AMPA Receptor Agonists: Synthesis, Protolytic Properties, and Pharmacology of 3-Isothiazolol Bioisosteres of Glutamic Acid

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Received October 15, 1996⊗

A number of 3-isothiazolol bioisosteres of glutamic acid (**1**) and analogs of the AMPA receptor agonist, (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA, **2a**), including (*RS*)-2-amino-3-(3-hydroxy-5-methylisothiazol-4-yl)propionic acid (thio-AMPA, **2b**), were synthesized. Comparative in vitro pharmacological studies on this series of 3-isothiazolol and the corresponding 3-isoxazolol amino acids were performed using a series of receptor binding assays (IC_{50} values) and the electrophysiological rat cortical slice model (EC_{50} values). Whereas **2a** ($IC_{50} = 0.04 \pm 0.005$ μM, $EC_{50} = 3.5 \pm 0.2$ μM) is markedly more potent than the *tert*-butyl analog ATPA (**3a**) ($IC_{50} = 2.1 \pm 0.16$ μM, $EC_{50} = 34 \pm 2.4$ μM) in [^{3}H]AMPA binding and electrophysiological studies, **2b** ($IC_{50} = 1.8 \pm 0.13$ μM, $EC_{50} = 15.0 \pm 2.4$ μM) was approximately equipotent with thio-ATPA (**3b**) ($IC_{50} = 0.63 \pm 0.07$ μM, $EC_{50} = 14 \pm 1.3$ μM). (*RS*)-2-Amino-3-(3-hydroxyisoxazol-5-yl)propionic acid (HIBO, **4a**) was approximately equipotent with its thio analog **4b**, whereas 4-Br-HIBO (**5a**) ($IC_{50} = 0.65 \pm 0.12$ μM, $EC_{50} = 22 \pm 0.6$ μM) turned out to be much more potent than the corresponding 3-isothiazolol **5b** ($IC_{50} = 17 \pm 2.2$ μM, $EC_{50} = 500 \pm 23$ μM). **2b** ($ED_{50} = 130$ μmol/kg) was more potent than **2a** (220 μmol/kg) as a convulsant after subcutaneous administration in mice. The protolytic properties of **2a,b**−**4a,b** were determined using ^{13}C NMR spectroscopy. For each pair of compounds, the α-amino acid groups showed similar protolytic properties, whereas the 3-isoxazolol moieties typically showed pK_a values 2 units lower than those of the 3-isothiazolols. Accordingly, calculations of ionic species distributions revealed pronounced differences between 3-isoxazolol and 3-isothiazolol amino acids. No simple correlation between activity as AMPA agonists in vitro and pK_a values of these compounds was apparent. On the other hand, the relative potencies of AMPA (**2a**) and thio-AMPA (**2b**) in vitro and in vivo may reflect that these compounds predominantly penetrate the blood−brain barrier as net uncharged diprotonated ionic species.

Introduction

Glutamic acid (**1**) (Figure 1), which is the main excitatory neurotransmitter in the central nervous system (CNS), and other excitatory amino acids (EAAs) operate through four different classes of receptors. In addition to the three heterogeneous classes of ionotropic EAA receptors (iGluRs), named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA, **2a**), and kainic acid receptors,[1−3] a heterogeneous class of metabotropic EAA receptors (mGluRs) has been shown to have important functions in the central excitatory neurotransmission processes.[4] It is now generally agreed that iGluRs as well as mGluRs play important roles in the healthy as well as in the diseased CNS and that all subtypes of these receptors are potential targets for therapeutic intervention in a number of diseases.[5,6]

EAA receptors are involved in the mechanisms of long-term potentiation, which is believed to play an important role in learning and memory functions, and the deficits of these functions in the Alzheimer patients may, to some extent, be caused by hypoactivity at iGluRs and/or mGluRs in the brain.[7−10] There is also a growing evidence of an implication of EAA receptors in schizophrenia.[11,12] As in Alzheimer's disease, the role of these receptors in the etiology and the clinical manifestations of schizophrenia is still very incompletely understood, but there is evidence to suggest that

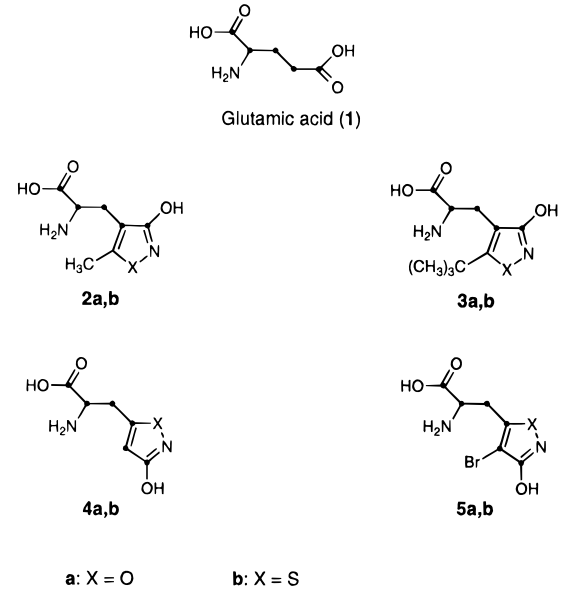

Figure 1. Structures of glutamic acid (**1**), the previously described AMPA receptor agonists AMPA (**2a**), ATPA (**3a**), HIBO (**4a**), and Br-HIBO (**5a**), and the new compounds thio-AMPA (**2b**), thio-ATPA (**3b**), **4b**, and **5b**.

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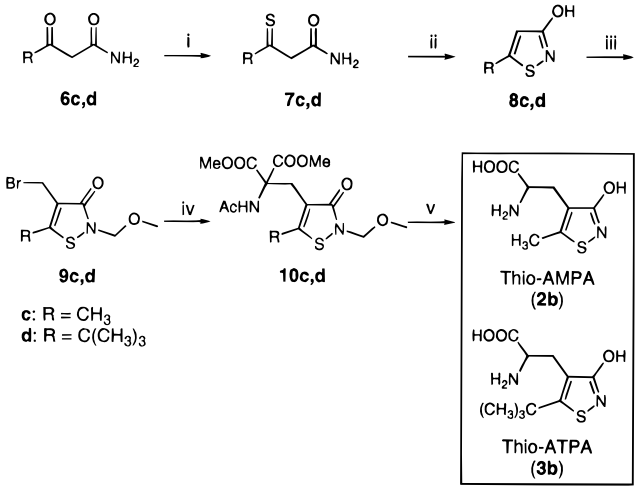
⊗ Abstract published in *Advance ACS Abstracts,* January 15, 1997.

