to be non-toxic until 25 μM concentration except **1** that presents a cytotoxic activity also at 12 μM and was tested at 6 μM.

No antiviral activity was observed against Herpes simplex virus 1 (HSV-1). Compounds **3** and **5** show a wider activity spectrum involving three viral strains; moreover they are able to abolish almost completely the viral growth of HHV-6.

Introduction of the pyridyl group into the 1-position of the 2,1,3-BTD heterocycle led to active compounds irrespectively of insertion point of this group (4 for compound **3** and 2 for compound **8**) and elongation of the link between the BTD heterocycle and the pyridyl group. On the contrary, the furylmethyl (compound **1**) and the *N*-methylphtalimide group (compounds **2** and **9**) lead to a total loss of antiviral activity.

The bulky antioxidant 2,6-di*tert*-butylphenol moiety on N-1 is compatible with antiviral activity against ADV (compounds **4** and **7**) and Cox-B5 (compound **7**); however the *N*-3-para-substitution (compound **6**) resulted in loss of the antiviral activity which might reflect a spatial restriction in the target site. The 7-chloro substitution on the benzene ring in the BTD scaffold improves activity against Cox-B5 viral strain (compare compound **7** with compound **4**).

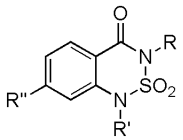
The middle anti-ADV activity of the 1,2,4-BTD derivative **10**, *N*-2-monosubstituted with a 4-hydroxybenzyl group, suggests that, in the case of this heterocyclic system and differently to 2,1,3-BTD ring, N,N double substitution is not indispensable for antiviral action.

The mechanism of action of these compounds and their viral target remain unknown; it is interesting to point out that compounds **3**, **5**, **7** and **8** are able to inhibit DNA and RNA viruses and this is in agreement with the hypothesis that they could interact to a common viral target.

In the case of 2,1,3-BTD derivatives, it could be important to have the ability to assume a butterfly like conformation, a structural characteristic of widely studied antiviral drugs.

Table 2

In vitro antiviral activity against ADV, HHV-6, Cox-B5 and H-CMV



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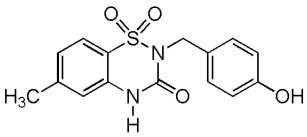

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Comp.	R	R'	R''	NCC μM [b]	TCID [c]		PFU [d]	% of IFA [e] positive cells
					ADV	CoxB5	H-CMV	HHV-6
C- [a]					$1.2x10^3$	$1.7x10^6$	45	56,8
1	benzyl	2-furylmethyl	H	6	$9x10^2$	$1.2x10^6$	48	59,0
2	benzyl	N-methylphtalimide	H	25	$1x10^3$	$1.5x10^6$	46	51,2
3	benzyl	4-pyridylmethyl	H	25	$1.1x10^3$	$1.2x10^5$	17	1.1
4	2-Cl-benzyl	3,5-di*tert*-butyl-4-OH-benzyl	H	25	$1.9x10^2$	$1.9x10^6$	43	50,8
5	2-Cl-benzyl	4-NO_2-benzyl	H	25	$2.2x10^2$	$7.3x10^5$	41	1.9
6	4-Cl-benzyl	3,5-di*tert*-butyl-4-OH-benzyl	H	25	$1.3x10^3$	$1.5x10^6$	48	61.2
7	2-Cl-benzyl	3,5-di*tert*-butyl-4-OH-benzyl	7-Cl	25	$4.8x10^2$	$1.4x10^5$	45	58.3
8	2-phenylethyl	2-pyridylethyl	H	25	$1.1x10^2$	$1.9x10^5$	44	66.5
9	2-phenylethyl	N-methylphtalimide	H	25	$1x10^3$	$1.6x10^6$	47	59.2
10	-	-	-	25	$1.3x10^2$	$1.4x10^6$	40	49.7

[a] negative control; [b] non cytotoxic concentrations; [c] Total cells infectious $dose_{50}$/mL: it is the virus dilution able, in theory, to infect the 50% cell cultures obtained by extrapolation from different values in a 96 well cell culture plate by means of the Reed and Muench formula which provides the exact exponent of the 10 fold dilution; [d] Plaque Forming Unit/mL; [e] Immunofluorescence Assay.

