

## Communications to the Editor

### Biarylpropylsulfonamides as Novel, Potent Potentiators of 2-Amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic Acid (AMPA) Receptors

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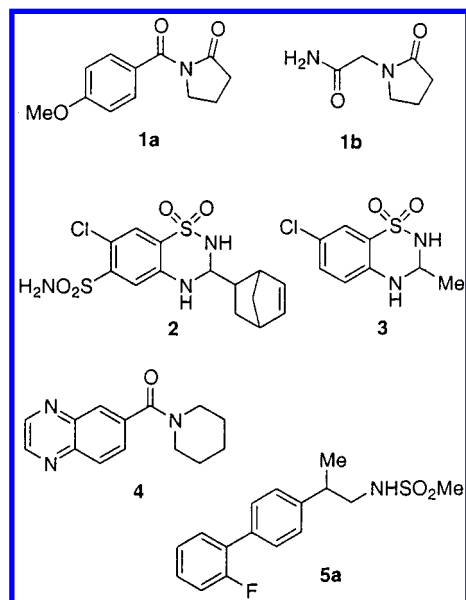
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**Introduction.** Glutamic acid is the major excitatory neurotransmitter in the central nervous system, exerting its actions at multiple subtypes of excitatory amino acid (EAA) receptors.<sup>1</sup> 2-Amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic acid (AMPA) receptors are a subtype of the ligand gated ion channel (ionotropic) family of EAA receptors, which may be composed of assemblies of four different receptor protein subunits, GluR1–4.<sup>2</sup> In addition, two splice variant forms of each of the four AMPA receptor proteins have been characterized, named flip and flop.<sup>3</sup> Signals are transduced at AMPA receptors through conductance of sodium and calcium ions into cells upon activation by glutamic acid.

Recent studies have identified pharmacological agents that enhance ion influx through AMPA receptors by positive allosteric modulation. Pyrrolidinones **1a** (aniracetam) and **1b** (piracetam) (Chart 1) are examples of compounds that potentiate AMPA receptor-mediated responses.<sup>4</sup> Furthermore, these compounds exhibit nootropic properties in animals and humans.<sup>4,5</sup> Subsequent studies have identified other compounds, such as the benzothiadiazides **2** (cyclothiazide)<sup>6</sup> and **3** (IDRA-21)<sup>7</sup> and the benzamide **4** (CX-516),<sup>8</sup> that are also AMPA receptor potentiators (Chart 1).

There is interest in the development of AMPA potentiators for the treatment of cognitive disorders. Compounds that potentiate AMPA receptor function facilitate performance in a wide variety of learning and memory tasks in rats<sup>8–11</sup> and primates.<sup>12</sup> Data has been reported on the use of AMPA potentiator **4** in human studies; however, its relatively weak potency and short half-life necessitated high doses.<sup>13,14</sup> Thus, there is significant need to develop AMPA potentiators with greater potency as therapeutic agents.

