Full Paper

1,2,4-Benzothiadiazine-1,1-dioxide Derivatives as Ionotropic Glutamate Receptor Ligands: Synthesis and Structure–Activity Relationships

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Ionotropic glutamate receptor (iGluR) modulators, specially AMPA receptor antagonists, are potential tools for numerous therapeutic applications in neurological disorders, including Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, epilepsy, chronic pain, and neuropathology ensuing from cerebral ischemia or cardiac arrest. In this work, the synthesis and binding affinities at the Gly/NMDA, AMPA, and kainic acid (KA) receptors of a new series of 1,2,4-benzothiadiazine-1,1-dioxide derivatives are reported. The results show that 1,2,4-benzothiadiazine-1,1-dioxide is a new scaffold for obtaining iGluR ligands. Moreover, this work has led us to the 7-(3-formylpyrrol-1-yl)-6-trifluoromethyl substituted compound **7**, which displays the highest AMPA receptor affinity and high selectivity versus the Gly/NMDA (90-fold) and KA (46-fold) receptors.

Keywords: AMPA receptor / AMPA receptor antagonists / 1,2,4-Benzothiadiazine-1,1-dioxide / Ionotropic glutamate receptors / Neurological diseases

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Introduction

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and plays primary roles in the control of motor function, cognition, and mood. The effects of Glu are mediated through a number of pre- and postsynaptic receptors [1, 2]. On the basis of pharmacological profile and ligand selectivity studies, Glu receptors have been grouped into two main classes: the fast-acting ionotropic receptors (iGluRs) and the G-protein coupled metabotropic receptors (mGluRs), which produce a significantly slower signal transduction through second messenger system. Within the class of mGluRs, to date, eight subtypes, subdivided in three groups have been defined and named: group I (subunits mGluR1, 5), group II (subunits mGluR2, 3), and group III (subunits mGluR4, 6–8) [1]. Likewise for the iGluRs three subgroups have been established on the basis of ligand affinity studies: the AMPA (subunits GluA1–4), kainic acid (KA) (subunits GluK1–5), and NMDA receptors (subunits GluN1, GluN2A–D, and GluN3A, B). The NMDA receptor complex possesses different binding sites including, as a unique feature, the glutamate co-agonist glycine binding site (Gly/NMDA) [1–3].

It has been well established that an overstimulation of iGluRs, specially AMPA receptor subtypes, induces an uncontrolled Ca^{2+} overload potentially leading to cell damage and death. Several neurological disorders such as Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, epilepsy, chronic pain, and neuropathology ensuing from cerebral ischemia or cardiac arrest, are, at least in part, linked to over-activation of AMPA receptors. Therefore, AMPA subtypes represent potential targets for therapeutic intervention in many neurological diseases [4, 5].

During a research program devoted to the development of new iGluRs ligands [6–17], we studied the 3-hydroxyquinazoline-2,4-dione (QZ) system, which was found to be a useful

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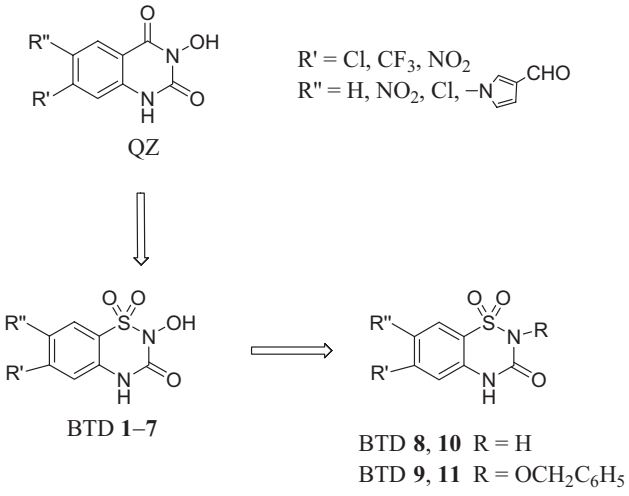


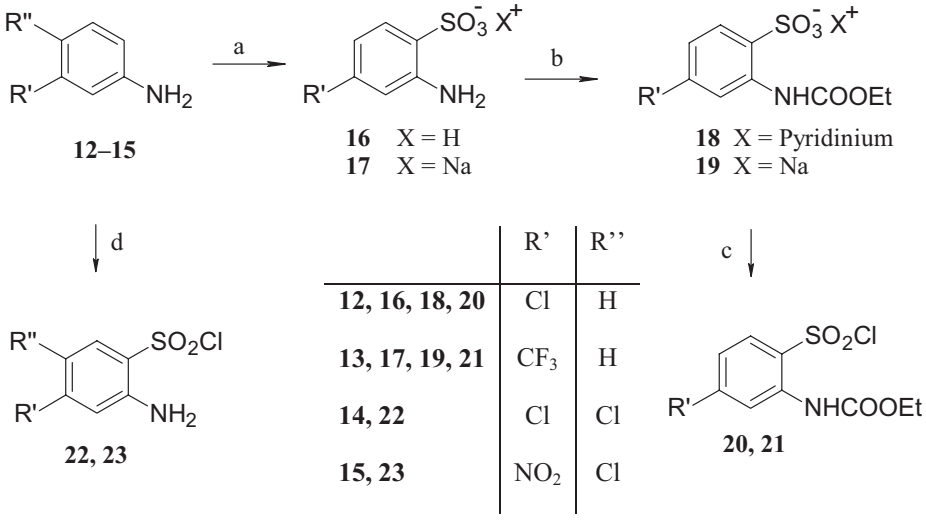
Figure 1. QZ and BTD derivatives.

scaffold to obtain selective iGluRs antagonists [13, 15, 17]. Continuing our research, in the present paper we disclose the results of our studies on a new series of 1,2,4-benzothiadiazine-1,1-dioxides (BTDs) as novel iGluRs ligands (Fig. 1). BTDs **1**–**7** share QZ derivatives features that turned out to be important structural requirements for Gly/NMDA, AMPA, and KA receptor recognition. Specifically: (i) a flat hydrophobic area represented by the fused benzo ring; (ii) a NH hydrogen bond donor that binds a proton acceptor of the receptors; (iii) a δ-negatively charged moiety represented by both the carbonyl group and the hydroxyl substituent able to engage a hydrogen bond with a cationic hydrogen bond donor of the receptor. Moreover, the planar 4-carbonyl group of QZ derivatives has been replaced with the tetrahedral 1-sulfonyl moiety in the BTDs. The latter represents the only structural difference between the two series. However, both the carbonyl and the sulfonyl moieties can behave as hydrogen bond accepting groups in a hydrogen bond interaction with a hydrogen donor of the binding site. To evaluate the importance of the 2-hydroxyl group for receptor ligand interaction, we synthesized the 2-unsubstituted BTDs **8** and **10**. Moreover, the 2-benzyloxy substituted BTDs **9** and **11** were tested in order to investigate the effect of a bulky substituent for the binding to the receptors. Finally, all the synthesized BTDs **1**–**11** bear, on the fused benzo moiety, substituents that proved to be useful to increase affinity and/or selectivity in the QZ series. In particular: (i) an electron-withdrawing substituent at position-6 (R′), such as a chlorine atom, a trifluoromethyl, or a nitro group, able to improve the binding affinity toward all three receptors; (ii) a substituent at position-7 (R″), such as a chlorine atom, a nitro group, or a 3-formyl-pyrrole ring, that seems to influence the selectivity profile toward AMPA and/or KA receptors [13, 15, 17].

