Amino Acids (2011) 40:913–922
DOI 10.1007/s00726-010-0713-1

ORIGINAL ARTICLE

Synthesis of C5-tetrazole derivatives of 2-amino-adipic acid displaying NMDA glutamate receptor antagonism

Fatimazohra Lenda · Nadine Crouzin · Mélanie Cavalier · Janique Guiramand · Fabien Lanté · Gérard Barbanel · Catherine Cohen-Solal · Jean Martinez · Farhate Guenoun · Frédéric Lamaty · Michel Vignes

Received: 9 February 2010 / Accepted: 27 July 2010 / Published online: 13 August 2010

Abstract Five derivatives of 2-amino-adipic acid bearing a tetrazole-substituted in C5 position were synthesized. These compounds displayed selective antagonism towards *N*-methyl-D-aspartate (NMDA) receptors compared with AMPA receptors, and they were devoid of any neurotoxicity. Among these five analogues, one exhibited a higher affinity for synaptic NMDA responses than the other four. Therefore, C5 tetrazole-substituted of 2-amino-adipic acid represent an interesting series of new NMDA receptor antagonists. This approach may be considered as a new strategy to develop ligands specifically targeted to synaptic or extra-synaptic NMDA receptors.

Keywords Glutamate · *N*-Methyl-D-Aspartate (NMDA) · Synaptic transmission · Extrasynaptic receptors

Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system of vertebrates. Besides its role in neurotransmission, glutamate is involved in several important neural processes, such as development, learning and memory, plasticity and neurotoxicity (Pin and Duvoisin 1995; Brauner-Osborne et al. 2000; Kew and Kemp 2005; Mayer 2005; Recasens et al. 2007). Glutamate activates two main classes of receptors, ionotropic (iGluR) and metabotropic (mGluR) receptors. Ionotropic glutamate receptors are subdivided into three subclasses: *N*-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionate/kainate (AMPA/KA) and KA receptors. Concerning mGluRs, three subgroups have been characterized: group I (mGluR1 and 5), group II (mGluR2 and 3) and group III (mGluR4 and 6–8).

The NMDA receptor subtypes are potential therapeutic targets to treat a broad range of brain pathologies. Indeed, neurodegenerative processes occurring during various pathological conditions such as stroke, or trauma for instance, result from a disturbance of glutamate homeostasis leading to an excessive activation of glutamate receptors, especially the NMDA subtype (Choi 1992; Rothman and Olney 1995). On the other hand, a hypofunctioning of these receptors, especially in the prefrontal cortex, is often associated with psychosis (Kristiansen et al. 2007). Discovery of selective NMDA receptor ligands, either agonists or antagonists, devoid of intrinsic toxicity, is still of interest to cure a large range of brain disorders (Paoletti and Neyton 2007). Different targets within the NMDA receptor channel have been identified, including the glutamate binding site, the glycine binding site, the polyamine binding site or the channel pore itself. Unfortunately, most of the molecules designed to interact with these targets trigger neuronal death and are therefore inappropriate for a therapeutic use (Olney et al. 1991; Ikonomidou et al. 1999). Memantine, a moderate affinity non-competitive NMDA receptor antagonist, is to date the only NMDA receptor ligand having received an agreement to treat Alzheimer's disease symptoms (Reisberg et al.

N. Crouzin and F. Lenda contributed equally to this study.

F. Lenda · N. Crouzin · M. Cavalier · J. Guiramand · F. Lanté · G. Barbanel · C. Cohen-Solal · J. Martinez · F. Lamaty · M. Vignes (✉)
Institut des Biomolécules Max Mousseron UMR 5247 CNRS-Universités Montpellier 1 et 2, Université Montpellier 2, Pl. E. Bataillon, cc2400, 34095 Montpellier Cedex 5, France
e-mail: Michel.Vignes@univ-montp2.fr

F. Lenda · F. Guenoun
Faculty of Sciences, University Moulay Ismail, B.P. 1201, Zitoune, Meknès, Morocco

2003; Johnson and Kotermanski 2006; Robinson and Keating 2006).

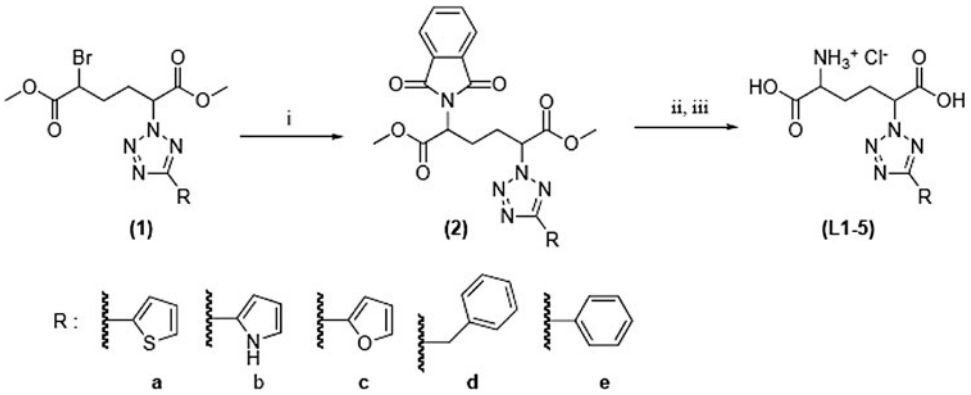
2-Amino-adipic acid (2-AA) is known for long as NMDA receptor ligand, its L-enantiomeric form exerting an agonist activity, while the D-enantiomer is an antagonist (Curtis and Watkins 1960; McLennan and Hall 1978; Watkins et al. 1990; Brauner-Osborne et al. 2000). Consequently, the 2-AA could be an interesting backbone to develop a new series of NMDA receptor ligands. L-2-AA also exhibits agonist effects on some mGluRs (Brauner-Osborne et al. 1996, 2000). However, substitutions at the C-5 position of the carbon backbone of 2-AA have been reported to decrease the potency of these agonist effects towards mGluRs (Guldbrandt et al. 2002). On the other hand, only a few glutamate receptor modulators bearing tetrazole substitutions have been synthesized so far. They include LY233053 reported as a potent NMDA receptor antagonist (Ornstein et al. 1991), and LY300020 an agonist of AMPA receptors (Monn et al. 1993). We therefore decided to test the idea that introduction of such tetrazole substitutions on C5 within the structure of 2-AA could enhance the affinity of the molecule towards NMDA receptors and eventually its selectivity owing to a sufficient steric hindrance.