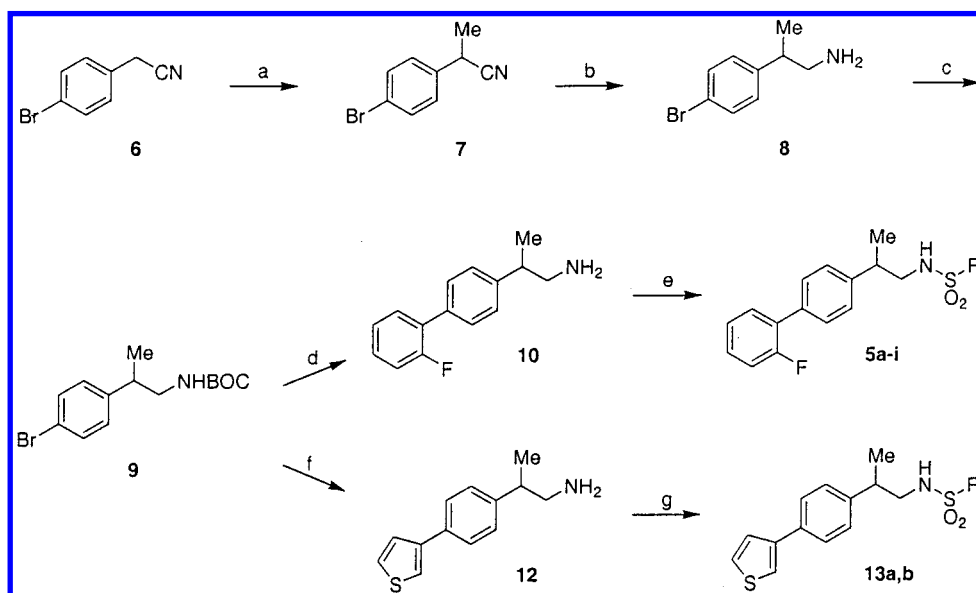
Chart 1



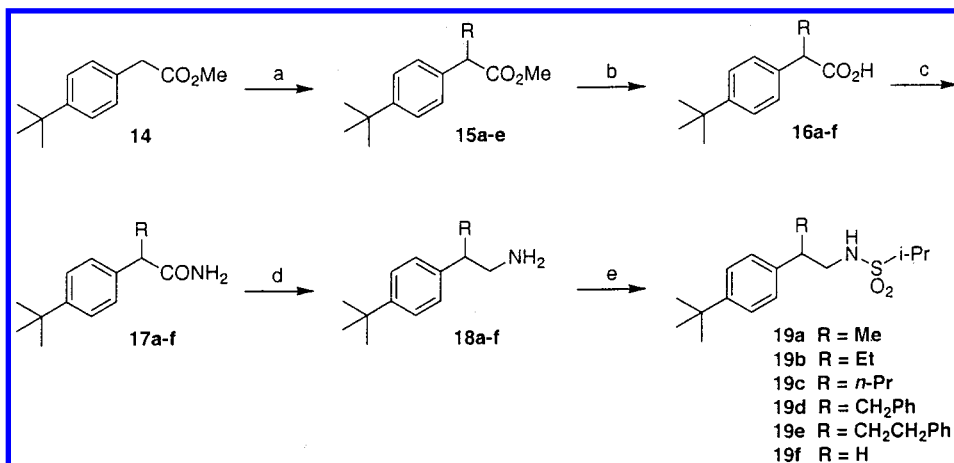
We have previously reported the cloning of AMPA receptor proteins and their homomeric expression in stable cell lines.<sup>15</sup> Using human GluR4 receptors expressed in HEK-293 cells, we developed an assay that measured responses mediated through AMPA receptors by determining changes in intracellular calcium concentrations. We identified compound **5a** as a novel AMPA receptor potentiator lead using this technology for high-throughput screening of the Lilly archival database. Key features of compound **5a** include a methanesulfonamide group connected to an aromatic ring by a two-methylene spacer, a methyl group on the carbon adjacent to the aromatic ring, and an *o*-fluorophenyl group attached to the aromatic ring para to the two-methylene spacer. These functional groups represent aspects of the structure–activity relationship (SAR) that we modified with the goal of increasing the AMPA potentiator potency of **5a**. In this Communication, we describe some of our initial SAR studies that allowed us to identify highly potent AMPA potentiators.

**Chemistry.** Analogues of **5a** were prepared as shown in Scheme 1. We converted 4-bromophenylacetonitrile **6** to 4-bromophenylpropionitrile **7** with potassium carbonate and dimethyl carbonate, then reduced the nitrile to amine **8** using borane–dimethyl sulfide complex. After protection of the amine as the *tert*-butoxycarbonyl (BOC) derivative **9**, Suzuki coupling with 2-fluorobenzeneboronic acid and removal of the BOC group gave the amine **10**. Reaction of **10** with a variety of sulfonyl chlorides using 2% cross-linked polyvinylpyridine then yielded the desired sulfonamides **5a–i**. If we performed the same sequence of reactions but omitted the methylation step and used isopropylsulfonyl chloride, we obtained sulfonamide **11** (see Table 1 for structure). Alternatively, reaction of **9** with 3-thienylboronic acid

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Scheme 1<sup>a</sup>

<sup>a</sup> (a)  $\text{Me}_2\text{CO}_3$ ,  $\text{K}_2\text{CO}_3$ , 180 °C, sealed vessel, 16 h; (b)  $\text{BH}_3\cdot\text{SMe}_2$ , THF, reflux, overnight, 5 N HCl, MeOH; (c)  $\text{BOC}_2\text{O}$ ,  $\text{CHCl}_3$ , satd  $\text{NaHCO}_3$ , rt, 1 h; (d) 2-fluorobenzeneboronic acid,  $\text{K}_2\text{CO}_3$ , toluene,  $\text{Pd}(\text{PPh}_3)_4$ , 90 °C, 18 h, 20% TFA/ $\text{CH}_2\text{Cl}_2$ , rt, 2 h; (e)  $\text{RSO}_2\text{Cl}$  (see Table 1 for R), 2% cross-linked polyvinylpyridine,  $\text{CH}_2\text{Cl}_2$ , rt, overnight; (f) 3-thienylboronic acid,  $\text{K}_2\text{CO}_3$ , toluene,  $\text{Pd}(\text{PPh}_3)_4$ , 80 °C, 20% TFA/ $\text{CH}_2\text{Cl}_2$ , rt, 2 h; (g)  $i\text{-PrSO}_2\text{Cl}$  (for R =  $i\text{-Pr}$ ) or  $\text{MeSO}_2\text{Cl}$  (for R = Me),  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , rt, overnight, aminomethylpolystyrene.