Results and discussion

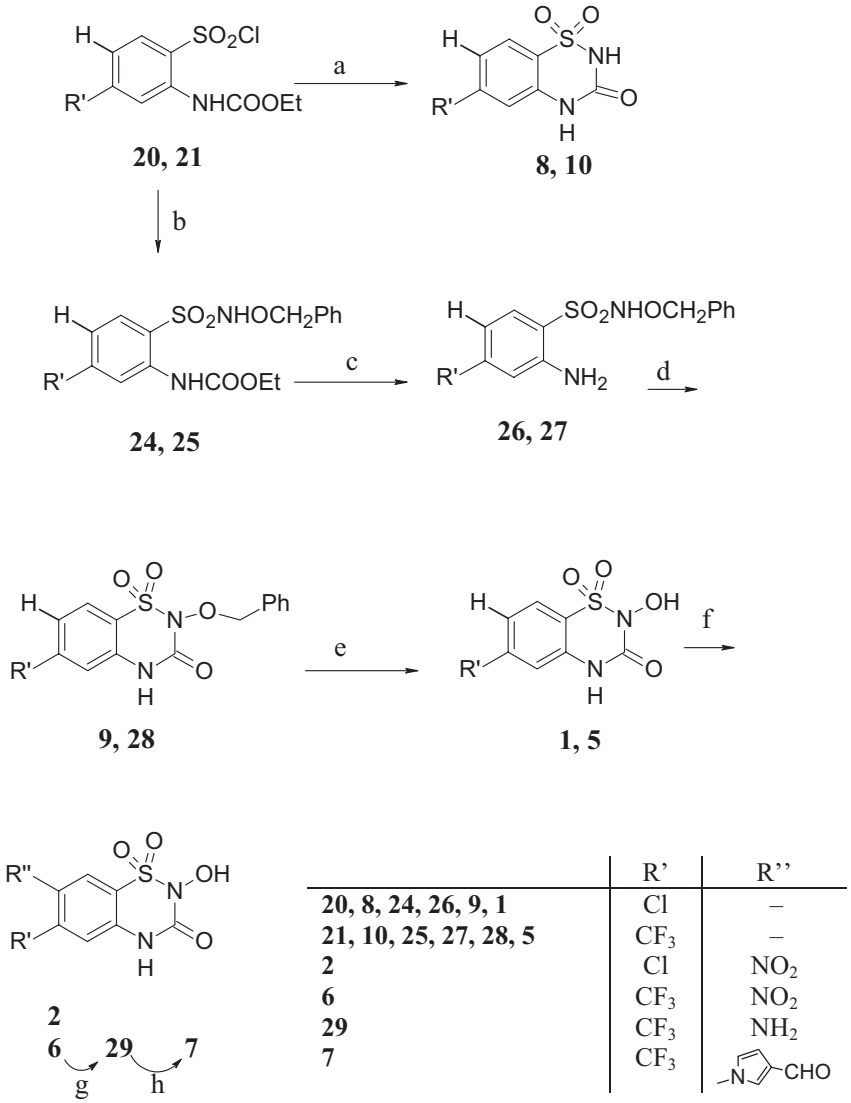
Chemistry

The BTDs **1**–**11** were prepared as outlined in Schemes 1–3 following, with modifications, the synthetic procedure reported by Wei et al. [18] for the preparation of other BTDs. First aim of the whole synthetic procedure was the synthesis of 2-amino-arylsulfonyl chlorides **20**–**23** following diverse



	R'	R''
12, 16, 18, 20	Cl	H
13, 17, 19, 21	CF_3	H
14, 22	Cl	Cl
15, 23	NO_2	Cl

Scheme 1. Reagents and conditions: (a) for **16**: (i) H_2SO_4 conc., (ii) 1,1,2,2-tetrachloroethane, MW 200°C; for **17**: (i) $ClSO_3H$ 98%, 1,1,2,2-tetrachloroethane, 125°C, (ii) Na_2CO_3, saturated water solution; (b) for **18**: ethylchloroformate, pyridine; for **19**: ethylchloroformate, H_2O; (c) $PCl_5/POCl_3$, 70°C; and (d) $ClSO_3H$ 98%, $SOCl_2$.

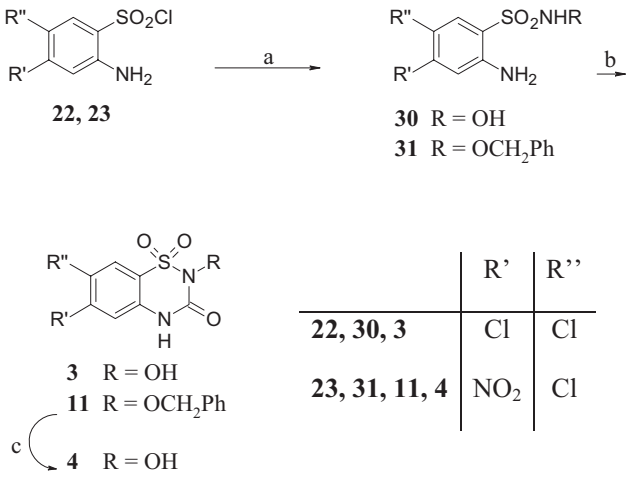


	R'	R''
20, 8, 24, 26, 9, 1	Cl	–
21, 10, 25, 27, 28, 5	CF_3	–
2	Cl	NO_2
6	CF_3	NO_2
29	CF_3	NH_2
7	CF_3	CHO

Scheme 2. (a) 33% NH_4OH, 60°C; (b) $NH_2OCH_2C_6H_5 \cdot HCl$, Et_3N, THF/H_2O; (c) 4% NaOH, 100°C; (d) triphosgene, THF; (e) BBr_3, CH_2Cl_2; (f) 100% HNO_3; (g) H_2, Pd/C, 40 psi; and (h) 2,5-dimethoxytetrahydrofuran-3-carbaldehyde, AcOH, 60°C.

procedures that depend on the benzo-substitution pattern (Scheme 1). Specifically, the 2-amino-4-chloro-benzenesulfonic acid **16** [19] was obtained in 90% yield by microwave-assisted thermal rearrangement of the 3-chloroaniline **12** hydrogen sulfate salt. The 2-amino-4-trifluoromethyl-benzenesulfonic acid sodium salt **17** was prepared by treatment of the corresponding aniline **13** with chlorosulfonic acid. The 2-amino group of **16**–**17** was protected using an excess of ethylchloroformate to give carbammates **18** and **19**, which, in the presence of $PCl_5/POCl_3$, easily provided the corresponding arylsulfonyl chlorides **20**–**21** [20]. Attempts to directly chlorinate compounds **16**–**17** were unsuccessful as an intermolecular condensation occurred (data not shown). Instead, the commercially available 3,4-dichloro- and 4-chloro-3-nitro anilines **14** and **15** were directly reacted with chlorosulfonic acid in the presence of thionyl chloride to provide the corresponding 2-amino-arylsulfonyl chlorides **22**–**23** [21].