In agreement with previous reports from our group, we have designed five derivatives of 2-AA bearing a tetrazole-substituted in position C-5 and developed a straightforward synthesis of these molecules (Lenda et al. 2005, 2007). As a first approach, all compounds (L1-L5) were further tested for their potential biological activity as stereoisomeric mixtures. To this aim, their actions on cell survival, NMDA receptor-associated currents and NMDA receptor-mediated synaptic transmission were evaluated in cultured rat hippocampal neurons.

Results

Synthesis of 2-amino-5-(5-substituted-2H-tetrazol-2-yl)hexanedioic acid hydrochloride (L1-5)

The diversely substituted starting materials, dimethyl 2-bromo-5-(5-substituted tetrazol-2-yl)hexanedioates **1** were prepared in a four-step synthesis as previously described (Lenda et al. 2005, 2007). Then, nucleophilic substitution of the bromine atom by potassium phthalimide was performed, yielding the protected amino derivatives **2a**–**2e** in a 70–90% yield depending on the nature of the R group (Scheme 1). **2** was refluxed in a mixture of 6 mol L^{-1} HCl and acetic acid for 10 h to afford after treatment free amino acids **L1**–**L5** in a good yield (70–80%, Scheme 1, Fig. 1). All products were identified by ^{1}H, ^{13}C NMR and mass spectrometry.



Scheme 1 General procedure for the synthesis of C5-derivatives of 2-AA. (*i*) potassium phthalimide, DMF, 90°C, 2 h, (*ii*) 6 N HCl, CH_3CO_2H, 70°C, (*iii*) propylene oxide/MeOH

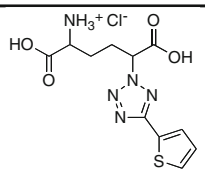
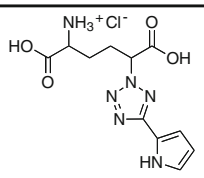
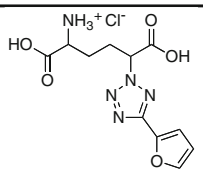
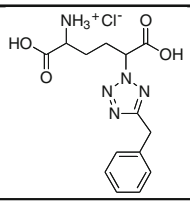
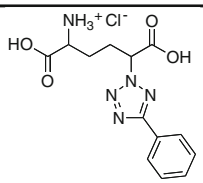
Compound	L1	L2	L3	L4	L5
Chemical structure					
Isolated yield	80%	77%	79%	70%	78%

Fig. 1 Chemical structure of 2-AA derivatives and their isolated yields

Functional characterization of 2-AA derivatives

First, we examined the potential antagonistic activity of the L1 derivative on NMDA and non-NMDA receptor-mediated responses. To this aim, increasing concentrations of this compound were tested on NMDA or KA-mediated currents in neurones voltage-clamped at −60 mV. The L1 compound antagonized the current elicited by the application of 30 μM NMDA with an IC_{50} of 229 μM as calculated with Jandel's Sigmaplot software^{TM} (Fig. 2a, d). Inhibition of the NMDA-induced current was also observed in neurons held at a voltage of +40 mV (Fig. 2b), indicating that the blockade exerted by L1 on NMDA receptors was voltage-independent. On average an inhibition of 68 ± 3% ($n = 5$) with 300 μM L1 was obtained on NMDA currents at +40 mV. By comparison, the L1 compound elicited a lesser inhibitory effect on currents elicited by applying 20 μM KA. On average, L1 inhibited KA currents by 17 ± 2% ($n = 5$) and by 28 ± 1% ($n = 5$) when applied at concentrations of 300 and 1,000 μM, respectively (Fig. 2c, d). The other 2-AA derivatives (L2–L5) also displayed an antagonism on NMDA-mediated responses with efficiency similar to that of L1 (Fig. 2e, f).

Next, the efficiency of L1–L5 compounds was evaluated on synaptic NMDA receptor-mediated responses. To this aim, the spontaneous excitatory transmission elicited by postsynaptic NMDA receptors ($sEPSC_{NMDA}$) was monitored in the presence of these compounds. Spontaneous $EPSC_{NMDA}$ were isolated in the presence of picrotoxin (25 μM) to block the spontaneous inhibitory transmission, in combination with NBQX (5 μM) to block the activation of AMPA receptors, and by removing Mg^{2+} ions from the extracellular medium supplemented with glycine (10 μM) to facilitate the activation of NMDA receptors (Shen et al. 1996). The application of L1 did not elicit any inward current, but inhibited the $sEPSC_{NMDA}$ in a concentration-dependent manner (Fig. 3a, b). Complete blockade of the $sEPSC_{NMDA}$ was achieved with 1 mM L1. The compounds, L2–L5, also inhibited the $sEPSC_{NMDA}$ (Fig. 3c, d). Interestingly, we found that L1 was more efficient than the other derivatives in blocking the $sEPSC_{NMDA.}$ Indeed, at the concentration of 150 μM, L1 inhibited the $sEPSC_{NMDA}$ amplitude by 76 ± 3% ($n = 3$), while L2, L3, L4 and L5 inhibition reached only 40 ± 3% ($n = 3$), 41 ± 6% ($n = 3$), 42 ± 4% ($n = 3$) and 39 ± 5% ($n = 3$), respectively (Fig. 3c, d). Therefore, L1 seemed to exhibit a higher affinity for the NMDA receptors involved in synaptic responses than its congeners.

Finally, we have analysed whether these compounds exerted any excitotoxic action on cultured hippocampal neurons. As evaluated with the MTT assay, the application of 2-AA derivatives up to 500 μM, for 24 h did not affect cell survival (Table 1).

Discussion

The main objective of this study was to develop and test the biological activity of some C5-tetrazole derivatives of 2-AA. We, interestingly, found that such a substitution affected the biological activity of the molecule, leading to the development of a new series of NMDA receptor antagonists, which might differ in their affinity towards synaptic versus extra-synaptic NMDA receptors.

The synthetic scheme that was developed is based on the functionalization of a diacid synthon. Such a diacid can be easily transformed into a dibromo derivative. We have already shown that by successive transformations and intramolecular reactions, original molecules, such as substituted-pipecolid acids or -prolines, could be efficiently prepared (Lenda et al. 2004, 2005, 2007). In the current study, we have extended our previous results by performing successive nucleophilic substitutions on a dibromo derivative to prepare new compounds bearing a heterocyclic structure (Scheme 2). By choosing as the first nucleophile a substituted tetrazole (Nu_1 = tetrazole) and as the second one a reactive amino nucleophile such as potassium phthalimide (Nu_2 = PG-N, PG = protecting group), we efficiently obtained a family of C5 tetrazole-substituted 2-AA. Each tetrazole-substituted amino acid was obtained as a mixture of stereoisomers (1:1 ratio of L and D amino acids) and tested as such.