Scheme 2<sup>a</sup>

<sup>a</sup> (a)  $\text{LiN}(\text{SiMe}_3)_2$ , THF, -78 °C, then RBr (see Table 1 for R; MeI for R = Me), 2 h; (b) LiOH,  $\text{H}_2\text{O}$ , MeOH, THF, rt, overnight; (c)  $(\text{ClCO})_2$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 2 h, 28%  $\text{NH}_4\text{OH}$ , MeOH, rt, overnight; (d)  $\text{BH}_3\cdot\text{THF}$ , THF, rt, overnight, MeOH, THF, 5 N NaOH, rt; (e)  $i\text{-PrSO}_2\text{Cl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , rt, overnight.

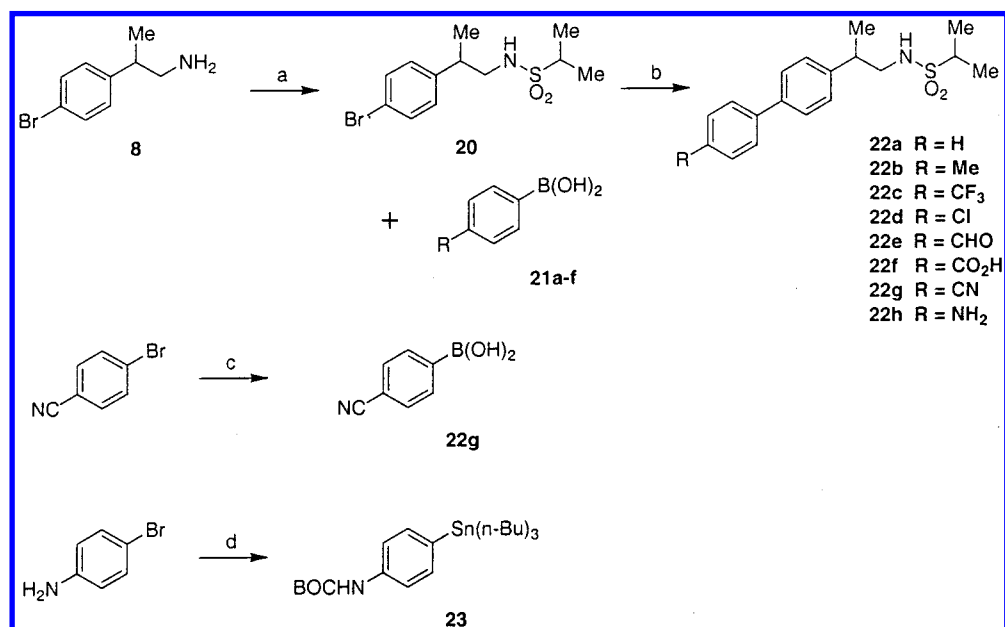
followed by removal of the BOC group afforded amine **12**, which upon reaction with either methyl- or isopropylsulfonamide afforded sulfonamides **13a,b**, respectively (Scheme 1).

To examine the effects of changing the benzylic substituent, we used a slightly different platform, the synthesis being shown in Scheme 2. Alkylation of the lithium enolate from methyl (4-*tert*-butylphenyl)acetate **14** with a variety of alkyl bromides (except for methyl, where we used the iodide) afforded the derivatives **15a-e**. Hydrolysis to the acids **16a-e** followed by formation of the corresponding acid chlorides and then reaction with aqueous ammonia gave the amides **17a-e**. Reduction with borane yielded the amines **18a-e**, and reaction with isopropylsulfonamide afforded the desired sulfonamides **19a-e**. If we performed this sequence of reactions but omitted the alkylation step, we obtained the unsubstituted derivative **19f** (R = H).

