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Synthesis, Pharmacological and Structural Characterization, and Thermodynamic Aspects of GluA2-Positive Allosteric Modulators with a 3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide Scaffold

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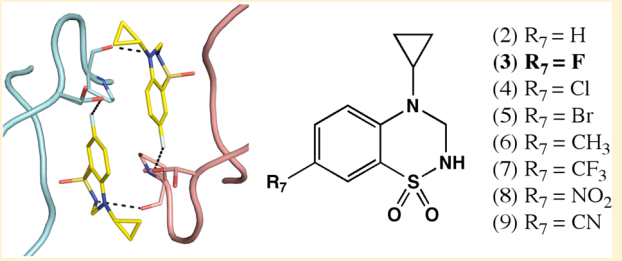
ABSTRACT: Positive allosteric modulators of ionotropic glutamate receptors are potential compounds for treatment of cognitive disorders, e.g., Alzheimer's disease. The modulators bind within the dimer interface of the ligand-binding domain (LBD) and stabilize the agonist-bound conformation, thereby slowing receptor desensitization and/or deactivation. Here we describe the synthesis and pharmacological testing at GluA2 of a new generation of 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides. The most potent modulator **3** in complex with GluA2-LBD-L483Y-N754S was subjected to structural analysis by X-ray crystallography, and the thermodynamics of binding was studied by isothermal titration calorimetry. Compound **3** binds to GluA2-LBD-L483Y-N754S with a K_d of 0.35 μM (ΔH = −7.5 kcal/mol and $-T\Delta S$ = −1.3 kcal/mol). This is the first time that submicromolar binding affinity has been achieved for this type of positive allosteric modulator. The major structural factor increasing the binding affinity of **3** seems to be interactions between the cyclopropyl group of **3** and the backbone of Phe495 and Met496.



INTRODUCTION

Ionotropic glutamate receptors (iGluRs) mediate the majority of fast excitatory neurotransmission in the mammalian central nervous system (CNS). iGluRs are divided into three subtypes: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, kainic acid receptors, and *N*-methyl-D-aspartic acid (NMDA) receptors. All three subtypes have been shown to be involved in long-term potentiation (LTP) and long-term depression (LTD), including learning and memory formation (see ref 1 and references therein). iGluR agonists function by direct activation of the receptors and, thus, may cause severe side effects, e.g., neurotoxicity. On the contrary, positive allosteric modulators of iGluRs only potentiate the effects of (*S*)-glutamate and therefore lead to a fine-tuning of receptor signaling. Several studies support positive allosteric modulation of AMPA receptors as a promising therapeutic strategy in the treatment of dysfunctions in the CNS, such as attention deficit hyperactivity disorder (ADHD),[2] Alzheimer's disease,[3] and schizophrenia.[4]

iGluRs form homo- or heterotetrameric ligand-gated ion channels, where each of the four subunits is composed of an extracellular part with an *N*-terminal domain and a ligand-binding domain (LBD), a membrane-spanning region, and finally an intracellular *C*-terminal domain.[1] The structure of the full-length homotetrameric AMPA receptor GluA2 showed that the LBDs are arranged as a dimer of dimers.[5] While the endogenous neurotransmitter (*S*)-glutamate binds at the agonist-binding site in the LBD,[6] positive allosteric modulators bind at the LBD dimer interface.[7,8] Positive allosteric modulators can potentiate GluA2 currents in two ways: (i) by stabilizing the (*S*)-glutamate-bound conformation and thereby slowing deactivation or (ii) by stabilizing the interface between two subunits and slowing the entrance into the desensitized state, which involves a rearrangement of the interface and closure of the ion channel with (*S*)-glutamate still bound to the receptor.

In 1998, Pirotte et al.[9] reported a series of 4*H*-1,2,4-pyridothiadiazine 1,1-dioxides and 2,3-dihydro-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides with various alkyl and aryl substituents in the 2-, 3-, and 4-positions as positive allosteric modulators of AMPA receptors. This study was later followed up by synthesis and pharmacological testing of new derivatives in an attempt to enhance modulatory activity, e.g., by

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addressing lipophilicity to obtain in vivo active compounds as well as the steric and electronic impact of different substituents.[10−12] In a recent study, we employed isothermal titration calorimetry (ITC) to determine the binding affinity of compound **1** (Figure 1) at the GluA2 LBD,[13] as **1** is the most

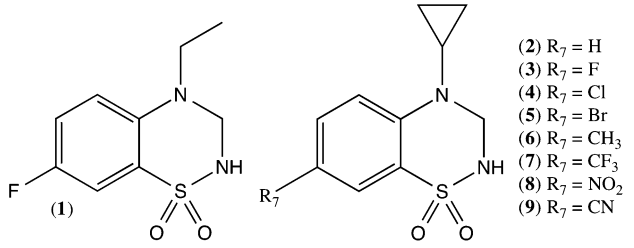


Figure 1. Chemical structure of the parent compound **1** and a general formula of the newly synthesized 4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **2**−**9**.

potent compound within the series of 1,2,4-benzothiadiazine 1,1-dioxides.[10−12] It was noticed that the ethyl substituent of **1** forms van der Waals contacts to GluA2 residues, primarily to backbone atoms of Met496.

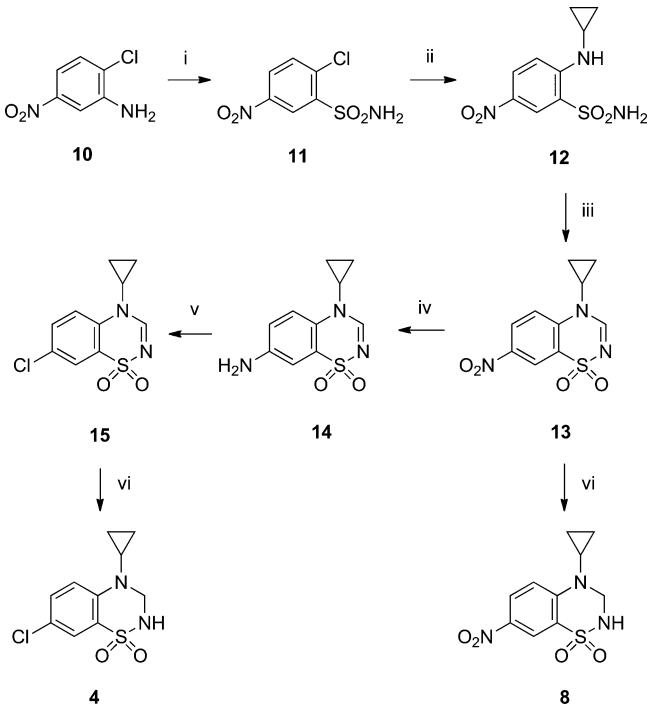
Here, we report the synthesis and pharmacological characterization of eight compounds in which the 4-ethyl substituent has been substituted with a 4-cyclopropyl group and with various substituents introduced in the 7-position (Figure 1). We show that the introduction of a cyclopropyl group leads to an approximately 10-fold improvement of potency at GluA2 compared to that of ethyl, using an in vitro test on rat primary brain cells measuring the effect of the new compounds on AMPA-evoked membrane depolarization (fluorescence assay). The most potent compound **3**, containing a fluorine atom in the 7-position, was selected for studies of thermodynamics of binding at GluA2 LBD using ITC. Together with an X-ray structural analysis, these studies provide a molecular explanation for the improved binding affinity of the new generation of 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides.

RESULTS AND DISCUSSION

Synthesis. Access to the diversely 7-substituted 4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **2**−**9** is described in Schemes 1 and 2.

The synthesis of the 7-chloro- and 7-nitro-substituted compounds **4** and **8** was initially performed according to the pathway reported in Scheme 1. The commercially available 2-chloro-5-nitroaniline (**10**) was converted into the corresponding benzenesulfonamide **11** according to the Meerwein variation of the Sandmeyer reaction.[14] Nucleophilic substitution of the chlorine atom by cyclopropylamine, providing intermediate **12**, was facilitated by the fact that the halogen atom was located at the *ortho* and *para* positions of two electron-withdrawing groups. Ring closure of **12** into the corresponding 4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **13** was achieved by heating **12** in triethyl orthoformate. Reduction of the nitro group of **13** into the corresponding 7-amino-substituted 4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**14**) by catalytic hydrogenation, followed by diazotization of **14** in the presence of cuprous chloride provided 7-chloro-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**15**). The latter intermediate was converted into the 7-chloro-substituted target compound **4** after saturation of the N2−C3 double bond by means of sodium borohydride.