EXPERIMENTAL

Melting points determined in open capillary tubes (Büchi 510 capillary apparatus) were uncorrected. Both ^1H and ^{13}C nmr spectra were recorded with a Brucker DPX 200 spectrometer; chemical shifts (δ) are reported as ppm downfield from tetramethylsilane as external standard, and the ^{13}C chemical shifts were referenced to the solvent peaks: dimethylsulfoxide-d_6 (39.6 ppm). Coupling constants (J) are in Hertz (Hz), multiplicities are abbreviated as follows: s, singlet; d, doublet; t, triplet; dd, double doublet; ddd, double double doublet; dt, double triplet; m, a more complex multiplet or overlapping multiplets; b, indicates a broadening of the signal. Ir spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer as Nujol mull. The spectra were in agreement with the proposed structures. The purity of compounds was checked by thin layer chromatography performed on aluminium sheets silica gel 60 F_{254}, 0.2 mm thick. Flash chromatography was performed on Silica gel Merck (230-400 mesh). UV spectra were acquired on a Cary 50 Bio UV-VISIBLE Spectrophotometer Varian. Elemental analyses for C, H and N were performed in Microanalysis Laboratory of Dipartimento di Scienze Farmaceutiche of Modena University on a Carlo Erba Elemental Analyzer 1106 apparatus. Reagents and solvents were purchased from common commercial suppliers and used as received.

3-(2-chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**12**).

General Procedure.

To a stirred and cooled (0 °C) solution of 2-chlorobenzylamine (5.00 g, 35 mmol) in dichloromethane (50 mL), chlorosulfonic acid (1.36 g, 12 mmol) was added drop by drop and the reaction mixture was stirred for an additional hour after being warmed to room temperature gradually. The resultant white salt between 2-chlorobenzylsulfamic acid and 2-chlorobenzylamine was collected, dissolved in toluene (50 mL), and treated with phosphorus pentachloride (3.00 g, 14.4 mmol). The mixture was refluxed for 1 hour and the inorganic by-products removed by filtration. The filtrate was evaporated *in vacuo* to give the *N*-2-chlorobenzylsulfamoyl chloride as an oily residue that was used in the next synthetic step without further purification.

To a solution of methyl 2-aminobenzoate (1.81 g, 12.5 mmol) and triethylamine (1.92 g, 19 mmol) in toluene (50 mL) was slowly added a solution of *N*-2-chlorobenzylsulfamoyl chloride (2.89 g, 12 mmol) in toluene (10 mL) and the resulting mixture was heated at 80 °C for 1 hour. The resultant triethylamine hydrochloride was filtered and the filtrate was evaporated *in vacuo* to give an oily residue. The residue was dissolved in 50 mL of a freshly prepared 0.5 *M* sodium methoxide methanolic solution. After stirring for 2

hours at 40 °C the solvent was evaporated under reduced pressure and the residue dissolved in water. After cooling the aqueous solution gave compound **12** on acidification with hydrochloric acid as a white solid (2.55 g, 66%); mp 142-144 °C (DMF/water); ^{1}H nmr (dimethylsulfoxide-d_6): δ 10.00 (bs, 1H, NH deuterium oxide exchangeable), 7.97 (dd, 1H, J=1.5, 7.8 Hz, H-5), 7.65 (dt, 1H, J=1.5, 7.6 Hz, H-7), 7.47 (m, 1H, aromatic), 7.28 (m, 4H, aromatic), 7.13 (dd, 1H, J=1.0, 7.6 Hz, H-8), 5.08 (s, 2H, CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.5 (C), 138.7 (C), 136.1 (CH), 133.6 (C), 132.0 (C), 130.1 (CH), 129.8 (CH), 129.5 (CH), 128.4 (CH), 127.8 (CH), 125.0 (CH), 120.7 (CH), 118.3 (C), 42.8 (CH_2); (ir (nujol) 3172, 1640, 1361, 1175, 1049, 750 cm^{-1}.

Anal. Calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10%; H, 3.44%; N, 8.68%. Found: C, 52.18%; H, 3.58%; N, 8.94%.

The already known 3-benzyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**11**) [20] and compounds **13**-**15** were prepared using the procedure described above. The physical and spectral data for **13**-**15** are listed below.

3-(4-Chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**13**).

