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Design and development of 2,3-benzodiazepine (CFM) noncompetitive AMPA receptor antagonists

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Abstract

2,3-Benzodiazepines represent a class of heterocyclic compounds that interact with AMPA-type glutamate receptors in a noncompetitive manner. These compounds have attracted great interest for their pharmacological effects against acute and chronic neurodegenerative diseases, such as ischemia and epilepsy. We synthesized a large number of 2,3-benzodiazepine derivatives, which showed anticonvulsant properties in different seizure models and a noncompetitive blockade of AMPA receptor. This article will briefly mention our work in this field and the main SAR considerations. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: AMPA-antagonists; 2,3-Benzodiazepines; Anticonvulsant agents

1. Introduction

L-Glutamate (Glu) is the major excitatory neurotransmitter in the vertebrate brain and plays an important role in neuronal activity via different receptor systems. Glutamate receptors (GluRs) are involved in fundamental physiological processes and in a variety of neurological diseases. This has resulted in an interest in development of GluR ligands as research tools and potential therapeutic agents [1–3].

GluRs are divided into metabotropic (mGluRs) and ionotropic receptors (iGluRs) (Fig. 1). Ionotropic glutamate receptors mediate most of the fast excitatory synaptic transmission in the central nervous system, playing a key role in synaptic plasticity. Based on their agonist affinities, iGluRs have been classified into three major pharmacological subtypes: *N*-methyl-D-aspartic acid (NMDA) receptor, kainic acid (KA) receptor and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptor [4,5].

The iGluRs are formed by homo- or heteromeric assemblies of subunits surrounding a central cation-conducting pore. Glutamate binding induces conformational changes which open the channel pore and lead to influx of cations into postsynaptic cells, but also cause the receptor to desensitize thus preventing excitotoxic processes or brain damage. It has previously been proposed that the ionotropic glutamate receptors operate as pentamer complexes like acetylcholine receptors, but biochemical and electrophysiological studies now suggest that iGluRs have a tetrameric structure, similar to most voltage-gated ion channels [6].

There are 14 distinct ionotropic glutamate receptor subunits with a different distribution in the brain, moreover some of these subunits are developmentally

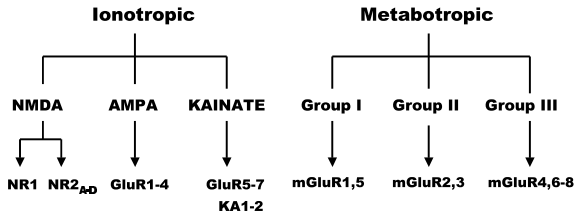

Fig. 1. Classification of glutamate receptors.

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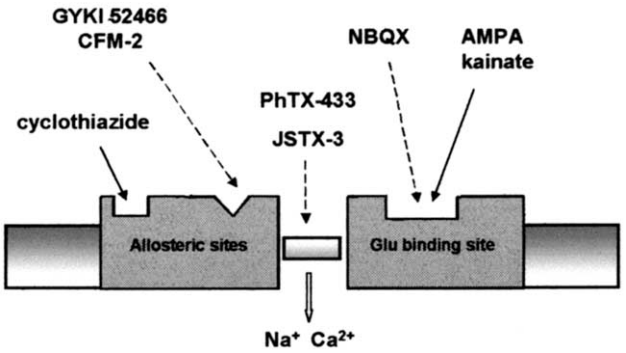


Fig. 2. Schematic representation of the AMPA-receptor.

regulated in an age-related manner. AMPA receptor types are composed of combinations of GluR1→4 subunits (Fig. 1), existing in "flip" and "flop" splice variants, which mediate fast excitatory postsynaptic potentials by the flux of Na^+ and Ca^{2+} [7].

AMPA receptor antagonists have been reported in literature and show promise in terms of their therapeutic potential for the prevention and treatment of a broad range of acute and chronic neurological diseases [8–13]. In particular, AMPA receptors are considered key mediators of seizure spread in the central nervous system and represent promising targets for antiepileptic drugs [11].

