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5′-Alkyl-benzothiadiazides: A New Subgroup of AMPA Receptor Modulators with Improved Affinity

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Abstract—AMPA receptors form a major subdivision of the glutamate receptor family that mediates excitatory synaptic transmission in the brain. Currents through AMPA receptors can be up- or down-regulated by compounds that allosterically modulate receptor kinetics through binding sites distinct from that for glutamate. One of those modulators is the benzothiadiazide IDRA-21 which has been reported to enhance synaptic transmission and be effective in behavioral tests, but typically requires threshold concentrations of at least 100 μM to be active in vitro. In this study, new benzothiadiazides were developed with IDRA-21 as lead compound and examined for their potency in modulating AMPA receptor kinetics. A significant increase in drug affinity was obtained by alkyl substitution at the 5′-position of IDRA-21; substitutions at other positions of the benzothiadiazide core generally did not yield a further gain in affinity and in some cases abolished drug binding. The 5′-ethyl derivative exhibited an EC_{50} value in the order of 22 μM which represents about a 30-fold gain in affinity over that of IDRA-21. The EC_{50} value is comparable to that of cyclothiazide, the most potent benzothiadiazide drug, but the effects on AMPA receptors differed substantially between these two compounds in that the 5′-ethyl derivative of IDRA-21 greatly increased the binding affinity for receptor agonists whereas cyclothiazide is known to reduce agonist binding. The structure–activity relationships reported here thus offer to provide new insights how receptor kinetics is linked to particular aspects of receptor–drug interactions.

Introduction

L-Glutamate is a ubiquitous excitatory neurotransmitter in the mammalian central nervous system, playing a key role in synaptic transmission and in plasticity processes vital to early neuronal development and encoding of memory. Receptor-selective agonists and antagonists have been instrumental to characterize the main subclasses of glutamate receptors [i.e., *N*-methyl-D-aspartate (NMDA), kainic acid, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and metabotropic receptors].[1] Selective inactivation or activation of these receptors have also been targeted for intervention in various neurological disorders.[2] A convincing case has been made that excessive glutamate receptor activation occurs during stroke and similar insults, mainly due to uncontrolled release of glutamate, and that this subsequently leads to neuronal degeneration.[3] Major efforts are accordingly being made to develop receptor antagonists which afford protection during those critical periods.[3,4]

Insufficient activation of glutamate receptors may be of similar clinical importance, but is more likely associated with chronic disorders. One example may be the progressive loss of synapses or neurons in age and age-related disorders.[5,6] Likewise, schizophrenia according to recent insights may involve subnormal excitatory activity in critical frontal regions of the brain.[7] Lower than normal excitatory drive would probably not cause cellular dysfunction but would compromise the integrative functions of the brain which depend on a balanced activity of its component parts. An obvious approach to correct the deficit would be to strengthen the remaining functional connections. Compounds that enhance glutamate receptor currents through allosteric

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modulation seem particularly suitable for this purpose because they would be expected to increase the gain of synaptic transmission without disrupting the spatiotemporal patterning of the information flow. The feasibility of this approach has been documented in behavioral studies which showed that up-modulators of AMPA receptors may alleviate memory deficits in aged rodents and humans[8,9] and that they potentiate the effects of antipsychotics in an experimental schizophrenia model.[10]

The first such modulator was discovered by Ito et al.[11] who showed that aniracetam selectively enhances currents through AMPA receptors by binding to a site distinct from that for glutamate. This was followed by the discovery of other compounds that allosterically alter AMPA receptor function[12–19] and by efforts to characterize the physiological and behavioral consequences of such modulation.[20–22] Among the first of these compounds were diazoxide and cyclothiazide, both of which are benzothiadiazides and hence structurally distinct from aniracetam (Fig. 1). Both have been in clinical use for peripheral actions unrelated to AMPA receptors. Diazoxide was found to stimulate AMPA receptors at concentrations (100–500 μM) similar to those needed for its known action on certain potassium channels.[12] Cyclothiazide is still one of the most potent modulators in that it effectively blocks AMPA receptor desensitization in excised-patch experiments at 10–30 μM;[13] however, it produces small effects on synaptic responses, presumably because desensitization is too slow to affect receptor deactivation during the very brief synaptic glutamate transients.[23–25] Cyclothiazide is markedly less effective in this regard than other types of modulators, including aniracetam and benzoylpiperidine compounds,[16–18,24] which probably act more prominently on channel closing and agonist dissociation than on receptor desensitization. The utility of cyclothiazide as a probe to study the behavioral consequences of AMPA receptor modulation may also be limited because of its clinically important action on renal function, a limited diffusion across the blood–brain barrier, and the fact that cyclothiazide consists of eight stereoisomers, only some of which are active.[26]

Various investigators have therefore sought new benzothiadiazides that are potent yet capable of enhancing glutamatergic synaptic transmission. Yamada and Tang[13] tested clinically active analogues of cyclothiazide and concluded that the 3′-norbornenyl substituent is essential for AMPA receptor affinity. Bertolino et al.[27] examined instead a series of analogues of diazoxide and discovered that the compound IDRA-21 is about 3 times more potent, with the added benefit of reduced peripheral activity. Minor variants of IDRA-21 were included in their tests but proved less effective. IDRA-21 was found to be superior to cyclothiazide in enhancing synaptic responses in hippocampal slices[28] and it produced desirable effects in behavioral tasks.[22] However, its EC_{50} remains far below that of cyclothiazide and concentrations of 200–500 μM are typically needed in physiological experiments.[28]

The present study used IDRA-21 as a lead compound because of its proven efficacy in enhancing synaptic responses. In this two-stage study, previously unexplored substitutions were made to the benzothiadiazide core structure, in particular on the aromatic ring, in hopes of improving the affinity of these compounds. In the first stage, the most promising among 64 compounds was identified using an indexed combinatorial library approach. Analogues of this compound were then synthesized in the second stage to establish more comprehensive structure–activity relationships. Drugs

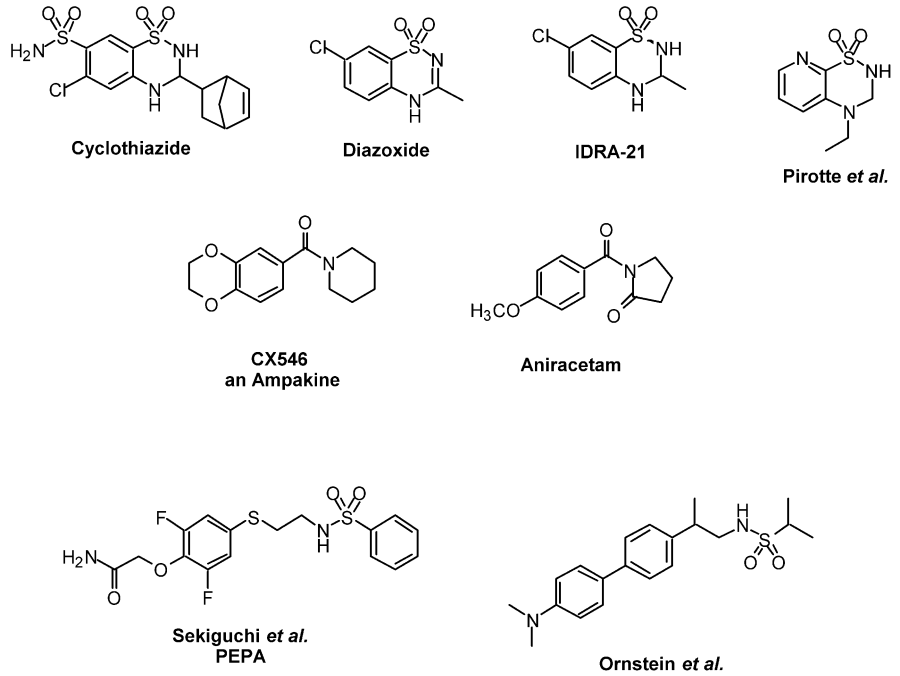
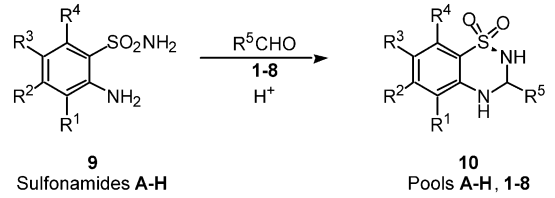


Figure 1. Structures of known AMPA receptor modulators. Depicted are eight known AMPA receptor modulators.

were characterized by measuring their effect on AMPA receptor currents in excised-patches and on the binding of radiolabeled agonists. The compounds discovered in this study showed greatly improved affinity and distinct effects on AMPA receptor kinetics that set them apart from earlier benzothiazides. While this study was in progress, Pirotte et al.[29] showed that certain pyridothiazine analogues of IDRA-21 exhibit similar gains in AMPA receptor affinity.



Scheme 1.

Results

Background on the indexed library approach

An indexed combinatorial library approach was employed in order to expedite the discovery of new, more potent IDRA-21 analogues.[30,31] Pirrung et al. first described this unique combinatorial approach for the identification of acetylcholinesterase inhibitors, wherein the compounds of the library are represented as a two-dimensional matrix where each matrix element is the resultant compound derived from the building blocks envisioned on the *x*- and *y*-axes, respectively. For this study, eight aldehydes (**1**–**8**) and eight sulfonamides (**A**–**H**) were used to conceptualize an 8 × 8 grid, where each matrix element represents the benzothiadiazide produced from the reaction between a sulfonamide (**A**–**H**, the *x*-axis) and an aldehyde (**1**–**8**, the *y*-axis). Rather than preparing all members of the library individually (64 in all in this study) and assaying them individually, the indexed approach allows one to identify new 'active hits' through preparing and assaying compound pools. Here 16 pools (i.e., mixtures), containing eight new benzothiazide compounds in each pool, were to be prepared from combinations of the eight aldehydes organized along the *y*-axis (**1**–**8**) and the eight sulfonamides organized along the *x*-axis (**A**–**H**). Following the preparation of the compound pools, neurobiological assays would be employed to determine whether a certain pool contained any active hits. Each member of the library is therefore tested twice, once each as a component of a pool from the *x*-axis and from the *y*-axis. Thus, if the library does contain hits, their structures would be identified at the intersection of an active pool from the *x*-axis and an active pool from the *y*-axis. A hypothetical example shows the intersection of an active pool produced from the sulfonamide **C** and an active pool produced from the aldehyde **5**; the structure of the hit would be postulated to be the individual compound produced by these two building blocks (Fig. 2). Resynthesis and testing of this particular would confirm the assumption. As a general rule, for this approach to be successful, the reactions need to be general, high yielding, and very clean. If these criteria are not met, then impurities in the reaction pools could make the assay results inconclusive (Scheme 1, Table 1).

Library synthesis

The individual sulfonamides **A**–**H** were chosen based on their availability from commercial starting materials and their relation to the corresponding substructures of cyclothiazide and IDRA-21. The resultant benzothiadiazides would resemble cyclothiazide or IDRA-21, and make it possible to examine the effects of various other functional groups, namely bromide, chloride, nitro, sulfonamide, and alkyl at various positions on the aromatic ring. The commercially available aldehydes, **1**–**8**, were selected in order to explore the tolerance for various functional groups at the 3′-position. Aldehydes **1**–**3** yield IDRA-like compounds with different size alkyl substituents; aldehydes **4**–**8** yield larger substituents that are similar in size to the 3′-norbornenyl moiety of cyclothiazide.

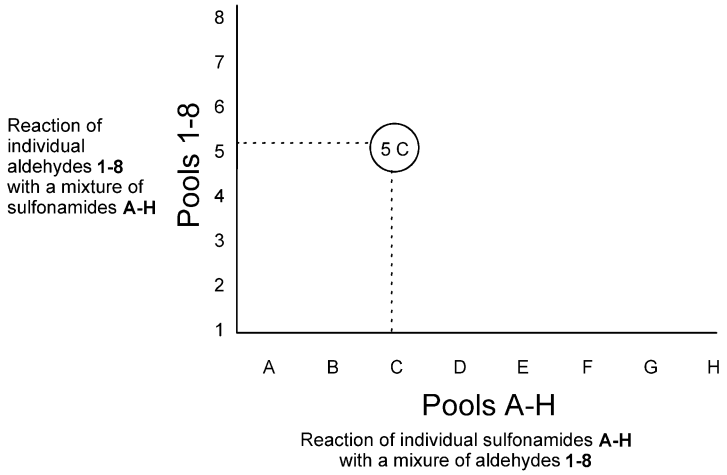
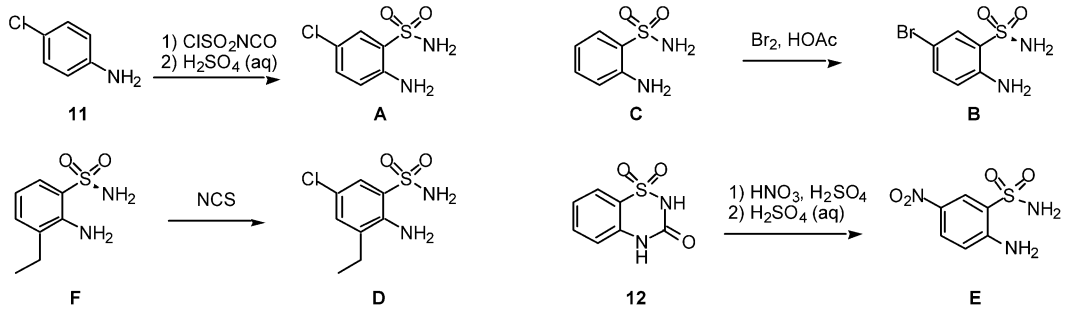


Figure 2. Hypothetical indexed combinatorial library depicted as a two-dimensional matrix. The figure depicts the hypothetical result (5C) from a completed indexed library search. As stated in the text, the most active compound(s) in a library are identified at the vertex of two pools of compounds that share activity. Chemical synthesis of the individual compound should confirm the results from the library search.



Scheme 2.

The sulfonamides were synthesized according to known literature procedures (Scheme 2).[32] Compounds **A** and **F** were synthesized from *p*-chloroaniline (**11**) and *o*-ethylaniline, respectively, via treatment with chlorosulfonylisocyanate (CSI) followed by acid hydrolysis. Commercially available 2-aminobenzenesulfonamide (**C**) was treated with bromine in acetic acid to give the brominated product **B**. Sulfonamide **F** was treated with *N*-chlorosuccinimide in refluxing acetonitrile to give **D**. Compound **12** (the initial adduct of aniline and CSI) was treated with nitric acid in refluxing sulfuric acid and then hydrolyzed in 50% sulfuric acid to give **E**.

The compound pools were prepared in order to expedite the identification of new potential lead structures by the indexed approach discussed earlier. Pools **A**–**H** were prepared by treating individual sulfonamides with a solution containing all eight aldehydes in equimolar concentration to produce the pools of compounds that make up the vertical columns. Pools **1**–**8** were prepared by treating individual aldehydes with solutions equimolar in all sulfonamides to produce the pools of compounds that make up the horizontal rows.

Biological testing of the indexed library

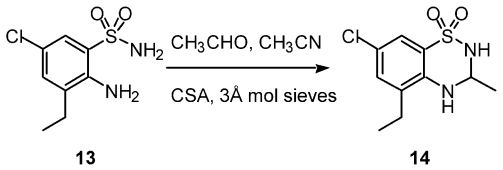
The various pools were prepared as discussed previously and tested as mixtures of eight compounds. In order to ensure that the reactions were complete, TLC and ^{1}H NMR analyses were performed on each pool. Since AMPA receptor modulators differ considerably in the way they alter receptor kinetics,[17,18] multiple test criteria were used to characterize the pools. We specifically tested the effects on: (i) peak and steady-state currents and rate of desensitization after step application of glutamate to excised patches from rat hippocampal pyramidal cells; (ii) response deactivation after a 1-ms glutamate pulse to the excised patches; (iii) field excitatory post-synaptic potentials (EPSP's) in CA1 of hippocampal slices; (iv) binding of [^{3}H]AMPA to membranes obtained from rat brain.

