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Design and Synthesis of Enantiomers of 3,5-Dinitro-*o*-tyrosine: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropanoic Acid (AMPA) Receptor Antagonists

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The *R*- and *S*-isomers of 3,5-dinitro-*o*-tyrosine (**6a**,**b**) have been synthesized through the use of chemoenzymatic synthesis and shown to bind differentially with the α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA, **3**) receptors. The phenolic functional group of these *o*-tyrosine analogues was designed to act as a bioisostere of the γ-carboxyl group of glutamate. The *S*-isomer of 3,5-dinitro-*o*-tyrosine (**6b**) was 6.5 times more potent than the *R*-isomer (**6a**) in inhibiting [^{3}H]AMPA binding with IC_{50} values of 13 ± 7 and 84 ± 26 μM, respectively. The phenolic group was important for binding affinity since the methoxy compound **7** was less potent than the phenolic compound **6** in inhibiting the binding of AMPA. The free amino group was also shown to be important since the *N*-acetyl analogue **15** and the *N*-*t*-BOC compounds **16** and **17** exhibited very low affinity for the AMPA receptors. AMPA receptor functional tests showed that the *o*-tyrosine analogues are antagonists and that the *S*-isomer **6b** (IC_{50} = 630 ± 140 μM) was more potent than the racemate **6** (IC_{50} = 730 ± 88 μM) while the *R*-isomer **6a** was inactive up to 1 mM concentration, which is consistent with the *S*-isomer having higher binding affinity than the *R*-isomer.

Introduction

Glutamate (**1**) (Chart 1) and to a lesser extent aspartate (**2**) have been established as excitatory neurotransmitters in the mammalian central nervous system (CNS), exerting their action on most neurons via excitatory amino acid (EAA) receptors.[1] In analogy to other neurotransmitter receptors, heterogeneity among EAA receptors has been observed. The EAA receptors are classified based on electrophysiological, radioligand binding, and biochemical studies into five subtypes: *N*-methyl-D-aspartic acid (NMDA), (*R*,*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA, **3**), kainate, (*S*)-2-amino-4-phosphonobutanoic acid (AP4), and metabotropic receptors.[2] EAA receptors appear to be involved in a number of neuronal growth and maturation processes. However, several lines of evidence indicate that hyperactivation of EAA receptors may be involved in the pathophysiology of a number of neurological diseases such as ischemia, stroke, epilepsy, dementia of the Alzheimer type, and Parkinsonism.[3,4] Furthermore, it has been shown that endogenous glutamate activity within the nucleus accumbens (NAC) modulates the psychomotor activation induced by cocaine and heroin.[5] Thus, the development of EAA antagonists might be useful as therapeutic intervention in neurodegenerative diseases as well as providing a new pharmacological strategy for treating the problems associated with drug addiction.

Psychostimulant drugs such as amphetamine and cocaine are believed to produce their behavioral stimulation through activation of dopaminergic neurotransmission in the NAC.[6] Our biological studies have shown that in addition to the well-documented dopamine component, AMPA receptors (named after AMPA, **3**, a very selective and potent agonist) in the NAC and ventral pallidum (VP) of rats are important in the psychostimulant actions of amphetamine and cocaine. We have shown that AMPA antagonists, such as (γ-glutamylamino)methanesulfonic acid (GAMS, **4**) and 6,7-dinitroquinoxaline-2,3-dione (DNQX, **5**), block the locomotor activity elicited by systemic administration of amphetamine and/or cocaine.[7] This experiment strongly suggests that activation of AMPA receptors in the NAC is important for the effects of amphetamine.

To extend the structure−activity relationship studies for AMPA receptor ligands, 3,5-dinitro-*o*-tyrosine (**6**) was synthesized.[8] The rationale behind this design was that the phenolate anion (pK_a = 3.4)[9] in **6** might serve as a bioisostere for the γ-carboxyl anion of glutamate (**1**), as the 3-hydroxyisoxazole anion of AMPA (**3**) presumably does. However, compound **6** was found to be an antagonist at AMPA receptors, inhibiting the locomotor stimulation produced by amphetamine. Although **6** is less potent than AMPA (**3**) in inhibiting specific [^{3}H]-AMPA binding in rat brain homogenate, it shows much higher selectivity than other antagonists for AMPA vs kainate receptors (approximately 20-fold more selective for AMPA than for kainate receptors).[8] In addition, in an attempt to determine the importance of the phenolate in **6** in AMPA receptor binding, compound **7** was designed and synthesized in which the phenolate was masked with a methoxy group.

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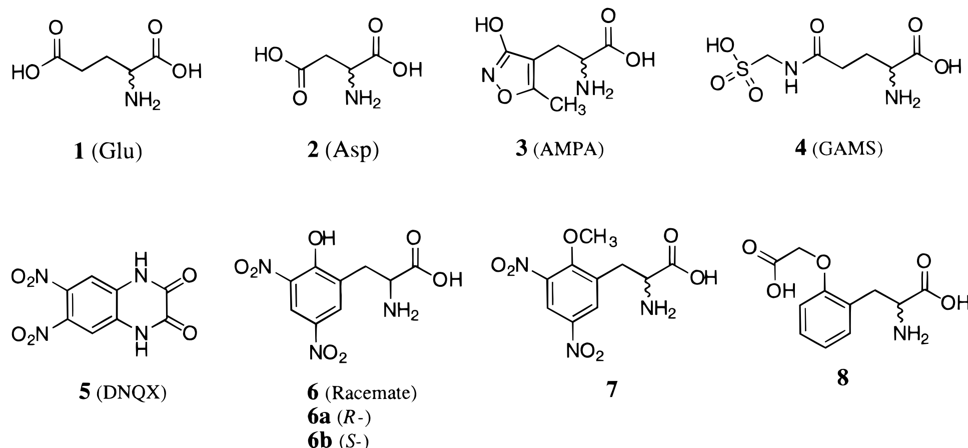
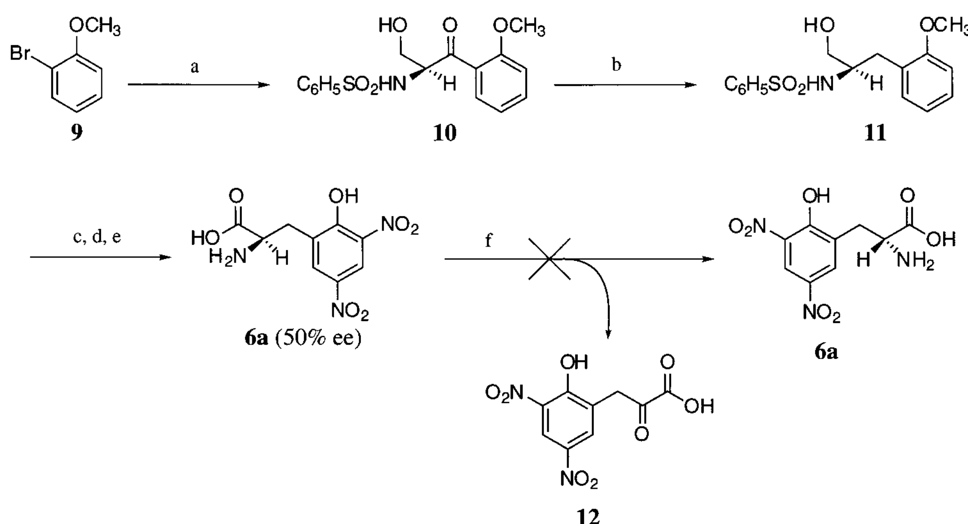
Chart 1



Scheme 1[a]



[a] Reagents: (a) (1) *n*-BuLi, THF, −78 °C, (2) *N*-(phenylsulfonyl)-L-serine; (b) Et_3SiH, CF_3COOH; (c) CrO_3/H_2SO_4, acetone, room temperature; (d) 48% HBr, phenol; (e) NO_2BF_4, CH_3CN, 0−5 °C; (f) L-amino acid oxidase, O_2, catalase, tris-maleate buffer.