Regarding the biological activity, the introduction of heterocyclic moieties on the C5 position of 2-AA yielded compounds with antagonist activity towards NMDA receptors. While the 5 derivatives of 2-AA synthesized so far had similar apparent IC_{50} (*circa* 230 μM) determined against 30 μM NMDA application, the L1 compound displays a higher inhibitory effect than its congeners on the NMDA receptor-mediated synaptic responses ($sEPSC_{NMDA}$) and thus shows a higher selectivity than the others towards synaptic NMDA receptors. This could be due to an additional use-dependent antagonistic action of L1 on NMDA receptors. Indeed, a use-dependent effect is observed at synaptic sites because of the sustained presence of glutamate (Rosenmund et al. 1995) and it is a hallmark of non-competitive NMDA channel blockers such as MK-801 or PCP (Paoletti and Neyton 2007). The major difference between the L1 compound and the other derivatives is the presence of a thiophenyl residue in its structure. To date, there are rather few NMDA receptor antagonists bearing such a substitution, except 1-[1-(2-thiophenyl)cyclohexyl] piperidine (TCP) whose affinity towards NMDA receptor is better than that of its phenyl congener (PCP; Espaze et al. 2000; Hirbec et al. 2002). One can thus hypothesize that the presence of this residue could confer a partial non-competitive use-dependent antagonistic property to L1, revealed when monitoring synaptic NMDA responses.

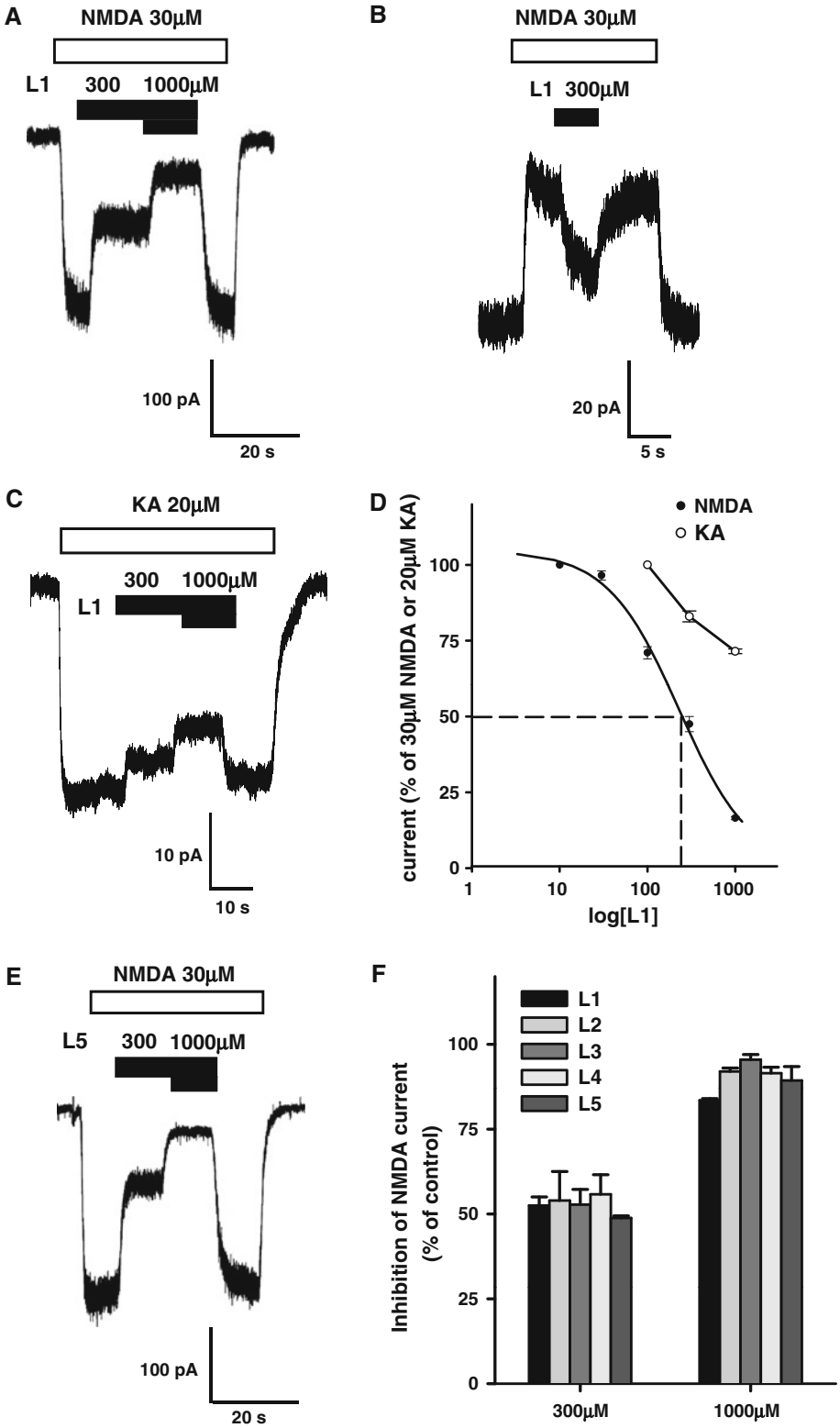


Fig. 2 Effect of 2-AA derivatives on NMDA and non-NMDA receptor-mediated currents. **a** A representative recording showing the concentration-dependent blockade of NMDA-evoked currents by L1 in neurons voltage-clamped at −60 mV. **b** Sample trace depicting the inhibitory effect of L1 (300 μM) on NMDA current at a holding voltage of +40 mV. **c** A representative recording showing the concentration-dependent blockade of KA-evoked currents by L1 in neurons voltage-clamped at −60 mV. **d** Concentration-dependent effect of L1 on the NMDA (30 μM, *filled circles*)- and KA (20 μM, *open circles*)-evoked currents. The L1 IC_{50} (229 μM) for the blockade of NMDA currents has been obtained by fitting the experimental curve with the SigmaPlot 9.0 software. **e** A representative recording showing the concentration-dependent blockade of NMDA-evoked currents by L5. **f** Recapitulative plot of the inhibitory actions of L1, L2, L3, L4 and L5 on NMDA-evoked current. Data are expressed as percentages of inhibition of the NMDA (30 μM) current and are means ± SEM of at least three independent determinations