We also wanted to explore the effects of substitution

on the 4-position of the aromatic ring distal to the sulfonamide (Scheme 3). Conversion of **8** to the isopropylsulfonamide **20** followed by palladium-mediated coupling with phenylboronic acid **21a** afforded the unsubstituted biphenyl analogue **22a**. Alternatively, coupling of **20** with various 4-substituted phenylboronic acids **21b-g** afforded the biphenyl analogues **22b-g**. To prepare the 4-amino derivative **22h**, 4-bromoaniline was first protected as the *N*-BOC derivative and then converted to the stannane **23**. Palladium-mediated coupling of **23** with **20** followed by deprotection gave **22h**.

**Pharmacology.** We evaluated all new compounds for their ability to potentiate responses mediated by 100  $\mu\text{M}$  L-glutamate in HEK-293 cells expressing iGluR4 flip.<sup>16</sup> The activities of test compounds at various concentrations were expressed as a percentage of responses evoked by 100  $\mu\text{M}$  cyclothiazide (**2**), and  $\text{EC}_{50}$  values were calculated; this data is shown in Table 1.

Scheme 3<sup>a</sup>

<sup>a</sup> (a) *i*-PrSO<sub>2</sub>Cl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to rt, overnight; (b) **21a–g**, K<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, dioxane/water, 100 °C, overnight; (c) 1.6 M *n*-BuLi, THF, –85 °C, (*i*-PrO)<sub>3</sub>B, –85 °C to rt, 1.5 h, 5 N HCl, 2.5 h; (d) NaSnMe<sub>3</sub>, THF, (BOC)<sub>2</sub>O, rt, 1 h, Et<sub>3</sub>N, (*n*-Bu)<sub>6</sub>Sn<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, 100 °C, 5 h.

Table 1. EC<sub>50</sub> Values for Novel AMPA Potentiators Using Homomerically Expressed iGluR4 Receptors Expressed in HEK-293 Cells

compd <sup>a</sup>	R	EC <sub>50</sub> ± SEM (μM) <sup>b</sup>	compd <sup>a</sup>	R	EC <sub>50</sub> ± SEM (μM) <sup>b</sup>	compd <sup>a</sup>	R	EC <sub>50</sub> ± SEM (μM) <sup>b</sup>
<b>2</b> <sup>c</sup>		3.8 ± 0.4	<b>5i</b>	NMe <sub>2</sub>	4.0 ± 0.3	<b>19f</b>	H	12.8 ± 3.0
<b>4</b> <sup>c</sup>		>1000	<b>10</b> <sup>c</sup>		>100	<b>22a</b>	H	1.0 ± 0.1
<b>5a</b>	Me	19.6 ± 3.0	<b>11</b>		7.2 ± 0.9	<b>22b</b>	Me	0.27 ± 0.09
<b>5b</b>	CF <sub>3</sub>	32.9 ± 4.9	<b>13a</b>	Me	4.5 ± 0.4	<b>22c</b>	CF <sub>3</sub>	>3
<b>5c</b>	Et	5.4 ± 0.5	<b>13b</b>	<i>i</i> -Pr	0.66 ± 0.16	<b>22d</b>	Cl	>3
<b>5d</b>	<i>n</i> -Pr	23.8 ± 2.2	<b>19a</b>	Me	1.2 ± 0.4	<b>22e</b>	CHO	0.25 ± 0.012
<b>5e</b>	<i>i</i> -Pr	4.4 ± 0.6	<b>19b</b>	Et	2.0 ± 0.5	<b>22f</b>	CO <sub>2</sub> H	1.4 ± 0.5
<b>5f</b>	<i>n</i> -Bu	>100	<b>19c</b>	<i>n</i> -Pr	27.9 ± 0.9	<b>22g</b>	CN	0.29 ± 0.1
<b>5g</b>	Ph	62.5 ± 16.8	<b>19d</b>	CH <sub>2</sub> Ph	>100	<b>22h</b>	NH <sub>2</sub>	0.13 ± 0.017
<b>5h</b>	CH <sub>2</sub> Ph	>100	<b>19e</b>	CH <sub>2</sub> CH <sub>2</sub> Ph	>100			

<sup>a</sup> All compounds are racemic. <sup>b</sup> EC<sub>50</sub> values ± standard error of the mean (SEM) for potentiation of responses mediated by 100 μM L-glutamate in HEK-293 cells expressing iGluR4 flip, relative to that of 100 μM **2** (cyclothiazide). <sup>c</sup> See Chart 1 for the structures of **2** and **4**. See Scheme 1 for the structure of **10**.

