Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 2200–2211
Bioorganic & Medicinal Chemistry

Structure–activity study of 2,3-benzodiazepin-4-ones noncompetitive AMPAR antagonists: Identification of the 1-(4-amino-3-methylphenyl)-3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-one as neuroprotective agent

Nicola Micale,[a,*,†] Simona Colleoni,[b,c,†] Giovanna Postorino,[a] Alessia Pellicanò,[a] Maria Zappalà,[a] John Lazzaro,[d] Valentina Diana,[c] Alfredo Cagnotto,[c] Tiziana Mennini[c] and Silvana Grasso[a]

[a]*Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Messina, Italy*
[b]*Istituto di Chimica Farmaceutica, Università di Milano, Italy*
[c]*Istituto di Ricerche Farmacologiche 'Mario Negri', Milano, Italy*
[d]*Pfizer Global Research and Development, Groton, CT, USA*

Received 20 July 2007; revised 22 November 2007; accepted 30 November 2007
Available online 5 December 2007

Abstract—In the search for AMPA receptor (AMPAR) antagonists, 2,3-benzodiazepines represent a family of specific noncompetitive antagonists with anticonvulsant and neuroprotective properties. We have previously shown that 2,3-benzodiazepin-4-ones possess marked anticonvulsant properties and high affinity for the noncompetitive binding site of the AMPAR complex. In this paper, we report the synthesis and pharmacological characterization of a full set of 2,3-benzodiazepin-4-ones in order to better define the structure–activity relationship (SAR) of this class of compounds. Binding assays and functional tests were performed to evaluate the antagonistic activity at the AMPARs. Through these results we have identified a potent AMPAR antagonist, 1-(4-amino-3-methylphenyl)-3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-one (**5c**). This compound noncompetitively inhibited AMPAR-mediated toxicity in primary mouse hippocampal cultures with an IC_{50} of 1.6 μM and blocked kainate-induced calcium influx in rat cerebellar granule cells with an IC_{50} of 6.4 μM. Thus, **5c** has the in vitro potential as therapeutic drug in the treatment of various neurological disorders.

1. Introduction

α-Amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors (AMPARs) are glutamate-gated ion channels which mediate the majority of fast excitatory synaptic transmissions in the mammalian brain. They belong to a larger family of ionotropic glutamate receptors including the closely related kainate (KA) and *N*-methyl-D-aspartic acid (NMDA) receptors.[1] Hyperactivation of AMPARs is implicated in a broad range of acute and chronic neurodegenerative and neuropsychiatric conditions, for example, cerebral ischemia, epilepsy, and Alzheimer's disease.[2] Thus, the modulation of AMPARs as potential targets for therapeutic intervention in many neurological disorders, and the development of selective competitive and noncompetitive antagonists, is one of the main goals in neuropharmacology.

Among the competitive agents, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline (NBQX) has been shown to be antiepileptic and neuroprotective in a variety of tests of cerebral ischemia and neurodegeneration. However, NBQX and its analogues have been recently shown to increase γ-amino-butyric acid (GABA) transmission in the cerebellum by non-AMPA-dependent mechanism, to depolarize hippocampal, and also to act at the KA receptors, implying that these agents may not be selective.[3] Noncompetitive AMPAR antagonists have the theoretical advantage to counteract excitotoxicity even at high

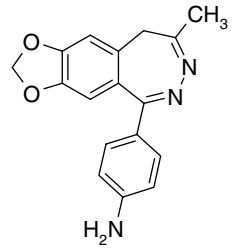
Keywords: 2,3-Benzodiazepin-4-ones; AMPAR affinity; Binding assay.
* Corresponding author. Tel.: +39 090 6766466; fax: +39 090 355613; e-mail: nmicale@pharma.unime.it
† Authors contributed equally to this work.

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.11.080

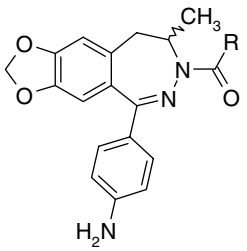
concentration of glutamate and to show less side-effects than competitive antagonists.[4] To date, there are a number of pharmacological agents that affect AMPAR function through interactions outside of the agonist-binding domain.[5] Among them there are 2,3-benzodiazepines (2,3-BZs) noncompetitive AMPAR antagonists whose prototype, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466) (Fig. 1), demonstrated significant anticonvulsant and neuroprotective action.[6] These antagonists bind at the interface between the S1 and S2 glutamate binding core and channel transmembrane domains, specifically interacting with S1-M1 and S2-M4 linkers, thereby disrupting the transduction of agonist binding into channel opening.[7]

Highly active analogues of GYKI 52466 have been found, for example, 3,4-dihydro-3-*N*-methylcarbamoyl (GYKI 53655) and 3,4-dihydro-3-*N*-acetyl (GYKI 53405) derivatives (Fig. 1).[8] The enantioselectivity of these derivatives has been evaluated, and the eutomer turned out to be the (4*R*)-enantiomer.[6] Subsequently, the eutomer (4*R*)-(+) of GYKI 53405 (Talampanel) was chosen as a drug candidate and is now in clinical investigation as an anticonvulsant and antiischemic.[9]

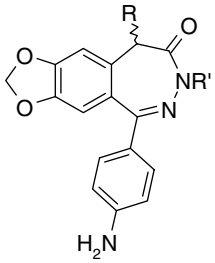
In the course of our studies we synthesized 1-(4-aminophenyl)-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-one (**1a**) and its 3-*N*-methylcarbamoyl derivative (**1b**) (Fig. 1), which have been shown to interact with the same allosteric AMPAR binding site of GYKI 52466.[10] Furthermore, kinetic investigations of the mechanism of inhibition performed by using a laser-pulse photolysis technique put in evidence that this class of compounds preferably inhibits the open-channel



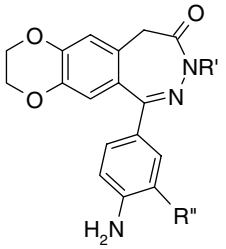
GYKI 52466



GYKI 53655, R=NHCH$_3$
GYKI 53405, R=CH$_3$



1a, R=R'=H
1b, R=H, R'=CONHCH$_3$
2a, R=CH$_3$, R'=H



5a, R'=R"=H
5b, R'=CONHCH$_3$, R" = H
5c, R'=H, R" = CH$_3$
5f, R'=H, R" = Cl

Figure 1.

state of the GluR2Q$_{flip}$, a key subunit that controls the calcium permeability of heteromeric AMPARs and thus is involved in the etiology of neurodegenerative disorders.[11]

By introducing a methyl group at C-5 of the diazepine nucleus, we have recently demonstrated a stereoselective interaction of **2a** (Fig. 1) with the noncompetitive binding site of the AMPARs and that (5*S*)-(−)-**2a** resulted to be the eutomer. Noteworthy, the configuration of (5*S*)-(−)-**2a** is opposite to that of Talampanel. However, a superimposition of the most populated conformation of the two molecules reveals an excellent overlapping of the common functional groups.[12] Moreover, in a recent paper we reported preliminary results that showed an improvement toward AMPAR affinity of 2,3-benzodiazepin-4-ones by substituting the dioxole nucleus with the dioxane homologue (i.e., **5a**, **5c**, and **5f**).[13]

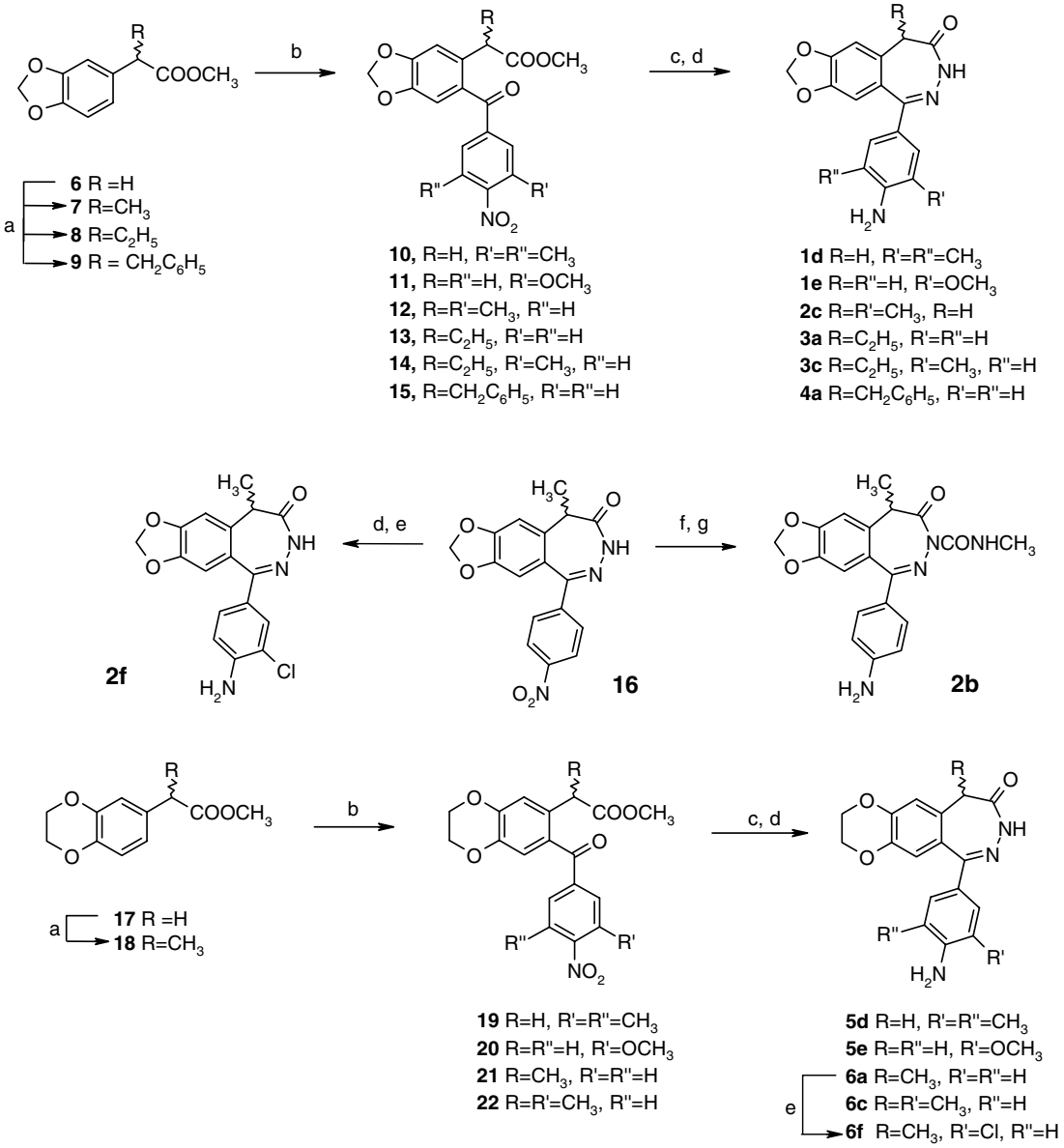
All these interesting results prompted us to synthesize a complete set of 2,3-benzodiazepin-4-ones in which we conveyed into our lead structures **1a**, **2a**, and **5a** the most remarkable modifications observed throughout our previous research efforts with the aim to gain more information from SAR studies and optimize the pharmacological profile of this class of noncompetitive AMPAR antagonists.

