Synthesis and Evaluation of Pharmacological Properties of Novel Annelated 2,3-Benzodiazepine Derivatives

Rosaria Gitto,*,# Valérie Orlando,# Silvana Quartarone,# Giovambattista De Sarro,§ Angela De Sarro,† Emilio Russo,§ Guido Ferreri,§ and Alba Chimirri#

Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy, Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi Magna Græcia di Catanzaro, Catanzaro, Italy, and Istituto di Farmacologia, Facoltà di Medicina e Chirurgia, Università di Messina, Messina, Italy

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New cyclofunctionalized 2,3-benzodiazepines characterized by a triazolone or triazindione ring fused on the "c" edge of the heptatomic nucleus have been prepared. These compounds were evaluated as potential anticonvulsant agents, and some of them proved to be more potent noncompetitive 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate (AMPA) receptor antagonists. In particular, 8,9-dimethoxy-6-(4-bromophenyl)-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-one (**5b**) was almost 10-fold more active than GYKI-52466 and 3.5-fold more active than Talampanel. Furthermore, **5b** potently reduced AMPA-evoked currents in electrophysiological experiments.

Introduction

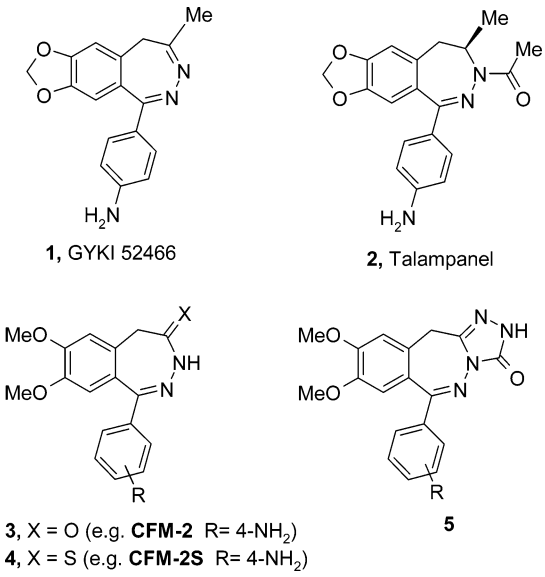
Excessive activation of the ionotropic glutamate receptors (iGluRs) may be involved in acute and chronic neurological diseases and in particular in the etiology of many forms of epilepsy. iGluRs are subdivided into three primary families based on the agonist that preferentially activates the receptor: *N*-methyl-D-aspartate (NMDA), kainate (KA), and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate (AMPA).[1−3]

A selective noncompetitive blockade of the AMPA receptor (AMPAR) was shown by some 2,3-benzodiazepines,[4,5] such as the 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (**1**, GYKI 52466) (Chart 1) and in particular the 3-*N*-acetyl-1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-2,3-benzodiazepine (**2**, Talampanel), which aroused great interest as an anticonvulsant agent and whose phase II/III clinical trials are underway.[6]

In our search for new AMPAR negative modulators,[7−14] we dentified 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**3**, CFM), and thiocarbonyl analogues **4** (Chart 1) showing anticonvulsant effects mediated through the AMPAR in a selective and noncompetitive fashion.[7−8]

To optimize the potency of our initial lead structures and provide tools through which to define the requirements for negative allosteric AMPAR ligands, we obtained a variety of related compounds such as phthalazines[9] and annelated 2,3-benzodiazepines.[10,11] SAR studies revealed that an important structural requirement necessary to retain anticonvulsant activity consisted of the presence of suitable chemical functionalities able to engage in hydrogen bonding and that the pharmacological profile was deeply influenced by the substituents on the phenyl moiety and the nature of the fused ring on the heptatomic nucleus. In particular, the 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-one derivatives (**5**) showed interesting properties and allowed us to clarify some SAR aspects.[10]

Chart 1. 2,3-Benzodiazepine Derivatives



In an attempt to further examine the role of cyclofunctionalization and to gain more information from SAR studies, we report in this paper the synthesis and pharmacological studies of other triazolo-2,3-benzodiazepines **5** and 2,12-dihydro-9,10-dimethoxy[1,2,4]-triazino[4,3-*c*][2,3]benzodiazepine-3,4-diones **7** with a six-membered ring fused to the heptatomic skeleton.