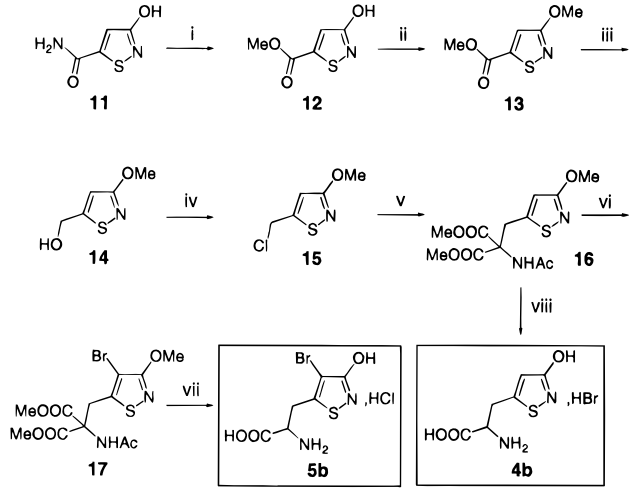
S0022-2623(96)00721-2 CCC: $14.00

Scheme 1[a]



[a] (i) H_2S, HCl; (ii) I_2, K_2CO_3; (iii) 1,3,5-trioxane, 62% aqueous HBr, MeOH; (iv) $AcNHCH(COOMe)_2$, NaH; (v) 1 M aqueous CF_3COOH.

Scheme 2[a]



[a] (i) MeOH, HCl; (ii) MeI, K_2CO_3; (iii) $NaBH_4$; (iv) $SOCl_2$; (v) $AcNHCH(COOMe)_2$, NaH; (vi) Br_2; (vii) 4 M HCl; (viii) 48% aqueous HBr.

hypoactivity at EAA receptors also is a factor of importance in the latter CNS disorder.[12−14]

In Alzheimer's disease as well as in schizophrenia, EAA receptor agonists, partial agonists, or functional partial agonists may have therapeutic interest.[15,16] While we have previously described the principles for the establishment of functional partial agonism at iGluRs,[10,15−18] attempts to develop truly partial agonists at these classes of EAA receptors have so far been unsuccessful.[15] Thus, structure−activity studies on a comprehensive series of analogs of AMPA (**2a**) containing alkyl groups of different sizes and polarities show either full AMPA agonism or inactivity.[19−25] Furthermore, although there is circumstantial evidence to suggest that the *tert*-butyl analog of AMPA, (*RS*)-2-amino-3-(5-*tert*-butyl-3-hydroxyisoxazol-4-yl)propionic acid (ATPA, **3a**), shows the capacity to enter the CNS after systemic administration,[26,27] principles for the design of EAA receptor ligands capable of penetrating the blood−brain barrier (BBB) have not been developed yet.

In the 4-aminobutyric $acid_A$ ($GABA_A$) agonist field, the ability to penetrate the BBB is largely determined by the protolytic properties of the zwitterionic compounds.[28−30] Although the 3-isothiazolol unit is markedly less acidic than the 3-isoxazolol unit, both of these heterocyclic systems are effective bioisosteres of the carboxyl group in this CNS transmitter system.[29,31] These aspects prompted us to synthesize the 3-isothiazolol analogs **2b**−**5b** of the previously described 3-isoxazolol AMPA receptor agonists **2a**−**5a**[19,32−36] and to describe the protolytic and pharmacological properties of these new compounds. The in vitro pharmacological studies comprise investigations of the relationship between AMPA receptor affinity and agonist potency and efficacy. The potencies of AMPA (**2a**) and thio-AMPA (**2b**) as convulsants after systemic administration in mice were compared.

Results

Chemistry. Thio-AMPA (**2b**) and thio-ATPA (**3b**) were synthesized as outlined in Scheme 1. Compound **8c** has previously been synthesized from **6c** via **7c**, the latter being converted into **8c** by oxidation with bromine.[37] In preliminary experiments, these reaction conditions resulted in the formation of 4-bromo-5-methylisothiazol-3-ol in varying quantities. With iodine as oxidizing agent, **7c** was converted into **8c** without formation of iodinated byproducts, and the reaction conditions for the conversion of **6c** into **8c** were successfully applied to the synthesis of **8d** from **6d**, although the yield of the intermediate **7d** (20%) was lower than that of **7c** (36%). In both cases, the desired reactions were accompanied by the formation of substantial amounts of decomposition products. Steric hindrance by the *tert*-butyl group may explain the particularly low yield of **7d**. Although the oxa analog of **9c** has been prepared in almost quantitative yield from 5-methylisoxazol-3-ol,[38] the same reaction only provided **9c**,**d** in yields of 27% and 39%, respectively. In attempts to increase these yields, prolonged reaction times and higher reaction temperatures led to extensive decomposition, whereas reduction of reaction time and/or temperature resulted in incomplete bromomethylation. Deprotection under acidic conditions of intermediates **10c**,**d** provided thio-AMPA (**2b**) and thio-ATPA (**3b**) in yields of 28% and 31%, respectively.

Compounds **4b** and **5b** were synthesized according to Scheme 2. Methylation of **12**, prepared by methanolysis of **11**,[39] provided **13** (68%) and the corresponding *N*-methylated product (not shown) in 26% yield. Compound **13** was reduced[40] to **14**, which was further converted into fully protected compound **16**. The deprotection of **16**, which required concentrated hydrobromic acid, was accompanied with marked decomposition of the product and provided **4b**·HBr in a yield of only 25%. Loss of bromine was observed after treatment of the brominated analog of **16**, compound **17**, with concentrated hydrobromic acid. Deprotection of **17** without significant loss of bromine was achieved by reflux in 4 M HCl for a period of less than 4 h, but, again, pronounced decomposition resulted in a low yield (20%) of **5b**·HCl.

Acidity Constants and Ionic Species Distribution. The use of ^{13}C NMR for determination of protolytic properties is based upon the measurement of chemical shift changes as a function of pH.[41] The pK_a values for **2a,b**, **3a,b**, **4a,b**, and **5a,b** (Table 1) were determined by nonlinear fitting of the NMR titration

Table 1. pK_a Values, Molar Fraction of Diprotonated Ionic Species (x_2), Receptor Binding, and Electrophysiological Data

compound	pK_a values	x_2, pH 7.4	IC_{50} (μM), [^{3}H]AMPA[a]	EC_{50} (μM), electrophysiology[a]
AMPA (**2a**)	1.94 ± 0.05,[h] 5.12 ± 0.03,[f] 10.09 ± 0.01[j]	0.01	0.04 ± 0.005[b]	3.5 ± 0.2[b]
thio-AMPA (**2b**)	1.84 ± 0.05,[j] 7.00 ± 0.01,[f] 10.27 ± 0.01[j]	0.28	1.8 ± 0.13	15.0 ± 2.4
ATPA (**3a**)	2.27 ± 0.13,[j] 4.76 ± 0.09,[g] 10.17 ± 0.02[j]	0.01	2.1 ± 0.16[c]	34 ± 2.4[d]
thio-ATPA (**3b**)	2.31 ± 0.05,[h] 7.03 ± 0.08,[h] 10.56 ± 0.07[j]	0.30	0.63 ± 0.07	14 ± 1.3
HIBO (**4a**)	1.90 ± 0.04,[i] 5.07 ± 0.16,[f] 8.81 ± 0.02[h]	0.01	1.5 ± 0.8[e]	370 ± 10[e]
4b	1.82 ± 0.05,[i] 6.69 ± 0.03,[g] 9.01 ± 0.05[g]	0.16	5.8 ± 1.4	380 ± 22
Br-HIBO (**5a**)	1.75 ± 0.06,[h] 3.49 ± 0.03,[f] 8.70 ± 0.02[h]	0.01	0.65 ± 0.12	22 ± 0.6
5b	1.64 ± 0.04,[h] 5.35 ± 0.04,[f] 8.77 ± 0.03[h]	0.01	17 ± 2.2	500 ± 23
glutamic acid (**1**)	2.23,[k] 4.37,[k] 9.53[k]	0.01		