To evaluate the AMPAR antagonism, all compounds have been screened against KA-induced Ca^{2+} uptake, which is mediated by AMPARs, in primary cultures of rat cerebellar granule neurons, and their interaction with the noncompetitive binding site labeled by [^{3}H]CP-526,427 has been assessed. Several active compounds were found, among which 1-(4-amino-3-methylphenyl)-3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-one (**5c**) disclosed the highest potency and so it was selected for a study as neuroprotective agent. Therefore, we have established an in vitro model of AMPAR-mediated excitotoxicity utilizing mouse embrionic hippocampal cultures exposed to KA.

2. Results and discussion

2.1. Chemistry

The synthesis of target compounds was accomplished according to the reaction sequence reported in Scheme 1. Methyl 3,4-methylenedioxyphenylacetate (**6**) was converted in high yield into the corresponding α-methyl **7**[12] or α-ethyl derivatives **8** by α-alkylation with the appropriate alkyl iodide conducted in THF and in the presence of potassium hydride. A hindered strong base such as potassium hexamethyldisilazane (KHMDS) was necessary to efficiently form the kinetic enolate of the carbonyl group and to introduce thereby the benzyl group (**9**). Intermediate ketoesters **10**–**15** were easily prepared via acylation of derivatives **6**–**9** with the appropriate 4-nitrobenzoic acid in the presence of an excess of phosphorus pentoxide. The treatment of **10**–**15** with hydrazine, followed by reduction with Raney-Ni/ammonium formate, gave 1-(4-aminophenyl)-3,5-dihydro-7,8-



Scheme 1. Reagents and conditions: (a) CH_3I or C_2H_5I and KH, or BnBr and KHMDS, THF anhydrous, −70 °C, rt, 16 h; (b) ArCOOH, P_2O_5, $(CH_2Cl)_2$, rt, 16 h; (c) $NH_2NH_2{\cdot}H_2O$, ethanol or *n*-butanol, Δ, 20 h; (d) $HCOONH_4$, Ni-Raney, EtOH, Δ, 2 h; (e) NCS, DMF, rt, 20 h; (f) CH_3NCO, CH_2Cl_2, Et_3N, rt, 24 h; (g) H_2/5% Pd–C, $CHCl_3$, rt, 3 h.

methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**1d**–**1e**, **2c**, **3a**, **3c**, **4a**) in good yields.

Compound **16** by reduction with Raney-Ni/ammonium formate and successive treatment with *N*-chlorosuccinimide (NCS) afforded compound **2f**, whereas by treatment with an excess of methyl isocyanate in the presence of triethylamine yielded the corresponding 3-*N*-methylcarbamoyl derivative, which furnished compound **2b** by catalytic hydrogenation with 5% Pd/C. Following analogous procedures, 7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-ones **5d**, **5e**, **6a**, **6c**, and **6f** were synthesized starting from methyl 3,4-ethylenedioxyphenylacetate/propionate (**17**, **18**), via ketoesters **19**–**22**, as shown in Scheme 1. Physical and spectral data (^{1}H NMR) of the synthesized compounds are in agreement with the proposed structures.

2.2. Pharmacology

2.2.1. Radioligand binding assay and structure–activity relationships. We have examined compounds **1**–**6** for their ability to displace [^{3}H]CP-526,427 from the corresponding binding site of the AMPAR complex. [^{3}H]CP-526,427 binds in a saturable manner to rat forebrain membranes with K_d value 10.47 nM and B_{max} value 4.2 pmol/mg of protein. The inhibition of [^{3}H]CP-526,427 specific binding (3 nM) to rat forebrain membranes was evaluated as previously described.[14] In this assay, CP-465,022 was used as a positive control; its IC_{50} values ranged from 20 to 40 nM. Most of the compounds show an ability to inhibit specific binding similar to that of GYKI 52466 (**1b**, **1c**, **2a**, **2c**, **2f**, **3a**, **3c**, **5a**, **5b**, **6a**); whereas some of them have a higher affinity (**2b**, **5c**, **5f**, **6c**) (Table 1).

A survey of these results put in evidence that the introduction of a methyl or ethyl group at C-5 of the diazepine nucleus enhances the affinity toward the noncompetitive binding site of AMPARs (e.g., **1a** IC_{50} 32 μM vs **2a** IC_{50} 12.9 μM and **3a** IC_{50} 8.37 μM), suggesting that a hydrophobic substituent at C-5 could be suitable for a better fit with the binding site. To evaluate the presence in the 2,3-BZ binding site of a pocket capable to accommodate groups more sterically hindered, we synthesized 5-benzyl derivative **4a**. The results clearly indicate that the insertion of a bulkier substituent strongly decreases the affinity (**4a** $IC_{50} > 100$ μM), suggesting the presence of a hydrophobic pocket of limited size in the binding site. Considering that both the introduction of a methyl group at C-5 and the ring enlargement of the dioxole ring to dioxane homologue brought to an increase in binding affinity (**1a** IC_{50} 32 μM vs **5a** IC_{50} 9.0 μM), we try to improve the interactions between our compounds and the AMPAR binding site by inserting concomitantly these two modifications. However, the insertion of a methyl group at C-5 in the 7,8-ethylenedioxy derivatives **6** does not seem to affect significantly the binding affinity (e.g., **5a** IC_{50} 9.0 μM vs **6a** IC_{50} 6.96 μM).

When we add a methyl or chlorine substituent into phenyl ring at C-1 close to the amino group, we generally observe an improvement of affinity both in the dioxole series (e.g., **1a** IC_{50} 32 μM vs **1c** IC_{50} 6.3 μM) and in the dioxane analogues (e.g., **5a** IC_{50} 9.0 μM vs **5c** IC_{50} 1.84 μM and **5f** IC_{50} 2.05 μM). Noteworthy, this structural modification should also slow down the first path metabolism (acetylation of the 4-amino group), as reported for a number of 2,3-BZ analogues that have a longer duration of action.[15]

Owing to the good AMPAR affinity of compounds **1c**, **5c**, and **5f**, we synthesized a number of derivatives 3,5-dimethyl or 3-methoxy substituted. However, the presence of two methyl groups decreases the receptor affinity (e.g., **1d** and **5d** $IC_{50} > 100$ μM) probably for a steric hindrance that completely shielded amino function, an essential structural requirement involved in the binding with the noncompetitive site of AMPARs. Similarly the lack of affinity of the 3-methoxy substituted derivatives (**1e** and **5e**) might be due to steric effects. Finally, a methylcarbamoyl moiety at N-3 determines an improvement of affinity in the dioxane series (e.g., **5a** IC_{50} 9.0 μM vs **5b** IC_{50} 7.78 μM), as well as within the dioxole series (e.g., **1a** IC_{50} 32 μM vs **1b** IC_{50} 12 μM and **2a** IC_{50} 12.9 μM vs **2b** IC_{50} 4.01 μM).

2.2.2. Intracellular calcium influx. Functional antagonism was determined by evaluating the ability of compounds **1**–**6** to inhibit KA-induced increase of the $[Ca^{2+}]_i$ in primary cultures of rat cerebellar granule cells (CGC) which express AMPARs (Table 1); GYKI 52466 was used as the control.[16]

With the exception of **1d** and **5d**, inactive also in the binding assay, all compounds completely inhibited the KA-induced Ca^{2+} uptake in a concentration-dependent manner with IC_{50} values ranging from 1.23 to 31 μM (Table 1).

Their ability to reduce Ca^{2+} uptake is similar or better than that shown by GYKI 52466.