Chemistry

The cyclofunctionalized 2,3-benzodiazepines **5** and **7** were prepared through a multistep synthetic pathway (Scheme 1) using as intermediates compounds **3**, **4**, and **6** obtained according to our previously reported strategies.[7−9] In particular, 3,5-dihydro-4*H*-2,3-benzo-

* To whom correspondence should be addressed. Phone: +39 090 6766413. Fax: +39 090 355613. E-mail: gittoros@pharma.unime.it.
# Dipartimento Farmaco-Chimico, Università di Messina.
§ Università degli Studi Magna Græcia di Catanzaro.
† Istituto di Farmacologia, Università di Messina.

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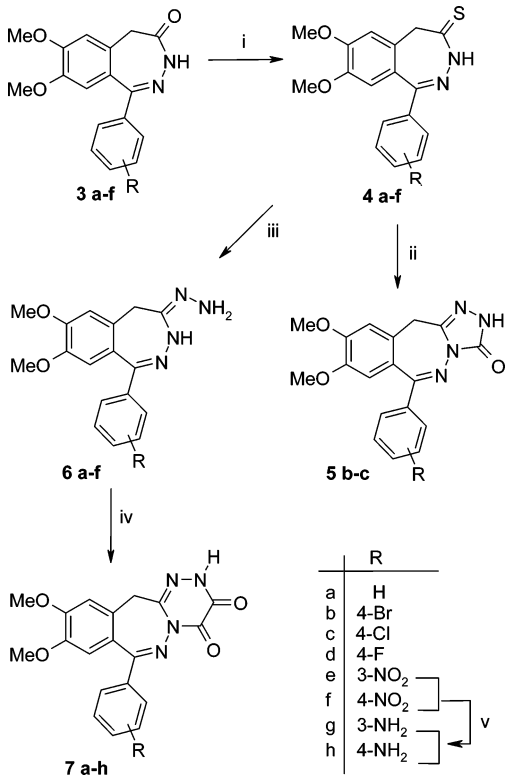
Scheme 1[a]



[a] Reagents: (i) Lawesson's reagent, dry toluene, △, 90−120 min; (ii) H_2NNHCO_2Et, n-BuOH, △, 24 h; (iii) $N_2H_4 \cdot H_2O$, THF, room temp, 60 min; (iv) $Cl_2C_2O_2$, dry toluene, room temp, 120 min; (v) H_2/Raney Ni, MeOH, room temp, 60 min.

diazepin-4-ones **3** were activated by transformation into the corresponding thiocarbonyl derivatives **4** and successively converted either into 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-one derivatives **5** or 3,5-dihydro-4*H*-2,3-benzodiazepine-4-hydrazinyl derivatives **6**. By reflux of hydrazinyl derivatives **6** with oxalyl chloride, a series of new 2,12-dihydro-9,10-dimethoxy-[1,2,4]triazino[4,3-*c*][2,3]benzodiazepine-3,4-diones **7** was synthesized. Aminophenyl-substituted derivatives **7g** and **7h** were prepared by reduction of the corresponding nitro analogues **7e** and **7f**, respectively. The structures of the compounds obtained were supported by elemental analyses and spectroscopic measurements (^{1}H NMR).

Results and Discussion

The anticonvulsant efficacy of the novel 2,3-benzodiazepines **5b**−**c** and **7a**−**h** was evaluated, 30 min after intraperitoneal (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. The results were compared with those of GYKI 52466 (**1**), Talampanel (**2**), CFM-2 (**3h**), and 6-(4-aminophenyl)-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-one (**5h**) already reported.[10] Compounds **7** are generally less potent than the corresponding **5** and possess an anticonvulsant potency that is influenced by the substituent on the phenyl ring. Anticonvulsant screening of triazolo-2,3-benzodiazepines **5b** and **5c** indicated that the introduction of a halogen atom on the phenyl ring led to potent anticonvulsant agents and in particular that the newly synthesized compound 6-(4-bromophenyl)-8,9-dimethoxy[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-one (**5b**) is almost 10-fold more active than GYKI 52466 and 3.5-fold more active than CFM-2 and Talampanel.