[a] Mean ± SEM. [b] Reference 22. [c] Reference 21. [d] Reference 14. [e] Reference 36. [f–j] Based on carbon atoms C-3, C-4, α-CH, CH_2, and COOH, respectively. [k] Reference 43.

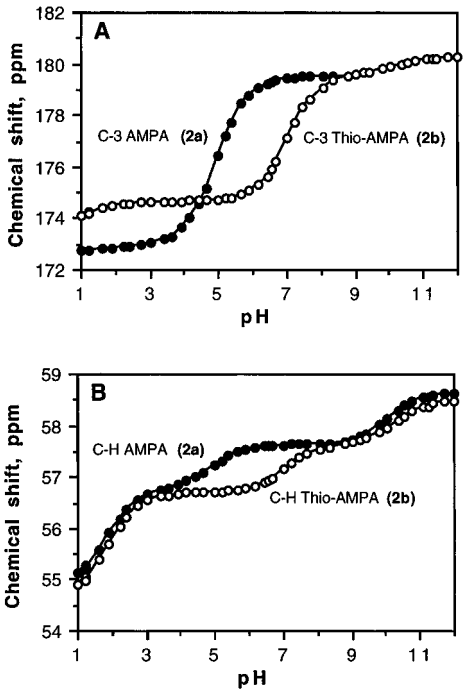


Figure 2. ^{13}C NMR titration curves for AMPA (**2a**) and thio-AMPA (**2b**) in D_2O (25 °C, ionic strength 1 M): (A) for C-3 carbon atoms and (B) for methine (CH) carbon atoms.

curve, defined by eq 1, to the experimental data for appropriate carbon atoms (Figure 2). In eq 1 δ_i is the observed chemical shift of the ith site, δ_i^0, δ_i^1, δ_i^2, and δ_i^3 are the chemical shifts of the ith site in non-, mono-, di-, and triprotonated ionic species, and K_1, K_2, and K_3 are the equilibrium constants for the successive deprotonations of fully protonated forms (Figure 3).

$$\delta_i = (\delta_i^0 K_1K_2K_3 + \delta_i^1 K_1K_2[H^+] + \delta_i^2 K_1[H^+]^2 + \delta_i^3[H^+]^3)/([H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3) \quad (1)$$

The NMR titration curves were obtained at relatively high salt concentration (1 M) to maintain a constant ionic strength during the experiment. The p$K_a$values of the amino and the caboxyl groups were closely similar for the 3-isoxazolol and the 3-isothiazolol analogs, whereas the pK_a values of the 3-hydroxyl group were 1.62−2.27 pK_a units lower in the 3-isoxazolol series than in the 3-isothiazolol series (Table 1). Introduction of the α-amino acid moiety and alkyl substituents lowered the pK_a values of the hydroxyl group by approximately 0.5 pK_a unit compared to the unsubstituted 3-isoxazolol and 3-isothiazolol units.[42] Replacement of the hydrogen atom in the 4-position of HIBO and **4b** with a bromine

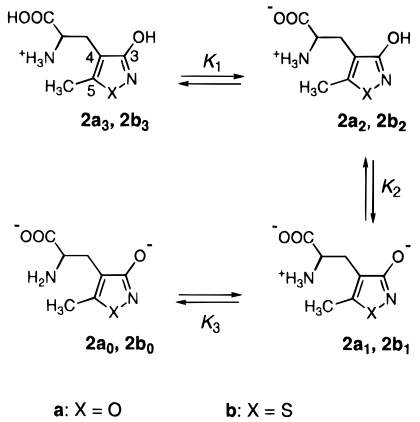


Figure 3. Structures of the triprotonated (**2a**$_3$, **2b**$_3$), diprotonated (**2a**$_2$, **2b**$_2$), monoprotonated (**2a**$_1$, **2b**$_1$), and nonprotonated (**2a**$_0$, **2b**$_0$) ionic species of AMPA (**2a**) and thio-AMPA (**2b**).

atom to give **5a**,**b**, respectively, decreased the pK_a value of the hydroxyl group by about 1.5 pK_a units as expected. Except for **5a**, the pK_a values of the two heterocyclic bioisosteres were higher than the pK_a value of the distal carboxyl group in glutamic acid.[43]

Based upon the pK_a values obtained, ionic species distribution curves (Figure 4) were calculated for the amino acids **2a,b**, **3a,b**, **4a,b**, and **5a,b** using eqs 2−6, where x_0, x_1, x_2, and x_3 are the molar fractions of non-, mono-, di-, and triprotonated species present.

$$x_0 = K_2K_3/[H^+]^2F \quad (2)$$

$$x_1 = K_2/[H^+]F \quad (3)$$

$$x_1 = 1/F \quad (4)$$

$$x_3 = [H^+]^2/K_1F \quad (5)$$

$$\text{where } F = [H^+]/K_1 + 1 + K_2/[H^+] + K_2K_3/[H^+]^2 \quad (6)$$

The molar fractions at pH = 7.4 of the net uncharged, diprotonated ionic species, x_2, in which the amino group as well as the 3-hydroxyl group are protonated and the carboxyl group is ionized, are shown in Table 1. The 3-isoxazolol analogs **2a**−**5a** as well as the 3-isothiazolol analog **5b** exist mainly as the negatively charged, monoprotonated ionic species at physiological pH, the hydroxyl and carboxyl groups being completely deprotonated. By contrast, the calculation of the molar fraction of the diprotonated species present in the solution of **2b**−**4b** gave values in the range of 0.16−0.30 (Table 1), suggesting that the net uncharged,