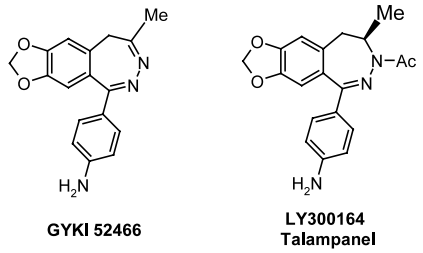
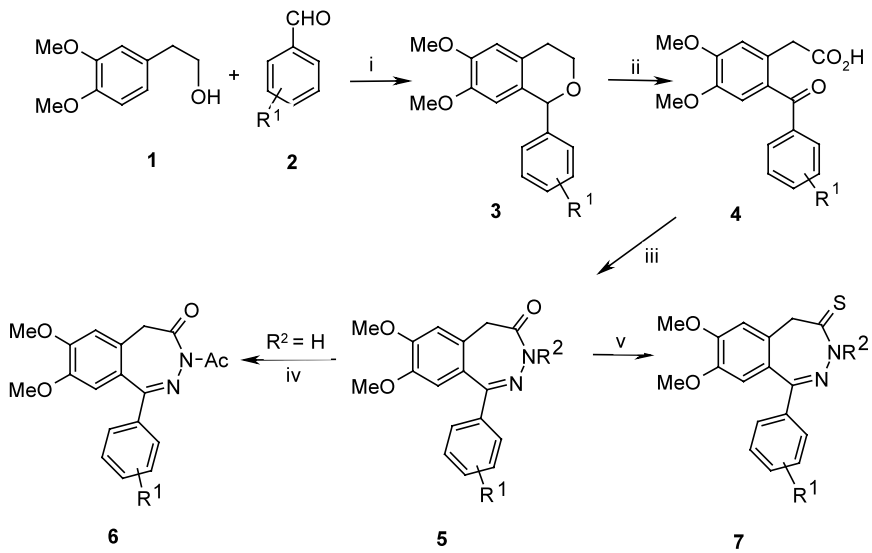


Fig. 3.

The AMPA receptor complex has at least three separate binding sites at which antagonists can act: (a) the glutamate binding site for competitive antagonists, (b) an allosteric site at which noncompetitive antagonists can bind; and (c) a polyamine site within the ion channel [13] (Fig. 2).

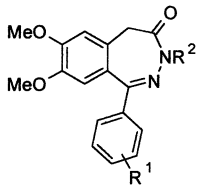
A selective and noncompetitive blockade of AMPA receptor was shown by some 2,3-benzodiazepine derivatives, such as GYKI 52466 and talampanel (Fig. 3), which possess potent anticonvulsant properties but, contrary to classical 1,4-benzodiazepines, lack sedative–hypnotic activity; in particular, talampanel represents one of the most clinically advanced agents of this class of highly selective AMPA antagonists [14–20].

In this article we report the results of our researches on 2,3-benzodiazepine derivatives, structurally related to GYKI 52466 and talampanel, as negative modulators of AMPA receptor.



Scheme 1.

Table 1
Anticonvulsant activity of compounds **5** against audiogenic seizures in DBA/2 mice



		ED_{50}, μmol/kg (±95% confidence limits)	
R^1	R^2	clonic phase	tonic phase
H	H	33.9 (26.0-44.2)	31.8 (24.8-40.6)
3-NH_2	H	19.3 (16.9-22.0)	18.3 (16.0-20.8)
3-NO_2	H	>120	>120
4-NH_2	H	15.0 (9.01-24.0)	12.6 (8.01-19.0)
4-Br	H	110 (79.3-151)	82.5 (58.6-116)
4-Cl	H	102 (76.1-137)	75.3 (60.7-93.2)
4-CN	H	>120	>120
4-F	H	78.0 (46.0-132)	57.2 (41.5-78.8)
4-NO_2	H	>120	>120
H	Me	37.8 (23.7-60.1)	26.7 (14.7-48.2)
3-NH_2	Me	50.2 (34.6-73.0)	43.7 (31.3-61.0)
4-Br	Me	63.0 (33.0-121)	38.0 (20.0-72.0)
4-CN	Me	>120	>120
4-NO_2	Me	>120	>120
H	Ac	101 (52.0-194)	72.1 (47.6-109)
4-NAc_2	Ac	56.8 (39.3-82.1)	43.9 (31.2-61.9)
4-CN	Ac	>120	>120
GYKI 52466		35.8 (24.4-52.4)	25.3 (16.0-40.0)

