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Synthesis and Pharmacology of Mono-, Di-, and Trialkyl-Substituted 7-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides Combined with X-ray Structure Analysis to Understand the Unexpected Structure–Activity Relationship at AMPA Receptors

Anja Probst Larsen,[†,∥] Pierre Francotte,[‡,∥] Karla Frydenvang,[†] Daniel Tapken,[†] Eric Goffin,[‡] Pierre Fraikin,[‡] Daniel-Henri Caignard,[§] Pierre Lestage,[§] Laurence Danober,[§] Bernard Pirotte,[‡,⊥] and Jette Sandholm Kastrup*[,†,⊥]

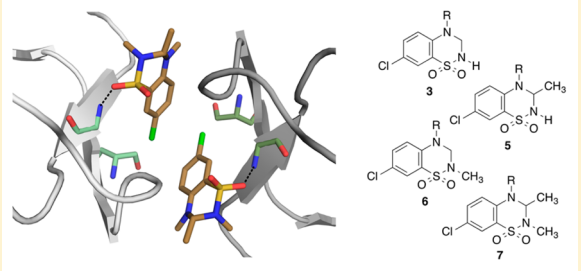
[†]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken, 2, DK-2100 Copenhagen, Denmark

[‡]Department of Medicinal Chemistry, Center for Interdisciplinary Research on Medicines (CIRM), University of Liege, Avenue de l'Hôpital, 1, B36, B-4000 Liège, Belgium

[§]Institut de Recherches Servier, Chemin de Ronde, 125, F-78290 Croissy-sur-Seine, France

Supporting Information

ABSTRACT: Positive allosteric modulators of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA)-type ionotropic glutamate receptors are promising compounds for treatment of neurological disorders, for example, Alzheimer's disease. Here, we report synthesis and pharmacological evaluation of a series of mono-, di-, or trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides, comprising in total 16 new modulators. The trisubstituted compounds **7b**, **7d**, and **7e** revealed potent activity ($EC_{2\times}$ = 2.7–4.3 μM; concentration of compound responsible for a 2-fold increase of the AMPA mediated response) as AMPA receptor potentiators in an in vitro cellular fluorescence assay (FLIPR). The 4-cyclopropyl compound **7f** was found to be considerably less potent ($EC_{2\times}$ = 60 μM), in contrast to previously described 4-monoalkyl-substituted benzothiadiazine dioxides for which the cyclopropyl group constitutes the best choice of substituent. **7b** was subjected to X-ray structural analysis in complex with the GluA2 ligand-binding domain. We propose an explanation of the unexpected structure–activity relationship of this new series of mono-, di-, and trialkyl-substituted 1,2,4-benzothiadiazine 1,1-dioxide compounds. The methyl substituent in the 3-position directs the binding mode of the 1,2,4-benzothiadiazine 1,1-dioxide (BTD) scaffold. When a methyl substituent is present in the 3-position of the BTD, additional methyl substituents in both the 2- and 4-positions increase potency, whereas introduction of a 4-cyclopropyl group does not enhance potency of 2,3,4-alkyl-substituted BTDs. A hydrogen bond donor in the 2-position of the BTD is not necessary for modulator potency.



KEYWORDS: *Positive allosteric modulators, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid receptor, ligand-binding domain, synthesis, crystal structure, structure–activity relationship*

INTRODUCTION

L-Glutamic acid is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), activating metabotropic glutamate receptors (coupled to G-proteins) and ionotropic glutamate receptors (iGluRs). The iGluRs are divided into three subtypes: *N*-methyl-D-aspartic acid (NMDA) receptors, kainic acid receptors, and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptors.[1] Of the three, AMPA receptors mediate the majority of fast excitatory postsynaptic signaling and are involved in expression of long-term potentiation, a phenomenon closely linked to learning and memory formation.[2,3] Therefore, AMPA receptors represent an interesting target for the development of cognitive enhancers. Although AMPA receptor agonists are potential drug candidates, they may cause severe, unfavorable side effects, for example, neurotoxicity, because they activate the receptors directly. By contrast, positive allosteric modulators of AMPA receptors (AMPA-PAMs) only potentiate the receptor-mediated currents after release of the endogenous ligand and thereby function by fine-tuning receptor signaling. Several studies suggest a role for AMPA-PAMs in the therapeutic strategy to treat CNS disorders, such as schizophrenia,[4] Alzheimer's disease,[5] and attention-deficit/hyperactivity disorder (ADHD).[6]

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iGluRs assemble as homo- or heterotetrameric receptors to form ligand-gated ion channels. Each subunit comprises an extracellular part with an N-terminal domain and a ligand-binding domain (LBD), a transmembrane region and an intracellular C-terminal domain.[1,7,8] The four LBDs of the functional receptor are arranged as dimer-of-dimers[8] with glutamate binding in the orthosteric site within each LBD, whereas AMPA-PAMs bind in the dimer interface of two LBDs. Thereby, AMPA-PAMs potentiate the glutamate-evoked response by stabilizing the activated state of the receptor via two different mechanisms: (i) by stabilizing the glutamate-bound conformation and thereby slowing deactivation,[9] or (ii) by stabilizing the LBD dimer interface and thereby slowing the structural rearrangement that leads to receptor desensitization with glutamate still bound.[10]

In recent years, several structures of the LBD of the ionotropic glutamate receptor A2 (GluA2) in complex with chemically diverse AMPA-PAMs have been reported (e.g., reviewed by Pøhlsgaard et al.[11] and by Kumar and Mayer[12]). Classical examples of modulators are cyclothiazide (CTZ, compound **9**, Figure 1) and IDRA 21 (compound **5a**, Figure 1). Both modulators have a 1,2,4-benzothiadiazine 1,1-dioxide (BTD) scaffold, but comprise different substituents in the 3-position of the thiadiazine ring. This difference leads to a shifted binding mode of **5a** compared to that of **9** (Figure 2), as previously reported.[13] Compound **5a** has been an important lead compound for clinical trials because it is active in vivo.[14]

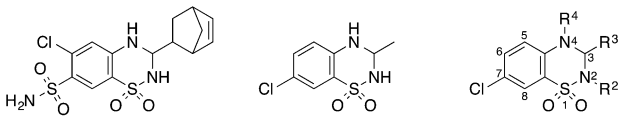

Figure 1. Chemical structures of two representatives of AMPA-PAMs, cyclothiazide (CTZ, **9**) and IDRA 21 (**5a**), and a general formula of the newly synthesized series of mono-, di-, and trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides.

We have previously described AMPA-PAMs belonging to BTDs[15−17] and 1,2,4-pyridothiadiazine 1,1-dioxides (PTDs).[18−20] Structural analysis of selected compounds has revealed how the nature of the 4-alkyl substituent in the thiadiazine ring greatly affects the modulator potency.[17] To further investigate the structure−activity relationship (SAR) for various substituents in the thiadiazine ring, we have designed and synthesized a series of mono-, di-, and trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (Figure 1), based on our previous studies on pyrido-, thieno-, and benzothiadiazine dioxides as AMPA-PAMs. The new compounds have been evaluated as AMPA-PAMs in two in vitro assays: a voltage clamp assay (VC) on *Xenopus laevis* oocytes and a fluorescence assay (FLIPR) on rat brain cells. Furthermore, the X-ray structure of one compound within this series, **7b**, in complex with the LBD of the target protein GluA2 has been solved. Based on this structure, we propose a likely explanation of the SAR of this new series of mono-, di-, and trialkyl-substituted 1,2,4-benzothiadiazine 1,1-dioxide compounds.

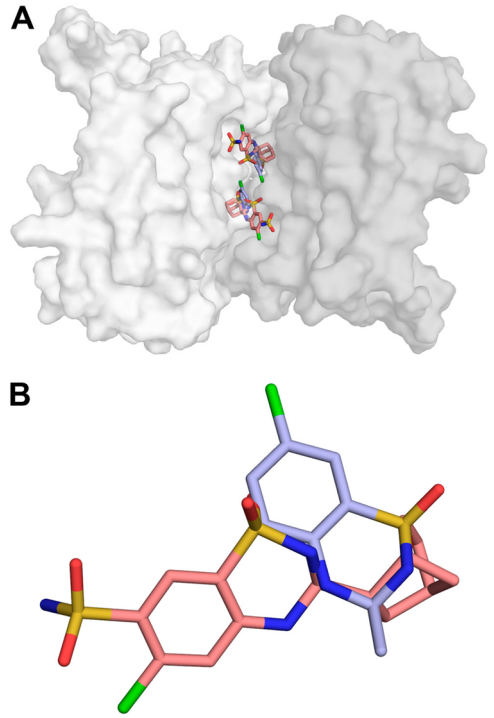

Figure 2. Binding mode of cyclothiazide (CTZ, **9**) and of the shifted thiazide IDRA 21 (**5a**) in GluA2 LBD. (A) Dimeric GluA2 LBD (mol*A* in white and mol*B* in light gray surface representation) with two molecules of **5a** (carbon in light blue sticks, PDB ID 3IL1) and **9** (carbon in salmon sticks, PDB ID 3TDK), respectively, bound in the dimer interface. (B) The benzothiadiazine dioxide scaffold of **5a** is shifted compared to that of **9**. Chlorine atoms are shown in green, nitrogen atoms in blue, oxygen atoms in red and sulfur atoms in yellow.

RESULTS AND DISCUSSION

Chemistry. The synthetic route to the mono-, di-, and trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides is described in Scheme 1. As previously reported, 4-alkyl-7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**3**) can be prepared from the corresponding unsaturated compounds (**2**) after reaction with sodium borohydride in isopropanol. Except for 7-chloro-4-cyclopropylmethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **3g**, the synthesis of the other 4-alkyl-substituted compounds (**3a−f**) has been published elsewhere.[15−17] The unsaturated intermediate **2g** was obtained after alkylation of 7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**1**)[15] with cyclopropylmethyl bromide in acetonitrile in the presence of potassium carbonate.