Excitatory neurotransmitter ([^{3}H]AMPA) binding was enhanced by less than 10% by all pools (125 μM maximal concentration of each element) except **D**, **H**, **1** and **4** (Fig. 3 A). The matrix component **H4** is similar to cyclothiazide with a cyclohexyl ring substituting for the norbornenyl group present in the latter; it is shown elsewhere that this analogue is equipotent to cyclothiazide.[33] The latter reduces [^{3}H]AMPA binding by about half at the concentration used here[34] and thus the effects of pools **H** and **4** can be explained by the presence of the cyclothiazide analogue **H4**. The large increase in [^{3}H]AMPA binding by pools **D** and **1** was of greater interest because no other compound pools had caused a binding increase under the assay conditions employed. The fact that both pools produced an equal size increase suggested that the effect might be produced by a single component, the matrix element leading to **D1**.

Pools **D** and **1** also showed superior efficacy in most other tests (Fig. 3B). Pool **D** prolonged deactivation of fast responses to glutamate more than 4-fold (500 μM), the only other pool among **A**–**H** that produced some effect being **F** which has a 5-ethyl group in common with **D**. Pool **1** increased deactivation 2.6-fold; this effect decreased as the size of the substituent on the heterocyclic ring increased and disappeared with aromatic substitutions. Pools **D**, **F**, and **1** also effectively reduced receptor desensitization (not shown), along with pools **H** and **4**, which contain the cyclothiazide analogue. Effects on synaptic receptors were assessed for pools **A**–**H** (250 μM) from the change in the half-width of the field EPSP, which is a more reliable indicator of AMPA receptor modulation than response amplitude. The response width was increased by about

Table 1. Library components for indexed combinatorial library

Sulfonamide	R^1	R^2	R^3	R^4	Aldehyde	R^5
A	H	H	Cl	H	**1**	CH_3
B	H	H	Br	H	**2**	$CH(CH_3)_2$
C	H	H	H	H	**3**	$CHCHCH_3$
D	CH_2CH_3	H	Cl	H	**4**	$CH(CH_2)_5$
E	H	H	NO_2	H	**5**	C_6H_5
F	CH_2CH_3	H	H	H	**6**	3-Pyrido
P	H	Cl	H	H	**7**	4-$CH_3OC_6H_4$
H	H	Cl	SO_2NH_2	H	**8**	2,5-$(CH_3O)_2C_6H_3$



Scheme 3.

50% by group **D** and by less than 15% by the other pools (not shown); the relative lack of effect of **H** and **A** was expected given that cyclothiazide produces only small changes in synaptic responses and IDRA-21 (matrix element **A1**) needs concentrations of at least 500 μM to be effective.[35]

The indexed library approach led to the identification of a new compound inferred to be **D1** that appeared to have greatly improved affinity for AMPA receptors compared to IDRA-21 and yet differed from cyclothiazide in important aspects. Individual synthesis of this compound (Scheme 3) and biological testing confirmed this result. Compound **D1** enhances [^{3}H]AMPA binding with an EC_{50} of 22 μM (Fig. 4), which is similar in magnitude to that of cyclothiazide (31 μM),[34] but it increases binding instead of decreasing it; a more detailed analysis of the effects of **D1** on physiology and binding will be given elsewhere. Since the effect of an ethyl substituent at the 5′-position gave such pronounced effects, examination of additional analogues with similar substitution patterns was performed in the second phase of this project.

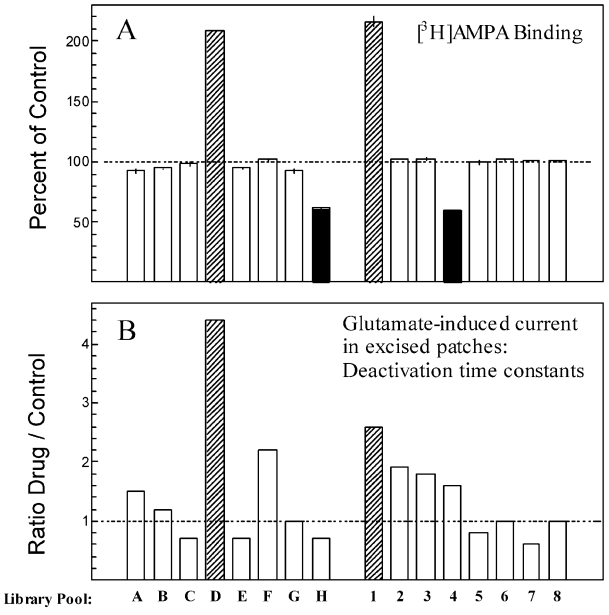


Figure 3. Effects of library pools on the binding of [^{3}H]AMPA (A) and on deactivation rate constants of patch responses (B). Aqueous solutions of pools **A**–**H** and **1**–**8** were prepared by dissolving the reaction product in DMSO (dimethylsulfoxide) to a concentration of 100 or 400 mM and then diluting these solutions 100-fold into the assay buffer to give nominal concentrations of approximately 1 or 4 mM for the total of all eight compounds, or 125 or 500 jiM for each individual component of the pool. Precipitates in the final dilutions were removed through 0.25 μm filters; no efforts were made to estimate actual concentrations and thus the values given represent upper limits. (A) Specific binding of [^{3}HJAMPA to brain membranes was measured at 25 °C in the presence of 50 mM KSCN and either 1% DMSO (control) or one of the pool mixtures containing up to 125 μM of each component. Binding in the presence of the compounds was expressed as a percentage of control binding (means and SEM of triplicate determinations). (B) Pulses of 10 mM L-glutamate were applied for one millisecond to excised patches in the absence and in the presence of a pool mixture at a nominal concentration of 500 μM per compound. For each condition, a single exponential was fitted to the decay phase of the response. The time constants of these exponentials ('deactivation time constants') were then used to calculate the ratio 'with' versus 'without' compound plotted on the *y*-axis.

Synthesis of D1 analogues and SAR

In the previous stage of this investigation, it was discovered that a simple alkyl substituent (ethyl) at the 5′-position of IDRA-21 led to enhanced binding and activity. Since this previously untested substitution had such a pronounced effect, a structure–activity study was conducted by synthesizing and testing analogues of **D1**. Three families of second-generation analogues were examined: (1) halide and alkyl substitutions at various positions on the aromatic ring, (2) alkyl substitutions at the 3′-position, and (3) alkyl substitutions on the 2′- and 4′-nitrogens. Screening new compounds primarily relied on binding assays to determine affinity; if a compound were reasonably potent, the more time-consuming patch current measurements (electrophysiology) were performed to verify physiological efficacy. By performing these substitutions on the core structure of the benzothiadiazide, a clearer understanding of the structure–activity relationships (SARs) for these compounds was developed.

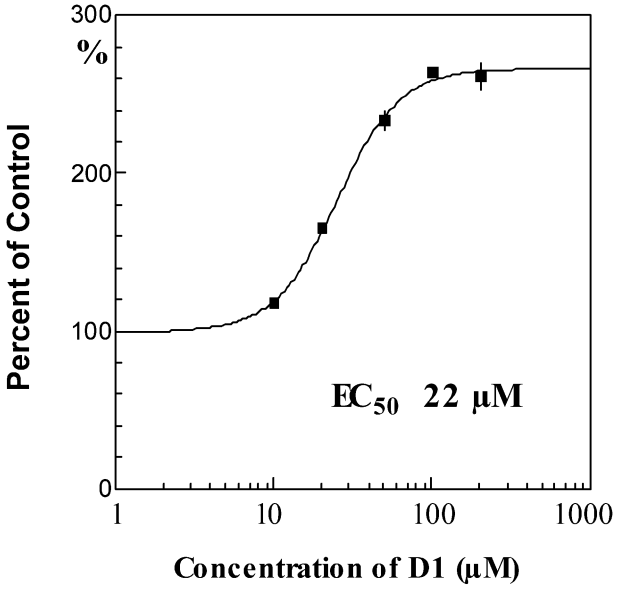
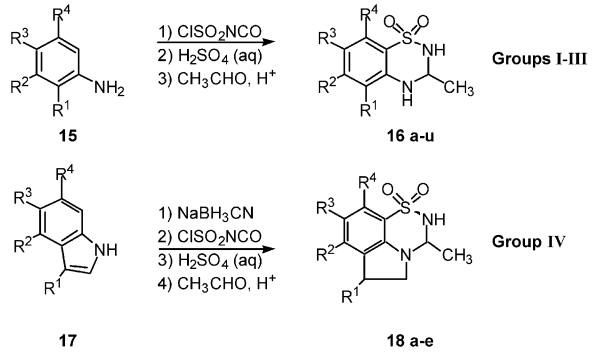


Figure 4. Effect of Dl on [^{3}H]AMPA binding. Binding of 50 nM [^{3}H]AMPA to rat brain membranes (25 °C, with 50 mM KSCN) was measured in the presence of the Dl concentrations indicated on the *x*-axis and expressed as a percentage of the binding in the absence of compound. A four-point logistic equation was fitted through to the data points through nonlinear regression. Average values (mean and SEM) from 11 separate experiments are: EC_{30} 26.6 ± 1.8 μM: maximal binding: 269 ± 6% of control; Hill coefficient: 2.2 ± 0.1.



Scheme 4.

Groups I–IV

The first goal of this second stage was to gain a better understanding of the role the halide and alkyl substituents play in the binding and activity of IDRA-21 and **D1**. Since results from prior studies,[13] and from the indexed library approach, suggested that longer alkyl chain and aromatic substitutions at the 3′-position lead to a loss in activity, only compounds with a methyl substituent at this position were synthesized. The analogues were organized into groups based on their similar aromatic substitution patterns: (1) Group I analogues have an alkyl substituent at the 5′-position and either a hydrogen or a halide at the 7′-position; (2) Group II analogues have a methyl at the 5′-position and contain either a methyl or a chloride or both at various positions; (3) Group III analogues have either a halide, methoxide, or hydrogen at the 5′-position and various other substituents at the 7′-position; and (4) Group IV analogues are tricyclic analogues derived from indolines and contain either a hydrogen or a halide at the 7′-position. All members of Groups I–III (**16a**–**u**) were prepared from commercially available anilines by treating the aniline with chlorosulfonyl isocyanate (CSI), followed by acid hydrolysis[32] and condensation with acetaldehyde (Scheme 4). Compounds **18a**–**e** (Group IV) were synthesized similarly from their requisite indolines.

Biological testing of Groups I–IV

The agonist [^{3}H]-fluorowillardine was used in binding tests because it allows binding to be measured without non-physiological additives; EC_{50} values obtained with this ligand are slightly lower than those from tests using [^{3}H]AMPA (Table 2). Table 2 shows the EC_{50} and the amount of binding at saturating compound concentration, expressed as a percentage of that without compound. If it was not possible to establish a reliable concentration–effect relation by fitting a four-point logistic equation, the percent of control binding at a single concentration, usually near the solubility limit, is given.

Group I

Group I compounds bear alkyl substituents at R^1 of varying lengths, in combination with chloride, fluoride and hydrogen substituents at R^3. It is evident that binding is affected by the substituent at the R^1 position.

Table 2. Structures and effects of Groups I–IV

Group	Compd	R^1	R^2	R^3	R^4	EC_{50}[c] (μM)	Max (%)	Concn (μM)	% effect
	D1[c]	CH_3CH_2	H	Cl	H	17	315		
I	**16a**	CH_3CH_2	H	F	H	29	290		
	16b	CH_3CH_2	H	H	H	101	265		
	16c	$CH(CH_3)_2$	H	Cl	H	34	405		
	16d	$CH(CH_3)_2$	H	H	H	>200			
	16e	$CH_3CH_2CH_2$	H	Cl	H	170	420		
	16f	$CH_3CH_2CH_2$	H	H	H			200	N.E.
	16g	CH_3	H	Cl	H	96	190		
	16h	CH_3	H	F	H	90	150		
II	**16i**	CH_3	H	CH_3	H	115	179		
	16j	CH_3	CH_3	Cl	H			50[a]	94
	16k	CH_3	CH_3	H	H			200	N.E.
	16l	CH_3	Cl	H	H			75[a]	N.E.
	16m	CH_3	H	H	Cl			80[a]	N.E.
III	**16n**	F	H	H	H			500	N.E.
	16o	F	H	Cl	H			400	N.E.
	16p	Cl	H	CH_3	H	141	137		
	16q	Cl	H	CH_3CH_2	H			120	N.E.
	16r	OCH_3	H	H	H			400	114
	16s	OCH_3	H	OCH_3	H			400	120
	16t	H	H	CH_3CH_2	H			200	N.E.
	16u	H	H	F	H			1000[b]	68
IV	**18a**	H	H	H	H	247	203		
	18b	H	H	F	H	76	254		
	18c	H	H	Cl	H	85	184		
	18d	CH_3	H	H	H	67	223		
	18e	CH_3	H	Cl	H	18	320		

[a]Solubility limit.
[b]Assay with [^{3}H]AMPA as radioligand.
[c]Binding parameters are from typical experiments and were replicated within a factor of 1.3 in at least one additional experiment. The EC_{50} for **D1** is the mean value from nine experiments.
N.E., no effect.

Methyl substituted compounds (**16g** and **16h**) allowed binding of the agonist to increase to 150–200%, ethyl substituted compounds (**D1** and **16a,b**) to 265–315%, and propyl/isopropyl substituted compounds (**16c–16f**) to about 400% of control. The highest binding affinity was obtained with **D1** [17.4 ± 1.2 μM (SEM), $n = 9$] and its fluorinated analogue **16a** (29 μM), with the isopropyl bearing analogue **16c** being slightly lower (34 μM). Interestingly, when the ethyl substituent of **D1** was replaced with a propyl substituent, the affinity declined precipitously (**16e**, 170 μM). When the 5′-ethyl of **D1** was replaced with a methyl group (**16g**), binding affinity was reduced about 5-fold (97 ± 7 μM, $n = 3$). This compound group also shows that a halide substituent at R^3 confers a 5- to 10-fold gain in binding affinity and that F and Cl are similarly effective.

Excised patch experiments and EPSP measurements in hippocampal slices confirmed that the length of the 5′-alkyl group plays a substantial role in the activity of these compounds (Table 5). These latter tests also indicated, however, that methyl and ethyl substituents had different effects on amplitude and duration of EPSPs and the 5′-methyl compounds (**16g** and **16h**) may provide certain advantages over longer chain analogues despite the lower affinity. For this reason, most of the compounds hereafter bear the 5′-methyl group and hence the affinities should be compared with those of compounds **16g** or **16h**.

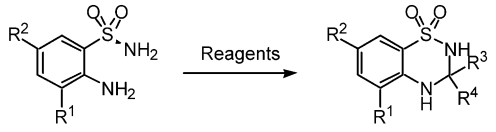
Group II

Group II analogues indicate that replacement of the 7-halide with a methyl leads to a loss in affinity. Halides or methyl groups at R^2 and R^4 have even lower activity than their R^3-substituted counterparts. Compound **16j** (which bears an additional methyl group at R^2) appears to have reduced affinity but the extent of that change could not be assessed due to poor solubility.

