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Imidazothiadiazine Dioxides: Synthesis and Antiviral Activity

Ana Martínez,[a,*] Ana I. Esteban,[a] Angela Herrero,[a] Carmen Ochoa,[a] Graciela Andrei,[b] Robert Snoeck,[b] Jan Balzarini[b] and Erik De Clercq[b]

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

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Abstract—A new series of imidazothiadiazine dioxides, including the first acyclonucleosides derived from this heterocycle moiety, has been synthesized. A wide-spectrum antiviral screening was performed. Some of the *N*-1 benzyl imidazothiadiazines and the new acyclonucleosides showed interesting anti-CMV or anti-HIV activity. These structures could be considered as new lead compounds for antiviral drug research.

Introduction

The last past years have witnessed a revival of interest in analogues of nucleosides following the discoveries that several of such compounds exhibit powerful antiviral effects.[1] The introduction of acyclovir[2] as a clinically useful anti-herpetic drug has spurred interest in other acyclic nucleosides as potential chemotherapeutic agents. These compounds belong to a series of open-chain chemical structures derived by cleavage of at least one C–C bond or deletion of one or more carbon atoms of the sugar ring.[3] The nature of the heterocyclic base has also been modified since it has a pronounced effect on the antiviral activity.[4]

Continuing with our work in this field,[5,6] we report here the synthesis of the first imidazothiadiazine dioxide acyclonucleosides and the antiviral activities found for compounds related to this heterocyclic system which can be considered as SO_2-purine analogues.

Results and Discussion

The imidazothiadiazine dioxides were synthesized according to a described procedure,[7] which involves the reaction of aldehydes in very careful conditions[8] with 3,4,5-triamino-1,2,6-thiadiazine 1,1-dioxide (Scheme 1). This last compound was previously obtained in three steps from cyclocondensation of sulfamide and malononitrile.[9]

The synthesis of the acyclonucleosides was achieved using the silylation procedure.[10] Thus, treatment of imidazothiadiazine dioxides with hexamethyldisilizane and pyridine as co-solvent, followed by reaction with acetoxymethyl ether[11] in dichloromethane and boron trifluoride as catalyst afforded the *N*-1 acyclonucleosides **1** and **2**. Deprotection was performed following a high efficient lipase mediated deacylation, which has been previously reported by our group,[12] yielding compound **3** in quantitative yield (Scheme 1, route i). To prepare the acyclonucleosides substituted in the imidazole ring a benzylation-debenzylation synthetic strategy which has been successfully employed in riboside synthesis[13] was followed (Scheme 1, route ii). Alkylation reaction of imidazothiadiazine dioxides with benzyl chloride in alkaline medium yielded the 1-benzyl derivatives **4**–**7**. These *N*-1 blocked compounds were first silylated with hexamethyldisilizane and afterwards treated with acetoxymethyl ether to yield the *N*-1, *N*-7-disubstituted compounds **8** and **9**. Hydrogenolysis of the benzyl moiety afforded acyclonucleoside **10**, which can be considered an SO_2-analogue of acyclovir.

The analytical and spectroscopic data of all the new compounds are collected in the Experimental section. NOE experiments and HMBC (Heteronuclear Multiple Bond Correlation) sequences were used to unequivocally determined the site of glycosilation.

The new imidazothiadiazine dioxides *N*-1 benzyl derivatives together with their first acyclonucleosides (compounds **1**–**10**) were evaluated for their antiviral activity in a wide variety of assay systems:[14] herpes simplex virus type 1 (strains KOS, F. McIntyre), herpes simplex virus

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* Corresponding author.