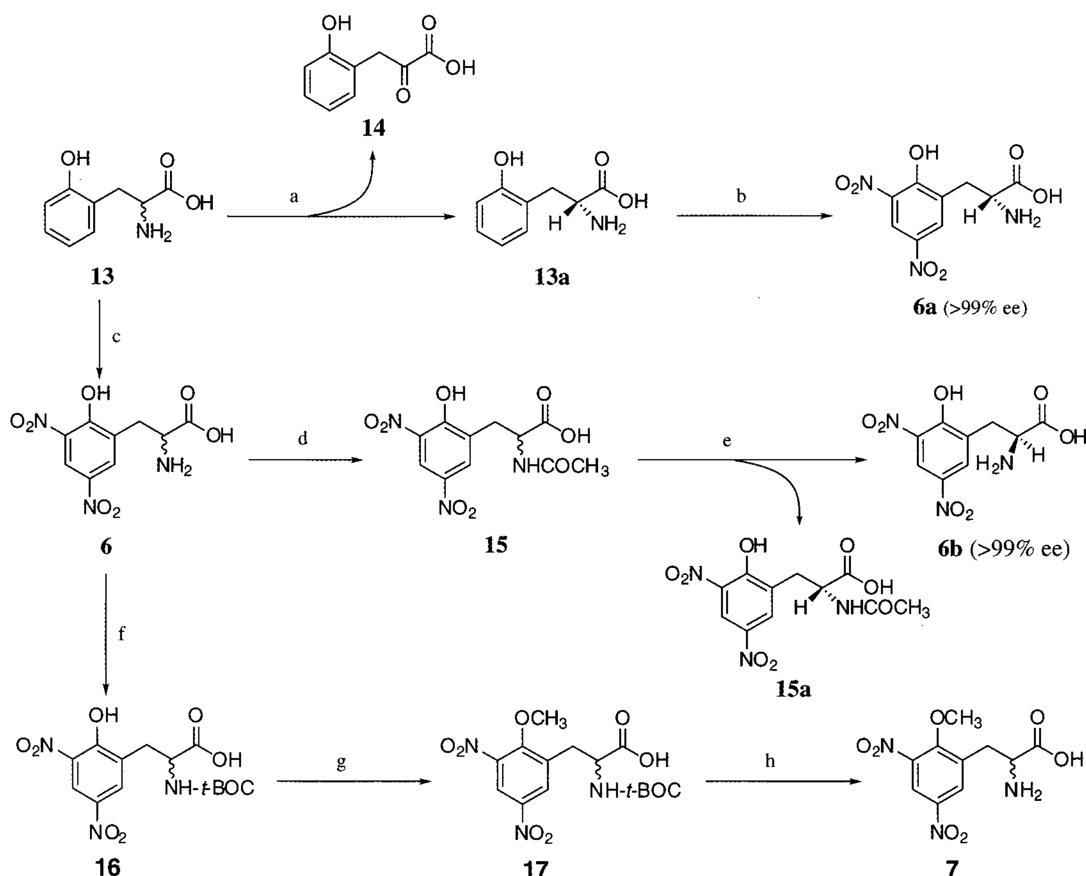
The AMPA receptor shows a pronounced stereoselectivity toward the L-enantiomers of agonists. For example, L-AMPA shows 4000 times higher affinity for AMPA receptors than D-AMPA.[10] The neuroexcitatory effects of two other AMPA agonists, 4-bromohomoibotenic acid and 3-hydroxy-4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridine-5-carboxylate also reside only with the *S*-enantiomers. Therefore, one of our objectives was to synthesize the optical isomers of 3,5-dinitro-*o*-tyrosine, **6a**,**b**, and to determine the enantioselectivity of the AMPA receptors for binding and functional activity for these AMPA ligands.

Homologation is a classical approach used to convert agonists to antagonists. It was therefore our objective to use *o*-tyrosine (*o*-tyrosine itself is unable to inhibit specific [^{3}H]AMPA binding in rat forebrain homogenates) as a flexible template for designing AMPA antagonists, and *β*-[2-(carboxymethoxy)phenyl]-α-alanine (**8**) was designed to probe the distance between the two carboxylic groups (six atoms apart in comparison with a three-atom separation in Glu, **1**, or AMPA, **3**).

Chemistry

Our initial attempt to synthesize *R*-enantiomeric isomer **6a** was based on the methodology developed by Rapoport *et al.*[11] as outlined in Scheme 1, in which L-serine is utilized as the chiral source. The *S*-isomer of **6a** can also be synthesized by using D-serine as the starting material. *N*-(Phenylsulfonyl)-L-serine was synthesized according to the procedure of Rapoport et al.[11] Treatment of *o*-bromoanisole (**9**) with *n*-BuLi and the lithium salt of *N*-(phenylsulfonyl)-L-serine at −78 °C gave (*S*)-2-methoxy-α-[(phenylsulfonyl)amino]-*β*-hydroxypropiophenone (**10**) in 35−50% yield. The carbonyl group in **10** was reduced with Et_3SiH in CF_3COOH,[11] resulting in the formation of (*R*)-2-[(phenylsulfonyl)-amino]-3-(*o*-methoxyphenyl)propan-1-ol (**11**) with a 59% yield. Inverse addition of an acetone solution of **11** to Jones reagent at 0 °C gave (*R*)-2-[(phenylsulfonyl)-amino]-3-(*o*-methoxyphenyl)propionic acid (59%) which was refluxed with 48% HBr in phenol[11] followed by nitration with NO_2BF_4[12] at 0−5 °C to give **6a**. However, the product had an optical purity of only 50% ee, possibly due to partial racemization in the oxidation (step c) and/or deprotection (step d) under strong acidic conditions. The nitration reaction should not cause racemization since under the same conditions an optically pure **6a** was obtained when using **13a** as the precursor, as demonstrated in Scheme 2.

Scheme 2[a]



[a] Reagents: (a) L-amino acid oxidase, O_2, 38 °C, 72 h, tris-maleate buffer; (b) NO_2BF_4, CH_3CN, 0−5 °C; (c) same as b; (d) (1) $(CH_3CO)_2O$, NaOH, (2) 2 N NaOH, room temperature, 30 min; (e) *Aspergillus* acylase I, pH 7.5−8.0, 40 °C; (f) $[(CH_3)_3COCO]_2O$, NaOH, THF; (g) CH_3I, K_2CO_3, DMSO; (h) CF_3CO_2H, 45 min, room temperature.