Group III

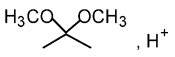
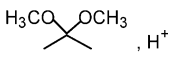
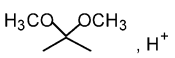
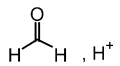
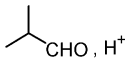
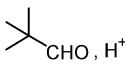
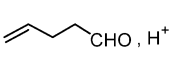
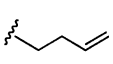
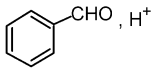
Compounds with a fluoro- (**16n**, **16o**) or methoxy- (**16r**, **16s**) substituent at R^1 instead of an alkyl group have

Table 3. Group V compounds



19a $R^1 = CH_3CH_2$, $R^2 = H$
19b $R^1 = CH_3CH_2$, $R^2 = Cl$
19c $R^1 = CH_3$, $R^2 = F$

20 a-i

Compd	R^1	R^2	Reagents	R^3	R^4	EC_{50} (μM)	Max (%)	Concn (μM)	% effect
20a	CH_3CH_2	H		CH_3	CH_3			200	N.E.
20b	CH_3CH_2	Cl		CH_3	CH_3	>400			
20c	CH_3	F		CH_3	CH_3			300	N.E.
20d	CH_3	F		H	H	127	145		
20e	CH_3	F			H	178	119		
20f	CH_3	F			H			100[a]	N.E.
20g	CH_3	F			H			20[a]	N.E.
20h	CH_3	F			H			40[a]	N.E.
20I	CH_3	F			H			200	N.E.

[a]Solubility limit.
N.E., no effect.

little activity at 400–500 μM. It should be noted that IDRA-21 itself, which has an EC_{50} value of about 1000 μM in physiological tests (not shown), produced only small binding changes (~25% at 1 mM), and thus binding testes are not suitable to rank the potencies of these low affinity compounds. Interestingly, when R^1 is substituted with a chloride substituent in combination with a methyl group at R^3, the compound was nearly as effective as **16g**; however, the R^3-ethyl substituted analogue **16q** had reduced affinity. Finally, compounds **16t** and **16u** are IDRA-21 analogues in which the 7′-Cl was replaced with either a fluoride or an ethyl group; neither of those substitutions seemed to produce a significant improvement over IDRA-21 according to the binding tests.

Group IV

The indolyl-analogues in Group IV can be considered conformationally constrained D1 analogues. Compound **18e** is thus similar to D1 in regards to the length of the alkyl chain, and **18c** is equally comparable in this regard to **16g**. Interestingly, the effects on binding of **18b** and **18c** are almost identical to those of **16g** and **16h** with only a slight gain in affinity. The effects of compound **18e** were identical to those of **D1** but not those of **16c**. This indicates that the binding conformation of **D1** may resemble that of the conformationally constrained **18e**.

Group V compounds

Several 3′-substituted benzothiadiazides were prepared with the same aromatic substitution pattern as **D1** in the indexed combinatorial library study discussed earlier, but only the 3′-methyl substituted compound produced any activity. A closer look at the effects that other 3′-substituents would have on binding and biological activity was taken; by doing so, SAR would be developed to help define the substitution limits of this position. Several new analogues were prepared that contained various 3′-alkyl substituents (Group V). Furthermore, there was a particular interest in examining achiral **D1** analogues. It is known that both cyclothiazide and IDRA-21 have enantiospecific activity,[26,28] but if an achiral analogue has similar activity, then enantiospecific syntheses and/or resolution of racemic mixtures could be avoided. Thus, the gem-dimethyl achiral analogues (**20a**–**c**) were prepared from the requisite sulfonamide precursors by treatment with 2,2-dimethoxypropane (Table 3). Also, the achiral dihydro analogue **20d** was obtained similarly by treatment with formaldehyde. The other Group V analogues examined the effects that larger alkyl chains have on activity.

Biological testing of Group V compounds

It was found that 3′-substitutions other than a methyl group lead to a reduction in binding. For instance, when the methyl group was replaced with a hydrogen substituent (as in the case for **20d**), a modest loss in affinity was observed compared to its methyl counterpart (EC_{50} 127 μM vs 90 μM of **16 h**). A nearly 2-fold drop in affinity (178 μM, **20e**) was observed in the 3′-ethyl substituted analogue. Analogues with larger substituents at the 3′-position, such as the isopropyl (**20f**), *t*-butyl (**20g**), *n*-butenyl (**20 h**), and phenyl (**20i**) groups, showed no activity at concentrations below their solubility limit of 100, 20, 40, and 200 μM, respectively. A 3′-carboxyl analogue had no effect up to 1 mM (not shown).

The achiral gem-dimethyl analogues (**20a**–**c**) of **D1** and **16h** exhibited a drastic loss in affinity. Compound **20b** had barely detectable effects at 200–400 μM and crude estimates indicate an EC_{50} on the order of 400–600 μM. Its affinity is thus at least 30 times lower than that of **D1**; the implications of these results will be discussed later.

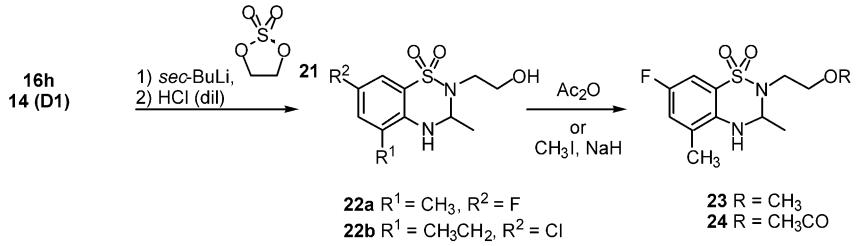
Group VI

Groups I–IV showed the effects of substituting the aromatic ring at various positions, from which it was concluded that a methyl group at the 5′-position and a halide at the 7′-position are optimal. The 3′-methyl substituent was confirmed as the optimal group in the Group V study. At this point in the research project, we pursued analogues where the heterocyclic nitrogens were substituted with the expectation of improving binding affinity, physiological efficacy, and water solubility (Group VI). Analogues that contain an alcohol, ether, ester, carboxylic acid, or a nitrile were chosen as synthetic targets because they could potentially enhance the water solubility of the parent benzothiadiazide, and would also diversify our SAR.

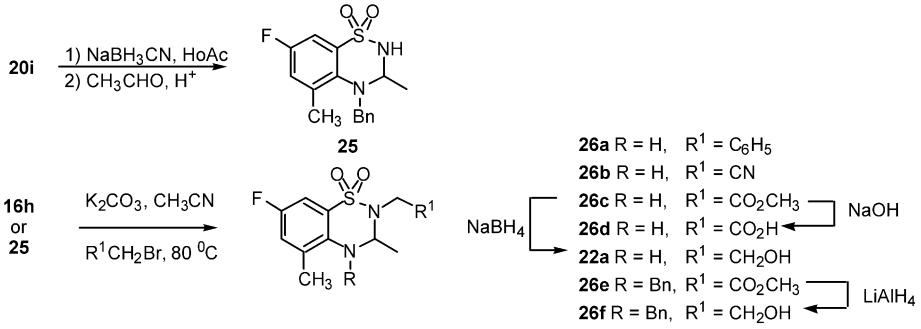
Synthesis of Group VI compounds

Two synthetic routes were employed in order to obtain the new analogues. In the first route, benzothiadiazides **16h** and **D1** were treated with *sec*-butyllithium at −78 °C, followed by the electrophile **21**. The intermediate sulfates were then hydrolyzed with aqueous acid to give **22a** and **22b**, respectively; however, this reaction sequence was problematic. For example, if the temperature of the reaction and the rate of addition were not carefully controlled, direct addition of *sec*-butyllithium to the heterocycle would result. In cases where the deprotonation and addition of **21** were successful to produce the required alkylated intermediate, the hydrolysis of the sulfate with dilute acid led to concomitant hydrolysis of the heterocycle, giving the aminobenzenesulfonamide as the major side-product. Several other bases were also examined such as sodium hydride, potassium carbonate, and triethylamine, but only *sec*-butyllithium gave the desired alkylated product. Nevertheless, this sequence was successful enough to allow for the preparation of suitable quantities of material for testing and for the preparation of the methyl ether (**23**) and the acetate (**24**) analogues (Scheme 5). A more general set of conditions would need to be discovered in order to improve this route for multiple analogue syntheses.

Despite the initial problems with *sec*-butyllithium as the base, we were able to successfully alkylate the heterocycle with several activated electrophiles. For example,



Scheme 5.



Scheme 6.

treatment of **16h** with *s*-butyllithium followed by addition of methyl bromoacetate, bromoacetonitrile, and benzyl bromide led to **26a**–**c**, respectively. The *N*-4 benzyl analogue (**25**) was also treated with *s*-butyllithium followed by **21** to give **26f**. While our work was in progress, Pirotte et al. reported that pyridothiadiazides could be alkylated in good yield with potassium carbonate as the base in refluxing acetonitrile.[29] When their procedure was applied to our system with methyl bromoacetate, bromoacetonitrile, and benzyl bromide as the electrophiles, clean conversion into the alkylated products resulted (Scheme 6). This second method proved to be an effective alternative to the *sec*-butyllithium procedure and was therefore adopted for further syntheses of **26a**–**c**,**f**. Additionally, analogue **26d** was obtained via saponification of **26c** and **26c** was also reduced with sodium borohydride in methanol to yield alcohol **22a**.

We also discovered that a completely regioselective reduction of compound **20i** to the corresponding *N*-4 benzyl product with sodium cyanoborohydride in acetic acid at 0 °C could be performed. The reduced product was then condensed with acetaldehyde under standard conditions to yield the *N*-4 benzylated analogue **27**. Alkylation with methyl bromoacetate gave ester **26e** and subsequent reduction with lithium aluminum hydride yielded the alcohol **26f**. The *N*-2 benzyl compound **26a** was obtained from **20i** by regioselective benzylation with sodium borohydride in methanol, followed by condensation with acetaldehyde. Although these two reductions were convenient, they were only effective with the phenyl-substituted compounds.

Results for Group VI analogues

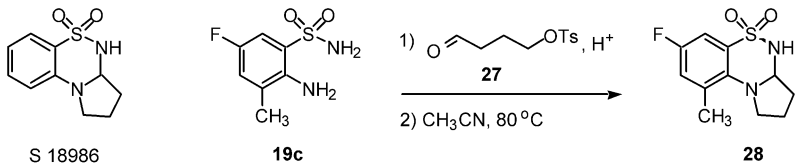
The binding data show that many of the Group VI compounds have lower binding affinities than their parent compounds (Table 4). Compound **22a**, the hydroxyethyl derivative of **16h**, has an EC_{50} of 115 μM (versus 90 μM) and it had a slightly lower effect on increasing agonist binding compared to the parent **16h** (23% vs 50%). Similar results were obtained with the acetate (**24**) derivative of **22a**. Introducing a methyl carboxy group at the *N*-2 position, either as the free acid (**26d**) or as the methyl ester (**26c**), completely abolished any activity, but the corresponding nitrile (**26b**) was as potent as the parent compound **16h**.

While this study was in progress, Desos et al. reported that S 18986 (Scheme 7) was active at inhibiting AMPA receptor desensitization in *Xenopus oocytes*.[36] An analogue of S 18986 was synthesized based on the structure of **16h**. This tricyclic compound (**28**) was obtained by treating **19c** with the tosyl aldehyde **27** followed by heating to reflux. This compound had no effect on binding below 100 μM and caused only a small (13%) increase at 400 μM. It thus appears to be considerably less potent than **16h** and the 3-ethyl derivative **20e**.

Table 4. Binding data for Group VI compounds

Compd	EC_{50} (μM)	Max (%)	Concn (μM)	% effect
22a	115	123		
22b	69	477		
23	360[b]	40		
24	143	120		
25			200	N.E.
26a			15[a]	N.E.
26b	89	211		
26c			500	N.E.
26d			1 mM	N.E.
26e			50[a]	N.E.
26f			200	N.E.
28	> 200			

[a]Solubility limit.
[b]Assay with [^{3}H]AMPA as radioligand.
N.E., no effect.



Scheme 7.

Table 5. Patch data for selected compounds

Compd	Test concn (μM)	Steady state/peak (%)
D1	30	70
	200	100
16a	50	95
	200	100
16b	30	70
16c	200	83
16d	200	48
16e	200	29
16f	200	52
16g	200	100
16h	200	100
16i	200	100
16j	50	20
16l	100	40
16m	100	23
16n	200	N.E.
16o	80	20
16p	200	88
16q	200	30
16r	200	38
16s	200	23
16t	200	40
16u	200	49
18a	30	14
18b	30	21
18c	30	14

N.E., no effect.

Electrophysiology

Patch clamp experiments using patches excised from hippocampal pyramidal cells were performed on several of the compounds in Groups I–IV (Table 5). Application of 1 mM glutamate to such a patch typically results in an inward current that reaches a peak within a few milliseconds and then decays due to a progressive desensitization to a steady-state level which is 5–10% of the peak current. The magnitude of the steady-state current depends on the rates of desensitization and resensitization, but it is also influenced by other kinetic parameters such as the channel opening/closing rates and thus is a sensitive indicator for the efficacy of any receptor modulator. The patch experiments were used to monitor the reliability of binding tests; a more complete analysis of the effects of **D1** on AMPA receptor currents will be presented elsewhere. Most tests were done at a compound concentration of 200 μM which in the case of **D1** completely blocked desensitization. At a concentration of 30 μM, **D1** raised to steady-state level to 70% which is in qualitative agreement with an EC_{50} values of 17–25 μM determined in binding tests. It is evident that the other compounds with reasonably high potency in binding, such as **16a–c**, **16h–j**, and **16p**, produced a commensurate blockade of desensitization at 200 μM whereas compounds inactive in binding produced only small effects.

Discussion

The present study shows that alkyl substitution at the 5′-position of the aromatic ring on the benzothiadiazide core leads to substantial increases in binding affinity and biological activity. **D1** has an EC_{50} on the order of 15–30 μM, which is similar to that of cyclothiazide in patch clamp experiments (10–30 μM)[13] and binding tests (31 μM),[34] and about 30 times more potent than IDRA-21. **D1** itself completely blocked desensitization of glutamate-induced responses at 100–200 μM and the binding affinity of **D1** analogues was highly dependent on the size of the 5-alkyl group, as expected. EC_{50} values were on the order of 100 μM for compounds with a methyl substituent (**16g**) and as low as 170 μM for compounds with a propyl substituent (**16e**). Binding affinities were similar for Group I and Group IV analogues. Modifications at other positions confirmed findings of earlier studies: (i) a halide at the 7′-position, (ii) the absence of a substituent at the 6′-position, and (iii) the presence of a methyl group at the 3′-position were all important for optimal compound affinity (Fig. 5).

Our data suggest that there is a substantial preference for the 3′-methyl group, an idea that is supported by the abrupt loss in affinity for compounds **20a–i**. The results

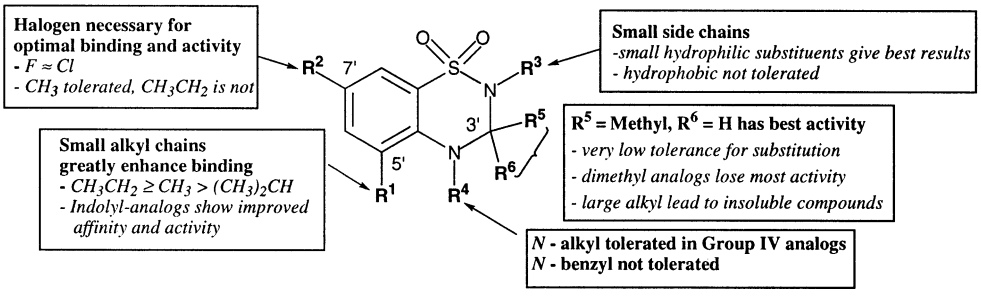


Figure 5. SAR summary.

from the achiral analogues **20a–c** provide further evidence that these compounds may bind stereospecifically. When the 3′-methyl group was embedded in a five-membered ring (**29**), an abrupt loss in affinity was also observed. Collectively, these observations suggest that there may be very little space in the receptor-binding site for substituents larger than a methyl group.

