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Synthesis and pharmacological properties of new 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines

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Abstract

A series of new 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines was synthesized starting from the corresponding bicyclic 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (CFMs), previously described as noncompetitive AMPA-type glutamate receptor antagonists, more potent than GYKI 52466. New synthesized compounds proved to be able to protect against seizures induced by means of auditory stimulation in DBA/2 mice and compound **8f** the most active of the series showed anticonvulsant properties comparable to GYKI 52466.

Keywords: AMPA receptor antagonists; 3-Ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines; Anticonvulsant agents

1. Introduction

Glutamate (Glu) is the main excitatory neurotransmitter in the vertebrate brain and plays an important role in neuronal activity via different receptor systems. It acts through a heterogeneous family of two major types of receptors: ionotropic (iGluRs) and metabotropic receptors (mGluRs).

Ionotropic glutamate receptors (iGluRs) are a family of ligand-gated ion channels that open in response to the binding of Glu. The neurotransmitter is released presynaptically and binds to a postsynaptic receptor gating a cation-selective channel, thus depolarizing the postsynaptic cells. These receptors are involved in fundamental processes such as neuronal development, learning and memory, but their aberrant overactivation is a key step in the cascade of events leading to neuronal death after ischemia, seizures or in neurodegenerative states [1–4].

It is well known that 2-amino-3-(3-hydroxy-methylisoxazol-4-yl)propionic acid (AMPA) receptor type of iGluRs may play a role in epileptogenesis and several AMPA antagonists, reported in the literature, show promise in terms of their therapeutic potential for the prevention and treatment of epilepsy [5–8].

In particular, a selective and noncompetitive blockade of AMPA receptor was shown by 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**, GYKI 52466), which possesses potent anticonvulsant properties [9–11].

In our ongoing search for new compounds that noncompetitively antagonize AMPA receptor function, we identified 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**2**, CFM) and thiocarbonyl analogs **3** (Fig. 1) which have shown marked antiepileptic properties in various seizure models and do not bind to the GABAergic benzodiazepine receptors [12–16]. Electrophysiological experiments carried out on the lead compound **2f** (R=4-NH_2, CFM-2) and some of its derivatives have confirmed that their anticonvulsant effects, analogous to GYKI 52466, are mediated through the AMPA subtype of the iGluR complex in a selective and noncompetitive fashion [13,16].

Extensive structure–activity relationship studies [17], aimed at exploring the effects of structural modifications of the heptatomic system of derivatives **2** and at

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developing AMPA receptor antagonists with increased potency and selectivity, longer-lasting activity and improved pharmacokinetic features, led to the synthesis of cyclofunctionalized 2,3-benzodiazepines. 11*H*-Tetrazolo[1,5-*c*][2,3]benzodiazepines (**4**) [18] and 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines (**5**) [19] showed weaker anticonvulsant effects than their parent compounds **2**–**3**; on the contrary 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-ones (**6**) demonstrated comparable or higher anticonvulsant potency than the corresponding derivatives **2** and we suggested that annelated 2,3-benzodiazepines **6** are active both for the presence of hydrogen bonding moieties and because they undergo biotransformation into the corresponding bicyclic derivatives **2** [20].

In pursuing our research in the area of AMPA receptor antagonists, we planned the synthesis of new 11H-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines (**8**) in which an exocyclic ethoxycarbonyl moiety is present on triazole ring. Herein, we report the chemical and anticonvulsant properties of these compounds.

2. Experimental

2.1. Chemistry

Melting points (m.p.) were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70–230 mesh). ^{1}H NMR spectra were measured in $CDCl_3$ with a Varian Gemini 300 spectrometer; chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hz. All exchangeable protons were confirmed by addition of D_2O. Compounds **3a**–**d** and **7a**–**d** were prepared according to a previously described procedure [16,18].