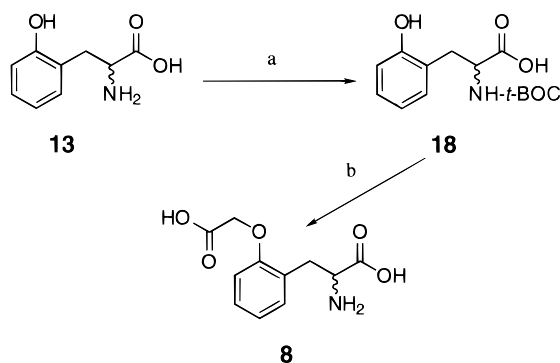
Since de novo synthesis of **6a** as well as **6b** gave only 50% ee, we decided to take a different approach, biological resolution of racemic amino acids with appropriate enzymes, to obtain our target compound(s) with higher optical purity. Hooker and Schellman[13] obtained (*R*)-*o*-tyrosine by using the L-amino acid oxidase (L-AAOx) method of Parikh et al.[14] for the resolution of the racemic mixture of amino acids. L-AAOx is a flavoenzyme that catalyzes the oxidation of L-amino acids utilizing FAD as its redox coenzyme. The resulting $FADH_2$ is reoxidized by O_2. Oxidation of 1 mol of amino acid results in 1 mol of keto acid, NH_3, and H_2O_2, with consumption of 1 mol of O_2.[15] Phenylalanine is an excellent substrate for this enzyme, and examples from the literature show that even substituted phenylalanines (-NO_2, -F, -OCH_3, -CH_3, -CF_3) still undergo oxidation with L-AAOx.[16]

Kinetic Resolution of (*R*)-3,5-Dinitro-*o*-tyrosine (6a) and (*R*)-*o*-Tyrosine by L-AAOx. Encouraged by the literature reports,[13] we decided to resolve **6a** from the combined **6a** and **6b** product obtained by the procedure described in Scheme 1 or from racemate **6**, using L-AAOx as a chemoenzymatic agent. However, we were unable to obtain optically pure *R*-isomer **6a** by this method. We do not know why the enzyme L-AAOx does not oxidize **6b** into the α-keto acid **12**. The two nitro groups on the benzene ring somehow block the oxidation (by steric bulk and/or electronic effect?). Since this attempt failed, we decided to resolve (*R*)-*o*-tyrosine (**13a**) from the racemic mixture of commercially available *o*-tyrosine (**13**) and nitrate it to obtain **6a**, as outlined in Scheme 2. In this experiment, the *S*-enantiomer of **13** was readily oxidized to α-keto acid **14** and removed, and **6a** was obtained in 31% yield with high optical purity (>99% ee).

Pirrung et al. have suggested that D-amino acid oxidase (D-AAOx) could be used to obtain L-isomers of aromatic amino acids.[17] However Parikh et al. have shown that attempts to prepare the L-isomers of phenylalanine, tryptophan, tyrosine, and hydroxyproline by using D-AAOx led to products largely contaminated with the respective D-isomers.[14] Hellermann et al.[18] have shown that D-AAOx is considerably inhibited by many aromatic compounds such as benzoic acid, phenylpyruvic acid, and indole-2-carboxylic acid. Therefore, we predicted that the oxidation of (*R*)-*o*-tyrosine with the D-AAOx would be effectively hindered by the products of the reaction.

Kinetic Resolution of (*S*)-3,5-Dinitro-*o*-tyrosine (6b) by *Aspergillus* Acylase I. Chemoenzymatic synthesis of enantioisomer **6b** is outlined in Scheme 2. Racemic *o*-tyrosine was nitrated with NO_2BF_4 in CH_3CN to give **6**, and acylation of **6** under Schotten−Baumann conditions gave *N*-acetyl-3,5-dinitro-*o*-tyrosine (**15**)[19,20] as a racemic mixture. The method of Whitesides[21] was employed to obtain **6b** in 41% yield with optical purity of >99% ee. Progress of the reaction of **15** with *Aspergillus* acylase I (Sigma) was monitored by quantitative HPLC analysis of the amount of produced **6b** (t_R = 4.32 min), using **13** as internal standard

Scheme 3[a]



[a] Reagents: (a) $[(CH_3)_3COCO]_2O$, NaOH, H_2O, THF; (b) (1) $BrCH_2CO_2Et$, K_2CO_3, acetone, (2) 2 N HCl.

(t_R = 2.22 min) [HPLC conditions: column, ODS 4.8-mm i.d. × 15-mm L; eluent, 8% CH_3CN in acetate buffer (0.02 M, pH = 4.0); flow rate, 2 mL/min; UV detector, λ = 280 mm; sample injected, 10 μL]. The completion of the conversion took approximately 30 h. The *S*-amino acid **6b** was separated from nonhydrolyzed *N*-acetyl amino acid by extracting the latter with EtOAc. An attempt to obtain **6a** was also made by hydrolyzing (*R*)-*N*-acetyl-3,5-dinitro-*o*-tyrosine (**15a**). However, hydrolysis in 2 N HCl for 70 min gave **6a** in only 80% ee. Basic hydrolysis (2.5 N NaOH) destroyed the product completely.

The synthesis of β-3,5-dinitro-2-(methoxyphenyl)-α-alanine (**7**) is also outlined in Scheme 2. Treatment of **6** with di-*tert*-butyl dicarbonate under Schotten–Boumann conditions gave (*R,S*)-*N*-(*tert*-butoxycarbonyl)-3,5-dinitro-*o*-tyrosine (**16**).[22,23] Methylation of **16** with CH_3I in the presence of K_2CO_3 in DMSO gave *N*-(*tert*-butoxycarbonyl)-β-(3,5-dinitro-2-methoxyphenyl)-α-alanine (**17**). However, when a stronger base (e.g., NaOH, KOH) was used, methylation of the protected amino group took place. The deprotection of the amino group of compound **17** was carried out in CF_3COOH, and purification by ion-exchange column afforded **7**.

Scheme 3 shows the synthesis of β-[(2-carboxymethoxy)phenyl]-α-alanine (**8**). Reaction of **13** with di-*tert*-butyl dicarbonate gave (*R,S*)-*N*-(*tert*-butoxycarbonyl)-*o*-tyrosine (**18**).[22,23] Treatment of **18** with ethyl bromoacetate and K_2CO_3 in acetone followed by reflux in 2 N HCl gave the product **8**.

Determination of Optical Purity. The optical purity of **6a**,**b** was determined by diastereoisomer formation with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) and analyzed by HPLC.[24,25] Parallel reaction with the racemic 3,5-dinitro-*o*-tyrosine allowed us to develop the HPLC method for separation of diastereoisomers. The blank probe (containing only Marfey's reagent) was used to determine the retention time for the hydrolyzed reagent (1-hydroxy-2,4-dinitrophenyl-5-L-alanine amide).