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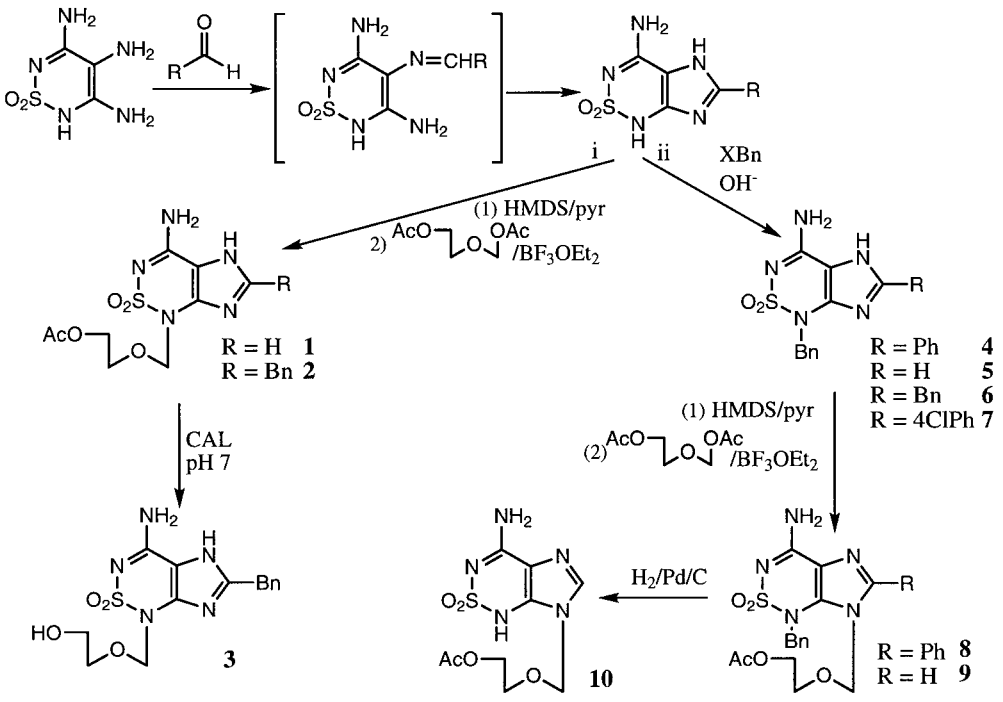


Scheme 1.

type 2 (strains G, 196, Lyons), thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains B 2006 and 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, Punta Toro virus and Semliki forest virus in Vero Cells. However, no antiviral activity was noted against any of these viruses at compound concentrations up to 400 μg/mL.

The imidazothiadiazine derivatives **1**–**10** were also evaluated for their activity against cytomegalovirus (CMV, strains AD-169 and Davis), varicella-zoster virus (VZV, strains OKA, YS, 07/1 and YS/R) in human embryonic lung (HEL) cells and human immunodeficiency virus (HIV) types 1 and 2 in human T-lymphocyte (CEM) cells. From these results (Table 1) new lead compounds for antiviral drug research emerged. Compounds **6** and **7** showed activity against CMV and VZV at a concentration that was not toxic to the host cells (although the IC_{50} for derivative **7** could not adequately be interpreted at concentrations >5 μg/mL because of interference with the normal cell morphology at these concentrations), while imidazothiadiazine acyclonucleoside **2** showed activity against HIV-1 and HIV-2 at a non-cytotoxic concentration. The selectivity (CC_{50}/IC_{50}) of **6** for CMV and VZV was approximately threefold to sixfold, while the selectivity index of compound **2** showed for HIV ranged between 4 and 14. In both

Table 1. Antiviral activity and cytotoxicity of representative imidazothiadiazine dioxides against human cytomegalovirus (CMV) and varicella-zoster virus (VZV) in human embyonic lung (HEL) cells and against human immunodeficiency virus (HIV-1 and HIV-2) in human T-lymphocyte (CEM) cells

Compd	Antiviral activity (HEL) IC_{50} (μg/mL)[a]						Cytotoxicity CC_{50} (μg/mL)[b]	Antiviral activity (CEM) EC_{50} (μg/mL)[c]		Cytotoxicity CC_{50} (μg/mL)[d]
	CMV		TK^+ VZV		TK^- VZV			HIV-1 (III_B)	HIV-2 (ROD)	
	AD-169	Davis	OKA	YS	07/1	YS/R				
2	>50	>50	>50	>50	>50	>50	>50	29±16	8±0	111±36
5	>50	>50	>50	>50	>50	>50	>50	30±14	≥100	91.3±12.4
6	9.2	6.1	12	15	12	10.2	35	>4	≥4	9.6±0.4
7	>5	>5	>5	4.1	>5	4.0	30	>4	>4	9.9±2.3
Acyclovir	–	–	0.73	0.78	24	26	>200	–	–	–
Ganciclovir	1.2	1.9	–	–	–	–	>50	–	–	–

[a] 50% inhibitory concentration, or compound concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU) for CMV and 20 PFU for VZV.
[b] 50% cytotoxic concentration, or compound concentration required to reduce cell growth by 50%.
[c] 50% effective concentration, or compound concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%.
[d] 50% cytotoxic concentration, or compound concentration required to reduce CEM cell viability by 50%. All assays were performed in at least two independent experiments.

cases, and in view of the unique chemical structures of compounds **2** and **6**, these results could serve as a guideline for further synthesis starting from **2** and **6** as the prototype compounds, to improve the potential antiviral activity and/or to lower the toxicity.

