[^{3}H]*N*-2-(4-(*N*-Benzamido)phenyl)propyl-2-propanesulfonamide: A Novel AMPA Receptor Potentiator and Radioligand

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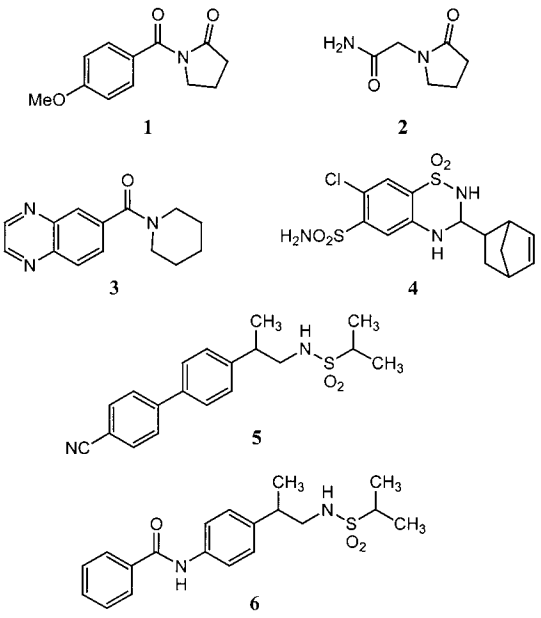
Introduction. The AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtype of ionotropic glutamate receptor mediates fast excitatory neurotransmission at the majority of synapses in the mammalian central nervous system. Native AMPA receptors are likely constituted as heterooligomers, composed of one or more of the AMPA receptor subunits GluR1, 2, 3, and 4.[1] Like other ligand-gated ion channels, AMPA receptor function can be affected by subunit composition,[2] alternative splicing,[3] RNA editing,[4] and the presence of allosteric modulatory sites.[5]

Activation of AMPA receptors is critical for expression and maintenance of long-term potentiation,[6] an enduring form of synaptic plasticity that may be associated with certain forms of learning and memory. Moreover, converging lines of evidence are consistent with the hypothesis that AMPA receptors play an important role in learning and memory processes. For example, rapid changes in radioligand binding to AMPA receptors have been evinced in rodent hippocampus following a variety of training (e.g. inhibitory avoidance) procedures.[7] Furthermore, compounds that potentiate AMPA receptor function facilitate performance in a wide variety of learning and memory tasks.[8] Despite the value of such compounds as tools for understanding AMPA receptor physiology and pharmacology, the utility of the previously described chemical classes [pyrrolidinones (e.g. aniracetam, **1**; piracetam, **2**; CX-516, **3**) and benzothiadiazines (e.g. cyclothiazide, **4**)][8−10] is compromised by relatively weak potency (Chart 1).

We have recently described a class of biarylpropylsulfonamides (e.g. **5**) as AMPA receptor potentiators.[11] The potency of this class of compounds is significantly higher than that of the previously discussed classes of AMPA receptor potentiators. In the present report, we describe the development of a novel AMPA receptor potentiator, *N*-2-(4-(*N*-benzamido)phenyl)propyl-2-propanesulfonamide, **6**, and compare its biological activity with that of the prototype benzothiadiazine, cyclothiazide, **4** (Chart 1). We have also identified [^{3}H]**6** as a novel radioligand that binds with high affinity to a putative AMPA receptor potentiator site on native receptors prepared from rat cerebral cortex. Thus, both **6** and [^{3}H]-

Chart 1



6 represent important new tools for understanding AMPA receptor pharmacology.

Chemistry. Amide **6** was prepared according to the procedure shown in Scheme 1. 4-Aminophenylacetonitrile **7** was converted to dibenzylamine analogue **8** upon treatment with potassium carbonate and subsequently benzyl bromide**.** Alkylation of the lithium enolate of **8** with methyl iodide resulted in product **9** which was refluxed with borane methyl sulfide, and the corresponding amine was converted to its hydrochloride salt **10**. Sulfonylation of **10** with isopropylsulfonyl chloride resulted in sulfonamide **11** which was deprotected by hydrogenation with ammonium formate and 10% palladium on carbon. Aniline **12** was then reacted with benzoyl chloride to give amide **6**. Aniline **12** was also converted to amide **13** via reaction with *p*-iodobenzoyl chloride. The radioligand [^{3}H]**6** was prepared from the corresponding amide **13** by catalytic tritiation as shown in Scheme 2.

Pharmacology. Acutely isolated cerebellar Purkinje neurons were prepared according to previously described methods.[12] Dissociated cells were plated onto poly-L-lysine-coated glass coverslips (50 μg/mL) and used within 24 h of isolation.