Scheme 1[a]

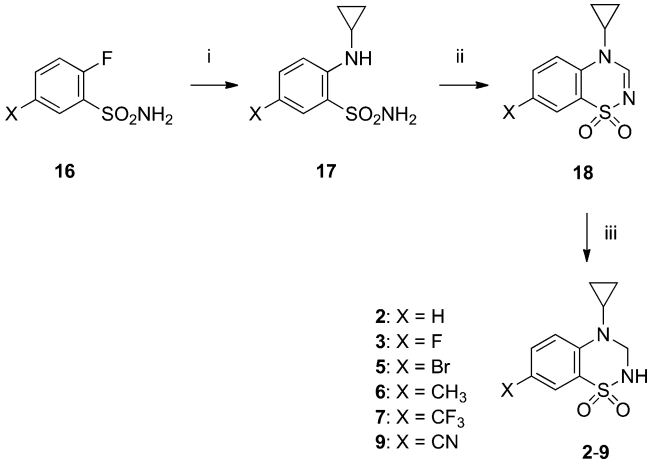


[a]Reagents and conditions: (i) (1) HNO_2, −5 °C; (2) SO_2, $CuCl_2$, 15 min; (3) NH_4OH (50−55%); (ii) cyclopropylamine, 100 °C, 24 h (90%); (iii) $HC(OEt)_3$, 130 °C, 3 h (85%); (iv) H_2, Pd/C (10%), rt, 30 min (80%); (v) (1) HNO_2, 0 °C; (2) Cu_2Cl_2, rt, 30 min (75%); (vi) $NaBH_4$, 2-propanol, 55 °C, 10 min (60%).

The use of the same reagent on intermediate **13** gave rise to the corresponding 7-nitro-substituted target compound **8**.

The other final compounds (**2**, **3**, **5**, **6**, **7**, and **9**) were obtained by means of an original pathway starting from commercially available 2-fluorobenzenesulfonamides **16** (Scheme 2). Independent of the nature of the substituent at the 5-position of 2-fluoro-substituted benzenesulfonamides, nucleophilic substitution of the fluorine atom by cyclopropylamine occurred, providing the corresponding 2-(cyclopropyla-

Scheme 2[a]



[a]Reagents and conditions: (i) cyclopropylamine, dioxane, 100−110 °C, 24−96 h (85−95%); (ii) $HC(OEt)_3$, 130−150 °C, 1−24 h (70−75%); (iii) $NaBH_4$, 2-propanol, 50−55 °C, 5−10 min (80%).

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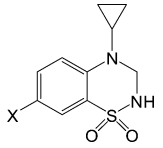
mino)-substituted benzenesulfonamides **17**. Such a reaction may be due to the greater lability to nucleophilic substitution of the aromatic fluorine atom compared to other halogen atoms, provided that only the electron-withdrawing sulfonamide group was present at the *ortho* position. Ring closure of **17** by means of triethyl orthoformate provided intermediates **18**, which were converted into the target compounds (**2**, **3**, **5**, **6**, **7**, and **9**) after saturation of the N2−C3 double bond of **18** by reaction with sodium borohydride.

Pharmacological Testing. The newly synthesized 4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides **2**−**9** were evaluated in vitro as AMPA potentiators by means of a fluorescence assay, performed on primary cultures of neurons from rat embryonic cortex, measuring the effect of the modulators on AMPA-evoked membrane depolarization. For each compound, the $EC_{2\times}$ value was determined, which corresponds to the concentration of modulator giving a 2-fold increase of the fluorescence induced by AMPA (300 μM) (Table 1). Moreover, the maximum effect of each modulator (E_{max} value normalized to unity for AMPA-evoked response taken as ×1) and its EC_{50} value (concentration of modulator responsible for 50% of the maximal effect) were also determined (Table 1).

Table 1. Effects of 7-Substituted 4-Cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides on the Fluorescence Induced by 300 μM AMPA on Primary Cultures of Neurons from Rat Embryonic Cortex



compd	X	$EC_{2\times}$ [a] (μM)	EC_{50} [b] (μM)	E_{max} [c]
2	H	0.73 ± 0.15 (3)	4.17 ± 0.60 (3)	× 8.5 ± 0.8 (3)
3	F	0.27 ± 0.03 (3)	0.90 ± 0.10 (3)	× 15.0 ± 2.6 (3)
4	Cl	0.75 ± 0.21 (4)	3.13 ± 0.72 (4)	× 10.9 ± 2.7 (4)
5	Br	1.27 ± 0.37 (3)	4.83 ± 1.17 (3)	× 9.3 ± 0.7 (3)
6	CH_3	5.13 ± 1.67 (3)	nd	× 8.7 ± 1.9 (3)
7	CF_3	>100 (3)	nd	nd
8	NO_2	14.0 ± 3.6 (2)[d]	22.5 (1/2)[d]	× 7 (1/2)[d]
9	CN	0.47 ± 0.03 (3)	2.50 ± 0.50 (3)	× 9.2 ± 0.4 (3)

[a] $EC_{2\times}$ = modulator concentration giving a 2-fold increase of the fluorescence induced by AMPA (300 μM) (mean ± SEM (*n*)). [b] EC_{50} = modulator concentration responsible for 50% of the maximal effect (mean ± SEM (*n*)). [c] E_{max} = maximal effect normalized to unity for AMPA-evoked response (×1). [d] FLIPR (fluorescence imaging plate reader). The principle of this technique is identical to that of the fluorescence assay (use of a fluorescent probe sensitive to variation of the membrane potential), but it uses another plate reader (FLIPR, Molecular Devices, US).

Compared to the voltage clamp assay on *Xenopus laevis* oocytes used in our previous studies for the evaluation of AMPA potentiators (measurement of AMPA-induced currents on *X. laevis* oocytes injected with rat cortex poly(A^+) mRNA), the fluorescence assay on primary neuronal cultures isolated from rat embryonic cortex was found to provide similar $EC_{2\times}$ values (personal observation). For comparison purposes, compound **4** was found to express an $EC_{2\times}$ value of 0.8 μM in the voltage clamp assay (data not shown), while it expressed an $EC_{2\times}$ value of 0.75 μM in the fluorescence assay (see Table 1).

In a previous work, we reported that compound **1**, the 4-ethyl analogue of compound **3**, markedly potentiated the effect of AMPA on its receptors expressed in *Xenopus* oocytes with an $EC_{2\times}$ value of 3.2 μM.[10] The replacement of the ethyl chain at the 4-position of compound **1** by a cyclopropyl moiety, leading to compound **3**, markedly increased the effect on AMPA receptors. The $EC_{2\times}$ value of **3**, determined in the fluorescence assay on rat neuronal cells, was found to be 0.27 μM, corresponding to a 10-fold increase in potency.

Interestingly, the fluorine atom appeared to be the best choice of halogen atom at the 7-position of 4-cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides. The rank order of efficacy as AMPA potentiators was found to be F > Cl > Br (see compounds **3**, **4**, and **5**, Table 1). Moreover, the fluoro compound **3** also expressed the highest maximal effect since this compound was able to increase by 15-fold the AMPA-evoked response (Table 1).

The presence of a hydrogen atom at the 7-position (see compound **2**) was found to produce the same effect as that of a chlorine atom and clearly appeared to be more suitable than the presence of a methyl group (see compound **6**). Considering the introduction of an electron-withdrawing group, such as a trifluoromethyl, nitro, or cyano group, a great variability was observed. The 7-cyano-substituted compound **9** was the most potent on AMPA receptors with an $EC_{2\times}$ value of 0.47 μM, not far from that of the most potent 7-fluoro-substituted compound **3**. However, introduction of a trifluoromethyl group led to a complete loss of activity, which was the same trend as the one already observed with 4-ethyl-substituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides.[10]

Thermodynamic Details of Binding. As compound **3** was shown to be a highly effective positive allosteric modulator of GluA2 with submicromolar potency, it was selected for studies of the thermodynamics of binding to GluA2 using isothermal titration calorimetry. We used the rat GluA2-LBD-L483Y-N754S double mutant,[13] which exists as a dimer in solution due to the L483Y mutation (numbering without the signal peptide). The preformed dimer enables measurement of modulator binding affinity independently of the dimerization process. In the original rat GluA2 structure reported by Armstrong and Gouaux,[6] an asparagine is present at position 754, corresponding to the flop-splice isoform of GluA2. In the flip-splice isoform, a serine is located at this position. Ser754 was introduced in the protein construct as it has previously been shown that a similar compound, cyclothiazide, displays preference for the flip-splice isoform.[15] This preference has been attributed to sterical hindrance in the flop-splice isoform.[7]

The ITC experiments showed that the binding of **3** to GluA2-LBD-L483Y-N754S is an exothermic process (Figure 2). The binding affinity (K_d) of **3** at GluA2-LBD-L483Y-N754S is 0.35 μM with ΔH = −7.5 kcal/mol and $-T\Delta S$ = −1.3 kcal/mol (Table 2). This means that the complex formation is primarily enthalpy-driven. Binding of compound **1** to GluA2-LBD-L483Y-N754S was also found to be enthalpy-driven[13] (Table 2). The increased binding affinity of **3** compared to **1** is seen to be primarily due to a large enthalpy gain combined with a small entropy loss.