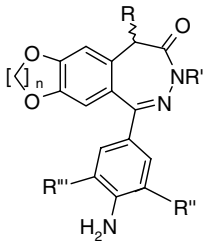
The results of this investigation are roughly in accordance with the data obtained in the binding experiments. It is clear that compounds containing a 7,8-ethylenedioxy fragment (**5**–**6**) possess a higher antagonist activity at the AMPAR than the corresponding 7,8-methylenedioxy homologues (**1**–**2**). In fact, compounds **5a**, **5b**, and **6c** are more potent than the corresponding derivatives **1a**, **1b**, and **2c** (**5a** IC_{50} 2.9 μM, **5b** IC_{50} 5.4 μM, **6c** IC_{50} 1.23 μM vs **1a** IC_{50} 12 μM, **1b** IC_{50} 11 μM, **2c** IC_{50} 18.8 μM, respectively).

2.2.3. Kainate-induced toxicity. We have also characterized the excitotoxicity of KA in primary cultures of mouse hippocampal neurones from mouse embryos (E13) at 7 days in vitro (DIV). Exposure of cultured hippocampal cells to various concentrations of KA (5–500 μM) for 48 h induced concentration-dependent neurotoxicity, as revealed by the MTT assay. Nonlinear regression analysis of viability values yielded an EC_{50} of 17.3 μM (Fig. 2).

Evidence for KA-induced excitotoxicity through AMPA-preferring receptors is that NBQX and GYKI 52466 completely blocked this toxicity (KA 30 μM) (Fig. 3) with an IC_{50} 11.9 ± 0.07 μM and 10.8 ± 0.25 μM, respectively. Using this test, we evaluated the neuroprotective effects of compound **5c** (Fig. 3), which possesses the highest affinity for AMPAR (IC_{50} 1.84 μM) and a good in vivo anticonvulsant activity, as previously reported.[13] The results presented in Figure 4A show that **5c** inhibited KA-induced toxicity completely and concentration-dependently. Compound **5c** induced, dose-dependently, a shift of the KA responses (Fig. 4B); higher KA concentrations than those shown in Figure 4B were not used because of its poor solubility and nonspecific effects. Noteworthy, compound **5c** showed higher neuroprotective activity than NBQX and GYKI 52466 with an IC_{50} value of 1.6 ± 0.02 μM.

Previous studies were carried out on the neuroprotective efficacy of GYKI 52466, LY300164 (Talampanel, eutomer of GYKI 53405), and LY303070 (eutomer of GYKI 53655) in an embryonic rat hippocampal culture model of AMPA receptor-mediated excitotoxicity.[17,18] These compounds attenuated the KA-excitotoxicity in a dose-dependent manner with LY300164 and LY303070 being 2.25 and 5.4 times more potent than GYKI 52466, respectively. Although absolute comparison of the IC_{50} values cannot be done due to species differences (the data that we have obtained came from embryonic mouse hippocampal culture), in our study **5c** is 6.75 times more potent than GYKI 52466. These findings suggest that **5c** has neuroprotective efficacy comparable to LY303070 and higher than LY300164, a promising neuroprotective drug candidate.[19]

Table 1. Pharmacological data of compounds **1**–**6** and GYKI 52466



Compound	*n*	R	R′	R″	R‴	[^{3}H]CP-526,427 IC_{50} (μM)	KA-[Ca^{2+}]$_i$ IC_{50} (μM)
1a	1	H	H	H	H	32[a]	12[a]
1b	1	H	$CONHCH_3$	H	H	12[a]	11[a]
1c	1	H	H	CH_3	H	6.3	2.5
1d	1	H	H	CH_3	CH_3	>100	>100
1e	1	H	H	OCH_3	H	>100	31
2a	1	CH_3	H	H	H	12.9	12[a]
2b	1	CH_3	$CONHCH_3$	H	H	4.01	15.8
2c	1	CH_3	H	CH_3	H	9.62	18.8
2f	1	CH_3	H	Cl	H	11.4	7.0
3a	1	C_2H_5	H	H	H	8.37	17.1
3c	1	C_2H_5	H	CH_3	H	15.6	4.34
4a	1	CH_2Ph	H	H	H	>100	ND
5a	2	H	H	H	H	9.0	2.9[a]
5b	2	H	$CONHCH_3$	H	H	7.78	5.4[a]
5c	2	H	H	CH_3	H	1.84[a]	6.4
5d	2	H	H	CH_3	CH_3	>100	>100
5e	2	H	H	OCH_3	H	81.1	12
5f	2	H	H	Cl	H	2.05[a]	2.0
6a	2	CH_3	H	H	H	6.96	ND
6c	2	CH_3	H	CH_3	H	4.5	1.23
6f	2	CH_3	H	Cl	H	21.6	18.0
GYKI 52466					H	12.6[a]	22[a]

IC_{50} are representative values of three different experiments. Variability is less than 10%.
[a] Data taken from Refs. 12 and 13.

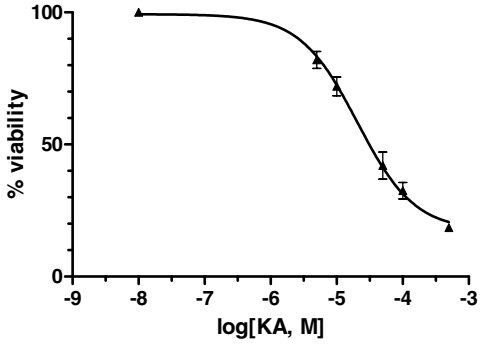


Figure 2. Dose–response of KA toxicity in mouse hippocampal cultures. Cultures (7 DIV) were treated with KA at the indicated concentrations and culture viability was assessed 48 h later. Cell viability was measured by MTT assay. Each point is the mean ± SEM of five–six experiments, carried out in duplicate.

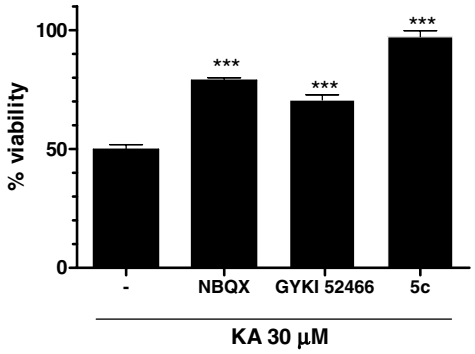


Figure 3. Protective effect of AMPAR antagonist on KA-induced toxicity. Mouse hippocampal neurones were treated with the compounds (10 μM NBQX, 10 μM GYKI 52466, and 3 μM **5c**) concomitantly with KA addition (30 μM) for 48 h. The data represent means ± SEM of two experiments carried out in triplicate. The statistical analysis was carried out with the one-way ANOVA followed by Tukey's test $^{***}p < 0.001$ versus KA.

3. Conclusion

In summary, SAR study of the complete set of 2,3-benzodiazepinen-4-ones reveals compound **5c** as the most potent AMPAR antagonist. **5c** significantly inhibits [^{3}H]CP-526,427 specific binding, strongly blocks AMPAR-mediated toxicity in primary mouse hippocampal cell cultures, and completely reduces calcium influx in rat cerebellar granule neurones. Thus, **5c** has the in vitro potential as therapeutic drug in the treatment of various types of neurological disorders.

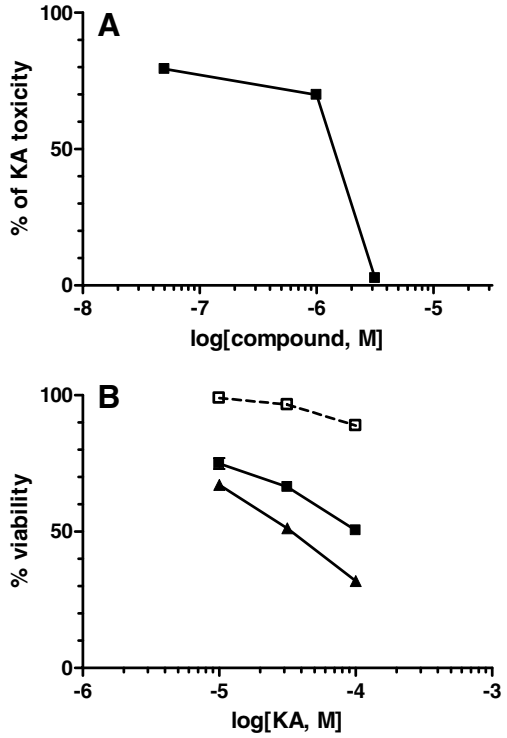


Figure 4. Effect of **5c** on KA toxicity in mouse hippocampal cultures. (A) Cultures (8 DIV) were treated with 30 μM KA in the presence of the indicated concentration of **5c** (■). (B) Cultures (8 DIV) were treated with the indicated concentration of KA either alone (▲) or in the presence of 1 μM (■) or 3 μM (□) **5c**. Culture viability was assessed 48 h later. Data are means ± SEM of three independent experiments.

4. Experimental

4.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were carried out on a C. Erba Model 1106 (elemental analyzer for C, H, and N) and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used as analytical TLC; column chromatography was performed on Merck silica gel 60 (70–230 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 by means of a Varian Gemini 300 spectrometer. Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hz. Compounds **1a**–**1c**, **2a**, **5a**–**5c**, and **5f** were synthesized as reported in the literature.[12,13,20,21]

4.2. Synthesis

4.2.1. Methyl 2-(3,4-methylenedioxyphenyl)butyrate (8). To a stirred solution of methyl 3,4-methylenedioxyphenylacetate **6** (3 g, 15.45 mmol) in anhydrous THF (50 mL) at −70 °C was added potassium hydride (682 mg, 17 mmol). After 30 min was added ethyl iodide (1.36 mL, 2.65 g, 17 mmol), keeping the temperature at −70 °C for many hours. The cooling bath was removed and the reaction mixture was stirred for further 16 h, then diluted with 50% acetic acid, poured into water, and extracted with diethyl ether (2 × 150 mL). The combined organic layers were washed with a saturated solution of Na_2CO_3 and dried over Na_2SO_4. The solvent was then removed and the residue was purified by a silica gel column chromatography (cyclohexane/diethyl ether 60:40) to afford compound **8** (2.9 g, 85%) as a colorless oil. R_f = 0.68 (cyclohexane/diethyl ether 60:40). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 0.87 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.67–2.06 (m, 2H, CH_2CH_3), 3.37 (t, 1H, J = 7.7 Hz, CH), 3.65 (s, 3H, OCH_3), 5.95 (s, 2H, OCH_2O), 6.70–6.87 (m, 3H, Ar). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.71; H, 6.47.

