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Benzothiadiazine dioxide human cytomegalovirus inhibitors: synthesis and antiviral evaluation of main heterocycle modified derivatives

Ana Martinez[1]*, Carmen Gil[1], Ana Castro[1], Ana M Bruno[1], Concepción Pérez[1], Columbiana Prieto[2] and Joaquin Otero[2]

[1]Instituto de Química Médica (CSIC), Madrid, Spain
[2]Unidad de Virologia, Servicio de Microbiologia, Hospital 'Doce de Octubre', Madrid, Spain

*Corresponding author: Tel: +34 91 562 29 00; Fax: +34 91 564 48 53; E-mail: amartinez@neuropharma.es

The benzothiadiazine dioxide derivatives are potent non-nucleoside human cytomegalovirus (HCMV) inhibitors. As part of our comprehensive structure–activity relationship (SAR) study of these compounds, we have now proposed structural modifications on the heterocyclic moiety both on the number and the nature of the fused heterocycle and on the kind of heteroatoms pre-sent on it. Synthesis of these new compounds (benzyl derivatives of thiadiazines, thienothiadiazines, benzothienothiadiazines and quinazolines) and the antiviral evaluation against HCMV has been performed. SAR investigation on this class of compounds has defined the structural requirements for potency and toxicity. They have revealed two important clues: i) a fused ring to the thiadiazine framework is necessary to maintain the anti-HCMV action, and ii) the sulfamido moiety in the main heterocycle is crucial to avoid cytotoxicity.

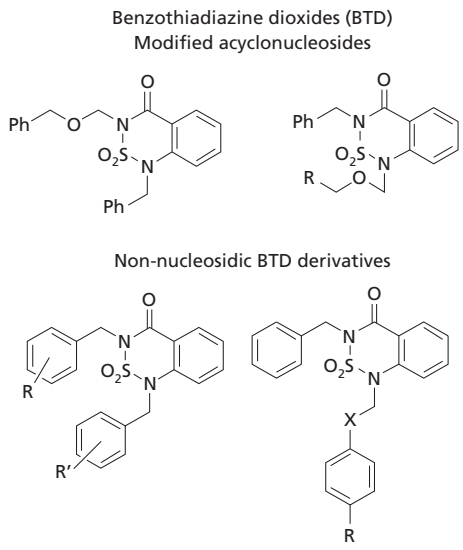
Introduction

Human cytomegalovirus (HCMV), a highly prevalent member of herpesvirus family, rarely causes disease in immunocompetent persons. However, reactivation of virus is of significant concern in the immunocompromised individual, most notably transplant recipients and patients with acquired immune deficiency syndrome (AIDS) (Snydman, 2001; Levin *et al.*, 2001). The interest in HCMV has increased with the escalation in the number of patients undergoing immunosuppressive therapy following organ and bone marrow transplantation, as well as the increasing number of AIDS patients. Furthermore, HCMV may have an important role in the development of vascular diseases such as arteriosclerosis, restenosis after coronary angioplasty and transplant vascular sclerosis (chronic rejection) (Levi, 2001; Horvath *et al.*, 2000; Van der Bij & Speich, 2001).

Antiviral agents currently licensed for the treatment of HCMV infection include ganciclovir, foscarnet, cidofovir and fomivirsen (Villarreal, 2001; Griffiths, 2002). All of them either directly or indirectly inhibit viral polymerase, or are able to reduce viral replication in patients who deve-lop the clinical symptoms associated with HCMV disease. However, toxicity associated with these drugs, poor oral bioavailability and high relapse rates have made their use less than optimal (Emery, 2001; Chou, 1999). Moreover, with the advent of the virus resistance to current drugs (Limaye, 2002), new drugs that act by a new mechanism of action may be highly desirable for the treatment of HCMV infection (Martinez *et al.*, 2001; Castro & Martinez, 2000).

In our search for new antiviral agents (Martinez *et al.*, 1999a, 2000a), we discovered the benzothiadiazine dioxide (BTD)-modified acyclonucleosides, which showed a marked activity against HCMV and varicella-zoster virus (Martinez *et al.*, 1997). The structure of these compounds is unique, not only for the nature of the heterocyclic base but also for the lack of the 5′-OH mimetic group pre-sent in ganciclovir and other current anti-HCMV drugs, which points to a different mechanism of action (Martinez *et al.*, 1999b). These factors were considered in the first optimization step performed on this family of compounds leading to the BTD dibenzylderivatives as potent non-nucleoside HCMV inhibitors, active against some current drug-resistant strains (Martinez *et al.*, 1999c). 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 (Martinez *et al.*, 2000b). As the viral target is yet unknown, a CoMFA analysis were performed to obtain further insights into the structural requirements for the biological activity of BTD. 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. From these results, new series of BTD derivatives were synthesized to

Figure 1. Benzothiadiazine dioxide derivatives



explore the sterics' requirement for their biological activity (Martinez *et al.*, 2000c, 2003) (Figure 1).