Data are also included in Table 1 for compounds **2** and **4**. All new compounds are racemic.

We first examined the effect of varying the sulfonamide group (present as methylsulfonamide in **5a**). For this part of our studies, we kept the (*o*-fluorobiphenyl)-propyl portion of the molecule intact. The trifluoromethanesulfonamide analogue **5b** was about 2-fold less potent than the parent **5a**. The ethyl- and isopropylsulfonamides **5c,e** were significantly more potent (about 4-fold) than **5a**, the *n*-propyl compound **5d** was equipotent, and the *n*-butyl compound **5f** was considerably less active. While a phenylsulfonamide was modestly

tolerated (**5g**), the benzylsulfonamide (**5h**) was less active. The sulfamide **5i** was also a particularly potent compound, better than **5a** and comparable to **5c** and **5e**. This is not surprising in light of the isosteric nature of the *N,N*-dimethylsulfamide relative to the isopropylsulfonamide. The des-sulfonamido compound **10** was inactive, speaking to the importance of this functional group for AMPA potentiation.

We next turned our attention to gauging the effects of changing substitution on the benzylic position. For this aspect of our SAR studies, we used a 4-*tert*-butylphenyl in lieu of the *o*-fluorobiphenyl and combined

this with the more optimal isopropylsulfonamide. Early in our SAR studies we discovered that the platform in which the distal phenyl of, e.g. **5e**, was replaced with a *tert*-butyl group also provided potent AMPA potentiators. We hypothesized that the distal aromatic ring might have a lipophilic interaction with the receptor protein, and therefore *tert*-butyl could be a viable replacement for this group. Our results with compounds such as **19a** appear to confirm our suspicions. The ready availability of methyl (4-*tert*-butylphenyl)acetate as a substrate for alkylation facilitated this aspect of the SAR. We prepared compounds **19a–e** which possess respectively a methyl, ethyl, *n*-propyl, benzyl, and phenylethyl substituent. Analogues **19a,b**, having either a methyl or ethyl, were comparably active; the *n*-propyl compound **19c** was less active; and the two aromatic substituted compounds **19d,e** were significantly less active.

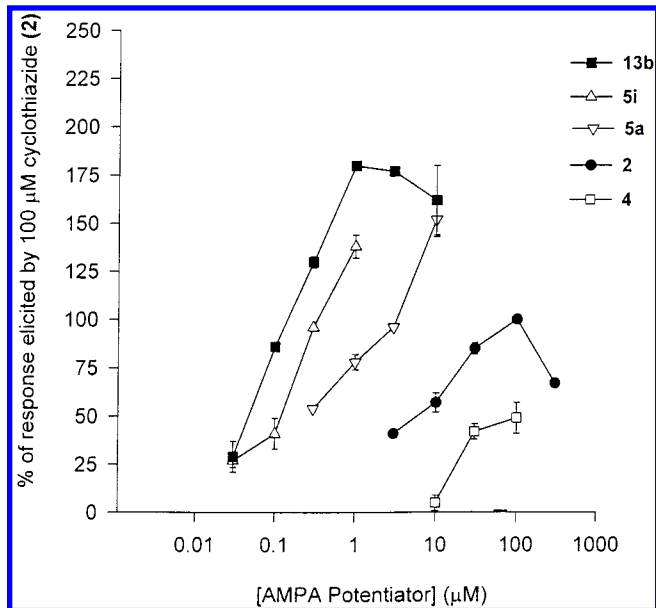
We prepared analogues of compounds **5e** and **19b**, which were identical except that they lacked the methyl substituent adjacent to the aromatic ring (**11** and **19f**). We found **11** and **19f** were less active than **5e** and **19b**, respectively, indicating the relative importance of the 2-arylpropylsulfonamide substructure found in our lead compound **5a**.

We examined replacement of the distal phenyl group with the well-documented isosteric 3-thienyl group. To our delight, we found that the activity of **13a** or **13b** was significantly greater than their counterparts **5a** or **5e**, respectively, with **13b** being nearly 7-fold more potent than **5e** and almost twice as potent as **22a**.