2.1.1. General procedure for the synthesis of 3-ethoxycarbonyl-11H-[1,2,4]triazolo[4,5-c][2,3]-benzodiazepines (*8a*–*d*)

To a cooled (5 °C) solution of **7a**–**d** (1 mmol) in toluene (15 ml) was added dropwise ethyl oxalyl chloride (1 mmol). This mixture was stirred for 15 min at room temperature, then refluxed for 90 min. The solution was evaporated to dryness, the oil crystallized by adding a small amount of suitable solvent.

*2.1.1.1. 8,9-Dimethoxy-3-ethoxycarbonyl-6-phenyl-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepine (**8a**).* M.p. 255–257 °C (EtOH), yield 62%. ^{1}H NMR: 1.48 (t, 3H, $J = 7.1$, CH_3), 3.68 (s, 3H, CH_3O-8), 3.97 (s, 3H, CH_3O-9), 4.14 (bs, 2H, CH_2), 4.53 (q, 2H, $J = 7.1$, CH_2) 6.68 (s, 1H, H-7), 6.91 (s, 1H, H-10), 7.48–7.86 (m, 5H, ArH). *Anal.* $C_{21}H_{20}N_4O_4$ (C, H, N).

*2.1.1.2. 8,9-Dimethoxy-3-ethoxycarbonyl-6-(4-fluorophenyl)-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepine (**8b**).* M.p. 211–212 °C (MeOH), yield 80%. ^{1}H NMR: 1.49 (t, 3H, $J = 7.1$, CH_3), 3.71 (s, 3H, CH_3O-8), 3.98 (s, 3H, CH_3O-9), 4.15 (bs, 2H, CH_2), 4.53 (q, 2H, $J = 7.1$, CH_2) 6.67 (s, 1H, H-7), 6.92 (s, 1H, H-10), 7.20 (dd, 2H, $J_{HH} = 8.5$ and $J_{HF} = 8.8$, H-3′,5′), 7.87 (dd, 2H, $J_{HF} =$

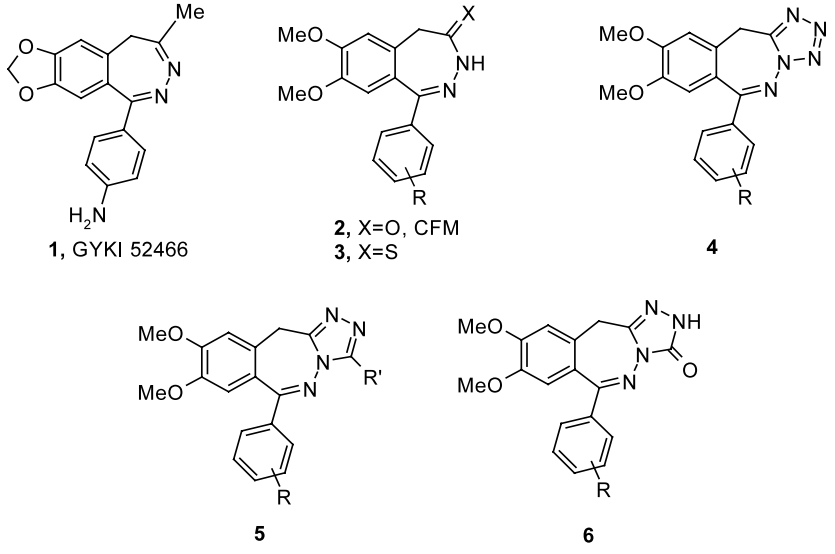


Fig. 1. 2,3-Benzadiazepine derivatives.

5.3 and $J_{HH} = 8.5$, H-2′,6′). *Anal.* $C_{21}H_{19}FN_4O_4$ (C, H, N).