The arylsulfonyl chlorides **20**–**21** were easily cyclized to the corresponding 2-unsubstituted BTDs **8** [22] and **10** [23] by reacting with aqueous ammonia solution (33%) (Scheme 2). Otherwise, the 2-amino-protected arylsulfonyl chlorides **20**–**21** were reacted with *O*-benzylhydroxylamine to give sulfonamides **24**–**25**, which were deprotected by treatment with 4% NaOH solution. The 2-amino-*N*-benzyloxy-benzenesulfonamides **26**–**27** underwent cyclization to the corresponding BTDs **9** and **28** with an excess of triphosgene. Removal of the



Scheme 3. (a) For **30**: $NH_2OH \cdot HCl$, Et_3N, 1,4-dioxane/H_2O; for **31**: $NH_2OCH_2C_6H_5 \cdot HCl$, Et_3N, THF/H_2O; (b) triphosgene, H_2O; and (c) BBr_3, CH_2Cl_2.

benzyl group with BBr_3 yielded the 2-hydroxy derivatives **1** and **5**, which, regioselectively nitrated with 100% HNO_3, provided the 7-nitro derivatives **2** and **6**, respectively. Finally, the 7-nitro-6-trifluoromethyl derivative **6** was catalytically reduced to the corresponding 7-amino **29**, which, by reacting with 2,5-dimethoxytetrahydrofuran-3-carbaldehyde, yielded the 7-(3-formylpyrrol-1-yl)-derivative **7**.

The synthesis of the 6,7-dichloro compound **3** and the 7-chloro-6-nitro BTDs **11** and **4** is shown in Scheme 3. Reaction of 2-amino-4,5-dichloro-benzenesulfonyl chloride **22** with hydroxylamine afforded the 2-amino-*N*-hydroxy-benzenesulfonamide **30** [21] that was cyclized with triphosgene to yield the corresponding 2-hydroxy-benzothiadiazine **3**. Instead, the 2-amino-5-chloro-4-nitro-benzenesulfonyl chloride **23** was reacted with *O*-benzylhydroxylamine to give the corresponding 2-amino-*N*-benzyloxy-benzenesulfonamide **31**. Cyclization of the latter with triphosgene afforded the 2-benzyloxy-substituted compound **11** that was debenzylated with BBr_3 to give the desired 2-hydroxy derivative **4**.

In vitro studies

The BTDs **1**–**11** were tested for their ability to displace tritiated glycine, AMPA, and KA from their specific binding sites in rat cortical membranes. The binding data are shown in Table 1 together with those of previously reported QZ derivatives **A**–**F** included as reference compounds.

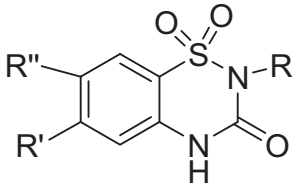
Results indicate that we have disclosed a new scaffold for the development of iGluRs ligands. In fact, BTDs **1**–**7**, bearing a hydroxyl group at position-2, show, in general, combined AMPA, KA, and Gly/NMDA receptor binding affinities ranging from micromolar to submicromolar values. Despite this, the 7-(3-formylpyrrol-1-yl)-6-trifluoromethyl substituted compound **7** displays the highest AMPA receptor affinity and also high selectivity versus Gly/NMDA (90-fold) and KA (46-fold) receptors.

Elimination of the 2-hydroxyl group of derivatives **1** and **5** yielded the 2-unsubstituted BTDs **8** and **10**, respectively, which show a reduced affinity toward the three receptors. Replacement of the 2-hydroxyl group of BTDs **1** and **4** with a benzyloxy moiety (see compounds **9** and **11**) leads to a collapse of affinities probably due to the steric hindrance of the 2-substituent. These results indicate that the 2-hydroxyl group is essential for ligand interaction with all three receptors, thus confirming the data obtained in the QZ series.

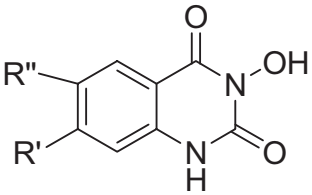
The herein reported binding data indicate that AMPA and KA receptor affinities of BTDs **1**–**3**, **5**–**7** are comparable to those of QZ derivatives **A**–**F**. Thus, it is easy to argue that replacement of the planar 4-carbonyl group of QZ derivatives with a tetrahedral sulfone moiety is tolerated by both AMPA and KA receptors. On the contrary, BTDs **1**–**3**, **5**–**7** show Gly/NMDA receptor affinities more than 10-fold lower than those of the corresponding QZ derivatives **A**–**F**, except compound **5**, which is only twofold less active than **D**.

As regards the substitution pattern on the fused benzo ring, the binding data clearly indicate that it influences affinity and selectivity of the reported compounds toward the three receptors. These results are in accordance with those previously obtained in the QZ series. In fact, the 7-unsubstituted 6-chloro compound **1** and the 6-trifluoromethyl derivative **5** show comparable AMPA and Gly/NMDA binding activities while being scarcely active at the KA receptor. Introduction of a nitro group at position-7 of **1** and **5** yields compounds **2** and **6**, respectively, which combine an increased AMPA receptor binding activity to a reduced affinity for the Gly/NMDA site. Indeed, BTDs **2** and **6** are selective AMPA receptor ligands. The 7-chloro-6-nitro derivative **4** maintains the same selectivity profile of its regioisomer 6-chloro-7-nitro **2**, but shows a lower AMPA receptor affinity, thus indicating that for AMPA receptor–ligand interaction, the concomitant presence of a chlorine atom at position-6 and a nitro group at position-7 is preferred. The 6,7-dichloro substitution (compound **3**) decreased potency and or selectivity of binding at the three receptors. In fact, while derivative **3** is equiactive to the 6-chloro parent **1** at the KA receptor, the affinities at the AMPA and Gly/NMDA ones are, respectively, 4- and 16-fold lower. Finally, the 6-trifluoromethyl substituted compound **7**, bearing a (3-formylpyrrol-1-yl) moiety at position 7, is very interesting. This compound was designed as a selective AMPA receptor ligand since it is well know that the presence of a nitrogen containing heterocycle at such position is particularly important for obtaining high affinity and/or selectivity for this receptor [8, 10, 13, 15, 17]. The choice of the 3-formylpyrrole moiety as the

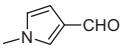
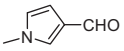
Table 1. Binding affinity at AMPA, Gly/NMDA and kainate receptors.