The addition of the 5′-methyl or 5′-ethyl groups may enhance the van der Waals interaction between the ligand and its receptor. An energy gain of $\sim$36 cal/A^2 has been estimated for contact of an alkyl substituent with its receptor.[37] With estimated van der Waals surface areas for methyl and ethyl substituents of 40 and 60 A^2, respectively,[37] the predicted increases in binding energies due to these additional interactions would be approximately 1.4 and 2.2 kcal/mol.[38] Taking these energies into account, an increase in binding affinity over that of IDRA-21 by a factor of about 10 (for **16g**) and 40 (for **D1**) would be expected. Indeed, the values are similar to those actually measured, suggesting that if the observed gain in binding affinity is due to this effect, it has approached its theoretical maximum. Since the 5′-ethyl group in **D1** occupies a major fraction of the 3′–6′ pocket of the binding site, then analogues would be confined to substitutions at the 7′, 8′, and *N*-2′ positions. Since 2′-substituted analogues were already prepared (Group VI), substitutions at the 7′- and 8′-positions remain to be examined.

Given that cyclothiazide and the simple benzothiadiazides such as IDRA-21 and the compounds described here contain the same core structure, it would be logical to assume that they dock in a similar manner to the AMPA receptor; however, structure–activity comparisons indicate that this may not be the case. We prepared a 3′-norbornenyl substituted analogue of **16b** (not shown), and found it be inactive at 100 μM, whereas **16b** itself increased binding by 80% at 100 μM. Conversely, in cyclothiazide the 3′-norbornenyl group is essential for binding and activity, illustrated by the fact that closely related analogues that lacked this group required millimolar concentrations to produce receptor activation.[13] Bertolino et al.[27] tested a compound (IDRA 22) that was identical to IDRA-21 except that the aromatic ring was substituted with the same 6′-chloride and 7′-sulfonamide groups present in cyclothiazide, and found that this compound had no effect in their patch experiments. It thus seems likely that cyclothiazide and the compounds shown here bind differently to the modulator site or that they bind to two distinct sites on the receptor. This may also account for the observations that cyclothiazide and **D1** produced very different changes in many receptor properties. For one, **D1** increased binding of [^{3}H]AMPA nearly 3-fold whereas cyclothiazide reduces binding under the same assay conditions by up to 90%.[34] **D1** also prolonged the rate at which responses to 1-ms glutamate pulses deactivate receptors by more than 4-fold, whereas cyclothiazide had relatively small effects.[24] It will be of considerable interest to examine whether or not these two types of benzothiadiazides interact with each other in a competitive manner.

Interestingly, while our work was in progress, Pirotte et al.[29] reported that pyridothiadiazides had affinities and physiological effects similar to those of IDRA-21 and that, among over 50 derivatives tested, conferred the most significant improvement in potency (Fig. 1). It seems likely that the interaction of their compound with the receptor may be equivalent to **18b**, in which the *N*-4 ethyl group of the Pirotte compound is similar to the *N*-4 substituent of **18b**. In fact, the potency of the Pirotte compound in blocking desensitization appears to be of similar magnitude to our compounds in that it increased the steady-state current through AMPA receptors by a factor of five at a concentration of 19 μM.

Sekiguchi et al.[19] and Ornstein et al.[39] recently described new potent AMPA receptor modulators (Fig. 1). Unlike the mostly compact and rigid benzothiadiazides and ampakines,[17] these compounds are elongated and rather flexible compounds. Whether their compounds and benzothiadiazides interact with the same binding site on the AMPA receptor remains to be determined; however, if they do bind to the same site as the benzothiadiazides and ampakines, then incorporating **D1** into a larger elongated structure might confer additional gains in potency. This may be possible via attachment to the *N*-2 nitrogen of the heterocycle.

Experimental

General

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX 500 (500 MHz), a DRX 400 (400 MHz), a GN 500 (500 MHz), or a QE 300 (300 MHz). For ^{1}H NMR spectra acquired in $CDCl_3$, data are reported in ppm and are calibrated according to internal $CHCl_3$ (7.26 ppm); spectra measured in D_2O are calibrated according to internal HOD (4.80 ppm); spectra measured in DMSO-d_6 were referenced to internal TMS (0.0 ppm). For ^{13}C NMR spectra measured in $CDCl_3$, data are reported in ppm and are calibrated according to internal $CDCl_3$ (77.0 ppm) unless otherwise noted. Data are reported as follows: chemical shift, multiplicity (app = apparent, par obsc = partially obscured, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, abq = ab quartet), coupling constant, and integration. Infrared (IR) spectra were obtained on a Perkin-Elmer Model 1600 series FTIR spectrophotometer and are reported in inverse-wavenumbers (cm^{-1}). Melting points (mp) were obtained from a Laboratory Devices Mel-Temp melting-point apparatus and are uncorrected. High resolution mass spectrometry were obtained from the Departmental Mass Spectrometry facility. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck pre-coated silica gel plates (60 F-254), and silica gel chromatography was performed using ICN 200–400 mesh silica gel. Inert atmosphere operations were conducted under nitrogen passed through a two Drierite drying tubes in oven or flame-dried glassware. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), and diethyl ether were filtered through two

columns of activated basic alumina and transferred under Ar (g) in a solvent purification system designed and manufactured in house by J. C. Meyer. Dry toluene was obtained similarly on the system by filtering through two columns of Q5. Unless otherwise noted, all crude reactions were processed as follows: organics were dried over solid magnesium sulfate ($MgSO_4$), filtered and concentrated ein vacuo on a rotary evaporator. Dry dimethylformamide (DMF) was obtained by passing through two columns of activated molecular sieves. Triethylamine (TEA), hexamethylphosphoramide (HMPA), hexamethyldisilazane (HMDS), and MeOH (MeOH) were purified by distillation from calcium hydride. Abbreviations: EtOAc (EtOAc), camphorsulfonic acid (CSA), *N*-methylmorpholine-*N*-oxide (NMO), tetrapropylammonium perruthenate (TPAP), magnesium sulfate ($MgSO_4$). All other reagents were used as purchased from Aldrich, Sigma-Aldrich, or Acros unless otherwise stated.

General procedure for the preparation of 2-aminobenzenesulfonamides (general procedure A)

To a stirring solution of chlorosulfonyl isocyanate (0.45 mL, 4.6 mmol) in nitroethane (8 mL) at −42 °C is added the aniline (4 mmol) dropwise. The solution is stirred until the intermediate chlorosulfonyl urea precipitates. Aluminum chloride (612 mg, 4.6 mmol) is then added in one portion and followed by warming to room temperature over 1 h. The reaction is then heated to 110 °C for 1 h, then cooled to room temperature and decanted into ice water. The resulting precipitate is filtered off, or the oil is extracted with ethyl acetate and dried over anhydrous $MgSO_4$, to yield the crude product. The crude product is then heated to 130–140 °C in a solution of 50% H_2SO_4 (aq) (10–15 mL) for 2 h. The homogenous reaction mixture is cooled to room temperature and neutralized with 10 N NaOH. Extraction with ethyl acetate (2 × 20 mL) is followed the usual processing and flash chromatography yields the pure compound.

5-Chloro-2-aminobenzenesulfonamide (A). Compound **A** was prepared by the general method, mp 151–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (1H, d, J = 2.5 Hz), 7.40 (2H, s), 7.27 (1H, dd, J = 8.8, 2.5 Hz), 6.82 (1H, d, J = 8.8 Hz), 6.00 (2H, s). HRMS calcd for $C_6H_7ClN_2O_2S$ [M^+] 205.9917, found 205.9916.

5-Bromo-2-aminobenzenesulfonamide (B). Compound **B** was prepared according to the literature.[40] Isolated by column chromatography (30% ethyl acetate/hexanes) as a white solid (0.694 g, 48%), mp 178–180 °C (lit. 179 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 (1H, d, J = 1.2 Hz), 7.39 (3H, m), 6.77 (1H, d, J = 8.8 Hz), 6.02 (2H, s). HRMS calcd for $C_6H_7BrN_2O_2S$ [M^+] 249.9412, found 249.9404.

3-Ethyl-2-aminobenzenesulfonamide (F). Yield 53% for two steps by general method; mp 118–120 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.47 (1H, d, J = 7.9 Hz), 7.25 (2H, s), 7.16 (1H, d, J = 7.1 Hz), 6.60 (1H, t, J = 7.7 Hz), 5.62 (2H, s), 2.51 (2H, q, J = 7.3 Hz), 1.15 (3H, t, J = 7.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 142.8, 131.8, 129.1, 125.8, 124.5, 115.0, 23.5, 13.0. FT-IR (KBr) 3401, 3342, 1619-, 1437, 1325, 1185, 1143, 1096 cm^{-1}. HRMS calcd for $C_8H_{12}N_2O_2S$ [M^+] 200.0619, found 200.0623.

5-Chloro-3-ethyl-2-aminobenzensulfonamide (D). To a solution of **F** (504 mg, 2.52 mmol) in 10 mL acetonitrile was added *N*-chlorosuccinimide (316 mg, 2.37 mmol). The mixture was heated to reflux for 12.5 h, cooled, filtered and concentrated in vacuo. Reaction was incomplete by ^{1}H NMR; the purple solides were redissolved in acetonitrile and more NCS (102 mg, 0.76 mmol) added. The mixture was refluxed for 15 min and worked up as before. Column chromatography (40% ethyl acetate/hexanes) gave pure **D** (522 mg, 94%); mp 134–135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.45 (3H, m), 7.19 (1H, d, J = 2.1 Hz), 5.76 (2H, s), 2.53 (2H, q, J = 7.3 Hz), 1.14 (3H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 141.9, 131.8, 131.2, 125.4, 124.8, 118.1, 23.4, 12.7. FT-IR (KBr) 3458, 3428, 3358, 3269, 3080, 2970, 1631, 1556, 1541, 1452, 1312, 1188 cm^{-1}. HRMS calcd for $C_8H_{11}ClN_2O_2S$ [M^+] 234.0230, found 234.0227.

5-Nitro-2-aminobenzenesulfonamide (E). To a 0 °C solution of 60% HNO_3 (14 mL) and H_2SO_4 was added **12** (1.98 g, 10.0 mmol), prepared by the general method A from aniline, portion-wise during a 1 h period. The mixture was stirred for 20 h at room temperature and quenched by pouring onto 100 g ice. The white-grey solid was collected by filtration and stirred in 50 mL 50% H_2SO_4 and heated to 130 °C until homogenous. The reaction was cooled to room temperature and poured onto 200 mL cold water. Neturalization was achieved with 40% NaOH and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried, filtered and concentrated to give pure **E** (1.257 g, 58% for two steps); mp 218–219 °C (lit. 214–215 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (1H, s), 8.11 (1H, dd, J = 9.4, 1.4 Hz), 7.62 (2H, s), 7.14 (2H, br s), 6.90 (1H, d, J = 8.8 Hz). HRMS calcd for $C_6H_7N_3O_4S$ [M^+] 217.0157, found 217.0516.

Indexed combinatorial synthesis

Stock solution of aldehydes. A stock solution containing 1.3 mmol of the following aldehydes was prepared in 0.87 mL acetonitrile to give a solution 0.75 M in each aldehyde (acetaldehyde, isobutyraldehyde, crotonaldehyde, cyclohexylcarboxaldehyde, benzaldehyde, 3-pyridine carboxaldehyde, *p*-anisaldehyde, and 2,5-dimethoxy benzaldehyde). The solution was used in 0.1 mL portions which are 0.075 mmol in each aldehyde.

Stock solution of 2-aminobenzensulfonamides. A stock solution of 2-aminobenzenesulfonamdies was prepared as follows: 0.9 mmol of compounds **A**–**G** in 30 mL acetonitrile to give a solution 0.03 M in each compound. The solution was used in 2.5 mL portions which contain 0.075 mmol of each compound.

Standard procedure for combinatorial reactions. Pools **A**–**G:** To a solution of 2-aminobenzenesulfonamide (0.9 mmol, 1.5 equiv per aldehyde) in 2.5 mL acetonitrile was added 3 Å molecular sieves; the solution was stirred in

an ice bath for 5 min when the stock solution of aldehydes (0.1 mL, 0.075 mmol per aldehyde) was added. A catalytic amount of (±) 10-camphorsulfonic acid was added and the reaction mixture stirred to 2 h at 0 °C and 2–16 h at room temperature. If reaction looked incomplete by TLC and NMR, the reactions were heated for 2–24 h at 80 °C in an oil bath. The reaction was worked up by filtration through Celite. Concentration of organics gave a crude mixture that was used for biological testing. Pools **1**–**8** A similar procedure to **A**–**G** was used to prepare pools **1**–**8**: except that the stock solution of 2-aminobenzensulfonamides (2.5 mL, 0.075 mmol in each compound) was stirred in the ice bath with molecular sieves, followed by addition of a particular aldehyde (0.62 mmol, 1.05 equiv per sulfonamide).

General procedure for chlorination with *N*-Chloro-succinimide (general procedure B)

The aminobenzenesulfonamide (prepared by the general procedure A), or the benzothiadiazide (prepared by the general procedures A and C) is dissolved in acetonitrile, treated with *N*-chlorosuccinimide (1.3 equiv), and heated to reflux for 5–12 h. The reaction is then cooled, concentrated, and purified by flash chromatography to afford the chlorinated product.

7-Chloro-5-ethyl-3-methyl-3,4-dihydro-2*H*-benzo[1,2,4]-thiadiazine 1,1-dioxide (D1) (general procedure C). A solution of 5-chloro-3-ethyl-2-aminobenzenesulfonamide (407 mg, 1.74 mmol) containing 3 Å molecular sieves in acetonitrile (8.5 mL) was to 0 °C. Acetaldehyde (0.15 mL, 2.68 mmol) and (±) 10-camporsulfonic acid (catalytic) were then added and the reaction stirred for 1 h. After the starting material was completely consumed, the reaction was filtered through Celite and the solvent was evaporated to give the crude **D1**. Purification by filtering through a narrow pad of silica gel gave pure **D1** (443 mg, 98%) as a white solid; mp 181–183 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (d, J = 11.8 Hz, 1H), 7.35 (d, J = 2.2 Hz, 1H), 7.21 (d, J = 2.2 Hz, 1H), 6.24 (s, 1H), 4.80 (m, 1H), 2.52 (m, 2H), 1.48 (d, J = 7.1 Hz, 3H), 1.13 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 140.2, 132.4, 131.1, 122.2, 120.5, 120.0, 62.2, 23.2, 20.1, 12.7. FTIR (KBr) 3406, 3233, 3002, 2943, 2884, 1491, 1439, 1310, 1282 cm^{-1}. HRMS calcd for $C_{10}H_{14}N_2O_2S$ [M$^+$] 260.0386, found 260.0386.

