Benzothiadiazine Dioxide Dibenzyl Derivatives as Potent Human Cytomegalovirus Inhibitors: Synthesis and Comparative Molecular Field Analysis

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The benzothiadiazine dioxide (BTD) derivatives are potent nonnucleoside human cytomegalovirus (HCMV) inhibitors. As part of our comprehensive structure−activity relationship study of these compounds, we have now synthesized *N*,*N*- and *N*,*O*-dibenzyl derivatives with different *para*-substituents (alkyl, phenyl, electron-donating, electron-withdrawing) in the phenyl ring of the benzyl moieties. The antiviral activity against HCMV (AD-169 strain) was also experimentally measured showing IC_{50} values between 2.5 and 50 μM. Comparative molecular field analysis (CoMFA) was employed to generate a model, based upon 32 diverse BTD derivatives, to delineate structural and electrostatic features important for enhanced activity against HCMV. The steric (van der Waals) interactions with the receptor majoritary describes the variation in antiviral activity among the inhibitors. Finally, the CoMFA model was used to design two sets of novel BTD derivatives. Synthesis and subsequent anti-HCMV evaluation of these compounds enabled us to maintain the activity of this new kind of HCMV inhibitors.

Introduction

Human cytomegalovirus (HCMV) has been recognized as one of the most important pathogens in immunocompromised hosts, particularly transplant recipients and patients with acquired immune-deficiency syndrome (AIDS).[1,2] HCMV infection gives little, if any, complications in immunocompetent individuals but can cause serious disease in infants infected before birth.[3]

The chemotherapy of HCMV has entered a new area. In recent years, various compounds have been described which achieve a selective inhibition of HCMV replication by inhibition of the viral DNA polymerase. Ganciclovir,[4] foscarnet,[5] cidofovir,[6] and recently fomivirsen[7] have been approved for the treatment of this viral infection. Unfortunately, toxicity associated with these drugs, poor oral bioavailability, and high relapse rates have made their use less than optimal.[8] In addition, concomitant with the increased use of antiviral drugs, an increased emergence of drug-resistant HCMV strains has been reported.[9] Considering the severity of HCMV infections in immunocompromised patients, more effective and/or less toxic drugs which act by new mechanisms to circumvent resistance are still required.

In our search for antiviral agents,[10,11] we have recently discovered new leads for the development of drugs against HCMV and Varicella-Zoster virus (VVZ) infection.[12] The benzothiadiazine dioxide (BTD) modified acyclonucleosides showed a marked activity against both viruses.[13] The structure of these compounds is quite unique, not only for the nature of the heterocyclic base but also for the lack of the 5′-OH mimetic group present in ganciclovir and other current anti-HCMV drug, which points to a different mechanism of action. Preliminary structure−activity analysis (SAR) showed the necessity of a double substitution in the heterocycle together with the lipophilicity in the acyclic side chain. These factors were considered in the first optimization step performed on this family of compounds leading to the chlorophenylmethyl BTD derivatives as potent nonnucleoside HCMV inhibitors active against some actual drugs resistant strains.[14]

As part of our comprehensive SAR study of those BTD derivatives, we have now synthesized *N*,*N*- and *N*,*O*-dibenzyl derivatives with different *para*-substituents (alkyl, phenyl, electron-donating, electron-withdrawing) in the phenyl ring of the benzyl moieties. The antiviral activity against HCMV (AD-169 strain) was also experimentally measured.

To obtain further insights into the structural requirements for the biological activity of BTD as new anti-HCMV drugs, we have employed the comparative molecular field analysis (CoMFA) method. The CoMFA methodology is based in the assumption that the interaction between an inhibitor and the enzyme (receptor) is primarily noncovalent in nature and shape-dependent and identifies the quantitative influence on potency of specific chemical features at particular regions in space.[15] One advantage of this approach when the 3D structure of the receptor is unknown is the graphical representation of the results of the analysis as 3D grids where the steric and electrostatic contributions of the activities are displayed.[16] The CoMFA model here derived was then used to design two different novel

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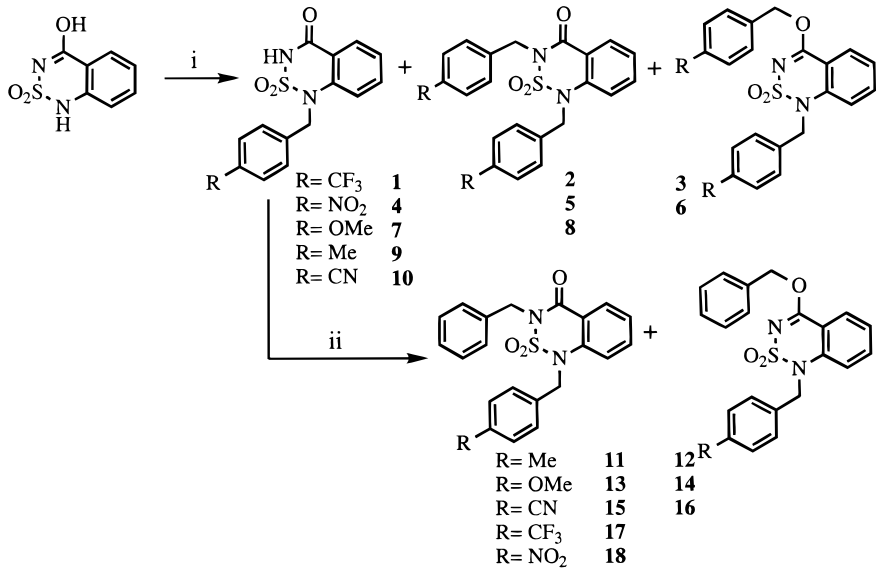
Scheme 1[a]



[a] Reagents: (i) $H_2O/NaHCO_3/R\text{-}C_6H_4\text{-}CH_2X$ (X = Cl or Br); (ii) $DMF/NaH/C_6H_5\text{-}CH_2Br$.

sets of compounds. One of this new series has been proved to be more potent HCMV inhibitors than those previously prepared.