Biological Results

Racemic 3,5-dinitro-*o*-tyrosine (**6**), β-(3,5-dinitro-2-methoxyphenyl)-α-alanine (**7**), β-[2-(carboxymethoxy)-phenyl]-α-alanine (**8**), and optical isomers **6a**,**b** were examined for the inhibition of [^{3}H]AMPA binding to rat brain membranes. In addition to these analogues, intermediates with protected amino groups [*N*-acetyl-3,5-dinitro-*o*-tyrosine (**15**), (*R,S*)-*N*-(*tert*-butoxycarbonyl)-3,5-dinitro-*o*-tyrosine (**16**), and *N*-(*tert*-butoxycarbonyl)-β-(3,5-dinitro-2-methoxyphenyl)-α-alanine (**17**)] were examined for binding to AMPA receptors. The reason for testing of the latter compounds was to examine the necessity of the free amino group in the binding to the AMPA receptors. Table 1 summarizes the effect of a 100 μM concentration of different 3,5-dinitro-*o*-tyrosine and *o*-tyrosine analogues on [^{3}H]-AMPA binding to brain homogenates. The results clearly demonstrate that an amino acid function on the side chain and two nitro groups on the ring are important for optimum binding activity. Concentration–response experiments (Figure 1) yielded IC_{50} values for **6b**,**a** of 13 ± 7 and 84 ± 26 μM, respectively. Thus the *S*-isomer is 6.5-fold more potent than the *R*-isomer. The reference compound DNQX had an IC_{50} value of 0.93 ± 0.42 μM. The racemic mixture of β-(3,5-dinitro-2-methoxyphenyl)-α-alanine (**7**), which lacks phenolate anion, was less potent than **6**. It was surprising that this compound still significantly inhibited [^{3}H]AMPA binding (66 ± 4% inhibition). At this time we do not have an explanation for the activity observed with **7**. β-[2-(Carboxymethoxy)phenyl]-α-alanine (**8**) did not show significant inhibition of specific [^{3}H]AMPA binding. A possibility for the low activity of **8** is that the two carboxy groups may be separated by too large of a distance. Intermediates **15**–**17**, all of which have protected amino groups, did not inhibit [^{3}H]-AMPA binding at 10^{-4} M. Thus the free amino group appears necessary in order for these compounds to bind to AMPA receptors.

The two enantiomers **6a**,**b** and the racemic compound **6** were examined for functional activity in an assay in

Table 1. Percent Displacement of [^{3}H]AMPA Binding to Rat Brain Membranes by a 100 μM Concentration of Dinitro-*o*-tyrosine and *o*-Tyrosine Analogues

compd	config	substitutions	[^{3}H]AMPA
6	racemate	R = OH	76 ± 3
6a	*R*	R = OH	58 ± 8
6b	*S*	R = OH	78 ± 5
7	racemate	R = OCH_3	66 ± 4
8	racemate		20 ± 11
15	racemate		14 ± 7
16	racemate		17 ± 7
17	racemate		26 ± 5
DNQX			99 ± 0.4

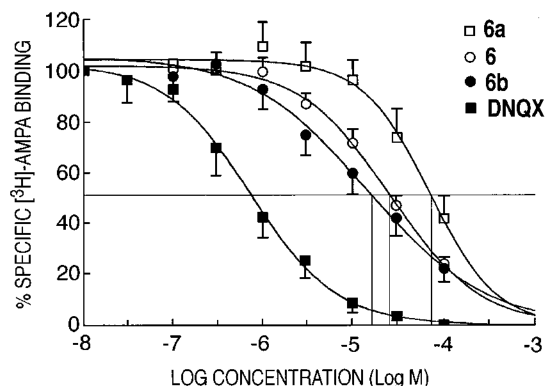


Figure 1. Effects of DNQX, (*R*)- and (*S*)-dinitro-*o*-tyrosine (**6a**,**b**), and their racemate (**6**) on specific [^{3}H]AMPA binding (IC_{50} values of DNQX, **6**, **6a**, and **6b** are 0.93 ± 0.42, 29 ± 6, 84 ± 26, and 13 ± 7 μM, respectively).

which AMPA agonists promote release of [^{3}H]norepinephrine from hippocampal slices and AMPA antagonists inhibit the AMPA-stimulated release. These compounds proved to be antagonists, and the *S*-isomer **6b** (IC_{50} = 630 ± 140 μM) was more potent than the racemate (**6**) (IC_{50} = 730 ± 88 μM) while the *R*-isomer **6a** was inactive up to 1 mM which is consistent with the *S*-isomer having higher binding affinity than the *R*-isomer (the reference compound DNQX had an IC_{50} value of 41 ± 6 μM). This relative order of binding potency for the isomers follows the same trend as found for the AMPA receptor agonists, AMPA and glutamate. This could imply that the tyrosine antagonists bind in a manner similar to agonists but do not produce any intrinsic activity. In conclusion we are reporting that the *S*-isomer **6b** is an AMPA receptor antagonist and represents a lead for a new structural class of AMPA antagonists.[26]

Experimental Section

General. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared data were collected on an Analect RFX-40 FTIR spectrophotometer (The Ohio State University, College of Pharmacy) or on a Perkin-Elmer 2000 FT-IR spectrophotometer (University of Tennessee, College of Pharmacy). The NMR spectra were obtained either on an IBM AF-250 FTNMR spectrometer (250 MHz) at The Ohio State University, College of Pharmacy, or on an Bruker ARX 300 FT NMR spectrometer (300 MHz) at the University of Tennessee, College of Pharmacy. ^{1}H NMR chemical shifts are reported in ppm relative to $CDCl_3$ (δ 7.24, or TMS), acetone-d_6 (δ 2.04), and DMSO-d_6 (δ 2.49). ^{13}C chemical shifts are reported in ppm relative to $CDCl_3$ (δ 77.00, or TMS), acetone-d_6 (δ 29.8), and DMSO-d_6 (δ 39.5). Mass spectra were obtained at the College of Pharmacy by use of a Kratos MS25RFA mass spectrophotometer or at The Ohio State University Campus Chemical Instrumentation Center by use of a VG 70-250S or Kratos MS-30 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, or by Atlantic Microlab, Inc., Norcross, GA, and were within ±0.4% of the theoretical values for the elements indicated. The solvents used in these experiments were purchased and were not further purified unless otherwise stated. Tetrahydrofuran was dried by refluxing with and distillation from sliced sodium and distilled under Ar, using benzophenone as an indicator for dryness. Ion-exchange resin Dowex 50 × 8 200, as well as Dowex 1 × 8 200, was purified prior to use. Enzymes acylase I (EC 3.5.1.14; 0.47 units/mg of solid), L-amino acid oxidase (EC 1.4.3.2; 0.53 units/mg of solid) and catalase (EC 1.11.1.6; 1600 units/mg of solid) were purchased from Sigma Chemical Co. Derivatizing reagent *N*-α-(2,4-dinitro-5-fluorophenyl)-L-alanine amide was purchased from TCI America, Inc. The pH values were determined with Fisher Scientific Accumet pH meter 15. Optical rotations were recorded on an Autopol III at λ = 589 nm at room temperature. High-performance liquid chromatography was performed on a Waters HPLC system equipped with a model Waters 510 Waters 501 pump, a U6K injector, and a model Waters 486 detector. All solvents for HPLC operation were HPLC grade and were filtered prior to use. The chromatograph was operated isocratically.