5-Ethyl-7-fluoro-3-methyl-3,4-dihydro-2*H*-benzo[1,2,4]-thiadiazine-1,1-dioxide (16a). Compound **16a** was synthesized from 2-ethyl-4-fluoroaniline by the general procedures A and C to give an off-white solid (69%, three steps); mp 156–157 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.65 (d, J = 11.8 Hz, 1H), 7.20 (dd, J = 7.6, 2.8 Hz, 1H), 7.13 (dd, J = 9.6, 2.8 Hz, 1H), 6.00 (s, 1H), 4.80 (m, 1H), 2.55 (quintet, J = 7.4 Hz, 2H), 1.50 (d, J = 6.1 Hz, 3H), 1.15 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.6 (d, J = 236.3 Hz), 138.5, 133.1 (d, J = 7.9 Hz), 121.7 (d, J = 8.7 Hz), 119.3 (d, J = 24.4 Hz), 107.1 (d, J = 23.6 Hz), 62.5, 23.5, 20.4, 12.9. FTIR (KBr) 3409, 3212, 2989, 2931, 1654, 1508, 1449, 1308, 1284 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{13}FN_2O_2S$ [M$^+$] 244.0682, found 244.0691.

5-Ethyl-3-methyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (16b). Compound **16b** was prepared from 2-ethylaniline by the general procedures A and C to give an off-white powder (55%, three steps); mp 166–168 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (d, J = 11.9 Hz, 1H), 7.36 (d, J = 7.7 Hz, 1H), 7.20 (d, J = 7.2 Hz, 1H), 6.73 (dd, J = 7.6 Hz, 1H), 6.04 (s, 1H), 4.82 (m, 1H), 2.53 (app q, J = 7.4 Hz, 2H), 1.50 (d, J = 6.2 Hz, 3H), 1.14 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.1, 131.2, 129.3, 121.4, 121.3, 116.3, 62.0, 23.1, 20.1, 12.9. FTIR (KBr) 3223, 2976, 2940, 2875, 1598, 1497, 1382, 1314, 1289 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{14}N_2O_2S$ [M$^+$] 226.0768, found 226.0768.

7-Chloro-5-isopropyl-3-methyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (16c). Compound **16c** was prepared from **16d** by the general procedure B to give an off-white powder in 100% yield; mp 176–178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (d, J = 11.8 Hz, 1H), 7.37 (d, J = 2.4 Hz, 1H), 7.27 (d, J = 2.5 Hz, 1H), 4.8 (m, 1H), 3.15 (septet, J = 6.8 Hz, 1H), 1.50 (d, J = 6.3 Hz, 3H), 1.17 (d, J = 6.9 Hz, 3H), 1.13 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 138.3, 136.3, 129.5, 123.7, 123.4, 121.7, 62.4, 27.5, 22.1, 21.9, 21.0. FTIR (KBr) 3389, 3213, 2969, 2872, 1596, 1491, 1449, 1420, 1316, 1290 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{15}ClN_2O_2S$ [M$^+$] 274.0543, found 274.0534.

5-Isopropyl-3-methyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16d). This was prepared from 2-isopropylaniline by the general procedures A and C to give a white powder (54%, three steps); mp 202–204 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.50 (d, J = 11.7 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H), 6.76 (dd, J = 7.7 Hz, 1H), 6.08 (s, 1H), 4.81 (m, 1H), 3.13 (septet, J = 6.5 Hz, 1H), 1.50 (d, J = 6.0 Hz, 3H), 1.16 (d, J = 6.6 Hz, 3H), 1.13 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 140.6, 134.1, 128.7, 121.8, 121.4, 116.6, 62.1, 25.9, 22.6, 22.4, 20.3. FTIR (KBr) 3404, 3192, 2964, 2868, 1598, 1578, 1501, 1447, 1419, 1320, 1292 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{16}N_2O_2S$ [M$^+$] 240.0932, found 240.0930.

7-Chloro-3-methyl-5-propyl-3,4-dihydro-2H-benzo[1,2,4]-thiadiazine 1,1-dioxide (16e). This was prepared from **16f** by the general procedure **B** to give an off-white powder in 67% yield; mp 150–151 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (d, J = 11.8 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.22 (d, J = 3.4 Hz, 1H), 6.24 (s, 1H), 4.81 (m, 1H), 2.53 (obscured by NMR solvent, 2H), 1.54 (sextet, J = 7.6 Hz, 2H), 1.50 (d, J = 6.0 Hz, 3H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 132.3, 131.2, 122.6, 120.8, 120.1, 62.5, 32.1, 21.3, 20.4, 14.0. FTIR (KBr) 3395, 3225, 2963, 2932, 2873, 1597, 1496, 1383, 1312, 1243 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{15}ClN_2O_2S$ [M$^+$] 274.0543, found 274.0554.

3-Methyl-5-propyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16f). This was prepared from 2-propylaniline by the general procedures A and C to give a tan powder (33%, three steps); mp 179–180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.52 (d, J = 11.9 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.17 (d, J = 7.4 Hz, 1H), 6.71 (dd,

$J=7.5$ Hz, 1H), 6.02 (s, 1H), 4.81 (m, 1H), 2.50 (obscured by NMR solvent, 2H), 1.55 (m, 2H), 1.50 (d, $J=6.3$ Hz, 3H), 0.93 (d, $J=7.3$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.6, 132.7, 128.2, 122.0, 121.8, 116.5, 62.4, 37.4, 21.4, 20.66, 14.1. FTIR (KBr) 3386, 3212, 2957, 2939, 2873, 1654, 1599, 1499, 1449, 1384, 1314, 1290 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{16}N_2O_2S$ [M$^+$] 240.0932, found 240.0931.

7-Chloro-3,5-dimethyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16g). This was prepared from 4-chloro-2-methylaniline by the general procedures A and C to give a white powder (33%, three steps); mp 256–258 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (d, $J=11.8$ Hz, 1H), 7.36 (s, 1H), 7.30 (d, $J=1.5$ Hz, 1H), 6.21 (s, 1H), 4.83 (m, 1H), 2.16 (s, 3H), 1.50 (d, $J=6.0$ Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 141.3, 133.3, 127.4, 122.4, 120.8, 120.1, 62.6, 20.5, 18.0. FTIR (KBr) 3418, 3222, 2988, 2940, 1601, 1497, 1466, 1449, 1420, 1383, 1313 cm^{-1}. HRMS for $C_9H_{11}ClN_2O_2S$ [M$^+$] calcd 246.0230, found 246.0230.

7-Fluoro-3,5-dimethyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16 h). This was prepared from 4-fluoro-2-methyl aniline by the general procedures A and C to give a white solid (60%, three steps); mp 211–214 °C. ^{1}H (500 MHz, DMSO-d_6) δ 7.67 (d, $J=11.9$ Hz, 1H), 7.21 (s, 1H), 7.18 (s, 1H), 5.97 (s, 1H), 4.80 (m, 1H), 2.17 (s, 3H), 1.49 (d, $J=6.1$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 154.0 (d, $J=236.3$ Hz), 139.8, 128.3 (d, $J=8.0$ Hz), 122.1, 122.0 (d, $J=23.9$ Hz), 107.9 (d, $J=23.6$ Hz), 63.3, 21.2, 18.7. FTIR (KBr) 3394, 3223, 2924, 1499, 1384, 1316, 1238 cm^{-1}. HRMS (CI) calcd for $C_9H_{11}FN_2O_2S$ [M$^+$] 230.0525, found 230.0524.

3,5,7-Trimethyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16i). This was prepared from 2,4-dimethylaniline by the general procedures A and C to give a tan powder (16%, three steps); mp 225–230 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.48 (d, $J=11.9$ Hz, 1H), 7.16 (s, 1H), 7.04 (s, 1H), 5.83 (s, 1H), 4.79 (m, 1H), 2.18 (s, 3H), 2.12 (s, 3H), 1.48 (d, $J=6.1$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 140.1, 134.9, 125.6, 124.6, 122.0, 121.3, 62.6, 20.7, 20.2, 18.1. FTIR (KBr) 3399, 3191, 1618, 1501, 1448, 1384, 1314, 1291 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{14}N_2O_2S$ [M$^+$] 226.0776, found 226.0773.

7-Chloro-3,5,6-trimethyl-3,4-dihydro-2H-benzo[1,2,4]-thiadiazine 1,1-dioxide (16j). This was prepared from **16k** by the general procedure B to give an off-white powder in 100% yield; mp 251–253 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.67 (d, $J=11.7$ Hz, 1H), 7.38 (s, 1H), 6.10 (s, 1H), 4.81 (m, 1H), 2.30 (s, 3H), 2.13 (s, 3H), 1.50 (d, $J=6.1$ Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 141.7, 139.0, 125.9, 122.6, 121.6, 121.5, 63.2, 21.2, 18.2, 15.4. FTIR (KBr) 3415, 3194, 2923, 1591, 1499, 1458, 1388, 1302, 1285 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{13}ClN_2O_2S$ [M$^+$] 260.0386, found 260.0375.

3,5,6-Trimethyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16k). This was prepared from 2,3-dimethylaniline by the general procedures A and C to give a tan powder (46%, three steps); mp 251–253 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.45 (d, $J=11.8$ Hz, 1H), 7.26 (d, $J=8.1$ Hz, 1H), 6.63 (d, $J=8.1$ Hz, 1H), 5.91 (s, 1H), 4.79 (m, 1H), 2.22 (s, 3H), 2.04 (s, 3H), 1.49 (d, $J=6.2$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.2, 140.9, 122.4, 121.1, 120.4, 119.2, 62.6, 21.1, 20.1, 13.6. FTIR (KBr) 3407, 3198, 2990, 1596, 1507, 1474, 1447, 1384, 1301, 1284 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{14}N_2O_2S$ [M$^+$] 226.0776, found 226.0780.

6-Chloro-3,5-dimethyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16l). This was prepared from 3-chloro-2-methylaniline by the general procedures A and C to give a tan powder (72%, three steps); mp 184–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.67 (d, $J=11.8$ Hz, 1H), 7.39 (d, $J=8.6$ Hz, 1H), 6.85 (d, $J=8.5$ Hz, 1H), 6.34 (s, 1H), 4.85 (m, 1H), 2.22 (s, 3H), 1.51 (d, $J=6.2$ Hz, 3H); ^{13}C (75 MHz, DMSO-d_6) δ 138.0, 123.6, 122.5, 121.4, 118.2, 63.4, 21.2, 15.4. FTIR (KBr) 3400, 3220, 2992, 1578, 1499, 1445, 1384, 1319, 1288 cm^{-1}. HRMS (CI) calcd for $C_9H_{11}ClN_2O_2S$ [M$^+$] 246.0230, found 246.0230.

8-Chloro-3,5-dimethyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16m). This was prepared from 5-chloro-2-methyl aniline by the general procedures A and C to give a tan powder (42%, three steps); mp 219–220 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (d, $J=8.8$ Hz, 1H), 7.15 (d, $J=7.9$ Hz, 1H), 6.74 (d, $J=7.9$ Hz, 1H), 6.20 (s, 1H), 4.77 (m, 1H), 2.12 (s, 3H), 1.49 (d, $J=6.2$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 144.2, 133.0, 127.5, 123.3, 120.0, 117.9, 61.1, 19.8, 17.7. FTIR (KBr) 3397, 3210, 2923, 1594, 1560, 1499, 1459, 1380, 1320, 1300, 1239 cm^{-1}. HRMS (CI) calcd for $C_9H_{11}ClN_2O_2S$ [M$^+$] 246.0230, found: 246.0233.

5-Fluoro-3-methyl-3,4-dihydro-2H-benzo[1,2,4]thiadiazine 1,1-dioxide (16n). This was prepared from 2-fluoroaniline by the general procedures A and C to give a yellowish powder; mp 208–210 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73 (d, $J=11.7$ Hz, 1H), 7.34 (d, $J=7.9$ Hz, 1H), 7.29 (dd, $J=11.4$, 8.2 Hz, 1H), 6.99 (s, 1H), 6.73 (dd, $J=7.8$, 4.7 Hz, 1H), 4.86 (m, 1H), 1.48 (d, $J=6.1$ Hz, 3H). FTIR (KBr) 3367, 3215, 1618, 1500, 1315, 1248 cm^{-1}. HRMS (CI) calcd for $C_8H_9FN_2O_2S$ [M$^+$] 216.0368, found 216.0368.

7-chloro-5-fluoro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (16o). This was prepared from **16n** by the general procedure B to give a brown solid; mp 194–196 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (d, $J=11.6$ Hz, 1H), 7.53 (dd, $J=11.3$, 1.8 Hz, 1H), 7.41 (s, 1H), 7.22 (s, 1H), 4.87 (m, 1H), 1.48 (d, $J=6.1$ Hz, 3H). FTIR (KBr) 3365, 3240, 2924, 1499, 1337, 1252 cm^{-1}. HRMS (CI) calcd for $C_8H_8ClFN_2O_2S$ [M$^+$] 249.9978, found 249.9972.

5-chloro-3,7-dimethyl-3,4-dihyrdo-2H-1,2,4-benzothiadiazine-1,1-dioxide (16p). This was prepared from 2-chloro-4-methylaniline by the general procedures A and C to give an off-white powder (54%, three steps); mp 239–242 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.76 (d, $J=11.7$ Hz, 1H), 7.37 (s, 1H), 7.34 (s, 1H), 6.31 (s, 1H), 4.86 (m, 1H), 2.22 (s, 3H), 1.50 (d, $J=6.1$ Hz, 3H); ^{13}C

NMR (125 MHz, DMSO-d_6) δ 138.8, 134.3, 127.3, 123.8, 123.4, 119.5, 63.2, 21.0, 20.2. FTIR (KBr) 3370, 3204, 2980, 1501, 1325, 1309, 1223 cm^{-1}. HRMS (CI) calcd for $C_9H_{11}ClN_2O_2S$ [M^+] 246.0230, found 246.0235.

5-Chloro-7-ethyl-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (16q). Compound **16q** was prepared from **16t** by the general procedure B to give an off-white solid in 60% yield; mp 189–191 °C. ^1H NMR (500 MHz, DMSO-d_6) δ 7.76 (d, $J=11.5$ Hz, 1H), 7.41 (d, $J=1.6$ Hz, 1H), 7.35 (s, 1H), 6.35 (s, 1H), 4.86 (m, 1H), 2.54 (q, $J=7.5$ Hz, 2H), 1.50 (d, $J=6.1$ Hz, 3H), 1.12 (t, $J=7.5$ Hz, 3H). FTIR (KBr) 3367, 3214, 2967, 1612, 1507, 1458, 1318, 1261, cm^{-1}. HRMS (CI) calcd for $C_{10}H_{13}ClN_2O_2S$ [M^+] 260.0386, found 260.0386.

5-Methoxy-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (16r). Compound **16r** was prepared from *o*-anisidine by the general procedures A and C to give an off-white powder (49%, three steps); mp 172–173 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 7.54 (d, $J=11.8$ Hz, 1H), 7.08 (d, $J=8.0$ Hz, 1H), 7.00 (d, $J=7.9$ Hz, 1H), 6.71 (t, $J=8.0$ Hz, 1H), 6.25 (s, 1H), 4.81 (m, 1H), 3.83 (s, 3H), 1.47 (d, $J=6.1$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 146.6, 134.6, 116.2, 115.5, 112.9, 62.6, 56.3, 20.5. FTIR (KBr) 3397, 3320, 3219, 2990, 1605, 1582, 1506, 1458, 1412, 1386, 1321, 1289, 1251 cm^{-1}. HRMS (CI) calcd for $C_9H_{12}N_2O_3S$ [M^+] 228.0569, found 228.0560.