In the present work, new structural modifications have been proposed to improve the knowledge about the structural requirements for the anti-HCMV activity of BTD derivatives. The benzothiadiazine main framework is now modified. Elimination and modifications of the number and the nature of the fused heterocyclic system are here described. As thiophene is the classical bioisoster analogue of benzene, we here considered thienothiadiazine system a good starting point. We also studied analogues in which the fused heterocycle to the thiadiazine ring has been elimina-ted. The three fused system as the benzothienothiadiazine was assayed as potential HCMV inhibitors. On the other hand, to asses the influence of the sulfamido group in the antiviral activity of these compounds, substitution of this group by urea or thiourea moieties is also described.

Material and methods

Chemistry

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 d values (ppm) relative to internal Me4Si and J values are reported in Hertz. The analytical department at C.N.Q.O. (CSIC) performed elemental analyses and the results obtained were within ±0.4% of the theoretical values.

1-[(4-Chlorophenyl)methyl]-thieno[3,2-c][1,2,6]-thiadiazin-4(3H)-one 2,2-dioxide ***(3)***

To a solution of thienothiadiazine dioxide **1** (Martinez *et al.*, 2000a) (0.30 g, 1.4 mmol) in aqueous saturated solution of sodium bicarbonate (20 ml), 4-chlorophenylmethyl chloride (0.35 g, 2.1 mmol) was added. The reaction mixture was refluxed for 24 h. After cooling to room temperature, the aqueous phase was washed with CH_2Cl_2 (1x10 ml). The aqueous phase was cooled at −4°C and the pro-duct was isolated by filtration of aqueous phase. Purification: recrystallization from toluene/MeOH, yield 0.28 g (60 %) as a solid; mp 325–327°C.

δ_H (DMSO-d_6) 4.89 (2H, s, NCH_2), 6.58 (1H, d, J_{H6H7} 5.3 Hz, H-7), 7.40–7.46 (4H, m, Ar-H), 7.57 (1H, d, H-6); δ_C (DMSO-d_6) 47.81 (NCH_2), 114.85 (C-4a), 117.17 (C-7), 128.28, 129.16, 131.60, 137.24 (Ar-C), 129.55 (C-6), 144.81 (C-7a), 162.62 (C-4). Anal. for $C_{12}H_9N_2O_3S_2Cl$ (C, H, N, S).

1-[(3,4-Dichlorophenyl)methyl]-thieno[3,2-c][1,2,6]-thiadiazin-4(3H)-one 2,2-dioxide ***(4)***

To a solution of thienothiadiazine dioxide **1** (Martinez *et al.*, 2000a) (0.40 g, 2.0 mmol) in aqueous saturated solution of sodium bicarbonate (20 ml), 3,4-dichlorophenylmethyl chloride (0.58 g, 2.9 mmol) was added. The reaction mixture was refluxed for 24 h. After cooling to room temperature, the aqueous phase was washed with CH_2CI_2 (1x10 ml). The aqueous phase was cooled at −4°C and the pro-duct was isolated by filtration of aqueous phase. Purification: recrystallization from toluene/MeOH, yield 0.40 g (56 %) as a solid; mp 246–248°C.

δ_H (DMSO-d_6) 4.89 (2H, s, NCH_2), 6.69 (1H, d, J_{H6H7} 5.1 Hz, H-7), 7.39–7.57 (3H, m, Ar-H), 7.55 (1H, d, H-6); δ_C (DMSO-d_6) 47.40 (NCH_2), 114.93 (C-4a), 117.00 (C-7), 127.59, 129.15, 129.53, 129.60, 130.84, 139.52 (Ar-C), 130.45 (C-6), 144.58 (C-7a), 162.47 (C-4). Anal. for $C_{12}H_8N_2O_3S_2C_{l2}$ (C, H, N, S).

1-[(4-Chlorophenyl)methyl]-3-benzyl-thieno[3,2-c][1,2,6]-thiadiazin-4-one 2,2-dioxide ***(5)***

To an equimolecular suspension of sodium hydride in DMF (25 ml) were added thienothiadiazine **3** (0.07 g, 0.2 mmol) and benzyl bromide (0.05 g, 0.3 mmol). The reaction

mixture was refluxed for 10 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with CH_2Cl_2 (2x10 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 CH_2Cl_2:hexane (1:1) as eluent. Compound **5** was obtained (0.006 g, 7%) as a solid; mp 85–86°C.

δ_H ($CDCl_3$) 4.81 (2H, s, N_1CH_2), 4.97 (2H, s, N_3CH_2), 6.70 (1H, d, J_{H6H7} Hz, H-7), 6.97–7.36 (9H, m, Ar-H), 7.57 (1H, d, H-6); δ_C ($CDCl3$) 46.11 (N_3CH_2), 54.52 (N_1CH_2), 119.91 (C-7), 120.85 (C-4a), 128.15, 128.59, 128.96, 129.07, 129.28, 132.20, 134.60, 135.57 (Ar-C), 134.22 (C-6), 142.67 (C-7a), 157.74 (C-4). Anal. for $C_{19}H_{15}N_2O_3S_2Cl$: (C, H, N, S).