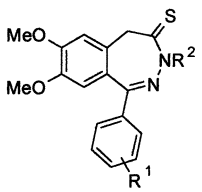
2. Results and discussion

The synthetic approach to obtain 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**5**–**6**, CFMs) is depicted in Scheme 1. The condensation of 3,4-dimethoxyphenethyl alcohol (**1**) with aromatic aldehydes **2** afforded 1-arylisochromans **3**, which were oxidized to 2-aroylphenylacetic acids **4** and condensed with hydrazine derivatives to give compounds **5**; moreover *N*-acetyl derivatives **6** were obtained from **5** by treatment with acetic anhydride [21,22].

These compounds have shown marked antiepileptic properties in various seizure models and do not bind the benzodiazepine receptors [21,22]. Table 1 reports the median effective dose (ED_{50}) values required to prevent clonic and tonic phases of sound-induced seizures in DBA/2 mice. At first, the structure–activity relationships in this series were examined by varying N-3 and C-1 aromatic ring substitutions. The introduction of a halogen atom, cyano or nitro group on the phenyl ring at C-1 negatively influences the activity, whereas the presence of an amino group increases the activity. The aminophenylderivatives proved to be more potent than GYKI 52466. The presence of a methyl group at N-3 generally is detrimental to anticonvulsant properties. Nevertheless, time-course of anticonvulsant activity and HPLC study suggest that a metabolic activation might take place in vivo and that some N-substituted derivatives undergo biotransformation by loss of the *N*-methyl substituent and show the same potency of the parent unsubstituted compounds [23–26].

After the first promising results and in an attempt to explore the effects of the lipophilicity on the anticonvulsant activity, we synthesized [27] a series of 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepine-4-thiones (**7**) by treatment with Lawesson's reagent of compounds **5** (Scheme 1). This modification generally led to potent

Table 2
Anticonvulsant activity of compounds **7** against audiogenic seizures in DBA/2 mice



		ED_{50}, μmol/kg (±95% confidence limits)	
R^1	R^2	clonic phase	tonic phase
H	H	19.7 (13.1-29.7)	15.0 (8.60-26.1)
3-NH_2	H	18.8 (8.70-36.5)	9.10 (3.70-22.3)
3-NO_2	H	ND	ND
4-NH_2	H	6.30 (2.60-15.4)	3.30 (1.30-8.30)
4-Br	H	52.1 (23.3-118)	33.3 (15.2-73.1)
4-Cl	H	82.4 (35.2-193)	55.0 (36.0-84.2)
4-F	H	36.2 (20.1-65.1)	17.1 (7.66-38.0)
4-NO_2	H	93.4 (64.7-135)	69.8 (51.4-94.8)
H	Me	30.6 (19.6-44.7)	24.7 (14.3-42.6)
4-NH_2	Me	29.8 (21.4-41.4)	20.3 (13.6-30.5)
4-Br	Me	105 (52.0-194)	60.4 (23.6-154)
4-NO_2	Me	103 (73.6-144)	81.5 (63.3-105)

anticonvulsant agents (Table 2); in particular, 1-(4′-aminophenyl) derivative was 2.5-fold more active than the corresponding carbonyl isostere. Moreover, compounds **7** showed longer-lasting activity and less toxicity than compounds **5** and GYKI 52466 [27].

Electrophysiological experiments confirmed that the anticonvulsant effects of compounds **5** and **7**, analogous to GYKI 52466, are mediated through the AMPA receptor complex in a selective and noncompetitive fashion [22,27].