Chemistry

On the basis of our preliminary results,[13,14] *N*,*N*- or *N*,*O*-dibenzyl BTD derivatives **2, 3, 5, 6**, and **8** were synthesized. The new disubstituted derivatives was obtained by reaction of 4-hydroxy-2,1,3-benzothiadiazine dioxide[17] with 2 equiv of the corresponding *para*-substituted benzyl halides in aqueous bicarbonate (Scheme 1). In these conditions, a mixture of mono- and disubstituted compounds were obtained. *N*,*N*-Dialkylation predominated in all cases here assayed over the *N*,*O*-disubstitution, as we have previously observed for di(biphenylmethyl) derivatives.[18,19] These compounds could be separated and isolated by circular thin-layer chromatography.

As the chlorophenylmethyl BTD family[14] showed an increase in the biological activity with substitution in the *para*-position of the benzyl group attached to *N*1, while no chlorine atom in the benzyl group linked to *N*3 enhanced the antiviral inhibition, the benzyl moiety was introduced in the *N*3 position of the benzothiadiazine ring starting from the *N1*-monobenzyl BTD derivatives **1, 4, 7, 9,** and **10**. In this case, benzylation took place in a polar, nonprotic solvent such as DMF in the presence of a strong, nonnucleophilic base such as NaH (Scheme 1). *N*,*N*-Dibenzyl derivatives **11, 13, 15, 17,** and **18** were obtained in good yields although traces of *N*,*O*-dialkylation was also observed. In some cases, the isolation and characterization of these minor compounds **12, 14,** and **16** were possible.

The structures of all new compounds were elucidated from their analytical and spectroscopic data (^1H and ^{13}C NMR) which are collected in Table 1 and in the Experimental section. Unequivocal assignment of all chemical shifts (^1H 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 NOE experiments and sequences of HMBC for long distance proton/carbon correlation. Thus, *N*1-CH_2 correlated exclusively with the quaternary carbon C-8a, while *N*3-CH_2 or *O*-CH_2 correlated with the heterocyclic carbon C-4. In this last case, a deshielding in both proton and carbon signals is observed (Table 1), which additionally confirmed the *O*-substitution.

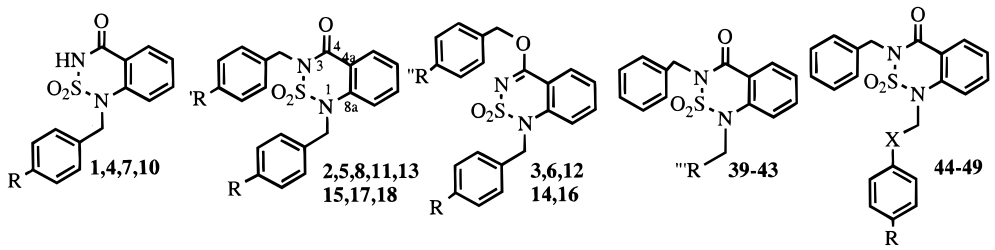
Biological Results

The new *N*,*N*- and *N*,*O*-dibenzyl BTD derivatives **2, 3, 5, 6, 8,** and **11**−**18** here described were evaluated for their activity against the laboratory strain of HCMV AD-169. Antiviral activity was determined by 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 >50 μM. Most of the compounds exhibit antiviral activity against HMCV, some of them being equiactive with the standard reference ganciclovir (Table 2). The activity against HCMV is shown at concentrations that were 3−10-fold lower than the concentration that was toxic for the host cells, which confirmed that *N*,*N*-dibenzyl BTD derivatives show a specific antiviral effect against HCMV.[20] However, the *N*,*O*-dibenzyl derivatives show IC_{50} values similar to CC_{50} results. It is worth mentioning that monobenzyl BTD derivatives were also evaluated as anti-HCMV agents, but no inhibition was found confirming that double heterocyclic substitution is indispensable for antiviral action.

The antiviral data of biphenylmethyl BTD derivatives **19**−**23** previously synthesized[18,19] are gathered in Table 2. We can note that despite the smaller size of the viral plaque observed in these assays, the IC_{50} values determined for these compounds are greater than those of ganciclovir. Steric factors and/or liphophilic factors can be responsible for this decrease in the antiviral activity.