*2.1.1.3. 8,9-Dimethoxy-3-ethoxycarbonyl-6-(3-nitrophenyl)-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepine (**8c**).* M.p. 246–248 °C (MeOH), yield 48%. ^{1}H NMR: 1.50 (t, 3H, $J = 7.1$, CH_3), 3.69 (s, 3H, CH_3O-8), 4.00 (s, 3H, CH_3O-9), 4.17 (bs, 2H, CH_2), 4.55 (q, 2H, $J = 7.1$, CH_2), 6.61 (s, 1H, H-7), 6.95 (s, 1H, H-10), 7.74 (t, 1H, $J = 8.0$, H-5′), 8.38 (m, 2H, H-2′,6′), 8.62 (d, 2H, $J = 1.8$, H-2′). *Anal.* $C_{21}H_{19}N_5O_6$ (C, H, N).

*2.1.1.4. 8,9-Dimethoxy-3-ethoxycarbonyl-6-(4-nitrophenyl)-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepine (**8d**).* M.p. 254–256 °C (EtOAc), yield 56%. ^{1}H NMR: 1.49 (t, 3H, $J = 7.1$, CH_3), 3.69 (s, 3H, CH_3O-8), 3.99 (s, 3H, CH_3O-9), 4.17 (bs, 2H, CH_2), 4.53 (q, 2H, $J = 7.1$, CH_2), 6.57 (s, 1H, H-7), 6.94 (s, 1H, H-10), 8.07 (d, 2H, $J = 8.9$, H-2′,6′), 8.30 (d, 2H, $J = 8.9$, H-3′, 5′). *Anal.* $C_{21}H_{19}N_5O_6$ (C, H, N).

*2.1.2. General procedure for the synthesis of aminophenyl derivatives **8e** and **8f***

A mixture of nitro derivative **8c** or **8d** (0.2 mmol) and $SnCl_2 \cdot 2H_2O$ (1 mmol) in EtOH (20 ml) was heated to 70 °C for 90 min. The mixture was cooled, poured in water, neutralized with a solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was dried over Na_2SO_4, the solvent was evaporated and from the crude residue compounds **8e** and **8f** were purified by crystallization with EtOAc.

*2.1.2.1. 6-(3-Aminophenyl)-8,9-dimethoxy-3-ethoxycarbonyl-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepine (**8e**).* M.p. 138–141 °C, yield 69%. ^{1}H NMR: 1.48 (t, 3H, $J = 7.1$, CH_3), 3.71 (s, 3H, CH_3O-8), 3.88 (bs, 2H, NH_2), 3.97 (s, 3H, CH_3O-9), 4.15 (bs, 2H, CH_2), 4.52 (q, 2H, $J = 7.1$, CH_2), 6.72 (s, 1H, H-7), 6.85 (m, 1H, H-4′), 6.89 (s, 1H, H-10), 7.05 (m, 1H, H-6′), 7.26 (m, 2H, H-2′,5′). *Anal.* $C_{21}H_{21}N_5O_4$ (C, H, N).

*2.1.2.2. 6-(4-Aminophenyl)-8,9-dimethoxy-3-ethoxycarbonyl-11H-[1,2,4]triazolo[4,5-c][2,3]benzodiazepine (**8f**).* M.p. 176–177 °C, yield 61%. ^{1}H NMR: 1.45 (t, 3H, $J = 7.1$, CH_3), 3.70 (s, 3H, CH_3O-8), 3.94 (s, 3H, CH_3O-9), 4.12 (bs, 2H, NH_2), 4.13 (bs, 2H, CH_2), 4.49 (q, 2H, $J = 7.1$, CH_2), 6.71 (d, 2H, $J = 8.6$, H-3′,5′), 6.76 (s, 1H, H-7), 6.91 (s, 1H, H-10), 7.64 (d, 2H, $J = 8.6$, H-2′,6′). *Anal.* $C_{21}H_{21}N_5O_4$ (C, H, N).