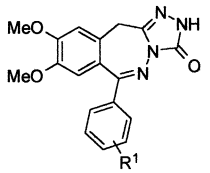
To better define the structure–activity relationships of CFM series and with the goal of developing more potent and selective compounds, we also synthesized various structurally related CFM analogues.

The synthesis of annelated 2,3-benzodiazepine derivatives **9**–**11** was performed starting from 3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones (**5**), which were activated by transformation into the corresponding thiocarbonyl derivatives **7**. The reaction with hydrazine hydrate furnished corresponding 2,3-benzodiazepin-4-ylhydrazine intermediates **8**, which were treated with sodium nitrite in acidic medium to afford 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (**9**) (Scheme 2) [28]. Instead, by refluxing 3,5-dihydro-4*H*-2,3-benzodiazepine-4-thiones (**7**) with suitable hydrazides or ethyl carbazate, respectively, 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]-benzodiazepines (**10**) and 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]-benzodiazepin-3(2*H*)-ones (**11**) were synthesized (Scheme 2) [29,30]. Aminophenyl-substituted derivatives were prepared by reduction of the corresponding nitro analogues with tin(II) chloride.

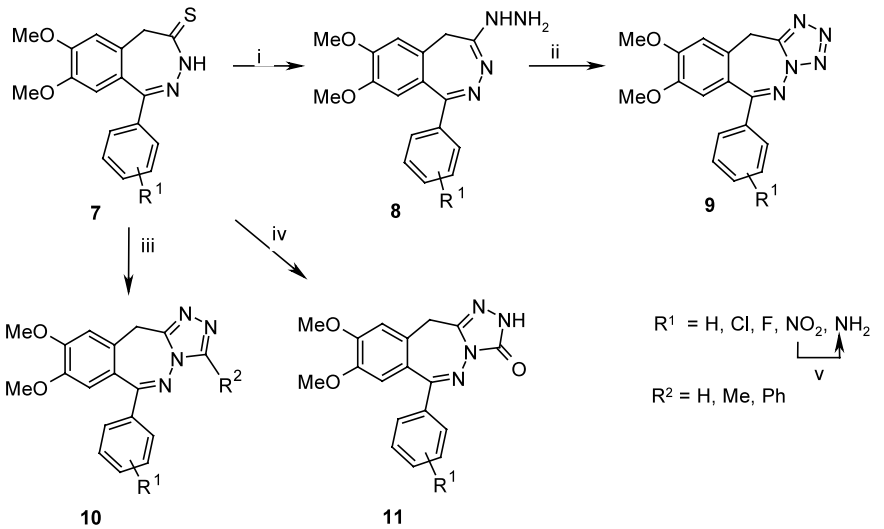
The anticonvulsant properties of the new tricyclic compounds were evaluated against audiogenic seizures to test the effect of the replacement of the (thio)amide function of parent compounds with a heterocyclic nucleus on the pharmacological profile.

11*H*-Tetrazolo[1,5-*c*][2,3]benzodiazepines (**9**) and 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines (**10**) showed anticonvulsant effects weaker than that of their parent compounds **5**. On the contrary, a comparison between the biological results of compounds 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-ones (**11**) (Table 3) and those of the parent bicyclic derivatives **5** (Table 1) revealed that the introduction of the triazolone nucleus on the diazepine skeleton leads to

Table 3
Anticonvulsant activity of compounds **11** against audiogenic seizures in DBA/2 mice