The 2-AA derivatives may help to discriminate between synaptic and non-synaptic effects of NMDA receptor stimulation. Indeed, it is now clear that NMDA receptors exist at both synaptic and extra-synaptic sites (Hardingham et al. 2002), while their exact subunit composition, especially in the hippocampus remains controversial (Thomas et al. 2006). Moreover, the direct activation of either of these two populations of receptors does not trigger similar intracellular pathways. Interestingly, synaptic NMDA receptors most likely activate plasticity and survival pathways, while

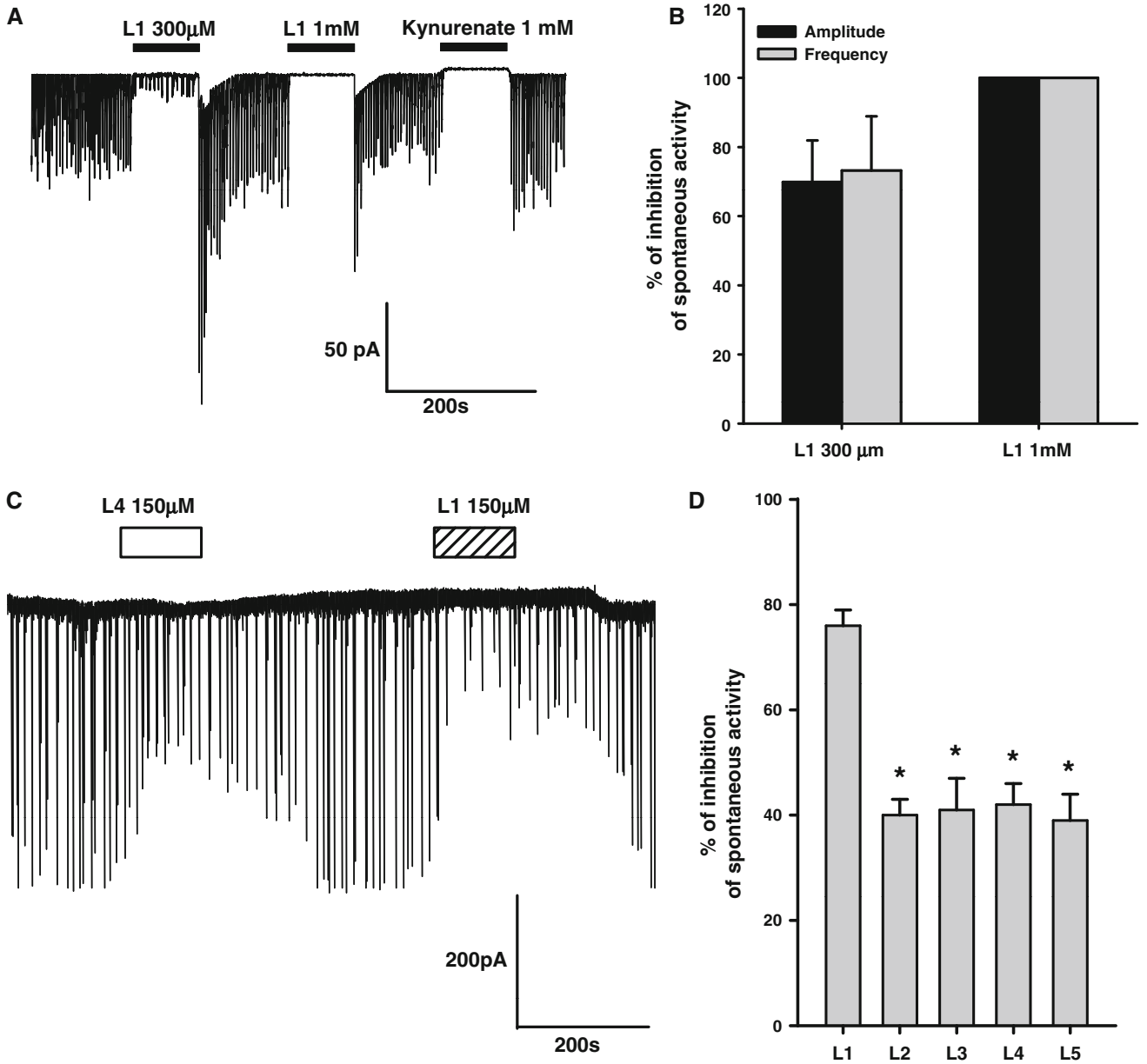


Fig. 3 Effects of 2-AA derivatives on NMDA receptor-mediated spontaneous synaptic transmission ($sEPSC_{NMDA}$) in cultured hippocampal neurons. **a** Concentration-dependent inhibition of $sEPSC_{NMDA}$ by L1. The broad-spectrum ionotropic glutamate receptor antagonist, kynurenic acid (1 mM) also blocked $sEPSC_{NMDA}$. **b** Pooled data of the effect of different concentrations of L1 on both the frequency and the amplitude of $sEPSC_{NMDA}$. Data are expressed as the percentages of inhibition of $sEPSC_{NMDA}$ frequency or amplitude and are means ± SEM of at least three independent determinations. **c** A representative recording of $sEPSC_{NMDA}$ in the presence of 150 µM L4 and then in the presence of the same concentration of L1 is shown. **d** Pooled data of the inhibitory effects on $sEPSC_{NMDA}$ amplitude induced by 150 µM of L1, L2, L3, L4 or L5. Data, expressed as percentages of inhibition, are means ± SEM of at least three independent determinations. $^*P < 0.05$

extrasynaptic NMDA receptors are associated with neuronal death pathways and may be likely involved in the general neurotoxic effect of NMDA (Hardingham et al. 2002; Bengtson et al. 2008). Designing specific molecules selective for one NMDA receptor subpopulation (synaptic versus extra-synaptic) is thus a great challenge of high therapeutic interest.

The distinction between synaptic and extrasynaptic receptors relies so far on the utilization of use-dependent NMDA channel antagonists such as MK-801 (Ivanov et al. 2006) which are supposed to preferentially block those synaptically activated NMDA receptors (Rosenmund et al. 1995). Unfortunately, use-dependent NMDA channel antagonists are often neurotoxic by themselves, hence

Table 1 Effect of L1, L3 and L4 on hippocampal neuron survival

	MTT transformation (% of control)
Control	100
L1	103 ± 1
L3	94 ± 2
L4	100 ± 3

Cells were treated for 24 h with the drugs at 500 μM. Cell survival was determined by the measurement of mitochondrial activity using MTT assay, as described in "Material and methods". Data are expressed as percentages of control values obtained in untreated cultures and they are means ± SEM of three values

difficult to use over long periods of time. Treatment with more selective and less toxic drugs is therefore necessary to more clearly assess the role of extrasynaptic NMDA receptors. Interestingly, the 2-AA derivatives that were synthesized in this study are devoid of intrinsic neurotoxic effect. This suggests also that these compounds are not likely to interfere with glutamate uptake mechanisms. Indeed, glutamate transport blockers have been shown to be particularly excitotoxic in this preparation (Guiramand et al. 2005).