Whole-cell voltage clamp recordings were made from isolated cells using extracellular solutions composed of (in mM): NaCl, 138; $CaCl_2$, 5; KCl, 5; $MgCl_2$, 1; HEPES, 10; glucose, 10; pH to 7.4 with NaOH; osmolality, 310 mosm/kg; and intracellular solutions composed of (in mM): CsCl, 140; $MgCl_2$, 1; diTris creatine phosphate, 14; creatine phosphokinase, 50 U/mL; HEPES, 10; BAPTA, 15; pH to 7.15 with CsOH; and osmolality, 295 mosm/kg. Drugs were applied by bath perfusion. Experiments were performed at room temperature (20−22 °C).

The binding of [^{3}H]**6** to rat cerebral cortical membranes was initially based on conditions used to assay [^{3}H]AMPA binding[13] and optimized in the following

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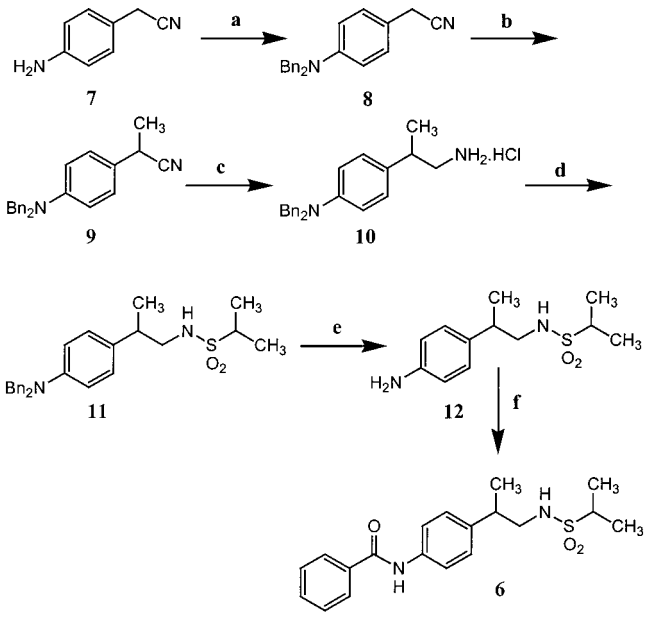
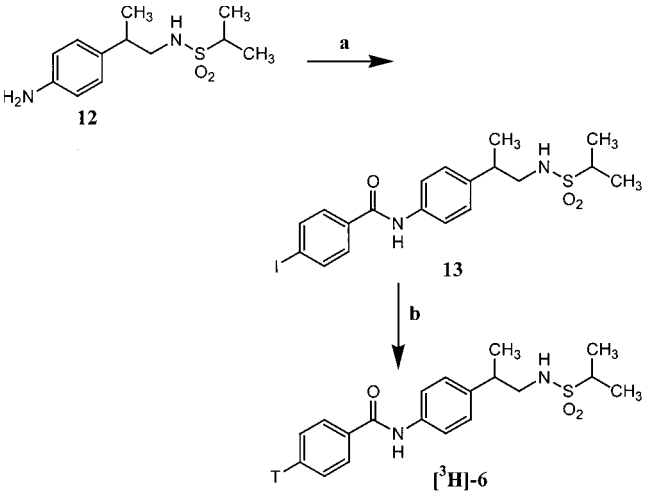
Scheme 1[a]



[a] (a) K_2CO_3, $PhCH_2Br$, KI, DMF, 25 °C; (b) $LiN(SiMe_3)_2$, THF, −78 °C, then MeI; (c) 1. BH_3·DMS, THF, reflux, 2. HCl/MeOH, Et_2O, 25 °C; (d) *i*-$PrSO_2Cl$, DBU, 0−25 °C; (e) $NH_4^+COO^-$, 10% Pd−C, H_2, EtOH, 25 °C; (f) PhCOCl, Et_3N, CH_2Cl_2, 25 °C.

Scheme 2[a]



[a] (a) IPhCOCl, Et_3N, CH_2Cl_2, 25 °C; (b) T_2, 10% Pd−C, *i*-Pr_2NEt, DMF, 25 °C.

modifications. In brief, assays (0.5−1 mL) were carried out in polystyrene deep well titer plates (Beckman Instrument, Fullerton, CA) or 13- × 100-mm glass test tubes (Fisher Scientific, Pittsburgh, PA) containing 30 mM Tris-HCl buffer (pH 7.4), 150−200 μg of cortical membrane protein suspension, and ∼10 nM [^{3}H]**6** (sp. act., 25 Ci/mmol; Amersham). Nonspecific binding was defined with 10 μM of **6**. Glutamate (500 μM) was included in the competition binding assays. The reactions were incubated at 0 °C for 2 h and terminated by rapid filtration (Brandel M-48R, Brandel Instruments, Garthersburg, MD) with 10 mL of ice-cold assay buffer through GF/B filters presoaked in 0.3% polyethylenimine.