3D-QSAR Studies. For this work, we have selected 32 *N*,*N*- and *N*,*O*-dibenzyl BTD derivatives whose anti-HCMV activity was measured in our laboratory (Table 2). All the compounds were initially modeling with the Tripos force field,[21] using the default bond distances and angles and Gastaiger−Marsili charges.[22] Since the

Table 1. Some Representative ^1H and ^{13}C NMR Data (chemical shifts in $CDCl_3$) of New BTD Derivatives



compd	R	R′	R″	R‴	X	*C*4	*C*4a	*C*8a	*N1*CH_2	*N3*CH_2	*O*CH_2	*N1*CH_2	*N3*CH_2	*O*CH_2
1	CF_3					165.64	119.64	141.96	46.11			5.06		
2	CF_3	CF_3				162.33	122.31	138.06	55.37	45.90		4.96	5.04	
3	CF_3		CF_3			165.61	112.37	138.121	49.13		69.72	5.25		5.56
4	NO_2					165.63	119.68	141.88	46.10			5.09		
5	NO_2	NO_2				161.81	122.01	139.56	55.39	45.66		4.96	5.04	
6	NO_2		NO_2			165.00	112.40	141.09	49.01		69.15	5.29		5.63
7	OMe					165.81	119.49	142.17	45.89			4.89		
8	OMe	OMe				162.09	123.19	139.71	56.17	46.04		4.84	4.96	
10	CN					165.67	119.60	141.87	46.28			5.03		
11	Me	H				162.15	122.88	139.83	56.09	46.54		4.79	4.93	
12	Me		H			164.73	112.77	140.93	49.16		70.74	5.14		5.48
13	OMe	H				162.14	123.17	139.79	55.17	46.52		4.76	4.92	
14	OMe		H			165.70	112.47	142.96	48.82		70.75	5.14		5.48
15	CN	H				161.83	122.34	139.40	55.19	46.42		4.88	4.97	
16	CN		H			165.76	111.27	142.57	49.04		71.05	5.24		5.50
17	CF_3	H				161.98	122.47	138.34	56.12	46.52		4.89	4.98	
18	NO_2	H				162.11	123.59	139.89	56.19	46.57		4.79	4.90	
39				C_6H_{11}		162.34	121.87	140.67	57.38	46.44		3.51	5.05	
40				1−naphthyl		162.31	122.45	139.98	54.79	46.57		5.19	5.13	
41				isopropyl		162.31	121.90	140.48	58.68	46.50		3.52	5.10	
42				propargyl		162.22	124.10	138.98	43.05	46.91		4.43	5.03	
43				3-pyridyl		161.81	122.87	139.38	53.72	46.46		4.86	4.98	
44	H				CH_2	162.18	122.68	139.86	54.07	46.36		3.99	5.00	
45	OMe				CH_2	162.23	122.71	140.03	54.24	46.40		3.89	4.96	
46	H				CH_2CH_2	162.28	122.54	139.98	52.01	46.38		3.77	5.10	
47	H				CO	162.32	122.69	139.84	56.62	47.00		5.30	5.16	
48	OMe				CO	162.35	122.56	139.91	56.20	46.98		5.17	5.06	
49	Me				CO	162.34	122.54	139.79	56.50	46.97		5.20	5.06	

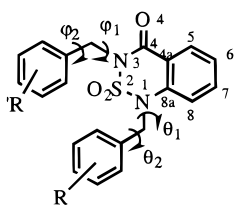
benzyl group orientation to the BTD heterocycle could be important for antiviral activity, we designated the torsional angles θ_1, θ_2 and φ_1, φ_2 or ϕ_1, ϕ_2, which rotate the benzyl groups, for conformational searching. To discern the conformational surface of dibenzyl BTD derivatives, we utilized GRIDSEARCH to rotate the four angles previously described over 360° in 30° increments. The low-energy conformation obtained was fully geometrically optimized with the Tripos force field. Geometry thus obtained for dibenzyl derivatives **24** and **25** was identical to that resulted from an ab initio calculation at the 6-31G* level with full optimization of the geometric parameters, so molecular mechanic calculations were chosen for the structure modeling of all the molecules of the training set. The atomic charges for all analogues were calculated by three different way: Gastaiger−Marsili algorithm, and Mulliken distribution and fitting point charges to electrostatic potential at the HF/6-31G* level via ab initio molecular orbital calculations using Gaussian 94.[23] This calculation level has been proved the best strategy for studying compounds containing the aminosulfonylamino moiety.[24] All of the compounds in the training set (Table 2) have identical BTD ring atoms, and these were used as the basis for an alignment rule (atoms N1, S2, N3, C4, O4, C5, C6, C7, and C8 were fit onto each other). Superimposition of all molecules is shown in Figure 1.

CoMFA, using default parameters, was calculated in the QSAR option of Sybyl 6.4 on a Silicon Graphics computer. The CoMFA grid space was 2.0 Å in the *x*, *y*, and *z* directions, and the grid region was automatically generated by the CoMFA routine to encompass all molecules with an extension of 4.0 Å in each direction. An sp^3 carbon and a charge of +1.0 were used as probes to generate the interaction energies at each lattice point. The default value of 30 kcal·mol^{-1} was used as the maximum electrostatic and steric energy cutoff. Several CoMFA studies were generated considering the abovementioned fields in the alignment described using the different calculated charges in the molecular training set.

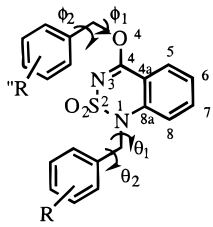
The PLS algorithm was initally used with the leave-one-out cross-validation option to obtain the optimal number of components needed for the subsequent analysis of the data. Final non-cross-validated models were only calculated for the two-field combinations chosen on the basis of the q^2 results. CoMFA results are summarized in Table 3. Models had fairly high predictive power ($q^2 > 0.5$); no significant improvements were achieved by changing the standard CoMFA settings for grid size and minimun σ.

