Antiviral Chemistry & Chemotherapy

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 develop 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 drug-resistant strains (Martinez et al., 1999c). 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 dominant 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...

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

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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, 2000).

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 develop 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 drug-resistant strains (Martinez et al., 1999c). 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 dominant 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...
explore the steric’s 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 eliminated. The three fused system as the benzothienothiadiazine was assayed as potential HCMV inhibitors. On the other hand, to assess 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, 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, data size 32 K. Chemical shifts are reported in δ values (ppm) relative to internal Me4Si and J values are reported in Hz. 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 CH2Cl2 (1x10 ml). The aqueous phase was cooled at –4°C and the product was isolated by filtration of aqueous phase. Purification: recrystallization from toluene/MeOH, yield 0.28 g (60 %) as a solid; mp 325–327°C.

$\delta^1$H (DMSO-d6) 4.89 (2H, s, NCH2), 6.58 (1H, d, JH6H7 5.3 Hz, H-7), 7.40–7.46 (4H, m, Ar-H), 7.57 (1H, d, H-6);
$\delta^{13}$C (DMSO-d6) 47.81 (NCH2), 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 C12H9N2O3S2Cl (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 CH2Cl2 (1x10 ml). The aqueous phase was cooled at –4°C and the product was isolated by filtration of aqueous phase. Purification: recrystallization from toluene/MeOH, yield 0.40 g (56 %) as a solid; mp 246–248°C.

$\delta^1$H (DMSO-d6) 4.89 (2H, s, NCH2), 6.69 (1H, d, JH6H7 5.1 Hz, H-7), 7.39–7.57 (3H, m, Ar-H), 7.55 (1H, d, H-6);
$\delta^{13}$C (DMSO-d6) 47.40 (NCH2), 114.93 (C-4a), 117.00 (C-7), 127.59, 129.15, 129.60, 129.53, 129.55 (C-6), 144.81 (C-7a), 162.62 (C-4). Anal. for C12H8N2O3S2Cl2 (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$_2$Cl$_2$ (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$_2$Cl$_2$:hexane (1:1) as eluent. Compound 5 was obtained (0.006 g, 7%) as a solid; mp 85–86°C.

δ$_{6}$(CDCl$_3$) 4.81 (2H, s, N$_1$CH$_2$), 4.97 (2H, s, N$_3$CH$_2$), 6.70 (1H, d, $J_{1H2-2H3}$ Hz, H-3), 6.97–7.36 (9H, m, Ar-H), 7.57 (1H, d, $J_{1H2-2H3}$ Hz, H-2), $\delta$ (CDCl$_3$) 46.11 (N$_1$CH$_2$), 54.52 (N$_3$CH$_2$), 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$_{14}$N$_2$O$_3$S$_2$Cl$_2$: (C, H, N, S).

The residue was chromatographed on silica gel column, using CH$_2$Cl$_2$:MeOH (50:1) as eluent. From the first fraction was isolated derivative 6: yield 0.01 g (2%) as a solid; mp 158–160°C.

δ$_{6}$(CDCl$_3$) 4.69 (2H, s, N$_1$CH$_2$), 4.94 (2H, s, N$_3$CH$_2$), 6.81–7.34 (8H, m, Ar-H), 7.31 (1H, t, $J_{1H2-2H3}$ Hz, H-7), 7.58 (1H, d, $J_{1H2-2H3}$ Hz, H-2), 7.82 (1H, d, $J_{1H2-2H3}$ Hz, H-9), 7.93 (1H, d, $J_{1H2-2H3}$ Hz, H-6), $\delta$ (CDCl$_3$) 45.62 (N$_1$CH$_2$), 56.09 (N$_3$CH$_2$), 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, 131.81, 134.25, 135.09 (Ar-C), 131.57 (C-5a), 137.15 (C-9b), 140.35 (C-9a), 158.47 (C-4). Anal. for C$_{19}$H$_{15}$N$_2$O$_3$S$_2$Cl: (C, H, N, S).