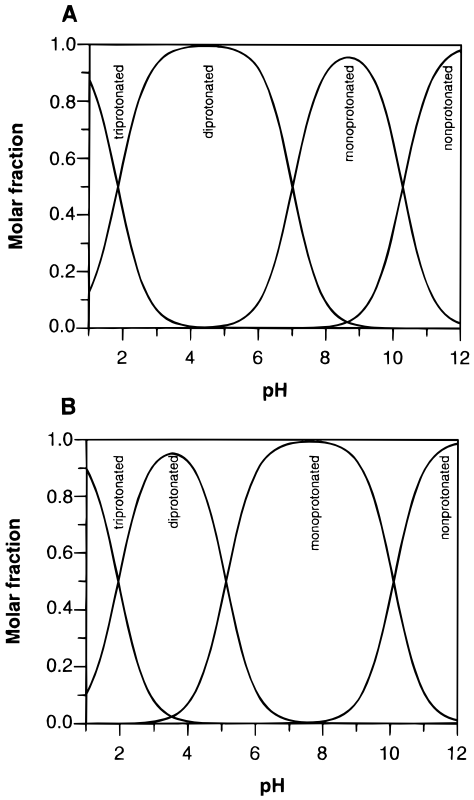


Figure 4. Ionic species distribution diagrams: (A) thio-AMPA (**2b**) and (B) AMPA (**2a**).

diprotonated ionic species are significant at physiological conditions.

In Vitro Pharmacology. The affinities of the new compounds for AMPA, NMDA, and kainic receptor sites were determined using [^{3}H]AMPA,[44] [^{3}H][3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid ([^{3}H]CPP),[45] and [^{3}H]kainic acid[46] as radioligands (Table 1). None of the compounds showed detectable affinity for [^{3}H]CPP- or [^{3}H]kainic acid-binding sites (IC_{50} > 100 μM). While AMPA (**2a**) is markedly more potent than ATPA (**3a**) in [^{3}H]AMPA binding, the affinities of thio-AMPA (**2b**) and thio-ATPA (**3b**) were comparable. Introduction of a bromine atom into the 4-position of the isoxazole ring of HIBO (**4a**) to give Br-HIBO (**5a**) is accompanied by an increase in affinity for AMPA binding sites. On the other hand, **5b** is proportionally weaker than **4b**, the 3-isothiazolol analog of **4a** (Table 1). With the exception of HIBO (**4a**) and **4b**, a relatively good correlation was observed between AMPA receptor affinity of the compounds and their agonistic effects (Figure 5) sensitive to the AMPA receptor antagonist 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX).[47] Compound **4b** and, in particular, **4a** showed weaker AMPA agonist effects than predicted from their AMPA receptor affinities. Interestingly, (*RS*)-2-amino-3-(3-hydroxyisoxazol-4-yl)propionic acid (demethyl-AMPA), which also has an unsubstituted position in the heterocyclic ring, is, like **4a**,**b**, a potent inhibitor of [^{3}H]AMPA binding but a very weak AMPA receptor agonist.[25] It has been hypothesized that the recognition site of the AMPA receptor contains a lipophilic cavity and that occupancy of this putative cavity by appropriately sized alkyl groups in the isoxazole ring of AMPA contributes to the stabilization of the agonist conformation of the AMPA receptor.[25] A similar effect may explain the relatively low agonist potency of **4a**,**b**.

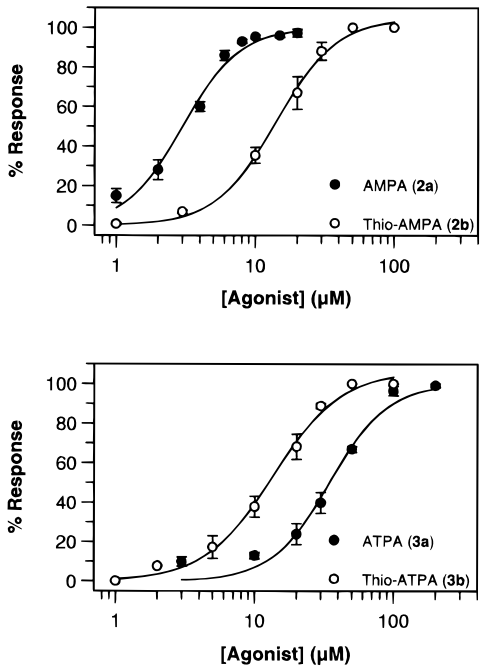


Figure 5. Dose−response curves as determined in the rat cortical wedge preparation for AMPA (**2a**) and analogs **2b** and **3a,b**. Values are mean values ± SEM relative to the maximal AMPA response. Data were fitted to the equation: % response = MAX × [Ago]^n/($EC_{50}{}^n$ + [Ago]^n), where MAX is the maximal response relative to the AMPA plateau response, [Ago] is the agonist concentration in μM, and n is the Hill slope, determined to be close to 2 for all compounds; 100% response is determined as the maximal response for AMPA (for details, see ref 16).

Table 2. Convulsive Potencies of AMPA (**2a**) and Thio-AMPA (**2b**) after Subcutaneous Administration in Mice

compound	ED_{50} (μmol/kg)[a]
AMPA (**2a**)	220
thio-AMPA (**2b**)	130

[a] Reference 27.

In Vivo Pharmacology. The convulsant activity of AMPA (**2a**) and thio-AMPA (**2b**) was studied in mice after subcutaneous administration. The results are shown in Table 2. Both AMPA (**2a**) and thio-AMPA (**2b**) induced convulsions, thio-AMPA (**2b**) being more potent than AMPA (**2a**).

Investigation of urine collected 30 min after the administration of 20 mg/kg **2b** by ^{1}H NMR strongly suggested that the compound is mainly excreted in unchanged form. Thus, the 400 MHz ^{1}H NMR spectrum of urine from the **2b**-treated mice contained a singlet at δ 2.40 (CH_3) not present in the urine from control mice (Figure 6). In addition, the eight lines of the AB part of the ABX system of the amino acid side chain (the methylene group) were readily recognizable around δ 3.0. The identity of the signals was confirmed by spiking with authentic **2b**.

Discussion

The synthesis and pharmacological characterization of the 3-isothiazolol amino acids **2b**−**5b**, which are analogs of the previously described 3-isoxazolol amino acids AMPA (**2a**),[32,33] ATPA (**3a**),[19,34] HIBO (**4a**),[32,36] and Br-HIBO (**5a**),[32,33] respectively (Table 1), served several purposes, including comparative in vitro pharmacological studies of these structurally related compounds. Like

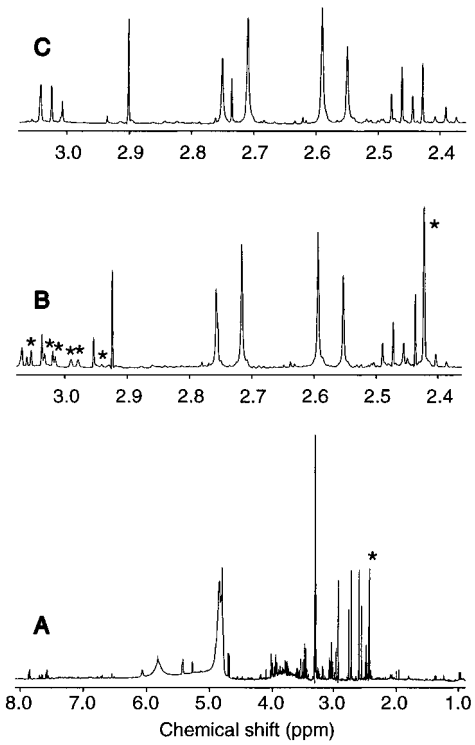


Figure 6. Detection of thio-AMPA (**2b**) in urine: (A) 400 MHz ^{1}H NMR spectrum of urine containing 10% D_2O, collected 30 min after subcutaneous administration of 20 mg/kg thio-AMPA (**2b**) to mice, (B) expansion of spectrum A showing signals of thio-AMPA (**2b**) (starred), and (C) corresponding region of the spectrum of urine collected from untreated mice.