The relative contributions to the derived CoMFA model (EPS charges, $q^2 = 0.544$) are 61.2% steric and 38.4% electrostatic, indicating that the variation in antiviral activity among the inhibitors is dominated by differences in the steric (van der Waals) interactions with the receptor. Correlation between calculated and experimental antiviral inhibition (log IC_{50}) is shown in

Table 2. Anti-HCMV (strain AD-169) Activity of *N*,*N*- and *N*,*O*-Dibenzyl BTD Derivatives



2, 5, 8, 11, 13
15, 17-19, 21
22, 24, 26-28
30, 32-36



3, 6, 12, 14
16, 20, 23, 25
29, 31, 37

compd	R	R′	R″	IC_{50} (μM)[a]	CC_{50} (μM)[b]	compd	R	R′	R″	IC_{50} (μM)[a]	CC_{50} (μM)[b]
2	4-CF_3	4-CF_3		15	>48	**23**[d]	4-Ph		4-Ph	47	>47
3	4-CF_3		4-CF_3	23	>48	**24**[e]	H	H		5.3	>66
5	4-NO_2	4-NO_2		53	>53	**25**[e]	H		H	7.9	>66
6	4-NO_2		4-NO_2	53	>53	**26**[e]	4-Cl	4-Cl		3.3	>56
8	4-OMe	4-OMe		57	>57	**27**[e]	2-Cl	2-Cl		33	>56
11	4-Me	H		2.5	>63	**28**[e]	3,4-Cl	3,4-Cl		15	>48
12	4-Me		H	10	>63	**29**[e]	3,4-Cl		3,4-Cl	48	>48
13	4-OMe	H		7	>61	**30**[e]	4-Cl	H		3.6	>60
14	4-OMe		H	61	>61	**31**[e]	3-Cl		H	36	>60
15	4-CN	H		8	>62	**32**[e]	H	4-Cl		6	>60
16	4-CN		H	29	>62	**33**[e]	2-Cl	H		4.8	>60
17	4-CF_3	H		3.3	>56	**34**[e]	H	2-Cl		12	>60
18	4-NO_2	H		8	>59	**35**[e]	3-Cl	H		3.6	>60
19[c]	2-Ph	2-Ph		47	>47	**36**[e]	3,4-Cl	H		4	>56
20[c]	2-Ph		2-Ph	47	>47	**37**[e]	3,4-Cl		H	26	>56
21[c]	2-Ph	H		44	>55	ganciclovir				5.9	>98
22[d]	4-Ph	4-Ph		47	>47						

[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. [c] Synthesis described in ref 19. [d] Synthesis described in ref 18. [e] Ref 14.

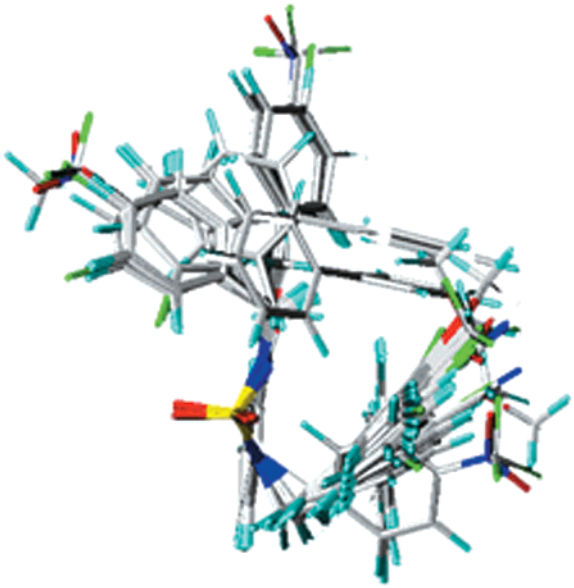


Figure 1. 3D-view of CoMFA training set. Compounds were aligned based on non-hydrogen atoms in the BTD ring.

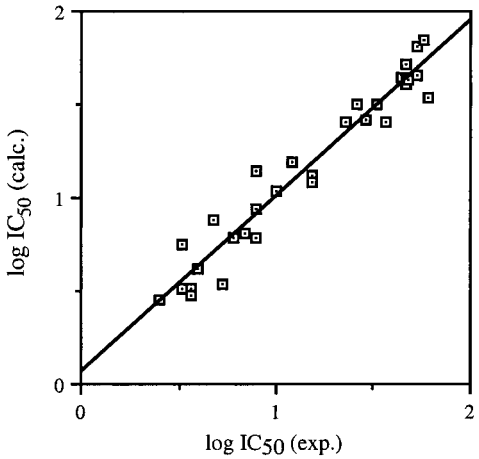


Figure 2. Plot of experimental versus calculated log IC_{50} values for training set compounds.

Table 3. CoMFA Analysis Results

	calcd charges in the training set		
	Gastaiger−Marsili	Mulliken	ESP
r^2 cross-validated	0.525	0.470	0.544
r^2 conventional	0.935	0.905	0.943
std error	0.125	0.151	0.121
no. of components	4	4	5
F value	101.05	66.85	82.78
contributions:			
steric	43.1	53.6	61.6
electrostatic	56.9	46.4	38.4

Figure 2, while graphical representation, using compound **30** as reference structure, is depicted in Figure 3. These color maps show the regions of space in which variation of the field considered has the most effect on the dependent variable, the inhibition of cytomegalovirus growth in our case.

An examination of the CoMFA steric field revealed that the *para*-positions of both benzyl substituents are the most sensitive ones to the steric properties. Therefore, bulky substituents on the benzyl group attached to *N*1 of BTD are predicted to enhance the activity against HCMV, while the opposite effect was observed in the benzyl group attached to *N*3.