4.2.2. Methyl 2-(3,4-methylenedioxyphenyl)-3-phenylpropionate (9). To a stirred solution of **6** (2 g, 10.3 mmol) in anhydrous THF (50 mL) at −70 °C were added KHMDS (14.23 mL, 10.8 mmol) and benzyl bromide (1.35 mL, 11.33 mmol) in more portions. The solution was stirred for 5 h increasing gradually the temperature until room temperature. The solution was quenched with sat. NH_4Cl (5 mL) and the aqueous phase was extracted with diethyl ether (2 × 150 mL). The combined organic layers were dried over Na_2SO_4. The solvent was then removed and the residue was purified by silica gel column chromatography (cyclohexane/diethyl ether 70:30) to afford compound **9** (726 mg, 25%) as a colorless oil. R_f = 0.61 (cyclohexane/diethyl ether 70:30). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.17 (AB of ABX, 2H, J_{AB} = 13.7 Hz, J_{AX} = 8.5 Hz, J_{BX} = 7.1 Hz, $\Delta\nu$ = 66.9 Hz, CH_2-Ph), 3.60 (s, 3H, CH_3), 3.76 (dd, 1H, J = 8.5 Hz, and J = 7.1 Hz, CH-Bn), 5.92–5.95 (m, 2H, OCH_2O), 6.72 (s, 2H, Ar), 6.85 (s, 1H, Ar), 7.11 (d, 2H, J = 6.6 Hz, Ar), 7.17–7.41 (m, 3H, Ar). Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.59; H, 5.48.

4.2.3. Methyl 2-[2-(3,5-dimethyl-4-nitrobenzoyl)-4,5-methylenedioxyphenyl]acetate (10). 3,5-Methyl-4-nitrobenzoic acid (331 mg, 1.69 mmol) and phosphorus pentoxide (2 g) were added to a stirred 1,2-dichloroethane solution (100 mL) of **6** (253 mg, 1.3 mmol). The mixture was further stirred at room temperature for 16 h, then water (30 mL) was cautiously added and the mixture extracted with chloroform (2 × 30 mL). The organic layer was separated and sequentially treated with 10% NaOH (30 mL), brine (30 mL), and water (2 × 30 mL). The organic phase was dried (Na_2SO_4) and the solvent removed under reduced pressure to yield crude **10** which was purified by column chromatography using diethyl ether/light petroleum (50:50) as eluant; pale yellow powder. Mp = 127–128 °C (300 mg, 62%) R_f = 0.51 (diethyl ether/light petroleum 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 2.35 (s, 6H, $2CH_3$), 3.64 (s, 3H, $COOCH_3$), 3.83 (s, 2H, CH_2), 6.06 (s, 2H, OCH_2O), 6.83 and 6.84 (2s, 2H, H-6 and H-3), 7.51–7.52 (m, 2H, H-2′,6′). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61; N, 3.77. Found: C, 61.73; H, 4.72; N, 3.52.

4.2.4. Methyl 2-[4,5-methylenedioxy-2-(3-methoxy-4-nitrobenzoyl)-phenyl]acetate (11). With a similar procedure, **11** was prepared from **6** (693 mg, 3.57 mmol), 3-methoxy-4-nitrobenzoic acid (914 mg, 4.64 mmol), and

phosphorus pentoxide (2 g); yellow soft powder. Mp = 152–153 °C (400 mg, 30%) R_f = 0.33 (diethyl ether/light petroleum 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 3.63 (s, 3H, $COOCH_3$), 3.84 (s, 2H, CH_2), 4.00 (s, 3H, OCH_3), 6.06 (s, 2H, OCH_2O), 6.84 (s, 2H, H-6 and H-3), 7.32 (dd, 1H, J_o = 8.2 Hz, J_m = 1.4 Hz, H-6′), 7.54 (d, 1 H, J_m = 1.4 Hz, H-2′), 7.83 (d, 1 H, J_o = 8.2 Hz, H-5′). Anal. Calcd for $C_{18}H_{15}NO_8$: C, 57.91; H, 4.05; N, 3.75. Found: C, 58.12; H, 4.21; N, 3.64.

4.2.5. Methyl 2-[4,5-methylenedioxy-2-(3-methyl-4-nitrobenzoyl)-phenyl]propionate (12). With a similar procedure, **12** was prepared from **7**[12] (826 mg, 3.97 mmol), 3-methyl-4-nitrobenzoic acid (934 mg, 5.16 mmol), and phosphorus pentoxide (2 g). Mp = 102–104 °C (387 mg, 26%) R_f = 0.40 (diethyl ether/light petroleum 40:60); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.50 (d, 3H, J = 7.1 Hz, CH_3-α), 2.63 (s, 3H, Ar-CH_3), 3.60 (s, 3H, OCH_3), 4.11 (q, 1H, J = 7.1 Hz, CH), 6.06 (s, 2H, OCH_2O), 6.75 (s, 1H, H-6), 7.00 (s, 1H, H-3), 7.71 (d, 1H, J = 8.2 Hz, H-6′), 7.77 (s, 1H, H-2′), 7.99 (d, 1H, J = 8.2 Hz, H-5′). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61; N, 3.77. Found: C, 61.26; H, 4.34; N, 3.95.

4.2.6. Methyl 2-[4,5-methylenedioxy-2-(4-nitrobenzoyl)-phenyl]butyrate (13). With a similar procedure, **13** was prepared from **8** (1.5 g, 6.75 mmol), 4-nitrobenzoic acid (1.47 g, 87.7 mmol), and phosphorus pentoxide (4 g). Mp = 179–181 °C (1.2 g, 48%) R_f = 0.51 (diethyl ether/light petroleum 40:60); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 0.86 (t, 3H, J = 7.4, CH_2CH_3) 1.70–1.86 and 2.01–2.10 (2m, 2H, CH_2CH_3), 3.60 (s, 3H, OCH_3), 3.90 (t, 1H, J = 7.4 Hz, CH), 6.05 (s, 2H, OCH_2O), 6.72 (s, 1H, H-6), 7.06 (s, 1H, H-3), 7.94 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.31 (d, 2H, J = 8.8 Hz, H-3′,5′). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61; N, 3.77. Found: C, 61.62; H, 4.50; N, 3.64.

4.2.7. Methyl 2-[4,5-methylenedioxy-2-(3-methyl-4-nitrobenzoyl)-phenyl]butyrate (14). With a similar procedure, **14** was prepared from **8** (2.8 g, 11.8 mmol), 3-methyl-4-nitrobenzoic acid (2.8 g, 15.3 mmol), and phosphorus pentoxide (6 g). The crude was purified by column chromatography using diethyl ether/light petroleum (60:40) as eluant. Mp = 110–112 °C (2.4 g, 56%) R_f = 0.6 (diethyl ether/light petroleum 60:40); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 0.86 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.65–2.18 (2m, 2H, CH_2CH_3), 2.63 (s, 3H, Ar-CH_3), 3.61 (s, 3H, OCH_3), 3.86 (t, 1H, J = 6.9 Hz, CH), 6.05 (s, 2H, OCH_2O), 6.74 (s, 1H, H-6), 7.06 (s, 1H, H-3), 7.65 (dd, 1H, J_o = 8.2 Hz and J_m = 1.8 Hz, H-6′), 7.76 (d, collapsed, 1H, H-2′), 7.99 (d, 1H, J = 8.2 Hz, H-5′). Anal. Calcd for $C_{20}H_{19}NO_7$: C, 62.33; H, 4.97; N, 3.64. Found: C, 62.21; H, 5.17; N, 3.75.

4.2.8. Methyl 2-[4,5-methylenedioxy-2-(4-nitrobenzoyl)-phenyl]-3-phenylpropionate (15). With a similar procedure, **15** was prepared from **9** (726 mg, 3.97 mmol), 4-nitrobenzoic acid (556 mg, 3.32 mmol), and phosphorus pentoxide (2 g); yellow crystals. Mp = 141–143 °C (670 mg, 60%) R_f = 0.50 (diethyl ether/light petroleum 40:60); ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.19 (AB of ABX, 2H, J_{AB} = 13.5 Hz, J_{AX} = 7.1 Hz, J_{BX} = 8.2 Hz, $\Delta\nu$ = 58.9 Hz, CH_2-Ph), 3.63 (s, 3H, CH_3), 4.48 (dd, 1H, J = 7.1;Hz and 8.2 Hz, CH-Bn), 6.03–6.09 (m, 2H, OCH_2O), 6.60 (s, 1H, H-6), 7.18 (s, 1H, H-3), 7.30–7.00 (m, 5H, Ar), 7.59 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.21 (d, 2H, J = 8.8 Hz, H-3′,5′). Anal. Calcd for $C_{24}H_{19}NO_7$: C, 66.51; H, 4.42; N, 3.23. Found: C, 66.82; H, 4.38; N, 3.14.