2a−**5a**, all the new compounds were shown to be selective AMPA receptor agonists, exhibiting no detectable affinity for NMDA or kainic acid receptor sites (Table 1). However, no simple correlation between structure and biological activity of these compounds was apparent. Thus, whereas **2b** was markedly weaker than **2a** as an AMPA agonist, the corresponding *tert*-butyl analogs **3b**,**a** showed the reverse relative potency, **3b** being some 3 times more potent than **3a**. Introduction of bromine atoms into the 4-position of the rings of the approximately equipotent compounds **4a**,**b** had opposite effects, **5a** being more potent than **4a** and **5b** less potent than **4b**. These structure−activity relationships indicate that a number of factors, including steric bulk of substituents, protolytic properties, and probably electrostatic parameters, have to be considered in studies of the structural requirements for binding to and activation of AMPA receptors.

One of our objectives of replacing the ring oxygen atoms of **2a**−**5a** by sulfur actually was to convert these full AMPA agonists into partial agonists. Since all of the new compounds **2b**−**5b** were shown to produce full AMPA receptor agonism, as exemplified in Figure 5, this attempt to reduce the relative efficacy of AMPA receptor agonists obviously was unsuccessful. Another attempt to convert the full AMPA receptor agonist AMPA (**2a**) into partial agonists by substitution of a hydrogen atom or a variety of alkyl groups for the methyl group in AMPA was equally unsuccessful.[25] Without exceptions, these analogs of AMPA were either full AMPA agonists or inactive.

A further goal of this project was to shed light on the relationship between protolytic properties and the ability to penetrate the BBB of this structural class of AMPA agonists. In general, the pK_a values of the carboxyl and amino groups in the 3-isothiazolol series were closely similar to those of the 3-isoxazolol analogs, although some minor differences were observed. As expected from the higher electronegativity of the oxygen atom compared to the sulfur atom, the pK_a values of the 3-isoxazolol groups of **2a**−**5a** were consistently lower by 1.5−2 pK_a units. From the point of view of systemic pharmacological activity, it is interesting to determine the ionic species of the test compounds (Table 1) present at physiological pH. The net uncharged, diprotonated species are believed to be capable of penetrating the BBB. Calculation of species distribution showed that the proportion of net uncharged, diprotonated species of thio-AMPA (**2b**), thio-ATPA (**3b**), and thio-HIBO (**4b**) becomes significant at physiological pH (Figure 4). Contrary to this, **2a**−**5a** and also **5b** mainly exist in the negatively charged, monoprotonated forms at physiological pH. The significance of the ionic species distribution for the systemic activity was elucidated by the comparison of the convulsive activities of **2a**,**b** after subcutaneous administration in mice. Although being intrinsically less potent, **2b** showed higher convulsant activity in mice than **2a** (Table 2).

The ^{1}H NMR spectra of urine collected from **2b**-treated mice revealed the presence of unchanged compound (Figure 6) and apparently no metabolites containing the 5-methyl group. To our knowledge, this is the first report about the metabolic fate of a glutamic acid analog containing the isothiazole moiety.

In conclusion, no simple correlation between pK_a values of 3-isoxazolol or 3-isothiazolol analogs of glutamic acid (**1**) and their potency as AMPA receptor agonists was apparent. All of the compounds studied, **2a**−**5a** and **2b**−**5b**, showed full AMPA agonism of widely different potency. Although thio-AMPA (**2b**) is markedly weaker than AMPA (**2a**) as an AMPA agonist, **2b** is the more potent convulsant after subcutaneous administration in mice. On the basis of analyses of ionic species distribution of **2a**,**b** at physiological pH, we suggest that these compounds penetrate the BBB in the net uncharged, diprotonated form.

Experimental Section

Chemistry. General Procedures. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mrs. K. Linthoe, Department of General and Organic Chemistry, University of Copenhagen, Mr. G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Denmark, or Analytical Research Department, H. Lundbeck A/S, Denmark, and are within 0.4% of the calculated values, unless otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 or AMX 400 WB spectrometer, using $CDCl_3$, D_2O (with 1,4-dioxane, $\delta = 3.69$, as an internal standard), H_2O-D_2O (9:1), or DMSO-d_6 as solvent. Drying of organic phases was performed using $MgSO_4$. Column chromatography (CC) and TLC were performed on silica gel 60 (70−230 mesh; Merck) and silica gel F_{254} plates (Merck), respectively. The compounds were visualized on TLC plates using UV light and $KMnO_4$ spraying reagent. Compounds containing amino groups were visualized using a ninhydrin-spraying reagent, whereas compounds containing the 3-isothiazolol unit were visualized using a $FeCl_3$-spraying reagent.

4,4-Dimethyl-3-oxopentanamide (6d). Aqueous NH_3 (25%, 645 mL) was added dropwise to ethyl 4,4-dimethyl-3-oxopentanoate[48] (64.3 g, 374 mmol) with ice cooling. The mixture was stirred for 72 h at room temperature. Evaporation and CC [eluent: toluene−EtOAc (9:1)] followed by re-

crystallization (EtOAc−light petroleum) afforded **6d** (33.7 g, 63%): mp 83−84 °C. ^{1}H NMR (DMSO-d_6): δ 7.37 (s, 1H), 7.00 (s, 1H), 3.38 (s, 2H), 1.07 (s, 9H). Anal. ($C_7H_{13}NO_2$) C, H, N.

4,4-Dimethyl-3-thioxopentanamide (7d). Dry EtOH (175 mL) was saturated with HCl and H_2S by passing HCl gas through it for 0.5 h followed by H_2S gas for 0.5 h at −6 °C. **6d** (15.0 g, 105 mmol) was added, and H_2S gas was passed through the solution for an additional 15 h, keeping the temperature at 0 °C. Evaporation and CC [eluent: toluene−EtOAc (2:1)] resulted in **7d** (3.3 g, 20%): mp 120−121 °C. ^{1}H NMR (DMSO-d_6): δ 10.90 (s, 1H), 7.77 (s, 1H), 7.56 (s, 1H), 5.99 (s, 1H), 1.16 (s, 9H). Anal. ($C_7H_{13}NOS$) H, N, S; C: calcd, 52.80; found, 53.27.