The key intermediates in the access way to the desired target compounds **5** (3,4-dialkyl-substituted benzothiadiazine dioxides) and **7** (2,3-dialkyl-substituted and 2,3,4-trialkyl-substituted benzothiadiazine dioxides) are the ortho-alkylaminobenzenesulfonamides **4**. The latter were obtained from the reaction of the commercially available 5-chloro-2-fluorobenzenesulfonamide **10** with the appropriate alkyl/cycloalkylamine or from the ring opening of the 4-alkyl-substituted unsaturated benzothiadiazine dioxides **2** under aqueous alkaline conditions. The ortho-alkylaminobenzenesulfonamides **4** reacted with acetaldehyde in the presence of a catalytic amount of camphorsulfonic acid to provide the diverse 4-alkyl-substituted 7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **5a−g**. It must be pointed out that some of the 3,4-dialkyl-substituted benzothiadiazine dioxides showed chemical instability (i.e., **5c**, **5f** and **5g**) and that it was not possible to

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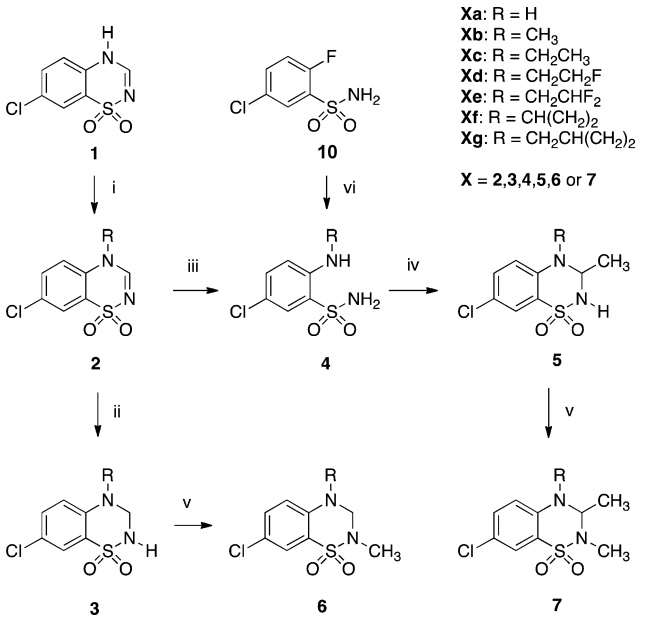
Scheme 1. [a]



[a]Reagents: (i) R–X, K_2CO_3, CH_3CN, reflux, 24 h (70–80%); (ii) $NaBH_4$, 2-propanol, 50 °C, 5 min. (80–85%); (iii) NaOH 5%, H_2O, 70 °C, 1 h (80–90%); (iv) CH_3CHO, H^+, CH_3CN, rt, 1–2 h (65–85%); (v) CH_3I, K_2CO_3, CH_3CN, 70–80 °C, 1–3 h (80–90%); (vi) R-NH_2, dioxane, 100–110 °C, 24 h (80–90%).

examine them in the biological assays on AMPA receptors. They were immediately engaged in the next step of the synthesis leading to the more stable 2,3,4-trialkyl-substituted benzothiadiazine dioxides **7b–g**. The greater stability of compounds **5a**, **5b**, **5d** and **5e** in aqueous medium could be explained by the lower tendency of the nitrogen atom at the 4-position to be protonated (lower basic character), as protonation is expected to facilitate the opening of the thiadiazine ring (Figure 3). Indeed, the availability of the lone

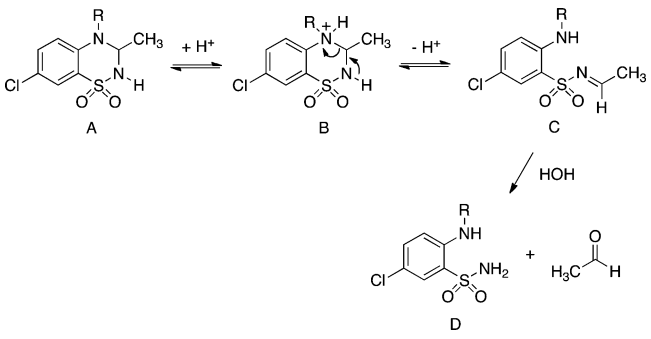


Figure 3. Protonation of the nitrogen atom at the 4-position and the presence of a hydrogen atom at the 2-position are expected to facilitate the opening of the thiadiazine ring in aqueous medium. The two first steps (A to B and B to C) are reversible, while the addition of a water molecule (C to D) irreversibly leads to the release of acetaldehyde.

pair of the nitrogen atom to form a bond with a proton could be influenced by the electron donating effect of the substituent linked to this atom, which is probably more pronounced with an alkyl or cycloalkyl chain of two or more carbon atoms (**5c**, **5f**, and **5g**) than with a hydrogen atom (**5a**), a methyl group (**5b**) or a fluoro-substituted ethyl group (**5d** and **5e**). When the 2-position of the benzothiadiazine dioxide bears a methyl (or any alkyl) group instead of a labile hydrogen atom of the weak acidic sulfonamide function, the rearrangement leading to ring opening is a less probable issue. Compounds of general formula **3** are not known to be unstable in aqueous medium, probably because the absence of an electron donating alkyl chain at the 3-position (only hydrogen atoms) reduces the basicity of the nitrogen atom and does not promote its protonation and afterward the breaking of the C3–N4 single bond of the 4-*N*-protonated intermediate B (Figure 3).

A more detailed study of the stability in aqueous medium of selected mono-, di-, and trimethyl-substituted BTDs is reported in the next section (see below).

Finally, the 2-methyl-substituted benzothiadiazine dioxides of general formula **6** (2,4-dialkyl-substituted compounds **6b–g** and the monoalkyl-substituted compound **6a**) and of general formula **7** (2,3,4-trialkyl-substituted compounds **7b–g** and the 2,3-dialkyl-substituted compound **7a**) were prepared by methylation of the target compounds **3** or **5**, respectively, by means of methyl iodide in acetonitrile in the presence of potassium carbonate.

Impact of the Mono-, Di-, or Trimethylation of the BTD Scaffold on Water Solubility and Aqueous Stability. Water solubility is an important physicochemical parameter to determine for a new compound in the perspective of a selection as a drug candidate. It is well established that very poorly water-soluble compounds are expected to exhibit poor oral bioavailability. Small-sized molecules with a favorable hydrophilic/lipophilic balance (reflected by their logP value) and satisfying water solubility are the best candidates. In Table 1, we report the water solubility determined in distilled water at room temperature (20 °C) of the mono-, di- and trimethyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **3b**, **5a**, **5b**, **6a**, **6b**, **7a**, and **7b**, as well as of two reference BTDs previously described, that is, compounds **3f** and **8**.

Logically, water solubility decreased when the number of methyl groups increased. The most water-soluble compound was found to be the monomethyl-substituted compound **3b** (554 μM; 129 μg/mL), while the trimethyl-substituted analog **7b** retained modest water solubility (38 μM; 10 μg/mL) combined with a calculated log *P* value of 2.07 ensuring appropriate hydrophilic/lipophilic balance for oral bioavailability and CNS distribution.[21] For comparison purposes, the very potent AMPA receptor potentiators **3f** and **8** previously described[17] also demonstrated appropriate physicochemical parameters for in vivo per os administration and blood brain barrier penetration (water solubility, calculated log *P*: **3f**: 31 μg/mL, 1.86; **8**: 70 μg/mL, 1.41).

As discussed in the previous section, 3,4-dialkyl-substituted BTDs showed chemical instability in aqueous medium (Figure 3). This was confirmed when studying the impact of the presence or absence of a methyl group at the 2- and/or the 3- and/or the 4-positions of the BTD scaffold on the stability in distilled water at room temperature (20 °C). The results shown in Table 1 and Figure S1 (Supporting Information) clearly indicate that the presence of a methyl group at the 3-position of BTD instead of hydrogen atoms creates a chemical instability, in particular when the 2-position is not substituted by a methyl group, thus bearing a labile sulfonamide-type hydrogen atom (compound **5b**, half-life = 3.9 h). All compounds bearing two hydrogen atoms at the 3-position were found to exhibit no significant degradation after 48 h in aqueous solution at room temperature (compounds **6a**, **3b**, **6b**, **3f**, and **8**). Compared to

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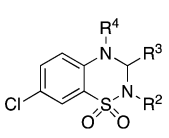
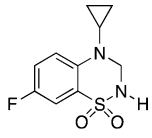
Table 1. Impact on Water Solubility and Aqueous Stability of the Introduction of a Methyl Group at the 2-, 3-, and/or 4-Positions of 7-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides





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compd	R^2	R^3	R^4	water solubility[a] μM	water solubility[a] μg/mL	avg log P[b]	aqueous stability half-life (h)[a]
6a	CH_3	H	H	213.4 ± 7.3	49.7 ± 1.7	1.54	n.e.[c]
5a[d]	H	CH_3	H	244.5 ± 8.6	56.9 ± 2.0	1.47	72.12
3b[d]	H	H	CH_3	554.0 ± 8.9	128.9 ± 2.1	1.30	n.e.[c]
7a	CH_3	CH_3	H	109.8 ± 3.3	27.1 ± 0.8	1.92	264.96
6b[d]	CH_3	H	CH_3	42.3 ± 1.2	10.4 ± 0.3	1.69	n.e.[c]
5b	H	CH_3	CH_3	n.d.[f]	n.d.[f]	1.70	3.86
7b	CH_3	CH_3	CH_3	37.5 ± 0.5	9.8 ± 0.1	2.07	176.54
3f[d]				120.9 ± 4.2	31.3 ± 1.1	1.86	n.e.[c]
8[e]				288.5 ± 3.8	69.9 ± 0.9	1.41	n.e.[c]

[a]Water solubility and aqueous stability in distilled water at room temperature (20 °C). Results of water solubility are expressed as means of six determinations ± standard deviation. [b]Average log *P*: calculated log *P* value obtained with the ALOGPS software (Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005). [c]n.e.: not established, but no significant degradation after 48 h in distilled water at room temperature (20 °C). [d]Published compounds.[15] [e]Published compound.[17] [f]n.d. not determined: rapid degradation in aqueous medium preventing the correct determination of the water solubility.

5b, when the 4-position is devoid of the methyl group and harbors a hydrogen atom, thus providing compound **5a**, the basicity of the nitrogen atom at the 4-position slightly decreased and, as expected by the proposed ring-opening mechanism (Figure 3), the chemical instability decreased (half-life of **5a** = 72.1 h). Compared to **5a**, the introduction of a methyl group at the 2-position, providing compound **7a**, greatly increased the chemical stability in aqueous solution, as expected by the absence of a labile hydrogen atom in this position (half-life of **7a** = 265.0 h). Logically, the 2,3,4-trimethyl-substituted compound **7b** was found to be more stable than the 3,4-dimethyl-substituted compound **5b** (introduction of a methyl group at the 2-position instead of the labile hydrogen atom), but less stable than the 2,3-dimethyl-substituted compound **7a** (increased basicity of the nitrogen atom at the 4-position) (half-life of **7b** = 176.5 h).

Finally, HPLC analysis of the degradation kinetics revealed that the major compound formed by ring opening followed by the hydrolysis of intermediate C was intermediate D (Figure 3), as certified by the use of authentic samples of the corresponding ortho-aminobenzenesulfonamides (intermediates D) injected in the same HPLC conditions.

The present study is consistent with a previous work published by Cannazza et al. on the stability of **5a** in aqueous medium at different pH showing a greater instability of **5a** in acidic medium (ring opening and release of acetaldehyde), and therefore supporting an acid-catalyzed mechanism.[22] Moreover, our work also supports previously published observations[23] that 3-methyl-substituted dihydrobenzothiadiazine dioxides are very sensitive to enantiomerization in aqueous medium, the latter process resulting from successive ring opening and ring closure reactions (Figure 3: steps A to B and B to C are reversible, while step C to D is irreversible).