This compound was obtained starting from methyl 2-aminobenzoate by reaction with 4-chlorobenzylsulfamoyl chloride as a white solid in 26% yield; mp 167-170 °C (methanol/water); ^{1}H nmr (dimethylsulfoxide-d_6): δ 10.30 (bs, 1H, NH deuterium oxide exchangeable), 7.98 (ddd, 1H, J=0.4, 1.6, 7.9 Hz, H-5), 7.65 (dt, 1H, J=1.6, 7.4 Hz, H-7), 7.39 (m, 4H, aromatic), 7.26 (dt, 1H, J=1.1, 7.4 Hz, H-6), 7.14 (1H, ddd, J= 0.4, 1.1, 7.4 Hz, H-8), 5.00 (2H, s, CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.4 (C), 138.4 (C), 136.0 (CH), 135.8 (C), 132.7 (C), 130.2 (2xCH), 130.0 (CH), 128.9 (2xCH), 125.1 (CH), 120.5 (CH), 118.3 (C), 44.4 (CH_2); (ir (nujol) 3165, 1655, 1610, 1329, 1175, 1017, 756 cm^{-1}.

Anal. Calcd for $C_{14}H_{11}ClN_2O_3S$: C, 52.10%; H, 3.44%; N, 8.68%. Found: C, 52.39%; H, 3.15%; N, 8.96%.

7-Chloro-3-(2-chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**14**).

This compound was obtained starting from methyl 2-amino-4-chlorobenzoate by reaction with 2-chlorobenzylsulfamoyl chloride as a white solid in 20% yield; mp 128-130 °C (methanol/water); ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.98 (bs, 1H, NH deuterium oxide exchangeable), 7.86 (dd, 1H, J=1.2, 7.8 Hz, aromatic), 7.43 (m, 1H, aromatic), 7.26 (m, 3H, aromatic), 7.03 (dd, 1H, J=1.9, 7.8 Hz, aromatic), 7.00 (m, 1H, aromatic), 5.03 (s, 2H, CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.2 (C), 142.5 (C), 139.9 (C), 133.9 (C), 131.9 (C), 131.6 (CH), 129.7 (CH), 129.4 (CH), 128.5 (CH), 127.8 (CH), 123.4 (CH), 120.3 (CH), 116.3 (C), 42.8 (CH_2); ir (nujol) 3117, 1666, 1602, 1325, 1161, 752 cm^{-1}.

Anal. Calcd. for $C_{14}H_{10}Cl_2N_2O_3S$: C, 47.07%; H, 2.82%; N, 7.84%. Found: C, 47.36%; N, 8.11%.

3-(2-Phenylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**15**).

This compound was obtained starting from methyl 2-aminobenzoate by reaction with 2-phenylethylsulfamoyl chloride as a white solid in 76% yield; mp138-140 °C (acetone/petroleum ether 40-60 °C); ^{1}H nmr (dimethylsulfoxide-d_6): δ 10.80 (bs, 1H, NH deuterium oxide exchangeable), 7.99 (dd, 1H, J=1.6, 7.6 Hz, H-5), 7.67 (dt, 1H, J=1.6, 7.6 Hz, H-6/7), 7.28 (m, 6H, aromatic H), 7.17 (dd, 1H, J=0.7, 8.1 Hz, H-8), 4.04 (m, 2H, NCH_2) 2.97 (m, 2H, CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.3 (C), 138.7 (C), 138.4 (C), 135.8 (CH), 129.8 (CH), 129.2 (2xCH), 129.0 (2xCH), 127.0 (CH), 124.6 (CH), 120.2 (CH), 118.1 (C), 43.1 (CH_2), 35.0 (CH_2); ir (nujol) 3127, 1638, 1606, 1176 cm^{-1}.

Anal. Calcd. for $C_{15}H_{14}N_2O_3S$: C, 59.59%; H, 4.67%; N, 9.27%. Found: C, 58.86%; H, 4.80%; N, 9.21%.

3-Benzyl-1-(2-furylmethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**1**).

General Procedure.