4.2.9. 1-(4-Amino-3,5-dimethylphenyl)-3,5-dihydro-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (1d). Hydrazine hydrate (0.13 mL, 2.59 mmol) and HCl 6N (0.4 mL) were added to a solution of compound **10** (300 mg, 0.81 mmol) in *n*-butanol (40 mL), then the mixture was heated at reflux and stirred for 20 h. After cooling at room temperature, the formed precipitate was filtered off, washed with MeOH, dried, and recrystallized from MeOH to give pure nitroderivative. Data of nitroderivative: mp > 300 °C (140 mg, 49%) R_f = 0.38 (EtOAc/cyclohexane 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 2.34 (s, 6H, $2CH_3$), 3.46 (s, 2H, CH_2), 6.05 (s, 2H, OCH_2O), 6.58 (s, 1H, H-6), 6.84 (s, 1H, H-9), 7.36 (s, 2H, H-2′,6′), 8.70 (br s, 1H, NH). A suspension of nitroderivative (140 mg, 0.4 mmol), Raney-Ni, and ammonium formate (100 mg, 1.58 mmol) in EtOH (40 mL) was stirred under reflux for 2 h and then filtered through Celite. The organic layer was evaporated under reduced pressure and the residue, dissolved in $CHCl_3$, was washed with saturated NaCl to remove ammonium formate. The organic layer, dried over Na_2SO_4, was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with EtOAc/MeOH 99:1 to afford **1d** as yellow soft powder. Data of **1d**: mp = 297–300 °C (dec) (55 mg, 43%) R_f = 0.73 (EtOAc/MeOH 99:1). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 2.19 (s, 6H, $2CH_3$), 3.42 (s, 2H, CH_2), 3.86 (br s, 2H, NH_2), 6.02 (s, 2H, OCH_2O), 6.71 (s, 1H, H-6), 6.82 (s, 1H, H-9), 7.19 (s, 2H, H-2′,6′), 8.52 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.67; H, 5.42; N, 13.21.

4.2.10. 1-(4-Amino-3-methoxyphenyl)-3,5-dihydro-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (1e). With a similar procedure, **1e** was prepared from **11** (400 mg, 1.07 mmol), hydrazine hydrate (0.17 mL, 3.43 mmol), and HCl 6N (0.52 mL) in *n*-butanol (40 mL). Data of nitroderivative: mp = 267–271 °C (80 mg, 21%) R_f = 0.22 (EtOAc/cyclohexane 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 3.48 (s, 2H, CH_2), 4.01 (s, 3H, OCH_3), 6.06 (s, 2H, OCH_2O), 6.59 (s, 1H, H-6), 6.85 (s, 1H, H-9), 7.13 (d, 1H, J_o = 8.5 Hz, H-6′), 7.49 (s, 1H, H-5′), 7.86 (d, 1H, J_o = 8.5 Hz, H-2′), 8.50 (br s, 1H, NH). Successive reduction was performed starting from nitro derivative (80 mg, 0.23 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **1e**: mp = 247–251 °C (47 mg, 64%) R_f = 0.73 (EtOAc/MeOH 99:1); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 3.43 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.08 (br s, 2H, NH_2), 6.02 (s, 2H, OCH_2O), 6.66 (d, 1H, J_o = 8.0 Hz, H-5′), 6.73 (s, 1H, H-6), 6.81 (s, 1H, H-9), 6.87 (dd,

1H, 7.13, J_o = 8.0 Hz, J_m = 1.6 Hz, H-6′), 7.25 (d, 1H, J_m = 1.9 Hz, H-2′), 8.34 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}N_3O_4$: C, 62.76; H, 4.65; N, 12.92. Found: C, 66.58; H, 4.56; N, 12.73.

4.2.11. 1-(4-Amino-3-methylphenyl)-3,5-dihydro-5-methyl-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (2c). With a similar procedure, **2c** was prepared from **12** (387 mg, 1.04 mmol), hydrazine hydrate (0.06 mL, 1.15 mmol) in *n*-butanol (40 mL); yellow powder. Data of nitroderivative: mp = 273–275 °C (99 mg, 27%) R_f = 0.54 (EtOAc/cyclohexane 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 1.63 (d, 3H, J = 6.9 Hz, CH_3-α), 2.64 (s, 3H, Ar-CH_3), 3.25 (q, 1H, J = 6.9 Hz, CH), 6.06 (s, 2H, OCH_2O), 6.56 (s, 1H, H-9), 6.90 (s, 1H, H-6), 7.58 (d, 1H, J = 8.5 Hz, H-6′), 7.64 (s, 1H, H-2′), 8.02 (d, 1H, J = 8.5 Hz, H-5′), 8.60 (br s, 1H, NH). Successive reduction was performed starting from nitroderivative (100 mg, 0.28 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **2c**: mp = 239–241 °C (74 mg, 82%) R_f = 0.55 (EtOAc/cyclohexane 80:20); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 1.59 (d, 3H, J = 6.6 Hz, CH_3-α), 2.19 (s, 3H, Ar-CH_3), 3.27 (q, 1H, J = 6.6 Hz, CH), 3.87 (br s, 2H, NH_2), 6.02 (s, 2H, OCH_2O), 6.67 (d, 1H, J = 8.2 Hz, H-5′), 6.70 (s, 1H, H-9), 6.86 (s, 1H, H-6), 7.26 (d collapsed, 1H, H-6′), 7.37 (s, 1H, H-2′), 8.29 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 67.07; H, 5.37; N, 12.87.

4.2.12. 1-(4-Aminophenyl)-3,5-dihydro-5-ethyl-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (3a). With a similar procedure, **3a** was prepared from **13** (700 mg, 1.89 mmol), hydrazine hydrate (0.12 mL, 2.45 mmol), and *n*-butanol (50 mL). Data of nitroderivative: mp = 247–250 °C (350 mg, 52%) R_f = 0.75 (EtOAc/cyclohexane 60:40). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 1.10 (t, 3H, CH_2CH_3), 1.96–2.05 and 2.36–2.43 (2m, 2H, CH_2CH_3), 2.89–2.93 (m, 1H, CH), 6.05 (d, 2H, OCH_2O), 6.55 (s, 1H, H-9), 6.85 (s, 1H, H-6), 7.85 (d, 2H, J = 8.8 Hz, H-3′,5′), 8.28 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.68 (br s, 1H, NH). Successive reduction was performed starting from nitroderivative (350 mg, 0.99 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **3a**: mp = 222–225 °C (101 mg, 31%) R_f = 0.41 (EtOAc/cyclohexane 60:40). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.07 (t, 3H, CH_2CH_3), 1.89–1.98 and 2.29–2.37 (2m, 2H, CH_2CH_3), 2.83–2.98 (m, 1H, CH), 3.93 (br s, 2H, NH_2), 5.98–6.02 (m, 2H, OCH_2O), 6.69 (d, 2H, J = 8.5 Hz, H-3′,5′), 6.70 (s, 1H, H-9) 6.81 (s, 1H, H-6), 7.46 (d, 2H, J = 8.5 Hz, H-2′,6′), 8.30 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.62; H, 5.48; N, 13.25.

4.2.13. 1-(4-Amino-3-methylphenyl)-3,5-dihydro-5-ethyl-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (3c). With a similar procedure, **3c** was prepared from **14** (2.4 g, 6.2 mmol), hydrazine hydrate (1 mL, 20 mmol) in EtOH (70 mL). Data of nitroderivative: mp = 280–282 °C (800 mg, 36%) R_f = 0.65 (EtOAc/cyclohexane 50:50). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm: 1.10 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.94–2.35 (2m, 2H, CH_2CH_3), 2.36–2.42 (m, 1H, CH), 2.64 (s, 3H, Ar-CH_3), 6.02–6.07 (m, 2H, OCH_2O), 6.56 (s, 1H, H-9), 6.90 (s, 1H, H-6), 7.85 (d, 1H, J = 8.5 Hz, H-6′), 7.64 (s, 1H, H-2′), 8.02 (d, 1H, J = 8.5 Hz, H-5′), 8.68 (br s, 1H, NH). Successive reduction was performed starting from nitroderivative (400 mg, 1.09 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **3c**: mp = 240–242 °C (360 mg, 92%) R_f = 0.3 (EtOAc/cyclohexane 50:50). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.08 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.92–2.37 (2m, 2H, CH_2CH_3), 2.18 (s, 3H, Ar-CH_3), 2.98 (m, 1H, CH), 3.87 (br s, 2H, NH_2), 5.98–6.05 (m, 2H, OCH_2O), 6.68 (d, 1H, J = 8.5 Hz, H-5′), 6.71 (s, 1H, H-9), 6.82 (s, 1H, H-6), 7.28 (d, J = 8.5 Hz 1H, H-6′), 7.39 (s, 1H, H-2′), 8.31 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46. Found: C, 67.49; H, 5.82; N, 12.36.