In order to begin building up a structure–activity relationship some other related imidazothiadiazine compounds **11**–**25** (Fig. 1), including SO_2-purine like ribosides,[13] were evaluated for their antiviral activity.

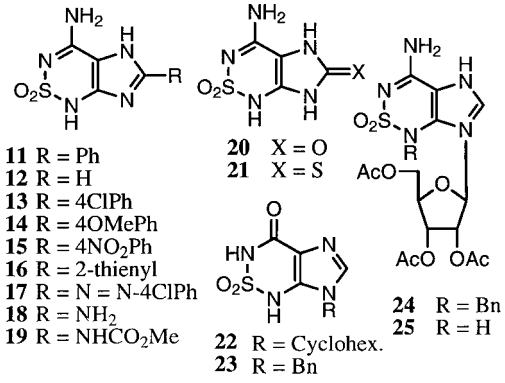
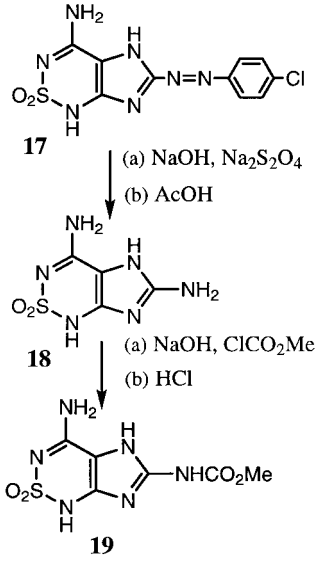


Figure 1.

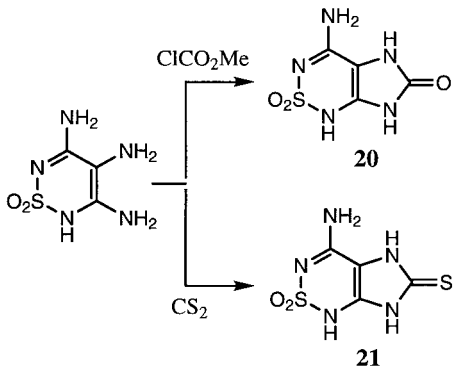
The great majority of these derivatives have been previously prepared,[7,15] but some of them were specially synthesized on this occasion. Thus, the 6-amino (**18**) and 6-methylcarbamate (**19**) derivatives of 4-amino-1*H*,5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxides were synthesized from its 6-[*p*-(chlorophenyl)azo] derivative (**17**), previously described.[16] Reduction of **17** with sodium dithionite afforded compound **18** and then, condensation of the 6-amino group of **18** with methyl chloroformate yielded compound **19** (Scheme 2).

Cyclocondensation reactions of 3,4,5-triamino-1,2,6-thiadiazine 1,1 dioxide with electrophiles, such as methyl choroformate and carbon disulfide, allowed us to prepare



Scheme 2.

the 6-oxo (**20**) and 6-thioxo (**21**) derivatives of 4-amino-1*H*,5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxides, respectively (Scheme 3).



Scheme 3.

No specific antiviral activity was noted with any of these new tested compounds, except for compound **23**[17] against Punta Toro virus (MIC = 20 μg/mL; MCC = 100 μg/mL) which is just on the borderline of antiviral activity.

From this study we can conclude that *N*-1 benzyl substituted imidazothiadiazine dioxides could be considered as new lead compounds for anti-CMV and anti-VZV drug research. It is worth mentioning that the benzyl moiety is present in the chemical structure of the recently described anti-CMV compounds.[18,19] A synthetic pathway for the synthesis of the first acyclonucleosides derived from this heterocyclic system has been explored, while the biological activity results could serve as a new guideline towards the development of new antiviral agents.