1–11



A–F

				K_i (μM)[a] or I%[b]		
Compd.	**R′**	**R″**	**R**	**[^{3}H]AMPA**	**[^{3}H]KA**	**[^{3}H]glycine**
1	Cl	H	OH	9.1 ± 3.7	66.2 ± 10.8	3.5 ± 0.5
2	Cl	NO_2	OH	0.79 ± 0.09	18.3 ± 2.7	11.4 ± 2.1
3	Cl	Cl	OH	38.7 ± 7.5	58.2 ± 5.2	56.5 ± 3.5
4	NO_2	Cl	OH	6.5 ± 1.6	52.4 ± 16.4	27.2 ± 6.1
5	CF_3	H	OH	2.6 ± 0.3	54%	1.9 ± 0.1
6	CF_3	NO_2	OH	1.8 ± 0.1	27.6 ± 3.2	42%
7	CF_3		OH	0.11 ± 0.02	5.1 ± 0.4	9.9 ± 1.1
8	Cl	H	H	35%	63%	44.6 ± 6.9
9	Cl	H	OCH_2Ph	0%	0%	0%
10	CF_3	H	H	35%	64%	24.6 ± 3.9
11	NO_2	Cl	OCH_2Ph	8%	9%	0%
A[c]	Cl	H	–	11.6 ± 1.9	140 ± 15	0.24 ± 0.02
B[c]	Cl	NO_2	–	1.3 ± 0.1	19.2 ± 1.0	1.1 ± 0.1
C[c]	Cl	Cl	–	12.5 ± 2.6	68 ± 7	0.3 ± 0.03
D[c]	CF_3	H	–	4.8 ± 0.4	37.6 ± 7.0	0.85 ± 0.09
E[c]	CF_3	NO_2	–	0.26 ± 0.02	7.0 ± 0.5	1.3 ± 0.06
F[c]	CF_3		–	0.72 ± 0.13	4.2 ± 0.3	0.8 ± 0.1

[a] K_i values are means ± SEM from three to five separate determinations in triplicate.
[b] Percentage of inhibition (I%) of specific binding at 100 μM concentration.
[c] Refs. [13, 15, 17].

nitrogen-containing heterocyclic substituent was made since the presence of the same substituent in the QZ series (QZ **F**) afforded a combined AMPA/Gly/NMDA receptor antagonist. Indeed, **7** shows not only the highest AMPA receptor affinity ($K_i = 0.11$ μM) but also the highest selectivity (AMPA vs. Gly/NMDA = 90; AMPA vs. KA = 46) among the reported compounds. Moreover, it has to be noted that compound **7** shows both AMPA receptor affinity and AMPA versus Gly/NMDA selectivity higher than those of its corresponding QZ **F**, which is a mixed AMPA/Gly/NMDA receptor antagonist.

Conclusion

In summary, the synthesis of BTDs **1–11** has led us to the discovery of a new class of iGluR ligands. Comparison of the binding data of QZs and BTDs reveals that replacement of the flat carbonyl group of QZs with the tetrahedral sulfonyl moiety of BTDs marginally affects the binding affinity while increasing, in some cases, the selectivity toward the AMPA receptor. In particular, compound **7**, bearing a 6-trifluoromethyl and a 7-(3-formylpyrrol-1-yl) substitution pattern, displays the highest AMPA receptor affinity and selectivity for the AMPA receptor.

Due to the chemical versatility of the 1,2,4-benzothiadiazine-1,1-dioxide scaffold, which allows introduction of various substituents in diverse positions, further exploration of BTD series are in progress to improve affinity and selectivity toward the AMPA receptor.

Experimental

Chemistry

Silica gel plates (Merck F254) and silica gel 60 (Merck, 70–230 mesh) were used for analytical and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N, and the results were within ±0.4% of the theoretical values, unless otherwise stated. All final compounds revealed a purity not less than 95%.

The IR spectra were recorded with a Perkin-Elmer Spectrum RX I spectrometer in Nujol mulls and are expressed in cm^{-1}. ^1H NMR spectra were recorded on a Bruker Avance 400 MHz

instrument. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent, which was always DMSO-d_6. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, and ar = aromatic protons.

*2-Amino-4-chlorobenzenesulfonic acid (**16**)*

Commercially available 3-chloroaniline **12** (0.1 mol) was added, drop-by-drop, at room temperature to a solution of concentrated sulfuric acid (6.9 mL) and water (50 mL). Filtration of the reaction mixture gave a light brown crystalline solid, which was suspended in 1,1,2,2-tetrachloroethane (10 mL) and microwave irradiated at 200°C for 45 min. The suspension was filtered and the solid was resuspended in fresh 1,1,2,2 tetrachloroethane (10 mL) and microwave irradiated again at 200°C for 45 min. The product was filtered off, washed with diethyl ether, and recrystallized. Yield: 90%; m.p. >300°C (ethanol), (lit. [18] >310°C). ^{1}H NMR: 6.72–6.74 (d, 1H, ar. J = 8.3 Hz), 6.83 (s, 1H, ar), 7.47–7.50 (d, 1H, ar, J = 8.3 Hz). IR: 1176, 1251, 1462, 1556, 2620, 3069. Anal. calcd. for $C_6H_6ClNO_3S$: C, 34.71; H, 2.91; N, 6.75. Found: C, 35.22; H, 3.66; N, 5.99.

*2-Amino-4-(trifluoromethyl)benzenesulfonic acid, sodium salt (**17**)*

A solution of chlorosulfonic acid (98%, 3.44 mL) in 1,1,2,2-tetrachloroethane (25 mL) was added, drop-by-drop, at 0°C, to a solution of commercially available 3-trifluoromethylaniline **13** (30 mmol) in 1,1,2,2-tetrachloroethane (100 mL). At the end of addition (1 h and 30 min), the solution was stirred at 125°C for 3 h. During this period, some precipitation occurred. The suspension was allowed to cool to room temperature, filtered, and the solid washed with diethyl ether, and then treated with saturated water solution of Na_2CO_3 (20 mL). The resulting sodium salt is filtered and used as such for the next step. Yield: 94%; ^{1}H NMR: 5.99 (s, 2H, NH_2), 6.72 (d, 1H, ar, J = 7.9 Hz), 6.92 (s, 1H, ar), 7.58 (d, 1H, ar, J = 7.9 Hz).

*4-Chloro-2-[(ethoxycarbonyl)amino]benzenesulfonic acid, pyridinium salt (**18**)*

Ethylchloroformate (14.2 mmol) was portionwise added to a suspension of the acid **16** (11.8 mmol) in pyridine (10 mL). The mixture was kept at room temperature for 2 h and 45 min. Evaporation at reduced pressure of the solvent yielded a solid, which was treated with ethanol (1–2 mL) and collected by filtration. Yield: 80%; m.p. 133–136°C (ethanol). ^{1}H NMR: 1.23–1.27 (t, 3H, CH_3, J = 7.1 Hz), 4.12–4.17 (q, 2H, CH_2, J = 7.1 Hz), 7.05–7.07 (d, 1H, ar. J = 8.3 Hz), 7.63–7.65 (d, 1H, ar, J = 8.3 Hz), 7.95–7.98 (t, 2H, ar, J = 8.4 Hz), 8.14 (s, 1H, ar); 8.45–8.49 (t, 1H, ar, J = 7.8 Hz), 8.86–8.88 (d, 2H, ar, J = 5.8 Hz), 9.87 (s, 1H, NH). IR: 1167, 1239, 1724, 3085, 3064, 3369.