4.2.14. 1-(4-Aminophenyl)-5-benzyl-3,5-dihydro-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (4a). With a similar procedure, **4a** was prepared from **15** (670 mg, 1.55 mmol), hydrazine hydrate (0.24 mL, 4.95 mmol) in *n*-butanol (40 mL); yellow powder. Data of nitroderivative: mp = 212–216 °C (72 mg, 11%) R_f = 0.54 (CH_2Cl_2/EtOAc 90:10); ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.44 (dd, 1H, J = 5.8 and 8.0 Hz, CH-Bn), 3.52 (AB of ABX, 2H, J_{AB} = 13.7 Hz, J_{AX} = 8.0 Hz, J_{BX} = 5.8 Hz, $\Delta\nu$ = 75.8 Hz, CH_2-Ph), 6.02–6.08 (m, 2H, OCH_2O), 6.57 (s, 1H, H-6), 6.95 (s, 1H, H-9), 7.36–7.00 (m, 5H, Ar), 7.85 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.22 (d, 2H, J = 8.8 Hz, H-3′,5′), 8.59 (br s, 1H, NH). Successive reduction was performed starting from nitroderivative (72 mg, 0.17 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **4a**: mp = 149–153 °C (30 mg, 46%) R_f = 0.56 (EtOAc/diethyl ether/petroleum ether 60:20:20); ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 3.49 (dd, 1H, J = 6.0 and 8.5 Hz, CH-Bn), 3.50 (AB of ABX, 2H, J_{AB} = 14.3 Hz, J_{AX} = 8.5 Hz, J_{BX} = 6.0 Hz, $\Delta\nu$ = 74.7 Hz, CH_2-Ph), 5.95–6.04 (m, 2H, OCH_2O), 6.68 (d, 2H, J = 8.5 Hz, H-3′,5′), 6.71 (s, 1H, H-9), 6.90 (s, 1H, H-6), 7.36–7.00 (m, 5H, Ar), 7.45 (d, 2H, J = 8.5 Hz, H-2′,6′), 8.54 (br s, 1H, NH). Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.67; H, 4.97; N, 10.90. Found: C, 71.84; H, 4.86; N, 11.07.

4.2.15. 1-(4-Amino-3-chlorophenyl)-3,5-dihydro-5-methyl-7,8-methylenedioxy-4H-2,3-benzodiazepin-4-one (2f). A suspension of **16** (75 mg, 0.22 mmol) and an excess of Raney-Ni in EtOH (30 mL) was stirred with ammonium formate (250 mg, 4.1 mmol) to afford compound **2a** as previously described.[12] NCS (28 mg, 0.21 mmol) was added to a solution of **2a** (65 mg, 0.21 mmol) in DMF (2.5 mL) and the reaction mixture was stirred at room temperature for 20 h. The mixture was diluted with EtOAc (40 mL), washed with water and brine, dried over Na_2SO_4, and evaporated in vacuo. The crude was purified by a silica gel column chromatography using EtOAc/cyclohexane 70:30 as eluant. Mp = 154–156 °C (58 mg, 80%) R_f = 0.65 (EtOAc/cyclohexane 70:30); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.59 (d, 3H, J = 6.9 Hz, CH_3-α), 3.25 (q, 1H, J = 6.9 Hz, CH), 4.33 (br s, 2H, NH_2), 6.03 (m, 2H, OCH_2O), 6.68 (s, 1H, H-9), 6.77 (d, 1H, J_o = 8.2 Hz, H-5′), 6.87 (s, 1H, H-

6), 7.35 (dd, 1H, J_o = 8.2 Hz, J_m = 1.6 Hz, H-6′), 7.55 (d, 1H, J_m = 1.6 Hz, H-2′), 8.50 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{14}ClN_3O_3$: C, 59.39; H, 4.11; N, 12.23. Found: C, 59.57; H, 3.97; N, 12.42.

4.2.16. 1-(4-Aminophenyl)-3,5-dihydro-5-methyl-3-*N*-methylcarbamoyl-7,8-methylendioxy-4H-2,3-benzodiazepin-4-one (2b). To a solution of **16**[12] (300 mg, 0.88 mmol) in CH_2Cl_2 (50 mL) were added triethylamine (1.15 mL, 8.23 mmol) and methyl isocyanate (253 mg, 4.42 mmol). The reaction mixture was stirred at room temperature for 24 h and then washed with slightly acid solution and brine, and extracted with chloroform. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The resulting residue was purified by treatment with diethyl ether and used at the next step without further purification. The subsequent hydrogenation was carried out at atmospheric pressure by adding 5% Pd/C (50 mg) to a chloroformic solution (50 mL) of the nitro derivate. The mixture was shaken under hydrogen for 3 h and the Pd/C was filtered out through a Celite pad. The solution was washed with water and brine, and extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, evaporated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/EtOAc 50:50) to afford **2b** as a white solid. Further purification was obtained by trituration of the powder with ethyl ether. Mp = 186–188 °C (109 mg, 34%) R_f = 0.35 ($CHCl_3$/EtOAc 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.56 (d, 3H, J = 6.6 Hz, CH_3-α), 2.89 (d, 3H, J = 4.7 Hz, *NH*CH_3), 3.49 (q, 1H, J= 6.6 Hz, CH), 3.97 (br s, 2H, NH_2), 6.00–6.07 (m, 2H, OCH_2O), 6.68 (d, 2H, J = 8.8 Hz, H-3′,5′), 6.72 (s, 1H, H-9), 6.86 (s, 1H, H-6), 7.58 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.66 (q collapsed, 1H, NH*CH*$_3$). Anal. Calcd for $C_{19}H_{19}N_4O_4$: C, 62.28; H, 4.95; N, 15.29. Found: C, 62.41; H, 4.78; N, 15.18.

4.2.17. Methyl 2-(3,4-ethylenedioxyphenyl)propionate (18). With a procedure similar to that reported for **7**,[12] **18** was prepared from methyl 3,4-ethylenedioxyphenylacetate **17**[13] (1.85 g, 9 mmol), potassium hydride (400 mg, 10 mmol), and methyl iodide (1.4 g, 10 mmol) in anhydrous THF (40 mL). Compound **18** was isolated as colorless oil (1.07 g, 54%) by silica gel column chromatography (cyclohexane/EtOAc 70:30). R_f = 0.54 (cyclohexane/EtOAc 70:30). ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.47 (d, 3H, J = 6.9 Hz, CH_3-α), 3.66 (m, 4H, CH and OCH_3), 4.22 (s, 4H, OCH_2CH_2O), 6.79–4–6.84 (m, 3H, Ar). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.65; H, 6.52.

4.2.18. Methyl 2-[2-(3,5-dimethyl-4-nitrobenzoyl)-4,5-ethylenedioxy-phenyl]propionate (19). With a procedure similar to that reported for **10**, **19** was prepared from **17**[13] (201 mg, 0.97 mmol), 3,5-methyl-4-nitrobenzoic acid (247 mg, 1.26 mmol), and phosphorus pentoxide (2 g). White crystals. Mp = 143–144 °C (300 mg, 80%) R_f = 0.38 (diethyl ether/light petroleum 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 2.35 (s, 6H, 2ArCH_3) 3.62 (s, 3H, $COOCH_3$), 3.84 (s, 2H, CH_2), 4.27–4.32 (m, 4H, OCH_2CH_2O), 6.85 (s, 1H, H-6), 6.94 (s, 1H, H-3), 7.53 (m, 2H, H-2′,6′). Anal. Calcd for $C_{20}H_{19}NO_7$: C, 62.33; H, 4.97; N, 3.64. Found: C, 62.27; H, 4.89; N, 3.71.

4.2.19. Methyl 2-[4,5-ethylenedioxy-2-(3-methoxy-4-nitrobenzoyl)-phenyl]propionate (20). With a similar procedure, **20** was prepared from **17**[13] (345 mg, 1.67 mmol), 3-methoxy-4-nitrobenzoic acid (427 mg, 2.17 mmol), and phosphorus pentoxide (2 g); light yellow crystals. Mp = 156–158 °C (400 mg, 62%) R_f = 0.37 (diethyl ether/light petroleum 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 3.64 (s, 3H, $COOCH_3$), 3.86 (s, 2H, CH_2), 4.00 (s, 3H, OCH_3), 4.24–4.37 (m, 4H, OCH_2-CH_2O), 6.86 (s, 1H, H-6′), 6.94 (s, 1H, H-3′), 7.33 (dd, 1H, J_o = 8.2 Hz, J_m = 1.6 Hz, H-6′), 7.54 (d, 1H, J_m = 1.6 Hz, H-2′), 7.84 (d, 1H, J_o = 8.2 Hz, H-5′). Anal. Calcd for $C_{19}H_{17}NO_8$: C, 58.91; H, 4.42; N, 3.62. Found: C, 58.78; H, 4.57; N, 3.49.

4.2.20. Methyl 2-[4,5-ethylenedioxy-2-(4-nitrobenzoyl)-phenyl]propionate (21). With a similar procedure, **21** was prepared from **18** (1.07 g, 4.8 mmol), 4-nitrobenzoic acid (1.02 g, 6.3 mmol), and phosphorus pentoxide (2 g). The product was purified by silica gel column chromatography (cyclohexane/EtOAc 70:30) Mp = 113–115 °C (841 mg, 48%) R_f = 0.38 (cyclohexane/EtOAc 70:30); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.52 (d, 3H, J = 7.2 Hz, CH_3-α), 3.59 (s, 3H, OCH_3), 4.22–4.34 (m, 5H, CH and OCH_2CH_2O) 6.84 (s, 1H, H-6), 7.01 (s, 1H, H-3), 7.94 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.30 (d, 2H, J = 8.8 Hz, H-3′,5′). Anal. Calcd for $C_{19}H_{17}NO_7$: C, 61.45; H, 4.61; N, 3.77. Found: C, 61.28; H, 4.57; N, 3.91.