5-*tert*-Butyl-3-isothiazolol (8d). A solution of I_2 (6.1 g, 24 mmol) in EtOH (35 mL) was added dropwise to a mixture of **7d** (2.9 g, 18 mmol) and K_2CO_3 (9.6 g, 69 mmol) in EtOH (35 mL) with ice cooling. After stirring for 48 h at room temperature, H_2O (100 mL) was added and pH was adjusted to 3 using 1 M H_2SO_4. Extraction of the aqueous phase with Et_2O (4 × 200 mL), drying of the combined organic extracts, evaporation, CC [eluent: toluene−EtOAc (2:1), 1% AcOH], and recrystallization (toluene) gave **8d** (2.2 g, 77%): mp 125−127 °C. ^{1}H NMR ($CDCl_3$): δ 10.21 (s, 1H), 6.31 (s, 1H), 1.36 (s, 9H). Anal. ($C_7H_{11}NOS$) C, H, N, S.

4-(Bromomethyl)-2-(methoxymethyl)-5-methylisothiazolin-3-one (9c). 5-Methyl-3-isothiazolol (**8c**)[37] (6.0 g, 52 mmol) and 1,3,5-trioxane (7.0 g, 78 mmol) were stirred in 62% aqueous HBr (60 mL) at 60 °C for 3 h. The mixture was extracted with CH_2Cl_2 (5 × 50 mL). MeOH (75 mL) was added to the combined organic extracts, and the mixture was stirred for 1 h at room temperature. CH_2Cl_2 (100 mL) was added, and the mixture was washed with H_2O (3 × 200 mL), dried, and evaporated. CC [eluent: toluene−EtOAc (1:1)] gave crude **9c** (3.5 g, 27%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 5.31 (s, 2H), 4.40 (s, 2H), 3.36 (s, 3H), 2.45 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 168.2 (C-3), 155.1 (C-5), 120.0 (C-4), 74.4 (NCH_2O), 56.6 (OCH_3), 21.8 (CH_2Br), 13.2 (CH_3).

4-(Bromomethyl)-5-*tert*-butyl-2-(methoxymethyl)isothiazolin-3-one (9d). Compound **9d** was synthesized as described for **9c** using 5-*tert*-butyl-3-isothiazolol (**8d**) (3.0 g, 19 mmol), 1,3,5-trioxane (2.6 g, 28 mmol), and 62% aqueous HBr (20 mL) at 80 °C for 6.5 h. Purification by CC [eluent: toluene−EtOAc (2:1)] gave crude **9d** (2.2 g, 39%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 5.18 (s, 2H), 4.48 (s, 2H), 3.38 (s, 3H), 1.47 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 179.2 (C-3), 177.0 (C-5), 117.9 (C-4), 74.4 (NCH_2O), 56.8 (OCH_3), 35.8 (quart C), 29.5 [$(CH_3)_3$], 24.0 (CH_2Br).

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-[2-(methoxymethyl)-5-methyl-3-oxoisothiazolin-4-yl]propionate (10c). A 60% suspension of NaH in mineral oil (560 mg, 14 mmol) was added to a solution of dimethyl acetamidomalonate (2.7 g, 14 mmol) in dry DMF (35 mL). After stirring for 30 min, a solution of **9c** (3.5 g, 14 mmol) in dry DMF (10 mL) was added, and stirring was continued at room temperature for 12 h. AcOH (1.3 mL) was added, and the reaction mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with H_2O (100 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic extracts were dried and evaporated. CC [eluent: toluene−EtOAc (1:1)] followed by recrystallization (Et_2O−light petroleum) gave **10c** (3.5 g, 70%): mp 129−130 °C. ^{1}H NMR ($CDCl_3$): δ 7.25 (s, 1H), 5.11 (s, 2H), 3.81 (s, 6H), 3.42 (s, 2H), 3.32 (s, 3H), 2.30 (s, 3H), 2.01 (s, 3H). Anal. ($C_{14}H_{20}N_2O_7S$) C, H, N, S.

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-[5-*tert*-butyl-2-(methoxymethyl)-3-oxoisothiazolin-4-yl]propionate (10d). Compound **10d** was synthesized as described for **10c** using a 60% suspension of NaH in mineral oil (671 mg, 17 mmol), dimethyl acetamidomalonate (2.9 g, 15 mmol), and **9d** (4.5 g, 15 mmol) in dry DMF (100 mL). Purification by CC [eluent: toluene−EtOAc (1:1)] followed by recrystallization (Et_2O−light petroleum) gave **10d** (4.4 g, 71%): mp 120−122 °C. ^{1}H NMR ($CDCl_3$): δ 7.66 (s, 1H), 5.12 (s, 2H), 3.80 (s, 6H), 3.61 (s, 2H), 3.34 (s, 3H), 1.96 (s, 3H), 1.39 (s, 9H). Anal. ($C_{17}H_{26}N_2O_7S$) C, H, N, S.

(*RS*)-2-Amino-3-(3-hydroxy-5-methylisothiazol-4-yl)-propionic Acid Monohydrate (Thio-AMPA, 2b). A solution of **10c** (2.2 g, 6.1 mmol) in 1 M aqueous CF_3CO_2H (45 mL, 45 mmol) was refluxed for 12 h and evaporated to dryness. The residue was dissolved in H_2O (10 mL) and subjected to ion exchange chromatography (IRA-400) using 1 M AcOH as the eluent. Evaporation of the ninhydrin-positive fractions and recrystallization (EtOH−H_2O) gave **2b** (350 mg, 28%): mp 180−184 °C dec. ^{1}H NMR (D_2O): δ 4.14 (t, 1H, J = 6.4 Hz), 2.92 (d, 2H, J = 6.5 Hz), 2.26 (s, 3H). ^{13}C NMR [D_2O−H_2O (1:9), pH = 12.5]: δ 185.2 (CO_2H), 180.3 (C-3), 159.0 (C-5), 123.7 (C-4), 58.5 (CH), 34.2 (CH_2), 15.2 (CH_3). ^{13}C NMR [D_2O−H_2O (1:9), pH = 1.08]: δ 174.2 (C-3), 174.1 (CO_2H), 160.4 (C-5), 118.6 (C-4), 55.0 (CH), 28.3 (CH_2), 15.1 (CH_3). Anal. ($C_7H_{10}N_2O_3S\cdot1H_2O$) C, H, N, S.