Results and Discussion. Both the amide **6** and cyclothiazide **4** produced concentration-dependent increases in AMPA receptor-mediated inward currents (Figure 1A). Thus, co-application of **4** typically increased

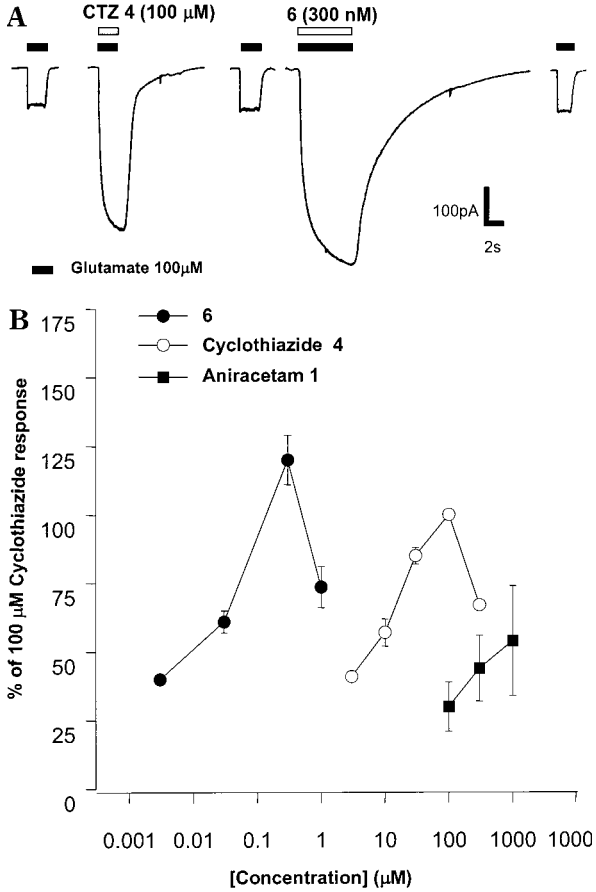


Figure 1. A,B: Potentiation of AMPA receptor-mediated inward currents in acutely isolated cerebellar Purkinje neurons. Cells were voltage-clamped at −70 mV, and steady-state glutamate (100 μM)-induced inward currents were evoked in the absence and presence of **6**. In each cell, the effect of **6** (●) and aniracetam **1** (■) was compared to the effect of cyclothiazide **4** (100 μM) (○) on glutamate (100 μM)-evoked inward currents (expressed as % of 100 μM cyclothiazide **4** response). Data points represent the mean ± SEM for at least 3 determinations at each concentration of compound. Experiments were performed at room temperature.

steady-state responses to 100 μM glutamate by 4−6-fold (Figure 1B) with an EC_{50} ∼ 10 μM. Moreover, **6** is a more efficacious potentiator than the prototype **4**; responses with **6** were typically 20% greater at 300 nM than those observed with a maximally effective concentration of **4** (100 μM). Because cerebellar Purkinje neurons contain heterogeneous AMPA receptor populations, the greater maximal responses produced by **6** may represent activation of a specific receptor subpopulation-(s) insensitive to **4**, in addition to possible differences in intrinsic efficacy between these compounds.

The specific binding of [^{3}H]**6** to rat cerebral cortical membranes was modest in the absence of glutamate or (*S*)-AMPA, typically representing less than 20% of the total binding. Addition of glutamate or AMPA produced a concentration-dependent increase in [^{3}H]**6** binding, typically increasing the specific binding of this radioligand 5−10-fold (Figure 2A). The EC_{50} values for (*S*)-AMPA (5.5 ± 1.5 μM) and glutamate (31 ± 6 μM) to increase [^{3}H]**6** binding are in the concentration range reported to activate AMPA receptors in a variety of preparations.[12,14] This enhancement in [^{3}H]**6** binding exhibited a high degree of stereoselectivity, since (*R*)-AMPA (the biologically inactive enantiomer) produced