*2-[(Ethoxycarbonyl)amino]-4-(trifluoromethyl)-benzenesulfonic acid, sodium salt (**19**)*

An excess of ethylchloroformate (7 mmol) was portionwise added to a suspension of the sodium salt **17** (3.8 mmol) in water (50 mL) while keeping alkaline the pH by adding solid $NaHCO_3$. At the end of addition, heavy precipitation occurred. The solid was collected and dried. Yield 80%. ^{1}H NMR: 1.26 (t, 3H, CH_3, J = 7.0 Hz), 4.16 (q, 2H, CH_2, J = 7.0 Hz), 7.36 (d, 1H, ar, J = 8.1 Hz), 7.85 (d, 1H, ar, J = 8.1 Hz), 8.46 (s, 1H, ar), 9.94 (br s, 1H, NH).

*4-Chloro-2-[(ethoxycarbonyl)amino]benzenesulfonyl chloride (**20**)*

PCl_5 (10.6 mmol) was added to a suspension of the pyridinium salt **18** (6.9 mmol) in $POCl_3$ (8 mL) and the mixture was refluxed for 1 h and 30 min. The solvent was evaporated at reduced pressure and the residue was treated with brine (20 g). The solid was collected, washed with iced water, and recrystallized. Yield 99%; m.p. 57–59°C (cyclohexane), (lit. [20] 56–58°C (cyclohexane)). ^{1}H NMR: 1.24 (t, 3H, CH_3, J = 7.1 Hz), 4.14 (q, 2H, CH_2, J = 7.1 Hz), 7.05 (dd, 1H, ar, J = 8.3, 1.8 Hz), 7.64 (d, 1H, ar, J = 8.3 Hz), 8.13 (d, 1H, ar, J = 1.8 Hz), 9.86 (s, 1H, NH). IR: 1241, 1529, 1587, 1715, 3388. Anal. calcd. for $C_9H_9Cl_2NO_4S$: C, 36.26; H, 3.04; N, 4.70. Found: C, 35.32; H, 3.68; N, 5.87.

*2-[(Ethoxycarbonyl)amino]-4-trifluoromethyl-benzenesulfonyl chloride (**21**)*

PCl_5 (20 mmol) was added to a suspension of the sodium salt **19** (10 mmol) in $POCl_3$ (16 mL). The suspension was stirred at 70°C for 1 h and 30 min and then allowed to cool to room temperature, poured in small portions into ice (30 g) and extracted with diethyl ether (3 × 30 mL). The organic layer was washed twice with water (60 mL), dried (Na_2SO_4), and concentrated under reduced pressure to yield an oily residue, which was pure enough to be used in the next step. Yield 75%. ^{1}H NMR: 1.24 (t, 3H, CH_3, J = 7.0 Hz), 4.15 (q, 2H, CH_2, J = 7.0 Hz), 7.35 (d, 1H, ar, J = 8.0 Hz), 7.84 (d, 1H, ar, J = 8.0 Hz), 8.44 (s, 1H, ar), 9.91 (s, 1H, NH).

*General procedure for the synthesis of 2-amino-benzenesulfonyl chlorides (**22** and **23**)*

Commercially available aniline derivative **14** or **15** (39 mmol) was added in small portions to a chilled and well-stirred solution of $ClSO_3H$ (98%, 18 mL). During the addition, the temperature was held below 10°C, then it was raised to 150°C for 1 h. After cooling at room temperature, $SOCl_2$ (5 mL) was dropwise added. The mixture was heated at 150°C for 30 min (compound **22**) or for 90 min (compound **23**) and then was poured onto chopped ice (50 g) and extracted with ethyl acetate (3 × 100 mL). Evaporation of the dried (Na_2SO_4) organic layers afforded a solid, which was collected and used as such for the next step.

*2-Amino-4,5-dichlorobenzenesulfonyl chloride (**22**)*

Yield 60% (lit. [21] 130–131°C (cyclohexane/C_6H_6)). ^{1}H NMR: 7.04 (s, 1H, ar), 7.58 (s, 1H, ar). IR: 1353, 1462, 3388, 3499.

*2-Amino-5-chloro-4-nitro-benzenesulfonyl chloride (**23**)*

Yield 50%; ^{1}H NMR: 7.26 (s, 1H, ar), 7.56 (s, 1H, ar). IR: 1352, 1462, 3388, 3481.

*General procedure for the synthesis of 2H-1,2,4-benzothiadiazine-1,1-dioxides (**8** and **10**)*

Aryl sulfonyl chloride **20** or **21** (1.5 mmol) was dissolved in aqueous ammonia solution (33%, 20 mL). The solution was heated at 60°C for 2 h. The alkaline mixture was filtered and the filtrate acidified with 6 N HCl. The precipitate was collected by filtration, washed with water, and recrystallized.

*6-Chloro-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**8**)*

Yield 50%; m.p. 326–328°C (water). ^{1}H NMR: 7.26 (s, 1H, ar), 7.34 (d, 1H, ar, $J = 8.2$ Hz), 7.81 (d, 1H, ar, $J = 8.2$ Hz), 11.35 (br s, 1H, NH). IR: 1370, 1736, 3292. Anal. calcd. for $C_7H_5ClN_2O_3S$: C, 36.14; H, 2.17; N, 12.04. Found: C, 35.21; H, 3.66; N, 11.00.

*6-Trifluoromethyl-3-oxo-2H-1,2,4-benzothiadiazine-1,1-dioxide (**10**)*

Yield 52%; m.p. 227–228°C (water). ^{1}H NMR: 7.54 (s, 1H, ar), 7.60 (d, 1H, ar, $J = 8.2$ Hz), 8.00 (d, 1H, ar, $J = 8.2$ Hz), 11.41 (br s, 1H, NH). IR: 1340, 1736, 3514, 3554. Anal. calcd. for $C_8H_5F_3N_2O_3S$: C, 36.10; H, 1.89; N, 10.52. Found: C, 38.22; H, 2.50; N, 9.02.

*General procedure for the synthesis of N-benzyloxy-2-[(ethoxycarbonyl)amino]benzenesulfonamides (**24** and **25**)*

A solution of triethylamine (8.0 mmol) and *O*-benzylhydroxylamine hydrochloride (8.1 mmol) in a mixture tetrahydrofuran/water (7:3, 10 mL), was portionwise added to a suspension of sulfonyl chloride **20**, **21** (4.0 mmol) in tetrahydrofuran (5 mL). At the end of addition, the mixture was stirred at room temperature for 24 h. Then, evaporation of the solvent and dilution with water (15 mL) yielded a mixture, which was adjusted to pH 1 with 6 N HCl. After 2 h stirring, the precipitate was collected by filtration, washed with water, and recrystallized.

*N-Benzyloxy-4-chloro-2-[(ethoxycarbonyl)amino]-benzenesulfonamide (**24**)*

Yield 80%; m.p. 79–81°C (cyclohexane). ^{1}H NMR: 1.26 (t, 3H, CH_3, $J = 7.1$ Hz), 4.18 (q, 2H, CH_2, $J = 7.1$ Hz), 4.89 (s, 2H, CH_2), 7.31–7.39 (m, 6H, ar), 7.83 (dd, 1H, ar, $J = 7.6$, 2.1 Hz), 8.97 (s, 1H, NHCOO), 10.90 (s, 1H, SO_2NH). IR: 1376, 1461, 1734, 3192, 3357. Anal. calcd. for $C_{16}H_{17}ClN_2O_5S$: C, 49.94; H, 4.45; N, 7.28. Found: C, 51.16; H, 4.07; N, 9.77.