1-[(3,4-Dichlorophenyl)methyl]-3-benzyl-thieno[3,2-c][1,2,6]-thiadiazin-4-one 2,2-dioxide ***(6)***

To an equimolecular suspension of sodium hydride in DMF (25 ml) were added thienothiadiazine **4** (0.10 g, 0.2 mmol) and benzyl bromide (0.05 g, 0.3 mmol). The reaction mixture was refluxed for 5 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with CH_2Cl_2 (2x10 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 CH_2Cl_2:hexane (1:1) as eluent. Compound **6** was obtained (0.02 g, 18%) as a solid; mp 102–104°C.

δ_H (CDC_{l3}) 4.78 (2H, s, N_1CH_2), 4.98 (2H, s, N_3CH_2), 6.70 (1H, d, J_{H6H7} 5.2 Hz, H-7), 7.18–7.36 (8H, m, Ar-H), 7.60 (1H, d, H-6); δ_C ($CDCl_3$) 46.20 (N_3CH_2), 53.83 (N_1CH_2), 119.60 (C-7), 120.65 (C-4a), 127.00, 128.19, 128.62, 128.87, 129.77, 130.95, 132.96, 133.09, 134.17, 135.52 (Ar-C), 134.48 (C-6), 142.61 (C-7a), 157.63 (C-4). Anal. for $C_{19}H_{14}N_2O_3S_2Cl_2$: (C, H, N, S).

1-[(4-Chlorophenyl)methyl]-benzothieno[3,2-a][1,2,6]-thiadiazin-4(3H)-one 2,2-dioxide ***(7)*** *and 1,3-bis[(4-chlorophenyl)methyl]-benzothieno[3,2-a][1,2,6]-thiadiazin-4-one 2,2-dioxide* ***(8)***

To a solution of benzothienothiadiazine dioxide **2** (Martinez *et al.*, 2000d) (0.30 g, 1.1 mmol) in aqueous sa-turated solution of sodium bicarbonate (20 ml), 4-chlorophenylmethyl chloride (0.28 g, 1.7 mmol) was added. The reaction mixture was refluxed for 6 h. After cooling to room temperature, the aqueous phase was extracted with CH_2Cl_2 (4×10 ml). The organic phase was dried over sodium sulphate and the solvent evaporated under reduced pressure. The residue was chromatographed on silica gel column, using CH_2Cl_2:MeOH (50:1) as eluent. From the first fraction was isolated derivative **8**: yield 0.01 g (2%) as a solid; mp 158–160°C.

δ_H ($CDCl_3$) 4.79 (2H, s, N_3CH_2), 4.94 (2H, s, N_1CH_2), 6.81–7.34 (8H, m, Ar-H), 7.51 (1H, t, J_{H8H7} 7.0 Hz, H-7), 7.58 (1H, t, H-8), 7.82 (1H, d, J_{H8H9} 8.0 Hz, H-9), 7.93 (1H, d, J_{H7H6} 7.3 Hz, H-6); δ_C ($CDCl_3$) 45.62 (N_3CH_2), 56.09 (N_1CH_2), 122.99 (C-9), 124.15 (C-6), 125.40 (C-4a), 126.00 (C-7), 128.78 (C-8), 128.83, 129.91, 130.66, 130.92, 133.81, 134.25, 135.08 (Ar-C), 131.57 (C-5a), 137.15 (C-9b), 140.35 (C-9a), 158.47 (C-4). Anal. for $C_{23}H_{16}N_2O_3S_2Cl_2$: (C, H, N, S).

From the second fraction, derivative **7** was isolated: yield 0.13g (30%) as a white solid; mp 230–232°C.

δ_H (CDC_{l3}) 5.02 (2H, s, N_1CH_2), 7.25–7.30 (5H, m, Ar-H, H-7), 7.37 (1H, t, J_{H7H8} 7.1 Hz, H-8), 7.68 (1H, d, J_{H8H9} 8.0 Hz, H-9), 7.87 (1H, d, J_{H7H6} 7.6 Hz, H-6); δ_C ($CDCl_3$) 51.94 (N_1CH_2), 122.88 (C-9), 123.75 (C-6), 122.13 (C-4a), 124.44 (C-7), 126.49 (C-8), 128.10, 128.94, 131.53, 137.00 (Ar-C), 132.46 (C-5a), 139.10 (C-9b), 137.00 (C-9a), 164.32 (C-4). Anal. for $C_{16}H_{11}N_2O_3S_2Cl$: (C, H, N, S).