All of these reactions were carried out under a nitrogen atmosphere. To a stirred and cooled (0 °C) solution of 3-benzyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**11**) (1.00 g, 3.2 mmol), 2-furylmethanol (0.21 g, 2.1 mmol) and triphenylphosphine (1.10 g, 4.2 mmol) in dry THF (20 mL), diisopropylazodicarboxylate (0.85 g, 4.2 mmol) was added dropwise in five minutes and the reaction mixture was allowed to stand at that temperature for an additional 30 minutes. The orange-red colour of diisopropylazodicarboxylate disappears immediately. After being gradually warmed to room temperature, the mixture was stirred overnight. Removal of the solvent *in vacuo* gave a residue, which was washed with petroleum ether 40-60 °C, then purified by silica gel flash column chromatography using cyclohexane/ethyl acetate (6:4, v/v) as eluent to afford **1** (0.38 g, yield 49%); mp 115°C (acetone/petroleum ether 40-60 °C); ^{1}H nmr (dimethylsulfoxide-d_6): δ 7.99 (dd, 1H, J=1.6, 7.9Hz, H-5), 7.81 (ddd, 1H, J=1.6, 7.4, 8.0 Hz, H-7), 7.65 (dd, 1H, J=1.2, 8.0 Hz, H-8), 7.50 (dd, 1H, J=0.9, 1.9 Hz, H-5 furyl), 7.49 (ddd, 1H, J=1.2, 7.4, 7.9 Hz, H-6), 7.39-7.25 (m, 5H, benzyl), 6.30 (dd, 1H, J=1.9, 3.2Hz, H-4 furyl), 6.17 (dd, 1H, J=0.9, 3.2 Hz, H-3 furyl), 5.08 (s, 2H, CH_2 furyl), 4.95 (s, 2H, CH_2 benzyl); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.0 (C-4), 148.0 (C-2' furyl), 144.3 (C-5' furyl), 140.0 (C-8a), 136.4 (C-1' benzyl), 135.8 (C-7), 129.3 (C-5), 129.0 (C-3' and C-5' benzyl), 128.4 (C-2' and C-6' benzyl), 128.2 (C-4' benzyl), 127.6 (C-6), 124.2 (C-8), 123.0 (C-4a), 111.2 (C-3' furyl), 111.1 (C-4' furyl), 49.1 (CH_2 benzyl), 46.8 (CH_2 furyl); ir (nujol): 1673, 1379, 1180, 1010, 603 cm^{-1}.

Anal. Calcd. for $C_{19}H_{16}N_2O_4S$: C, 61.94%; H, 4.38%; N, 7.60%. Found: C, 62.18%; H, 4.15%; N, 7.81%.

The compounds **2-10** were prepared using the procedure described above. Their physical and spectral data are listed below.

2-[(3-Benzyl-2,2-dioxido-4-oxo-3,4-dihydro-1*H*-2,1,3-benzothiadiazin-1-yl)methyl]-1*H*-isoindole-1,3(2*H*)dione (**2**).

This compound was obtained starting from 3-benzyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**11**) by reaction with 2-(hydroxymethyl)-1*H*-isoindole-1,3(2*H*)-dione. Flash column chromatography: eluent chloroform/acetone (9.5:0.5, v/v). Yield 82%; mp 148-150 °C (acetone/petrol ether 40-60 °C); ^{1}H nmr (dimethylsulfoxide-d_6): δ 7.96 (m, 1H, aromatic), 7.84 (m, 5H, aromatic), 7.79 (dd, 1H, J=1.5, 8.2 Hz, aromatic), 7.51 (ddd, 1H, J=2.2,6.3, 6.5 Hz, aromatic), 7.29 (m, 5H, aromatic H), 5.64 (s, 2H, NCH_2N), 5.00 (s, 2H, NCH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 167.1 (2xC), 162.1 (C), 138.5 (C), 136.3 (C), 135.7 (CH), 135.5 (2xCH), 131.3 (2xC), 129.8 (CH), 128.9 (2xCH), 128.3 (2xCH), 128.2 (CH), 128.1 (CH), 125.3 (CH), 124.1 (2xCH), 123.6 (C), 54.2 (CH_2), 47.0 (CH_2); ir (nujol): 1782, 1737, 1688, 1025, 891cm^{-1}.

Anal. Calcd. for $C_{23}H_{17}N_3O_5S$: C, 61.74%; H, 3.83%; N, 9.39%. Found: C, 62.14%; H, 3.52%; N, 9.58%.

3-Benzyl-1-(4-pyridiylmethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**3**).