5,7-Dimethoxy-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (16s). Compound **16s** was prepared from 2,4-dimethoxyaniline by the general procedures A and C to give an off white solid; ^1H NMR (300 MHz, DMSO-d_6) δ 7.53 (d, $J=11.9$ Hz, 1H), 6.66 (d, $J=2.3$ Hz, 1H), 6.56 (d, $J=2.3$ Hz, 1H), 5.85 (s, 1H), 4.76 (m, 1H), 3.82 (s, 3H), 3.71 (s, 3H), 1.44 (d, $J=6.3$ Hz, 3H). FTIR (KBr) 3379, 3228, 1500, 1319, 1286 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{14}N_2O_4S$ [M^+] 258.0674, found 258.0670.

7-Ethyl-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-1,1-dioxide (16t). Compound **16t** was prepared from 4-ethylaniline by the general procedures A and C to give an off-white powder (38%, three steps); mp 191–195 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 7.40 (d, $J=11.8$ Hz, 1H), 7.26 (s, 1H), 7.16 (dd, $J=8.6$, 1.6 Hz, 1H), 6.93 (s, 1H), 6.71 (d, $J=8.5$ Hz, 1H), 4.77 (m, 1H), 2.50 (obscured by NMR solvent, 2H), 1.40 (d, $J=6.1$ Hz, 3H), 1.12 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.3, 133.1, 132.2, 122.4, 121.2, 116.4, 62.3, 27.4, 20.5, 16.2. FTIR (KBr) 3352, 3206, 2964, 1617, 1508, 1320, 1288 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{14}N_2O_2S$ [M^+] 226.0776, found 226.0774.

7-Fluoro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine-1,1-dioxide (16u). Compound **16u** was prepared from 4-fluoroaniline by the general procedures A and C to give a light gray powder (46%, three steps); mp 200–202 °C. ^1H NMR (500 MHz, DMSO-d_6) δ 7.57 (d, $J=11.7$ Hz, 1H), 7.31 (dd, $J=8.0$, 2.9 Hz, 1H), 7.22 (ddd, $J=8.8$, 2.9 Hz, 1H), 7.10 (s, 1H), 6.82 (dd, $J=9.2$, 4.5 Hz, 1H), 4.79 (m, 1H), 1.41 (d, $J=6.1$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.1 (d, $J=240.2$ Hz), 140.6, 120.8 (d, $J=23.6$ Hz), 120.4 (d, $J=7.9$ Hz), 117.6 (d, $J=7.9$ Hz), 109.3 (d, $J=23.6$ Hz), 61.9, 19.8. FTIR (KBr) 3378, 3278, 3063, 2993, 1499, 1449, 1427, 1380, 1319, 1286, 1262, 1216, 1151 cm^{-1}. HRMS (CI) calcd for $C_8H_9FN_2O_2S$ [M^+] 216.0369, found 216.0367.

3-Methyl-1,2,3,4-tetrahydro-5-thia-2a,4-diaza-acenaphthylene 5,5-dioxide (18a). Compound **18a** was prepared from indoline by the general procedures A and C to give a beige solid (27% yield, three steps); mp 193–196 °C. ^1H NMR (500 MHz, DMSO-d_6) δ 7.92 (d, $J=12.4$ Hz, 1H), 7.23 (d, $J=7.4$ Hz, 2H), 6.73 (dd, $J=7.6$ Hz, 1H), 4.60 (dq, $J=11.7$, 5.9 Hz, 1H), 3.61 (t app, $J=8.7$ Hz, 1H), 3.24 (q app, $J=8.9$ Hz, 1H), 3.02 (m, 2H), 1.41 (d, $J=6.2$ Hz, 3H). FTIR (KBr) 3112, 2874, 2851, 1735, 1604, 1480, 1461, 1438 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{12}N_2O_2S$ [M^+] 224.0619, found 224.0613.

7-Fluoro-3-methyl-1,2,3,4-tetrahydro-5-thia-2a,4-diaza-acenaphthylene 5,5-dioxide (18b). Compound **18b** was prepared from 5-fluoroindoline by the general procedures A and C to give a beige solid (23% yield, four steps); mp 193–197 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.05 (d, $J=11.0$ Hz, 1H), 7.20 (dd, $J=8.8$, 0.6 Hz, 1H), 7.13 (d, $J=8.5$ Hz, 1H), 4.59 (dq, $J=11.4$, 5.8 Hz, 1H), 3.60 (app t, $J=7.5$ Hz, 1H), 3.23 (q app, $J=9.7$ Hz, 1H), 3.05 (m, 2H), 1.40 (d, $J=6.1$ Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.2 ($J=239.7$), 145.8, 134.3 ($J=8.9$ Hz), 119.7 ($J=6.5$ Hz), 116.7 ($J=23.5$ Hz) 106.0 ($J=25.3$ Hz), 67.2, 49.7, 28.2, 18.6. FTIR (KBr) 3112, 2985, 2849, 1730, 1599, 1480, 1460, 1438 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{11}FN_2O_2S$ [M^+] 242.0525, found 242.0526.

7-Chloro-3-methyl-1,2,3,4-tetrahydro-5-thia-2a,4-diaza-acenaphthylene 5,5-dioxide (18c). Compound **18c** was prepared from **18a** by the general procedure B to give an beige solid (76% yield); mp 205–207 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.07 (d, $J=11.3$ Hz, 1H), 7.29 (s, 2H), 4.64 (dq, $J=11.4$, 6.1 Hz, 1H), 3.61 (td, $J=8.5$, 3.0 Hz, 1H), 3.30 (q app, $J=8.9$ Hz, 1H), 3.06 (m, 2H), 1.41 (d, $J=6.2$ Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 148.3, 134.7, 128.46, 122.1, 121.1, 119.9, 67.2, 49.9, 28.5, 19.0. FTIR (KBr) 3278, 2865, 1604, 1588, 1491, 1466, 1437 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{11}ClN_2O_2S$ [M^+] 258.0230, found 258.0227.

1,3-Dimethyl-1,2,3,4-tetrahydro-5-thia-2a,4-diaza-acenaphthylene 5-oxide (18d). Compound **18d** was prepared as per **18b** and isolated as a 2:1 mixture of unassigned diastereomers; mp 168–173 °C. ^1H NMR (500 MHz, DMSO-d_6) δ 7.93 (d, $J=11.5$ Hz, 0.33H), 7.86 (d, $J=11.3$ Hz, 0.67 Hz), 7.26–7.23 (m, 2H), 6.78 (t, $J=7.6$ Hz, 0.33H), 6.73 (t, $J=7.6$ Hz, 0.67H), 4.66 (app sextet, $J=6.1$ Hz, 0.67H), 4.55 (app sextet, $J=6.1$ Hz, 0.33H), 3.73 (t, $J=8.4$ Hz, 0.33H), 3.48 (t, $J=8.7$ Hz, 0.67H), 3.40–3.37 (m, 1H), 3.22 (dd, $J=8.7$, 2.4 Hz, 0.67H), 2.83 (dd, $J=11.1$, 8.5, 0.33H), 1.42–1.40 (m, 3H), 1.30 (d, $J=6.7$ Hz, 1H), 1.20 (d, $J=7.0$ Hz, 2H). FTIR (KBr) 3434, 3323, 2959, 2853, 1597, 1488, 1449 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{14}N_2O_2S$ [M^+] 238.0776, found 238.0776.

7-Chloro-1,3-dimethyl-1,2,3,4-tetrahydro-5-thia-2a,4-diaza-acenaphthylene 5-oxide (18e). Compound **18e** was prepared from **18d** by the general procedure B; mp 184–187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (m, 1H), 7.09 (app t, J = 1.6 Hz, 1H), 4.68 (dq, J = 12.5, 6.1 Hz, 1H), 4.56 (d, J = 12.6 Hz, 1H), 3.74 (app t, J = 8.3 Hz, 1H), 3.47–3.40 (m, 1H), 2.80 (dd, J = 11.3, 8.3 Hz, 1H), 1.52 (d, J = 6.0 Hz, 3H), 1.36 (d, J = 6.8 Hz, 3H). HRMS (CI) calcd for $C_{11}H_{13}ClN_2O_2S$ [M^+] 272.0386, found 272.0380. FTIR (KBr) 3214, 2967, 2874, 1491, 1466, 1322 cm^{-1}.

5-Ethyl-3,3-dimethyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20a). Compound **19a** (308.0, 1.54 mmol) was dissolved in acetonitrile (3 mL) and treated with 2,2-dimethoxypropane (171.0 mg, 1.60 mmol) and camphorsulfonic acid (48.6 mg, 0.21 mmol) and stirred for 1 h. The reaction was then treated with saturated $NaHCO_3$ (3 mL) and extracted with ethyl acetate (2 × 5 mL). The organic layer was processed in the usual way to give the title compound (360.7 mg, 98%) as an off-white solid; mp 143–145 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 (s, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.17 (d, J = 7.3 Hz, 1H), 6.69 (dd, J = 7.8, 7.5 Hz, 1H), 5.98 (s, 1H), 2.50 (obscured by NMR solvent, 2H), 1.54 (s, 6H), 1.13 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.2, 132.4, 130.3, 122.5, 121.8, 121.8, 117.0, 69.8, 29.4, 24.5, 14.1. FTIR (KBr) 3354, 3191, 2977, 1598, 1570, 1560, 1491, 1447 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{16}N_2O_2S$ [M^+] 240.0932, found 240.0923.

7-Chloro-5-ethyl-3,3-dimethyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20b). Compound **19b** was subjected to the same conditions as **19a** to give the title compound in quantitative yield; mp 145–148 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.82 (s, 1H), 7.35 (s, 1H), 7.21 (s, 1H), 2.50 (obscured by NMR solvent, 2H) 1.54 (s, 6H), 1.13 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 140.1, 135.2, 131.9, 122.3, 121.3, 120.4, 69.9, 29.1, 24.2, 13.6. FTIR (KBr) 3385, 3225, 2973, 1596, 1498, 1458 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{15}ClN_2O_2S$ [M^+] 274.0543, found 274.0544.

7-Fluoro-3,3,5-trimethyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20c). Same procedure as **20b** with **19c** (100%); mp 171–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (s, 1H), 7.18–7.16 (m, 2H), 5.90 (s, 1H), 2.15 (s, 3H), 1.53 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.6 (d, J = 236.0 Hz), 138.7, 128.0 (d, J = 6.1 Hz), 122.0 (d, J = 23.0 Hz), 121.1, 107.6 (d, J = 23.3 Hz), 69.8, 29.1, 18.8. FTIR (KBr) 3378, 3280, 3215, 2987, 2932, 1505, 1478, 1412 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{13}FN_2O_2S$ [M^+] 244.0682, found 244.0683.

7-Fluoro-5-methyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20d). Same as general procedure C, except paraformaldehyde was used and the crude product required additional refluxing in ethanol with catalytic sulfuric acid to give the title compound; mp 212–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (t, J = 8.0 Hz, 1H), 7.20–7.17 (m, 2H), 6.38 (s, 1H), 4.61 (dd, J = 8.0, 3.6 Hz, 2H), 2.11 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.8 (d, J = 236.3 Hz), 138.7, 127.2 (d, J = 6.8 Hz), 121.4 (d, J = 6.8 Hz), 120.9 (d, J = 23.0 Hz), 106.9 (d, J = 23.9 Hz), 54.5, 17.6. FTIR (KBr) 3421, 3204, 3063, 2943, 1502, 1384, 1380, 1302 cm^{-1}. HRMS (CI) calcd for $C_8H_9N_2SO_2F$ [M^+] 216.0369, found 216.0369.

3-Ethyl-7-fluoro-5-methyl-3,4-dihydro-2*H*-benzo[1,2,4]-thiadiazine 1,1-dioxide (20e). Same as general procedure C, except propionaldehyde was used, mp 185–186 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (d, J = 11.8 Hz, 1H), 7.19 (m, 2H), 5.84 (s, br, 1H), 4.57–4.54 (m, 1H), 2.18 (s, 3H), 1.97 (m, 1H), 1.76 (m, 1H), 0.99 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.1 (d, J = 236.4 Hz), 138.7, 127.6 (d, J = 7.0 Hz), 121.7 (d, J = 6.1 Hz), 120.9 (d, J = 23.0 Hz), 106.8 (d, J = 23.4 Hz), 67.5, 26.6, 17.7, 9.3. FTIR (KBr) 3386, 3298, 3215, 3073, 2980, 1490, 1473 cm^{-1}. HRMS (CI) calcd for $C_{10}H_{13}FN_2O_2S$ [M^+] 244.0682, found 244.0677.

7-Fluoro-3-isopropyl-5-methyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20f). Same as general procedure C, except isovaleradehyde was used, mp 178–183 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.35 (d, J = 12.1 Hz, 1H), 7.22–7.18 (m, 2H), 5.60 (d, J = 2.64 Hz, 1H), 4.43 (ddd, J = 12.1, 6.0, 3.0 Hz, 1H), 2.18–2.15 (m, 4H), 1.05 (d, J = 6.7 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H);); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.2 (d, J = 237.7 Hz), 138.8, 128.1 (d, J = 7.6 Hz), 122.3 (d, J = 7.2 Hz), 121.0 (d, J = 22.9 Hz), 107.0 (d, J = 24.3 Hz),70.8, 30.7, 18.7, 17.6, 17.0. FTIR (KBr) 3417, 3238, 3204, 2969, 2880, 1495, 1422, 1344, 1311, 1232 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{15}FN_2O_2S$ [M^+] 258.0855, found 258.0849.

3-*tert*-Butyl-7-fluoro-5-methyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20g). Same as general procedure C, except pivaldehyde was used, mp 252–253 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.30 (d, J = 12.4 Hz, 1H), 7.26–7.23 (m, 2H), 4.89 (d, J = 3.3 Hz, 1H), 4.38 (dd, J = 12.4, 3.4 Hz, 1H), 2.24 (s, 3H), 1.0 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.6 (d, J = 236.3 Hz), 138.6, 128.8 (d, J = 7.5 Hz), 123.2 (d, J = 7.5 Hz), 121.0 (d, J = 22.5 Hz), 107.0 (d, J = 22.5 Hz),73.5, 35.1, 25.4, 17.4. FTIR (KBr) 3427, 3247, 3078, 2974, 1496, 1478 cm^{-1}. HRMS (CI) calcd for $C_{12}H_{17}FN_2O_2S$ [M^+] 272.0995, found 272.0991.

3-(But-3-enyl)-7-fluoro-5-methyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine 1,1-dioxide (20 h). Same as general procedure C, except 4-butenal was used, mp 151–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J = 11.8 Hz, 1H), 7.21–7.18 (m, 2H), 5.92–5.82 (m, 2H), 5.09 (d, J = 17.2 Hz, 1H), 5.02 (d, J = 9.3 Hz, 1H), 4.65 (m, 1H), 2.27–2.20 (m, 5H), 2.12–2.01 (m, 1H), 1.91–1.82 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.2 (d, J = 230.0 Hz), 138.7, 137,6, 127.7 (d, J = 10.0 Hz), 121.8 (d, J = 10.0 Hz), 121.1 (d, J = 20.0 Hz), 115.5, 106.9 (d, J = 20.0 Hz), 65.6, 32.5, 28.4, 17.8. FTIR (KBr) 3317, 3118, 3062, 2929, 1477 cm^{-1}. HRMS (CI) calcd for $C_{12}H_{15}FN_2O_2S$ [M^+] 270.0838, found 270.0846.