On the other hand, the CoMFA electrostatic contour maps revealed that a high electron density (blue polyhedra) around the aminosulfonylamino moiety region enhances the antiviral activity, which could explain the lack of HCMV inhibition found in monobenzyl BTD derivatives. Moreover a low electron density was local-

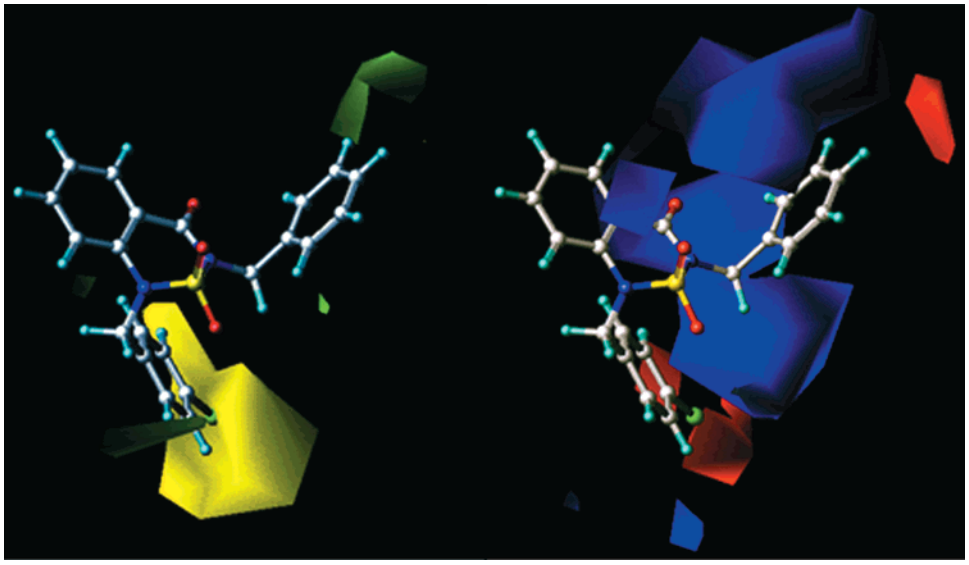
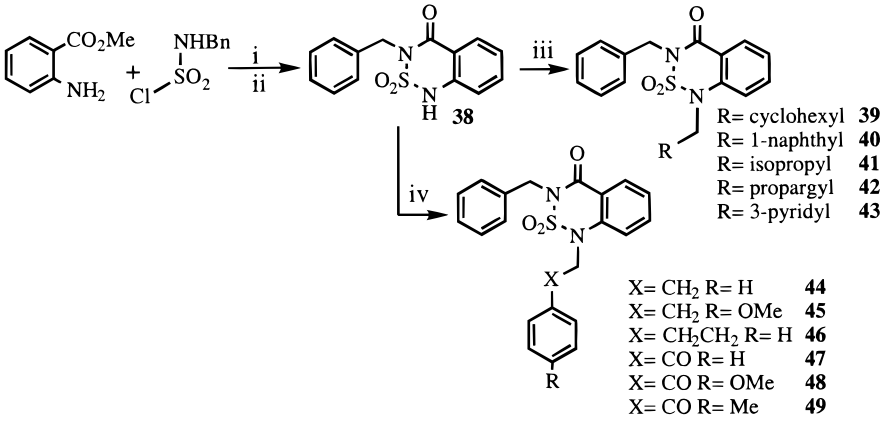


Figure 3. Views of CoMFA steric and electrostatic contour plots. Regions where increased steric bulk is associated with enhanced activity are indicated in yellow, while regions where increased bulk is associated with diminished activity are indicated in green. Regions where increased positive charge is favorable for activity are indicated in red, while regions where increased negative charge is favorable for activity are indicated in blue.

Scheme 2[a]



[a] Reagents: (i) $CH_3C_6H_5$; (ii) 6 N NaOH; (iii) DMF/NaH/R-CH_2Br; (iv) DMF/NaH/R-C_6H_4-X-CH_2Br.

ized around the *N*1 position. This fact could suggest that electron-withdrawing groups should improve the antiviral activity.

On the basis of these results, we propose the synthesis of compounds bearing a benzyl group as optimal substitution at the *N*3 position. By contrast, we suggest two modifications at the *N*1 position which allow to explore the 3D requirements. The first one, based on steric results, is elongation of the link between the BTD heterocycle and the phenyl group. The second will be compounds bearing different electrostatic environments such as naphthalene, pyridyl, cyclohexyl, propargyl, or isopropyl ones in that position. Thus, new designed compounds were synthesized, and their activity against HCMV was measured.

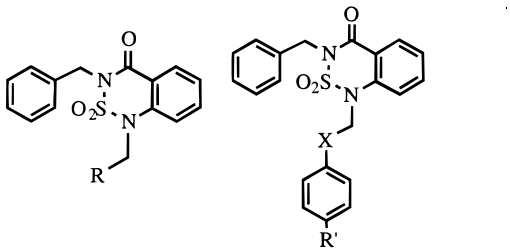
Optimization Step. For the preparation of the newly designed BTD derivatives **39**−**49**, an unambiguous synthetic pathway was planned to avoid a mixture of *N*,*N*- and *N*,*O*-disubstitution. Thus, we chose as starting material 3-benzyl BTD **38** unequivocally prepared in an efficient two-step synthesis following the Cohen and Klarberg procedure[17] from methyl anthranilate and *N*-benzylsulfamoyl chloride.[25] Alkylation of compound **38** in basic medium (DMF/NaH) afforded in good yields exclusively the *N*,*N*-disubstituted BTD derivatives **39**−**49** (Scheme 2). Some spectroscopic data (^{1}H and ^{13}C NMR) of these new compounds are collected in Table 1.