Structure Determination. Cocrystallization of compound **3** with the GluA2 LBD and examination of the interactions at the level of the modulator-binding site are expected to help the understanding of the unique impact of the cyclopropyl group

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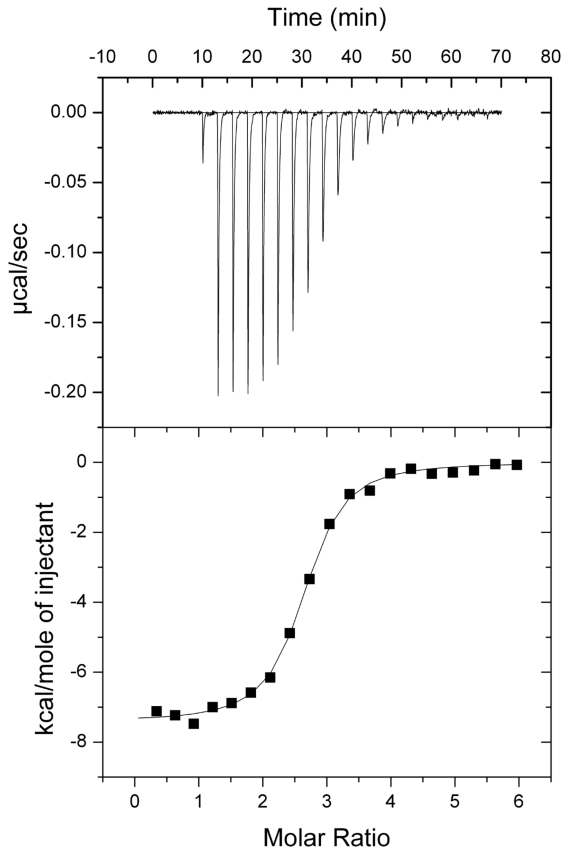

Figure 2. Isothermal titration calorimetry study of binding of compound **3** to GluA2-LBD-L483Y-N754S. The raw data (top panel) and isotherm (bottom panel) are presented. The graphs show that heat is developed after each injection (exothermic reaction) and that the signal is diminished when the protein becomes saturated with **3**. The experiment shown is a representative of three experiments.

Table 2. Isothermal Titration Calorimetry Using GluA2-LBD-L483Y-N754S

	3	**1**[a]
K_d (μM)	0.35 ± 0.02[b]	5.6 ± 0.9
ΔH (kcal/mol)	−7.5 ± 0.2	−4.9 ± 0.4
$-T\Delta S$ (kcal/mol)	−1.3 ± 0.2	−2.3 ± 0.4
n_H	2.7 ± 0.1	2.3 ± 0.1

[a]The values are from Krintel et al.[13] [b]Standard deviation.

and fluorine atom on the improvement of the binding affinity for AMPA receptors. To get such detailed information on the binding mode of **3**, we determined a high-resolution structure of GluA2-LBD-L483Y-N754S in complex with (*S*)-glutamate and **3**. Data collection and refinement statistics are shown in Table 3. The complex crystallizes as a dimer with (*S*)-glutamate located at the agonist-binding site and two molecules of **3** at the dimer interface (Figure 3A). We have previously shown that the L483Y mutation does not alter the GluA2 dimer interface and binding mode of cyclothiazide,[13] and as Tyr483 is located approximately 9 Å from compound **3**, it most likely does not affect the binding mode of the modulator. Two different dimers are observed; one dimer is composed of molecule A and a symmetry-related molecule A and the second dimer of molecules B and C. The agonist-binding site is positioned in a cavity between the so-called D1 and D2 lobes that are connected via a hinge region. Upon (*S*)-glutamate binding, the

Table 3. X-ray Data Collection and Refinement Statistics

Data Collection	
space group	$P2_12_12$
a, *b*, *c* (Å)	113.9, 164.0, 47.3
resolution (Å)	29.13−1.87 (1.97−1.87)[a]
no. of unique reflns	74220
redundancy	4.1 (4.1)
completeness (%)	99.9 (100)
R_{merge}[b] (%)	5.7 (38.5)
$I/\sigma I$	10.9 (2.0)
Refinement	
R_{work}[c]/R_{free}[d] (%)	17.5/21.1
no. of non-hydrogen atoms	7134
no. of residues (molecules A/B/C)	262/260/262
no. of (*S*)-glutamate/**3**/water/acetate/cacodylate/glycerol/zinc ions	3/3/687/13/1/6/10
RMS deviations	
bond lengths (Å)	0.007
bond angles (deg)	1.1
no. of residues in allowed areas of Ramachandran plot[e] (%)	100
av *B* values (Å^2)	
molecules A/B/C	21.2/26.2/25.3
(*S*)-glutamate/**3**/water/acetate/cacodylate/glycerol/zinc ions	16.4/14.3/31.5/38.4/22.6/38.0/42.0

[a]Values in parentheses correspond to the outermost resolution shell. [b]$R_{merge} = \sum_{hkl}\sum_i|I_i(hkl) - I(hkl)|/\sum_{hkl}\sum_i|I_i(hkl)|$, where $I_i(hkl)$ is the intensity of an individual measurement of the reflection with Miller indices *hkl* and $I(hkl)$ is the intensity from multiple observations. [c]$R_{work} = \sum_{hkl}|F_{obsd} - F_{calcd}|/\sum_{hkl}|F_{obsd}|$, where $|F_{obsd}|$ and $|F_{calcd}|$ are the observed and calculated structure factor amplitudes. [d]R_{free} is equivalent to R_{work}, but calculated with reflections omitted from the refinement process (5% of reflections omitted). [e]The Ramachandran plot was calculated according to PROCHECK.[30]

LBD adopts a closed clamshell-like structure as a result of movement of lobe D2 of ca. 20° compared to the unliganded (apo) structure of GluA2 LBD.[6] In the present structure, the closure of lobe D2 toward D1 is 20.7°, 19.4°, and 20.2° for molecules A, B, and C, respectively, compared to the apo structure (PDB code 1FTO, molecule A). These values are similar to those of agonist-bound structures without modulators.[16]

The core of **3** binds with the same contacts to the protein as observed for **1**.[13] The primary polar contacts of **3** to GluA2 residues are to Pro494 and Ser497 (Figure 3B). The N2 atom of **3** forms a hydrogen bond to the carbonyl oxygen atom of Pro494. No hydrogen bonds from **3** to water molecules are seen.

As seen from Figure 3C, shape complementarities are present between the GluA2 modulator-binding site and compound **3**. The N4 cyclopropyl moiety is at van der Waals distance from the backbone atoms of Phe495 and Met496. In addition, there is an extensive water molecule network (Figure 4A), also at van der Waals distance from the cyclopropyl group. Analysis of the binding-site water molecules shows 5−7 water molecules are located within a 4 Å distance of compound **3**. However, these water molecules do not form hydrogen-bonding interactions with **3**, but are engaged in the formation of hydrogen bonds to residues Phe495, Ser497, Ser729, Lys730, Ser754, and Asp760.

Compared to the GluA2-LBD-L483Y-N754S with **1** bound, small differences in the pocket accommodating the cyclopropyl

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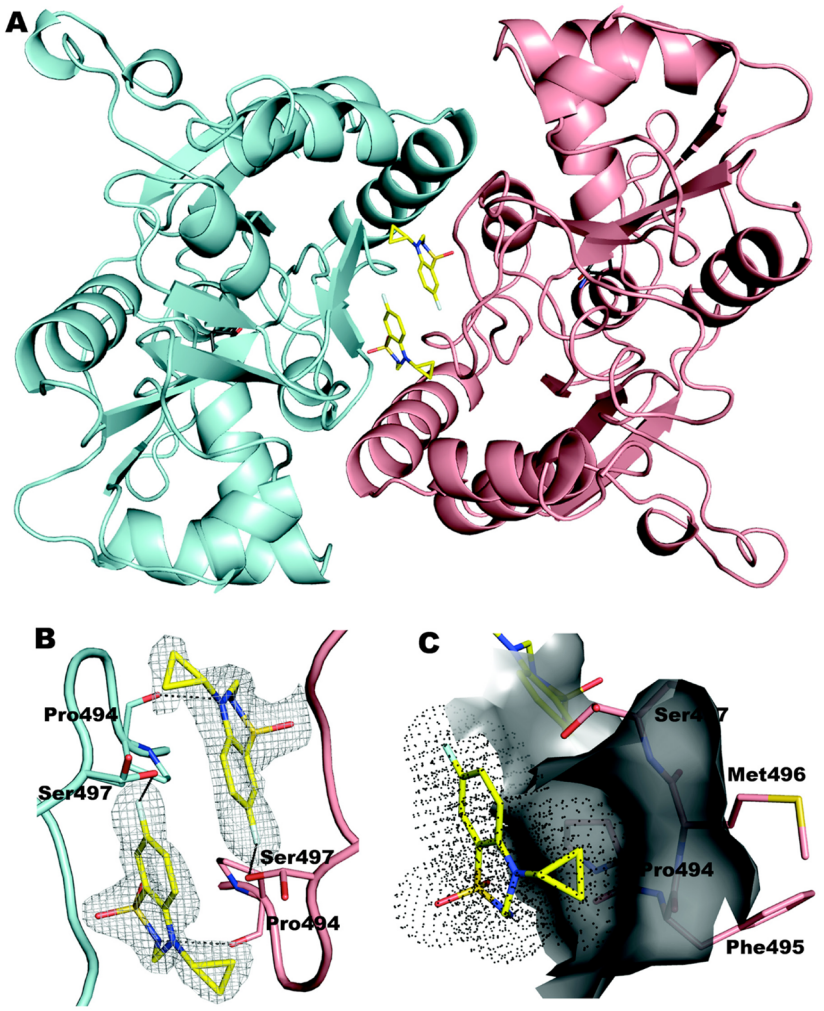