4.2.21. Methyl 2-[4,5-ethylenedioxy-2-(3-methyl-4-nitrobenzoyl)phenyl]propionate (22). With a similar procedure, **22** was prepared from **18** (1.23 g, 5.6 mmol), 3-methyl-4-nitrobenzoic acid (1.31 g, 7.2 mmol), and phosphorus pentoxide (2 g). The product was purified by silica gel column chromatography (diethyl ether/light petroleum 60:40). Mp = 117–119 °C (858 mg, 49%) R_f = 0.53 (diethyl ether/light petroleum 60:40); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.51 (d, 3H, J = 7.1 Hz, CH_3-α), 2.63 (s, 3H, Ar-CH_3), 3.68 (s, 3H, OCH_3), 4.15–4.35 (m, 5H, CH and OCH_2CH_2O) 6.85 (s, 1H, H-6), 7.01 (s, 1H, H-3), 7.70 (dd, J_o = 8.2 Hz and J_m = 1.6 Hz, H-6′), 7.76 (d collapsed, 1H, H-2′), 7.99 (d, 1H, J_o = 8.5 Hz, H-5′). Anal. Calcd for $C_{20}H_{19}NO_7$: C, 62.33; H, 4.97; N, 3.64. Found: C, 62.52; H, 4.81; N, 3.77.

4.2.22. 1-(4-Amino-3,5-dimethylphenyl)-3,5-dihydro-7,8-ethylenedioxy-4H-2,3-benzodiazepin-4-one (5d). With a procedure similar to that reported for **1d**, **5d** was prepared from **19** (300 mg, 0.78 mmol), hydrazine hydrate (0.12 mL, 2.49 mmol), and HCl 6 N (0.38 mL). Data of nitroderivative: mp > 300 °C (200 mg, 70%) R_f = 0.34 (EtOAc/cyclohexane 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 2.34 (s, 6H, 2CH_3), 3.47 (s, 2H, CH_2), 4.24–4.34 (m, 4H, OCH_2CH_2O), 6.67 (s, 1H, H-5), 6.89 (s, 1H, H-9), 7.37 (s, 2H, H-2′ and H-6′), 8.56 (br s, 1H, NH). Successive reduction was performed starting from nitroderivative (200 mg,

0.55 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **5d**: mp = 239–242 °C (dec) (50 mg, 27%) R_f = 0.69 (EtOAc/MeOH 99:1); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 2.18 (s, 6H, $2CH_3$), 3.42 (s, 2H, CH_2), 3.85 (br s, 2H, NH_2), 4.22–4.34 (m, 4H, OCH_2CH_2O), 6.80 (s, 1H, H-5), 6.85 (s, 1H, H-8), 7.20 (s, 2H, H-2′,6′), 8.48 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46. Found: C, 67.38; H, 5.74; N, 12.52.

4.2.23. 1-(4-Amino-3-methoxyphenyl)-3,5-dihydro-7,8-ethylenedioxy-4H-2,3-benzodiazepin-4-one (5e). With a procedure similar to that reported for **1e**, **5e** was prepared from **20** (400 mg, 1.03 mmol), hydrazine hydrate (0.16 mL, 3.30 mmol), and HCl 6 N (0.5 mL). Data of nitroderivative: Mp = 234–236 °C (156 mg, 41%) R_f = 0.22 (EtOAc/cyclohexane 50:50); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 3.49 (s, 2H, CH_2), 4.01 (s, 3H, OCH_3), 4.19–4.41 (m, 4H, OCH_2CH_2O), 6.68 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.13 (dd, 1H, J_o = 8.5, J_m = 1.6, H-6′), 7.50 (d, 1H, J_m = 1.6, H-5′), 7.86 (d, 1H, J_o = 8.5, H-2′), 8.62 (br s, 1H, NH). Successive reduction was performed starting from nitro derivative (156 mg, 0.42 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **5e**: mp = 250–252 °C (76 mg, 53%) R_f = 0.69 (EtOAc/MeOH 99:1); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 3.43 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.09 (br s, 2H, NH_2), 4.22–4.34 (m, 4H, OCH_2CH_2O), 6.66 (d, 1H, J_o = 8.0 Hz, H-5′), 6.82 (s, 1H, H-6), 6.85 (s, 1H, H-9), 6.87 (dd, 1H, J_o= 8.0 Hz, J_m = 1.6 Hz, H-6′), 7.26 (d, 1H, J_m= 1.9 Hz, H-2′), 8.31 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.56; H, 5.18; N, 12.41.

4.2.24. 1-(4-Aminophenyl)-3,5-dihydro-7,8-ethylenedioxy-5-methyl-4H-2,3-benzodiazepin-4-one (6a). With a procedure similar to that reported for **2c**, **6a** was prepared from **21** (841 mg, 2 mmol), hydrazine hydrate (0.12 mL, 2 mmol). Data of nitroderivative: mp > 280 °C (150 mg, 18%) R_f = 0.51 (cyclohexane/EtOAc 50:50); ^{1}H NMR (DMSO-d_6): 1.45 (d, 3H, J = 6.3 Hz, CH_3-α), 3.31 (q, 1H, J = 6.3 Hz, CH), 4.28–4.34 (m, 4H, OCH_2CH_2O), 6.63 (s, 1H, H-9), 6.89 (s, 1H, H-6), 7.83 (d, 2H, J = 8.3 Hz, H-2′,6′), 8.33 (d, 2H, J = 8.3 Hz, H-3′,5′), 11.25 (br s, 1H, NH). Successive reduction was performed starting from nitro derivative (150 mg, 0.42 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **6a**: mp > 280 °C (31 mg, 24%) R_f = 0.48 (EtOAc/cyclohexane 70:30); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.56 (d, 3H, J = 6.9 Hz, CH_3-α), 3.30 (q, 1H, J = 6.9 Hz, CH), 3,85 (br s, 2H, NH_2), 4.18–4.31 (m, 4H, OCH_2-CH_2O), 6.67 (d, 2H, J = 8.8 Hz, H-3′,5′), 6.79 (s, 1H, H-9), 6.86 (s, 1H, H-6), 7.43 (d, 2H, J = 8.8 Hz, H-2′,6′), 8.57 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 67.08; H, 5.13; N, 13.18.

4.2.25. 1-(4-Amino-3-methylphenyl)-3,5-dihydro-7,8-ethylenedioxy-5-methyl-4H-2,3-benzodiazepin-4-one (6c). With a similar procedure, **6c** was prepared from **22** (858 mg, 2.2 mmol), hydrazine hydrate (0.34 mL, 7.1 mmol). Data of nitroderivative: mp = 259–260 °C (165 mg, 20%) R_f= 0.78 (CH_2Cl_2/EtOH 95:5); ^{1}H NMR (DMSO-d_6): 1.41 (d, 3H, J = 6.3 Hz, CH_3-α), 2.54 (s, 3H, Ar-CH_3), 3.20–3.32 (m, 1H, CH), 4.22–4.30 (m, 4H, OCH_2CH_2O), 6.59 (s, 1H, H-9), 6.84 (s, 1H, H-6), 7.56 (d, 1H, J = 8.5 Hz, H-6′), 7.63 (s, 1H, H-2′), 8.06 (d, 1H, J = 8.5 Hz, H-5′), 11.16 (br s, 1H, NH). Successive reduction was performed starting from nitro derivative (165 mg, 0.45 mmol), Raney-Ni as catalyst, and an excess of ammonium formate. Data of **6c**: Mp = 260–263 °C (60 mg, 36%) R_f = 0.5 (CH_2Cl_2/EtOH 95:5); ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.57 (d, 3H, J = 5.5 Hz, CH_3-α), 2.18 (s, 3H, Ar-CH_3), 3.24–3.37 (m, 1H, CH), 3,86 (br s, 2H, NH_2), 4.24–4.32 (m, 4H, OCH_2CH_2O), 6.66 (d, 1H, J = 8.2 Hz, H-5′), 6.8 (s, 1H, H-9), 6.87 (s, 1H, H-6), 7.25 (d, J = 8.2 Hz 1H, H-6′), 7.39 (s, 1H, H-2′), 8.23 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46. Found: C, 67.78; H, 5.81; N, 12.27.

4.2.26. 1-(4-Amino-3-chlorophenyl)-3,5-dihydro-7,8-ethylenedioxy-5-methyl-4H-2,3-benzodiazepin-4-one (6f). With a procedure similar to that reported for **2f**, **6f** was obtained starting from **6a** (110 mg, 0.34 mmol) and NCS (46 mg, 0.34 mmol). Mp = 148–151 °C (40 mg, 36%) R_f = 0.64 (diethyl ether/light petroleum 90:10) ^{1}H NMR (300 MHz, $CDCl_3$), δ ppm 1.57 (d, 3H, J = 5.5 Hz, CH_3-α), 3.22–3.31 (m, 1H, CH), 4.26–4.32 (m, 4H, OCH_2CH_2O), 4.71 (br s, 2H, NH_2), 7.41–7.63 (m, 3H, Ar), 6.76 (s, 1H, H-9), 6.89 (s, 1H, H-6), 8.40 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{16}ClN_3O_3$: C, 60.40; H, 4.53; N, 11.74. Found: C, 60.28; H, 4.67; N, 11.95.

4.3. Pharmacology

4.3.1. Animals. Procedures involving animals and their care were conducted in conformity with the Institutional Guidelines that comply with national (D.L. n.116, G.U., suppl. 40 Feb. 18, 1992) and international (EEC Council Directive 86/609, OJL 358, 1, Dec. 12, 1987; NIH Guide for Care and Use of Laboratory Animals, U.S. National Research Council, 1996) laws and policies.