In conclusion, the C5 tetrazole-substituted 2-AA derivatives synthesized herein, which, at least in vitro, do not present neurotoxicity, could be potentially interesting therapeutic molecules against some brain pathologies, such as some neurodegenerative diseases. In addition, the chemical approach developed here may be considered as an interesting way to synthesize new series of antagonists and/or agonists specifically targeted to synaptic or extrasynaptic NMDA receptors, which remains to date a great pharmacological challenge. Further purification is now requested to isolate active stereoisomers. This will probably generate more efficient and selective antagonists targeted to inhibit either extrasynaptic or synaptic NMDA-associated biological responses.

Materials and methods

Chemistry

Chemical analyses

^{1}H and ^{13}C NMR analyses were performed with 200- and 400-MHz NMR spectrometers. Resonances were characterized as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Mass spectra (electrospray ionization mode, ESIMS) were recorded with a Platform II quadrupole mass spectrometer fitted with an electrospray interface. The mass spectrometer (Micromass, Manchester, UK) was calibrated in the positive-ion ESI mode. The samples were dissolved in H_2O/CH_3CN (50:50 by volume). The high-resolution mass spectra were measured with a JEOL JMS-SX-102A mass spectrometer.

General procedure for the synthesis of dimethyl 2-(1, 3-dioxoisoindolin-2-yl)-5-(5-substituted-2H-tetrazol-2-yl)hexanedioate (2)

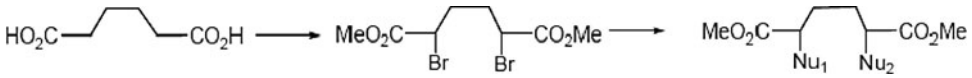
A mixture of dimethyl 2-bromo-5-(5-substituted tetrazol-2-yl)hexanedioates (Lenda et al. 2005) and potassium phthalimide (3 eq.) was heated in dimethylformamide (30 ml) under argon at 90°C for 2 h. Dimethylformamide was evaporated under reduced pressure. The solid residue was diluted with water (50 ml) and extracted three times with chloroform (3 × 50 ml). The organic layer was quickly washed with sodium hydroxide (0.2 N, 50 ml) and with water until neutral pH (2 × 100 ml). The organic layer was decanted, dried over sodium sulphate, filtered and concentrated under reduced pressure. Column chromatography (silica gel, hexane/diethyl ether: 70/30) afforded the title compound.

Dimethyl 2-(1,3-dioxoisoindolin-2-yl)-5-(5-(5-methylthiophen-2-yl)-2H-tetrazol-2-yl) hexanedioate (2a)

The general procedure was used with dimethyl 2-bromo-5-[5-(thiophen-2-yl)tetrazol-2-yl]hexanedioate (402 mg, 1 mmol) and potassium phthalimide (555 mg, 3 mmol) to afford 321 mg (80%) of the title compound as a greenish oil. ^{1}H NMR ($CDCl_3$, $SiMe_4$): δ 1.9 (m, 1H), 2.2 (m, 3H), 3.5 (s, 3H), 3.6 (s, 3H), 4.7 (m, 1H), 5.4 (m, 1H), 6.9 (m, 1H), 7.2 (m, 1H), 7.5 (m, 1H), 7.6 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$, $SiMe_4$): δ 25.93, 28.18, 51.83, 53.35, 53.81, 65.40, 124.12, 124.13, 128.34, 128.61, 128.66, 129.11, 132.04, 134.80, 134.85, 161.82, 167.72, 167.99, 169.28 ppm. HRMS calculated for $C_{21}H_{20}O_6N_5S$ 470.1134; found 470.1145.

Dimethyl 2-(5-(1H-pyrrol-2-yl)-2H-tetrazol-2-yl)-5-(1,3-dioxoisoindolin-2-yl) hexanedioate (2b)

The general procedure was applied to dimethyl 2-bromo-5-[5-(1H-pyrrol-2-yl)tetrazol-2-yl]hexanedioate (385 mg,



Scheme 2 General procedure for the synthesis of **2**. The procedure is based on the transformation of adipic acid in a dibromo derivative, followed by two sequential nucleophilic substitutions. Nu_1 = tetrazole and Nu_2 = PG-N (PG = protecting group)

1 mmol) and potassium phthalimide (555 mg, 3 mmol) to afford 288 mg (77%) of the title compound as a greenish oil. ^{1}H NMR ($CDCl_3$, $SiMe_4$): δ 2 (m, 1H), 2.2 (m, 3H), 3.5 (s, 3H), 3.6 (s, 3H), 4.7 (m, 1H), 5.4 (m, 1H), 6.1 (m, 1H), 6.7 (d, 1H, $J = 2$ Hz), 6.8 (d, 1H, $J = 9.2$ Hz), 7.5 (m, 2H), 7.65 (m, 2H), 10.1 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, $SiMe_4$): δ 25.77, 28.09, 51.88, 53.38, 53.79, 65.11, 110.59, 110.76, 119.63, 121.40, 124.13, 132.00, 134.84, 134.87, 160.44, 167.91, 168.10, 169.35, 169.37 ppm. HRMS calculated for C_{21} H_{21} O_6 N_6 453.1523; found 453.1648.

Dimethyl 2-(1,3-dioxoisoindolin-2-yl)-5-(5-(furan-2-yl)-2H-tetrazol-2-yl)hexanedioate (2c)

The general procedure was applied to dimethyl 2-bromo-5-[5-(furan-2-yl)tetrazol-2-yl] hexanedioate (386 mg, 1 mmol) and potassium phthalimide (555 mg, 3 mmol) to afford 289 mg (75%) of the title compound as a greenish oil. ^{1}H NMR ($CDCl_3$, $SiMe_4$): δ 2.2 (m, 1H), 2.5 (m, 3H), 3.6 (s, 3H), 3.7 (s, 3H), 4.9 (m, 1H), 5.7 (m, 1H), 6.5 (m, 1H), 7.15 (m, 1H), 7.75 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$, $SiMe_4$): δ 25.87, 28.13, 51.76, 53.07, 53.32, 65.77, 112.37, 112.23, 124.07, 131.94, 131.99, 134.78, 134.83, 142.99, 144.92, 158.84, 167.60, 167.88, 169.26 ppm. HRMS calculated for C_{21} H_{20} O_7 N_5 454.1363; found 454.1396.