Effect of the New Compounds on AMPA Receptors. The new mono-, di- and trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides of general formulas **3**, **5**, **6** and **7** were evaluated as AMPA receptor potentiators in an in vitro fluorescence assay (FLIPR) on rat brain cells cultures, and for some of them (**3g**, **5d**, **5e**, **6d**, **7d**, and **7e**) in a voltage clamp assay (VC) on *Xenopus* oocytes (Figure S2, Supporting Information). Both assays allowed the evaluation of AMPA-PAMs on cells expressing AMPA receptors, by measuring the effect of compounds either on the current passing through AMPA receptors (VC) or on the depolarization induced by AMPA receptor activation using fluorescent membrane potential dyes (FLIPR). Compounds **5a** and **9** were used as reference compounds in the two assays. It must be pointed out, however, that **9** carries four asymmetric carbon atoms and that this commercially available reference compound is a mixture of eight stereoisomers expressing different activities on AMPA receptors.[24,25]

For the tested compounds, the $EC_{2\times}$ and EC_{50} values (corresponding to the concentrations of compound responsible for a 2-fold increase of the AMPA mediated response and for 50% of the maximal effect, respectively) and the maximal effect (potentiation) determined using either the VC or FLIPR assay are reported in Table 2. Previous work on 4-monoalkyl-substituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides revealed that a cyclopropyl group introduced at the 4-position of the heterocycle was one of the best choices of alkyl chains for activity on AMPA receptors as positive allosteric modulators.[17] Thus, compound **3f** emerged as the lead compound with $EC_{2\times}$ values in the low micromolar range independent of the biological assay used for its evaluation (VC: $EC_{2\times}$ = 0.75 μM;[17] FLIPR: $EC_{2\times}$ = 2.3 μM, Table 2). The homologue **3g** bearing an additional methylene moiety compared to **3f** was characterized by a drastic decrease of the potency as an allosteric modulator of AMPA receptors in both VC and FLIPR assays (Table 2), indicating that very small structural modifications at the level of the 4-position (cyclopropylmethyl instead of cyclopropyl) could be responsible for a dramatic impact on activity.

Within the series of 3,4-dialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **5b**−**e**, all bear-

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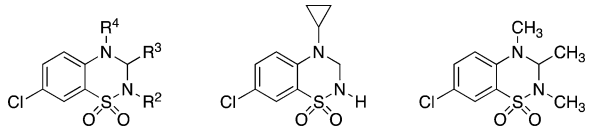
Table 2. Effects of Mono-, Di-, and Trialkyl-Substituted 7-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides on AMPA Receptors



3f **7b**

				voltage clamp			FLIPR		
compd	R^2	R^3	R^4	$EC_{2\times}$ (μM)[a]	EC_{50} (μM)[a]	maximal effect (fold increase versus AMPA)[b]	$EC_{2\times}$ (μM)[c]	EC_{50} (μM)[c]	maximal effect (fold increase versus AMPA)[d]
3b[e]	H	H	CH_3	6.0 [3.8; 9.3]	22.1 [7.5; 64.9]	17.4×	n.d.	n.d.	n.d.
3c[e]	H	H	CH_2CH_3	5.8 [3.9; 8.6]	31.8 [21.6; 47.0]	36.8×	n.d.	n.d.	n.d.
3d[f]	H	H	CH_2CH_2F	6.4 [4.1; 9.8]	37.2 [28.4; 48.6]	29.5×	18.5 [1.3; 260]	21.6 [2.4; 197]	7.3×
3e[f]	H	H	CH_2CHF_2	13.4 [8.0; 22.6]	n.d.	>30.0×	9.7 [7.1; 13.3]	11.6 [10.1; 13.3]	5.3×
3f[fg]	H	H	$CH(CH_2)_2$	0.8 [0.07; 9.6]	3.9 [0.2; 71.4]	25.7×	2.3 [2.1; 2.6]	3.3 [0.04; 254]	4.2×
3g	H	H	$CH_2CH(CH_2)_2$	68.4	n.d.	>8.0×	67.7 [49; 95]	n.d.	>2.2×
5a[e,f]	H	CH_3	H	133.9 [106.2; 168.9]	n.d.	>7.8×	260	398	4.2×
5b	H	CH_3	CH_3	n.d.[h]	n.d.	n.d.	55.7 [21.1; 147]	n.d.	>2.7×
5d	H	CH_3	CH_2CH_2F	21.2	n.d.	>19.0×	>100	n.d.	n.d.
5e	H	CH_3	CH_2CHF_2	12.9 [3.0; 56.3]	n.d.	>12.0×	68.5	n.d.	>2.0×
6a	CH_3	H	H	n.d.	n.d.	n.d.	72	n.d.	>2.1×
6b[e]	CH_3	H	CH_3	5.9	n.d.	>19.5×	n.d.	n.d.	n.d.
6c[e]	CH_3	H	CH_2CH_3	16.9 [2.2; 126.5]	n.d.	>11.0×	n.d.	n.d.	n.d.
6d	CH_3	H	CH_2CH_2F	25.6 [10.8; 60.7]	n.d.	>29.0×	n.d.	n.d.	n.d.
6e	CH_3	H	CH_2CHF_2	n.d.	n.d.	n.d.	(3.2[i])	n.d.	n.d.
6f	CH_3	H	$CH(CH_2)_2$	n.d.	n.d.	n.d.	13.4 [4; 45]	n.d.	>4.2×
6g	CH_3	H	$CH_2CH(CH_2)_2$	n.d.	n.d.	n.d.	51.5	n.d.	>2.0×
7a	CH_3	CH_3	H	n.d.	n.d.	n.d.	46 [7; 302]	n.d.	>2.9×
7b	CH_3	CH_3	CH_3	n.d.	n.d.	n.d.	4.3 [0.8; 23] (0.83[i])	8.3 [0.9; 78.3]	5.8×
7c	CH_3	CH_3	CH_2CH_3	n.d.	n.d.	n.d.	6 [1; 30]	6 [2; 20]	2.6×
7d	CH_3	CH_3	CH_2CH_2F	2.7 [0.7; 10.0]	19.8 [4.4; 88.9]	36.0×	2.7	9.3	6.7×
7e	CH_3	CH_3	CH_2CHF_2	6.3	15.1	14.8×	3.8	n.d.	>8.0×
7f	CH_3	CH_3	$CH(CH_2)_2$	n.d.	n.d.	n.d.	60	n.d.	>2.0×
7g	CH_3	CH_3	$CH_2CH(CH_2)_2$	n.d.	n.d.	n.d.	15.1 [4; 57]	16.2 [4.3;61.6]	4.2×
Cyclothiazide (**9**)[ef]				1.7 [1.0; 2.7]	7.0 [4.7; 10.2]	8.4×	4.5 [2.9; 7.0]	5.6 [3.6; 8.9]	5.5×

[a] $EC_{2\times}$: concentration of modulator giving a 2-fold increase of the amplitude of the current induced by AMPA using voltage clamp recordings on *Xenopus laevis* oocytes (n = 2−6). EC_{50}: concentration of modulator responsible for 50% of the maximal effect. [b] Maximum effect (potentiation) of the modulator on the AMPA-evoked current (normalized to unity versus the current evoked by AMPA in absence of compound). For new compounds **3g**, **5d**, **5e**, and **6d**, the maximal effect was not reached at the highest concentration of modulator used. The maximal effects indicated in the table correspond to the potentiation obtained at the highest tested dose. [c] $EC_{2\times}$: concentration of modulator giving a 2-fold increase of the fluorescence induced by AMPA with the FLIPR method on rat primary brain cultures (n = 2−3); EC_{50}: concentration of modulator responsible for 50% of the maximal effect. $EC_{2\times}$ and EC_{50} are expressed as a geometric mean for n = 2 and as a geometric mean and 95% confidence intervals (in brackets) for n > 2 experiments. [d] Maximum effect (potentiation) is expressed as arithmetic mean. For new compounds **3g**, **5b**, **5e**, **6a**, **6f**, **6g**, **7a**, **7e**, and **7f**, the maximal effect was not reached at the highest concentration of modulator used. The maximal effects indicated in the table correspond to the potentiation obtained at the highest tested dose. [e] Published by Francotte et al.[15] [f] Published by Francotte et al.[16] [g] Published by Nørholm et al.[17] [h] n.d.: not determined. [i] $EC_{2\times}$ values obtained by the FDSS fluorescence assay, a variant of the FLIPR fluorescence assay using another plate reader.[17] For compound **6e** tested in the FDSS system, the $EC_{2\times}$ value is given as follows: 3.2 μM (n = 2) with maximum effect > 3.3×. For compound **7b**, the $EC_{2\times}$ value is given as follows: 0.83 μM [0.63; 1.07] (n = 5) with maximum effect > 3×.

ing a methyl group at the 3-position, it is evident that the introduction of a short alkyl group at the 4-position of the reference compound **5a** improved the potency at AMPA receptors (compare **5a** versus **5b**, **5d** and **5e** in the FLIPR assay, Table 2). However, compared to their respective monosubstituted homologues of general formula **3**, the presence of a methyl chain at the 3-position in most cases provoked a decrease of the potentiation on AMPA receptors. Due to their chemical instability in aqueous solution, compounds **5** are of limited interest as putative drug candidates. Lastly, these compounds are racemic mixtures due to the presence of a chiral carbon at the 3-position (including the reference compound **5a**).

The 2,4-dialkyl-substituted compounds of general formula **6** are all characterized by the presence of a methyl group at the 2-position of the thiadiazine ring. The 2,4-dialkyl-substituted compounds **6** were generally found to exert a potentiation of the effect on AMPA receptors with $EC_{2\times}$ values in similar concentration ranges to those of their respective unmethylated counterparts **3** (i.e., compare **6b** versus **3b** in the VC assay and **6g** versus **3g** in the FLIPR assay, Table 2). However, in some cases, the methylation at the 2-position was responsible for a loss of potency. For example, regarding the 4-cyclopropyl-substituted benzothiadiazine dioxides **3f** and **6f**, the 2,4-dialkylated compound **6f** was clearly less potent than its 4-

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monoalkyl-substituted counterpart **3f** (FLIPR: **3f** $EC_{2\times}$ = 2.3 μM; **6f** $EC_{2\times}$ = 13.4 μM).

Lastly, the 2,3,4-trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides of general formula **7**, obtained after methylation of the corresponding 3,4-dialkyl-substituted compounds **5**, were found to exhibit a stronger activity on AMPA receptors (FLIPR, Table 2) than the unmethylated compounds **5**, independent of the nature of the alkyl group located at the 4-position (compare **7a**, **7b**, **7d**, and **7e** versus **5a**, **5b**, **5d**, and **5e** in the FLIPR assay). Some of these compounds (**7b**, **7c**, **7d**, and **7e**) reached the activity of **9** in the FLIPR assay.