Table 1. Anticonvulsant Activity of 2,3-Benzodiazepine Derivatives against Audiogenic Seizures in DBA/2 Mice

	ED_{50},[a] μmol/kg	
compd	clonic phase	tonic phase
1[b]	35.8 (24.4−52.4)	25.3 (16.0−40.0)
2[b]	13.4 (10.1−17.8)	9.70 (7.00−13.4)
3h[b]	15.0 (9.01−24.0)	12.6 (8.01−19.0)
5b	3.65 (2.51−5.31)	1.34 (1.01−1.78)
5c	28.8 (18.3−45.3)	14.1 (9.17−21.8)
5h[c]	16.1 (11.8−22.0)	10.2 (8.67−11.9)
7a	33.6 (22.6−49.9)	17.9 (11.8−27.1)
7b	76.6 (60.8−96.7)	54.6 (41.5−71.8)
7c	37.4 (24.6−57.0)	33.0 (24.0−45.4)
7d	39.2 (24.9−61.7)	28.0 (15.3−51.1)
7e	>120	88.9 (69.2−114)
7f	60.0 (45.1−79.9)	25.7 (15.9−41.6)
7g	74.5 (60.1−92.4)	57.7 (45.7−76.1)
7h	59.3 (44.5−78.9)	52.8 (40.6−68.8)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[16] 95% confidence limits are given in parentheses. [b] Reference 14. [c] Reference 10.

Table 2. ED_{50} Values against MES and PTZ Induced Seizures in Swiss Mice

	ED_{50},[a] μmol/kg	
compd	MES	PTZ
1[b]	35.7 (29.3−43.4)	68.3 (56.2−83.1)
2[c]	28.8 (23.5−35.3)	56.3 (34.2−92.7)
3h[b]	15.9 (7.30−33.5)	22.6 (11.7−43.8)
5b	5.93 (3.54−9.93)	13.8 (8.54−22.4)
5c	ND[d]	33.1 (17.5−62.5)
7a	36.6 (21.6−62.1)	57.6 (34.3−96.7)
7d	37.8 (27.6−51.8)	63.2 (45.6−87.6)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[16] 95% confidence limits are given in parentheses. [b] Reference 8. [c] Reference 14 [d] ND = not determined.

Furthermore, it is noteworthy to observe that the typical aminophenyl substituent that characterizes some active 2,3-benzodiazepines (e.g., **1**, **2**, **3h**) is not always the best for the activity but other functionalities, such as electron-withdrawing substituents on the phenyl moiety, might be useful for optimizing the pharmacological properties. This possibility had also been suggested by our pharmacophore model of noncompetitive AMPAR, in which one of the main structural features consists of an aromatic ring without taking the phenyl substituent into consideration.[13]

The most active compounds of series **5** and **7** were further evaluated against seizures induced by MES and PTZ in Swiss mice (Table 2) and compared with 2,3-benzodiazepines **1**, **2**, and **3h**. As shown in Table 2, MES and PTZ induced seizures were significantly reduced 45 min after ip administration of the studied compounds, with the same rank order observed in the audiogenic seizures test, and **5b** retained anticonvulsant effects higher than those of GYKI 52466 (**1**), Talampanel (**2**), and CFM-2 (**3h**).