(*S*)-2-Methoxy-α-[(phenylsulfonyl)amino]-*β*-hydroxypropiophenone (10). Method A: 2-Bromoanisole (**9**) (22.4 g, 120 mmol) was dissolved in dry THF (100 mL) with stirring under argon atmosphere, and the flask was then cooled to −78 °C with a dry ice−acetone bath. *n*-BuLi (53 mL, 133 mmol) was added via a syringe over a period of 5 min, and stirring was continued for an additional 1 h at −78 °C. The solution of *N*-(phenylsulfonyl)-L-serine (4.9 g, 20 mmol) in dry THF (400 mL) was added *via* a dropping funnel over 50 min at −78 °C. The cooling bath was removed after completion of addition and the mixture was allowed to warm to room temperature. The mixture was then poured into ice-cold 1.2 N HCl (500 mL) and extracted with Et_2O (3 × 300 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (300 mL) and brine (300 mL) and dried over Na_2SO_4. After filtration, the solution was concentrated under reduced pressure, and the product was crystallized from EtOAc/Hex to give slightly yellow crystals of **10**: yield 3.39 g (51%); mp 109−110.5 °C; ^{1}H NMR ($CDCl_3$, TMS, 250 MHz) δ 7.88−7.84 (dd, 2H, J = 1.3, 8.1 Hz, Ar*H*), 7.58−7.55 (dd, 1H, J = 1.8, 7.75 Hz, Ar*H*), 7.54−7.40 (m, 4H, Ar*H*), 7.00−6.90 (m, 2H, Ar*H*), 6.17−6.14 (d, 1H, J = 7.0 Hz, N*H*), 5.13−5.07 (m, 1H, C*H*), 3.99−3.94 (dd, 1H, J_{vic} = 3.3 Hz, J_{gem} = 11.6 Hz, CH_2), 3.84 (s, 3H, OCH_3), 3.74−3.67 (dd, 1H, J_{vic} = 4.3 Hz, CH_2), 2.32−2.27 (t, 1H, J = 6.8 Hz, O*H*); IR (KBr, cm^{-1}) 3540 (OH stretching), 3270 (NH stretching), 1693 (C=O stretching); MS (EI) *m/z* 336 (M^+), 200 (M^+ − Ph(OCH_3)CO), 135 (base); $[\alpha]^{25}_D$ = +94.2° (*c* 1.0, acetone). Anal. ($C_{16}H_{17}NO_5S$) C, H, N.

Method B: *N*-(Phenylsulfonyl)-L-serine (2.45 g, 10 mmol) in freshly distilled THF (100 mL) was cooled to -78 °C in a dry ice−acetone bath and stirred under nitrogen atmosphere. *n*-BuLi (8 mL, 20 mmol) was added dropwise via a syringe, and the mixture was stirred for an additional 30 min at −78 °C. The mixture was then treated with 2-methoxyphenylmagnesium bromide in THF. Stirring was continued at room temperature for another 40 h. It was then poured into 1 N HCl (150 mL) which was cooled in an ice−bath and extracted with ether (3 × 100 mL). The combined extracts were washed with saturated $NaHCO_3$ (250 mL) and brine (250 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure on a rotavapor. Crystallization of the residue from EtOAc/Hex gave **10** as a white crystalline solid: yield 1.76 g (53%); mp 109−111 °C.

(*R*)-2-[(Phenylsulfonyl)amino]-3-(*o*-methoxyphenyl)-propan-1-ol (11). To a solution of **10** (5 g, 15 mmol) in CF_3-COOH (34 g, 298 mmol) was added triethylsilane (12.4 g, 66 mmol) via a dropping funnel while stirring and cooling in an ice bath. The mixture was then stirred and heated on an oil bath at 50−55 °C for 2 h. After the mixture to cooled room temperature, a mixture of H_2O (20 mL) and MeOH (20 mL) was added and stirring was continued overnight. The mixture was neutralized with $NaHCO_3$ and extracted with EtOAc (3 × 100 mL). Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a yellow oil. This residue was chromatographed on a silica gel column (mobile phase Hex/EtOAc, 8/2 and then 1/1). The product **11** was crystallized from EtOAc/Hex in the form of white crystals: yield 2.84 g (59%); mp 77−79 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.63−7.59 (dd, 2H, J = 1.3, 7.5 Hz, Ar*H*), 7.49−7.44 (m, 1H, Ar*H*), 7.36−7.30 (t, 2H, J = 7.9, 8.2 Hz, Ar*H*), 7.19−7.12 (td, 1H, J = 1.7, 7.7 Hz, Ar*H*), 6.95−6.91 (dd, 1H, J = 1.7, 7.4 Hz, Ar*H*), 6.82−6.72 (q, 2H, J = 7.4, 8.7 Hz, Ar*H*), 5.36−5.33 (d, 1H, J = 7 Hz, N*H*), 3.75 (s, 3H, OCH_3), 3.55 (s, 2H, CH_2), 3.49−3.39 (m, 1H, C*H*), 2.80−2.72 (t, 2H, J = 5.8, 7.9, 6.6 Hz, CH_2OH), 2.67 (br s, 1H, O*H*); IR (KBr, cm^{-1}) 3509.8 (OH stretching), 3299.6 (NH stretching); MS(EI) *m/z* 321 (M^+), 290 (M^+ − OCH_3), 200 (M^+ − C_6H_5-(OCH_3)CH_2), 77 (base) (C_6H_5); $[\alpha]^{25}_D$ = +66.5° (*c* 1.0, acetone). Anal. ($C_{16}H_{19}NO_4S$) C, H, N.

(*R*)-3,5-Dinitro-*o*-tyrosine (6a). Method A: To a solution of Jones reagent (0.118 g of CrO_3/mL in 1.5 M H_2SO_4) (12 mL) was added a solution of **11** (1.0 g, 3.11 mmol) in acetone (30 mL) via a dropping funnel over a period of 30 min at 5−10 °C. The mixture continued to stir at room temperature for 24 h, was poured into Et_2O (70 mL), and was extracted with brine (3 × 100 mL). The Et_2O layer was then washed with 1 N NaOH, and the combined basic extract was acidified with 10 M H_2SO_4 and extracted with Et_2O (3 × 100 mL). The Et_2O extracts were combined, washed with H_2O (2 × 100 mL) and brine (150 mL), dried over Na_2SO_4, and concentrated under reduced pressure on a rotavapor to give a brown oil. The product was crystallized from EtOAc/Hex in the form of slightly yellow crystals which were identified as (*R*)-2-[(phe-

nylsulfonyl)amino]-3-(*o*-methoxyphenyl)propionic acid: yield 0.62 g (59%); mp 146−147 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.59−7.56 (dd, 2H, J = 1.4, 8.1 Hz, Ar*H*), 7.50−7.44 (m, 1H, Ar*H*), 7.44−7.30 (m, 2H, Ar*H*), 7.22−7.15 (td, 1H, J = 1.6, 8.0 Hz, Ar*H*), 6.99−6.95 (dd, 1H, J = 1.7, 7.5 Hz, Ar*H*), 6.83−6.71 (m, 2H, Ar*H*), 5.39−5.35 (d, 1H, J = 8.4 Hz, N*H*), 4.2−4.11 (m, 1H, C*H*), 3.69 (s, 3H, OC*H*$_3$), 3.02−2.99 (d, 2H, J = 6.8 Hz, C*H*$_2$); IR (KBr, cm^{-1}) 3334 (OH stretching), 3004 (br COOH stretching), 1727.9 (C═O stretching); MS(EI) *m/z* 335 (M^+), 290 (M^+ − COOH), 121 (base) ($C_6H_5(OCH_3)CH_2$); $[\alpha]^{25}_D$ = +38.9° (*c* 1.0, acetone). Anal. ($C_{16}H_{17}NO_5S$) C, H, N.