From the diverse mono-, di-, or trimethyl-substituted compounds studied (**3b**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**), one of the most powerful potentiators seemed to be compound **7b** (low $EC_{2\times}$ and high maximal potentiation values), an example of a compound with a methyl group at all possible positions of the thiadiazine ring. This compound was selected for further examination in cocrystallization experiments with the GluA2 LBD. Due to the presence of a chiral carbon atom at the 3-position, compound **7b**, like the other 3-substituted compounds (including **5a**), is a racemic mixture of the *R*- and the *S*-isomer (Figure 4). It is expected that the more active

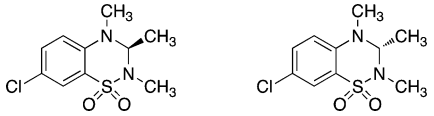

Figure 4. Structures of (*R*)-**7b** (left) and (*S*)-**7b** (right).

enantiomer of compound **7b** could more favorably cocrystallize with the GluA2 LBD. The 4-cyclopropyl compound **7f** was found to be considerably less potent than its corresponding 4-methyl analog **7b**, in contrast to the observation made with the previously described 4-monoalkyl-substituted benzothiadiazine dioxides for which the cyclopropyl group constitutes the best choice of substituent. This unexpected observation suggests that the binding mode of the trialkyl-substituted benzothiadiazine dioxides at the dimer interface could be different from that of the 4-monoalkyl-substituted compounds previously described.

Structure of 7b Bound to GluA2 LBD. For crystallization of compound **7b** with GluA2 LBD, the GluA2 LBD-L483Y-N754S double mutant was used. This mutant has previously been shown to form a dimer in solution without altering the dimer interface compared to the wild type structure.[17,27] Asn754 was mutated to serine in order to create a flip-like splice isoform containing a slightly larger modulator binding site. The soluble GluA2 LBD can be used for gaining information on the modulator binding mode of importance for future design of AMPA-PAMs, but less on deactivation/desensitization profiles.

The structure of GluA2 LBD-L483Y-N754S in complex with L-glutamate and **7b** was solved to 2.07 Å resolution with two molecules of GluA2 LBD-L483Y−N754S in the asymmetric unit of the crystal (Figure 5A). The complex crystallized with L-glutamate bound in the orthosteric binding site and two molecules of **7b** were located in the dimer interface positioned around a pseudo-2-fold symmetry axis. Domain closures relative to the apo structure of GluA2 LBD (PDB ID 1FTO, mol*A*)[28] of 20.5° and 20.9° for mol*A* and mol*B*, respectively, were seen and found to be within the expected range.[11] Statistics from data processing and structure refinement are given in Table 3.

Only the *R*-enantiomer of **7b** is observed to bind in the structure even though a racemic mixture of the compound was used for crystallization and functional characterization. A conformational search using *MAESTRO* (v.9.4; Schrödinger, LLC) identified one low-energy conformation of the *R*-enantiomer of **7b** that could unambiguously be modeled in the electron density at the dimer interface (Figure 5B). The two molecules of **7b** were refined to occupancy of 0.76/0.74, with putative sulfate ions alternatively present (0.24/0.26, see Figure S3). The partial presence of **7b** leads to two conformations of Ser497: when **7b** is present the side chain of Ser497 is pointing away from **7b** in order to avoid unfavorable steric interaction (distance of 2.7 Å (mol*A*) and 2.5 Å (mol*B*) between hydroxyl group of Ser497 and chlorine atom of **7b** when pointing toward **7b**), while the side chain is oriented toward the binding site in the absence of **7b**. A single polar contact is formed between **7b** and the protein: The backbone NH of Gly731 establishes a hydrogen bond to one of

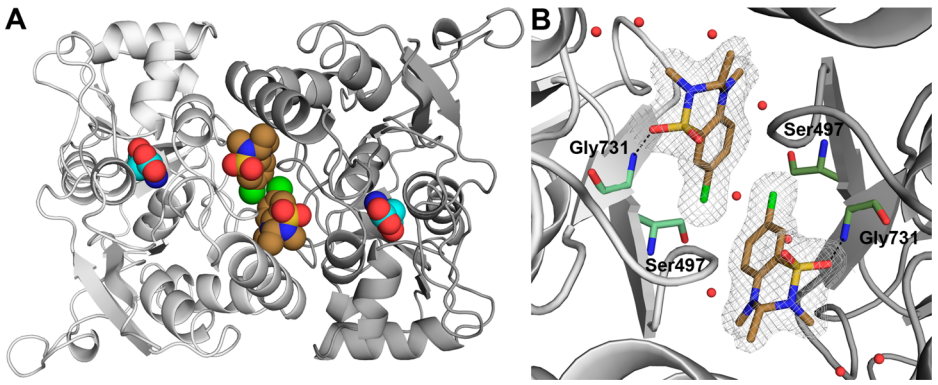

Figure 5. Structure of the dimeric GluA2 LBD-L483Y-N754S in complex with glutamate and **7b**. (A) Cartoon representation of the GluA2 LBD dimer. Two molecules of **7b** (spheres representation with carbon in sand) bind at the dimer interface and glutamate (spheres representation with carbon in cyan) binds in the orthosteric binding site. The protein molecules are shown in white (mol*A*) and gray (mol*B*). (B) Zoom on the modulator binding site. The final $2F_o - F_c$ map (in gray; contoured at 1 σ level, carved at 2.0 Å around **7b**) is shown. The $mF_o - DF_c$ map after molecular replacement is shown in Figure S3. Potential hydrogen bonds within 3.5 Å between **7b** and surrounding residues are shown as black, dashed lines. Water molecules in the dimer interface within 4 Å from the modulators are shown as red spheres. The side chain of Ser497 is shown in the conformation observed when **7b** is present. Atomic coloring scheme from Figure 2 has been used.

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Table 3. Crystal Data, Data Collection, and Refinement Statistics of GluA2 LBD-L483Y-N754S in Complex with L-Glutamate and 7b

crystal data	
PDB ID	5BUU
space group	$P2_12_12$
unit cell: *a*, *b*, c (Å)	99.21, 122.27, 47.65
molecules in au[a]	2
data collection	
wavelength (Å)	0.9100
resolution (Å)	47.65−2.07 (2.18−2.07)[b]
no. of unique reflections	36199 (5184)
avg redundancy	4.1 (4.1)
completeness (%)	100.0 (100.0)
R_{merge}[c]	0.057 (0.332)
$I/\sigma(I)$	9.0 (2.1)
Wilson B (Å^2)	27
refinement	
amino acid residues (mol A/mol B)[d]	260/254
L-glutamate/**7b**/ethylene glycol/sulfate/water	2/2/5/5/275
R_{work}[e]/R_{free}[f] (%)	20.4/25.0
avg *B* values (Å^2) for:	
amino acid residues (mol A/mol B)	39/50
L-glutamate/**7b**/ethylene glycol/sulfate/water	26/18/64/57/42
rms deviation bond length (Å)/angles (deg)	0.003/0.7
Ramachandran outliers/favored (%)[g]	0.0/97.9
Rotamer outliers (%)/Cβ outliers (%)/Clash score[g]	1.6/0/1.9

[a]au: asymmetric unit of the crystal. [b]Values in parentheses correspond to the outermost resolution shell. [c]R_{merge} is calculated as follows: $I_i(hkl)$ is the intensity of an individual measurement of the reflection with Miller indices *hkl*, and $I(hkl)$ is the intensity from multiple observations. $R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(hkl) - I(hkl)|/\Sigma_{hkl}\Sigma_i|I_i(hkl)|$. [d]Rat GluA2 LBD,[28] comprising segment S1 residues 392−506, a GT linker and segment S2 residues 632−776 (numbering without signal peptide) was used. [e]$R_{work} = \Sigma_{hkl}|F_{obs} - F_{calc}|/\Sigma_{hkl}|F_{obs}|$ where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively, for reflection *hkl*. [f]R_{free} is equivalent to R_{work}, but calculated with 5% of reflections omitted from the refinement process. [g]MolProbity statistics.[42]

the oxygen atoms of the sulfonamide group of **7b**. No hydrogen bonds between **7b** and water molecules are seen in the structure. Van der Waals interactions (within 4 Å) are established between **7b** and surrounding residues in both protomers (Ile481*B*, Lys493*A*, Pro494*A*/*B*, Ser497*B*, Ser729*B*, Lys730*B*, Gly731*B*, Val750*A*, Leu751*A*, and Ser754*A*; vice versa for the other molecule of **7b**) as well as surrounding water molecules. The two molecules of **7b** additionally form van der Waals contacts within 4 Å with each other, between the chlorine atom of one molecule and the C6 and C7 atoms of the other.

As it appears from Figure 6A, a methyl group in the 3*S*-position of **7b** would lead to steric clash with the protein. No additional density suggesting that the *S*-enantiomer might bind in a different site than the *R*-enantiomer was seen in the structure. This suggests that the *R*-enantiomer is the bioactive species of **7b**. The saturated analog **5a** also contains a methyl group in the 3-position. The structure of **5a** bound to GluA2 LBD has previously been reported,[13] showing the *S*-enantiomer of **5a** to bind to GluA2 LBD-N754S (Figure 6B). However, the *R*- and *S*-enantiomer can equally well be fitted into the electron density (R. Oswald, personal communication), questioning the bioactive form of **5a**.

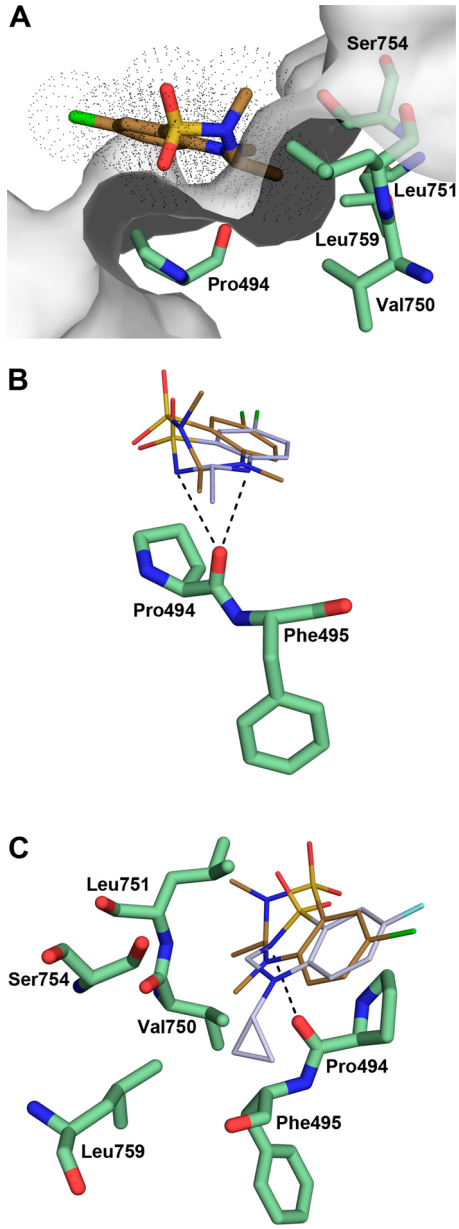

Figure 6. Binding mode of **7b** in GluA2 LBD-L483Y-N754S in comparison with IDRA 21 (**5a**) and BPAM 344 (**8**). (A) Shape complementarity of the 3*R*-methyl group of **7b** (carbon atoms in sand sticks) and GluA2 LBD residues (carbon atoms in pale green sticks). A surface representation of the protein is shown as white solid surface and of **7b** as black dots. (B) Overlay of the structures of (*R*)-**7b** and (*S*)-**5a** (carbon atoms in light blue sticks; PDB ID 3IL1). The geometry for potential hydrogen bonds (black, dashed lines) from **5a** to Pro494 is seen to be nonoptimal. Residues of the **7b** complex only are shown for clarity. (C) Overlay of the structures of **7b** and BPAM 344 (carbon atoms in light blue sticks; PDB ID 4N07) bound to GluA2 LBD-L483Y-N754S. One potential hydrogen bond from the N2 atom of BPAM 344 to the carbonyl O atom of Pro494 is seen (black, dashed line). Atomic coloring scheme from Figure 2 has been used.