This compound was obtained starting from 3-benzyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (11) by reaction with 4-(hydroxymethyl)pyridine. Flash column chromatography: eluent chloroform/acetone (8:2, v/v). Yield 69%; mp 100 °C (acetone/petroleum ether 40-60 °C); ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.46 (bd, 2H, J=6.0 Hz, pyridine H-2 and H-6), 8.06 (dd, 1H, J=1.6, 7.8 Hz, H-5), 7.78 (ddd, 1H, J=1.6, 7.5, 8.0 Hz, H-7), 7.43 (m, 7H, aromatic), 7.15 (bd, 2H, J=6.0 Hz, pyridine H-3 and H-5), 5.18 (s, 2H, CH_2Py), 5.03 (s, 2H, CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.0 (C), 150.1 (2xCH), 144.2 (C), 139.6 (C), 136.2 (C and CH), 130.2 (CH), 129.0 (2xCH), 128.6 (2xCH), 128.3 (CH), 126.9 (CH), 123.0 (2xCH), 122.1 (CH), 121.6 (C), 53.5 (CH_2), 46.6 (CH_2); ir (nujol) 1694, 1603, 1313, 1173, 703 cm^{-1}.

Anal. Calcd. for $C_{20}H_{17}N_3O_3S$: C, 63.31%; H, 4.52%; N, 11.07%. Found: C, 63.44%; H, 4.57%; N, 10.98%.

3-(2-Chlorobenzyl)-1-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**4**).

This compound was obtained starting from 3-(2-chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**12**) by reaction with 2,6-di-*tert*-butyl-4-(hydroxymethyl)phenol. Flash column chromatography: eluent cyclohexane/ethyl acetate (6:4, v/v). Yield 17%; mp183-185 °C (acetone/petroleum ether 40-60 °C; ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.00 (dd, 1H, J=1.5, 7.9 Hz, H-5), 7.92 (dd, 1H, J=1.5, 7.3 Hz, aromatic), 7.82 (bd, 1H, J=8.4 Hz, aromatic), 7.60 (dt, 1H, J=1.3, 7.5 Hz, aromatic), 7.50 (m, 1H, aromatic), 7.38-7.25 (m, 2H, aromatic), 7.13 (m, 1H, aromatic), 7.02 (s, 1H, OH, deuterium oxide exchangeable), 6.68 (s, 2H, aromatic), 4.98 (s, 2H, CH_2Ph), 4.67 (s, 2H, CH_2Ph), 1.26 (s, 18H, *tert*-butyl); ^{13}C nmr (dimethylsulfoxide-d_6): δ 161.7 (C), 154.7 (C), 140.1(C), 139.3 (2xC), 136.2 (CH), 133.1 (C), 131.8 (C), 129.9 (CH), 129.8 (CH), 129.7 (CH), 128.3 (CH), 127.9 (CH), 125.7 (2xCH), 125.5 (CH), 124.6 (CH), 123.6 (C), 58.7 (CH_2), 44.7 (CH_2), 34.7 (2xC), 30.4 (6xCH_3); ir (nujol): 3585, 1677, 1603, 1604, 1326, 1188, 1052 cm^{-1}.

Anal. Calcd. for $C_{29}H_{33}ClN_2O_4S$: C, 64.37%; N, 5.18%. Found: C, 64.63%; N, 5.41%.

3-(2-Chlorobenzyl)-1-(4-nitrobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**5**).

This compound was obtained starting from 3-(2-chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**12**) by reaction with 4-nitrobenzyl alcohol. Flash column chromatography: eluent cyclohexane/ethyl acetate (7:3, v/v). Yield 67%; mp 108-110 °C (methanol); ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.20 (bd, 2H, J=8.8 Hz, 4-nitrobenzyl H-3 and H-5), 8.08 (dd, 1H, J=1.6, 7.8 Hz, H-5), 7.83 (1H, ddd, J=1.6, 7.4, 8.0 Hz, aromatic), 7.53 (m, 3H, H aromatic), 7.50 (bd, 2H, J=8.8 Hz, 4-nitrobenzyl H-2 and H-6) 7.30 (m, 3H, H aromatic), 5.30 (s, 2H, CH_2Ph), 5.11 (s, 2H, CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 162.0 (C), 147.8 (C), 143.0 (CH), 139.8 (C), 136.4 (C), 133.2 (CH), 132.2 (C), 130.4 (CH), 129.9 (CH), 129.8 (CH), 129.5 (2xCH), 129.0 (CH), 127.8 (CH), 127.2 (C), 124.3 (2xCH), 122.4 (CH), 121.7 (C), 54.2 (CH_2), 44.4 (CH_2); ir (nujol) 1681, 1603, 1518, 1175, 1050, 759 cm^{-1}.