1-[(4-Chlorophenyl)methyl]-3-benzyl-benzothieno[3,2-a][1,2,6]-thiadiazin-4-one 2,2-dioxide ***(9)***

To an equimolecular suspension of sodium hydride in DMF (25 ml) were added benzotienothiadiazine **7** (0.08 g, 0.2 mmol) and benzyl bromide (0.05 g, 0.3 mmol). The reaction mixture was refluxed for 6 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 thin layer chromatography, using CH_2Cl_2:hexane (1:1) as eluent. Compound **9** was obtained (0.01 g, 14%) as a solid; mp 167–168°C.

δ_H ($CDCl_3$) 4.86 (2H, s, N_3-CH_2), 4.93 (2H, s, N_1-CH2), 6.82–7.42 (9H, m, Ar-H), 7.49 (1H, t, H-7), 7.57 (1H, t, JH7H8 8.0 Hz, H-8), 7.81 (1H, d, JH8H9 8.0 Hz, H-9), 7.92 (1H, d, JH7H6 7.5 Hz, H-6); δ_C ($CDCl_3$) 46.51 (N_3CH_2), 56.05 (N_1CH_2), 122.91 (C-9), 124.12 (C-6), 125.48 (C-4a), 125.91 (C-7), 128.66 (C-8), 128.60, 128.79, 129.23, 129.92, 131.12, 134.93 (Ar-C), 131.67 (C-5a), 137.17 (C-9b), 140.30 (C-9a), 158.53 (C-4). Anal. for $C_{23}H_{17}N_2O_3S_2Cl$: (C, H, N, S).

3-[(4-Chlorophenyl)methyloxy]-6-[4-(chlorophenyl)methyl]-1,2,6-thiadiazin 1,1-dioxide (11) and 2,6-bis[4-(chlorophenyl)methyl]-1,2,6-thiadiazin-3(2H)-one 1,1-dioxide ***(12)***

To an equimolecular suspension of sodium hydride in DMF (25 ml) were added the thiadiazine dioxide **10** (Goya & Stud, 1978; Su *et al.*, 1981) (0.10 g, 0.6 mmol) and 4-chlorophenylmethyl chloride (0.27 g, 1.6 mmol). The reaction mixture was refluxed for 48 h. The solvent

was evaporated under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with CH_2Cl_2 (4×10 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 (2:1) as eluent. From the first fraction, derivative **11** was isolated: yield 0.02g (9%) as a white solid; mp 111–113°C.

δ_H ($CDCl_3$) 4.84 (2H, s, N6CH2), 5.26 (2H, s, OCH_2), 5.51 (1H, d, JH5H4 8.0 Hz, H-4), 6.94 (1H, d, H-5), 7.29–7.36 (8H, m, Ar-H); δ_C ($CDCl_3$) 51.29 (N_6CH_2), 69.13 (OCH_2), 92.65 (C-4), 146.03 (C-5), 128.90, 129.36, 129.92, 130.10, 132.72, 132.78, 134.79, 134.90 (Ar-C), 167.51 (C-3). Anal. for $C_{17}H_{14}N_2O_3SCl_2$: (C, H, N, S).

From the second fraction, derivative **12** was isolated: yield 0.01g (4%) as syrup.

δ_H ($CDCl_3$) 4.77 (2H, s, N_6CH_2), 4.94 (2H, s, N_2CH_2), 5.67 (1H, d, JH5H4 8.3 Hz, H-4), 6.80 (1H, d, H-5), 7.13–7.30 (8H, m, Ar-H); δ_C ($CDCl_3$) 44.77 (N_2CH_2), 52.36 (N_6CH_2), 104.82 (C-4), 139.68 (C-5), 128.73, 129.39, 129.44, 130.22, 132.06, 133.96, 134.06 (Ar-C), 161.71 (C-3). Anal. for $C_{17}H_{14}N_2O_3SCl_2$: (C, H, N, S).

General procedure for the N^1-alkylation of 3-benzyl quinazoline derivatives

To a suspension of sodium bicarbonate in excess in DMF (25 ml), N^3-benzyl quinazolinedione **13** (Singh & Bhandari, 1976) or thioxoquinazolinone **14** (Wagner & Rothe, 1969) and the corresponding alkylant agent (1.5 mmol) was added. 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 (2:1) as eluent.

1-[(4-Chlorophenyl)methyl]-3-benzyl-quinazolin-2, 4-dione ***(15)***

Reagents: quinazolinedione 13 (0.11 g, 0.4 mmol), 4-chlorophenylmethyl chloride (0.11 g, 0.6 mmol). Yield: 0.07 g (40%) as a white solid: mp 148–150°C.

δ_H ($CDCl_3$) 5.32 (2H, s, N_1-CH_2), 5.32 (2H, s, N_3-CH_2), 7.03 (1H, d, JH7H8 8.5, H-8), 7.16–7.55 (10H, m, Ar-H, H-6), 7.50 (1H, t, JH6H7 7.3, H-7), 8.24 (1H, d, JH6H5 7.9, H-5); δ_C ($CDCl_3$) 45.17 (N_3-CH_2), 46.81 (N_1-CH_2), 114.09 (C-8), 115.82 (C-4a), 123.23 (C-6), 127.66, 127.88, 128.45, 128.98, 129.14, 133.53, 134.18, 136.91 (Ar-C), 129.28 (C-5), 135.12 (C-7), 139.71 (C-8a), 151.37 (C-2), 161.61 (C-4). Anal. for $C_{22}H_{17}N_2O_2Cl$: (C, H, N).