Five structures of GluA2 LBD-L483Y-N754S have previously been reported in complex with positive allosteric modulators containing a BTD or PTD scaffold similar to **7b**: **5a**,[13] BPAM 97,[27] BPAM 344 (compound **8**; referred to as compound 3 by Nørholm et al.[17]), BPAM 25 (referred to as compound 6 by Francotte et al.[20]) and BPAM 37 (referred to as compound 5

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by Francotte et al.[20]). These compounds adopt similar binding modes and belong to the shifted thiazide class of AMPA receptor modulators.[13] However, a small rotation of ∼30° of the BTD scaffold of **5a** around an axis perpendicular to the ring plane is seen, which is also evident in the structure with **7b** (Figure 6C). This rotation is important for avoiding steric clashes with the protein. The methyl group in the 3-position of **7b** is located in a hydrophobic pocket lined by Val750, Leu751, Ser754, and Leu759 (Figure 6A), similar to what was previously observed for **5a**. The shift in binding mode allows the 3-methyl group to establish van der Waals contacts within 4 Å to Val750$^{C\gamma}$, Leu751$^{C\delta}$, and Ser754$^{C\beta}$. Given the shape complementarity of the 3-methyl group and the hydrophobic pocket (Figure 6A), as well as the fact that without a shift in the position of the BTD scaffold the 3-methyl group would most likely clash with the protein, it seems that introduction of a methyl group in the 3-position directs the binding mode of the BTD scaffold.

Although a hydrogen bond from N2 of, for example, BPAM 97 or **8** (Figure 6C) to the carbonyl oxygen of Pro494 is likely to contribute significantly to the enthalpy-driven binding affinity (K_d) of these two compounds for the GluA2 LBD,[17,27] such a polar contact does not seem to be necessary in order to obtain modulator potency. In **7b**, a methyl substituent in the N2 position prevents a polar contact to be established to Pro494, yet the potency of **7b** (FDSS: $EC_{2\times}$ = 0.83 μM, FLIPR: $EC_{2\times}$ = 4.3 μM, Table 2) lies between those of BPAM 97 (FLIPR: $EC_{2\times}$ = 16.8 μM[16]) and **8** (FDSS: $EC_{2\times}$ = 0.27 μM[17]). Likewise, the 2,4-dimethyl substituted **6b** shows the same potency (VC: $EC_{2\times}$ = 5.9 μM, Table 2) as the monosubstituted 4-methyl analog **3b** (VC: $EC_{2\times}$ = 6.0 μM, Table 2). This suggests that it is not critically important for modulator potency to have a hydrogen bond donor in the 2-position of the BTD scaffold.

Based on the compounds presented in Table 2, it appears that 2,3,4-trimethyl substitution (**7b**; FLIPR: $EC_{2\times}$ = 4.3 μM) is better tolerated than 2,3-dimethyl (**7a**; FLIPR: $EC_{2\times}$ = 46 μM) or 3,4-dimethyl (**5b**; FLIPR: $EC_{2\times}$ = 56 μM) substitution, while 3-methyl substitution alone is even less tolerated (**5a**; FLIPR: $EC_{2\times}$ = 260 μM). For **7b** no direct hydrogen bond to Pro494 is possible, but the additional van der Waals interactions between the methyl group in the 2-position and Lys730$^{C\beta}$ (mol*A*), Leu751$^{C\delta}$ (mol*B*) and the side chain hydroxyl group of Ser754 (mol*B*) are likely to counteract the loss of a hydrogen-bonding donor in the sulfonamide. Likewise, additional van der Waals interactions are established between the 4-methyl group of **7b** and the carbonyl oxygen atom of Ser729 (mol*A*), the side chain hydroxyl group of Ser497 (mol*B*) and the carbonyl oxygen of Pro494 (mol*B*). Furthermore, as the van der Waals interaction partners of the 2- and 4-methyl groups are located on both sides of the dimer interface they may contribute to stabilization of the GluA2 LBD dimer, and thus increase the modulator potency of **7b** and other 2,3- and 3,4-dimethyl analogs compared to **5a**. From the structure of GluA2 LBD with **5a**, it appears that the two NH groups of **5a** are positioned in a way leading to unfavorable geometry for hydrogen bonding to the carbonyl oxygen atom of Pro494 (Figure 6B). The same trend of higher potency for 2,3,4-substituted versus 3,4-substituted compounds is also seen within the series of compounds having larger 4-substituents (**7d** versus **5d** and **7e** versus **5e**).

For the 4-monosubstituted compounds (**3b**, **3c**, and **3f**), there is a trend that increasing the bulkiness of the 4-substituent to a cyclopropyl group enhances the modulator potency, whereas the size of the 4-substituent seems less important for 2,4-substituted compounds (**6b**−**6g**). Surprisingly, for the corresponding compounds from the series of 2,3,4-substituted BTDs (**7b**, **7c**, and **7f**) this trend is not evident. Having a cyclopropyl group in the 4-position (**7f**) decreases the modulator potency 10-fold or more compared to an ethyl (**7c**) or a methyl group (**7b**) in the 4-position. Based on the structure presented here, one likely explanation is that introduction of a cyclopropyl group in the 4-position of the 2,3,4-substituted BTD would result in a clash with backbone atoms of residues Pro494-Phe495 and C$^{\beta}$ of Ser754.

CONCLUSION

We have synthesized and pharmacologically evaluated a new series of mono-, di- and trialkyl-substituted 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides as positive allosteric modulators at AMPA receptors. The trialkyl-substituted compounds **7b**, **7d** and **7e** revealed an unexpected strong activity as AMPA receptor potentiators. The X-ray crystal structure of **7b** bound to the ligand-binding domain of the target protein GluA2 was solved. We observed that only the *R*-enantiomer of **7b** binds in the structure even though a racemic mixture of the compound was used for crystallization. A small rotation of ∼30° of the BTD scaffold of **7b** relative to **8** around an axis perpendicular to the ring plane is seen of importance for avoiding steric clashes with the protein. 2,3,4-Trimethyl substitutions were seen to be better tolerated than 2,3-dimethyl or 3,4-dimethyl substitution due to additional van der Waals interactions. On the basis of the structure we propose a likely explanation of the unexpected SAR of this new series of compounds, supported by four main observations: (i) the methyl substituent in the 3-position directs the binding mode of the BTD scaffold, (ii) a hydrogen bond donor in the 2-position of the BTD is not necessary for modulator potency, (iii) when having a methyl substituent in the 3-position of the BTD additional methyl substituents in both the 2- and 4-positions increase potency, and (iv) introduction of a 4-cyclopropyl group does not enhance potency of 2,3,4-substituted BTDs.

METHODS

Chemistry. All commercial chemicals (Sigma-Aldrich, Belgium; Appolo Scientific, United Kingdom and Fluorochem, United Kingdom) and solvents were reagent grade and used without further purification. Melting points were determined on a Stuart SMP3 apparatus in open capillary tubes and are uncorrected. NMR spectra were recorded on a Bruker Avance 500 spectrometer (^{1}H: 500 MHz; ^{13}C: 125 MHz) using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard; chemical shifts are reported in δ values (ppm) relative to internal TMS. The abbreviation s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and bs = broad signal are used throughout. Elemental analyses (C, H, N, S) were carried out on a Thermo Flash EA 1112 series elemental analyzer and were within ±0.4% of the theoretical values, except for three compounds where larger than 0.4% difference for the sulfur atom content were seen (**5b**, 0.48%; **7b**, 0.69%; **7c**, 0.65%). The technique used for our compounds is known to be less accurate in the determination of the sulfur content. This analytical process ensured for each target compound a purity equal to or greater than 95%. All reactions were followed by thin layer chromatography (TLC) (silica gel 60F$_{254}$ Merck) and visualization was accomplished with UV light (254 or 366 nm).

Synthetic Pathway to 2-Alkylamino-5-chlorobenzenesulfonamides 4. The 2-alkylamino-5-chlorobenzenesulfonamides **4b**, **4c**, and **4e** were obtained according to reported procedures.[16,29] 5-Chloro-

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2-cyclopropylaminobenzenesulfonamide **4f** was prepared from the reaction of 5-chloro-2-fluorobenzenesulfonamide **10** with cyclopropylamine as previously described.[20] 5-Chloro-2-(2-fluoroethylamino)-benzenesulfonamide **4d** and 5-chloro-2-cyclopropylmethylaminobenzenesulfonamide **4g** were obtained from 4-(2-fluoroethyl)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **2d**[16] and 4-cyclopropylmethyl-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **2g** (see below) after ring opening in alkaline hydrolytic conditions.

5-Chloro-2-cyclopropylaminobenzenesulfonamide (4f). White solid; m.p.: 180−182 °C; ^{1}H NMR (DMSO-d_6) δ 0.51 (m, 2H, CH(CH_2)$_2$), 0.80 (m, 2H, CH(CH_2)$_2$), 2.46 (m, 1H, CH(CH$_2$)$_2$), 6.15 (s, 1H, N*H*), 7.15 (d, *J* = 8.9 Hz, 1H, 3-*H*), 7.46 (dd, *J* = 8.9 Hz/2.6 Hz, 1H, 4-*H*), 7.50 (s, 2H, SO_2NH_2), 7.57 (d, *J* = 2.6 Hz, 1H, 6-*H*); ^{13}C NMR (DMSO-d_6) δ 7.3 (CH(CH_2)$_2$), 24.6 (CH(CH$_2$)$_2$), 114.8 (C-3), 118.7 (C-5), 126.1 (C-1), 127.2 (C-6), 132.8 (C-4), 144.2 (C-2).