Anal. Calcd. for $C_{21}H_{16}ClN_3O_5S$: C, 55.09%; H, 3.52%; N, 9.18%. Found: C, 55.45%; H, 3.90%; N, 9.53%.

3-(4-Chlorobenzyl)-1-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**6**).

This compound was obtained starting from 3-(4-chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**13**) by reaction with 2,6-di-*tert*-butyl-4-(hydroxymethyl)phenol. Flash column chromatography: eluent cyclohexane/ethyl acetate (1:1, v/v). Yield 53%; mp 142-145 °C (methanol/water); ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.00 (dd, 1H, J=1.4, 7.9 Hz, H-5), 7.88 (ddd, 1H, J=1.4, 7.9, 8.1 Hz, H-7), 7.82 (dd, 1H, J=1.0, 8.1 Hz, H-8), 7.54 (ddd, 1H, J=1.0, 7.9, 8.1 Hz, H-6), 7.40 (bd, 2H, J=8.5 Hz, 4-chlorobenzyl H-3 and H-5), 7.27 (bd, 2H, J=8.5 Hz, 4-chlorobenzyl H-2 and H-6), 7.02 (s, 1H, OH, deuterium oxide exchangeable), 6.71 (s, 2H, H aromatic), 4.98 (s, 2H, CH_2Ph), 4.64 (s, 2H, CH_2Ph), 1.26 (s, 18H, *tert*-butyl); ^{13}C nmr (dimethylsulfoxide-d_6): δ 161.8 (C), 154.7 (C), 140.1 (C), 139.3 (2xC), 139.2 (C), 136.0 (CH), 135.2 (C), 132.9 (C), 130.1 (2xCH), 129.8 (CH), 129.0 (2xCH), 125.5 (2xCH), 124.8 (CH), 124.2 (C), 123.9 (C), 57.8 (CH_2), 46.0 (CH_2), 34.7 (2xC), 30.5 (6xCH_3); ir (nujol) 3577, 1677, 1605, 1326, 1176 cm^{-1}.

Anal. Calcd. for $C_{29}H_{33}ClN_2O_4S$: C, 64.37%; H, 6.15%; N, 5.18%. Found: C, 64.62%; H, 6.24%; N, 5.48%.

7-Chloro-3-(2-chlorobenzyl)-1-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**7**).

This compound was obtained starting from 7-chloro-3-(2-chlorobenzyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**14**) by reaction with 2,6-di-*tert*-butyl-4-(hydroxymethyl)phenol. Flash column chromatography: eluent cyclohexane/ethyl acetate (9:1, v/v). Yield 40%; mp 165-168 °C (methanol/water); ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.00 (dd, 1H, J=1.5,7.9 Hz, H-5), 7.66 (dd, 1H, J=2.0, 8.5 Hz, H-6), 7.50 (m, 1H, aromatic), 7.32 (m, 2H, aromatic), 7.15-7.11 (m, 1H, aromatic), 7.06 (s, 1H, OH, deuterium oxide exchangeable), 6.74 (s, 2H, aromatic), 5.04 (s, 2H, CH_2Ph), 4.74 (s, 2H, CH_2Ph), 1.28 (s, 18H, *tert*-butyl); ir (nujol) 3575, 1684, 1596, 1326, 1321, 1185 cm^{-1}.

Anal. Calcd. for $C_{29}H_{32}Cl_2N_2O_4S$: C, 60.42%; H, 5.60%; N, 4.87%. Found: C, 60.54%; H, 5.74%; N, 4.84%.

3-(2-Phenylethyl)-1-(2-pyridylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (**8**).