Experimental

Chemical procedures

Melting points were measured on a Reichert–Jung Thermovar and are uncorrected. Reagents were purchased from commercial suppliers and used without further purification. Reaction solvents were distilled from an appropriate drying agent before use. Chromatographic separations were performed on silica gel, using the following techniques: flash column chomatography (Kieselgel 60 Merck of 230–400 mesh) and preparative centrifugal circular thin layer chromatography (CCTLC, on a circular plate coated with a 1 mm layer of Kieselgel 60 PF254 gishalting, Merck, using a Chromatotron®). Compounds were detected with UV light (254 nm). ^{1}H NMR spectra were obtained at 298 K on a Varian-Gemini 200 and a Varian XL-300, operating at 200 and 300 MHz, respectively. ^{13}C NMR spectra were recorded with a Varian-Gemini 200 and a Bruker AM-200, operating at 50 MHz. 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 departament at CNQO (CSIC)

and the results obtained were within ±0.4% of the theoretical values. *Candida antarctica* lipase used was the Novo Nordisk's immobilized preparation Novozym 435.

General procedure for glycosylation

To a solution in dichloromethane (25 mL) of the silyl derivative of the corresponding imidazothiadiazine dioxides (1 mmol) prepared by refluxing the base in hexamethyldisilazane (5 mL) and pyridine as co-solvent under nitrogen, 2-acetoxyethyl acetoxymethyl ether[11] (1 mmol) dissolved in dichloromethane (25 mL) was added. The mixture was cooled, and $BF_3.Et_2O$ (1.4 mmol) was added under vigorous stirring and exclusion of moisture. The resulting mixture was stirred at room temperature for 2 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 first on a silica gel column and then by CCTLC, using them as eluent mixtures of solvents in the proportions indicated for each particular case.

1-[(2-Acetoxyethoxy)methyl]-4-amino-(5*H*)-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (1). Following the general procedure, the silyl derivative of 4-amino-1*H*,5*H*-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide[20] (0.1 g, 0.5 mmol) (co-solvent: pyridine (1 mL)), was treated with 2-acetoxyethyl acetoxymethyl ether (0.09 g, 0.5 mmol), at room temperature for 2 h. After work up, the syrupy residue obtained was chromatographed first on a silica gel column and by CCTLC after using dichlorometane/methanol (25/1) as eluent. The acyclonucleoside **1** (0.01 g, 7%) was obtained as a white foam. ^{1}H NMR (200 MHz, CD_3OD) δ 7.97 (s, 1H, H-6), 5.54 (s, 2H, N-CH_2-O), 4.36 (m, 2H, CH_2-CO), 4.03 (m, 2H, CH_2-O), 2.18 (s, 3H, CH_3-CO); ^{13}C NMR (50 MHz, CD_3OD) δ 170.97 (CO), 159.53 (C-4), 149.87 (C-7a), 143.45 (C-6), 104.44 (C-4a), 76.78 (OCH_2N), 67.96 (CH_2-O), 64.41 (CH_2-CO), 20.69 (CH_3-CO)). Anal. calcd for $C_9H_{13}N_5O_5S$: C, 35.61; H, 4.29; N, 23.08; S, 10.57. Found: C, 35.72; H, 4.29; N, 23.13; S, 10.65.

1-[(2-Acetoxyethoxy)methyl]-4-amino-6-benzyl-(5*H*)-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (2). According to the general procedure, the silyl derivative of 4-amino-6-benzyl-1*H*,5*H*-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide[7] (0.1 g, 0.4 mmol) (co-solvent: pyridine (1 mL)), was treated with 2-acetoxyethyl acetoxymethyl ether (0.07 g, 0.4 mmol) at room temperature for 2 h. After work up, the syrupy residue obtained was chromatographed first on a silica gel column and then by CCTLC after using dichlorometane:methanol 50:1 as eluent. Compound **2** (0.04 g, 26%) was obtained as a white foam. ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.61–7.27 (m, 5H, H-Ph), 5.21 (s, 2H, N-CH_2-O), 4.70 (s, 2H, CH_2-Ph), 4.09 (m, 2H, CH_2-CO), 3.75 (m, 2H, CH_2-O), 1.97 (s, 3H, CH_3-CO); ^{13}C NMR (50 MHz, DMSO-d_6) δ 167.98 (CO), 154.21 (C-4), 151.13 (C-7a), 149.39 (C-6), 137.07, 129.30, 129.32, 127.53 (C-Ph), 104.84 (C-4a), 76.04 (OCH_2N), 66.96 (CH_2-O), 63.34 (CH_2-CO), 35.08 (CH_2-Ph), 30.07 (CH3-CO)). Anal. calcd for $C_{16}H_{19}N_5O_5S$: C, 48.80; H, 4.80; N, 17.79; S, 8.15. Found: C, 48.77; H, 4.63; N, 17.54; S, 8.27.