(*RS*)-2-Amino-3-(5-*tert*-butyl-3-hydroxyisothiazol-4-yl)-propionic Acid Hemihydrate (Thio-ATPA, 3b). Compound **3b** was synthesized as described for **2b** using **10d** (370 mg, 0.9 mmol) in 1 M aqueous CF_3CO_2H (10 mL, 10 mmol). Recrystallization (H_2O) gave **3b** (70 mg, 31%): mp 216 °C dec. ^{1}H NMR (D_2O/CF_3CO_2D): δ 4.07 (dd, 1H, J = 5.0, 8.3 Hz), 3.25 (dd, 1H, J = 5.2, 16.6 Hz), 3.01 (dd, 1H, J = 8.7, 16.6 Hz), 1.36 (s, 9H). ^{13}C NMR [D_2O−H_2O (1:9), pH = 12.3]: δ 185.0 (CO_2H), 181.4 (C-3), 173.6 (C-5), 122.1 (C-4), 58.4 (CH), 37.0 (quart C), 34.9 (CH_2), 32.5 [$(CH_3)_3$]. ^{13}C NMR [D_2O−H_2O (1:9), pH = 0.84]: δ 173.8 (CO_2), 173.7 (C-3), 174.6 (C-5), 117.6 (C-4), 54.8 (CH), 37.5 (quart C), 31.5 [$(CH_3)_3$], 29.7 (CH_2). Anal. ($C_{10}H_{16}N_2O_3S\cdot0.5H_2O$) C, N; H: calcd, 6.76; found, 6.25. S: calcd, 12.65; found, 13.12.

Methyl 3-Hydroxyisothiazole-5-carboxylate (12). 3-Hydroxyisothiazole-5-carboxamide (**11**)[39] (14.2 g, 99 mmol) was added to an ice-cooled solution of methanolic HCl prepared from MeOH (460 mL) and acetyl chloride (92 mL). Stirring for 12 h at 50 °C, evaporation, CC [eluent: toluene−2-propanol (10:1)], and recrystallization (toluene) gave **12** (7.1 g, 45%): mp 170−172 °C. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 7.06 (s, 1H), 3.90 (s, 3H), 10.10 (s, 1H). Anal. ($C_5H_5NO_3S$) C, H, N, S.

Methyl 3-Methoxyisothiazole-5-carboxylate (13). Methyl iodide (16.3 mL, 72 mmol) was added dropwise to a suspension of **12** (2.7 g, 17 mmol) and K_2CO_3 (5.9 g, 43 mmol) in acetone (160 mL). Stirring for 24 h in the dark, evaporation, and CC (eluent: EtOAc) gave the *O*-alkylated compound **13** and the *N*-alkylated compound methyl 2-methyl-3-oxoisothiazoline-5-carboxylate. Fractions containing this undesired reaction product were discarded. Sublimation (45 °C, 0.5 mmHg) of the *O*-alkylated compound gave **13** (2.0 g, 68%): mp 54−55 °C. ^{1}H NMR ($CDCl_3$): δ 7.11 (s, 1H), 4.03 (s, 3H), 3.98 (s, 3H). Anal. ($C_6H_7NO_3S$) C, H, N, S.

5-(Hydroxymethyl)-3-methoxyisothiazole (14). Sodium borohydride (642 mg, 18 mmol) was added to a solution of **13** (2.2 g, 13 mmol) in dry THF (70 mL). MeOH (13 mL) was added dropwise to the refluxing reaction mixture over 30 min. After cooling to room temperature, 1 M HCl (30 mL) was added. Extraction with Et_2O (3 × 100 mL), drying, evaporation, and CC [eluent: toluene−EtOAc (3:1)] resulted in a yellow oil. Kugelrohr distillation gave **14** (1.6 g, 87%): bp 150 °C, 1 mmHg.^{1}H NMR ($CDCl_3$): δ 6.42 (s, 1H), 4.83 (s, 2H), 4.15 (s, 1H), 3.95 (s, 3H). Anal. ($C_5H_7NO_2S$) C, H, S; N: calcd, 9.66; found, 9.14.

5-(Chloromethyl)-3-methoxyisothiazole (15). Thionyl chloride (10 mL) was added dropwise to ice-cooled **14** (1.5 g, 10.3 mmol). The mixture was refluxed for 1 h, evaporated, and poured into H_2O (35 mL). The solution was extracted with CH_2Cl_2 (2 × 50 mL). CC [eluent: toluene−EtOAc (8:1)] followed by Kugelrohr distillation gave **15** (1.3 g, 77%): bp 150 °C, 0.5 mmHg. ^{1}H NMR ($CDCl_3$): δ 6.57 (br s, 1H), 4.70 (d, 2H, J = 0.8 Hz), 3.99 (s, 3H). Anal. (C_5H_6ClNOS) C, H, N, S; Cl: calcd, 21.70; found, 22.13.

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-(3-methoxyisothiazol-5-yl)propionate (16). A 60% suspension of NaH in mineral oil (1.2 g, 31 mmol) was added to a solution of dimethyl acetamidomalonate (5.2 g, 28 mmol) in dry DMF (50 mL). After stirring for 30 min, a solution of **15** (4.5 g, 28 mmol) in dry DMF (25 mL) was added. Stirring at room temperature was continued for 12 h. The mixture was acidified with AcOH (2.5 mL) and evaporated to dryness. The

residue was dissolved in CH_2Cl_2 (150 mL), washed with H_2O (50 mL), dried, and evaporated. CC [eluent: toluene−EtOAc (2:1)] followed by recrystallization (Et_2O−light petroleum) gave **16** (4.4 g, 50%): mp 104−105 °C. ^{1}H NMR ($CDCl_3$): δ 6.80 (s, 1H), 6.26 (s, 1H), 3.96 (s, 3H), 3.89 (s, 2H), 3.80 (s, 6H), 2.09 (s, 3H). Anal. ($C_{12}H_{16}N_2O_6S$) C, H, N, S.

Methyl 2-Acetamido-2-(methoxycarbonyl)-3-(4-bromo-3-methoxyisothiazol-5-yl)propionate (17). Bromine (68 mL, 1.3 mol) was added dropwise to **16** (1.5 g, 5 mmol) with ice cooling. Stirring at room temperature for 6 h, evaporation, CC [eluent: toluene−EtOAc (4:1)], and recrystallization (EtOAc−light petroleum) gave **17** (1.2 g, 65%): mp 167−169 °C. ^{1}H NMR ($CDCl_3$): δ 6.78 (s, 1H), 4.05 (s, 3H), 3.96 (s, 2H), 3.82 (s, 6H), 2.10 (s, 3H). Anal. ($C_{12}H_{15}BrN_2O_6S$) C, H, Br, N, S.