Figure 3. X-ray structure of dimeric GluA2-LBD-L483Y-N754S in complex with compound **3**. (A) Cartoon representation of the GluA2 dimer (molecules B and C). Two molecules of **3** (in yellow stick representation) bind at the dimer interface, and (*S*)-glutamate (in black stick representation) binds at the agonist-binding site. The protein molecules are shown in cyan (molecule B) and salmon (molecule C). (B) Zoom on the modulator-binding site. A simulated annealing $2F_o - F_c$ OMIT electron-density map for **3** (in dark gray, contoured at the 1σ level and carved 2.0 Å around **3**) is shown. Possible hydrogen-bonding interactions between GluA2 residues and **3** within 3.5 Å are shown as dashed lines. (C) Surface representation of the modulator-binding site, showing shape complementarities between GluA2 and **3**.

group of **3** or the ethyl group of **1** are seen. In particular, the cyclopropyl ring seems to form stacking interactions with the peptide backbone (Figure 4A), probably as a result of the fact that the cyclopropyl ring has some π-character.[17] All these nonbonded interactions are likely to contribute to the better affinity and enthalpy of binding of **3** compared to **1**.

The oxygen atoms of the sulfonamide are also engaged in van der Waals interactions, one oxygen to the side chains of Lys493 and Pro494 and the other oxygen to the side chain of Lys730 (not shown). The fluorine atom in the 7-position of **3** is directed toward the side chain of Ser497, which was located in two conformations. In one conformation, a fluorine hydrogen bond to the side-chain hydroxyl group of Ser497 (3.2−3.3 Å) is seen, Figure 3B. The occupancy of this conformation has been refined to 0.42, 0.33, and 0.46 for molecules A, B, and C, respectively, whereas compound **3** is present with full occupancy. As the fluorine hydrogen-bonding distance is above 3.2 Å and as Ser497 is present in two conformations, this hydrogen bond is not very strong. Analysis of the hydrogen-bonding pattern of the other side-chain conformation of Ser497 shows the hydroxyl group to be engaged in a water-mediated contact to Ser729 through two water molecules at the B−C dimer interface and in a water-mediated contact to Ser497 of a symmetry-related molecule A through one water molecule in the A−A(sym) dimer. Thus, both conformations of the side chain of Ser497 will contribute to stabilization of the dimer interface.

Among compounds **2**−**9**, most of the substituents in the 7-position are of electron-withdrawing character, and generally, such substituents will enhance the binding due to more favorable π-stacking interactions of **3** to the peptide backbone of Lys730 and Gly731 and the other molecule of **3** in the binding site.[18] There is, however, not much space in this position since the cyclopropyl group of the other molecule of **3** is within 4.5 Å of the substituent. To accommodate larger substituents in the 7-position than, e.g., a cyano group, such compounds will need to adopt another binding mode to avoid steric clashes.

Compound **3** shows a binding mode similar to that of IDRA-21, which belongs to the shifted thiazide class.[19] Compound **3** is a much more potent and efficacious compound than IDRA-21 (the concentration of IDRA-21 giving a 2-fold increase of

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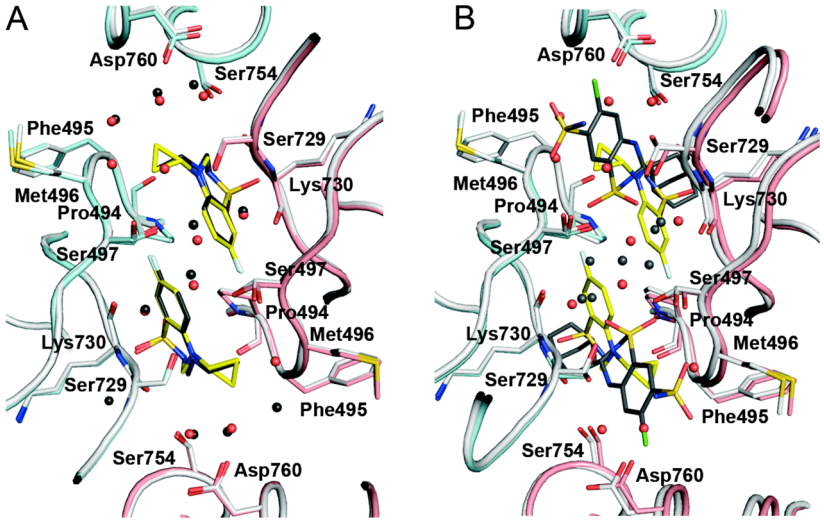

Figure 4. Binding mode of **3** compared to that of **1** (A) and cyclothiazide (B) at GluA2-LBD-L483Y-N754S. The structures of GluA2-LBD-L483Y-N754S in complex with **3** (modulator shown in yellow and protein molecules in cyan and salmon for molecules B and C, respectively), **1** (residues in light gray and **1** in dark gray, PDB code 3TDJ, molecules A and B), and cyclothiazide (residues in light gray and cyclothiazide in dark gray, PDB code 3TKD, molecules A and B) have been superimposed on lobe D1 residues. Selected residues are shown. Water molecules are shown as spheres, in red for the structure with **3**, in dark gray for the structure with **1**, and in dark gray for the structure with cyclothiazide.

the magnitude of the current induced by (*S*)-AMPA being 134 μM[10]). A comparison of the binding mode of compound **1** and IDRA-21 has previously been reported,[13] suggesting that the major structural factors increasing the potency of **1** over IDRA-21 are increased van der Waals contacts. Compound **3** shows a different binding mode compared to cyclothiazide, which belongs to the classical thiazide class (Figure 4B). However, in both classes, two modulator molecules are bound at the dimer interface.

CONCLUSION

We report the synthesis of eight new allosteric modulators containing a 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide scaffold. Compound **3** with a cyclopropyl group in the 4-position and fluorine in the 7-position was found in a functional study to potentiate AMPA-evoked currents by a factor of 15 with an EC_{50} of 0.90 μM. The binding affinity of **3** determined by isothermal titration calorimetry was seen to be in the submicromolar range, with a K_d of 0.35 μM. The detailed molecular interactions of **3** at GluA2-LBD-L483Y-N754S were studied by X-ray crystallography. Compared to compound **1**, the cyclopropyl group was found to possess shape complementarity to the GluA2 LBD and to form stacking interactions with the peptide backbone. On the basis of these compounds, new routes of synthesis of related compounds might lead to positive allosteric modulators with an improved activity profile in future pharmacological and structural studies.

EXPERIMENTAL SECTION

General Procedures. Melting points were determined on a Büchi Tottoli capillary apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance (500 MHz) instrument using deuterated dimethyl sulfoxide (d_6-DMSO) as the solvent with tetramethylsilane (TMS) as an internal standard; chemical shifts are reported in δ values (ppm) relative to that of internal TMS. The abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and br = broad are used throughout. Elemental analyses (C, H, N, S) were realized on a Thermo Scientific Flash EA 1112 elemental analyzer and were within ±0.4% of the theoretical values. This analytical method certified a purity of ≥95% for each tested compound. All reactions were routinely checked by TLC on silica gel Merck 60 F_{254}.

General Synthetic Pathway to 7-Substituted 4-Cyclopropyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxides 2−9. Finely ground $NaBH_4$ (1 g, 26.4 mmol) was added to a solution of the appropriate 7-substituted 4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **13** or **18a−f** (1.9 g, 6.3−8.6 mmol) or **15** (1.2 g, 4.68 mmol) in 2-propanol (40−50 mL) and then heated at 50−55 °C for 5−10 min. The solvent was removed by evaporation under reduced pressure. The residue was taken up in water (50 mL) and brought to acidic pH by adding 6 N HCl. The title compound was extracted with dichloromethane (3 × 30 mL). The organic phase was dried over $MgSO_4$ and filtered. The filtrate was evaporated to dryness, and the residue was recrystallized from methanol/water (1/1) (60 mL) (yield 80%) except for compound **4**, which was purified by chromatography on a silica column (mobile phase chloroform) and then recrystallized from methanol (yield 60%).