7-[(2-Acetoxyethoxy)methyl]-4-amino-1-benzyl-6-phenyl-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (8). Following the general procedure, the silyl derivative of 4-amino-1-benzyl-6-phenyl-(5*H*)-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (**4**)[7] (0.3 g, 1 mmol) (cosolvent: pyridine (1 mL)), was treated with 2-acetoxyethyl acetoxymethyl ether (0.17 g, 1 mmol), at room temperature for 12 h. After work up, the syrupy residue obtained was chromatographed first on a silica gel column and then by CCTLC after using dichlorometane:methanol 200:3 as eluent. The acyclonucleoside (**8**) (0.01 g, 2%) was obtained as a white foam. ^{1}H NMR (200 MHz, CD3OD) δ 7.71–7.26 (m, 10H, H-Ph), 5.01 (s, 2H, N–CH_2–O), 5.08 (s, 2H, CH_2-Ph), 4.21 (m, 2H, CH_2-CO), 3.49 (m, 2H, CH_2-O), 1.98 (s, 3H, CH_3-CO); ^{13}C NMR (50 MHz, CD_3OD) δ 170.59 (CO), 158.64 (C-4), 147.08 (C-7a), 143.07 (C-6), 130.56, 129.08, 128.72, 128.94, 128.11, 127.40 (C-Ph), 109.16 (C-4a), 73.65 (OCH_2N), 66.33 (CH_2-O), 62.79 (CH_2-CO), 55.50 (CH_2-Ph), 20.76 (CH_3-CO)). Anal. calcd for $C_{22}H_{23}N_5O_5S$: C, 56.29; H, 4.90; N, 14.92; S, 6.84. Found: C, 56.40; H, 4.92; N, 14.99; S, 6.70.

7-[(2-Acetoxyethoxy)methyl]-4-amino-1-benzyl-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (9). According to the general procedure, the silyl derivative of 4-amino-1-benzyl-(5*H*)-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (**5**)[15] (0.1 g, 0.4 mmol) (co-solvent: pyridine (1 mL)), was treated with 2-acetoxyethyl acetoxymethyl ether (0.07 g, 0.4 mmol), at room temperature for 2 h. After work up, the syrupy residue obtained was chromatographed first on a silica gel column and then by CCTLC after using dichlorometane:methanol (200:3) as eluent. Compound **9** (0.02 g, 11%) was obtained as a white foam. ^{1}H NMR (200 MHz, acetone-d_6) δ 7.38–7.11 (m, 5H, H-Ph), 5.62 (s, 2H, N-CH_2-O), 4.92 (s, 2H, CH_2-Ph), 3.78 (m, 2H, CH_2-CO), 4.10 (m, 2H, CH_2-O), 1.78 (s, 3H, CH_3-CO); ^{13}C NMR (50 MHz, acetone-d_6) δ 170.94 (CO), 153.93 (C-4), 149.67 (C-7a), 143.47 (C-6), 138.34, 129.04, 128.97, 128.20 (C-Ph), 108.06 (C-4a), 72.24 (OCH_2N), 67.87 (CH_2-O), 63.18 (CH_2-CO), 48.30 (CH_2-Ph), 20.53 (CH3-CO)). Anal. calcd for $C_{16}H_{19}N_5O_5S$: C, 48.80; H, 4.80; N, 17.79; S, 8.15. Found: C, 48.63; H, 4.71; N, 18.18; S, 8.10.

4-Amino-6-benzyl-1-[(2-hydroxyethoxy)methyl]-(5*H*)-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (3). A solution of the acetylated acyclonuceloside **2** (0.001 g, 0.02 mmol) in *t*-BuOH/buffer pH 7 (90/10) was incubated with 10 mg mL^{-1} of *Candida antarctica* lipase (CAL), at 45°C and 250 rpm in an orbital shaker. When all the starting material had disappeared, the enzyme was removed by filtration and washed with methanol. The filtrate was evaporated in vacuo yielding derivative **3** (0.008 g, 95%) as a syrup. ^{1}H NMR (200 MHz, CD_3OD) δ 7.58–7.48 (m, 5H, H-Ph), 5.55 (s, 2H, N-CH_2-O), 4.38 (s, 2H, CH2-Ph), 3.87 (m, 4H, CH_2-CH_2); ^{13}C NMR (50 MHz, CD_3OD) δ 154.73 (C-4), 153.05 (C-7a), 150.48 (C-6), 137.37, 130.10, 129.97, 128.45 (C-Ph),