The anticonvulsant properties of **5b** and **5c** were also evaluated after pretreatment with aniracetam, a positive allosteric modulator of AMPAR, reversing the anticonvulsant properties of some 2,3-benzodiazepines.[15] As expected, the icv injection of aniracetam (50 nmol) 60 min before testing reduced the efficacy of **5b** and **5c** (Table 3), indicating an involvement of AMPA-mediated

Table 3. ED_{50} Values against Audiogenic Seizures after Pretreatment with Aniracetam in DBA/2 Mice

	ED_{50},[a] μmol/kg pretreatment with aniracetam	
compd	clonic phase	tonic phase
1[b]	134 (88.8−203)	100 (63.4−158)
3h[b]	65.4 (44.5−96.2)	56.2 (43.4−77.9)
5b	18.6 (10.1−34.2)	7.87 (4.61−13.4)
5c	87.8 (63.4−121)	71.8 (53.2−96.9)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[16] 95% confidence limits are given in parentheses. [b] Reference 8.

Table 4. ED_{50} Values against AMPA-Induced Seizures in DBA/2 Mice

	ED_{50},[a] μmol/kg AMPA-induced seizures	
compd	clonic phase	tonic phase
1[b]	57.5 (43.5−76.0)	40.5 (26.3−60.8)
2[c]	44.2 (34.6−56.5)	29.1 (22.6−37.5)
3h[b]	32.1 (23.2−44.3)	25.0 (16.5−30.0)
5b	17.7 (13.6−23.0)	13.4 (7.10−25.0)
5c	46.8 (38.2−57.3)	39.9 (18.6−85.5)

[a] All data were calculated according to the method of Litchfield and Wilcoxon.[16] 95% confidence limits are given in parentheses. [b] Reference 8. [c] Reference 14.

neurotransmission in the anticonvulsant properties. As shown in Table 4, the ip administration of **5b** or **5c** was also able to protect against AMPA-induced seizures.

Finally, electrophysiological studies were carried out on **5b**, the most active compound of the series, measuring AMPA receptor-mediated currents (Figure 1) under voltage clamp in Sprague−Dawley rat olfactory cortical neurons in vitro (Supporting Information). The results confirmed that the responses evoked by 0.25−5 μM AMPA were consistently abolished by 1 μM **5b** (Figure 1A), a dose 50-fold lower than those of compounds **1** and **3h**.[8] The mean peak amplitude of the AMPA-evoked inward currents was suppressed by ∼7−57% over the AMPA dose range (Figure 1A). Moreover, from the clear depression of the apparent maximum of the AMPA dose−response relation (Figure 1B), it is likely that **5b** was acting via a noncompetitive-type blocking mechanism at the AMPA receptor/ion channel complex.

In conclusion, we have identified new cyclofunctionalized 2,3-benzodiazepines **5** and **7** that showed anticonvulsant activity in different seizure models acting as noncompetitive AMPAR antagonists. In particular, derivative **5b** proved to be in vivo and in vitro more active than GYKI 52466 (**1**), CFM-2 (**3h**), and Talampanel (**2**). Furthermore, the results herein reported provide evidence that, in contrast to SAR findings reported until now, electron acceptor substituents are useful for optimizing the AMPAR antagonist activity.

Experimental Section

Chemistry. Melting points 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. ^{1}H NMR spectra were measured with a Varian Gemini 300 spectrometer. Chemical shifts are expressed in δ (ppm) relative to TMS as the internal standard, and coupling constants (*J*) are in hertz. All exchangeable protons were confirmed by addition of D_2O.