1-[(3,4-Dichlorophenyl)methyl]-3-benzyl-quinazolin-2,4-dione ***(16)***

Reagents: quinazolinedione **13** (0.15 g, 0.6 mmol), 3,4-dichlorophenylmethyl chloride (0.17 g, 0.9 mmol). Yield: 0.05 g (21%) as a white solid: mp 85–86°C.

δ_H ($CDCl_3$) 5.21 (2H, s, N_1-CH_2), 5.24 (2H, s, N_3-CH_2), 6.98 (1H, d, JH7H8 8.1, H-8), 6.92–7.50 (10H, m, Ar-H, H-6, H-7), 8.17 (1H, d, JH6H5 7.9, H-5); δ_C ($CDCl_3$) 45.14 (N_3-CH_2), 46.34 (N_1-CH_2), 113.83 (C-8), 115.74 (C-4a), 123.38 (C-6), 125.81, 127.68, 128.45, 128.91, 129.35, 131.82, 133.12, 135.94, 136.74 (Ar-C), 130.90 (C-5), 135.23 (C-7), 139.43 (C-8a), 151.27 (C-2), 161.48 (C-4). Anal. for $C_{22}H_{16}N_2O_2Cl_2$: (C, H, N).

1-[(4-Nitrophenyl)methyl]-3-benzyl-quinazolin-2,4-dione ***(17)***

Reagents: quinazolinedione **13** (0.15 g, 0.6 mmol), 4-nitrophenylmethyl bromide (0.19 g, 0.9 mmol). Yield: 0.07 g (29%) as a yellow solid: mp 153–154°C.

δ_H ($CDCl_3$) 5.27 (2H, s, N_1-CH_2), 5.39 (2H, s, N_3-CH_2), 6.91 (1H, d, JH7H8 8.5, H-8), 7.15–8.15 (11H, m, Ar-H, H-6, H-7), 8.21 (1H, d, JH6H5 7.9, H-5); δ_C ($CDCl_3$) 45.21 (N_3-CH_2), 46.88 (N_1-CH_2), 113.71 (C-8), 115.89 (C-4a), 123.50 (C-6), 124.20, 127.27, 127.72, 128.47, 128.95, 136.77, 143.16, 147.56 (Ar-C), 129.52 (C-5), 135.24 (C-7), 139.46 (C-8a), 151.33 (C-2), 161.42 (C-4). Anal. for $C_{22}H_{17}N_3O_4$: (C, H, N).

1-{[(4-Trifluoromethyl)phenyl]methyl}-3-benzyl-quinazolin-2,4-dione ***(18)***

Reagents: quinazolinedione **13** (0.15 g, 0.6 mmol), 4-trifluoromethylphenylmethyl chloride (0.17 g, 0.9 mmol). Yield: 0.09 g (38%) as a white solid: mp 160–161°C.

δ_H ($CDCl_3$) 5.34 (2H, s, N_1-CH_2), 5.41 (2H, s, N_3-CH_2), 7.01 (1H, d, JH7H8 8.4, H-8), 7.19–7.59 (11H, m, Ar-H, H-6, H-7), 8.26 (1H, d, JH6H5 7.9, H-5); δ_C ($CDCl_3$) 45.12 (N_3-CH_2), 46.91 (N_1-CH_2), 113.91 (C-8), 115.75 (C-4a), 123.26 (C-6), 125.82 (CF3), 125.88, 126.67, 127.62, 128.40, 128.90, 136.81, 139.77 (Ar-C), 129.26 (C-5), 135.13 (C-7), 139.57 (C-8a), 151.30 (C-2), 161.49 (C-4). Anal. for $C_{23}H_{17}N_2O_2F_3$: (C, H, N).

1-[(4-Chlorophenyl)methyl]-3-benzyl-2-thioxo-quinazolin-4-one ***(19)***

Reagents: thioxoquinazolinone **14** (0.15 g, 0.5 mmol), 4-chlorophenylmethyl chloride (0.13 g, 0.7 mmol). Yield: 0.09 g (48%) as a white solid: mp 96–98°C.