*Data for 4-Cyclopropyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**2**).* White solid. Mp: 165−166 °C. ^{1}H NMR (DMSO-d_6): δ 0.65 (m, 2H, CH(CH_2)$_2$), 0.91 (m, 2H, CH(CH_2)$_2$), 2.48 (m, 1H, CH(CH$_2$)$_2$), 4.66 (s, 2H, 3-CH$_2$), 6.86 (t, J = 7.5 Hz, 1H, 7-H), 7.27 (d, J = 8.5 Hz, 1H, 5-H), 7.45 (t, J = 7.9 Hz, 1H, 6-H), 7.53 (dd, J = 7.9 Hz, 0.9 Hz, 1H, 8-H), 7.85 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.4 (CH(CH$_2$)$_2$), 29.6 (CH(CH$_2$)$_2$), 61.0 (C-3), 114.5 (C-5), 117.3 (C-7), 123.2 (C-8a), 124.2 (C-8), 132.9 (C-6), 144.1 (C-4a). Anal. Calcd for $C_{10}H_{12}N_2O_2S$: C, 53.55; H, 5.39; N, 12.49; S, 14.29. Found: C, 53.49; H, 5.30; N, 12.63; S, 14.04.

*Data for 4-Cyclopropyl-7-fluoro-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**3**).* White solid. Mp: 163−164 °C. ^{1}H NMR (DMSO-d_6): δ 0.64 (m, 2H, CH(CH_2)$_2$), 0.90 (m, 2H, CH(CH_2)$_2$), 2.49 (m, 1H, CH(CH$_2$)$_2$), 4.65 (s, 2H, 3-CH$_2$), 7.31 (dd, J = 9.3 Hz, 4.5 Hz, 1H, 5-H), 7.38 (m, 1H, 6-H), 7.41 (m, 1H, 8-H), 7.99 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.4 (CH(CH$_2$)$_2$), 29.8 (CH(CH$_2$)$_2$), 61.0 (C-3), 110.3 (d, J = 24 Hz, C-8), 116.6 (d, J = 7 Hz, C-5), 120.5 (d, J = 23 Hz, C-6), 123.1 (d, J = 6 Hz, C-8a), 141.1 (C-4a), 152.9−154.8 (d, J = 238 Hz, C-7). Anal. Calcd for $C_{10}H_{11}FN_2O_2S$: C, 49.57; H, 4.58; N, 11.56; S, 13.23. Found: C, 49.34; H, 4.66; N, 11.70; S, 13.12.

*Data for 7-Chloro-4-cyclopropyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**4**).* White solid. Mp: 159−161 °C. ^{1}H NMR (DMSO-d_6): δ 0.66 (m, 2H, CH(CH_2)$_2$), 0.92 (m, 2H, CH(CH_2)$_2$), 2.53 (m, 1H, CH(CH$_2$)$_2$), 4.68 (s, 2H, 3-CH$_2$), 7.29 (d, J = 9.1 Hz, 1H, 5-H), 7.50 (dd, J = 9.1 Hz, 2.6 Hz, 1H, 6-H), 7.53 (d, J = 2.5 Hz, 1H, 8-H), 8.01 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.4

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($CH(CH_2)_2$), 29.7 ($CH(CH_2)_2$), 60.9 (C-3), 116.7 (C-5), 120.9 (C-7), 123.3 (C-8), 123.9 (C-8a), 132.8 (C-6), 143.0 (C-4a). Anal. Calcd for $C_{10}H_{11}ClN_2O_2S$: C, 46.42; H, 4.29; N, 10.83; S, 12.39. Found: C, 46.44; H, 4.22; N, 11.01; S, 12.82.

*Data for 7-Bromo-4-cyclopropyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (**5**).* White solid. Mp: 178−179 °C. ^{1}H NMR (DMSO-d_6): δ 0.66 (m, 2H, $CH(CH_2)_2$), 0.91 (m, 2H, $CH(CH_2)_2$), 2.52 (m, 1H, $CH(CH_2)_2$), 4.68 (s, 2H, 3-CH_2), 7.24 (d, J = 9 Hz, 1H, 5-H), 7.60 (dd, J = 9 Hz, 2.4 Hz, 1H, 6-H), 7.63 (d, J = 2.2 Hz, 1H, 8-H), 8.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.4 ($CH(CH_2)_2$), 29.6 ($CH(CH_2)_2$), 60.9 (C-3), 108.0 (C-7), 117.1 (C-5), 124.4 (C-8a), 126.0 (C-8), 135.5 (C-6), 143.3 (C-4a). Anal. Calcd for $C_{10}H_{11}BrN_2O_2S$: C, 39.62; H, 3.66; N, 9.24; S, 10.57. Found: C, 39.69; H, 3.67; N, 9.38; S, 10.58.

*Data for 4-Cyclopropyl-7-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**6**).* White solid. Mp: 144−145 °C. ^{1}H NMR (DMSO-d_6): δ 0.63 (m, 2H, $CH(CH_2)_2$), 0.88 (m, 2H, $CH(CH_2)_2$), 2.24 (s, 3H, CH_3), 2.44 (m, 1H, $CH(CH_2)_2$), 4.61 (s, 2H, 3-CH_2), 7.18 (d, J = 8.6 Hz, 1H, 5-H), 7.27 (dd, J = 8.7 Hz, 1.9 Hz, 1H, 6-H), 7.34 (d, J = 2 Hz, 1H, 8-H), 7.82 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.3 ($CH(CH_2)_2$), 19.6 (CH_3), 29.6 ($CH(CH_2)_2$), 61.1 (C-3), 114.7 (C-5), 123.2 (C-8a), 123.9 (C-8), 126.5 (C-7), 133.7 (C-6), 142.0 (C-4a). Anal. Calcd for $C_{11}H_{14}N_2O_2S$: C, 55.44; H, 5.92; N, 11.76; S, 13.45. Found: C, 55.41; H, 5.94; N, 11.92; S, 13.05.

*Data for 4-Cyclopropyl-7-trifluoromethyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**7**).* White solid. Mp: 155−157 °C. ^{1}H NMR (DMSO-d_6): δ 0.71 (m, 2H, $CH(CH_2)_2$), 0.96 (m, 2H, $CH(CH_2)_2$), 2.64 (m, 1H, $CH(CH_2)_2$), 4.78 (s, 2H, 3-CH_2), 7.42 (d, J = 9.5 Hz, 1H, 5-H), 7.77 (m, 1H, 6-H/8-H), 8.10 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.4 ($CH(CH_2)_2$), 29.9 ($CH(CH_2)_2$), 60.9 (C-3), 115.3 (C-5), 116.7 (d, J = 33 Hz, C-7), 121.3 (d, J = 4 Hz, C-8), 122.4 (C-8a), 123.1−125.2 (d, J = 271 Hz, CF_3), 129.4 (d, J = 3 Hz, C-6), 146.7 (C-4a). Anal. Calcd for $C_{11}H_{11}F_3N_2O_2S$: C, 45.20; H, 3.79; N, 9.58; S, 10.97. Found: C, 45.41; H, 4.02; N, 9.67; S, 11.02.

*Data for 4-Cyclopropyl-3,4-dihydro-7-nitro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**8**).* White solid. Mp: 198−201 °C. ^{1}H NMR (DMSO-d_6): δ 0.77 (m, 2H, $CH(CH_2)_2$), 1.01 (m, 2H, $CH(CH_2)_2$), 2.76 (m, 1H, $CH(CH_2)_2$), 4.86 (s, 2H, 3-CH_2), 7.41 (d, J = 9.2 Hz, 1H, 5-H), 8.29 (m, 3H, 6-H/8-H/NH). ^{13}C NMR (DMSO-d_6): δ 8.4 ($CH(CH_2)_2$), 30.5 ($CH(CH_2)_2$), 61.1 (C-3), 115.1 (C-5), 120.4 (C-8), 121.6 (C-8a), 128.0 (C-6), 136.3 (C-7), 148.7 (C-4a). Anal. Calcd for $C_{10}H_{11}N_3O_4S$: C, 44.60; H, 4.12; N, 15.60; S, 11.91. Found: C, 44.75; H, 4.51; N, 16.00; S, 12.23.