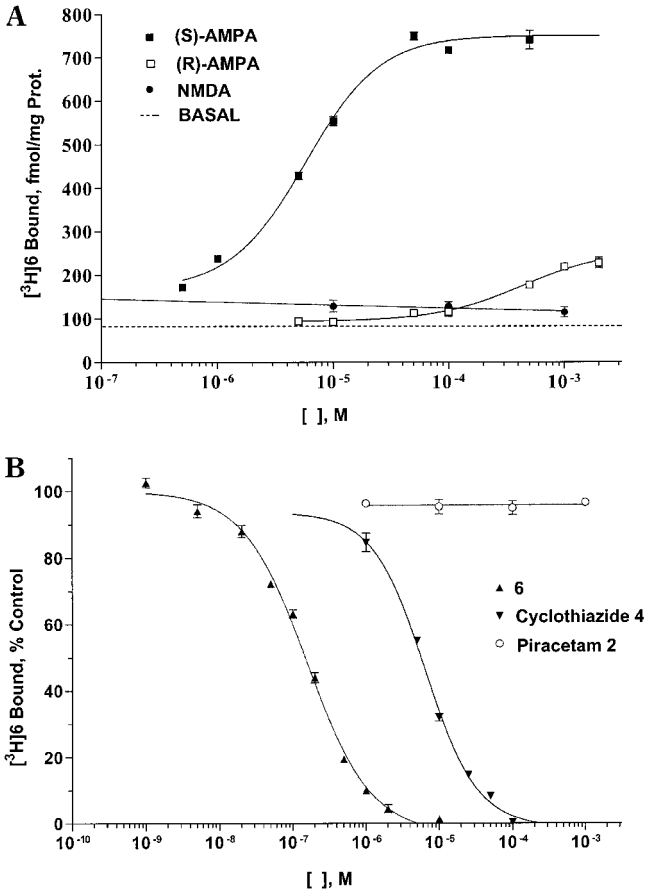


Figure 2. Characterization of [^{3}H]**6** binding to cerebral cortical membranes: (A) stereoselective enhancement by AMPA and (B) effects of AMPA receptor modulators. These are representative experiments repeated 3 times: (*S*)-AMPA (■), (*R*)-AMPA (□), NMDA (●), **6** (▲), cyclothiazide **4** (▼), piracetam **2** (○). Equilibrium binding assays were performed as described in the text, using ~10 nM [^{3}H]**6**. A: The specific binding (dotted line) was 85 fmol/mg protein, representing ≈44% of the total binding in the absence of (*S*)-AMPA (or glutamate). In this representative experiment, the EC_{50} value for (*S*)-AMPA was 5.5 μM; the EC_{50} value for (*R*)-AMPA could not be accurately determined. NMDA did not enhance the [^{3}H]-**6** binding at concentrations of up to 1 mM. B: The IC_{50} values for **6** and cyclothiazide **4** in this representative experiment were 0.17 and 6.5 μM, respectively. The Hill slopes for **6** and cyclothiazide **4** in this representative experiment were 1.0 and 1.2, respectively. Piracetam **2** had no effect on [^{3}H]**6** binding at concentrations up to 1 mM. All assays in panel B contained 500 μM glutamate.

only a modest enhancement in binding at 100-fold higher concentrations. The selective enhancement of [^{3}H]**6** binding by AMPA receptor ligands is further underscored by the failure of *N*-methyl-D-aspartate (NMDA) to affect radioligand binding at concentrations of up to 1 mM. [^{3}H]**6** binding (measured in the presence of maximally effective glutamate concentrations) was inhibited by **6** and **4** with IC_{50} values of 0.17 ± 0.03 and 6.1 ± 0.7 μM, respectively (Figure 2B). These values are well within the range that modulate AMPA-mediated currents in cerebellar Purkinje neurons, consistent with the hypothesis that [^{3}H]**6** labels a unique AMPA receptor potentiator site. Piracetam, **2**, which appears to modulate AMPA receptors via a mechanism distinct from **4**, had no effect on [^{3}H]**6** binding at concentrations of up to 1 mM (Figure 2B).

In summary, we have shown that *N*-2-(4-(*N*-benzamido)phenyl)propyl-2-propanesulfonamide, **6**, is greater than 2 orders of magnitude more potent than the prototype **4** in potentiating AMPA receptor-mediated responses in acutely isolated cerebellar Purkinje neurons.[12] Furthermore, [^{3}H]**6** is the first reported compound to label (with nanomolar affinity) a putative AMPA receptor potentiator site on heteromeric AMPA receptors prepared from rat cortical membranes. Both **6** and [^{3}H]**6** represent novel tools to better understand AMPA pharmacology and are prototypes of a novel series in this class that may be useful in the treatment of neuropsychiatric disorders ranging from age-associated memory impairment to schizophrenia.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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