104.96 (C-4a), 76.83 (OCH_2N), 71.89 (CH_2-O), 62.14 (CH_2-CO), 36.07 (CH_2-Ph). Anal. calcd for $C_{13}H_{15}N_5O_5S$: C, 52.17; H, 5.27; N, 21.73; S, 9.93. Found: C, 52.22; H, 5.47; N, 21.87; S, 9.55.

7-[(2-Acetoxyethoxy)methyl]-4-amino-(1*H*)-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (10). A solution of **9** (0.005 g, 0.02 mmol) in ethanol (5 mL), was hydrogenated with 50 psi of hydrogen in the presence of 10% palladium/charcoal catalyst at room temperature. After 2 h, the catalyst was filtered off and the solvent evaporated under reduced pressure. Debenzylated derivative **10** was obtained in quantitatively yield as a syrup. ^{1}H NMR (200 MHz, acetone-d_6) δ 5.69 (s, 2H, N-CH_2-O), 3.90 (m, 2H, CH_2-CO), 4.24 (m, 2H, CH_2-O), 1.78 (s, 3 H, CH_3-CO); ^{13}C NMR (200 MHz, CD_3OD) δ 5.01 (s, 2H, N-CH_2-O), 4.00 (m, 2H, CH_2-CO), 4.39 (m, 2H, CH_2-O), 1.97 (s, 3H, CH_3-CO); ^{13}C NMR (50 MHz, CD_3OD) δ 172.50 (CO), 155.11 (C-4), n.o. (C-7a), 143.71 (C-6), 104.30 (C-4a), 77.39 (OCH_2N), 66.06 (CH_2-O), 63.81 (CH_2-CO), 20.60 (CH_3-CO)). Anal. calcd for $C_9H_{13}N_5O_5S$: C, 35.61; H, 4.29; N, 23.08; S, 10.57. Found: C, 35.70; H, 4.33; N, 23.09; S, 10.70.

4-Amino-1,6-dibenzyl-5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (6). To a solution of 4-amino-6-benzyl-1*H*,5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide[7] (0.24 g, 1 mmol) in aqueous sodium bicarbonate (10 mL), benzyl bromide (0.15 g, 1.0 mmol) was added. The reaction mixture was refluxed for 8 h. After cooling, a white solid appeared which was filtered and purified by silica gel column chromatography using ethyl acetate: hexane (10:1) as eluent. Compound **6** (0.29 g, 92% yield) was obtained as white crystals, mp 263–265°C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.35 (s, 1H, NH, exchangeable with D_2O), 7.71–7.26 (m, 10H, H-Ph), 4.92 (s, 2H, NCH2-Ph), 4.38 (s, 2H, CCH_2-Ph); ^{13}C NMR (50 MHz, DMSO-d_6) δ 158.44 (C-4), 148.64 (C-7a), 144.09 (C-6), 131.65, 130.56, 129.08, 128.72, 128.94, 128.11, 127.56 (C-Ph), 107.45 (C-4a), 55.56 (NCH_2), 47.2 (CH_2). Anal. calcd for $C_{18}H_{17}N_5O_2S$: C, 61.17; H, 4.85; N, 15.85; S, 9.07. Found: C, 61.34; H, 4.53; N, 15.55; S, 9.32.