*Data for 7-Cyano-4-cyclopropyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide (**9**).* White solid. Mp: 259−261 °C. ^{1}H NMR (DMSO-d_6): δ 0.71 (m, 2H, $CH(CH_2)_2$), 0.97 (m, 2H, $CH(CH_2)_2$), 2.65 (m, 1H, $CH(CH_2)_2$), 4.79 (s, 2H, 3-CH_2), 7.35 (d, J = 9 Hz, 1H, 5-H), 7.82 (dd, J = 9 Hz, 2 Hz, 1H, 6-H), 8.02 (d, J = 2 Hz, 1H, 8-H), 8.13 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 8.4 ($CH(CH_2)_2$), 30.0 ($CH(CH_2)_2$), 60.9 (C-3), 98.1 (C-7), 115.4 (C-5), 118.6 (CN), 123.0 (C-8a), 128.8 (C-8), 135.9 (C-6), 146.9 (C-4a). Anal. Calcd for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86; S, 12.86. Found: C, 53.01; H, 4.35; N, 16.68; S, 13.12.

2-Chloro-5-nitrobenzenesulfonamide (11). A portion of glacial acetic acid (160 mL) was saturated for 30 min with gaseous sulfur dioxide. To the resulting solution, cooled on an ice bath, was added under stirring an aqueous solution of $CuCl_2$ (7 g in 20 mL) (suspension A). 2-Chloro-5-nitroaniline (**10**) (15 g, 87 mmol) was dissolved in a mixture of glacial acetic acid (160 mL) and 16 N HCl (40 mL). To the resulting solution, cooled in an ice−salt bath (−5 °C), was added dropwise under stirring an aqueous solution of $NaNO_2$ (8 g in 20 mL, 116 mmol). At the end of the addition, the resulting solution was slowly mixed with suspension A. After being stirred for 15 min, the suspension was poured onto ice (400 g). The resulting precipitate was collected by filtration, washed with water, and immediately redissolved in dioxane (150 mL). The solution obtained was added gradually, under stirring, to a concentrated ammonium hydroxide solution (300 mL) previously cooled on an ice bath. After the resulting solution was stirred for 30 min, the organic solvent and part of the ammonia were removed by evaporation under reduced pressure. The aqueous solution/suspension obtained was adjusted to neutral pH by adding 6 N HCl. The precipitate formed was collected by filtration and washed with water. The crude product was suspended in water (200 mL), and 10% NaOH was added until the pH was clearly alkaline. The suspension was gently heated to facilitate dissolution of the title product. The residual insoluble material was removed by filtration, and the cooled filtrate was adjusted to neutral or slightly acidic pH by adding 6 N HCl. The resulting precipitate was collected by filtration, washed with water, and dried. Yield: 50−55%. Mp: 180−183 °C. ^{1}H NMR (DMSO-d_6): δ 7.97 (d, J = 8.7 Hz, 1H, 3-H), 8.00 (s, 2H, SO_2NH_2), 8.43 (dd, J = 8.7 Hz, 2.7 Hz, 1H, 4-H), 8.67 (d, J = 2.7 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 123.6 (C-6), 127.8 (C-4), 133.3 (C-3), 137.1 (C-2), 142.1 (C-1), 145.9 (C-5).

2-(Cyclopropylamino)-5-nitrobenzenesulfonamide (12). 11 (5 g, 21 mmol) was introduced into a hermetically closed vessel containing a mixture of dioxane (70 mL) and cyclopropylamine (3.5 mL, 50 mmol). The hermetically closed vessel was placed in an oven at 100 °C for 24 h. After this time period, the solvent and the reagent were removed by distillation under reduced pressure. The residue was taken up in methanol (20 mL), and the insoluble material, which contains the title product, was collected by filtration, washed with methanol, and dried (yield: 90%). This compound was used in the next step without further purification. ^{1}H NMR (DMSO-d_6): δ 0.63 (m, 2H, $CH(CH_2)_2$), 0.91 (m, 2H, $CH(CH_2)_2$), 2.67 (m, 1H, $CH(CH_2)_2$), 6.96 (br s, 1H, NH), 7.28 (d, J = 9.3 Hz, 1H, 3-H), 7.72 (br s, 2H, SO_2NH_2), 8.28 (dd, J = 9.3 Hz, 2.7 Hz, 1H, 4-H), 8.48 (d, J = 2.7 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 7.4 ($CH(CH_2)_2$), 25.0 ($CH(CH_2)_2$), 113.1 (C-3), 124.5 (C-1), 125.0 (C-6), 128.7 (C-4), 135.5 (C-5), 150.1 (C-2).

4-Cyclopropyl-7-nitro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (13). In a round-bottom flask, a mixture of **12** (5 g, 19.4 mmol) and ethyl orthoformate (50 mL) was heated in the open state at 130 °C for 3 h. The resulting suspension was cooled on an ice bath, and the insoluble material was collected by filtration, washed with ether, and dried. Yield: 85%. Mp: 233−235 °C. ^{1}H NMR (DMSO-d_6): δ 1.08 (m, 2H, $CH(CH_2)_2$), 1.20 (m, 2H, $CH(CH_2)_2$), 3.46 (m, 1H, $CH(CH_2)_2$), 8.07 (d, J = 9.3 Hz, 1H, 5-H), 8.29 (s, 1H, 3-H), 8.56 (d, J = 2.6 Hz, 1H, 8-H), 8.60 (dd, J = 9.3 Hz, 2.6 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 7.5 ($CH(CH_2)_2$), 32.8 ($CH(CH_2)_2$), 118.9 (C-5), 120.2 (C-8), 122.0 (C-8a), 127.8 (C-6), 141.7 (C-7), 144.6 (C-4a), 152.5 (C-3).

7-Amino-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (14). To a solution of **13** (5 g, 18.7 mmol) in ethanol (180 mL) was added 10% palladium on carbon (500 mg). The suspension was placed in a hydrogenator under H_2 (10 atm) for 30 min at room temperature. The suspension was concentrated to dryness under reduced pressure. The residue was taken up in boiling acetone (300 mL). The insoluble material was removed by filtration in the hot state and was washed with boiling acetone. The filtrate was concentrated to dryness, and the residue was recrystallized from methanol. Yield: 80%. Mp: 283−285 °C. ^{1}H NMR (DMSO-d_6): δ 0.95 (m, 1H, $CH(CH_2)_2$), 1.11 (m, 1H, $CH(CH_2)_2$), 3.28 (m, 1H, $CH(CH_2)_2$), 5.72 (s, 2H, NH_2), 6.95 (d, J = 2.5 Hz, 1H, 8-H), 6.98 (dd, J = 9 Hz, 2.6 Hz, 1H, 6-H), 7.54 (d, J = 9 Hz, 1H, 5-H), 7.92 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.0 ($CH(CH_2)_2$), 31.9 ($CH(CH_2)_2$), 105.3 (C-8), 117.9 (C-5), 119.1 (C-6), 123.6 (C-8a), 125.8 (C-4a), 147.9 (C-7), 149.4 (C-3).

7-Chloro-4-cyclopropyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (15). A solution of $CuSO_4{\cdot}5H_2O$ (84 g, 336 mmol) and NaCl (22.5 g, 385 mmol) in water (200 mL) was cooled on an ice bath, and an aqueous solution of $Na_2S_2O_5$ (22.5 g in 100 mL, 118 mmol) was added dropwise. After the resulting solution was stirring for 15 min, the precipitate of Cu_2Cl_2 was collected by filtration and washed with water. A solution of **14** (3.48 g, 14.7 mmol) in 6 N HCl (40 mL) was cooled on an ice bath, and then an aqueous solution of $NaNO_2$ (2 g in 15 mL, 29 mmol) was added dropwise. The resulting solution was added gradually to a solution of Cu_2Cl_2 (3 g, 15.2 mmol) in concentrated HCl (30 mL). After the resulting solution was stirred for 30 min at room temperature, water (150 mL) was added to the reaction mixture, and the resulting precipitate was collected by

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filtration, washed with water, and dried. Yield: 75%. Mp: 229−231 °C. ^{1}H NMR (DMSO-d_6): δ 1.02 (m, 2H, CH(CH_2)$_2$), 1.16 (m, 2H, CH(CH_2)$_2$), 3.38 (m, 1H, CH(CH$_2$)$_2$), 7.87−7.90 (m, 2H, 5-H/6-H), 7.94 (d, J = 7.8 Hz, 1H, 8-H), 8.17 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.3 (CH(CH_2)$_2$), 32.3 (CH(CH$_2$)$_2$), 119.4 (C-5), 123.3 (C-8), 123.4 (C-8a), 130.6 (C-7), 133.3 (C-6), 135.4 (C-4a), 151.6 (C-3).

