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Chlorophenylmethyl Benzothiadiazine Dioxides Derivatives: Potent Human Cytomegalovirus Inhibitors

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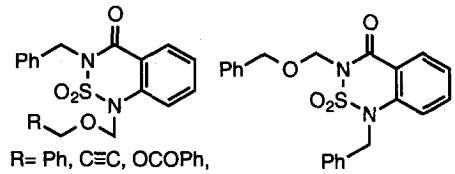
Abstract- Modifications of our previously reported lead compounds, acyclonucleosides derived from 2,1,3-benzothiadiazine dioxides, in the search for inhibitors of human cytomegalovirus (HCMV), lead us to identify the chlorophenylmethyl benzothiadiazine dioxides derivatives as potent HCMV inhibitors. The synthesis and antiviral data of this second-generation of benzothiadiazine dioxide compounds are reported.

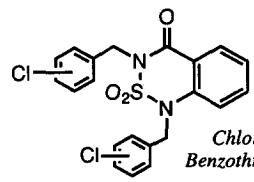
The human cytomegalovirus (HCMV), a highly prevalent member of herpesvirus family, is responsible for congenitally acquired infection and disease as well as significant morbidity and mortality among immunocompromised individuals, including transplant recipients and AIDS patients.[1]

The two most widely used agents for the treatment of HCMV are ganciclovir and foscarnet.[2,3] Cidofovir,[4] a new drug against HCMV, has recently been approved for use in the USA. Unfortunately, toxicity associated with these agents, poor oral bioavailability,[5] high relapse rates, and drug resistance[6] have made their use less than optimal.

We have recently discovered a new family of HCMV inhibitors: the 2,1,3-benzothiadiazine dioxide modified acyclonucleosides.[7] The preliminary structure-activity analysis of these compounds pointed to the necessity of a double substitution in the heterocycle together with the lipophilicity in the acyclic side chain.[8] These factors were considered when preparing this second-generation of benzothiadiazine dioxide derivatives here reported.



Benzothiadiazine Dioxides Acyclonucleosides



Chlorophenylmethyl Benzothiadiazine Dioxides

In order to assess the influence of the acyclo chain and the benzyl moiety in the antiviral activity of these compounds, dibenzyloxy and dibenzyl derivatives **1** and **2** were synthesized (Scheme 1) using experimental reaction conditions previously optimized by our group.[8] It was also possible to isolate the *N,O*-dibenzyl compound **3** by chromatography techniques. Moreover, some modified acyclonucleosides with chlorophenylmethyl fragments, **4** and **5,** were obtained following the silylation procedure.[9]

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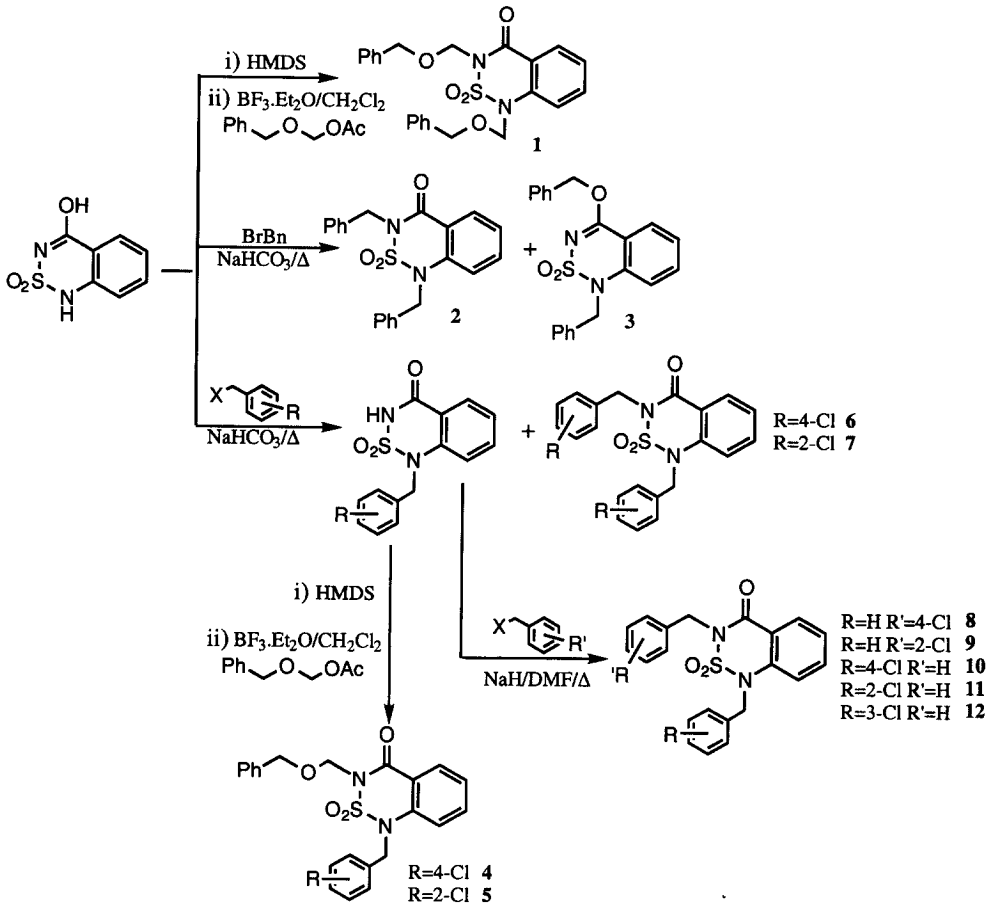
Chlorophenylmethyl derivatives **8-12** were obtained in a two step synthesis starting from 4-hydroxy-2,1,3-benzothiadiazine dioxide, following a method recently described.[10] Considering the different acidity of the two nitrogen atoms,[11] the first chlorophenylmethyl fragment was introduced in aqueous sodium bicarbonate, while sodium hydride was necessary for the benzylation of the second position (Scheme 1). Although, in the $NaHCO_3$ medium, the major compounds were the *N*-monobenzyl derivatives, after chromatographic techniques, the *N,N*-dialkylated compounds **6** and **7** could also be obtained.