A mixture of (*R*)-2-[(phenylsulfonyl)amino]-3-(*o*-methoxyphenyl)propionic acid (1.7 g, 4.7 mmol), phenol (1.8 g, 19 mmol), and freshly distilled 48% HBr (20.9 g, 258 mmol) was refluxed for 2.5 h. Progress of the reaction was monitored by assaying aliquots (0.5 mL) on HPLC (ODS column, mobile phase of 15% MeOH in H_2O, UV detector λ = 254 nm) in 30-min intervals. The reaction mixture was allowed to cool to room temperature, diluted with H_2O (50 mL), and extracted with EtOAc (50 mL). The water layer was purified over cation-exchange column Dowex 50 × 8 (H^+). The product was eluted from the column with 0.3 M NH_4OH. Solvent was removed under reduced pressure to give (*R*)-*o*-tyrosine as a white powder: yield 0.53 g (62%); mp 248−250 °C (lit. mp 249−250 °C[11]); ^{1}H NMR (D_2O, DSS, 300 MHz) δ 7.28−7.20 (m, 2H, Ar*H*), 6.97−6.92 (t, 2H, Ar*H*), 4.07−4.03 (m, 1H, C*H*), 3.38−3.32 (dd, 1H, J_{vic} = 5.0 Hz, J_{gem} = 14.0 Hz, C*H*$_2$), 3.08−3.00 (q, 1H, C*H*$_2$); $[\alpha]^{25}_D$ = +17.46° (*c* 1.0, 1 M HCl) [lit. $[\alpha]^{25}_D$ = +25.0° (*c* 0.84, 1 M HCl)[11]]. The nitration reaction of the obtained (*R*)-*o*-tyrosine with NO_2BF_4 in CH_3CN gave **6a** in 50% ee (detailed procedure is the same as and described in method B).

Method B: A racemic mixture of **13** (2.72 g, 15 mmol) was suspended in tris-maleate buffer (250 mL, 0.05 M, pH 7.8). To this mixture was added KCl (1.86 g, 25 mmol) followed by 40 mg of L-amino acid oxidase (Sigma; type I, activity 0.53 units/mg) and 10 mg of catalase. The reaction mixture was vigorously stirred in a water bath at 35−40 °C for 48 h. The mixture was then acidified to pH 6−7 with 1 N HCl, heated with Norite on a steam bath, and filtered over Celite. The filtrate was alkalized to pH 10−11 and purified over anion-exchange column Dowex 1 × 8 (OH^-), eluting the amino acid from the column with 1 M CH_3COOH followed by purification over cation-exchange column Dowex 50 × 8 (H^+). The column was washed with H_2O and EtOH, and the amino acid was eluted with 0.3 M NH_4OH. The solvent was removed under reduced pressure, and the product was crystallized from water to give (*R*)-*o*-tyrosine (**13a**) as white crystals: yield 1.2 g (88%); mp 249−251 °C; ^{1}H NMR was the same as for (*R*)-*o*-tyrosine obtained by method A; $[\alpha]^{25}_D$ = +31.5° (*c* 0.84, 1 M HCl).

A suspension of **13a** (0.41 g, 2.3 mmol) in CH_3CN (10 mL) was cooled in an ice−acetone bath at ~0−5 °C, and NO_2BF_4 (0.8 g, 5.8 mmol) was added in small portions over a period of 20 min while stirring. Progress of the reaction was monitored by assaying aliquots with HPLC (ODS column, mobile phase of 8% CH_3CN in 0.1% CF_3COOH in H_2O, flow rate 2 mL/min, UV detector λ = 254 nm). The reaction was completed after 8 h. The mixture was diluted with water (120 mL), stirred overnight, and filtered over Celite. The filtrate was purified by cation-exchange chromatography on Dowex 8 × 50 (H^+). After the column was loaded and washed it with H_2O (2 L) and EtOH (500 mL), the amino acid was eluted with 0.3 M NH_4OH. The eluent was concentrated under reduced pressure to 5 mL, pH was adjusted to ~3.5 with 1 N HCl, and **6a** was crystallized as yellow crystals which had optical purity of >99% ee: yield 0.19 g (31%); mp 235−240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.54−8.53 (d, 1H, J = 3.1 Hz, Ar*H*), 7.89−7.88 (d, 1H, Ar*H*), 4.14−4.11 (m, 1H, C*H*), 3.12−3.06 (dd, 1H, J_{vic} = 3.8 Hz, J_{gem} = 14.0 Hz, C*H*$_2$), 2.89−2.82 (dd, 1H, J_{vic} = 7.4 Hz, C*H*$_2$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.37 (*C*OOH), 162.33 (Ar*C*), 136.02 (Ar*C*), 134.61 (Ar*C*), 127.90 (Ar*C*), 127.76 (Ar*C*), 123.29 (Ar*C*), 54.61 (*C*HNH$_2$), 35.78 (*C*H$_2$); IR (KBr, cm^{-1}) 3500−2500 (COOH), 3278 (OH), 3083 (NH), 1637 (C═O); MS (FAB$^+$) *m/z* 272 (M^+ + H); $[\alpha]^{25}_D$ = −5.66° (*c* 2.0, 0.05 M HCl). Anal. ($C_9H_9N_3O_7$) C, H, N.

(*R*, *S*)-*N*-Acetyl-3,5-dinitro-*o*-tyrosine (15). A racemic mixture of 3,5-dinitro-*o*-tyrosine (**6**) (3.3 g, 9.8 mmol), obtained from the nitration of **13** by NO_2BF_4, was dissolved in H_2O (20 mL) and 2 N NaOH (5 mL) and cooled in an ice−acetone bath at 0 °C. Redistilled acetic anhydride (2 mL, 22 mmol) and 2 N NaOH (24 mL) were added in small equal portions. After completion of addition, stirring was continued at room temperature for additional 40 min. The mixture was acidified with 10 N H_2SO_4 and extracted with EtOAc (3 × 70 mL). Organic extracts were combined, washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure on a rotavapor. Crystallization of the residual material from acetone gave **15** as a yellow powder: yield 3.0 g (98%); mp 174−175 °C; ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.85−8.84 (d, 1H, J = 3.0 Hz, Ar*H*), 8.46−8.45 (d, 1H, Ar*H*), 7.51−7.49 (br d, 1H, J = 8.0 Hz, N*H*), 4.93−4.85 (m, 1H, C*H*), 3.57−3.51 (dd, 1H, J_{vic} = 5.0 Hz, J_{gem} = 14.0 Hz, C*H*$_2$), 3.17−3.09 (dd, 1H, J_{vic} = 9.0 Hz, C*H*$_2$), 1.84 (s, 3H, C*H*$_3$); MS(EI) *m/z* 313 (M^+), 268 (M^+ − COOH), 74 (base). Anal. ($C_{11}H_{11}N_3O_7$) C, H, N.