δ_H ($CDCl_3$) 4.35 (2H, s, N_1-CH_2), 5.25 (2H, s, N_3-CH_2), 7.49 (1H, d, JH7H8 8.1, H-8), 7.14–7.32 (10H, m, Ar-H, H-6), 7.61 (1H, t, JH6H7 7.0, H-7), 8.15 (1H, d, JH6H5 8.0, H-5); δ_C ($CDCl_3$) 35.84 (N1-CH2), 47.25 (N_3-CH_2), 119.36 (C-4a), 125.87 (C-8), 125.97 (C-6), 127.21 (C-5), 127.45, 127.65, 128.53, 128.62, 130.62,

133.30, 135.08, 135.35 (Ar-C), 134.49 (C-7), 147.23 (C-8a), 155.55 (C-2), 161.87 (C-4). Anal. for $C_{22}H_{17}N_2OSCl$: (C, H, N, S).

1-[(3,4-Dichlorophenyl)methyl]-3-benzyl-2-thioxo-quinazolin-4-one ***(20)***

Reagents: thioxoquinazolinone **14** (0.15 g, 0.5 mmol), 3,4-dichlorophenylmethyl chloride (0.13 g, 0.7 mmol). Yield: 0.08 g (36%) as a white solid: mp 129–131°C.

δ_H ($CDCl_3$) 4.41 (2H, s, N_1-CH_2), 5.35 (2H, s, N_3-CH_2), 7.58 (1H, d, JH7H8 8.0, H-8), 7.23–7.35 (8H, m, Ar-H), 7.40 (1H, t, JH6H7 7.1, H-6), 7.71 (1H, t, H-7), 8.24 (1H, d, JH6H5 8.0, H-5); δ_C ($CDCl_3$) 35.32 (N_1-CH_2), 47.31 (N_3-CH_2), 119.46 (C-4a), 125.96 (C-8), 126.02 (C-6), 127.30 (C-5), 127.46, 127.74, 128.60, 130.38, 131.29, 131.56, 132.40, 135.35, 137.14 (Ar-C), 134.58 (C-7), 147.22 (C-8a), 155.55 (C-2), 161.87 (C-4). Anal. for $C_{22}H_{16}N_2OSCl_2$: (C, H, N, S).

1-[(4-Nitrophenyl)methyl]-3-benzyl-2-thioxo-quinazolin-4-one ***(21)***

Reagents: thioxoquinazolinone **14** (0.15 g, 0.5 mmol), 4-nitrophenylmethyl bromide (0.16 g, 0.7 mmol). Yield: 0.01 g (7%) as a yellow solid: mp 145–148°C.

δ_H ($CDCl_3$) 4.48 (2H, s, N_1-CH_2), 5.29 (2H, s, N_3-CH_2), 7.35 (1H, t, JH7H6 7.0, H-6), 7.48–8.09 (11H, m, Ar-H, H-8, H-7), 8.17 (1H, d, JH6H5 7.9, H-5); δ_C ($CDCl_3$) 35.63 (N_1-CH_2), 47.37 (N_3-CH_2), 119.53 (C-4a), 125.95 (C-8), 126.16 (C-6), 127.40 (C-5), 123.68, 127.49, 127.80, 128.64, 134.63, 135.37, 144.73 (Ar-C), 130.06 (C-7), 147.18 (C-8a), 155.28 (C-2), 161.84 (C-4). Anal. for $C_{22}H_{17}N_3O_3S$: (C, H, N, S).

1-{[(4-Trifluoromethyl)phenyl]methyl}-3-benzyl-2-thioxo-quinazolin-4-one ***(22)***

Reagents: thioxoquinazolinone **14** (0.15 g, 0.5 mmol), 4-trifluoromethylphenylmethyl chloride (0.14 g, 0.7 mmol). Yield: 0.10 g (46%) as a white solid: mp 100–102°C.

δ_H ($CDCl_3$) 4.74 (2H, s, N_1-CH_2), 5.30 (2H, s, N_3-CH_2), 7.19–7.55 (11H, m, Ar-H, H-6, H-8), 7.60 (1H, t, JH6H7 7.0, H-7), 8.19 (1H, d, JH6H5 7.9, H-5); δ_C ($CDCl_3$) 35.88 (N_1-CH_2), 47.29 (N_3-CH_2), 119.41 (C-4a), 125.45 (C-8), 125.99 (CF_3), 126.01 (C-6), 127.72 (C-5), 125.40, 127.28, 127.46, 128.58, 129.59, 135.34, 140.94 (Ar-C), 134.57 (C-7), 147.21 (C-8a), 156.65 (C-2), 161.91 (C-4). Anal. for $C_{23}H_{17}N_2OSF_3$: (C, H, N, S).

Virology

Cells

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

Viruses

AD-169 strain of HCMV 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 the 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 proliferate for 24 h. Different concentrations of the test compounds were then added (in duplicate), and after 8 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%.

Results

The thienotiadiazine **1** (Martinez *et al.*, 2000a) and benzothienothiadiazine **2** (Martinez *et al.*, 2000d) dioxide rings were prepared following a synthetic strategy that involves the cyclocondensation of the corresponding functionalised heterocycles with sulphamoyl chloride as the key step.