Antiviral activity against HCMV (AD-169 strain) was determined by plaque reduction assay. Data are collected in Table 4. Compounds **45, 48,** and **49**, which have been previously designed from the steric requirements predicted in the CoMFA study, exhibit potent antiviral activity against HCMV, with IC_{50} values lower than those of previously described BTD derivatives and the standard reference ganciclovir. It is worthmentioning that substitution in the *para*-position of the phenyl ring enhances the anti-HCMV activity as was predicted.

Conclusions

Several *N*,*N*- and *N*,*O*-dibenzyl BTD derivatives were synthesized, and their antiviral activity was evaluated against HCMV. Most of them had good IC_{50} values confirming the specific antiviral action previously observed for this kind of nonnucleosidic compounds. 3D-QSAR (CoMFA) analyses identified the most significant steric and electrostatic interactions involved in anti-

Table 4. Anti-HCMV (strain AD-169) Activity of Newly Designed Dibenzyl BTD Derivatives



compd	R	X	R′	IC_{50} (μM)[a]	CC_{50} (μM)[b]
39	cyclohexyl			15.6	>65
40	1-naphthyl			11.6	>58
41	isopropyl			14.5	>72
42	propargyl			76.6	>77
43	3-pyridyl			13.1	>66
44		CH_2	H	7.6	>64
45		CH_2	OMe	4.0	>59
46		CH_2CH_2	H	18.4	>57
47		CO	H	24.6	>61
48		CO	OMe	3.2	>57
49		CO	Me	2.8	>59
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.

HCMV activity suggesting that the steric component is a predominant factor in the antiviral activity of these analogues with electrostatic factors playing a smaller yet significant role. Furthermore, the CoMFA model was shown to have potential utility for designing new agents. Thus, chemical modification of the *N*,*N*-dibenzyl BTDs enables us to maintain the activity in the new series of compounds belonging to this novel class of antiviral agents. From the reported series, compounds **48** and **49** emerged as the most potent and selective inhibitors of HCMV. Further studies are in progress to understand the mechanism and target of interaction of these compounds.

Experimental Section

Chemical Procedures. Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Flash column chomatography was carried out at medium pressure using silica gel (E. Merck, grade 60, particle size 0.040−0.063 mm, 230−240 mesh ASTM) with the indicated solvent as eluent. ^{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. NOE difference spectra were measured under the same conditions, using a presaturation time of 3 s. ^{13}C NMR experiments were carried out on the Varian Gemini-200 spectrometer operating at 50 MHz. Tha acquisiton 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 δ values (ppm) relative to internal Me_4Si and J values are reported in hertz (Hz). Elemental analyses were performed by the analytical departement at CSIC and the results obtained were within ±0.4% of the theoretical values.

1-{[4-(Trifluoromethyl)phenyl]methyl}-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (1), 1,3-Di{[4-trifluoromethyl)phenyl]methyl}-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (2), and 1-{[4-(Trifluoromethyl)phenyl]methyl}-4-{[4-(trifluoromethyl)phenyl]methyloxy}-2,1,3-benzothiadiazine 2,2-Dioxide (3). To an aqueous solution of sodium bicarbonate (20 mL) were added 4-hydroxy-2,1,3-benzothiadiazine dioxide[17] (0.20 g, 1.0 mmol) and 4-(trifluoromethyl)phenylmethyl chloride (0.48 g, 2.5 mmol). The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 (2 × 10 mL). The aqueous phase was cooled and compound **1** was isolated by filtration. Purification: recrystallization (toluene/MeOH), yield 0.27 g (76%) as a white solid; mp 265−267 °C. Anal. ($C_{15}F_3H_{11}N_2O_3S$) C, H, N, S.

The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure; the residue was chromatographed on circular thin-layer chromatography, using CH_2Cl_2:hexane (1:1) as eluent. From the first fraction derivative **2** was isolated: yield 0.007 g (1%) as a syrup. Anal. ($C_{23}F_6H_{16}N_2O_3S$) C, H, N, S.

From the second fraction derivative **3** was isolated: yield 0.004 g (0.7%) as a syrup. Anal. ($C_{23}F_6H_{16}N_2O_3S$) C, H, N, S.

1-[(4-Nitrophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (4), 1,3-Di[(4-nitrophenyl)methyl]-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (5), and 1-[(4-Nitrophenyl)methyl]-4-[(4-nitrophenyl)methyloxy]-2,1,3-benzothiadiazine 2,2-Dioxide (6). To an aqueous solution of sodium bicarbonate (20 mL) were added 4-hydroxy-2,1,3-benzothiadiazine dioxide[17] (0.50 g, 2.5 mmol) and 4-nitrophenylmethyl bromide (1.35 g, 6.2 mmol). The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 (2 × 10 mL). The aqueous phase was cooled and compound **4** was isolated by filtration. Purification: recrystallization (toluene/MeOH), yield 0.76 g (92%) as a white solid; mp 270−272 °C. Anal. ($C_{14}H_{11}N_3O_5S$) C, H, N, S.

