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SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF L-*TRANS*-4-TETRAZOLYLPROLINE (LY300020): A NOVEL SYSTEMICALLY-ACTIVE AMPA RECEPTOR AGONIST

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Abstract: LY300020, a conformationally constrained, ω-tetrazole-containing analog of L-glutamic acid was prepared and has been shown to be a relatively potent, highly selective, systemically-active AMPA receptor agonist. The activity of LY300020 was shown to be highly stereoselective as its enantiomer, LY301900, was devoid of binding affinity at ionotropic excitatory amino acid receptors.

L-Glutamic acid (Figure) is one of the principal endogenous ligands which indiscriminately activate excitatory amino acid (EAA) receptors (NMDA, AMPA, kainate, and metabotropic types) in the mammalian central nervous system.[1] Glutamate is a conformationally flexible molecule, possessing

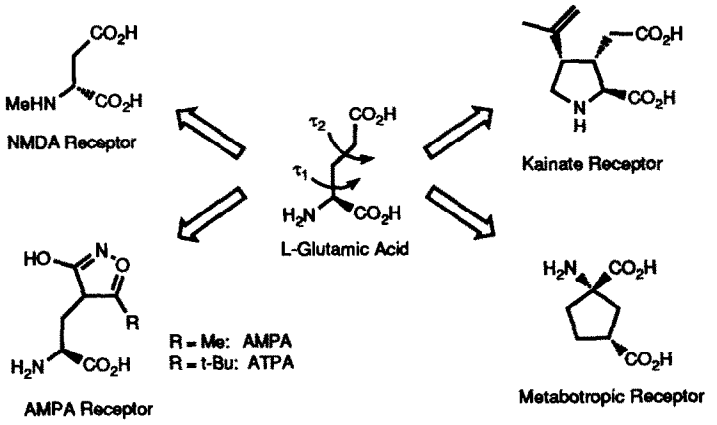


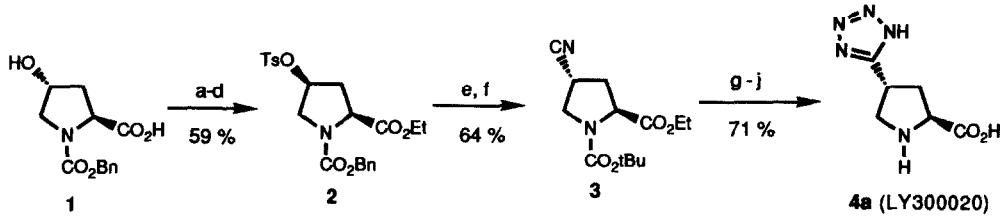
Figure. Pharmacological classification of excitatory amino acid receptors and structures of representative selective agonists

two key torsional angles (τ_1 and τ_2, Figure) which define the geometrical relationships between the α-amino acid and distal carboxylate functionalities. As part of our program aimed at understanding the effect of glutamic acid conformation on both potency at, and selectivity for, EAA receptors, we have prepared 2*S*,4*R*-4-(1H-tetrazol-5-yl)pyrrolidine-2-carboxylic acid (L-*trans*-4-tetrazolylproline, **4a**, LY300020) and 2*R*,4*S*-4-(1H-tetrazol-5-yl)pyrrolidine-2-carboxylic acid (D-*trans*-4-tetrazolyl-

proline, **4b**, LY301900) as conformationally restrained analogs of glutamate[2] possessing a tetrazole moiety as a bioisosteric replacement for the ω-carboxylate of the natural ligand.[3]

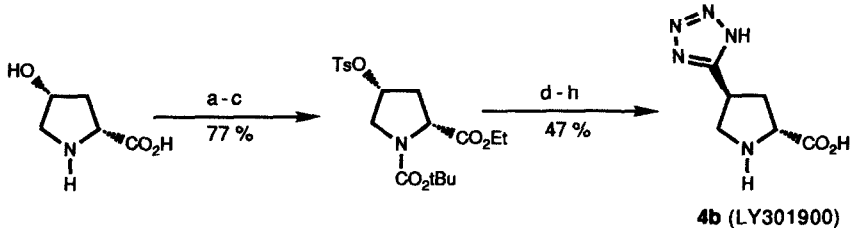
Chemistry. The synthesis of **4a** is depicted in Scheme 1. Commercially available 2*S*,4*R*-N-carbobenzyloxy-4-hydroxyproline (**1**) is converted in four steps to the fully protected tosyl derivative **2** (59%).[2f,4] In order to facilitate the expeditious conversion of **2** to **4a**, the N-protecting group was converted from carbobenzyloxy to t-butyloxycarbonyl (96%)[5] prior to cyanide displacement of the C4-tosylate (67%). Conversion of **3** to the C4-tetrazole derivative by warming at 90 °C with tributyltin azide, followed by sequential hydrolysis of the protecting groups and ion exchange chromatography afforded **4a** (71%).[6] The 2*R*,4*S*-isomer **4b** was prepared in a conceptually analogous manner from 2*R*,4*R*-hydroxyproline (Scheme 2).[6]

Scheme 1



Reagents: a. CrO_3, H_2SO_4, acetone 24 °C; b. $NaBH_4$, EtOH, H_2O, 5 °C - 24 °C; c. EtOH, p-TsOH, reflux; d. p-TsCl, pyridine; e. H_2 (60 psi), Pd-C, $(t\text{-BuOCO})_2O$, EtOH; f. NaCN, DMSO, 45 °C; g. Bu_3SnN_3, 90 °C; h. HCl (g), EtOH, 0 °C; i. NaOH, H_2O, THF, 24 °C; j. cation exchange (Dowex 50X8-100, 5 % pyridine/H_2O eluent).