7-Fluoro-5-methyl-3-phenyl-3,4-dihydro-2*H*-benzo[1,2,4]-thiadiazine 1,1-dioxide (20i). Compound **19c** (222 mg, 1.1 mmol) was dissolved in acetonitrile (9.0 mL) and treated with benzaldehyde (0.35 mL, 3.4 mmol) and camphorsulfonic acid (307 mg, 1.2 mmol) and stirred for 24 h. The crude product was filtered through a pad of Celite and concentrated in vacuo. Column chromatography (25% ethyl acetate/hexanes) gave the pure product (308 mg, 97%); mp 183–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 12.0 Hz, 1H), 7.66 (m, 2H), 7.45 (m, 3H), 7.26 (m, 2H), 6.20 (app. d, J = 1.5 Hz, 1H), 5.77 (d, J = 13.8 Hz, 1H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.5 (d, J = 241.4 Hz), 139.0, 137.6, 129.0, 128.6 (d, J = 6.8 Hz), 128.4, 127.6, 122.4 (J = 6.8 Hz), 121.3 (d, J = 22.9 Hz), 107.0 (d, J = 24.0 Hz), 68.7, 17.8. FTIR KBr) 3401, 3260, 3224, 3072, 1490, 1313 cm^{-1}. HRMS (CI) calcd for $C_{14}H_{13}FN_2O_2S$ [M$^+$] 293.0760, found 293.0760.

2-(7-Fluoro-3,5-dimethyl-1,1-dioxo-3,4-dihydro-1*H*-benzo[1,2,4]thiadiazin-2-yl)-ethanol (22a). Compound **16 h** (2.3 g, 9.9 mmol) was dissolved in THF (30 mL) and cooled to −78 °C. Then, *s*-butyllithium (12.0 mL, 1.1 M in hexanes, 10.9 mmol) was added slowly over 20 min. Ethylene glycol cyclic sulfate (1.84 g, 14.9 mmol) was dissolved in THF (10 mL) and added via cannula. The reaction stirred at −78 °C for 1 h, then room temperature for 1 h. Concentrated HCl (2.5 mL) was added, and the reaction stirred for an additional 18 h. Saturated $NaHCO_3$ (20 mL) was added and the reaction extracted with EtOAc (3 × 20 mL) and processed in the usual way. Flash chromatography (70% EtOAc/hexanes) gave the title compound (991 mg, 37% yield) as a white solid; mp 159–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27–7.22 (m, 2H), 6.06 (d, J = 4.0 Hz, 1H), 5.21 (dq, J = 6.4, 4.0 Hz, 1H), 3.54–3.44 (m, 2H), 2.98 (ddd, J = 14.5, 7.3, 7.3 Hz, 1H), 2.73 (ddd, J = 11.0, 11.0, 5.2 Hz, 1H), 2.21 (s, 3H), 1.52 (d, J = 6.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.6 (d, J = 238.0 Hz), 138.1, 128.2 (d, J = 7.5 Hz), 121.5 (d, J = 23.0 Hz), 119.5 (d, J = 6.0 Hz), 108.1, (d, J = 24.4 Hz), 65.7, 60.9, 43.3, 18.4, 17.1. FTIR (KBr) 3509, 3342, 3072, 2978, 1625, 1496, 1472, 1314 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{15}FN_2O_3S$ [M$^+$] 274.0787, found 274.0783.

2-(7-Chloro-5-ethyl-3-methyl-1,1-dioxo-3,4-dihydro-1*H*-benzo[1,2,4]thiadiazin-2-yl)-ethanol (22b). Same as for **22a**; mp 159–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (d, J = 2.0 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 6.35 (d, J = 3.0 Hz, 1H), 5.2 (m, 1H), 4.81 (t, J = 6.0 Hz, 1H), 3.51 (m, 2H), 2.96 (q, J = 7.0 Hz, 1H), 2.75 (q, J = 7.0 Hz, 1H), 2.55 (m, 2H), 1.53 (d, J = 7.0 Hz, 3H), 1.15 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 139.9, 133.4, 121.7, 122.0, 121.5, 121.1, 66.0, 61.3, 44.2, 23.4, 18.8, 12.9. FTIR (KBr) 3530, 3401, 2954, 2884, 1495, 1438, 1305, 1235, 1137, 1033, 859, 790, 686, 570 cm^{-1}. HRMS calcd for $C_{12}H_{17}SO_3N_2Cl$ [M$^+$] 305.0726, found 305.0724.

7-Fluoro-2-(2-methoxy-ethyl)-3,5-dimethyl-3,4-dihydro-2*H*-benzo[1,2,4]thiadiazine1,1-dioxide (23). Alcohol **22a** (122 mg, 0.45 mmol) was dissolved in THF (2.6 mL) and cooled to −78 °C. Then iodomethane (0.10 mL, 1.6 mmol) was added, followed by NaH (30 mg (60% dispersion in oil), 0.75 mmol). The reaction was monitored by TLC and quenched when starting material was no longer detected. The reaction was subsequently warmed to 0 °C and quenched with pH 7 buffer, followed by extraction with EtOAc (2 × 10 mL), brine washes (1 × 10 mL) and processing in the usual way. Column chromatography (50% ethyl acetate/hexanes) gave the title compound (74 mg, 58%) as a white solid; mp 105–107 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (m, 2H), 6.06 (d, J = 4.0 Hz, 1H), 5.22 (m, 1H), 3.44 (app t, J = 7.3, 6.7 Hz, 2H), 3.25 (s, 3H), 3.10 (m, 1H), 2.84 (m, 1H), 2.20 (s, 3H), 1.52 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.8 (d, J = 220 Hz), 138.0, 128.3 (d, J = 7.3 Hz), 121.5 (d, J = 23.0 Hz), 119.5 (d, J = 7.3 Hz), 108.2 (d, J = 24.0 Hz), 71.7, 65.7, 58.1, 40.7, 18.3, 17.1. FTIR (KBr) 3319, 3072, 2931, 2813, 1490, 1472, 1331 cm^{-1}. HRMS (CI) calcd for $C_{12}H_{17}FN_2O_3S$ [M$^+$] 288.0944, found 288.0947.

Acetic acid 2-(7-fluoro-3,5-dimethyl-1,1-dioxo-3,4-dihydro-1*H*-benzo[1,2,4]-thiadiazin-2-yl)-ethyl ester (24). Alcohol **22a** (147 mg, 0.56 mmol) was dissolved in pyridine (1 mL) and cooled to 0 °C, when acetic anhydride (0.20 mL, 0.21 mmol) was added dropwise. The reaction was warmed to room temperature and quenched with 1 N HCl (1 mL) and extracted with ethyl acetate, washed with brine and processed in the usual way. Column chromatography (65% ethyl acetate/hexanes) gave the title compound as a white solid (145 mg, 86%); mp 100–102 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (m, 2H), 6.10 (d, J = 3.8 Hz, 1H), 5.23 (m, 1H), 4.12 (m, 2H), 3.21 (m, 1H), 2.93 (m, 1H), 2.20 (s, 3H), 2.00 (s, 3H), 1.53 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 154.0 (d, J = 238 Hz), 138.3, 128.6 (d, J = 7.0 Hz), 122.0 (d, J = 23.0 Hz), 119.7 (d, J = 7.0), 108.5 (d, J = 24.3 Hz), 66.1, 63.5, 40.39, 21.0, 18.4, 18.0. FTIR (KBr) 3354, 3072, 2987, 2961, 1728, 1472, 1326 cm^{-1}. HRMS (CI) calcd for $C_{13}H_{17}FN_2O_4S$ [M$^+$] 316.0893, found 316.0891.

4-Benzyl-7-fluoro-2-(2-methoxy-ethyl)-3,5-dimethyl-3,4-dihydro-2*H*-benzo[1,2,4] thiadiazine 1,1-dioxide (25). Sodium cyanoborohydride (2.0 g, 32.0 mmol) was dissolved in glacial acetic acid (40.0 mL) and cooled to 0 °C. Then, **20i** (2.3 g, 7.7 mmol) was dissolved in THF (10 mL) and added slowly via cannula to the sodium cyanoborohydride solution. The reaction was stirred for 24 h and quenched with sat. $NaHCO_3$ (200 mL) at 0 °C and stirred for an additional 2 h. The reaction contents were extracted with ethyl acetate (3 × 20 mL), washed with brine (1 × 20), and processed in the usual way. Column chromatography (50% ethyl ether/hexanes) gave the title compound (2.049 g, 90%), which was immediately subjected to the next reaction.

To the above material (157 mg, 53 mmol) was added acetonitrile (4.0 mL) and 3 Å mol sieves. Excess acetaldehyde (1.0 mL) was added dropwise, followed by a catalytic amount of camphorsulfonic acid. After the reaction was stirred for 5–6 h, it was filtered through a pad of Celite and concentrated in vacuo to give the title compound (162 mg, 95%); mp 178–180 °C. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 8.55 (d, $J=6.2$ Hz, 1H), 7.52 (m, 2H), 7.46 (m, 3H), 7.40 (m, 3H), 7.30 (m, 1H), 4.48 (m, 1H), 4.26 (d, $J=16.2$ Hz, 1H), 4.20 (d, $J=16.2$ Hz, 1H), 2.36 (s, 3H), 1.30 (d, $J=6.9$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.2 (d, $J=244.8$ Hz), 140.7 (d, $J=2.5$ Hz), 138.3, 137.9 (d, $J=7.8$ Hz), 132.3 (d, $J=7.3$ Hz), 128.5, 127.4, 127.2, 122.2 (d, $J=22.1$ Hz), 108.0 (d, $J=24.5$ Hz), 66.3, 54.9, 19.9, 18.5. FTIR (KBr) 3237, 3060, 3001, 2943, 2849, 1601, 1472, 1331 cm^{-1}. HRMS (CI) calcd for $C_{16}H_{17}FN_2O_2S$ [M$^+$] 321.1073, found 321.1065.

2 - Benzyl - 7 - fluoro - 3,5 - dimethyl - 3,4 - dihydro - 2*H* - benzo[1,2,4]thiadiazine 1,1-dioxide (26a). Compound **16 h** (190 mg, 0.833 mmol) was dissolved in acetonitrile (10 mL) and treated with benzylbromide (0.2 mL, 1.7 mmol), K_2CO_3 (200 mg, 1.4 mmol), and refluxed overnight. Upon cooling, ethyl acetate (25 mL) was added and the solution was washed with water. The combined organic layers were processed in the usual way and the crude product was purified by flash chromatography (40% ethyl acetate: hexanes) to give the title compound (180 mg, 68%); mp 138–139 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (m, 7H), 6.25 (d, $J=4.0$ Hz, 1H), 5.37 (m, 1H), 4.15 (d, $J=16.0$ Hz, 1H), 4.07 (d, $J=16.0$ Hz, 1H), 2.23 (s, 3H), 1.42 (d, $J=7.0$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 154.7 (d, $J=238$ Hz), 139.8, 139.1, 129.3, 129.0 (d, $J=9$ Hz), 128.4, 128.1, 127.9, 122.6 (d, $J=23$ Hz), 109.1 (d, $J=24$ Hz), 67.1, 45.4, 19.6, 18.8. FTIR (KBr) 3365, 3072, 2983, 1617, 1476, 1330, 1238, cm^{-1}. HRMS calcd for $C_{16}H_{17}SO_2N_2F$ [M$^+$] 320.0995, found 320.0985.

(7 - Fluoro - 3,5 - dimethyl - 1,1 - dioxo - 3,4 - dihydro - 1*H* - benzo[1,2,4]thiadiazin-2-yl)-acetonitrile (26b). Compound **16 h** (325 mg, 1.4 mmol) was dissolved in acetonitrile (7 mL) and treated with K_2CO_3 (319 mg, 2.3 mmol), bromoacetonitrile (0.20 mL, 2.9 mmol) and refluxed. After 24 h, the reaction was cooled to room temperature, diluted with water, extracted with ethyl acetate, washed with brine, and processed in the usual way. Flash chromatography (40% ethyl acetate:hexanes) gave the title compound (315 mg, 62%); mp 162–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (m, 2H), 6.27 (d, $J=3.3$ Hz, 1H), 5.29 (m, 1H), 4.22 (d, $J=18.5$ Hz, 1H), 4.14 (d, $J=18.5$ Hz, 1H), 2.20 (s, 3H), 1.62 (d, $J=6.4$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.8 (d, $J=238.5$ Hz), 138.0, 128.4 (d, $J=7.0$ Hz), 122.1 (d, $J=23.1$ Hz), 119.2 (d, $J=7.4$ Hz), 116.7, 108.0 (d, $J=24.5$ Hz), 65.8, 30.0, 17.9, 17.7. FTIR (KBr) 3386, 3063, 2991, 2556, 1491, 1335, 1335 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{12}FN_3O_2S$ [M$^+$] 269.0634, found 269.0638.

(7 - Fluoro - 3,5 - dimethyl - 1,1 - dioxo - 3,4 - dihydro - 1*H* - benzo[1,2,4]thiadiazin-2-yl)-acetic acid methyl ester (26c) method A (*sec*-BuLi). Compound **16 h** (1.08 g, 4.7 mmol) was dissolved in THF (20 mL) and cooled to −78 °C. A solution of *sec*-butyllithium (7.0 mL, 0.73 M in hexanes, 5.1 mmol) was added dropwise. Then, the reaction was warmed to 0 °C for 15 min and subsequently cooled to −78 °C, when methylbromoacetate (0.50 mL, 5.3 mmol) was added dropwise. The reaction was stirred to room temperature overnight, then quenched with a saturated solution of NH_4Cl (20 mL), extracted with EtOAc, washed with brine (2 × 10 mL), and processed in the usual way to give the crude product. Flash chromatography (50% ethyl acetate/hexanes) gave the title compound (1.05 g, 74%).

Method B (K_2CO_3). Compound **16 h** (542 mg, 2.4 mmol) was dissolved in acetonitrile (10 mL) and treated with K_2CO_3 (503 mg, 3.6 mmol) and methylbromoacetate (0.35 mL, 3.7 mmol). The reaction was then heated to reflux until starting material was completely consumed. Then, the reaction was cooled to room temperature, diluted with water, extracted with ethyl acetate, washed with brine, and processed in the usual way to give the crude product. Flash chromatography (40% ethyl acetate/hexanes) gave the title compound (681 mg, 96%); mp 150–151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (m, 2H), 6.10 (d, $J=3.9$ Hz, 1H), 5.28 (m, 1H), 3.83 (d, $J=17.8$ Hz, 1H), 3.67 (s, 3H), 3.60 (d, $J=17.7$ Hz, 2H), 2.21 (s, 3H), 1.48 (d, $J=6.4$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 153.8 (d, $J=238.3$ Hz), 137.9, 128.6 (d, $J=7.0$ Hz), 121.8 (d, $J=23.1$ Hz), 119.9 d, $J=7.0$ Hz), 108.0 (d, $J=24.3$ Hz), 65.7, 52.1, 42.7, 17.8, 17.7. FTIR (KBr) 3386, 3079, 2958, 1744, 1493, 1328 cm^{-1}. HRMS (CI) calcd for $C_{12}H_{15}FN_2O_4S$ [M$^+$] 302.0736, found 302.0736.

(7 - Fluoro - 3,5 - dimethyl - 1,1 - dioxo - 3,4 - dihydro - 1*H* - benzo[1,2,4]thiadiazin-2-yl)-acetic acid (26d). Compound **26c** (453 mg, 1.5 mmol) was dissolved in THF (3 mL) and treated with a solution of sodium hydroxide (1 mL, 10 M, 10 mmol). The reaction was stirred for 1 h, then acidified with 1 M HCl, and extracted with ethyl acetate. The combined organics were processed in the usual way to give the title compound (429 mg, 99%); mp 164–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27 (m, 2H), 6.08 (d, $J=3.9$ Hz, 1H), 5.28 (m, 1H), 3.69 (d, $J=17.9$ Hz, 1H), 3.50 (d, $J=17.9$ Hz, 1H), 2.21 (s, 3H), 1.48 (d, $J=6.4$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 153.8 (d, $J=238.3$ Hz), 137.9, 128.6 (d, $J=7.0$), 121.7 (d, $J=23.0$ Hz), 120.1 (d, $J=7.0$ Hz), 108.0 (d, $J=24.0$ Hz), 65.6, 42.5, 17.9, 17.7. FTIR (KBr) 3384, 2942, 2755, 2662, 2557, 1732, 1488, 1324, cm^{-1}. HRMS (CI) calcd for $C_{11}H_{13}FN_2O_4S$ [M$^+$] 288.0580, found 288.0575.