Scheme 1

The new benzothiadiazine derivatives here reported (**1-12**) were evaluated for their activity against the laboratory strain of HCMV AD-169, plaque reduction assay in confluent human embryonic lung MRC-5 fibroblasts. Cytotoxicity measurements were based on the inhibition of cell growth and was in all cases > 25 $\mu g.ml^{-1}$. All the compounds exhibit a potent antiviral activity against HCMV, with IC_{50} values similar to that of the standard reference ganciclovir (Table 1). In the great majority of the cases, the activity against HCMV is shown at concentrations that were >10-fold lower than the concentration that was toxic for the host cells, so that, this activity may qualify as a specific antiviral effect. The compounds are relatively unstable hydrolytically and after 24 days, repetition of the antiviral assay with the testing media previously prepared, lead to the same

value of IC_{50} but a considerable increase of the CC_{50}. The structure of these active compounds are quite unique not only for the nature of the heterocyclic base, but also because of the lack of the 5'-OH mimetic group present in ganciclovir and others current anti-CMV drugs, which points to a different mechanism of action. These results allowed us to classify the chlorophenylmethyl derivatives of benzothiadiazine dioxides as new HCMV inhibitors.

First structure-activity relationships conclusions show the benzyl or chlorophenylmethyl moiety more efficient for the antiviral activity of this family of compounds than the benzyloxymethyl one (compounds **1**, **4** and **5** *versus* **2**, **3**, **6-12**). Additionally, the presence of the chlorine atom in the phenyl nucleous lead to more potent HMCV-inhibitors. This substitution is specially favourable in the *para* position of the aromatic ring.

Additionally, in order to assess the potential of the new compounds for treatment of human diseases, compound **10** was selected to evaluate its antiviral activity on three HCMV-clinical isolates. These wild strains were isolated from patients with different clinical conditions: congenital infection (strain A), mononucleosis syndrome in an immunocompetent child (strain B), and AIDS (strain C). In all cases, the chlorophenylmethyl derivative **10** assayed shows an effective antiviral inhibition (Table 2).

Table 1.- Anti-HCMV (strain AD-169) activity of chlorophenylmethyl benzothiadiazines **1-12**

Comp.	IC_{50} (μM)[a]	CC_{50} (μM)[b]
1	34	>57
2	5.2	>66
3	7.9	>66
4	6.7	>56
5	18	>56
6	3.6	>60
7	3.6	>60
8	6.0	>60
9	12.1	>60
10	3.6	>60
11	4.8	>60
12	3.6	>60
ganciclovir	5.9	>98

[a] 50% inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. Assays were performed in duplicate. [b] 50% cytotoxic concentration, or concentration required to reduce cell growth by 50%. Assays were performed in duplicate.

Table 2.- Anti-HCMV activity of compound **10** against clinical isolates of human origin

CMV-strain	IC_{50} (μM)[a]	
	ganciclovir	**10**
A	5.9	2.4
B	3.9	1.2
C	3.9	2.4

[a] 50% inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. Assays were performed in duplicate.

Moreover, the determination of the mechanism of action has been initiated. Initially, compound **10** was assayed in HCMV AD-169 artificially made resistant to ganciclovir (upon repeated passage in ganciclovir-containing medium), showing an IC_{50} = 3 μM while ganciclovir shows a IC_{50} = 31.5 μM in the same assay. This antiviral activity found, 10-fold more potent than the standard, confirm that this new family of HCMV-inhibitors exerted their viral inhibition by a different biological mechanism of action than ganciclovir.

According to all these data, we can conclude that the chlorophenylmethyl benzothiadiazine dioxide derivatives are promising candidate drugs for the chemotherapy of HMCV infection including for the actual

ganciclovir resistant strains. Further studies are in progress to understand the mechanism and target of interaction of these novel compounds, including the possible inhibition of herpes protease.

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