This compound was obtained starting from 3-(2-phenylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**15**) by reaction with 2-(2-hydroxyethyl)pyridine as a yellow oil (yield 86%). Flash column chromatography: eluent chloroform/acetone (9.8:0.2, v/v). ^{1}H nmr (dimethylsulfoxide-d_6): δ 8.41 (m, 1H, pyridine-H), 8.00 (dd, 1H, J=1.6, 7.8 Hz, H-5), 7.74 (dt, 1H, J=1.6, 7.8 Hz, H-7), 7.61 (dt, 1H, J=1.9, 7.8 Hz, pyridine H-4/5), 7.54 (bd, 1H, J=7.8 Hz, H-8), 7.46 (bt, 1H, J=7.8, H-6), 7.29-7.12 (m, 7H, H aromatic), 4.24 (t, 2H, J=7.1 Hz, NCH_2CH$_2$Py), 3.97 (m, 2H, NCH_2CH$_2$Ph), 2.95 (m, 4H, NCH$_2$CH_2Ph, NCH$_2$CH_2Py); ^{13}C nmr (dimethylsulfoxide-d_6): δ 161.9 (C), 157.6 (C), 149.4 (CH), 139.9 (C), 138.1 (C), 137.2 (CH), 135.8 (CH), 129.9 (CH), 129.2 (2xCH), 129.0 (2xCH), 127.1 (CH), 126.7 (CH), 124.1 (CH), 122.8 (CH), 122.4 (CH), 122.1 (C), 51.7 (CH_2), 44.2 (CH_2), 35.9 (CH_2), 34.8 (CH_2); ir (nujol) 1711, 1604, 1461, 1378, 1261, 1171 cm^{-1}.

Anal. Calcd. for $C_{22}H_{21}N_3O_3S$: C, 64.85%; H, 5.19%; N, 10.31%. Found: C, 65.11%; H, 5.41%; N, 10.50%.

2-{[2,2-Dioxido-4-oxo-3-(2-phenylethyl)-3,4-dihydro-1*H*2,1,3-benzothiadiazin-1-yl]methyl}-1*H*-isoindole-1,3-(2*H*)-dione (**9**).

This compound was obtained starting from 3-(2-phenylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (**5**) by reaction with *N*-(hydroxymethyl)phtalimide as a white powder. Flash column chromatography: eluent chloroform. Yield 62%; mp 128-130 °C (acetone/petroleum ether 40-60 °C); ^{1}H nmr (dimethylsulfoxide-d_6): δ 7.96 (bd, 1H, J=7.8 Hz, H-5), 7.87-7.76 (m, 6H, aromatic), 7.51 (dt, 1H, J=2.1,7.0, aromatic), 7.37-7.19 (m, 5H, aromatic), 5.60 (s, 2H, NCH_2N), 4.00 (t, 2H, J=8.0 Hz, NCH_2CH_2Ph), 2.89 (t, 2H, J=8.0 Hz, NCH_2CH_2Ph); ^{13}C nmr (dimethylsulfoxide-d_6): δ 167.1 (2xC), 161.8 (C), 138.4 (C), 138.1 (C and CH), 135.6 (2xCH), 131.2 (2xC), 129.6 (CH), 129.1 (2xCH), 129.0 (2xCH), 128.3 (CH), 127.1 (CH), 125.5 (CH), 124.1 (2xCH), 123.7 (C), 54.3 (CH_2), 44.7 (CH_2), 35.0 (CH_2); ir (nujol) 1776, 1720, 1606, 1302, 1160, 978 cm^{-1}.

Anal. Calcd. for $C_{24}H_{19}N_3O_5S$: C, 62.46%; H, 4.15%; N, 9.11%. Found: C, 62.71%; H, 4.09%; N, 9.35%.

2-(4-Hydroxybenzyl)-6-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-Dioxide (**10**).

This compound was obtained starting from 6-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (**6**) [21] by reaction with 4-(hydroxymethyl)phenol. Flash column chromatography: eluent cyclohexane/ethyl acetate (5:5, v/v). Yield 64%; mp 200-204 °C (acetone/petroleum ether 40-60 °C); ^{1}H nmr (dimethylsulfoxide-d_6): δ 11.30 (s, 1H, NH, deuterium oxide exchangeable), 9.35 (s, 1H, OH, deuterium oxide exchangeable), 7.72 (d, 1H, J=8.0 Hz, H-8), 7.14 (m, 3H, H-7 and 4-hydroxybenzyl H-2 and H-6), 7.05 (bs, 1H, H-5), 6.68 (m, 2H, 4-hydroxybenzyl H-3 and H-5), 4.82 (s, 2H, CH_2Ph), 2.37 (s, 3H, CH_3); ^{13}C nmr (dimethylsulfoxide-d_6): δ 157.4 (C), 150.2 (C), 146.0 (C), 134.9 (C), 130.0 (2xCH), 127.2 (C), 124.7 (CH), 122.4 (CH), 120.2 (C), 117.3 (CH), 115.5 (2xCH), 43.5 (CH_2), 21.8 (CH_3); ir (nujol): 3455, 3250, 1680, 1610, 1593, 1517, 1272, 1152 cm^1.