(4-Benzyl-7-fluoro-3,5-dimethyl-1,1-dioxo-3,4-dihydro-1*H*-benzo[1,2,4]thiadiazin-2-yl)-acetic acid methyl ester (26e). Method A afforded 581 mg (59%) of **26e**. Method B afforded the title compound (649 mg, 99%); mp 135–137 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (m, 7H), 4.55 (dd, $J=16.0$ Hz, 1H), 4.51 (q, $J=7.0$ Hz, 1H), 4.13 (dd, $J=16.0$ Hz, 1H), 4.01 (s, 2H), 3.65 (s, 3H), 2.37 (s, 3H), 1.39 (d, $J=6.8$ Hz, 3H). FTIR (KBr) 3069, 2979, 2868, 1762, 1604, 1476, 1437, 1364 cm^{-1}. HRMS (CI) calcd for $C_{19}H_{21}FN_2O_4S$ [M$^+$] 392.1206, found 392.1206.

2-(4-Benzyl-7-fluoro-3,5-dimethyl-1,1-dioxo-3,4-dihydro-1*H*-benzo[1,2,4]thiadiazin-2-yl)-ethanol (26f). Compound **26e** (300 mg, 0.77 mmol) was dissolved in THF (5.0 mL) and cooled to 0 °C. Lithium aluminumhydride

(0.0404 g, 1.1 mmol) was dissolved in THF (6.0 mL) and cooled to 0 °C in a separate flask. The solution of **26e** was then added dropwise to the stirring suspension of LAH via cannula. Then, water (4.0 mL) was added very slowly dropwise and stirred for 20 min. Next, a solution of 15% NaOH (4.0 mL) was added dropwise and stirred for an additional 20 min. Finally, water (12.0 mL) was added and stirred for an additional 20 min. The suspension was filtered to remove the inorganic salts and the filtrate was concentrated in vacuo to give the title compound (278 mg, 98%); mp 104–106 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (m, 7H), 4.83 (app. t, J = 5.0 Hz, 1H), 4.43 (q, J = 6.8 Hz, 1H), 4.27 (dd, J = 15.7 Hz, 1H), 4.09 (dd, J = 15.7 Hz, 1H), 3.50 (m, 2H), 3.26 (dd, J = 6.8 Hz, 2H), 2.39 (s, 3H), 1.37 (d, J = 6.8 Hz, 3H). HRMS (CI) calcd for $C_{18}H_{21}FN_2O_3S$ [M^+] 364.1257, found 364.1254. FTIR (KBr) 3495, 3049, 2990, 2931, 2861, 1601, 1467, 1449, 1290, 1208, 1149, 1102 cm^{-1}.

7-Fluoro-9-methyl-2,3,3a,4-tetrahydro-1*H*-5-thia-4,9b-diaza - cyclopenta[*a*]naphthalene-5,5-dioxide (28). The aldehyde (**27**) (190 mg, 0.79 mmol) was dissolved in acetonitrile (8 mL) and cooled to 0 °C. Then **19c** (158 mg, 0.77 mmol) and CSA (37 mg, 0.16 mmol) were added and the reaction stirred to room temperature overnight. The reaction was then refluxed for 4 h, then cooled to room temperature, concentrated in vacuo, and purified by flash chromatography (25% EtOAc/hexanes → 50% EtOAc/hexanes) to give the title compound (64%); mp 149–150 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73 (d, J = 11.2 Hz, 1H), 7.36 (dd, J = 7.5, 2.9 Hz, 1H), 7.30 (dd, J = 9.5, 2.9 Hz, 1H), 4.98 (dd, J = 11.1, 6.4 Hz, 1H), 3.68 (dd, J = 16.1, 7.5 Hz, 1H), 2.98 (ddd, J = 9.0, 9.0, 4.7 Hz, 1H), 2.35–2.17 (m, 2H), 2.29 (s, 3H), 1.92–1.89 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.6 (d, J = 242.0 Hz), 140.0, 134.0 (d, J = 7.2 Hz), 128.0 (d, J = 6.1 Hz), 122.1 (d, J = 22.8 Hz), 107.3 (d, J = 24.7 Hz), 71.8, 51.4, 31.2, 22.7, 19.9. FTIR (KBr) 3472, 3413, 1637, 1614, 1467, 1443 cm^{-1}. HRMS (CI) calcd for $C_{11}H_{13}FN_2O_3S$ [M^+] 256.0682, found 256.0680.

Biological Experimentals

Binding experiments

Binding experiments were carried out by Dr. Markus Kessler with membranes from rat brain and were prepared according to previously published procedures.[17] Binding assays were conducted at 25 °C with the centrifugation method. Aliquots of the membrane suspension containing 100 μg protein in 200 μL were incubated for 45 min with 50 nM [^{3}H]AMPA or 20 nM [^{3}H]fluorowillardiine, the compound to be tested, and appropriate additions. Initial tests with [^{3}H]AMPA as the radioligand were carried out in the presence of 50 mM KSCN), a chaotropic ion that greatly increases the affinity for AMPA and without which binding could not be reliable assayed; later assays utilized the more recent radioligand [^{3}H]fluorowillardiine which binds with high affinity and does require the inclusion of KSCN. Incubations were terminated by centrifugation (20 min at 25,000*g*). The supernatant was aspirated and the pellet quickly rinsed with ice-cold buffered saline containing 50 mM KSCN (wash buffer). Compounds were added from 100-fold concentrated solutions in dimethyl sulfoxide (DMSO); control samples received the equivalent concentration of DMSO. Prior tests determined the maximal solubility of each compound and verified that the dilution procedure used in the binding test does not result in compound precipitation. Background values ('non-specific binding') were measured by inclusion of 5 mM L-glutamate and subtracted from total binding; separate background values were determined for incubations with and without compound. Protein content was determined according to Bradford with the reagent available from Bio-Rad. Binding curves were fitted to the data points with non-linear regression (Prism, GraphPad; San Diego, CA, USA). [^{3}H]AMPA was purchased from NEN/Dupont (Boston, MA, USA) and [^{3}H]fluorowillardiine from Tocris Cookson, (St. Louis, MO, USA). All other reagents were commercially available.

EC_{50} values obtained in the agonist binding assays have generally been found to correspond to those from physiological tests, and thus binding provides an effective method to determine the affinity of AMPA receptor modulators. However, magnitude and direction of the binding change differs between compounds and, although some correlations have been found, there are no simple relationships with changes in physiological parameters. The size of the binding change also depends on the assay conditions, and thus an active compound might, in some circumstances, produce a zero-magnitude effect and appear inactive. To avoid such false negatives, basic compound tests were routinely carried out under two different assay conditions (e.g., [^{3}H]AMPA plus KSCN versus [^{3}H]fluorowillardiine without KSCN).

Patch-clamp recordings from pyramidal neurons in the field CA1

Patch clamp studies were carried out by Dr. Amy Arai with outside-out patches excised from CA1 pyramidal neurons of organotypic hippocampal slices. Slice cultures were prepared from 13–14-day-old Sprague–Dawley rats and grown for 2 weeks on cellulose membrane inserts (Millipore CM) in the incubator. For recording, a slice was transferred to a chamber and immersed in a medium containing: NaCl 125 mM, KCl 2.5 mM, KH_2PO_4 1.25 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, $NaHCO_3$ 5 mM, D-glucose 25 mM, and HEPES 20 mM (pH 7.3). A patch was excised and relocated to an adjacent recording chamber perfused with recording medium (NaCl 130 mM, KCl 3.5 mM, HEPES 20 mM, MK-801 0.01 mM, and D-AP5 0.05 mM). Patch pipettes had a resistance of 3–8 MOhm and were filled with a solution containing: CsF 65 mM, CsCl 65 mM, EGTA 10 mM, $MgCl_2$ 2 mM, ATP disodium salt 2 mM, and HEPES 10 mM (pH 7.3).

A piezo system was employed to rapidly switch solutions applied to the patch. In brief, background medium and glutamate containing medium were flowing

continuously through two lines of a bifurcated pipette. The excised patch was initially positioned in the background stream. Actuation of the piezo translater moved the patch into the glutamate containing flow line and returned it to the original position after 400–800 ms. Data were acquired with a patch amplifier (AxoPatch-1D) at filter frequency of 5 kHz and digitized at 10 kHz with PClamp/ Digidata 1200 (Axon Instrument). To test the effect of a compound, both flow lines were connected to different reservoirs delivering 'background medium + compound' and 'glutamate containing medium + compound'; after about 30 s equilibration, testing was resumed. Typically, five responses were collected and averaged for each condition and measurements with a given patch were alternated repeatedly between 'control condition' and 'test condition with compound'. Steady-state/peak ratios from two to six patches were averaged. Because of receptor desensitization, control responses typically decay with a time constant of about 12 ms until the current reaches a steady-state level of 5–10% of the peak current; compounds that effectively block desensitization raise the steady-state level to 100%. Holding potentials were 50 mV. Compound solutions were typically prepared from 500-fold stock solution in DMSO; the same final concentration of DMSO was included in all compound and control solutions.

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References and Notes

1. Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365.
2. Bridges, R. J., Chamberlin, A. R. In *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven: New York, 1993; p 231.
3. Doble, A. *Pharmacol. Ther.* **1999**, *81*, 163.
4. Kawasaki-Yatugi, S.; Ichiki, C.; Yatusugi, S.; Takahasahi, M.; Shimizu-Sasamata, M.; Yamaguchi, T.; Minematsu, K. *Neuropharmacology* **2000**, *39*, 211.
5. Masliah, E.; Mallory, M.; Hansen, L.; DeTeresa, R.; Terry, R. D. *Neurology* **1993**, *43*, 192.
6. Kordower, J. H.; Chu, Y.; Stebbins, G. T.; DeKosky, S. T.; Cochran, E. J.; Bennett, D.; Mufson, E. J. *Ann. Neurol.* **2001**, *49*, 202.
7. Tamminga, C. A. *Crit. Rev. Neurobiol.* **1998**, *12*, 21.
8. Granger, R.; Deadwyler, S.; Davis, M.; Moskovitz, B.; Kessler, M.; Rogers, G.; Lynch, G. *Synapse* **1996**, *22*, 332.
9. Lynch, G.; Granger, R.; Ambros-Ingerson, J.; Davis, C. M.; Kessler, M.; Schehr, R. *Exp. Neurol.* **1997**, *145*, 89.
10. Johnson, S. A.; Luu, N. T.; Herbst, T. A.; Lutz, D.; Rogers, G. A.; Lynch, G. *J. Pharmacol. Exper. Ther.* **1999**, *289*, 392.
11. Ito, I.; Tanabe, S.; Khoda, A.; Sugiyama, H. *J. Physiol.* **1990**, *424*, 533.
12. Yamada, K. A.; Rothman, S. M. *J. Physiol. (Lond.)* **1992**, *458*, 409.
13. Yamada, K. A.; Tang, C.-M. *J. Neurosci.* **1993**, *13*, 3904.
14. Yamada, K. A. *Neuro. Biol. Dis.* **1998**, *5*, 67.
15. Yamada, K. A. *Exp. Opin. Invest. Drugs* **2000**, *9*, 765.
16. Arai, A.; Kessler, M.; Xiao, P.; Ambros-Ingerson, J.; Rogers, G.; Lynch, G. A. *Brain Res.* **1994**, *638*, 343.
17. Arai, A.; Kessler, M.; Ambros-Ingerson, J.; Quan, A.; Yigiter, E.; Rogers, G.; Lynch, G. *Neuroscience* **1996**, *75*, 573.
18. Arai, A.; Kessler, M.; Rogers, G.; Lynch, G. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 627.
19. Sekiguchi, M.; Fleck, M. W.; Mayer, M. L.; Takeo, J.; Chiba, Y.; Yamashita, S.; Wada, K. *J. Neurosci.* **1997**, *17*, 5760.
20. Arai, A.; Lynch, G. *Brain Res.* **1992**, *598*, 173.
21. Granger, R.; Staubli, U.; Davis, M.; Perez, Y.; Nilsson, L.; Rogers, G. A.; Lynch, G. *Synapse* **1993**, *15*, 326.
22. Zivkovic, I., Thompson, D. M., Bertolino, M., Uzunov, D., Dibella, M., Costa, E., Guidotti, A. *J. Pharm. Exp. Ther.* **1995**, *272*, 300.
23. Larson, J.; Le, T.; Hall, R.; Lynch, G. *NeuroReport* **1994**, *5*, 389.
24. Arai, A.; Lynch, G. *Brain Res.* **1998**, *799*, 230.
25. Hjelmstad, G. O.; Isaac, J. T.; Nicoll, R. A.; Malenka, R. C. *J. Neurophysiol.* **1999**, *81*, 3096.
26. Hu, Y.; Yamada, K. A.; Chalmers, D. K.; Annavajjula, D. P.; Covey, D. F. *J. Am. Chem. Soc.* **1996**, *118*, 4550.
27. Bertolino, M.; Baraldi, M.; Parenti, C.; Braghiroli, D.; DiBella, M.; Vicini, S.; Costa, E. *Recept. Channels* **1993**, *1*, 267.
28. Uzunov, D. P.; Zivkovic, I.; Pirkle, W. H.; Costa, E.; Guidotti, A. *J. Pharmaceut. Sci.* **1995**, *84*, 937.
29. Pirotte, B.; Podona, T.; Diouf, O.; de Tullio, P.; Lebrun, P.; Dupont, L.; Somers, F.; Delarge, J.; Morain, P.; Lestage, P.; Lepagnol, J. M. S. *J. Med. Chem.* **1998**, *41*, 2946.
30. Pirrung, M. C.; Chau, J. H.-L.; Chen, J. *Chem. Biol.* **1995**, *2*, 621.
31. Pirrung, M. C.; Chen, J. *J. Am. Chem. Soc.* **1995**, *117*, 1240.
32. Girard, Y., Atkinson, J. G., Rokach, J. *J. Chem. Soc., Perkins Trans. 1* **1979**, 1043.
33. Kessler, M.; Rogers, G.; Arai, A. *Neurosci. Lett.* **2000**, *207*, 161.
34. Hall, R. A.; Kessler, M.; Quan, A.; Ambros-Ingerson, J.; Lynch, G. *Brain Res.* **1993**, *628*, 345.
35. Arai, A.; Guidotti, A.; Costa, E.; Lynch, G. *NeuroReport* **1996**, *7*, 2211.
36. Desos, P.; Serkiz, B.; Morain, P.; Lepagnol, J.; Cordi, A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3003.
37. Gavezzotti, A. *J. Am. Chem. Soc.* **1985**, *107*, 962.
38. Jackson, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2199.
39. Ornstein, P. L.; Zimmerman, D. M.; Arnold, M. B.; Bleisch, T. J.; Cantrell, B.; Simon, R.; Zarrinmayeh, H.; Baker, S. R.; Gates, M.; Tizzano, J. P.; Bleakman, D. *J. Med. Chem.* **2000**, *43*, 4354.
40. Parke, D. V.; Williams, R. T. *J. Chem. Soc.* **1950**, 1757.