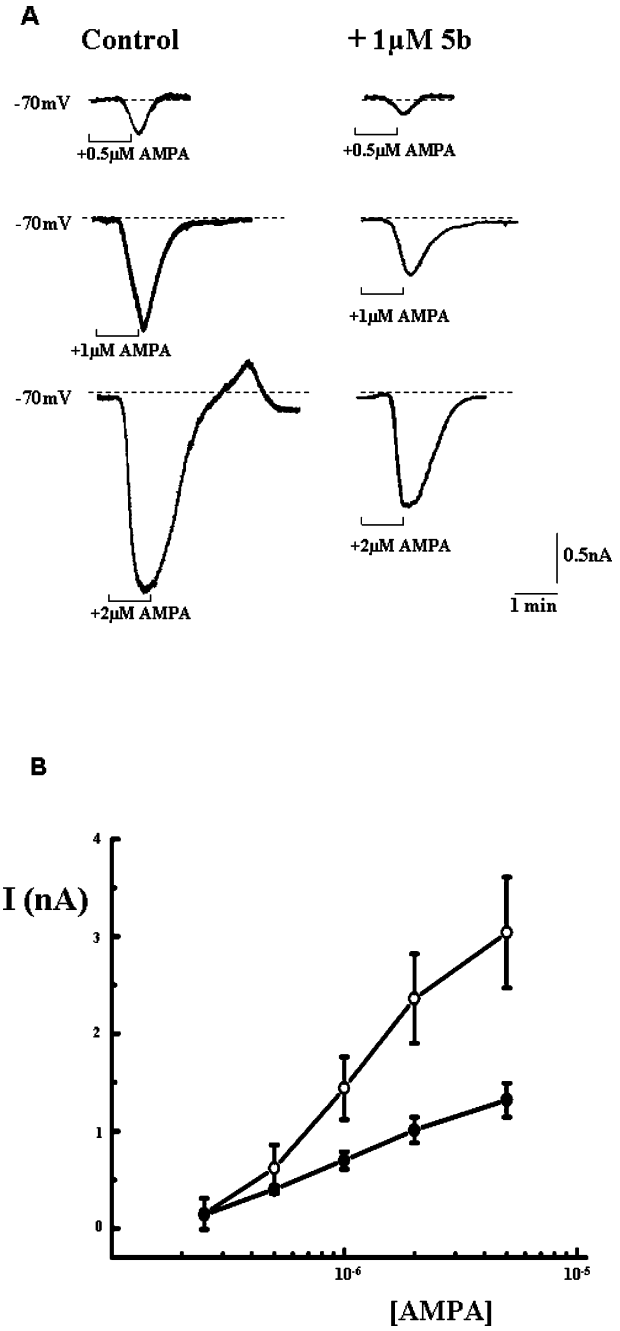


Figure 1. (A) Chart recordings from a single cortical neuron clamped at −70 mV (in TTX), showing inward currents evoked by 0.5, 1, and 2 μM AMPA (1 min bath applications) in control solution and after 15 min of preincubation with compound **5b** (1 μM). Peak currents were depressed by 52%, 56%, and 57%, respectively. Scale bars apply to all traces. (B) Noncompetitive-type depression of AMPA dose−response relation in the presence of **5b**. Peak inward membrane currents induced by AMPA were measured in rat olfactory cortical brain slice neurons voltage-clamped at −70 mV in the presence of 1 μM TTX. Points represent pooled means (±SEM, nA) plotted against applied AMPA concentration (0.25−5 μM, log scale) in the absence (○; $N = 7$) and presence (●; $N = 7$) of 0.5 μM **5b** (curves were fitted by eye).

Compounds **5b** and **5c** were prepared by refluxing 3,5-dihydro-4*H*-2,3-benzodiazepine-4-thiones (**4b** and **4c**) with ethyl carbazate in n-BuOH as previously reported in our paper.[10]

General Procedure for the Synthesis of 2,12-Dihydro-9,10-dimethoxy-7-aryl[1,2,4]triazino[4,3-*c*][2,3]benzodiazepine-3,4-diones (7a−f). To a cooled (5 °C) suspension of

compound **6** (0.28 mmol) in toluene (15 mL) was added oxalyl chloride (3.36 mmol). The mixture was stirred at room temperature for 2 h. The solution was evaporated under vacuum, and the crude product was crystallized from the suitable solvent. The starting compounds **6a**−**f** were easily prepared by treating thiocarbonyl derivatives **4a**−**f** with hydrazine hydrate according to a procedure previously described.[11b]

General Procedure for the Synthesis of Aminophenyl Derivatives (7g and 7h). The nitro derivatives (**7e** and **7f**, 0.36 mmol) were hydrogenated in vacuo by adding Raney Ni as catalyst to a MeOH solution (30 mL), and the mixture was stirred at room temperature for 1h. The Raney Ni was filtered out, and the solvent was removed under vacuum. The resulting residue was crystallized from $CHCl_3$.

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Supporting Information Available: Spectral data and experimental procedures for pharmacological evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

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