5-Chloro-2-cyclopropylmethylaminobenzenesulfonamide (4g). *Step 1. 4-Cyclopropylmethyl-7-chloro-4H-1,2,4-benzothiadiazine 1,1-Dioxide (2g).* The solution of 7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **1**[15] (1 g, 4.6 mmol) in acetonitrile (30 mL) was supplemented under stirring with potassium carbonate (2.5 g, 18.1 mmol) and cyclopropylmethyl bromide (0.75 mL, 7.7 mmol). The suspension was refluxed for 24 h and then concentrated under reduced pressure. The residue was suspended in water (50 mL) and the insoluble material of the title compound was collected by filtration, washed with water, and dried. The solid was suspended in hot ethyl acetate, collected by filtration, washed with ethyl acetate, and dried (yields: 72%). White solid; m.p.: 183−185 °C; ^{1}H NMR (DMSO-d_6) δ 0.43 (m, 2H, CH(CH_2)$_2$), 0.55 (m, 2H, CH(CH_2)$_2$), 1.27 (m, 1H, CH(CH$_2$)$_2$), 3.99 (d, *J* = 7.2 Hz, 2H, NCH_2), 7.78 (d, *J* = 9.2 Hz, 1H, 5-*H*), 7.85 (dd, *J* = 9.1 Hz/2.4 Hz, 1H, 6-*H*), 7.96 (d, *J* = 2.4 Hz, 1H, 8-*H*), 8.15 (s, 1H, 3-*H*); ^{13}C NMR (DMSO-d_6) δ 3.8 (CH(CH_2)$_2$), 9.8 (CH(CH$_2$)$_2$), 54.3 (NCH_2), 119.4 (C-5), 123.7 (C-8), 124.0 (C-7), 130.6 (C-8a), 133.4 (C-6), 133.9 (C-4a), 150.6 (C-3).

Step 2. 5-Chloro-2-cyclopropylmethylaminobenzenesulfonamide (4g). The suspension of 4-cyclopropylmethyl-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **2g** (0.4 g, 1.48 mmol) in a 5% m/v aqueous solution of NaOH (20 mL) was heated under stirring at 70 °C for 1 h. The resulting solution was cooled and adjusted to pH 2−3 by means of 6 N HCl. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried (yields: 93%). White solid; m.p.: 121−123 °C; ^{1}H NMR (DMSO-d_6) δ 0.25 (m, 2H, CH(CH_2)$_2$), 0.50 (m, 2H, CH(CH_2)$_2$), 1.11 (m, 1H, CH(CH$_2$)$_2$), 3.03 (dd, *J* = 6.5 Hz/5.4 Hz, 2H, NCH_2), 6.06 (t, *J* = 5 Hz, 1H, N*H*), 6.81 (d, *J* = 9.0 Hz, 1H, 3-*H*), 7.38 (dd, *J* = 8.9 Hz/2.6 Hz, 1H, 4-*H*), 7.52 (s, 2H, SO_2NH_2), 7.58 (d, *J* = 2.6 Hz, 1H, 6-*H*); ^{13}C NMR (DMSO-d_6) δ 3.4 (CH(CH_2)$_2$), 10.3 (CH(CH$_2$)$_2$), 47.3 (NCH_2), 113.8 (C-3), 117.8 (C-5), 125.8 (C-1), 127.4 (C-6), 132.9 (C-4), 143.8 (C-2).

5-Chloro-2-(2-fluoroethylamino)benzenesulfonamide (4d). The title compound was obtained from the alkaline hydrolysis of 4-(2-fluoroethyl)-7-chloro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **2d**[16] as described for **4g** (yields 82%). White solid; m.p.: 122−124 °C; ^{1}H NMR (DMSO-d_6) δ 3.53 (ddd, *J* = 27.0 Hz/10.3 Hz/5.2 Hz, 2H, NCH_2), 4.62 (dt, *J* = 47.5 Hz/4.9 Hz, 1H, CH_2F), 6.15 (t, *J* = 5.6 Hz, 1H, N*H*), 6.89 (d, *J* = 9.0 Hz, 1H, 3-*H*), 7.41 (dd, *J* = 8.9 Hz/2.6 Hz, 1H, 4-*H*), 7.54 (s, 2H, SO_2NH_2), 7.60 (d, *J* = 2.6 Hz, 1H, 6-*H*); ^{13}C NMR (DMSO-d_6) δ 43.0 (d, *J* = 20 Hz, NCH_2), 82.4 (d, *J* = 165 Hz, CH_2F), 67.4 (C-3), 113.9 (C-3), 118.4 (C-5), 126.4 (C-1), 127.4 (C-6), 132.9 (C-4), 143.6 (C-2).

Synthetic Pathway to 4-Alkyl-7-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxides 3. The 4-alkyl-7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **3b−f** were obtained as previously described.[15−17]

7-Chloro-4-cyclopropylmethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (3g). A solution of 7-chloro-4-cyclopropylmethyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**2g**) (0.35 g, 1.3 mmol) in 2-propanol (25 mL) was supplemented under stirring with sodium borohydride (0.1 g, 2.65 mmol). After the mixture was heated under stirring for 5 min at 50 °C, the solvent was removed by distillation under reduced pressure and the residue was suspended in water (25 mL). The alkaline suspension was adjusted to pH 6−7 by means of 6 N HCl and extracted 3-fold with chloroform (3 × 60 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the title compound was recrystallized in methanol/water, 1:2. White solid; m.p.: 106−109 °C; ^{1}H NMR (DMSO-d_6) δ 0.29 (m, 2H, CH(CH_2)$_2$), 0.47 (m, 2H, CH(CH_2)$_2$), 1.02 (m, 1H, CH(CH$_2$)$_2$), 3.30 (d, *J* = 6.7 Hz, 2H, NCH_2), 4.77 (s, 2H, 3−CH_2), 7.05 (d, *J* = 9.2 Hz, 1H, 5-*H*), 7.42 (dd, *J* = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.52 (d, *J* = 2.6 Hz, 1H, 8-*H*), 8.17 (bs, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 3.2 (CH(CH_2)$_2$), 8.6 (CH(CH$_2$)$_2$), 52.7 (NCH_2), 61.0 (C-3), 116.1 (C-5), 119.5 (C-7), 123.2 (C-8a), 123.5 (C-8), 133.0 (C-6), 142.0 (C-4a). Anal. ($C_{11}H_{13}ClN_2O_2S$) theoretical: C, 48.44; H, 4.80; N, 10.27; S, 11.75. Found: C, 48.38; H, 4.85; N, 10.43; S, 11.56.

Synthetic Pathway to 4-Alkyl-7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxides 5. The solution of the appropriate 2-alkylamino-5-chlorobenzenesulfonamide **4** (0.4 g, 1.5−1.8 mmol) in acetonitrile was supplemented with a catalytic amount of camphorsulfonic acid (5 mg) and acetaldehyde (0.5 mL; 8.9 mmol). The reaction mixture was stirred at room temperature in a closed vessel for 1−2 h. After completion of the reaction (followed by TLC), the solvent was removed by distillation under reduced pressure and the residue was dissolved in a small volume of methanol. The addition to this stirred solution of an equal volume of distilled water led to the precipitation of the title compound, which was collected by filtration, washed with water, and dried (yields: 65−85%). In some cases, due to its chemical instability in aqueous medium, the title compound was immediately engaged without further purification in the 2-methylation step (performed with compounds **5c**, **5f**, and **5g**).

According to this general synthetic pathway, the compounds listed below were obtained.

R/S-7-Chloro-3,4-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (5b). White solid; m.p.: 134−136 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (d, *J* = 6.5 Hz, 3H, CHCH_3), 2.91 (s, 3H, NCH_3), 4.83 (q, *J* = 6.4 Hz, 1H, CHCH$_3$), 6.92 (d, *J* = 9.1 Hz, 1H, 5-*H*), 7.45 (dd, *J* = 9.1 Hz/2.5 Hz, 1H, 6-*H*), 7.51 (d, *J* = 2.5 Hz, 1H, 8-*H*), 8.20 (bs, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 18.4 (CHCH_3), 34.6 (NCH_3), 68.1 (CHCH_3), 116.6 (C-5), 119.7 (C-7), 123.0 (C-8), 123.8 (C-8a), 133.1 (C-6), 142.9 (C-4a). Anal. ($C_9H_{11}ClN_2O_2S$) theoretical: C, 43.81; H, 4.49; N, 11.35; S, 12.99. Found: C, 43.42; H, 4.54; N, 11.39; S, 12.51.

R/S-7-Chloro-4-(2-fluoroethyl)-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (5d). White solid; m.p.: 134−136 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (d, *J* = 6.5 Hz, 3H, CHCH_3), 3.76 (m, 2H, NCH_2), 4.59 (m, 2H, CH_2F), 4.94 (q, *J* = 6.5 Hz, 1H, CHCH$_3$), 7.06 (d, *J* = 9.3 Hz, 1H, 5-*H*), 7.44 (dd, *J* = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.54 (d, *J* = 2.6 Hz, 1H, 8-*H*), 8.23 (bs, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 19.2 (CHCH_3), 46.8 (d, *J* = 21 Hz, NCH_2), 67.4 (CHCH$_3$), 82.3 (d, *J* = 167 Hz, CH_2F), 117.2 (C-5), 120.3 (C-7), 123.3 (C-8), 124.3 (C-8a), 133.0 (C-6), 142.2 (C-4a). Anal. ($C_{10}H_{12}ClFN_2O_2S$) theoretical: C, 43.09; H, 4.34; N, 10.05; S, 11.50. Found: C, 43.18; H, 4.33; N, 10.52; S, 11.63.

R/S-7-Chloro-4-(2,2-difluoroethyl)-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (5e). White solid; m.p.: 117−119 °C; ^{1}H NMR (DMSO-d_6) δ 1.53 (d, *J* = 6.5 Hz, 3H, CHCH_3), 3.91 (m, 2H, NCH_2), 4.97 (q, *J* = 6.5 Hz, 1H, CHCH$_3$), 6.24 (tt, *J* = 55.2 Hz/3.9 Hz, 1H, CHF_2), 7.14 (d, *J* = 9.2 Hz, 1H, 5-*H*), 7.47 (dd, *J* = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.58 (d, *J* = 2.6 Hz, 1H, 8-*H*), 8.33 (bs, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 18.9 (CHCH_3), 48.7 (t, *J* = 26 Hz, NCH_2), 67.7 (CHCH$_3$), 115.2 (t, *J* = 242 Hz, CHF_2), 117.8 (C-5), 121.3 (C-7), 123.4 (C-8), 124.8 (C-8a), 133.0 (C-6), 142.1 (C-4a). Anal. ($C_{10}H_{11}ClF_2N_2O_2S$) theoretical: C, 40.48; H, 3.74; N, 9.44; S, 10.80. Found: C, 40.74; H, 3.95; N, 9.47; S, 10.76.