*N-Benzyloxy-2-[(ethoxycarbonyl)amino]-4-trifluoromethyl-benzenesulfonamide (**25**)*

Yield 83%; m.p. 113–115°C (water/ethanol). ^{1}H NMR: 1.26 (t, 3H, CH_3, $J = 7.1$ Hz), 4.18 (q, 2H, CH_2, $J = 7.1$ Hz), 4.91 (s, 2H, CH_2), 7.32–7.37 (m, 5H, ar), 7.66 (d, 1H, ar, $J = 8.6$ Hz), 8.04 (d, 1H, ar, $J = 8.3$ Hz), 8.56 (s, 1H, ar), 9.05 (s, 1H, NHCOO), 11.07 (s, 1H, SO_2NH). IR: 1376, 1439, 1727, 3110, 3397. Anal. calcd. for $C_{17}H_{17}F_3N_2O_5S$: C, 48.80; H, 4.10; N, 6.70. Found: C, 47.21; H, 3.56; N, 7.68.

*General procedure for the synthesis of N-benzyloxy-2-amino-benzenesulfonamides (**26** and **27**)*

A solution of 2-[(ethoxycarbonyl)amino]benzenesulfonamides **24**, **25** (2.2 mmol) in an aqueous solution of NaOH (4%, 7 mL) was stirred at 100°C for 2 h. After cooling, the reaction mixture was diluted with water (20 mL) and acidified with 6 N HCl. The precipitate was collected by filtration, washed with water, and recrystallized.

*2-Amino-N-benzyloxy-4-chloro-benzenesulfonamide (**26**)*

Yield 93%; m.p. 83–85°C (water/ethanol). ^{1}H NMR: 4.83 (s, 2H, CH_2), 6.31 (br s, 2H, NH_2), 6.67 (d, 1H, ar, $J = 8.6$ Hz), 6.89 (s, 1H, ar), 7.34–7.37 (m, 5H, ar), 7.52 (d, 1H, ar, $J = 8.6$ Hz), 10.40 (s, 1H, SO_2NH). IR: 1375, 1464, 3192, 3392, 3488. Anal. calcd. for $C_{13}H_{13}ClN_2O_3S$: C, 49.92; H, 4.19; N, 8.96. Found: C, 47.55; H, 5.88; N, 7.55.

*2-Amino-N-benzyloxy-4-trifluoromethyl-benzene-sulfonamide (**27**)*

Yield 95%; m.p. 104–106°C (water/ethanol). ^{1}H NMR: 4.85 (s, 2H, CH_2), 6.49 (s, 2H, NH_2), 6.91 (d, 1H, ar, $J = 8.4$ Hz), 7.18 (s, 1H, ar), 7.32–7.37 (m, 5H, ar), 7.72 (d, 1H, ar, $J = 8.4$ Hz), 10.57 (s, 1H, SO_2NH). IR: 1349, 1449, 3210, 3368, 3458. Anal. calcd. for $C_{14}H_{13}F_3N_2O_3S$: C, 48.55; H, 3.78; N, 8.09. Found: C, 49.23; H, 5.01; N, 10.71.

*General procedure for the synthesis of 2-benzyloxy-3-oxo-1,2,4-benzothiadiazine-1,1-dioxides (**9** and **28**)*

Triphosgene (3.1 mmol) was added to a solution of 2-aminobenzenesulfonamide **26**, **27** (6.2 mmol) in anhydrous tetrahydrofuran (50 mL). The mixture was stirred at room temperature for 24–48 h (TLC monitoring: eluting system AcOEt/CHX/MeOH/NH_4OH 2:3:0.4:0.1). Evaporation of the solvent at reduced pressure yielded a solid, which was treated with water, collected by filtration, washed with water, and recrystallized.

*2-Benzyloxy-6-chloro-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**9**)*

Yield 98%; m.p. 228–229°C (ethanol). ^{1}H NMR: 5.11 (s, 2H, CH_2), 7.31 (d, 1H, ar, $J = 1.9$ Hz), 7.41–7.46 (m, 4H, ar), 7.48–7.51 (m, 2H, ar), 8.03 (d, 1H, ar, $J = 8.5$ Hz), 11.78 (s, 1H, NH). IR: 1376, 1462, 1715. Anal. calcd. for $C_{14}H_{11}ClN_2O_4S$: C, 49.64; H, 3.27; N, 8.27. Found: C, 50.85; H, 4.56; N, 9.62.

*2-Benzyloxy-3-oxo-6-trifluoromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**28**)*

Yield 94%; m.p. 215–216°C (ethanol). ^{1}H NMR: 5.12 (s, 2H, CH_2), 7.43–7.50 (m, 5H, ar), 7.59 (s, 1H, ar), 7.72 (d, 1H, ar, $J = 8.4$ Hz), 8.25 (d, 1H, ar, $J = 8.2$ Hz), 11.94 (s, 1H, NH). IR: 1339, 1462, 1728. Anal. calcd. for $C_{15}H_{11}F_3N_2O_4S$: C, 48.39; H, 2.98; N, 7.52. Found: C, 47.16; H, 3.16; N, 8.43.

*General procedure for the synthesis of 2-hydroxy-3-oxo-1,2,4-benzothiadiazine-1,1-dioxides (**1** and **5**)*

A solution of BBr_3 in CH_2Cl_2 (1 M, 11.3 mL) was, drop-by-drop, added to a cooled (−70°C) suspension of compounds **9**, **28** (5.7 mmol) in anhydrous dichloromethane (50 mL). At the end of addition, the suspension was allowed to warm at −20°C for 1 h and 30 min. The solution was diluted with water (50 mL) under vigourous stirring and, after 12 h, the resulting precipitate was collected by filtration, washed with water, and recrystallized.

*6-Chloro-2-hydroxy-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**1**)*

Yield 72%; m.p. 153–156°C (water). ^{1}H NMR: 7.29 (d, 1H, ar, $J = 1.8$ Hz), 7.40 (dd, 1H, ar, $J = 8.6$, 1.8 Hz), 7.96 (d, 1H, ar, $J = 8.6$ Hz), 11.17 (s, 1H, NH or OH), 11.57 (s, 1H, OH or NH). IR: 1714, 3482, 3578. Anal. calcd. for $C_7H_5ClN_2O_4S$: C, 33.81; H, 2.03; N, 11.27. Found: C, 35.12; H, 3.76; N, 12.36.

*2-Hydroxy-3-oxo-6-trifluoromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**5**)*

Yield 90%; m.p. 198–200°C (water). ^{1}H NMR: 7.57 (s, 1H, ar), 7.68 (d, 1H, ar, $J = 7.8$ Hz), 8.19 (d, 1H, ar, $J = 7.8$ Hz), 11.30 (s, 1H, NH or OH), 11.75 (s, 1H, OH or NH). IR: 1720, 3510, 3605. Anal. calcd. for $C_8H_5F_3N_2O_4S$: C, 39.05; H, 1.79; N, 9.93. Found: C, 41.41; H, 2.03; N, 11.24.