General Synthetic Pathway to 5-Substituted 2-(Cyclopropylamino)benzenesulfonamides 17. The solution of the appropriate 5-substituted 2-fluorobenzenesulfonamide **16** (3 g, 12−17 mmol) in dioxane (30 mL) supplemented with cyclopropylamine (3 mL, 43.1 mmol) was heated at 100−110 °C in a hermetically closed vessel for 24−96 h. The solvent and the excess amine were removed by distillation under reduced pressure, and the residue was dissolved in methanol (20 mL). The methanolic solution was cooled on an ice bath, and water (60 mL) was added. The resulting precipitate of the title compound was collected by filtration, washed with water, and dried. It was used in the next step without further purification. Yield: 85−95%. The following compounds were obtained.

*Data for 2-(Cyclopropylamino)benzenesulfonamide (**17a**).* Heating time: 96 h. ^{1}H NMR (DMSO-d_6): δ 0.51 (m, 2H, CH(CH_2)$_2$), 0.79 (m, 2H, CH(CH_2)$_2$), 2.46 (m, 1H, CH(CH$_2$)$_2$), 6.11 (s, 1H, NH), 6.72 (t, J = 7.5 Hz, 1H, 5-H), 7.14 (d, J = 8.3 Hz, 1H, 3-H), 7.30 (s, 2H, SO$_2$NH$_2$), 7.40 (t, 1H, 4-H), 7.60 (dd, J = 7.8 Hz, 0.8 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 7.25 (CH(CH_2)$_2$), 24.5 (CH(CH$_2$)$_2$), 112.9 (C-3), 115.4 (C-5), 125.0 (C-1), 128.0 (C-6), 133.2 (C-4), 145.4 (C-2).

*Data for 2-(Cyclopropylamino)-5-fluorobenzenesulfonamide (**17b**).* Heating time: 96 h. ^{1}H NMR (DMSO-d_6): δ 0.50 (m, 2H, CH(CH_2)$_2$), 0.78 (m, 2H, CH(CH_2)$_2$), 2.45 (m, 1H, CH(CH$_2$)$_2$), 5.98 (s, 1H, NH), 7.14 (dd, J = 9.1 Hz, 4.6 Hz, 1H, 3-H), 7.33 (td, J = 8.6 Hz, 3.1 Hz, 1H, 4-H), 7.38 (dd, J = 8.9 Hz, 3.1 Hz, 1H, 6-H), 7.46 (br s, 2H, SO$_2$NH$_2$). ^{13}C NMR (DMSO-d_6): δ 7.3 (CH(CH_2)$_2$), 24.8 (CH(CH$_2$)$_2$), 114.1 (d, J = 25 Hz, C-6), 114.3 (d, J = 7 Hz, C-3), 120.2 (d, J = 22 Hz, C-4), 125.3 (d, J = 6 Hz, C-1), 142.3 (C-2), 152.7 (d, J = 234 Hz, C-5).

*Data for 5-Bromo-2-(cyclopropylamino)benzenesulfonamide (**17c**).* Heating time: 24 h. ^{1}H NMR (DMSO-d_6) d 0.51 (m, 2H, CH(CH_2)$_2$), 0.80 (m, 2H, CH(CH_2)$_2$), 2.47 (m, 1H, CH(CH$_2$)$_2$), 6.16 (s, 1H, NH), 7.11 (d, J = 8.9 Hz, 1H, 3-H), 7.50 (s, 2H, SO$_2$NH$_2$), 7.56 (dd, J = 8.9 Hz, 2.4 Hz, 1H, 4-H), 7.69 (d, J = 2.4 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 7.26 (CH(CH_2)$_2$), 24.6 (CH(CH$_2$)$_2$), 105.8 (C-5), 115.3 (C-3), 126.5 (C-1), 129.9 (C-6), 135.6 (C-4), 144.6 (C-2).

*Data for 2-(Cyclopropylamino)-5-methylbenzenesulfonamide (**17d**).* Heating time: 72 h. ^{1}H NMR (DMSO-d_6): δ 0.48 (m, 2H, CH(CH_2)$_2$), 0.77 (m, 2H, CH(CH_2)$_2$), 2.43 (m, 1H, CH(CH$_2$)$_2$), 5.97 (s, 1H, NH), 7.05 (d, J = 8.4 Hz, 1H, 3-H), 7.23 (dd, J = 8.4 Hz, 1.8 Hz, 1H, 4-H), 7.43 (d, J = 2 Hz, 1H, 6-H), 7.54 (br s, 2H, SO$_2$NH$_2$). ^{13}C NMR (DMSO-d_6): δ 7.2 (CH(CH_2)$_2$), 19.8 (CH$_3$), 24.6 (CH(CH$_2$)$_2$), 113.1 (C-3), 124.1 (C-1), 124.9 (C-5), 128.0 (C-6), 133.8 (C-4), 143.3 (C-2).

*Data for 2-(Cyclopropylamino)-5-(trifluoromethyl)-benzenesulfonamide (**17e**).* Heating time: 24 h. ^{1}H NMR (DMSO-d_6): δ 0.58 (m, 2H, CH(CH_2)$_2$), 0.86 (m, 2H, CH(CH_2)$_2$), 2.58 (m, 1H, CH(CH$_2$)$_2$), 6.53 (s, 1H, NH), 7.29 (d, J = 8.8 Hz, 1H, 3-H), 7.58 (s, 2H, SO$_2$NH$_2$), 7.75 (dd, J = 8.8 Hz, 1.9 Hz, 1H, 4-H), 7.88 (d, J = 1.7 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 7.3 (CH(CH_2)$_2$), 24.6 (CH(CH$_2$)$_2$), 113.4 (C-3), 115.3 (C-5), 123.4−125.6 (d, J = 270 Hz, CF$_3$), 124.7 (C-1), 125.4 (d, J = 4 Hz, C-6), 129.9 (d, J = 3 Hz, C-4), 148.0 (C-2).

*Data for 5-Cyano-2-(cyclopropylamino)benzenesulfonamide (**17f**).* Heating time 24 h. ^{1}H NMR (DMSO-d_6): δ 0.57 (m, 2H, CH(CH_2)$_2$), 0.86 (m, 2H, CH(CH_2)$_2$), 2.58 (m, 1H, CH(CH$_2$)$_2$), 6.68 (s, 1H, NH), 7.24 (d, J = 8.8 Hz, 1H, 3-H), 7.59 (br s, 2H, SO$_2$NH$_2$), 7.81 (dd, J = 8.8 Hz, 2 Hz, 1H, 4-H), 7.91 (d, J = 2 Hz, 1H, 6-H). ^{13}C NMR (DMSO-d_6): δ 7.3 (CH(CH_2)$_2$), 24.6 (CH(CH$_2$)$_2$), 96.5 (C-5), 113.7 (C-3), 119.1 (CN), 125.5 (C-1), 132.5 (C-6), 136.4 (C-4), 148.3 (C-2).

General Synthetic Pathway to 7-Substituted 4-Cyclopropyl-4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides 18. In a round-bottom flask, a mixture of the appropriate 5-substituted 2-(cyclopropylamino)-benzenesulfonamide **17** (2.5 g, 8.6−11.8 mmol) and ethyl orthoformate (25 mL) was heated in the open state at 130−150 °C for 1−24 h. The resulting suspension was cooled on an ice bath, and the insoluble material was collected by filtration, washed with ether, and dried. The solid was redissolved in a hot mixture of acetone and methanol, and the hot solution was treated with charcoal and filtered. The filtrate was concentrated to dryness, and the residue was recrystallized from methanol. Yield: 70−75%. The following compounds were obtained.

*Data for 4-Cyclopropyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**18a**).* White solid. Mp: 191−193.5 °C. ^{1}H NMR (DMSO-d_6): δ 1.01 (m, 2H, CH(CH_2)$_2$), 1.17 (m, 2H, CH(CH_2)$_2$), 3.38 (m, 1H, CH(CH$_2$)$_2$), 7.57 (m, 1H, 7-H), 7.81−7.85 (m, 2H, 5-H/6-H), 7.88 (d, J = 7.8 Hz, 1H, 8-H), 8.15 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.3 (CH(CH_2)$_2$), 32.1 (CH(CH$_2$)$_2$), 116.8 (C-5), 122.3 (C-8a), 124.1 (C-7), 126.8 (C-8), 133.2 (C-6), 136.5 (C-4a), 151.5 (C-3).

*Data for 4-Cyclopropyl-7-fluoro-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**18b**).* White solid. Mp: 170−172 °C. ^{1}H NMR (DMSO-d_6): δ 1.02 (m, 2H, CH(CH_2)$_2$), 1.16 (m, 2H, CH(CH_2)$_2$), 3.39 (m, 1H, CH(CH$_2$)$_2$), 7.73 (m, 1H, 6-H), 7.79 (dd, J = 7.4 Hz, 2.9 Hz, 1H, 8-H), 7.92 (dd, J = 9.3 Hz, 4.4 Hz, 1H, 5-H), 8.14 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.3 (CH(CH_2)$_2$), 32.3 (CH(CH$_2$)$_2$), 110.2 (d, J = 25 Hz, C-8), 120.0 (C-5), 121.1 (d, J = 23 Hz, C-6), 123.3 (C-8a), 133.3 (C-4a), 151.3 (C-3), 158.4−160.4 (d, J = 248 Hz, C-7).