Synthetic Pathway to 4-Alkyl-7-chloro-2-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxides 6. The solution of the appropriate 4-alkyl-7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **3** (0.3 g, 1.1−1.3 mmol) in acetonitrile (20 mL) was supplemented with potassium carbonate (1 g, 7.2 mmol) and methyl iodide (0.5 mL, 8 mmol). The resulting suspension was heated at 70−

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80 °C under stirring for 1−3 h (until completion followed by TLC). The solvent was removed by distillation under reduced pressure and the residue was dissolved in a small volume of methanol. The addition to this stirred solution of an equal volume of distilled water led to the precipitation of the title compound, which was collected by filtration, washed with water, and dried (yields: 80−90%).

Compound **6a** devoid of an alkyl substituent at the 4-position is a known chemical substance[30] that was prepared according to the general synthetic pathway reported for compounds **6d−g** but using the commercially available 7-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**3a**) as the starting material.

Compounds **6b** and **6c** were obtained as previously described.[15]

*7-Chloro-2-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**6a**).* White solid; m.p.: 195−198 °C; ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3H, NCH_3), 4.80 (s, 2H, 3−CH_2), 6.88 (d, J = 9.0 Hz, 1H, 5-*H*), 7.38 (dd, J = 9.0 Hz/2.5 Hz, 1H, 6-*H*), 7.44 (s, 1H, N*H*), 7.49 (d, J = 2.5 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 34.3 (NCH_3), 60.4 (C-3), 118.0 (C-5), 118.3 (C-7), 120.0 (C-8a), 124.1 (C-8), 133.4 (C-6), 141.8 (C-4a). Anal. ($C_8H_9ClN_2O_2S$) theoretical: C, 41.29; H, 3.90; N, 12.04; S, 13.78. Found: C, 41.08; H, 4.03; N, 12.23; S, 13.65.

*7-Chloro-4-(2-fluoroethyl)-2-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**6d**).* White solid; m.p.: 112−114 °C; ^{1}H NMR (DMSO-d_6) δ 2.68 (s, 3H, NCH_3), 3.80 (dt, J = 27.1 Hz/4.8 Hz, 2H, NCH_2), 4.62 (dt, J = 47.4 Hz/4.8 Hz, 1H, CH_2F), 4.97 (s, 2H, 3−CH_2), 7.07 (d, J = 9.2 Hz, 1H, 5-*H*), 7.48 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.57 (d, J = 2.6 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 34.4 (NCH_3), 49.0 (d, J = 20 Hz, NCH_2), 67.4 (C-3), 82.0 (d, J = 165 Hz, CH_2F), 115.9 (C-5), 119.4 (C-7), 120.4 (C-8a), 124.8 (C-8), 133.6 (C-6), 141.0 (C-4a). Anal. ($C_{10}H_{12}ClFN_2O_2S$) theoretical: C, 43.09; H, 4.34; N, 10.05; S, 11.50. Found: C, 43.06; H, 4.35; N, 10.09; S, 11.45.

*7-Chloro-4-(2,2-difluoroethyl)-2-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**6e**).* White solid; m.p.: 143−145 °C; ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, NCH_3), 3.97 (td, J = 15.9 Hz/3.3 Hz, 2H, NCH_2), 5.00 (s, 2H, 3−CH_2), 6.30 (tt, J = 54.9 Hz/3.4 Hz, 1H, C*H*F$_2$), 7.15 (d, J = 9.2 Hz, 1H, 5-*H*), 7.52 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.60 (d, J = 2.6 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 34.4 (NCH_3), 50.4 (t, J = 24 Hz, NCH_2), 67.7 (C-3), 115.0 (t, J = 241 Hz, CHF$_2$), 116.4 (C-5), 120.0 (C-7), 121.3 (C-8a), 124.7 (C-8), 133.6 (C-6), 141.1 (C-4a). Anal. ($C_{10}H_{11}ClF_2N_2O_2S$) theoretical: C, 40.48; H, 3.74; N, 9.44; S, 10.80. Found: C, 40.33; H, 4.12; N, 9.55; S, 10.98.

*7-Chloro-4-cyclopropyl-2-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**6f**).* White solid; m.p.: 134−136 °C; ^{1}H NMR (DMSO-d_6) δ 0.70 (m, 2H, CH(CH_2)$_2$), 0.93 (m, 2H, CH(CH_2)$_2$), 2.59 (m, 1H, C*H*(CH$_2$)$_2$), 2.62 (s, 3H, NCH_3), 4.90 (s, 2H, 3−CH_2), 7.33 (d, J = 9.0 Hz, 1H, 5-*H*), 7.55 (dd, J = 9.0 Hz/2.6 Hz, 1H, 6-*H*), 7.57 (d, J = 2.4 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 8.3 (CH(CH_2)$_2$), 29.7 (NCH_3), 34.0 (C*H*(CH$_2$)$_2$), 66.8 (C-3), 116.6 (C-5), 120.4 (C-7), 121.3 (C-8a), 124.4 (C-8), 133.3 (C-6), 142.2 (C-4a). Anal. ($C_{11}H_{13}ClN_2O_2S$) theoretical: C, 48.44; H, 4.80; N, 10.27; S, 11.75. Found: C, 48.16; H, 4.87; N, 10.42; S, 11.36.

*7-Chloro-4-cyclopropylmethyl-2-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**6g**).* White solid; m.p.: 90−92 °C; ^{1}H NMR (DMSO-d_6) δ 0.31 (m, 2H, CH(CH_2)$_2$), 0.48 (m, 2H, CH(CH_2)$_2$), 1.06 (m, 1H, C*H*(CH$_2$)$_2$), 2.68 (s, 3H, NCH_3), 3.33 (m, 2H, NCH_2), 4.97 (s, 2H, 3−CH_2), 7.10 (d, J = 9.2 Hz, 1H, 5-*H*), 7.47 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.55 (d, J = 2.6 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 3.1 (CH(CH_2)$_2$), 8.7 (CH(CH_2)$_2$), 34.5 (NCH_3), 52.9 (NCH_2), 67.1 (C-3), 115.8 (C-5), 119.1 (C-7), 119.9 (C-8a), 124.8 (C-8), 133.6 (C-6), 141.1 (C-4a). Anal. ($C_{12}H_{15}ClN_2O_2S$) theoretical: C, 50.26; H, 5.27; N, 9.77; S, 11.18. Found: C, 49.67; H, 5.22; N, 9.76; S, 11.36.

*Synthetic Pathway to 4-Alkyl-7-chloro-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxides **7**.* The 4-alkyl-7-chloro-2,3-dimethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **7b−g** were obtained by methylation of the corresponding 4-alkyl-7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **5b−g** by means of methyl iodide in the experimental conditions used for the synthesis of compounds **6** (see above).

The non-4-alkylated compound 7-chloro-2,3-dimethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **7a** was also obtained by means of the same synthetic procedure starting from the reference compound **5a**.

*R/S-7-Chloro-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7a**).* White solid; m.p.: 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 1.45 (d, J = 6.4 Hz, 3H, CHCH_3), 2.46 (s, 3H, NCH_3), 5.25 (q, J = 6.4 Hz, 1H, C*H*CH$_3$), 6.87 (d, J = 9.0 Hz, 1H, 5-*H*), 7.39 (dd, J = 8.9 Hz/2.5 Hz, 1H, 6-*H*), 7.42 (bs, 1H, N*H*), 7.50 (d, J = 2.5 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 17.5 (CHCH_3), 27.5 (NCH_3), 64.7 (C*H*CH$_3$), 117.8 (C-5), 118.4 (C-7), 120.2 (C-8a), 124.2 (C-8), 133.3 (C-6), 141.9 (C-4a). Anal. ($C_9H_{11}ClN_2O_2S$) theoretical: C, 43.81; H, 4.49; N, 11.35; S, 12.99. Found: C, 43.41; H, 4.28; N, 11.24; S, 12.78.

*R/S-7-Chloro-2,3,4-trimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7b**).* White solid; m.p.: 126−128 °C; ^{1}H NMR (DMSO-d_6) δ 1.55 (d, J = 6.7 Hz, 3H, CHCH_3), 2.63 (s, 3H, NCH_3), 2.96 (s, 3H, NCH_3), 5.14 (q, J = 6.7 Hz, 1H, C*H*CH$_3$), 6.98 (d, J = 9.2 Hz, 1H, 5-*H*), 7.50 (dd, J = 9.1 Hz/2.5 Hz, 1H, 6-*H*), 7.56 (d, J = 2.5 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 17.8 (CHCH_3), 32.9 (NCH_3), 34.6 (NCH_3), 72.8 (C*H*CH$_3$), 116.4 (C-5), 120.3−120.5 (C-7/C-8a), 124.5 (C-8), 133.5 (C-6), 141.9 (C-4a). Anal. ($C_{10}H_{13}ClN_2O_2S$) theoretical: C, 46.06; H, 5.03; N, 10.74; S, 12.30. Found: C, 45.57; H, 5.03; N, 10.73; S, 11.61.

*R/S-7-Chloro-4-ethyl-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7c**).* White solid; m.p.: 70−71 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (t, J = 7 Hz, 3H, CH$_2$CH_3), 1.58 (d, J = 6.7 Hz, 3H, CHCH_3), 2.60 (s, 3H, NCH_3), 3.47 (m, 2H, NCH_2CH$_3$), 5.17 (q, J = 6.7 Hz, 1H, C*H*CH$_3$), 7.01 (d, J = 9.3 Hz, 1H, 5-*H*), 7.47 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.54 (d, J = 2.6 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 12.1 (CH$_2$CH$_3$), 18.3 (CHCH$_3$), 32.8 (NCH$_3$), 40.5 (NCH$_2$CH$_3$), 71.7 (CHCH$_3$), 116.1 (C-5), 119.9−120.1 (C-7/C-8a), 124.7 (C-8), 133.6 (C-6), 140.5 (C-4a). Anal. ($C_{11}H_{15}ClN_2O_2S$) theoretical: C, 48.08; H, 5.50; N, 10.20; S, 11.67. Found: C, 48.06; H, 5.51; N, 10.36; S, 11.02.

*R/S-7-Chloro-4-(2-fluoroethyl)-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7d**).* White solid; m.p.: 118−120 °C; ^{1}H NMR (DMSO-d_6) δ 1.59 (d, J = 6.7 Hz, 3H, CHCH_3), 2.63 (s, 3H, NCH_3), 3.80 (m, 2H, NCH_2), 4.61 (dt, J = 47.5 Hz/4.9 Hz, 1H, CH_2F), 5.23 (q, J = 6.7 Hz, 1H, C*H*CH$_3$), 7.09 (d, J = 9.3 Hz, 1H, 5-*H*), 7.48 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.59 (d, J = 2.6 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 18.2 (CHCH$_3$), 32.5 (NCH$_3$), 46.4 (d, J = 20 Hz, NCH$_2$), 72.1 (CHCH$_3$), 82.0 (d, J = 166 Hz, CH$_2$F), 116.7 (C-5), 120.8−120.9 (C-7/C-8a), 124.8 (C-8), 133.4 (C-6), 140.8 (C-4a). Anal. ($C_{11}H_{14}ClFN_2O_2S$) theoretical: C, 45.13; H, 4.82; N, 9.57; S, 10.95. Found: C, 45.07; H, 4.75; N,9.65; S, 10.71.