(*S*)-3,5-Dinitro-*o*-tyrosine (6b). Racemic *N*-acetyl-3,5-dinitro-*o*-tyrosine (**15**) (3.0 g, 9.6 mmol) was suspended in distilled H_2O (50 mL). 2 N KOH (10 mL) was added to make the final solution pH 7.5−8.0, and the solution was diluted with distilled H_2O to 100 mL total volume. *Aspergillus* acylase I (Sigma; 0.47 units/mg, 40 mg) and $CoCl_2$ (15 mg) were added to the solution. The mixture was stirred in a water bath at 40 °C. Progress of the reaction was monitored by assaying aliquots (0.5 or 0.25 mL) for free amino acid on HPLC. Resolution proceeded for 24 h, but reaction was stopped after 27 h. The pH of the reaction mixture was adjusted to ~5 with 1 N HCl. Norite (100 mg) was added, and the mixture was stirred at 40 °C for 30 min, filtered over Celite, cooled to room temperature, and acidified to pH ~1.5−2.0 with 1 N HCl. The filtrate was then extracted with EtOAc (3 × 80 mL) to remove unhydrolyzed starting material ((*R*)-*N*-acetyl-3,5-dinitro-*o*-tyrosine). The aqueous layer was purified by cation-exchange column Dowex 50 × 8 (H^+). After the sample was loaded, the column was washed with H_2O (2 L) and EtOH (500 mL), and the product was eluted with 0.3 M NH_4OH. The aqueous layer was concentrated under reduced pressure to 5 mL, pH was adjusted to ~3.5−4.2, and the product **6b** crystallized in the form of yellow crystals: yield 0.54 g (41%); mp 235 °C (decomposition starts), 243−245 °C (decomposed rapidly); ^{1}H NMR, FT-IR, and elemental analysis data same as for **6a**; $[\alpha]^{25}_D$ = +4.44° (*c* 1.92, 0.05 M HCl).

(*R*,*S*)-*N*-(*tert*-Butoxycarbonyl)-3,5-dinitro-*o*-tyrosine (16). To a solution of **6** (1.4 g, 4.2 mmol) in H_2O (100 mL) were added 2 N NaOH (30 mL) and THF (150 mL). Di-*tert*-butyl dicarbonate (1.1 g, 4.6 mmol) was added to the vigorously stirred solution, and stirring was continued at room temperature for 2 h. Silica gel TLC of the reaction mixture [R_f = 0.05 (starting material), R_f = 0.57 (product); eluting solvent, 1% MeOH in CH_2Cl_2 + 2 drops of AcOH] showed that the reaction was incomplete. Additional di-*tert*-butyl dicarbonate (2.0 g, 9 mmol) was added, and the solution was stirred at room temperature overnight. The mixture was then poured into H_2O (200 mL) and extracted with *n*-hexanes to remove excess of di-*tert*-butyl dicarbonate. Water layer was cooled in an ice−acetone bath (~0−5 °C), acidified with 4 N HCl to pH ~2, and extracted with EtOAc (3 × 100 mL). Organic extracts were combined, dried over Na_2SO_4, and filtered. Removal of solvent under reduced pressure on a rotavapor gave a yellow powder. Recrystallization of the powder from EtOAc/Hex gave beige crystals of **16**: yield 1.5 g (96%); mp 187−189 °C; ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.88−8.87 (d, 1H, J = 2.6 Hz, Ar*H*), 8.49−8.48 (d, 1H, Ar*H*), 6.36−6.33 (br d, J = 8.5 Hz, 1H, N*H*), 4.67−4.59 (m, 1H, C*H*), 3.62−3.56 (dd, J = 4.0, 14.0 Hz, C*H*$_2$), 3.16−3.08 (m, 1H, C*H*$_2$), 1.27 (s, 9H, 3 × C*H*$_3$). Anal. ($C_{14}H_{17}N_3O_9$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*β*-(3,5-dinitro-2-methoxyphenyl)-α-alanine (17).** To a suspension of K_2CO_3 (0.4 g, 2.7 mmol) in DMSO (5 mL) was added **16** (0.4 g, 1.1 mmol) followed immediately by the addition of methyl iodide (0.42 g,

2.2 mmol). The mixture was vigorously stirred overnight at room temperature. Completion of the reaction was confirmed by silica gel TLC [R_f = 0.5 (starting material), R_f = 0.64 (product); eluting solvent, 1% MeOH in CH_2Cl_2 + 2 drops of AcOH]. The mixture was poured into water (50 mL), acidified with 4 N HCl while cooling in an ice−acetone bath (0−5 °C), and extracted with EtOAc (3 × 50 mL). Organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness under reduced pressure on a rotavapor. Recrystallization of the material from acetone/hexane gave **17** as yellow crystals: yield 0.30 g (72%); mp 153−154 °C; ^1H NMR (acetone-d_6, 300 MHz) δ 8.88−8.87 (d, J = 2.8 Hz, 1H, Ar*H*), 8.47−8.46 (d, J = 2.8 Hz, 1H, Ar*H*), 6.44−6.41 (br d, J = 8.8 Hz, 1H, N*H*), 4.66−4.58 (m, 1H, C*H*), 3.72 (s, 3H, OCH_3), 3.55−3.49 (dd, J = 4.8 Hz, 1H, CH_2), 3.16−3.80 (m, 1H, CH_2), 1.27 (s, 9H, 3 × CH_3). Anal. ($C_{15}H_{19}N_3O_9$) C, H, N.

***β*-(3,5-Dinitro-2-methoxyphenyl)-α-alanine (7).** Compound **17** (0.6 g, 1.6 mmol) was dissolved in CF_3COOH and stirred at room temperature for 45 min. At this point TLC of the reaction mixture showed there was no starting material left [R_f = 0.79 (starting material), R_f = 0.40 (product) on silica gel TLC; eluting solvent, 5% MeOH in CH_2Cl_2 + 2 drops of AcOH]. The mixture was poured into H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). Organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo to ∼5 mL. Triethylamine was added to adjust the pH to ∼5, and addition of water afforded **7** purified by ion-exchange column to give orange crystals:[27] yield 0.248 g (56%); mp 227−228 °C dec; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.59 (br s, 3H, NH_3^+), 8.54−8.53 (d, J = 3.0 Hz, 1H, Ar*H*), 7.87−7.86 (d, J = 3.0 Hz, 1H, Ar*H*), 4.27 (s, 1H, C*H*), 3.66 (s, 3H, OCH_3), 3.11−3.05 (dd, J_{vic} = 4.2 Hz, J_{gem} = 14.0 Hz, 1H, CH_2), 2.93−2.86 (m, 1H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.76 (*C*OOH), 169.72 (Ar*C*), 136.58 (Ar*C*), 131.01 (Ar*C*), 128.78 (Ar*C*), 127.94 (Ar*C*), 124.06 (Ar*C*), 52.91 (O*C*H_3), 52.19 (*C*HNH_2), 33.47 (*C*H_2); MS(EI) *m/z* 285 (M^+), 88 (base). Anal. ($C_{10}H_{11}N_3O_7$) C, H, N.