The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure; the residue was chromatographed on circular thin-layer chromatography, using CH_2Cl_2:hexane (1:1) as eluent. From the first fraction derivative **5** was isolated: yield 0.01 g (1%) as a syrup. Anal. ($C_{21}H_{16}N_4O_7S$) C, H, N, S.

From the second fraction derivative **6** was isolated: yield 0.004 g (0.3%) as a syrup. Anal. ($C_{21}H_{16}N_4O_7S$) C, H, N, S.

1-[(4-Methoxyphenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (7) and 1,3-Di[(4-methoxyphenyl)-methyl]-2,1,3-benzothiadiazinone 2,2-Dioxide (8). To an aqueous solution of sodium bicarbonate (20 mL) were added 4-hydroxy-2,1,3-benzothiadiazine dioxide[17] (0.19 g, 1.0 mmol) and 4-methoxyphenylmethyl chloride (0.39 g, 2.5 mmol). The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 (2 × 10 mL). The organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure; the residue was chromatographed on circular thin-layer chromatography, using CH_2Cl_2:hexane (1:1) as eluent. Compound **8** was obtained (0.03 g, 8%) as a syrup. Anal. ($C_{23}H_{22}N_2O_5S$) C, H, N, S.

The aqueous phase was extracted with AcOEt (10 × 10 mL), the organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure; the residue was chromatographed on silica gel column using CH_2Cl_2/MeOH (50:1) as eluent. Compound **7** was obtained (0.13 g, 43%) as a white solid: mp 260−262 °C. Anal. ($C_{15}H_{14}N_2O_4S$) C, H, N, S.

1-[(4-Cyanophenyl)methyl]-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-Dioxide (10). To an aqueous solution of sodium bicarbonate (20 mL) were added 4-hydroxy-2,1,3-benzothiadiazine dioxide[17] (0.50 g, 2.5 mmol) and 4-cyanophenylmethyl bromide (1.22 g, 6.2 mmol). The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was extracted with AcOEt (10 × 10 mL), the organic phase was dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue was recrystallized from toluene/MeOH yielding compound **10** (0.56 g, 72%) as a white solid: mp 298−300 °C. Anal. ($C_{15}H_{11}N_3O_3S$) C, H, N, S.

General Procedure for the *N*3-Benzylation of Benzothiadiazine Derivatives. To an equimolecular suspension of sodium hydride in DMF (25 mL) were added the corresponding monosubstitued benzothiadiazines and benzyl bromide (1.5 mmol). The reaction mixture was refluxed for 2 h.

After cooling, 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 sulfate and the solvent evaporated under reduced pressure. The residue was chromatographed on circular thin-layer chromatography, using CH_2Cl_2: hexane (1:1) as eluent.

1-[(4-Methylphenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (11) and 1-[(4-Methylphenyl)-methyl]-4-benzyloxy-2,1,3-benzothiadiazine 2,2-Dioxide (12). Reagents: benzothiadiazine **9**[19] (0.07 g, 0.2 mmol), benzyl bromide (0.05 g, 0.3 mmol). From the first fraction derivative **11** was isolated as a syrup: yield 0.03 g (43%). Anal. ($C_{22}H_{20}N_2O_3S$) C, H, N, S.

From the second fraction derivative **12** was isolated as a syrup: yield 0.003 g (4%). Anal. ($C_{22}H_{20}N_2O_3S$) C, H, N, S.

1-[(4-Methoxyphenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (13) and 1-[(4-Methoxyphenyl)-methyl]-4-benzyloxy-2,1,3-benzothiadiazine 2,2-Dioxide (14). Reagents: benzothiadiazine **7** (0.13 g, 0.4 mmol), benzyl bromide (0.10 g, 0.6 mmol). From the first fraction derivative **13** was isolated as a white solid: yield 0.02 g (14%); mp 143−145 °C. Anal. ($C_{22}H_{20}N_2O_4S$) C, H, N, S.

From the second fraction derivative **14** was isolated as a syrup: yield 0.004 g (2%). Anal. ($C_{22}H_{20}N_2O_4S$) C, H, N, S.

1-[(4-Cyanophenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (15) and 1-[(4-Cyanophenyl)-methyl]-4-benzyloxy-2,1,3-benzothiadiazine 2,2-Dioxide (16). Reagents: benzothiadiazine **10** (0.20 g, 0.6 mmol), benzyl bromide (0.15 g, 0.9 mmol). From the first fraction derivative **15** was isolated as a white solid: yield 0.03 g (12%); mp 45−47 °C. Anal. ($C_{22}H_{17}N_3O_3S$) C, H, N, S.

From the second fraction derivative **16** was isolated as a syrup: yield 0.004 g (2%). Anal. ($C_{22}H_{17}N_3O_3S$) C, H, N, S.

1-{[4-(Trifluoromethyl)phenyl]methyl}-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (17). Reagents: benzothiadiazine **1** (0.15 g, 0.4 mmol), benzyl bromide (0.10 g, 0.6 mmol); yield 0.03 g (18%) as a syrup. Anal. ($C_{22}H_{17}N_2O_3SF_3$) C, H, N, S.

1-[(4-Nitrophenyl)methyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (18). Reagents: benzothiadiazine **4** (0.20 g, 0.6 mmol), benzyl bromide (0.15 g, 0.9 mmol); yield 0.01 g (8%) as a syrup. Anal. ($C_{21}H_{17}N_3O_5S$) C, H, N, S.