From the second fraction, derivative 7 was isolated: yield 0.13g (30%) as a white solid; mp 210–212°C.

δ$_{6}$(CDCl$_3$) 5.02 (2H, s, N$_1$CH$_2$), 7.25–7.30 (5H, m, Ar-H, H-7), 7.37 (1H, t, $J_{1H2-2H3}$ Hz, H-8), 7.68 (1H, d, $J_{1H2-2H3}$ Hz, H-6), $\delta$ (CDCl$_3$) 51.94 (N$_1$CH$_2$), 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$_{23}$H$_{16}$N$_2$O$_3$S$_2$Cl: (C, H, N, S).

To an equimolecular suspension of sodium hydride in DMF (25 ml) were added thienothiadiazine dioxide (50:1) as eluent. Compound 6 was obtained (0.02 g, 18%) as a solid; mp 102–104°C.

δ$_{6}$(CDCl$_3$) 4.78 (2H, s, N$_1$CH$_2$), 4.98 (2H, s, N$_3$CH$_2$), 6.70 (1H, d, $J_{1H2-2H3}$ Hz, H-7), 7.18–7.36 (9H, m, Ar-H), 7.60 (1H, d, H-6), $\delta$ (CDCl$_3$) 46.20 (N$_1$CH$_2$), 53.83 (N$_3$CH$_2$), 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$_2$O$_3$S$_2$: (C, H, N, S).

1-(3,4-Dichlorophenyl)(methyl)-3-benzyl-thieno[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 saturated solution of sodium bicarbonate (20 ml), 4-chlorophenyl(methyl) 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$_2$Cl$_2$ (4x10 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$_2$Cl$_2$: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.
was evaporated under reduced pressure. The residue was dissolved in water and the aqueous phase was extracted with CHCl₃ (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 CH₂Cl₂:hexane (2:1) as eluent. From the first fraction, derivative 11 was isolated; yield 0.02 g (4%) as a white solid; mp 111–113°C.

δ₂ (CDCl₃) 4.84 (2H, s, N₆CH₂), 5.26 (2H, s, OCH₂), 5.51 (1H, d, JHH₄ 8.0 Hz, H-4), 6.94 (1H, d, H-5), 7.20–7.36 (1H, m, Ar-H); δ₁ (CDCl₃) 51.29 (N₃CH₂), 69.13 (OCH₂), 92.65 (C-4), 146.03 (C-5), 128.90, 129.36, 129.92, 130.10, 132.72, 134.79, 139.40 (Ar-C), 161.71 (C-3). Anal. for C₁₇H₁₄N₂O₃SCl₂: (C, H, N, S).

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

δ₂ (CDCl₃) 4.77 (2H, s, N₆CH₂), 4.94 (2H, s, N₂CH₂), 5.67 (1H, d, JHH₄ 8.3 Hz, H-4), 6.80 (1H, d, H-5), 7.14–7.30 (8H, m, Ar-H, H-6), 7.50 (1H, t, JHH₆H₇ 7.3, H-7), 8.24 (1H, d, JHH₆H₅ 8.0, H-5), 8.26 (1H, t, JHH₆H₇ 7.3, H-7), 8.26 (1H, d, JHH₆H₅ 8.0, H-5), 8.44 (1H, t, JHH₆H₇ 7.3, H-7), 8.24 (1H, d, JHH₆H₅ 8.0, H-5), 8.26 (1H, t, JHH₆H₇ 7.3, H-7), 8.24 (1H, d, JHH₆H₅ 8.0, H-5), 8.26 (1H, t, JHH₆H₇ 7.3, H-7).

General procedure for the N₁-alkylation of 3-benzyl quinoxaline derivatives

To a suspension of sodium bicarbonate in excess in DMF (25 ml), N₁-benzyl quinoxalinedione 13 (0.15 g, 0.6 mmol) and the corresponding alkylating 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 CHCl₃ (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 CH₂Cl₂:hexane (2:1) as eluent.