4-Amino-1-benzyl-6-(4′-chlorophenyl)-5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (7). To a solution of 4-amino-6-(4′-chlorophenyl)-1*H*,5*H*-imidazo-[4,5-c]-1,2,6-thiadiazine 2,2-dioxide[7] (1 g, 3.4 mmol) in 2 N sodium hydroxide (10 mL) benzyl chloride (0.60 g, 3.4 mmol) was added. The reaction mixture was stirred over 1 h at room temperature. Then, the mixture was heated at 120–130°C for 1 h. After cooling, a white solid appeared which was filtered and washed with methanol. Compound **7** (0.66 g, 50% yield) was obtained as white crystals, mp 265°C (dec.) (MeOH/H_2O); IR (Nujol) (3450, 3300 (NH_2), 3150 (NH), 1360, 1170 cm^{-1} (SO_2); UV (MeOH) λ_{max} (log ε) 327 nm (4.17); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.29 (s, 1H, NH, exchangeable with D_2O), 7.89 (d, 2H, $^3J_{H\text{-}2',H\text{-}3'} = 8.5$ Hz, H-2′), 7.62 (d 2H, H-3′), 7.31 (m, 5H, Ph), 6.95 (s, 2H, NH_2, exchangeable with D_2O), 5.01 (s, 2H, CH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 153.0 (C-4), 150.8 (C-7a), 146.8 (C-6), 137.3 (C-4′), 134.8 (C-1′), 129.2, 128.2, 127.7, 127.5, 127.2 (C-2′, C-3′, C_6H_5), 104.5 (C-4a), 47.2 (CH_2). Anal. calcd for $C_{17}H_{14}N_5O_2SCl$: C, 52.64; H, 3.64; N, 18.06. Found: C, 52.31; H, 3.53; N, 17.55.

4,6-Diamino-1*H*,5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (18). To a solution of potassium salt of compound **17**[16] (1.0 g, 2.7 mmol) in water (25 mL) was dropwise added sodium dithionite (2.35 g, 13.5 mmol). The mixture was stirred at room temperature during 2 h. At the end of this time, the initial red solution turned to colourless. The reaction mixture was filtered and the resulting solution acidified at pH 5 with acetic acid. On cooling, a white precipitate appeared. The solid was filtered, washed with water, and dried. Compound **18** (0.26 g, 48% yield) was obtained as white needles, mp >270°C (H_2O); IR (Nujol) ν 3400, 3350, 3300 (NH_2), 3200 (NH), 1650, 1630 (C=N), 1320, 1240–1120 cm^{-1} (SO_2); UV (H_2O) λ_{max} (log ε) 204 (4.23), 217 (sh), (4.17), 308 nm (4.01); ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.50 (s, 2H, NH_2), 6.83 (s, 1H, NH), 6.64 (s, 1H, NH), 3.60 (b.s., 2H, NH), 6.10 (b.s., 3H, NH) all signals were exchanged with D_2O; ^{13}C NMR (50 MHz, DMSO-d_6) δ 153.0 (C-4), 152.6 (C-6), 151.0 (C-7a), 96.0 (C-4a). Anal. calcd for $C_4H_6N_6O_2S$ C, 23.76; H, 2.99; N, 41.56; S, 15.86. Found: C,.23.83 H, 3.01; N, 41.38; S, 15.77.

4-Amino-6-methoxycarbonylamino-1*H*,5*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (19). To a solution of compound **18** (0.32 g, 1.6 mmol) in 0.5 N sodium hydroxyde (6 mL) methyl chloroformate (0.20 g, 2.1 mmol) was added. The mixture was stirred at room temperature over 24 h. The solid that appeared was filtered, washed with water and acetone, and dried to obtain 0.34 g (81% yield) of compound **19**, mp 214–15°C (dec.) (H_2O, white needles); IR (KBr) ν 3450, 3400, 3300, 3100 (NH_2, NH), 1740 (C≒O), 1620 (C=N), 1280, 1170–1120 cm^{-1} (SO_2); UV (H_2O) λ_{max} (log ε) 238 (4.06), 308 nm (3.89); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.30 (b.s., 1H, NH, exchangeable with D_2O), 7.92 (b.s., 2H, NH_2, exchangeable with D_2O), 7.05 (b.s., 2H, 2 NH, exchangeable with D_2O), 3.86 (s, 3H, CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 167.4 (NHCO), 156.6 (C-4), 153.0 (C-6), 150.8 (C-7a), 94.3 (C-4a), 54.6 (CH_3). Anal. calcd for $C_6H_8N_6O_4S$: C, 27.69; H, 3.10; N, 32.29. Found: C,27.51; H, 3.19; N,31.94.