Dimethyl 2-(5-benzyl-2H-tetrazol-2-yl)-5-(1,3-dioxoisoindolin-2-yl)hexanedioate (2d)

The general procedure was applied to dimethyl 2-(5-benzyltetrazol-2-yl)-5 bromohexanedioate (411 mg, 1 mmol) and potassium phthalimide (555 mg, 3 mmol) to afford 287 mg (70%) of the title compound as a greenish oil. ^{1}H NMR ($CDCl_3$, $SiMe_4$): δ 2.1 (m, 4H), 3.7 (s, 3H), 3.8 (s, 3H), 4.3 (s, 2H), 4.8 (m, 1H), 5.7 (m, 1H), 7.3 (m, 5H), 7.7 (m, 2H), 7.9 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, $SiMe_4$): δ 25.23, 28.11, 35.8, 50.91, 53.40, 69.62, 124.10, 124.12, 129.21, 128.41, 125.52, 127.41, 132.00, 132.30, 137.71, 134.79, 134.83, 164.21, 167.92, 168.01, 172.02 ppm. HRMS calculated for C_{24} H_{24} O_6 N_5 478.1727; found 478.1716.

Dimethyl 2-(1,3-dioxoisoindolin-2-yl)-5-(5-phenyl-2H-tetrazol-2-yl)hexanedioate (2e)

The general procedure was applied to dimethyl 2-bromo-5-(5-phenyltetrazol-2-yl) hexanedioate (397 mg, 1 mmol) and potassium phthalimide (555 mg, 3 mmol) to afford 356 mg (77%) of the title compound as a greenish oil. ^{1}H NMR ($CDCl_3$, $SiMe_4$): δ 2.1 (m, 1H), 2.25 (m, 3H), 3.6 (s, 3H), 3.65 (s, 3H), 4.8 (m, 1H), 5.5 (m, 1H), 7.37 (m, 3H), 7.65 (m, 2H), 7.77 (m, 2H), 8.08 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, $SiMe_4$): δ 25.92, 28.25, 51.87, 53.33, 53.77, 65.50, 124.00, 124.12, 127.40, 127.48, 127.51, 129.26, 130.89, 132.06, 134.79, 134.83, 165.75, 167.85, 168.00, 169.29 ppm. HRMS calculated for C_{23} H_{22} O_6 N_5 464.1570; found 464.1598.

General procedure for the synthesis of 2-amino-5-(5-(substituted-2-yl)-2H-tetrazol-2-yl)hexanedioic acid hydrochloride (L)

A mixture of phthalimido derivative **2** (400 mg) and 6 N HCl (30 ml) and acetic acid (3 ml) was refluxed during 10 h. The reaction mixture was cooled to room temperature and concentrated. The residue was dissolved in water and extracted with AcOEt. Treatment of reaction in acidic conditions followed by addition of a large excess of propylene oxide in methanol afforded **L1**–**L5**.

2-Amino-5-(5-(thiophen-2-yl)-2H-tetrazol-2-yl)hexanedioic acid hydrochloride (L1)

The general procedure was applied to dimethyl 2-(1,3-dioxoisoindolin-2-yl)-5-(5-(5-methylthiophen-2-yl)-2H-tetrazol-2-yl) hexanedioate (400 mg, 0.85 mmol) to afford 318 mg (70%) of the title compound as a greenish oil. ^{1}H NMR (D_2O, $SiMe_4$): δ 1.5 (m, 1H), 185 (m, 1H), 2.27 (m, 2H), 38 (m, 1H), 5.48 (m, 1H), 6.8 (t, 1H, $J_1 = 3.74$ Hz, $J_2 = 1.29$ Hz), 7.25 (d, 1H, $J_1 = 1.09$ Hz, $J_2 = 3.94$ Hz), 7.4 (d, 1H, $J_1 = 1.06$ Hz, $J_2 = 2.63$ Hz) ppm. ^{13}C NMR (D_2O, $SiMe_4$): δ 25.33, 26.58, 52.52, 66.02, 127.28, 128.71, 129.22, 129.73, 161.33, 170.80, 171.67 ppm. HRMS calculated for C_{11} H_{14} O_4 N_5 S 312.0767; found 312.0762.

2-(5-(1H-pyrrol-2-yl)-2H-tetrazol-2-yl)-5-aminohexanedioic acid hydrochloride (L2)

The general procedure was applied to dimethyl 2-(5-(1H-pyrrol-2-yl)-2H-tetrazol-2-yl)-5-(1,3-dioxoisoindolin-2-yl) hexanedioate (400 mg, 0.87 mmol) to afford 307 mg (70%) of the title compound as a greenish oil. ^{1}H NMR (D_2O, $SiMe_4$): δ 1.5 (m, 1H), 1.8 (m, 1H), 2.3 (m, 2H), 3.75 (m, 1H), 5.43 (m, 1H), 5.95 (m, 1H), 6.5 (d, 1H, $J = 2.2$ Hz), 6.7 (s, 1H) ppm. ^{13}C NMR (D_2O, $SiMe_4$): δ 26.41, 26.50, 52.48, 65.61, 110.01, 110.65, 118.37, 122.70, 160.07, 170.99, 171.63 ppm. HRMS calculated for C_{11} H_{15} O_4 N_6 295.1155; found 295.1171.

2-Amino-5-(5-(furan-2-yl)-2H-tetrazol-2-yl)hexanedioic acid hydrochloride (L3)

The general procedure was applied to dimethyl 2-(1,3-dioxoisoindolin-2-yl)-5-(5-(furan-2-yl)-2H-tetrazol-2-yl)hexanedioate (400 mg, 0.88 mmol) to afford 316 mg (70%) of

the title compound as a greenish oil. ^{1}H NMR (D_2O, SiMe4): δ 1.5 (m, 1H), 1.9 (m, 1H), 2.3 (m, 2H), 3.8 (m, 1H), 5.5 (m, 1H), 6.29 (s, 1H), 6.8 (s, 1H), 7.35 (s, 1H) ppm. ^{13}C NMR (D_2O, $SiMe_4$): δ 26.44, 26.50, 52.45, 65.86, 112.34, 112.90, 141.45, 145.79, 158.28, 170.59, 171.58 ppm. HRMS calculated for C_{11} H_{14} O_5 N_5 296.0995; found 296.0986.