Anal. Calcd. for $C_{15}H_{14}N_2O_4S$: C, 56.59%; H, 4.43%; N, 8.80%. Found: C, 56.93%; H, 4.30%; N, 8.95%.

Radical Scavenging Effect on DPPH Radical.

An ethanolic solution (2 mL, 1x10^{-4} *M*) of the tested compound was added to 2 mL of a DPPH solution (1 x 10^{-4} *M*), and the reaction mixture was shaken vigorously and kept at 37 °C ± 0.02 (Haake F 3C Thermocriostat) in air. DPPH absorption was measured at 514 nm every fifteen minutes. The mean values were obtained from quadruplicate experiments.

Cells and Virus.

The green monkey kidney cell line VERO was used to grow HSV-1, Cox-B5 and ADV, while HHV-6 was grown in SupT-1 cells (a human T cell line) and H-CMV in MRC-5 (human fibroblasts). VERO and MRC-5 cells were cultured in Minimum Essential Medium (MEM) added with 10% (growth medium) or 5% (maintenance medium) fetal calf serum (FCS), penicillin (100U/mL), streptomycin (100µg/mL) and incubated at 37 °C with 5% CO_2. SupT-1 cells were grown with RPMI1640 medium added with 10 % heat inactivated FCS and antibiotics as for MEM.

HSV-1, ADV and Cox-B5 were clinical strains adapted to grow in cells with several *in vitro* passages. For H-CMV and HHV-6 the references strain Towne [22] and Z-29 [23] were used, respectively. A same stock of each virus kept frozen at –80 °C was used in all the experiments.

Cytotoxicity Assays.

The non-toxic concentrations of each compound evaluated in the antiviral assays were assessed by means of the MTT test [24]. Briefly, 24 hours growth VERO cells in a 24-well cell culture plate were placed in contact with different concentrations of the compounds under study dissolved in maintenance medium. After 48 hours incubation, the MTT stain was added to each well at a 0.5 mg/mL final concentration. MTT is a tetrazolium salt that is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase of live cells, so the colour formation is proportional to the number of live cells. After 3 hours incubation, adding 0.1 *N* HCl in absolute isopropanol the insoluble formazan developed in viable cells was solubilized. Absorbance of converted dye was measured at a wavelength of 570 nm with background subtraction at 630 nm. Each dilution was always tested in triplicate and in each set of experiments three control wells without drug were included.

Antiviral Assays.

Antiviral activity was ascertained by means of plaque assays for HSV-1 and H-CMV and by viral yield assays for ADV, Cox-B5 and HHV-6. Plaque assays were carried out as follows. For HSV-1, 24 hours growth VERO in 24-well tissue culture plates and 48 hours MRC-5 cells for H-CMV were infected with 50 PFU/well. After 1 hour adsorption, virus inoculum was removed, the plates washed with PBS, and the maintenance medium with or without (control cultures) the compounds under study. Then 0.6% human γ-globulin was added. After 48 hours incubation, the plates were fixed with methanol, stained with Giemsa and the plaques counted. Each compound was assayed in triplicate.

For ADV and Cox-B5, 24 hours growth VERO cells were infected at a multiplicity of infection (MOI) of 0.01 $TCID_{50}$/cell. After 1 hour adsorption at 37 °C, the inoculum was removed, the plates washed with PBS and the compounds added to the maintenance medium. After 2 days incubation, the plates were frozen and thawed three times and the viral yield was titred by the end-point titration. In this case, 10-fold dilutions of each cell lysate were seeded onto 24 hours growth VERO cells in a 96-well culture plate and after 2 days the titre, expressed as $TCID_{50}$/mL, was read, taking account of the final dilution which afforded the typical viral cytopathic effect and the results were elaborated using the Reed and Muench formula [25].

For HHV-6, 10^5 SupT-1 cells in 24-well plates were infected at a MOI of 0.01 $TCID_{50}$/cell and incubated for 2 hours and then the medium with the drugs added. After 6 days incubation, the virus growth was ascertained determining the percentage of infected cells by immunofluorescence assays as described elsewhere [26].

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