*Data for 7-Bromo-4-cyclopropyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**18c**).* White solid. Mp: 242−244 °C. ^{1}H NMR (DMSO-d_6): δ 1.02 (m, 2H, CH(CH_2)$_2$), 1.16 (m, 2H, CH(CH_2)$_2$), 3.37 (m, 1H, CH(CH$_2$)$_2$), 7.80 (d, J = 8.8 Hz, 1H, 5-H), 8.00−8.02 (m, 2H, 6-H/8-H), 8.18 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.25 (CH(CH_2)$_2$), 32.23 (CH(CH$_2$)$_2$), 118.2 (C-7), 119.6 (C-5), 123.6 (C-8a), 126.1 (C-8), 135.8 (C-4a), 136.1 (C-6), 151.6 (C-3).

*Data for 4-Cyclopropyl-7-methyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**18d**).* White solid. Mp: 192−195 °C. ^{1}H NMR (DMSO-d_6): δ 0.99 (m, 2H, CH(CH_2)$_2$), 1.15 (m, 2H, CH(CH_2)$_2$), 2.42 (s, 3H, CH$_3$), 3.35 (m, 1H, CH(CH$_2$)$_2$), 7.63 (dd, J = 8.7 Hz, 1.7 Hz, 1H, 6-H), 7.69 (d, J = 2 Hz, 1H, 8-H), 7.73 (d, J = 8.6 Hz, 1H, 5-H), 8.10 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.2 (CH(CH_2)$_2$), 20.2 (CH$_3$), 32.0 (CH(CH$_2$)$_2$), 116.7 (C-5), 122.2 (C-8a), 123.4 (C-8), 134.0 (C-6), 134.2 (C-7), 136.8 (C-4a), 151.1 (C-3).

*Data for 4-Cyclopropyl-7-(trifluoromethyl)-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**18e**).* White solid. Mp: 160−162 °C. ^{1}H NMR (DMSO-d_6): δ 1.06 (m, 2H, CH(CH_2)$_2$), 1.18 (m, 2H, CH(CH_2)$_2$), 3.43 (m, 1H, CH(CH$_2$)$_2$), 8.04 (d, J = 9.4 Hz, 1H, 5-H), 8.19 (m, 2H, 6-H/8-H), 8.25 (s, 1H, 3-H). ^{13}C NMR (DMSO-d_6): δ 7.4 (CH(CH_2)$_2$), 32.5 (CH(CH$_2$)$_2$), 118.5 (C-5), 121.7 (d, J = 4 Hz, C-8), 122.1−124.3 (d, J = 272 Hz, CF$_3$), 122.4 (C-8a), 126.7 (d, J = 33 Hz, C-7), 129.8 (d, J = 3 Hz, C-6), 139.6 (C-4a), 152.3 (C-3).

*Data for 7-Cyano-4-cyclopropyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (**18f**).* White solid. Mp: 268−270 °C. ^{1}H NMR (DMSO-d_6): δ 1.04 (m, 2H, CH(CH_2)$_2$), 1.18 (m, 2H, CH(CH_2)$_2$), 3.40 (m, 1H, CH(CH$_2$)$_2$), 7.98 (d, J = 8.9 Hz, 1H, 5-H), 8.23 (s, 1H, 3-H), 8.24 (dd, J = 8.9 Hz, 1.8 Hz, 1H, 6-H), 8.50 (d, J = 1.9 Hz, 1H, 8-H). ^{13}C NMR (DMSO-d_6): δ 7.4 (CH(CH_2)$_2$), 32.5 (CH(CH$_2$)$_2$), 109.1 (C-7), 117.2 (CN), 118.3 (C-5), 122.7 (C-8a), 129.4 (C-8), 136.3 (C-6), 139.8 (C-4a), 152.3 (C-3).

Effect on AMPA-Evoked Membrane Depolarization. This assay consisted of investigating AMPA-evoked membrane depolarization, measured by fluorescent membrane potential dyes and an imaging based plate reader (FDSS, Hamamatsu, JP), on rat primary brain cultures.[12] Dissociated rat primary brain cells were prepared from embryonic rat (E16) and were added to poly-D-lysine-coated 96-well culture plates for 18 days at 37 °C and 5% CO_2 (density 20 000 cells/well). On the day of the experiment, ground medium was removed from the cells and was replaced by 20 μL/well of membrane potential dye loading solution (Molecular Devices), reconstituted according to the manufacturer's instructions. The plates were incubated for 1 h at room temperature and then directly transferred to the fluorescence imaging based plate reader. Baseline fluorescence was monitored for 10 s followed by the addition of AMPA (300 μM)

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over 3 min and then the compound in the presence of AMPA over 3 min. Subsequent monitoring of fluorescence changes was performed during these two periods of 3 min. Responses were averaged over the last 15 s of each 3 min recording period. AMPA concentration−response curves were calculated in the absence or presence of different concentrations of the compound. The results are expressed as the area under the curve of the AMPA-mediated concentration−response effect in the absence or presence of the compound. $EC_{2\times}$ corresponds to the concentration of compound that evoked a 2-fold increase of all AMPA-mediated responses. EC_{50} corresponds to the concentration of compound that evoked 50% of the maximal effect of the compound.

Protein Expression and Purification. GluA2-LBD-L483Y-N754S was expressed and purified as previously described.[13] In brief, *Escherichia coli* Origami B (DE3) cells were transformed with the GluA2-LBD-L483Y-N754S pET-22b(+) plasmid. The cells were grown to an OD_{600} of 0.9 in LB medium and cooled to 20 °C. Protein expression was induced by addition of 0.5 mM isopropyl β-D-thiogalactopyranoside. After 18 h, the cells were harvested and lysed, and the protein was purified by Ni^{2+}-affinity chromatography. The *N*-terminal His tag was removed by trypsin digestion, and the cleaved protein was purified by anion-exchange chromatography and size-exclusion chromatography.

Crystallization and X-ray Structure Determination. The protein solution consisted of 6 mg/mL GluA2-LBD-L483Y-N754S in 10 mM HEPES, 20 mM NaCl, 1 mM EDTA, and 1 mM (*S*)-glutamate, pH 7.0. Solid compound **3** (0.5−1 mg) was added to 100 μL of protein solution and incubated at 4 °C for at least 24 h prior to the setup of the crystallization experiments.

The hanging drop vapor diffusion method was used with drops consisting of 1 μL of protein solution and 1 μL of reservoir solution. The crystallization conditions were 16.5% PEG4000, 0.15 M zinc acetate, and 0.1 M cacodylate, pH 6.5. The crystals were briefly soaked in cryoprotectant (reservoir solution with 20% glycerol) and flash-cooled in liquid nitrogen.

Diffraction data were collected at MAX-Lab, Lund, Sweden, on beamline I911-3. The data were processed using XDS[20] and SCALA within CCP4.[21]

The structure was solved by molecular replacement using Phaser[22] with the structure of GluA2-LBD-L483Y (PDB code 1LB8, molecule A) as search model. ARP/wARP[23] was used for initial automatic model building, and for manual model building the program Coot[24] was used. Topology and parameter files for **3** were obtained using PRODRG[25] and eLBOW.[26] Refinements were performed using PHENIX.[27] Domain closure was calculated using DynDom.[28] Figures 3 and 4 were prepared using PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.5, Schrödinger, LLC).

Isothermal Titration Calorimetry. ITC was performed at 25 °C on an ITC200 microcalorimeter (GE Healthcare) with a cell volume of 200 μL. The protein solution consisted of 17 μM GluA2-LBD-L483Y-N754S in 100 mM HEPES, 100 mM NaCl, 2 mM KCl, and 5 mM (*S*)-glutamate, pH 7.0. The protein concentration was determined by UV absorption. Compound **3** was dissolved in the same buffer as for the protein solution, and a concentration of 243 μM was used for titration. The experiments were performed as 20 injections with 3 min intervals, using 0.5 μL in the first injection and 2 μL in the following injections. Data analysis was performed with the Origin 7.0 software (MicroCal) using a single binding site model.[29] The baseline was manually corrected, and the first data point was discarded. The titration was repeated three times, and the reported ΔH, $-T\Delta S$, and K_d are mean values.

■ ASSOCIATED CONTENT

Accession Codes

The structure coordinates and corresponding structure factor file of GluA2-LBD-L483Y-N754S with **3** have been deposited in the Protein Data Bank under the accession code 4N07.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADHD, attention deficit hyperactivity disorder; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNS, central nervous system; iGluRs, ionotropic glutamate receptors; ITC, isothermal titration calorimetry; LBD, ligand-binding domain; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartic acid

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