4.3.2. [^{3}H]CP-526,427 binding[14]. The binding of [^{3}H]CP-526,427 was characterized in rat forebrain membranes. Forebrains of adult male Sprague–Dawley rats were homogenized in 0.32 M sucrose at 4 °C. The crude nuclear pellet was removed by centrifugation at 1000*g* for 10 min, and the supernatant centrifuged at 17,000*g* for 25 min. The resulting pellet was re-suspended in 5 mM Tris–acetate, pH 7.4, at 4 °C for 10 min to lyse cellular particles and again centrifuged at 17,000*g*. The resulting pellet was washed twice in Tris–acetate, re-suspended at 10 mg of protein/ml, and stored at −20 °C until use. Immediately before binding assays, membranes were thawed, homogenized, and diluted to 0.5 mg of protein/mL with 50 mM Tris·HCl, pH 7.4. For competition assays, compounds were added at various concentrations followed by 3 nM [^{3}H]CP-526,427 (specific activity, 24.36 Ci/mmol). After incubation for 20 min at 30 °C in a shaking water bath, samples were filtered onto Whatman GFB glass fiber filters using a MB-48R

Cell Harvester (Brandel Research and Development Laboratories, Gaithersburg MD). Filters were washed for 10 s with ice-cold Tris·HCl buffer and the radioactivity trapped on the filter quantified by liquid scintillation counting. Nonspecific binding for [^{3}H]CP-526,427 was determined in parallel incubations containing 10 μM unlabeled CP-526,427 or CP-465,022. Specific binding was defined as total binding minus nonspecific binding.

4.3.3. Primary cultures of rat cerebellar granule neurons. Primary cultures of rat cerebellar granule neurons were prepared as described previously.[16]

4.3.4. $^{45}Ca^{2+}$ uptake[14]. Neurons in poly-D-lysine-coated 96-well plates were preincubated for 30 min with different concentrations of compounds in balanced salt solution (BSS; 115 mM NaCl, 5.4 mM KCl, 0.96 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 11 mM *d*-glucose, and 25 mM Hepes, pH 7.3). They were then exposed at room temperature to 100 μM kainate or NMDA in BSS containing 10 μM glycine, 0.5 mM dithiothreitol, and 0.5 μCi $^{45}Ca^{2+}$ (final specific activity, 2.78 μCi/μmol) in a volume of 100 μl/well. After 10 min, the neurons were then rapidly washed five times with 200 μl/well of ice-cold BSS containing 5 mM EGTA. Neurons were then lysed in 30 μl/well of 0.6% Triton X-100 and radioactivity in aliquots of the lysate was measured with a TopCount microtiter scintillation counter (Packard Instrument Co., Downers Grove, IL).

4.3.5. Measurement of $[Ca^{2+}]_i$[14]. Neurons in 96-well, black/clear, poly-D-lysine-coated tissue culture plates were rinsed once with BSS and then incubated for 1 h in BSS containing 4 μM Fluo-4/AM (Molecular Probes, Inc., Eugene, OR). Fluo-4/AM was prepared immediately before use as a 1 mM stock solution in dimethylsulfoxide with 10% (w/v) pluronic acid. Cells were then washed three times and held in BSS at room temperature and used within 1 h. A fluorescent imaging plate reader (FLIPR; Molecular Devices, Sunnyvale, CA) was used for simultaneous imaging and fluid addition. Cells were preincubated with test compounds for approximately 6 min, then stimulated with 32 μM AMPA. Changes in fluorescent intensity were measured at a frequency of 1 sample/2 s after AMPA addition. Raw data are expressed in relative fluorescent units (RFUs) after the background fluorescence was subtracted.

4.3.6. Mouse hippocampal cell cultures. Primary cultures were obtained from hippocampus of 13 days old C57 BL/6N mouse embryos (E13) (Charles River, Calco, Italy). Hippocampus was removed and cleaned of meninges with microscopic surgical operations. Tissues collected were centrifuged at 700 rpm for 5 min, re-suspended in complete culture medium (CCM) composed by Neurobasal medium (Invitrogen GIBCO, Milan, Italy), 2% B27 (Invitrogen GIBCO, Milan, Italy), 0.5 mM L-glutamine (Sigma, Milan, Italy), 25 μM glutamate (Sigma, Milan, Italy), and 25 μM 2-mercaptoethanol, 1% penicillin–streptomycin (Sigma, Milan, Italy), 10 ng/mL BDNF (kind gift of Amgen, Thousand Oaks, CA, USA). After mechanical dissociation cells were plated at a density of 2×10^5 cells/well on poly-L-lysine (Sigma, Milan, Italy) coated 12-well plates. Cultures were grown at 37 °C in a humidified incubator with 5% CO_2. The same medium (without glutamate) was added on fourth and sixth day in vitro (DIV). Cultures prepared by this method were enriched in hippocampal neurons, as assessed by GFAP immunocytochemistry (data not shown). At 7th DIV drugs were dissolved in CCM without glutamate and neurons were exposed for 48 h at 37 °C in a humidified incubator with 5% CO_2. Neuronal survival was assayed by measuring conversion of yellow water-soluble tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide—MTT, Sigma, Milan, Italy) to blue water-insoluble formazan. Culture medium was removed and cells were incubated for 4 h with 10% MTT solution (5 mg/mL in NaCl) in fresh CCM at 37 °C. After incubation media were removed, cells were dissolved in dimethylsulfoxide (DMSO, Sigma, Milan, Italy), and solution was transferred to a 96-well plate to measure absorbance (570 nm). Viability is expressed as a percentage of absorbance measured in untreated cells.

Acknowledgments

We thank Dr. F.S. Menniti, Pfizer Global Research and Development, Groton, Connecticut, USA, for supplying with [^{3}H]CP-526,427 radioligand, and Dr. M. De Paola (IRFMN) for mouse hippocampal culture setting. This work was financially supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR-COFIN2005)-Rome, Italy.

References and notes

1. Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacol. Rev.* **1999**, *51*, 7.
2. (a) Lees, G. J. *Drugs* **2000**, *59*, 33; (b) Franciosi, S. *Cell. Mol. Life Sci.* **2001**, *58*, 921.
3. Gressens, P.; Spedding, M.; Gigler, G.; Kertezs, S.; Villa, P.; Medja, F.; Williamson, T.; Kapus, G.; Levay, G.; Szenassi, G.; Barkoczy, J.; Harsing, L. G., Jr. *Eur. J. Pharmacol.* **2005**, *519*, 58.
4. Parsons, G. C.; Danysz, W.; Quack, G. *Drug News Perspect.* **1998**, *11*, 523.
5. Kew, J. N.; Kemp, J. A. *Psychopharmacology (Berl.)* **2005**, *179*, 4.
6. Sólyom, S.; Tarnawa, I. *Curr. Pharm. Des.* **2002**, 913.
7. Balannik, V.; Menniti, F. S.; Paternain, A. V.; Lerma, J.; Stern-Bach, Y. *Neuron* **2005**, *48*, 279.
8. Szabados, T.; Gigler, G.; Gacsalyi, I.; Gyertan, I.; Levay, G. *Brain Res. Bull.* **2001**, *55*, 387.
9. Chappell, A. S.; Sander, J. W.; Brodie, M. J.; Chadwick, D.; Liedo, A.; Zhang, D.; Bjerke, J.; Kiesler, G. M.; Arroyo, S. *Neurology* **2002**, *58*, 1680.
10. Grasso, S.; De Sarro, G.; De Sarro, A.; Micale, N.; Zappalà, M.; Puja, G.; Baraldi, M.; De Micheli, C. *J. Med. Chem* **1999**, *42*, 4414.
11. Ritz, M.; Micale, N.; Li, G.; Grasso, S.; Niu, L. *Biochemistry* **2008**, *47*, doi:10.1021/bi700782x.
12. Zappalà, M.; Postorino, G.; Micale, N.; Caccamese, S.; Parrinello, N.; Roda, G.; Menniti, F. S.; Ferreri,

G.; De Sarro, G.; Grasso, S. *J. Med. Chem.* **2006**, *49*, 575.
13. Zappalà, M.; Pellicanò, A.; Micale, N.; Menniti, F. S.; Ferreri, G.; De Sarro, G.; Grasso, S.; De Micheli, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 167.
14. Menniti, F. S.; Chenard, M. B.; Collins, M. F.; Ducat, M. F.; Elliot, M. L.; Ewing, F. E.; Huang, J. I.; Kelly, K. A.; Lazzaro, J. T.; Pagnozzi, M. J.; Weeks, J. L.; Welch, W. M.; White, W. F. *Mol. Pharmacol.* **2000**, *58*, 1310.
15. Greff, Z.; Szabó, G.; Barkóczy, J.; Rátkai, Z.; Blaskó, G.; Simig, Gy.; Gigler, G.; Martonné Markó, B.; Lévay, Gy.; Tihanyi, K.; Egyed, A.; Simó, A. W.O. Patent 004122, 2001.
16. Parks, T. N.; Artman, L. D.; Alasti, N.; Nemeth, E. F. *Brain Res.* **1991**, *552*, 13.
17. May, P. C.; Robinson, P. M. *Neurosci. Lett.* **1993**, *152*, 169.
18. May, P. C.; Robinson, P. M.; Fuson, K. S. *Neurosci. Lett.* **1999**, *262*, 219.
19. Erdò, F.; Berzsenyi, P.; Nemet, L.; Andrasi, F. *Br. Res. Bull.* **2006**, *68*, 269.
20. Xia, H.; Cai, S. X.; Field, G.; Lan, N. C.; Wang, Y. U.S. Patent 5891871, 1999.
21. De Sarro, A.; De Sarro, G.; Gitto, R.; Grasso, S.; Micale, N.; Zappalà, M. *Farmaco* **1999**, *54*, 178.