Benzyl thienothiadiazine derivatives (**3–4**) were obtained by reaction of thienothiadiazine 1 with the appropriate benzyl chloride derivatives in aqueous bicarbonate. Disubstituted compounds (**5–6**) could be obtained by reaction of the corresponding monosubstituted derivatives with benzyl bromide in a polar non-protic solvent such as dimethylformamide (DMF), using sodium hydride (NaH) as base (Figure 2). The 4-chlorophenylmethyl and 3,4-dichlorophenylmethyl substituents attached to the N1, and benzyl substituent attached to the N_3 of these heterocycles,

were chosen because of their good anti-HCMV activity previously shown in the BTD series.

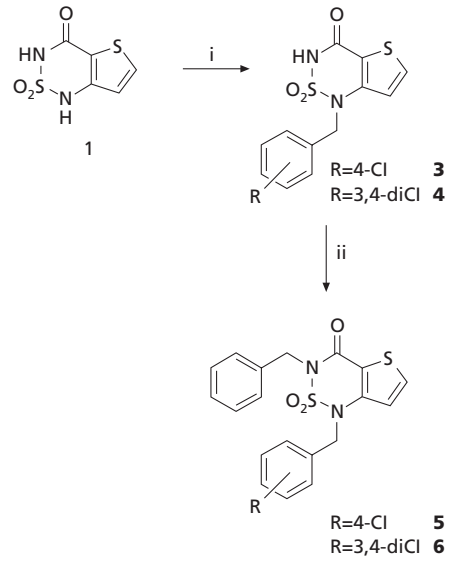
In the same way, we prepared the benzothienothiadiazine derivatives (**7–9**). In this case, a mixture of monoalkyl and dialkyl compounds was obtained when we alkylated **2** with 4-chlorobenzylchloride in aqueous bicarbonate. This could be separated by silica gel column chromatography (Figure 3).

The 1,2,6-thiadiazine 1,1-dioxide **10**, a SO_2-uracil related derivative in which the fused ring is eliminated, was prepared in four steps according to a procedure previously described (Goya & Stud, 1978; Su *et al.*, 1981). Disubstituted compounds (**11–12**) were obtained by alkylation with 4-chlorophenylmethyl chloride in DMF and NaH (Figure 4).

For the preparation of 1H,3H-quinazoline-2,4-diones and ^{1}H,^{3}H-quinazoline-2-thio-4-one derivatives, an unambiguous synthetic pathway was planned to obtain N_3-benzyl derivatives. We choose as starting material **13** and **14** unequivocally prepared from methylanthranilate and benzylisocyanate or benzylisothiocyanate, respectively (Wagner & Rothe 1969; Singh & Bhandari, 1976). Alkylation of compounds **13** and **14** in basic medium (DMF, $NaHCO_3$) afforded the required *N,N'*-disubstituted derivatives **15–22** (Figure 5).

The structure of all new compounds was elucidated from their analytical and spectroscopic data (^{1}H and ^{13}C NMR), which are collected in the experimental section. Unequivocal assignment of all chemical shifts (^{1}H and ^{13}C NMR) was done using bidimensional experiments such as COSY or HMQC for one bond correlation. The site of alkylation was determined from the chemical shifts of benzylic CH_2 signals and by means of n.O.e. experiments and sequences of HMBC for long distance proton/carbon correlation.

Figure 2. Synthesis of thienothiadiazine dioxide derivatives



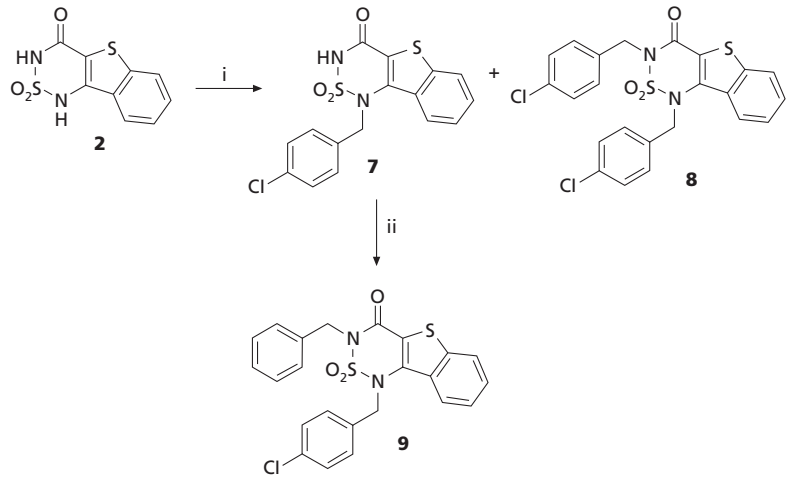
Reagents: (i) $RPhCH_2Cl/NaHCO_3/\Delta$; (ii) BrBn/DMF/NaH/Δ.

Discussion

The new disubstituted derivatives here described, were evaluated for their antiviral activity against the laboratory strain of HCMV AD-169. Antiviral activity was determined by plaque reduction assay in confluent human embryonic lung MRC-5 fibroblast. Cytotoxicity measurements were based on the inhibition of cell growth. The results are presented in Table 1.