Finally, we directed our attention to substitution on the distal aromatic ring of the biphenyl group, focusing on substitution in the 4'-position. We first prepared the unsubstituted biphenyl analogue **22a**; its activity was about 4-fold better than that of **5e** and 20-fold better than that of the lead **5a**. We explored a range of electron-withdrawing and electron-donating substituents, including methyl (**22b**), trifluoromethyl (**22c**), chloro (**22d**), formyl (**22e**), carboxy (**22f**), cyano (**22g**), and amino (**22h**). While the compounds with a chloro- or trifluoromethyl group were less active than the unsubstituted derivative **22a**, the carboxy analogue was about equal in activity to **22a**. Even greater potency was observed for the methyl-, formyl-, cyano-, and amino-substituted compounds, with **22b,e,g** about 4-fold more potent than **22a**, and **22h** about 8-fold more than **22a**. All told, we realized a 150-fold increase in AMPA potentiator potency versus our lead compound **5a**.

A select group of compounds from this SAR (**5a,i** and **13b**) were evaluated using whole-cell voltage clamp recordings on acutely isolated cerebellar Purkinje cells to determine their ability to potentiate AMPA responses on a native rat brain receptor population. Acutely isolated cerebellar Purkinje neurons were isolated according to methods previously described.<sup>17,18</sup>

Figure 1 shows a comparison of activities of compounds **5a,i** and **13b**, along with **2** (cyclothiazide) and **4** (CX516) with responses expressed as a percentage of those evoked by 100  $\mu$ M **2**. These biarylpropylsulfonamide AMPA potentiators showed the same rank order of potency in native AMPA receptors that we observed in iGluR4 flip receptors in HEK-293 cells, with **13b** >



**Figure 1.** Effects of AMPA receptor modulators on inward currents evoked by glutamate (100  $\mu$ M) in acutely isolated rat cerebellar Purkinje neurons under whole-cell voltage clamp recording conditions ( $V_h = -70$  mV). Currents were evoked every 30 s by 10-s applications of glutamate. Compound was applied, and 10-s applications of glutamate continued every 30 s until steady-state potentiation of the evoked currents was reached. Data at each concentration of compound is expressed relative to the potentiation observed with **2** (cyclothiazide; 100  $\mu$ M). For each data point values are from at least 3 separate cells.

**5i** > **5a**. We also observed that these compounds were significantly more potent than **2** and **4** in these native AMPA receptors. The thienyl-substituted compound **13b** was 100-fold more potent than **2** and at least 1000-fold more potent than **4** (when comparing the concentration of compound required to produce a similar percentage of the response elicited by cyclothiazide). Thus, these are the most potent AMPA potentiators described to date.

Herein we disclosed a novel series of biarylpropylsulfonamides that are very potent potentiators of responses mediated through AMPA receptors. SAR studies demonstrated significant changes in potency when the methylsulfonamide was changed to an isopropylsulfonamide and when the *o*-fluorophenyl group of the lead **5a** was changed to a *tert*-butyl, a 3-thienyl, or a 4-cyanophenyl group. Further studies with this series of compounds will be reported soon.

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- (16) 96-Well plates containing confluent monolayers of HEK-293 cells stably expressing human GluR4 AMPA receptors were prepared. Cells were incubated in buffer solution (10 mM glucose, 138 mM sodium chloride, 1 mM magnesium chloride, 5 mM potassium chloride, 5 mM calcium chloride, 10 mM *N*-2-hydroxyethyl-piperazine-*N*-2-ethanesulfonic acid, to pH 7.1–7.3) containing 20  $\mu$ M Fluo3-AM dye (obtained from Molecular Probes Inc., Eugene, OR) for 60 min. Cells were washed with buffer solution and fluorescence measurements made using a Fluoroskan II fluorimeter (Labsystems, Needham Heights, MA) that indicated changes in fluorescence upon influx of calcium into cells upon stimulation by glutamate (100  $\mu$ M) in the presence of cyclothiazide (100  $\mu$ M) or compound.
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- (18) Dissociated cells were plated onto poly-L-lysine-coated glass coverslips (50  $\mu$ g/mL). Whole-cell voltage clamp recordings were made from isolated cells using extracellular solutions composed of 138 mM NaCl, 5 mM CaCl<sub>2</sub>, 5 mM KCl, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, and 10 mM glucose, with the pH adjusted to 7.4 with NaOH, and osmolality of 310 mOsm/kg; intracellular solutions were composed of 140 mM CsCl, 1 mM MgCl<sub>2</sub>, 14 mM diTris creatine phosphate, 50 U/mL creatine phosphokinase, 10 mM HEPES, and 15 mM BAPTA, with the pH adjusted to 7.15 with CsOH and osmolality of 295 mOsm/kg. Drug application was by bath perfusion and experiments were performed at room temperature (20–22 °C).

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