4-Amino-6-oxo-1*H*,5*H*,7*H*-imidazo-[4,5-c]-1,2,6-thiadiazine 2,2-dioxide (20). A solution of 3,4,5-triamino-1,2,6-thiadiazine 1,1-dioxide[20] (1.8 g, 10 mmol) in a mixture of acetone (9 mL), water (5 mL) and triethylamine (2.68 g, 26.5 mmol) was cooled at 0°C and then methyl chloroformate (0.97 g, 10.3 mmol) was added. The reaction mixture was stirred at 0°C over 1 h, and at room temperature during two days more. The acetone was evaporated and the resulting solution acidified at pH 1 with concentrated hydrochloric acid. On cooling, a white precipitate was obtained which was filtered, washed with water and dried to yield 0.94 g (46%) of compound **20** as colourless prisms, mp 255°C (dec.) (MeOH/H_2O); IR (Nujol) ν 3400, 3300 (NH_2), 3150 (NH), 1720 (C=O), 1650 (C=N), 1310, 1130 cm^{-1} (SO_2); UV (H_2O) λ_{max} (log ε) 200 (4.21), 215 (sh) (4.10), 310 nm (4.02); ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.30

(b.s., 2H, NH_2, exchangeable with D_2O), 6.82 (b.s., 3H, NH, exchangeable with D_2O); ^{13}C NMR (50 MHz, DMSO-d_6) δ 152.6 (C-4), 150.4 (C-6), 145.4 (C-7a), 90.9 (C-4a). Anal. calcd for $C_4H_5N_5O_3S$: C, 23.65; H, 2.48; N, 34.47. Found: C,23.64; H, 2.51; N, 34.33.

4-Amino-6-thioxo-1*H*,5*H*,7*H*-imidazo-[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (21). To a solution of 3,4,5-triamino-1,2,6-thiadiazine 1,1-dioxide[20] (0.5 g, 2.8 mmol) in aqueous potassium hydroxyde (0.28 g of KOH and 5 mL of H_2O) carbon disulfide (1.7 mL) in ethanol (20 mL) was added. The solution was stirred over 12 h at room temperature. The white precipitate that appeared was filtered and washed with ethanol. The potassium salt obtained was dissolved in water and acidified with concentrated hydrochloric acid to pH 1 to obtain 0.35 g (59% yield) of compound **21**, mp >295°C (H_2O, white needles); IR (Nujol) ν 3400, 3300 (NH_2), 3150 (NH), 1650 (C=N), 1320, 1160–1120 cm^{-1} (SO_2); UV (H_2O) λ_{max} (log ε) 243 (4.27), 324 nm (4.30); ^1H NMR (200 MHz, DMSO-d_6) δ 12.60 (b.s., 1H, NH), 11.60 (s, 1H, NH), 7.32 (s, 2H, NH_2), 6.15 (b.s., 3H, NH) all signals were exchanged with D_2O; ^{13}C NMR (50 MHz, DMSO-d_6 + TFA) δ 164.2 (C-6), 148.2 (C-4), 147.3 (C-7a), 97.1 (C-4a). Anal. calcd for $C_4H_5N_5O_2S_2$: C, 21.91 H, 2.30; N, 31.94; S, 29.25. Found: C, 22.10; H, 2.43; N, 31.68; S, 29.51.

Biological methods

The compounds were evaluated for antiviral activity following established procedures, as reviewed in ref 14.

Cells

Human embryonic lung (HEL) fibroblast were propagated in Eagle's minimun 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 cell cultures. 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 activity assays

Confluent HEL cells grown in 96-well microtiter plates were infected with the different virus strains at 20 (VZV) or 100 (CMV) plaque forming units (PFU). After a 2 h incubation period, residual virus was removed and the infected cells were further incubated with MEM supplemented with 2% inactivated FCS, 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 stained with 2.5% Giemsa solution. Virus plaque formation (virus input: 20 PFU, (VZV)) or viral cytophatic effect (virus input: 100 PFU, (CMV)) was monitored microscopically. The antiviral activity is expressed as IC_{50} which represents the compound concentration required to reduce virus plaque formation or cytopathicity by 50%. IC_{50} values were calculated 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 determined.

Antiretroviral activity 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, Betheseda, MD). Virus stocks were prepared from supernatants of HIV-1-infected MT-4 cells. HIV-2 (strain ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France) and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells. CEM cells were obtained from the American Tissue Culture Collection (Rockville, MD) and were infected as follows: 4×10^5 cells/mL were exposed to HIV-1 or HIV-2 at ∼100 $CCID_{50}$ (50% cell culture infective dose) per 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 appropriate dilutions of the test compounds. After four days giant cell formation was recorded microscopically in the HIV-infected cell cultures. The EC_{50} was defined as the compound concentration required to reduce the number of giant cells by 50%.

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