Scheme 2



Reagents: a. $(t\text{-BuOCO})_2O$, $NaHCO_3$, 24 °C; b. EtI, K_2CO_3, DMF, 5 °C; c. p-TsCl, pyridine, 5 °C; d. NaCN, DMSO, 45 °C; e. Bu_3SnN_3, 90 °C; f. HCl (g), EtOH, 0 °C; g. NaOH, H_2O, THF, 24 °C; h. cation exchange (Dowex 50X8-100, 5 % pyridine/H_2O eluent).

Biochemistry. Compounds **4a** and **4b** were examined for their ability to displace [^{3}H]CGS19755 (10 nM)[7], [^{3}H]AMPA (5 nM)[8], and [^{3}H]kainate (5 nM)[9] binding in rat forebrain membranes. For comparison, 2*S*,4*R*-pyrrolidine-2,4-dicarboxylic acid[2f] (*trans*-PDC, Tocris Neuramin) was also evaluated. Neither *trans*-PDC nor **4b** displaced [^{3}H]CGS19755, [^{3}H]AMPA or [^{3}H]kainate binding at concentrations ≤100 μM. In contrast, **4a** displaced ^{3}H-AMPA (IC_{50} = 3.38 ± 0.48 μM) at

concentrations which do not significantly inhibit the binding of either ^{3}H-CGS19755 (IC_{50} > 100 μM) or ^{3}H-kainate (IC_{50} = 61.0 ± 6.6 μM).

Electrophysiology. Compound **4a** was evaluated in the cortical slice assay[10] for intrinsic agonist activity and for its ability to antagonize depolarizations induced by NMDA (40 μM), AMPA (40 μM) or kainate (10 μM). When **4a** (100 μM) was applied alone to the cortical slice, depolarizations approximately equivalent to those elicited by AMPA (40μM) were observed which could be antagonized by the AMPA antagonist NBQX[11] (1 μM), but not by the NMDA antagonist D-AP5 (20 μM). NBQX (1 μM) increased the concentration of **4a** required to produce control levels of depolarizations by approximately 10-fold, suggesting that the excitation elicited by **4a** is mediated via AMPA rather than the kainate receptors.[12] Compound **4a** had no antagonist effect on the depolarizations elicited by NMDA, AMPA, or kainate. Thus, **4a** is a selective AMPA agonist *in vitro.*

In Vivo Pharmacology. Compound **4a** was evaluated for systemic AMPA agonist activity in CF-1 mice (Charles Rivers Labs). At a dose of 25 mg/kg (i.v., n = 5), seizures of very short duration were observed, along with 100% survival. Increasing the dose of **4a** to 50 mg/kg (i.v., n = 5) produced tonic extensor seizures, in two of the mice, followed by death. The other three mice exhibited forelimb flexor seizures with no accompanying mortality. Within 5 sec after bolus injection of 100 mg/kg of **4a** (i.v., n = 5), seizures followed by 100% mortality was observed.

Discussion. As part of our ongoing program aimed at delineating the structural and conformational aspects of agonist and antagonist binding to excitatory amino acid receptors, we have prepared and evaluated enantiomeric *trans*-4-tetrazolylproline isomers **4a** and **4b**. Stereospecific binding to glutamate receptors labeled by ^{3}H-AMPA was observed for **4a** (IC_{50} = 3.38 μM). In addition, **4a** demonstrated negligible binding to either the NMDA receptor (IC_{50} > 100 μM) or the kainate receptor (IC_{50} = 61 μM). In contrast, isomeric **4b** and isosteric *trans*-PDC[2f] were devoid of binding affinity for any of the ionotropic EAA receptors. Compound **4a** has been characterized as an AMPA agonist *in vitro* , and its convulsant and lethal effects in the mouse are highly suggestive of functional activation of AMPA receptors *in vivo* when administered systemically.

Recent cloning and expression studies have defined at least eight different AMPA-preferring receptor proteins in the rat (GluR1-GluR4, flip and flop splice versions).[13] The discovery of novel medicinal agents capable of demonstrating AMPA receptor subtype selectivity as well as activity (agonist or antagonist) *in vivo* is a requisite next step toward ascribing function(s) to each member of the AMPA receptor family. The *in vivo* pharmacological effects elicited by **4a** (convulsions and death) are particularly interesting as they are quite distinct from the effect (muscular rigidity) observed after administration of another systemically active AMPA agonist, ATPA (Figure).[14] Thus, it is possible that **4a** is activating a population of AMPA receptors different from those activated by ATPA. If so, **4a** may prove to be an important pharmacological tool for establishing the function of these receptors and in the characterization of putative AMPA receptor antagonists *in vivo.*

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