*R/S-7-Chloro-4-(2,2-difluoroethyl)-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7e**).* White solid; m.p.: 119−121 °C; ^{1}H NMR (DMSO-d_6) δ 1.59 (d, J = 6.7 Hz, 3H, CHCH_3), 2.66 (s, 3H, NCH_3), 3.96 (m, 2H, NCH_2), 5.24 (q, J = 6.7 Hz, 1H, C*H*CH$_3$), 6.29 (tt, J = 55.0 Hz/3.7 Hz, 1H, C*H*F$_2$), 7.18 (d, J = 9.3 Hz, 1H, 5-*H*), 7.52 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-*H*), 7.62 (d, J = 2.6 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 18.0 (CHCH$_3$), 32.8 (NCH$_3$), 48.2 (t, J = 25 Hz, NCH$_2$), 72.6 (CHCH$_3$), 115.0 (t, J = 242 Hz, CHF$_2$), 117.2 (C-5), 121.4−121.7 (C-7/C-8a), 124.7 (C-8), 133.4 (C-6), 140.9 (C-4a). Anal. ($C_{11}H_{13}ClF_2N_2O_2S$) theoretical: C, 42.52; H, 4.22; N, 9.01; S, 10.32. Found: C, 42.68; H, 4.21; N,9.09; S, 10.73.

*R/S-7-Chloro-4-cyclopropyl-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7f**).* White solid; m.p.: 135−136 °C; ^{1}H NMR (DMSO-d_6) δ 0.66 (m, 2H, CH(CH_2)$_2$), 0.97 (m, 2H, CH(CH_2)$_2$), 1.57 (d, J = 6.9 Hz, 3H, CHCH_3), 2.51 (m, 1H, C*H*(CH$_2$)$_2$), 2.67 (s, 3H, NCH_3), 4.99 (q, J = 6.8 Hz, 1H, C*H*CH$_3$), 7.32 (d, J = 9.1 Hz, 1H, 5-*H*), 7.54 (dd, J = 9.1 Hz/2.6 Hz, 1H, 6-*H*), 7.57 (d, J = 2.5 Hz, 1H, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 7.7 (CH(CH$_2$)$_2$), 10.1 (CH(CH$_2$)$_2$), 17.4 (CHCH$_3$), 28.3 (NCH$_3$), 35.1 (CH(CH$_2$)$_2$), 73.9 (CHCH$_3$), 117.6 (C-5), 121.4−121.8 (C-7/C-8a), 124.2 (C-8), 133.2 (C-6), 141.3 (C-4a). Anal. ($C_{12}H_{15}ClN_2O_2S$) theoretical: C, 50.26; H, 5.27; N, 9.77; S, 11.18. Found: C, 50.30; H, 5.26; N, 9.97; S, 11.18.

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*R/S-7-Chloro-4-cyclopropylmethyl-2,3-dimethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7g**).* White solid; m.p.: 100−102 °C; ^{1}H NMR (DMSO-d_6) δ 0.34 (m, 2H, CH(CH_2)$_2$), 0.49 (m, 2H, CH(CH_2)$_2$), 1.04 (m, 1H, CH(CH$_2$)$_2$), 1.55 (d, J = 6.8 Hz, 3H, CHCH_3), 2.69 (s, 3H, NCH_3), 3.31 (m, 2H, NCH_2), 5.17 (q, J = 6.7 Hz, 1H, CHCH$_3$), 7.10 (d, J = 9.3 Hz, 1H, 5-H), 7.47 (dd, J = 9.2 Hz/2.6 Hz, 1H, 6-H), 7.56 (d, J = 2.6 Hz, 1H, 8-H); ^{13}C NMR (DMSO-d_6) δ 3.5 (CH(CH_2)$_2$), 9.1 (CH(CH_2)$_2$), 18.7 (CHCH$_3$), 34.4 (NCH_3), 50.7 (NCH_2), 72.8 (CHCH$_3$), 116.6 (C-5), 120.0−120.2 (C-7/C-8a), 124.7 (C-8), 133.5 (C-6), 140.6 (C-4a). Anal. ($C_{13}H_{17}ClN_2O_2S$) theoretical: C, 51.91; H, 5.70; N, 9.31; S, 10.66. Found: C, 52.43; H, 5.64; N, 9.49; S, 10.53.

Determination of Water Solubility and Aqueous Stability of Mono-, Di-, and Trimethyl-Substituted BTD Compounds. About 10 mg of the tested compound was suspended in 10 mL of distilled water and stirred at room temperature (20 °C) for 24 h. Three aliquots of the suspension were centrifuged (13 400 rpm, 5 min), and 1 mL of the clear supernatant was collected (three working solutions). A calibration curve was established with standard solutions of the tested compound in DMSO/water (2:98) (calibration standards at 25, 50, 100, and 250 μM). The different solutions (working solutions and calibration standards) were examined by HPLC in the following experimental conditions: equipment, Agilent 1100; column, Alltech Hypersil BDS (C18, 3 μm); mobile phase, acetonitrile/water (20:80, 25:75 or 30:70); detection at 254 nm; temperature, 20 °C; injection, 50 μL; debit, 0.5 mL/min). Each solution was injected two times. The final result is reported in Table 1 as the mean of six determinations given with the standard deviation.

For the stability study, a solution of the tested compound (between 20 and 50 μM, thus below the saturation concentration) was kept at room temperature and was injected at regular time intervals (0, 24, 48, 72, 96, 336 h) in the same HPLC conditions as reported above (three injections). The results (mean of the three injections) were expressed as the residual concentration of the modulator in % of the starting concentration considered being 100% (natural logarithm of the residual % of modulator versus time in hours). The half-life was determined from the linear regression equation obtained with the Excel software.

Effect on AMPA-Evoked Currents in *Xenopus laevis* Oocytes. Electrophysiological recordings were performed at room temperature on *Xenopus laevis* oocytes injected with rat cortex poly(A+) mRNA according to our previously described procedure (see Figure S2, Supporting Information).[16] The recordings were performed using 10 μM AMPA, inducing 90−100% of AMPA-mediated response.

Effect on AMPA-Evoked Membrane Depolarization (in Vitro Fluorescence Assay). This assay investigating AMPA-evoked membrane depolarization was performed on rat primary brain cultures using fluorescent membrane potential dyes and an imaging based plate reader (FLIPR, fluorescence imaging plate reader, Molecular Devices, US). The assay was performed following our previously published procedures (see Figure S2, Supporting Information).[16,26] The assay was performed using 300 μM AMPA, inducing 90−100% of AMPA-mediated response.

Crystallization. The double mutant of rat GluA2 LBD-L483Y-N754S, which forms a dimer in solution,[27] was expressed and purified as previously described.[17,27] Racemic **7b** was added as solid compound to GluA2 LBD-L483Y-N754S with L-glutamate (4.0 mg mL^{-1} in 10 mM HEPES pH 7.0, 20 mM NaCl, 1 mM EDTA, 8.5 mM L-glutamate). Prior to setting up drops, the mixture was left to equilibrate for 1 week at 6 °C, centrifuged, and new solid compound was added and mixed again. The protein in complex with L-glutamate and **7b** crystallized as rods after 5 days using the hanging-drop vapor-diffusion method at 6 °C, with 500 μL of reservoir solution (15.2% PEG4000, 0.2 M ammonium sulfate, 0.1 M phosphate-citrate pH 4.5). Drops consisted of 1 μL of the protein solution mixed with 1 μL of the reservoir solution. Crystals were briefly submerged in reservoir solution containing 25% glycerol as cryo protectant before flash cooling in liquid nitrogen.

X-ray Data Collection and Structure Determination. X-ray diffraction data were collected at beamline I911-3, MAX-lab, Lund, Sweden,[31] and processed using *XDS*[32] and *SCALA*[33] implemented in the CCP4 suite of programs.[34] The structure was solved by molecular replacement in *PHASER*[35] using the structure of GluA2 LBD-L483Y-N754S with L-Glu and **9** as search model (PDB ID 3TKD, molA). The program *AUTOBUILD*[36] in *PHENIX*[37] was used for initial automated model building, where also several water molecules were modeled. Iterative model building and structure refinements were performed in *COOT*[38] and *PHENIX*, respectively. Topology and parameter files for **7b** were obtained using *MAESTRO* (v.9.4; Schrödinger, LLC) and *eLBOW*.[39] Individual isotropic B-factors were used during refinements. During later rounds of refinement TLS was applied with groups generated by the TLSMD server.[40] Ser497 was modeled in two alternative conformations and these two conformations were linked to the modulator/sulfate pairs during refinements in phenix. Domain closure was calculated using DynDom,[41] and figures were prepared in PyMOL (v.1.7; The PyMOL Molecular Graphics System, V.S., LLC).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00318.

Degradation kinetics of compounds **5a**, **5b**, **7a**, and **7b** in solution in distilled water at room temperature (Figure S1); original data using voltage clamp recordings on *Xenopus laevis* oocytes and in vitro fluorescence assay (FLIPR) (Figure S2); zoom on the modulator binding site (Figure S3) (PDF)

Accession Codes

The structure coordinates and corresponding structure factor file of GluA2 LBD-L483Y−N754S with **7b** have been deposited in the Protein Data Bank under the accession code 5BUU.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: + 45 3533 6486. E-mail: jsk@sund.ku.dk.

Author Contributions

‖A.P.L. and P.F. contributed equally to the work.

Author Contributions

⊥B.P. and J.S.K. equally supervised the work.

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Notes

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■ ABBREVIATIONS USED

ADHD, attention-deficit/hyperactivity disorder; AMPA, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid; AMPA-PAMs, positive allosteric modulators of AMPA receptors; BTDs, benzothiadiazine dioxides; CNS, central nervous system; CTZ, cyclothiazide; EC_{2x}, the concentration of compound responsible for a 2-fold increase of the AMPA

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mediated response; EC_{50}, the concentration of compound responsible for 50% of the maximal effect of the AMPA mediated response; FDSS, functional drug screening system; FLIPR, fluorescence imaging plate reader; GluA2, ionotropic glutamate receptor A2; iGluRs, ionotropic glutamate receptors; LBD, ligand-binding domain; NMDA, *N*-methyl-D-aspartic acid; PTDs, pyridothiadiazine dioxides; SAR, structure−activity relationship; TLC, thin layer chromatography; TMS, tetramethylsilane; VC, voltage clamp assay

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