2-Amino-5-(5-benzyl-2H-tetrazol-2-yl)hexanedioic acid hydrochloride (L4)

The general procedure was applied to dimethyl 2-(5-benzyl-2H-tetrazol-2-yl)-5-(1,3-dioxoisoindolin-2-yl)hexanedioate (400 mg; 0.83 mmol) to afford 277 mg (70%) of the title compound as a greenish oil. ^{1}H NMR (D_2O, $SiMe_4$): δ 1.65 (m, 1H), 2.1 (m, 1H), 2.48 (m, 2H), 4 (m, 1H), 4.25 (s, 2H), 5.65 (m, 1H), 7.3 (m, 5H) ppm. ^{13}C NMR (D_2O, SiMe4) : δ 19.40, 30.16, 33.90, 56.52, 65.23, 126.59, 128.14, 128.33, 157.83, 161.51, 166.53 ppm. HRMS calculated for C_{14} H_{18} O_4 N_5 320.1359; found 320.1383.

2-Amino-5-(5-phenyl-2H-tetrazol-2-yl)hexanedioic acid hydrochloride (L5)

The general procedure was applied to dimethyl 2-(1,3-dioxoisoindolin-2-yl)-5-(5-phenyl-2H-tetrazol-2-yl)hexanedioate (400 mg; 0.86 mmol) to afford 310 mg (78%) of the title compound as a greenish oil. ^{1}H NMR (D_2O, $SiMe_4$): δ 1.7 (m, 1H), 2.1 (m, 1H), 2.5 (m, 2H), 3.95 (m, 1H), 5.7 (m, 1H), 7.4 (m, 3H), 7.88 (m, 2H) ppm. ^{13}C NMR (D_2O, $SiMe_4$): δ 26.69, 34.89, 52.67, 66.30, 126.23, 127.19, 129.61, 131.57, 165.47, 171.31, 171.88 ppm. HRMS calculated for C_{13} H_{16} O_4 N_5 306.1202; found 306.1219.

Biological tests

Biological tests were all undertaken on rat cultured hippocampal neurons. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/ECC). Sprague–Dawley rats were from the "Centre d'Elevage Depré" (France).

Drugs

Versene, antibiotics, and foetal calf serum (FCS) were purchased from Invitrogen. Culture media (DMEM and Ham F12) and Dulbecco's phosphate-buffered saline (Dulbecco's PBS) were from Eurobio. Culture dishes were from Nunc. Tetrodotoxin was purchased from Latoxan. NBQX (2,3-dihydro-6-nitro-7-sulphamoyl-benzo(f)quinoxaline was obtained from Ascent Scientific (UK). All other compounds were purchased from Sigma.

Primary cultures of rat hippocampal neurons

Primary neuronal cultures were prepared from 18-day-old embryonic rat hippocampi, as previously described, with minor modifications (Blanc et al. 1995, 1999; de Jesus Ferreira et al. 2005). After a pre-incubation with Versene, hippocampal cells were mechanically dissociated and plated at a density of either 5.10^5 cells/dish in 24-well plate or 2.10^6 cells/dish in 8-well plates. All culture plates have been previously coated with poly-L-lysine (15 µg/ml) and then with DMEM/HAM F12 containing 10% FCS. Cells were grown in a defined medium containing DMEM/HAM F12, supplemented with 33 mM glucose, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 5 mM HEPES, 13 mM sodium bicarbonate, 50 µg/ml transferrin, 87 µM insulin, 1 pM β-estradiol, 3 nM triiodothyronine, 20 nM progesterone, 46 nM sodium selenite and 100 µM putrescine. All experiments were performed on cultures grown for at least 2 weeks in vitro.

Cell viability assays

Experiments were performed on cells cultured in 24-well plates. Cells were treated for 24 h with the drugs. Treatments were performed using at least 100 times concentrated aqueous solutions of the drugs directly added to the culture medium. Cell viability was determined by measuring mitochondrial activity using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide), as previously described (Martin et al. 2003). Briefly, cells were washed with 500 µl of Krebs–Ringer buffer comprising 124 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM KH_2PO_4, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-Glucose and 10 mM HEPES/Tris (pH 7.4) (bubbled with O_2/CO_2: 95/5), and then MTT (250 µg/ml) was added. After incubation at 37°C, for 20 min, cells were lysed with DMSO and OD at 570 nm was measured. Data were expressed as the percentages of control OD obtained with untreated cells. Statistical analyses were performed using Student's *t* tests.

Electrophysiological measurements

For electrophysiological studies, neurons were cultured in 8-well dishes containing Thermanox^{TM} coverslips. On the day of the experiment, a coverslip was transferred to the recording chamber of an upright microscope (DMIRB, Leica). Cells were perifused with the extracellular solution containing 124 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-Glucose, 10 mM HEPES/Tris (pH 7.4) (bubbled with O_2/CO_2: 95/5), at room temperature. Whole-cell currents were recorded with glass microelectrodes (4–5 MΩ resistance)

filled with a solution comprising: 120 mM $CsMeSO_3$, 1 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 5 mM N-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314), 5 mM HEPES (pH = 7.3) and 4 mM Mg-ATP. Unless otherwise stated, all the experiments were performed by holding membrane potential at −60 mV. Currents were measured with patch-clamp amplifier (Axopatch 200 B, Axon Instruments, USA) and digitized (Digidata 1200 Interface, Axon Instruments, USA). Signals were filtered at 1 kHz and sampled at 10 kHz. Recording and analysis were performed with John Dempster's software ('WinEDR', Strathclyde University). Drugs were directly puffed on the recorded cell via a gravity-fed perfusion system (Harvard Apparatus). On graphs plotting pooled data, results are presented as mean ± SEM, and the statistical significance of the differences between experimental and control data were assessed using Student's *t* test ($P < 0.05$ considered significant and indicated by *).

NMDA or AMPA/KA receptor-mediated currents

For these recordings, tetrodotoxin (TTX) at 500 nM was added to the extracellular solution to block spontaneous activity. NMDA and KA currents were obtained by applying either 30 μM NMDA or 20 μM KA, respectively. 2-AA derivatives were tested at various concentrations in co-application with either NMDA or KA.

NMDA receptor-mediated excitatory spontaneous synaptic transmission ($sEPSC_{NMDA}$)

In order to record NMDA receptor-mediated excitatory spontaneous synaptic transmission, experiments were performed in an extracellular medium deprived of Mg^{2+} ions and in the presence of picrotoxin (25 μM), NBQX (5 μM) and glycine (10 μM).

Acknowledgments This work was supported by the French Ministry for Higher education and Research and Centre National de la Recherche Scientifique (CNRS). Fabien Lanté was the recipient of a grant from Fondation pour la Recherche Médicale (FRM).