*General procedure for the synthesis of 2-hydroxy-7-nitro-3-oxo-1,2,4-benzothiadiazine-1,1-dioxides (**2** and **6**)*

Compounds **1**, **5** (1 mmol) was portionwise added to HNO_3 (100%, 3.7 mL). The mixture was stirred at room temperature for 8 h, then the solution was poured onto ice (about 50 g) and extracted with diethyl ether (3 × 20 mL). The organic layer was washed with an aqueous solution of $NaHCO_3$ (3%, 30 mL) and then with water (3 × 20 mL). Then, it was dried over anhydrous Na_2SO_4, filtered, and concentrated to yield a solid, which was filtered and recrystallized.

*6-Chloro-2-hydroxy-7-nitro-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**2**)*

Yield 70%; m.p. 120–122°C (water). ^{1}H NMR: 7.45 (s, 1H, ar), 8.73 (s, 1H, ar), 11.11 (s, 1H, NH or OH), 11.47 (s, 1H, OH or NH). Anal. calcd. for $C_7H_4ClN_3O_6S$: C, 28.63; H, 1.37; N, 14.31. Found: C, 26.58; H, 2.97; N, 13.26.

*2-Hydroxy-3-oxo-7-nitro-6-trifluoromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**6**)*

Yield 70%; m.p. 217–220°C (water). ^{1}H NMR: 7.76 (s, 1H, ar), 8.82 (s, 1H, ar), 11.27 (s, 1H, NH or OH), 11.27 (s, 1H, OH or NH). Anal. calcd. for $C_8H_4F_3N_3O_6S$: C, 29.37; H, 1.23; N, 12.84. Found: C, 27.24; H, 2.98; N, 10.65.

*7-Amino-2-hydroxy-3-oxo-6-trifluoromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**29**)*

20% Pd/C was added to a solution of compound **6** (1.2 mmol) in ethanol (200 mL) and the mixture was hydrogenated in a Parr apparatus at 40 psi for 4 days. The catalyst was filtered off and the solvent was evaporated at reduced pressure to yield a solid that was recrystallized. Yield 50%; m.p. 240–243°C (water). ^{1}H NMR: 6.00 (s, 2H, NH_2), 7.28 (s, 1H, ar), 7.38 (s, 1H, ar), 11.01 (s, 1H, NH or OH), 11.07 (s, 1H, OH or NH). IR: 1701, 3388, 3492. Anal. calcd. for $C_8H_6F_3N_3O_4S$: C, 32.33; H, 2.03; N, 14.14. Found: C, 30.98; H, 3.47; N, 12.56.

*7-(3-Formylpyrrol-1yl)-2-hydroxy-3-oxo-6-trifluoromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**7**)*

A solution of 2,5-dimethoxytetrahydrofuran-3-carbaldehyde (1 mmol) in glacial acetic acid (1.5 mL) was dropwise added to a solution of the 7-amino derivative **29** (0.7 mmol) in glacial acetic acid (4 mL). The mixture was stirred at 60°C for 2 h. Evaporation of the solvent at reduced pressure yielded a solid, which was suspended in water (10 mL), collected and recrystallized. Yield 60%; m.p. >300°C (ethanol). ^{1}H NMR: 6.65 (s, 1H, ar), 7.16 (s, 1H, ar), 7.74 (s, 1H, ar), 7.91 (s, 1H, ar), 8.28 (s, 1H, ar), 9.78 (s, 1H, CHO), 11.41 (s, 1H, NH or OH), 11.96 (s, 1H, OH or NH). Anal. calcd. for $C_{13}H_8F_3N_3O_5S$: C, 41.61; H, 2.15; N, 11.20. Found: C, 43.21; H, 3.69; N, 13.12.

*2-Amino-4,5-dichloro-N-hydroxy-benzenesulfonamide (**30**)*

A solution of sulfonyl chloride **22** (7.7 mmol) in 1,4-dioxane (15 mL) was added dropwise to a stirred and chilled solution of hydroxylamine hydrochloride (15.4 mmol), triethylamine (17 mmol), and water (5 mL). The mixture was stirred at room temperature for 24 h. Evaporation at reduced pressure of the solvent then addition of water yielded a solid, which was collected, washed with water, and recrystallized. Yield 67%; m.p. 170–172°C (ethanol), (lit. [21] 171–172°C cyclohexane). ^{1}H NMR: 6.34 (s, 2H, NH_2), 7.09 (s, 1H, ar), 7.59 (s, 1H, ar), 9.65 (s, 1H, NH or OH), 9.67 (s, 1H, OH or NH). IR: 1309, 1462, 3203, 3255, 3389, 3442. Anal. calcd. for $C_6H_6Cl_2N_2O_3S$: C, 28.03; H, 2.35; N, 10.90. Found: C, 26.89; H, 3.77; N, 8.13.

*2-Amino-5-chloro-4-nitro-N-(phenylmethoxy)-benzenesulfonamide (**31**)*

The title compound was obtained by reacting sulfonyl chloride **23** (3.2 mmol) with *O*-benzylhydroxylamine hydrochloride (6.3 mmol) in the same reaction conditions described above to prepare compounds **24** and **25** from **20** and **21**, respectively. Yield 95%; m.p. 98–101°C (cyclohexane/ethyl acetate). ^{1}H NMR: 4.89 (s, 2H, CH_2), 6.73 (s, 2H, NH_2), 7.36–7.38 (m, 5H, ar), 7.46 (s, 1H, ar), 7.71 (s, 1H, ar), 10.73 (s, 1H, NH). IR: 1340, 1463, 3224, 3392, 3500. Anal. calcd. for $C_{13}H_{12}ClN_3O_5S$: C, 43.64; H, 3.38; N, 11.75. Found: C, 41.21; H, 4.59; N, 12.68.

*General procedure for the synthesis of 2-hydroxy-3-oxo-1,2,4-benzothiadiazine-1,1-dioxide (**3**) and 2-benzyloxy-3-oxo-1,2,4-benzothiadiazine-1,1-dioxide (**11**)*

The title compounds were obtained by reacting sulfonamides **30** or **31** (1.5 mmol) with triphosgene (0.8 mmol) in the same reaction conditions described above to prepare compounds **9** and **28** from **26** and **27**, respectively.

*6,7-Dichloro-2-hydroxy-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**3**)*

Yield 60%; m.p. 202–205°C (ethanol). ^{1}H NMR: 7.45 (s, 1H, ar), 8.31 (s, 1H, ar), 11.26 (s, 1H, NH or OH), 11.68 (s, 1H, OH or NH). IR: 1349, 1465, 1711, 3486, 3572. Anal. calcd. for $C_7H_4Cl_2N_2O_4S$: C, 29.70; H, 1.42; N, 9.90. Found: C, 31.20; H, 3.12; N, 11.22.

*2-Benzyloxy-7-chloro-6-nitro-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**11**)*

Yield 95%; m.p. 218–220°C (ethanol). ^{1}H NMR: 5.13 (s, 2H, CH_2), 7.42–7.44 (m, 3H, ar), 7.49–7.52 (m, 2H, ar), 7.89 (s, 1H, ar), 8.56 (s, 1H, ar), 12.15 (s, 1H, NH). IR: 1376, 1463, 1722. Anal. calcd. for $C_{14}H_{10}ClN_3O_6S$: C, 43.82; H, 2.63; N, 10.95. Found: C, 45.24; H, 3.46; N, 12.37.