2.2. Pharmacology

2.2.1. Testing of anticonvulsant activity

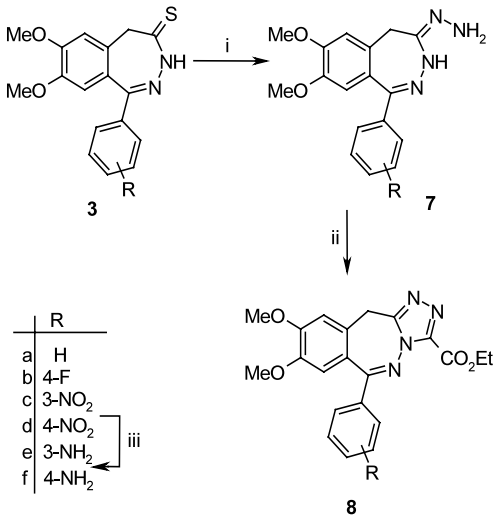
All experiments were performed with DBA/2 mice which are genetically susceptible to sound-induced seizures [21]. DBA/2 mice (8–12 g; 22–25-days-old) were purchased from Charles River (Calco, Como, Italy). Groups of ten mice of either sex were exposed to auditory stimulation 30 min following administration of vehicle or each dose of drugs studied. The compounds were given intraperitoneal (ip) (0.1 ml per 10 g of body weight of the mouse) as a freshly-prepared solution in 50% dimethyl sulfoxide (DMSO) and 50% sterile saline (0.9% NaCl). Individual mice were placed under a hemispheric perspex dome (diameter 58 cm), and 60 s were allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 60 s or until tonic extension occurred, and induced a sequential seizure response in control DBA/2 mice, consisting of an early wild running phase, followed by generalized myoclonus and tonic flexion and extension sometimes followed by respiratory arrest. The control and drug-treated mice were scored for latency to and incidence of the different phases of the seizures [22].

Seizures were also induced by icv injection of AMPA. The CD_{50} of AMPA for clonus was 1.76 (1.06–3.07) while that for tonus was 2.90 (1.83–4.58) nmol. For icv injection, mice were anesthetized with diethyl ether, and injections were made in the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to the bregma; depth 2.4 mm) using a 10 μl Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle as previously described [23]: injections of drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min. The animals were placed singly in a $30 \times 30 \times 30$ cm box, and the observation time was 30 min after the administration of AMPA. The icv microinjection of aniracetam was performed according to experimental procedures previously described for AMPA microinjection [23]. The dose of aniracetam (50 nmol icv) was administered to DBA/2 mice 60 min before auditory stimulation or 30 min before each compound.

The experimental protocol and all the procedures involving animals and their care were conducted in conformity with the institutional guidelines and the European Council Directive of laws and policies.

2.2.2. Statistical analysis

Statistical comparisons between groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases). The ED_{50} values of each phase of audiogenic seizures was determined for each dose of compound administered, and dose–response curves were fitted using a computer program by Litchfield and Wilcoxon's method [24].



Scheme 1. (i) $NH_2NH_2 \cdot H_2O$, EtOH or THF, Δ or r.t. respectively, 60–90 min; (ii) EtOCOCOCl, toluene, Δ, 90 min; (iii) $SnCl_2$, EtOH, Δ, 90 min.

Table 1
Anticonvulsant activity of compounds **1**, **2f** and **8a**–**e** against audiogenic seizures in DBA/2 mice

Comp.	ED_{50} (μmol kg^{-1}) [a] (±95% confidence limits)	
	Clonic phase	Tonic phase
1 [b]	35.8 (24.4–52.4)	25.3 (16.0–40.0)
2f [b]	15.0 (9.01–24.0)	12.6 (8.01–19.0)
8a	81.5 (63.3–105)	75.0 (49.4–114)
8b	58.1 (43.6–77.4)	40.6 (27.7–59.6)
8c	112 (69.9–179)	82.5 (58.6–116)
8d	145 (73.2–288)	124 (64.8–238)
8e	55.3 (38.1–80.3)	37.8 (24.0–59.3)
8f	35.1 (22.4–55.9)	26.8 (17.3–44.7)

[a] All data were calculated according to the method of Litchfield and Wilcoxon [24]; 95% confidence limits are given in parentheses. At least 32 animals were used to calculate each ED_{50}.
[b] Reference [13].