(*R,S*)-*N*-(*tert*-Butoxycarbonyl)-*o*-tyrosine (18). *o*-Tyrosine (**13**) (1.0 g, 5.5 mmol) was dissolved in a mixture of H_2O (30 mL), 2 N NaOH (10 mL), and THF (30 mL). Di-*tert*-butyl dicarbonate (1.32 g, 6.1 mmol) was added to the vigorously stirred mixture, and stirring was continued for 5 h at room temperature. The mixture was poured into water (100 mL) and extracted with *n*-hexanes to remove unreacted di-*tert*-butyl dicarbonate. Water layer was then acidified with 4 N HCl while cooling in an ice−acetone bath (0−5 °C) and extracted with EtOAc (3 × 50 mL). Organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated on a rotavapor to give a white solid. Recrystallization of the solid from acetone/hexane gave **18** as white crystals: yield 1.41 g (91%); ^1H NMR (acetone-d_6, 300 MHz) δ 7.18−7.15 (dd, J = 1.6, J = 7.5 Hz, 1H, Ar*H*), 7.08−7.03 (t, J = 7.0 Hz, 1H, Ar*H*), 6.92−6.70 (m, 2H, Ar*H*), 6.12−6.09 (br d, J = 7.0 Hz, 1H, N*H*), 4.48−4.41 (m, 1H, C*H*), 3.24−3.17 (dd, J = 4.7, 13.5 Hz, 1H, CH_2), 2.98−2.90 (m, 1H, CH_2), 1.33 (s, 9H, 3 × CH_3).

***β*-[2-(Carboxymethoxy)phenyl]-α-alanine (8).** Finely powdered K_2CO_3 (0.98 g, 7.1 mmol) and compound **18** (0.8 g, 2.8 mmol) were stirred in acetone at 50 °C in an oil bath for 1.5 h. Ethyl bromoacetate (0.52 g, 3.1 mmol) was added to the mixture, and stirred at 50 °C for 3 h. Silica gel TLC of the reaction mixture at this point showed the reaction was incomplete [R_f = 0.17 (compound **18**); eluting solvent, hexanes/EtOH (8/2) + 2 drops of AcOH]. Additional ethyl bromoacetate (0.94 g, 5.6 mmol) was added and the mixture stirred at 50 °C for another 4 h when all starting material disappeared. The mixture was allowed to cool to room temperature, poured into 50 mL of water, acidified with 4 N HCl (pH ∼2), and extracted with EtOAc (3 × 50 mL). Organic extracts were combined and concentrated under reduced pressure on a rotavapor to give a slightly yellow oil. This residue was suspended in 2 N HCl (50 mL), refluxed for 24 h, and cooled to room temperature. Removal of solvent under reduced pressure gave a white solid. Recrystallization of the solid from acetone/water/Et_2O afforded **8** as white crystals: yield 0.203 g (26%); mp 203−204 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.37 (br s, 3H, NH_3^+), 7.26−7.17 (m, 2H, Ar*H*), 6.93−6.85 (m, 2H, Ar*H*), 4.72 (s, 2H, OCH_2), 4.19−4.15 (t, 1H, J = 6.5 Hz, C*H*), 3.24−3.17 (dd, J_{vic} = 6.3 Hz, J_{gem} = 14.0 Hz, 1H, CH_2), 3.09−3.05 (m, 1H, CH_2); ^{13}C (DMSO-d_6, 75 MHz) δ 170.8 (*C*OOH), 170.6 (*C*OOH), 156.2 (Ar*C*), 131.6 (Ar*C*), 129.0 (Ar*C*), 123.5 (Ar*C*), 121.2 (Ar*C*), 111.9 (Ar*C*), 64.9 (O*C*H_2), 52.3 (*C*HNH_2), 31.6 (*C*H_2); MS (EI) *m/z* 221 (M^+ − H_2O), 107 (base). Anal. ($C_{11}H_{13}NO_5$·0.9HCl) C, H, N.

Derivatization and Determination of Enantiomeric Excess of (*S*)- and (*R*)-3,5-Dinitro-*o*-tyrosine. (*S*)-3,5-Dinitro-*o*-tyrosine (**6b**) (100 μL, 5 μmol) was dissolved in 100 μL of 0.5 M $NaHCO_3$. To the solution was added 200 μL of a 1% solution of Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) in acetone. The mixture was incubated at 40 °C for 90 min and allowed to cool to room temperature prior to the addition of 50 μL of 2 N HCl. The sample was allowed to degas and was diluted with EtOH (1.2 mL); 10-μL aliquots of the sample were used for HPLC injection. The *R*-enantiomer **6a** and racemic mixture **6** were derivatized in the same fashion, and **6** was used as a standard. The diastereoisomers were separated by reverse-phase HPLC on an ODS column. The enantiomeric excess of **6a**,**b** was calculated based on the peak integration.

Biological Methods. 1. Binding Studies. The interaction of compounds with AMPA receptors was assessed by inhibition of specific [^{3}H]AMPA binding in washed membrane preparations of rat brain following the procedure originally published by Honore et al.[28] Washed membranes were incubated with [^{3}H]AMPA (1.4 nM final concentration) and solutions of test compounds at appropriate concentrations in 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$ and 100 mM KSCN, pH 7.2. The mixture was incubated on ice for 60 min with periodic shaking, after which bound and free ligands were separated by rapid filtration through Whatman GF/C glass fiber filters. The resulting filter disks, containing membrane-bound radioligand, were dissolved in Scintiverse-E cocktail for scintillation counting. Nonspecific binding was determined in the presence of 1 mM glutamate. For determination of potency, a range of test compound concentrations (0.01−100 μM) was evaluated. IC_{50} values were calculated using non-linear regression analysis.

2. Neurotransmitter Release Assay. This assay is based on the fact that AMPA receptor activation induces norepinephrine release from hippocampal nerve endings.[29] The procedure used was adapted from the method of Desai et al.[30] Briefly, mouse hippocampi were dissected out and chopped into 0.3-mm × 0.3-mm slices. Slices were incubated with 0.2 μM [^{3}H]norepinephrine in Krebs buffer for 30 min and then transferred to superfusion chambers and washed for 60 min with warmed, oxygenated Krebs buffer at 0.3 mL/min. Following this, 10 5-min fractions were collected. After the third collection (i.e., fraction 3), buffer containing 50 μM cyclothiazide was introduced. The presence of cyclothiazide diminishes AMPA receptor desensitization, thus allowing for a more readily quantifiable AMPA response. After the collection of fraction 4, buffer containing drug, cyclothiazide, and 100 μM AMPA was introduced for 5 min followed by normal buffer until the end. For initial screening purposes, tissue slices were exposed to 1 mM test compound alone (test for possible agonist activity) run in parallel with tissue slices exposed to test compound plus 100 μM AMPA (test for antagonist). Each condition was run in triplicate. At least two experiments were conducted to generate an n = 6 for each treatment. For dose−response data, a minimum of four concentrations of each test compound were run against 100 μM AMPA (for antagonists) or alone (for agonists). Determination of IC_{50} values was accomplished utilizing median effect plot analysis.

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