1-[4-Chlorophenyl][methyl]-3-benzyl-2-thioxoquinazolin-4-dione (15)

Reagents: quinoxalinedione 13 (0.15 g, 0.6 mmol), 4-chlorophenylmethyl chloride (0.11 g, 0.6 mmol). Yield: 0.07 g (40%) as a white solid: mp 148–150°C.

δ₂ (CDCl₃) 5.32 (2H, s, N₁CH₂), 5.32 (2H, s, N₃CH₂), 7.03 (1H, d, JHH₄ 8.5, H-4), 7.16–7.55 (10H, m, Ar-H, H-6), 7.50 (1H, t, JHH₆H₇ 7.3, H-7), 8.24 (1H, d, JHH₆H₅ 7.9, H-5), δ₁ (CDCl₃) 45.17 (N₆CH₂), 46.81 (N₃CH₂), 114.09 (C-8), 115.82 (C-4a), 123.25 (C-6), 127.16, 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 C₁₇H₁₄N₃O₂Cl: (C, H, N).
133.30, 135.08, 135.35 (Ar-C), 134.49 (C-7), 147.23 (C-8), 155.55 (C-2), 161.87 (C-4). Anal. for C_{22}H_{17}N_{2}O_{3}S: (C, H, N, S).

1-[(3,4-Dichlorophenyl)methyl]-3-benzyl-2-thioxo-quinazolin-4-one (20)
Reagents: thioquinoxaline 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.

\[ \delta_{\text{H}} (\text{CDCl}_3) 4.41 (2H, s, N 1-CH_2), 5.25 (2H, s, N 3-CH_2), 7.59 (1H, d, J_{HH} 8.0, H-8), 7.23–7.35 (3H, m, Ar-H), 7.40 (1H, t, J_{HH} 7.1, H-6), 7.71 (1H, t, H-7), 8.24 (1H, d, J_{HH} 8.6, H-5), \delta_{\text{C}} (\text{CDCl}_3) 35.88 (N 1-CH_2), 47.29 (N 3-CH_2), 119.41 (C-4a), 125.96 (C-8), 126.01 (C-6), 127.72 (C-5), 127.85, 128.60, 130.38, 131.29, 131.56, 132.40, 135.35, 137.14 (Ar-C), 144.73 (Ar-C), 147.18 (C-8a), 155.28 (C-2), 161.84 (C-4).

Anal. for C_{22}H_{17}N_{2}O_{3}S: (C, H, N, S).

1-[(4-Nitrophenyl)methyl]-3-benzyl-2-thioxo-quinazolin-4-one (21)
Reagents: thioquinoxaline 14 (0.15 g, 0.5 mmol), 4-nitrophénylmethyl bromide (0.16 g, 0.7 mmol). Yield: 0.01 g (7%) as a yellow solid: mp 145–148°C.

\[ \delta_{\text{H}} (\text{CDCl}_3) 4.48 (2H, s, N 1-CH_2), 5.30 (2H, s, N 3-CH_2), 7.35 (1H, J_{HH} 7.0, H-6), 7.48–8.09 (11H, m, Ar-H), 8.17 (1H, d, J_{HH} 7.9, H-5), \delta_{\text{C}} (\text{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, 128.60, 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_{2}O_{3}S: (C, H, N, S).

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 10% inactivated fetal calf serum and 1% l-glutamine.

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.
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, a SO₂-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 1H,3H-quinazoline-2-thio-4-one derivatives, an unambiguous synthetic pathway was planned to obtain N₁-benzyl derivatives. We chose 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, NaHCO₃) 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 (1H and 13C NMR), which are collected in the experimental section. Unequivocal assignment of all chemical shifts (1H and 13C 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 CH₂ signals and by means of n.O.e. experiments and sequences of HMBC for long distance proton/carbon correlation.

**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,
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being some of them equipotent with the standard reference ganciclovir. The IC50 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 CC50 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 IC50 value and in cytotoxicity. From these results, we can conclude 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 (CC50<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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