3-Benzyl-2,1,3-benzothiadiazin-4(1*H*)-one 2,2-Dioxide (38). To a 0 °C cooled and stirred solution of benzylamine (10.7 g, 0.1 mol) in CH_2Cl_2 (100 mL) was added chlorosulfonic acid (3.49 g, 0.03 mol) cautiously. The resulting suspension was stirred for 0.5 h at room temperature and then filtered. The collected solids were dissolved in toluene (50 mL) and treated with phosphorus pentachloride (6.24 g, 0.03 mol). A midly exothermic reaction took place. The solution was refluxed for 1 h and the solid was filtered off. The filtrate was concentrate in vacuo and the syrupy residue (*N*-benzylsulfamoyl chloride) thus obtained was used in the next synthetic step without further purification.

Methyl anthranilate (2.11 g, 0.01 mol) was dissolved in toluene (10 mL). This mixture was added to an stirred solution of *N*-benzylsulfamoyl chloride (3 g) previously prepared in toluene (10 mL). After 3 h, 6 N sodium hydroxide solution (20 mL) was added. The aqueous layer was separated and made acidic with concentrated hydrochloridic acid. Upon cooling the mixture, a white crystalline solid precipitated: yield 2.71 g (67%); mp 250−251 °C (lit.[13] mp 250−251 °C).

General Procedure for *N*1-Alkylation of 3-Benzylbenzothiadiazine Dioxide. To an equimolecular suspension of sodium hydride in DMF (25 mL) were added *N*3-benzyl benzothiadiazine **38** and the corresponding aqueous agent (1.5 mmol). 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 sulfate and the solvent evaporated under reduced pressure. The residue was chromatographed on circular thin-layer chromatography, using CH_2Cl_2:hexane (1:1) as eluent.

1-(Cyclohexylmethyl)-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (39). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), cyclohexylmethyl bromide (0.07 g, 0.4 mmol); yield 0.02 g (24%) as a white solid; mp 100−101 °C. Anal. ($C_{21}H_{24}N_2O_3S$) C, H, N, S.

1-(1-Naphthylmethyl)-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (40). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), 1-naphthylmethyl chloride (0.07 g, 0.4 mmol); yield 0.04 g (29%) as a syrup. Anal. ($C_{25}H_{20}N_2O_3S$) C, H, N, S.

1-Isobutyl-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (41). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), isobutyl bromide (0.05 g, 0.4 mmol); yield 0.02 g (21%) as a syrup. Anal. ($C_{18}H_{20}N_2O_3S$) C, H, N, S.

1-Propargyl-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (42). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), propargyl bromide (0.05 g, 0.4 mmol); yield 0.06 g (50%) as a white solid; mp 119−120 °C. Anal. ($C_{17}H_{14}N_2O_3S$) C, H, N, S.

1-(3-Pyridylmethyl)-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (43). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), 3-(bromomethyl)pyridine hydrobromide (0.11 g, 0.4 mmol); yield 0.05 g (43%) as a syrup. Anal. ($C_{20}H_{17}N_3O_3S$) C, H, N, S.

1-Phenylethyl-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (44). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), phenylethyl bromide (0.07 g, 0.4 mmol); yield 0.02 g (20%) as a syrup. Anal. ($C_{22}H_{20}N_2O_3S$) C, H, N, S.

1-[(4-Methoxyphenyl)ethyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (45). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), 4-(methoxy)phenylethyl chloride (0.07 g, 0.4 mmol); yield 0.02 g (14%) as a syrup. Anal. ($C_{23}H_{22}N_2O_4S$) C, H, N, S.

1-Phenylpropyl-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (46). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), phenylpropyl chloride (0.07 g, 0.4 mmol); yield 0.01 g (8%) as a syrup. Anal. ($C_{24}H_{22}N_2O_3S$) C, H, N, S.

1-(Phenylcarbonylmethyl)-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (47). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), 2-bromoacetophenone (0.08 g, 0.4 mmol); yield 0.02 g (15%) as a white solid; mp 118−120 °C. Anal. ($C_{22}H_{18}N_2O_4S$) C, H, N, S.

1-[(4-Methoxyphenyl)carbonylmethyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (48). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), 2-bromo-4′-methoxyacetophenone (0.09 g, 0.4 mmol); yield 0.07 g (45%) as a white solid; mp 135−136 °C. Anal. ($C_{23}H_{20}N_2O_5S$) C, H, N, S.

1-[(4-Methylphenyl)carbonylmethyl]-3-benzyl-2,1,3-benzothiadiazin-4-one 2,2-Dioxide (49). Reagents: benzothiadiazine **38** (0.10 g, 0.3 mmol), 2-bromo-4′-methylacetophenone (0.08 g, 0.4 mmol); yield 0.05 g (32%) as a white solid; mp 130−132 °C. Anal. ($C_{23}H_{20}N_2O_4S$) C, H, N, S.

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

Viruses: Virus stocks were prepared in MRC-5 cells as infected cells. When 70% cytopathic effect was obtained, the cells were trypsinized, resuspended in medium containing 10% DMSO and stored in aliquots at −80 °C. The AD-169 strain of human cytomegalovirus was used. Virus stocks consisted of cell-free virus obtained from the supernatant of infected cell cultures that had 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 100 (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: 100 PFU/well) was monitored microscopically. The antiviral activity is expressed as IC_{50} which repre-

sents the compound concentration required to reduce virus plaque formation by 50%. IC_{50} values were stimated 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 in microtiter plates and allowed to prolifarate 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%.

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Supporting Information Available: ^1H and ^{13}C NMR data and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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