3. Results and discussion

The 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]-benzodiazepin-3(2*H*)-ones **(8)** (Scheme 1) were obtained starting from 3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones **(2)**, which were activated by transformation into the corresponding thiocarbonyl derivatives **3**, by reaction with Lawesson's reagent. By treatment with hydrazine hydrate, **3a**–**d** yielded 2,3-benzodiazepin-4ylhydrazines intermediates **7a**–**d**, which, as crude products, were refluxed with ethyl oxalyl chloride to afford 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-ones (**8a**–**d**). Aminophenyl-substituted derivatives **8e**–**f** were prepared by reduction of the corresponding nitro analogs **8c**–**d** with tin(II) chloride. The structures of the compounds obtained were supported by elemental analyses and spectroscopic measurements (^{1}H NMR).

The anticonvulsant properties of 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-ones (**8a**–**f**) were evaluated after ip administration against audiogenic seizures in DBA/2 mice (Table 1), which has been considered an excellent animal model for generalized epilepsy and for screening new anticonvulsant drugs [24].

The results were compared with those of GYKI 52466 (**1**) and CFM-2 (**2f**), well known noncompetitive AMPA receptor antagonists that show anticonvulsant properties in various seizure models [13].

It was observed that the 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-ones (**8**) demonstrate lower anticonvulsant potency with respect to the corresponding bicyclic derivatives CMFs, but they were more active than type **4** and **5** compounds. In particular, compound **8f**, the most active of this class of triazolo-2,3-benzodiazepines, showed anticonvulsant properties comparable to GYKI 52466 (Table 1), confirming the importance of the 4-aminophenyl substituent as already observed both in the CFM series (**2**, **3**) [13,16] and in other classes of cyclofunctionalized 2,3-benzodiazepines (**5**, **6**) [19,20].

The most active derivative **8f** was tested against AMPA-induced seizures in DBA/2 mice in order to elucidate whether the anticonvulsant properties are mediated by interaction with AMPA-receptor complex. As shown in Table 2, **8f** afforded protection against the clonic and tonic phases of the seizures induced by icv administration of AMPA even if the ED_{50} values were higher than those required to prevent audiogenic seizures. Furthermore, the influence of aniracetam, a positive allosteric modulator of AMPA/KA receptor [9], on the anticonvulsant activity of derivative **8f** in DBA/2 mice was evaluated (Table 2). The administration of aniracetam 30 min before the injection of the tested

Table 2
ED_{50} values of **1**, **2f** and **8f** against AMPA-induced seizures and against audiogenic seizures following pretreatment with aniracetam

Comp.	ED_{50} (μmol kg^{-1}) (±95% confidence limits) [a]			
	AMPA		Pretreatment with aniracetam	
	Clonic phase	Tonic phase	Clonic phase	Tonic phase
1 [b]	57.5 (43.5–76.0)	40.5 (26.3–60.8)	134 (88.8–203)	100 (63.4–158)
2f [b]	32.1 (23.2–44.3)	25.0 (16.5–30.0)	65.4 (44.5–96.2)	58.2 (43.4–77.9)
8f	36.6 (24.4–54.9)	32.8 (22.6–46.5)	52.2 (42.3–64.3)	39.7 (25.6–61.6)

compound reduced the anticonvulsant effects similar to that of GYKI 52466 (**1**) and CFM-2 (**2f**) [13].

In conclusion, in order to extend our SAR study about molecules containing 2,3-benzodiazepine framework, a new class of triazolo-2,3-benzodiazepines was synthesized.

Through the evaluation of preliminary screening of anticonvulsant properties of new derivatives **8** against sound-induced seizures in DBA/2 mice, it was demonstrated that this kind of structural modification negatively influences the activity if compared with CFMs. It is probably due to the loss of the lactam moiety responsible for a good pharmacological profile correlated to both chemical–physical properties and AMPA receptor interaction, as suggested in previous studies [13,16,20].

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