*7-Chloro-2-hydroxy-6-nitro-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (**4**)*

The title compound was obtained by reacting the 2-benzyloxy derivative **11** (1.3 mmol) with BBr_3 (1M CH_2Cl_2 solution, 2.6 mL) in the same reaction conditions described above to prepare compounds **1** and **5** from **9** and **28**, respectively. Yield 68%; m.p. 219–221°C (water). ^{1}H NMR: 7.86 (s, 1H, ar), 8.49 (s, 1H, ar), 11.40 (s, 1H, NH or OH), 11.97 (s, 1H, OH or NH). IR: 1376, 1462, 1754,

3103. Anal. calcd. for $C_7H_4ClN_3O_6S$: C, 28.63; H, 1.37; N, 14.31. Found: C, 26.341; H, 2.97; N, 12.98.

Pharmacology

All animal procedures were conducted according to the Italian Guidelines for Animal Care, DL 116/92, application of the European Communities Council Directive (86/609/EEC).

Binding assay

Rat cortical synaptic membrane preparation and [^{3}H]glycine, [^{3}H]-AMPA, and high-affinity [^{3}H]kainate binding experiments were performed following the procedures described in [6, 24], and [11], respectively.

Sample preparation and results calculation

A stock 1 mM solution of the tested compound was prepared in 50% DMSO. Subsequent dilutions were produced in appropriate buffer. Radioligand binding data curves were analyzed using Prism 5.02 (GraphPad Software, Inc., San Diego, CA). IC_{50} values, derived from three to five displacement curves based on six scalar concentrations of the test compounds were converted to K_i values by application of the Cheng–Prusoff equation [25]. Under our experimental conditions, the dissociation constants (K_D) for [^{3}H]glycine, [^{3}H]-DL-AMPA, and [^{3}H]kainate were 42.96 ± 10.96, 17.76 ± 2.89, and 2.21 ± 0.33 nM, respectively.

The authors have declared no conflict of interest.

References

[1] H. Bräuner-Osborne, J. Egebjerg, E. X. Nielsen, U. Madsen, P. Krogsgaard-Larsen, *J. Med. Chem.* **2000**, *43*, 2609–2645.

[2] S. F. Traynelis, L. P. Wollmuth, C. J. McBain, F. S. Menniti, K. M. Vance, K. K. Ogden, K. B. Hansen, H. Yuan, S. J. Myers, R. Dingledine, *Pharmacol. Rev.* **2010**, *62*, 405–496.

[3] P. Stawski, H. Janovjak, D. Trauner, *Bioorg. Med. Chem.* **2010**, *18*, 7759–7772.

[4] G. J. Lees, *Drugs* **2000**, *59*, 33–78.

[5] H. Mattes, D. Carcache, H. O. Kalkman, M. Koller, *J. Med. Chem.* **2010**, *53*, 5367–5382.

[6] V. Colotta, D. Catarzi, F. Varano, L. Cecchi, G. Filacchioni, A. Galli, C. Costagli, *Arch. Pharm. Pharm. Med. Chem.* **1997**, *330*, 129–134.

[7] D. Catarzi, V. Colotta, F. Varano, L. Cecchi, G. Filacchioni, A. Galli, C. Costagli, *J. Med. Chem.* **1999**, *42*, 2478–2484.

[8] D. Catarzi, V. Colotta, F. Varano, L. Cecchi, G. Filacchioni, A. Galli, C. Costagli, V. Carlà, *J. Med. Chem.* **2000**, *43*, 3824–3826.

[9] F. Varano, D. Catarzi, V. Colotta, L. Cecchi, G. Filacchioni, A. Galli, C. Costagli, *Eur. J. Med. Chem.* **2001**, *44*, 3157.

[10] D. Catarzi, V. Colotta, F. Varano, G. Filacchioni, A. Galli, C. Costagli, V. Carlà, *J. Med. Chem.* **2001**, *44*, 3157–3165.

[11] F. Varano, D. Catarzi, V. Colotta, G. Filacchioni, A. Galli, C. Costagli, V. Carlà, *J. Med. Chem.* **2002**, *45*, 1035–1044.

[12] D. Catarzi, V. Colotta, F. Varano, F. R. Calabri, G. Filacchioni, A. Galli, C. Costagli, V. Carlà, *J. Med. Chem.* **2004**, *47*, 262–272.

[13] V. Colotta, D. Catarzi, F. Varano, F. R. Calabri, G. Filacchioni, C. Costagli, A. Galli, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2345–2349.

[14] F. Varano, D. Catarzi, V. Colotta, F. R. Calabri, O. Lenzi, G. Filacchioni, A. Galli, C. Costagli, *Bioorg. Med. Chem.* **2005**, *13*, 5536–5549.

[15] V. Colotta, D. Catarzi, F. Varano, O. Lenzi, G. Filacchioni, C. Costagli, A. Galli, C. Ghelardini, N. Galeotti, P. Gratteri, J. Sgrignani, F. Deflorian, S. Moro, *J. Med. Chem.* **2006**, *49*, 6015–6026.

[16] F. Varano, D. Catarzi, V. Colotta, O. Lenzi, G. Filacchioni, A. Galli, C. Costagli, *Bioorg. Med. Chem.* **2008**, *16*, 2617–2626.

[17] V. Colotta, O. Lenzi, D. Catarzi, F. Varano, L. Squarcialupi, C. Costagli, A. Galli, C. Ghelardini, A. M. Pugliese, G. Maraula, E. Coppi, D. E. Pellegrini-Giampietro, F. Pedata, D. Sabbadin, S. Moro, *Eur. J. Med. Chem.* **2012**, *54*, 470–482.

[18] P. H. L. Wei, S. C. Bell, S. J. Childress, *J. Heterocyclic Chem.* **1966**, *3*, 1–4.

[19] J. Valgeirsson, E. X. Nielsen, D. Peters, C. Mathiesen, A. S. Kristensen, U. Madsen, *J. Med. Chem.* **2004**, *47*, 6948–6957.

[20] L. Raffa, M. Bella, A. Monzani, *Farmaco Sci.* **1960**, *15*, 716–725.

[21] N. D. Heindel, H. C. C. Ko, R. B. Birrer, J. R. Merkel, *J. Med. Chem.* **1972**, *15*, 118–120.

[22] B. Pirotte, P. De Tullio, Q. A. Nguyen, F. Somers, P. Fraikin, X. Florence, P. Wahl, J. Bondo Hansen, P. Lebrun, *J. Med. Chem.* **2010**, *53*, 147–154.

[23] M. Di Bella, A. Monzani, M. G. Andrisano, U. Fabio, G. P. Quaglio, *Farmaco Sci.* **1979**, *34*, 189–198.

[24] E. O. Nielsen, U. Madsen, K. Schaumburg, L. Brehm, P. Krogsgaard-Larsen, *Eur. J. Chem. – Chim. Ther.* **1986**, *21*, 433–437.

[25] Y.-C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