(*RS*)-2-Amino-3-(3-hydroxyisothiazol-5-yl)propionic Acid Hydrobromide (4b·HBr). A solution of **16** (500 mg, 1.6 mmol) in 48% aqueous HBr (30 mL) was refluxed for 10 min and evaporated. The residue was dissolved in H_2O (10 mL) and treated with charcoal. Filtration, evaporation, and recrystallization (EtOH−Et_2O) gave **4b**·HBr (105 mg, 25%): mp 159−166 °C dec. ^{1}H NMR (D_2O): δ 6.23 (s, 1H), 3.93 (t, 1H, J = 5.6 Hz), 3.30 (d, 2H, J = 5.6 Hz). ^{13}C NMR [D_2O−H_2O (1:9), pH = 13.1]: δ 183.9 (CO_2H), 180.8 (C-3), 164.5 (C-5), 117.2 (C-4), 59.0 (CH), 36.6 (CH_2). ^{13}C NMR [D_2O−H_2O (1:9), pH = 0.73]: δ 173.2 (CO_2H), 173.5 (C-3), 160.8 (C-5), 116.4 (C-4), 55.5 (CH), 31.5 (CH_2). Anal. ($C_6H_8N_2O_3S$·HBr) C, H, Br, N, S.

(*RS*)-2-Amino-3-(4-bromo-3-hydroxyisothiazol-5-yl)-propionic Acid Hydrochloride (5b·HCl). A solution of **17** (1.1 g, 3 mmol) in 4 M HCl (30 mL) was refluxed for 4.5 h and evaporated. The residue was dissolved in H_2O (25 mL) and extracted with EtOAc (2 × 25 mL). The aqueous phase was evaporated. Recrystallization (EtOH−Et_2O) gave **5b**·HCl (181 mg, 20%): mp 230−234 °C dec. ^{1}H NMR (D_2O): δ 4.27 (t, 1H, J = 6.0 Hz), 3.55 (dd, 1H, J = 5.6, 15.0 Hz), 3.48 (dd, 1H, J = 5.6, 15.0 Hz). ^{13}C NMR [D_2O−H_2O (1:9), pH = 12.9]: δ 183.6 (CO_2H), 176.0 (C-3), 157.9 (C-5), 105.1 (C-4), 58.4 (CH), 37.3 (CH_2). ^{13}C NMR [D_2O−H_2O (1:9), pH = 1.09]: δ 173.4 (CO_2H), 169.5 (C-3), 154.3 (C-5), 104.6 (C-4), 55.0 (CH), 32.1 (CH_2). Anal. ($C_6H_7BrN_2O_3S$·HCl) C, H, N, S; Br: calcd, 26.32; found, 25.56. Cl: calcd, 11.68; found, 10.80.

NMR Titrations. The following solutions in 0.6 mL of H_2O−D_2O (9:1), containing 1 M KCl, were titrated (1) thio-AMPA (**2b**) (6 mg, 0.03 mmol) and AMPA (**2a**)[32] (10 mg, 0.06 mmol), (2) thio-ATPA (**3b**) (10 mg, 0.04 mmol) and ATPA (**3a**)[19] (19 mg, 0.08 mmol), (3) **4b**·HBr (12 mg, 0.05 mmol) and HIBO (**4a**)[32] (17 mg, 0.09 mmol), and (4) **5b**·HCl (18 mg, 0.07 mmol) and Br-HIBO (**5a**)[32] (10 mg, 0.04 mmol). The solutions were alkalized with 1 M NaOH and titrated with 0.5 M HCl adjusted to an ionic strength of 1 M with KCl. Acidity was measured directly in the NMR tubes, before and after recording the NMR spectrum, using a glass microelectrode connected to a Radiometer PHM 83 pH meter. The ^{13}C{^{1}H} NMR spectra were recorded with a Bruker AMX 400 WB spectrometer, operating at 100.62 MHz for ^{13}C. Spectral parameters were adjusted to obtain digital resolution in the frequency domain of 0.8 Hz/data point. All spectra were recorded at 25.0 °C and standardized to internal sodium 3-(trimethylsilyl)propanesulfonate set to δ 0.0. The nonlinear curve fitting was carried out with the Ultrafit v.2.11 program (Biosoft, Cambridge, U.K.) using Levenberg−Marquardt algorithm.

Receptor Binding Assays. Affinity for NMDA, AMPA, and kainic acid receptors was determined using the ligands [^{3}H]CPP,[45] [^{3}H]AMPA,[44] and [^{3}H]kainic acid,[46] respectively. The membrane preparations used in all the receptor binding experiments were prepared according to the method of Ransom and Stec.[49]

In Vitro Electrophysiology. A rat cortical wedge preparation for determination of excitatory amino acid-evoked depolarizations described by Harrison and Simmonds[50] was used in a slightly modified version. Wedges (500 μm thick) of rat brain, containing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with an Ag/AgCl pellet electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{2+}-free (and Ca^{2+}-free for the corpus callosum) oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium and the potential difference between the electrodes recorded on a chart recorder. Applications of agonists were done for 90 s at each concentration tested. The sensitivity of agonist effects to the AMPA receptor antagonist NBQX (5 μM) was tested at agonist concentrations producing at least 50% of maximal responses. Under these conditions, all of the recorded agonist responses were reversibly reduced by at least 70%. In experiments designed to detect antagonist effects of AMPA (**2a**) analogs at 1 mM concentrations, the compounds were applied alone for 90 s followed by coapplication of agonist (**2a**, 5 μM) and potential antagonist for another 90 s.

In Vivo Convulsant Activity. Convulsant activity was determined in male mice (NMRI/BOM, SPF, Bomholtgaard, Denmark) weighing 24−26 g as described.[27] Thio-AMPA (**2b**) was dissolved in saline and given subcutaneously in doses of 1.25, 5, 20, and 40 mg/kg. The animals were observed for 30 min for the presence or absence of clonic/tonic convulsions. Each dose group consisted of 5 mice. ED_{50} values were calculated by log−probit analysis.

Metabolism Studies. Thio-AMPA (**2b**) was dissolved in saline and given subcutaneously at a dose of 20 mg/kg. Urine was collected from 20 male mice (NMRI/BOM, SPF, Bomholtgaard, Denmark), weighing 24−26 g, after 30 min. After addition of 10 vol % of D_2O, urine samples were analyzed directly by ^{1}H NMR. ^{1}H NMR spectra were obtained with low-power presaturation for elimination of the water signal. The presence of thio-AMPA (**2b**) was proved by the observation of signals at δ 2.40 (s, CH_3) and 2.94−3.07 (CH_2), the intensities of which were increased after addition of 1 mg of authentic material. Due to strong chemical noise around δ 4.3, the signal of CH could not be detected.

Acknowledgment. This work was supported by grants from the Lundbeck Foundation and the Danish State Biotechnology Program (1991−1995). We thank the Alfred Benzon Foundation for a grant for the purchase of the Bruker AMX 400 WB spectrometer. The secretarial assistance of Anne Nordly is gratefully acknowledged.

Supporting Information Available: Tables listing intrinsic chemical shift data for the ionic forms of **2a**−**5b** (8 pages). Ordering information is given on any current masthead page.

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JM9607212