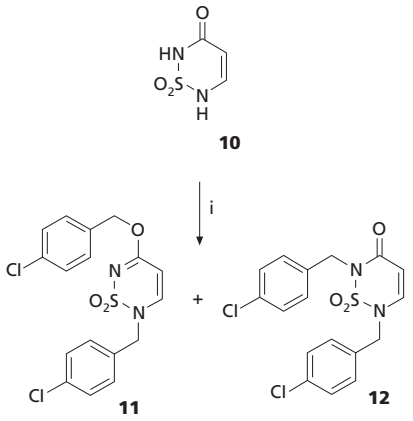
The thieno and benzothienothiadiazine derivatives (**5**, **6**, **8** and **9**), maintain antiviral activity against HCMV,

Figure 3. Synthesis of benzothienothiadiazine dioxide derivatives



Reagents: (i) $4\text{-}ClPhCH_2Cl/NaHCO_3/\Delta$; (ii) BrBn/DMF/NaH/$\Delta$.

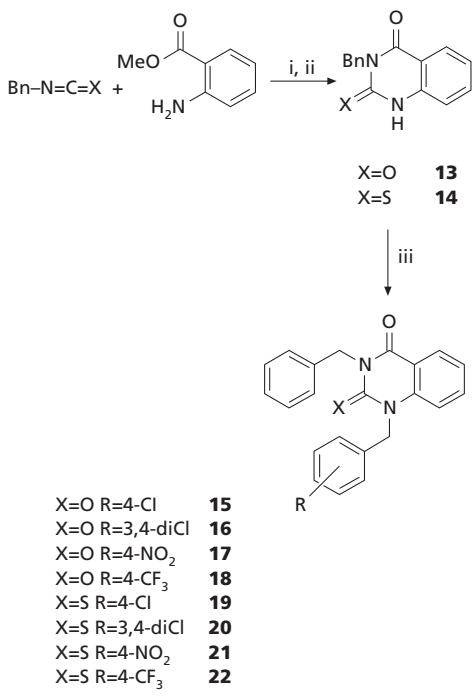
Figure 4. Synthesis of 1,2,6-thiadiazine 1,1-dioxide derivatives



Reagents: (i) 4-ClPhCH_2Cl/DMF/NaH/Δ.

being some of them equipotent with the standard reference ganciclovir. The IC_{50} values were in the same order than those previously obtained for their BTD analogues (Martinez *et al.*, 2000b) collected in Table 1, but now the CC_{50} value is slightly lower showing compounds a little more cytotoxic. Otherwise, in the 1,2,6-thiadiazine 1,1-dioxide derivatives (**11,12**) we observed increase both in the IC_{50} value and in cytotoxicity. From these results, we can conclude

Figure 5. Synthesis of quinazoline derivatives



Reagents: (i) Toluene, 10°C; (ii) NaOH 6N; (iii) RPhCH_2Cl/$NaHCO_3$/Δ.

Table 1. Anti-HCMV (strain AD-169) activity of thiadiazine dibenzyl derivatives

Compound	IC_{50} (μM)*	CC_{50} (μM)†
5	8.3	59.6
6	4	55
8	3.9	49.6
9	12.7	53.3
11	12.5	25.1
12	12.5	25.1
Ganciclovir	5.9	>98
di4Cl-BTD §	3.3	>56
4Cl-BTD §	3.6	>60

*50% inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. Assays were performed in triplicate.
† 50% cytotoxic concentration, or concentration required to reduce cell growth by 50%. Assays were performed in triplicate.
§ Martinez *et al.*, 2000b.

that the HCMV receptor for the BTD, tolerates modifications on the nature and the number of the fused heterocycles, but does not tolerate the lack of it.

When the anti-HCMV activity of quinazoline derivatives (**15–22**) were measured, we observed a high cytotoxic effect for all the compounds at the concentrations assayed (CC_{50}<5 μM) discarding this new series of compounds from any further development. This fact revealed that the sulfamido moiety is a key structural feature for cytotoxicity modulation. The lack of planarity present in the thiadiazine ring (Elguero *et al.*, 1990) regarding their oxo or thioxo analogues here described could provide a different binding mode to the cell and viral targets, respectively.

As a result of structural modifications carried out in the BTD main heterocycle, some structural requirements have been discovered that could help to modulate potency/toxicity of these derivatives. Two important structure-activity relationships have been delineated: i) a fused ring to the thiadiazine framework is necessary to maintain the anti-HCMV, action and ii) the sulfamido moiety in the main heterocycle is crucial to avoid cytotoxicity. Further research is in progress to determine the crucial HCMV receptor in which the BTD family of inhibitors exert its specific antiviral action.

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AM Bruno's permanent address: Centro de Síntesis y Estudio de Nuevos Compuestos Antineoplásicos, Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina.

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