References

Bengtson CP, Dick O, Bading H (2008) A quantitative method to assess extrasynaptic NMDA receptor function in the protective effect of synaptic activity against neurotoxicity. BMC Neurosci 9:11

Blanc EM, Vignes MH, Recasens M (1995) Excitatory amino acid-, except 1S, 3R-ACPD, induced transient high stimulation of phosphoinositide metabolism during hippocampal neuron development. Int J Dev Neurosci 13:723–737

Blanc EM, Jallageas M, Recasens M, Guiramand J (1999) Potentiation of glutamatergic agonist-induced inositol phosphate formation by basic fibroblast growth factor is related to developmental features in hippocampal cultures: neuronal survival and glial cell proliferation. Eur J Neurosci 11:3377–3386

Brauner-Osborne H, Slok FA, Skjaerbaek N, Ebert B, Sekiyama N, Nakanishi S, Krogsgaard-Larsen P (1996) A new highly selective metabotropic excitatory amino acid agonist: 2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid. J Med Chem 39:3188–3194

Brauner-Osborne H, Egebjerg J, Nielsen EO, Madsen U, Krogsgaard-Larsen P (2000) Ligands for glutamate receptors: design and therapeutic prospects. J Med Chem 43:2609–2645

Choi DW (1992) Excitotoxic cell death. J Neurobiol 23:1261–1276

Curtis DR, Watkins JC (1960) The excitation and depression of spinal neurones by structurally related amino acids. J Neurochem 6:117–141

de Jesus Ferreira MC, Crouzin N, Barbanel G, Cohen-Solal C, Recasens M, Vignes M, Guiramand J (2005) A transient treatment of hippocampal neurons with alpha-tocopherol induces a long-lasting protection against oxidative damage via a genomic action. Free Radic Biol Med 39:1009–1020

Espaze F, Hamon J, Hirbec H, Vignon J, Kamenka JM (2000) The low affinity PCP sites in the rat cerebellum not only bind TCP-like but also BTCP-like structures. Eur J Med Chem 35:323–331

Guiramand J, Martin A, de Jesus Ferreira MC, Cohen-Solal C, Vignes M, Recasens M (2005) Gliotoxicity in hippocampal cultures is induced by transportable, but not by nontransportable, glutamate uptake inhibitors. J Neurosci Res 81:199–207

Guldbrandt M, Johansen TN, Frydenvang K, Brauner-Osborne H, Stensbol TB, Nielsen B, Karla R, Santi F, Krogsgaard-Larsen P, Madsen U (2002) Glutamate receptor ligands: synthesis, stereochemistry, and enantiopharmacology of methylated 2-aminoadipic acid analogs. Chirality 14:351–363

Hardingham GE, Fukunaga Y, Bading H (2002) Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 5:405–414

Hirbec H, Mausset AL, Kamenka JM, Privat A, Vignon J (2002) Re-evaluation of phencyclidine low-affinity or "non-NMDA" binding sites. J Neurosci Res 68:305–314

Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 283:70–74

Ivanov A, Pellegrino C, Rama S, Dumalska I, Salyha Y, Ben-Ari Y, Medina I (2006) Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. J Physiol 572:789–798

Johnson JW, Kotermanski SE (2006) Mechanism of action of memantine. Curr Opin Pharmacol 6:61–67

Kew JN, Kemp JA (2005) Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl) 179:4–29

Kristiansen LV, Huerta I, Beneyto M, Meador-Woodruff JH (2007) NMDA receptors and schizophrenia. Curr Opin Pharmacol 7:48–55

Lenda F, Guenoun F, Tazi B, Ben larbi N, Martinez J, Lamaty F (2004) Synthesis of new triazole substituted pyroaminoadipic and pipecolic acid derivatives. Tetrahedron Lett 45:8905–8907

Lenda F, Guenoun F, Tazi B, Ben Larbi N, Allouchi H, Martinez J, Lamaty F (2005) Synthesis of new tetrazole-substituted pyroaminoadipic and pipecolic acid derivatives. Eur J Org Chem 2:326–333

Lenda F, Guenoun F, Martinez J, Lamaty F (2007) Synthesis of new tetrazole and triazole substituted pyroglutamic acid and proline derivatives. Tetrahedron Lett 48:805–808

Martin A, Recasens M, Guiramand J (2003) DNQX-induced toxicity in cultured rat hippocampal neurons: an apparent AMPA receptor-independent effect? Neurochem Int 42:251–260

Mayer ML (2005) Glutamate receptor ion channels. Curr Opin Neurobiol 15:282–288

McLennan H, Hall JG (1978) The action of D-alpha-aminoadipate on excitatory amino acid receptors of rat thalamic neurones. Brain Res 149:541–545

Monn JA, Valli MJ, True RA, Schoepp DD, Leander JD, Lodge D (1993) Synthesis and pharmacological characterization of l-trans-4-tetrazolylproline (ly300020): a novel systemically-active ampa receptor agonist. Bioorg Med Chem Lett 3:95–98

Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA (1991) NMDA antagonist neurotoxicity: mechanism and prevention. Science 254:1515–1518

Ornstein PL, Schoepp DD, Arnold MB, Leander JD, Lodge D, Paschal JW, Elzey T (1991) 4-(Tetrazolylalkyl)piperidine-2-carboxylic acids. Potent and selective N-methyl-D-aspartic acid receptor antagonists with a short duration of action. J Med Chem 34:90–97

Paoletti P, Neyton J (2007) NMDA receptor subunits: function and pharmacology. Curr Opin Pharmacol 7:39–47

Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34:1–26

Recasens M, Guiramand J, Aimar R, Abdulkarim A, Barbanel G (2007) Metabotropic glutamate receptors as drug targets. Curr Drug Targets 8:651–681

Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ (2003) Memantine in moderate-to-severe Alzheimer's disease. N Engl J Med 348:1333–1341

Robinson DM, Keating GM (2006) Memantine: a review of its use in Alzheimer's disease. Drugs 66:1515–1534

Rosenmund C, Feltz A, Westbrook GL (1995) Synaptic NMDA receptor channels have a low open probability. J Neurosci 15:2788–2795

Rothman SM, Olney JW (1995) Excitotoxicity and the NMDA receptor—still lethal after eight years. Trends Neurosci 18:57–58

Shen M, Piser TM, Seybold VS, Thayer SA (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 16:4322–4334

Thomas CG, Miller AJ, Westbrook GL (2006) Synaptic and extrasynaptic NMDA receptor NR2 subunits in cultured hippocampal neurons. J Neurophysiol 95:1727–1734

Watkins JC, Krogsgaard-Larsen P, Honore T (1990) Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. Trends Pharmacol Sci 11:25–33