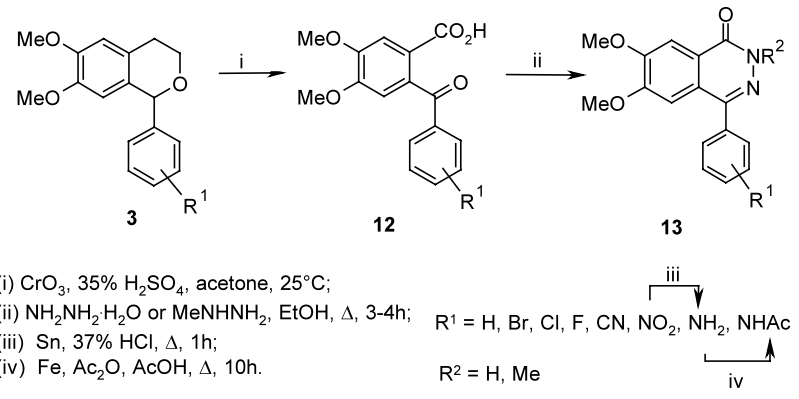


R^1	ED_{50}, μmol/kg (±95% confidence limits)	
	clonic phase	tonic phase
H	44.6 (32.5-61.2)	27.5 (15.4-49.4)
3-NH_2	20.1 (10.5-38.2)	13.1 (8.04-21.3)
3-NO_2	56.4 (39.2-81.1)	43.2 (32.5-57.4)
4-NH_2	16.1 (11.8-22.0)	10.2 (8.67-11.93)
4-F	32.1 (16.3-63.0)	21.8 (14.3-31.9)
4-NO_2	74.1 (60.3-91.0)	58.3 (41.3-82.3)



(i) $NH_2NH_2{\cdot}H_2O$, THF, rt, 1h; (ii) $NaNO_2$/HCl, rt, 2h; (iii) $R^2CONHNH_2$, n-BuOH, Δ, 24-30h; (iv) $EtOCONHNH_2$, n-BuOH, Δ, 20-25h; (v) $SnCl_2$, EtOH, Δ, 1.5 h.

Scheme 2.



Scheme 3.

compounds with anticonvulsant potency comparable or higher than the corresponding derivatives **5**.

HPLC studies put in evidence that these tricyclic derivatives **11** were converted in vivo into the corresponding **5**, likely the agents mainly responsible for the anticonvulsant properties observed [30]. Studies are in progress to clarify the mechanism of biotransformation. A possible metabolic pathway might be the cleavage of triazolone ring with subsequent decarboxylation and hydrolytic processes.

In attempt to better understand the structural requirements for binding with the allosteric site of AMPA receptor complex, we also synthesized [31] the 4-aryl-6,7-dimethoxyphthalazin-1(2*H*)-ones **13** wherein the phthalazine six-membered framework substitutes the diazepine nucleus and maintains the structural features essential for anticonvulsant activity: (a) dimethoxybenzene moiety; (b) lactam functionality; (c) 3′- or 4′-aminophenyl group.

The synthetic approach to 4-aryl-6,7-dimethoxyphthalazin-1(2*H*)-ones **13** is outlined in Scheme 3. By treatment with CrO_3 in 35% H_2SO_4 at room temperature or anyhow at a temperature over 5 °C, derivatives **3** were oxidized to 2-aroylbenzoic acids **12**. Successively, the reaction of derivatives **12** with hydrazine or monomethylhydrazine in refluxing EtOH afforded 4-aryl-6,7-dimethoxyphthalazin-1(2*H*)-ones **13** in good yields. Aminophenylderivatives were prepared by reduction of the corresponding nitroanalogues with tin in 37% HCl, whereas the reductive acetylation with iron powder in glacial acetic acid gave acetylamino-derivative.

The 4-aryl-6,7-dimethoxyphthalazin-1(2*H*)-ones **13** do not show significant anticonvulsant effects against audiogenic seizures. In fact, the most active derivatives of the series are about two-fold less active than the GYKI 52466 and four-fold less active than corresponding 2,3-benzodiazepines. Nevertheless, analogously to the 2,3-benzodiazepine series, the presence of an aminophenylgroup at C-4 positively influences the anticonvulsant effect.

3. Conclusions

This article summarizes the main findings of our research in the field of noncompetitive AMPA receptor antagonists containing 2,3-benzodiazepine skeleton. We synthesized different classes of 2,3-benzodiazepine derivatives starting from the 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (**5**, CFMs) template. Several of these compounds demonstrated to prevent seizures in different animal models and showed higher and longer-lasting activity as well as less toxicity than GYKI 52466. Their mechanism of action is mediated by noncompetitive AMPA-receptor antagonism. The results reported here furnished significant information about the main structural requirements for the anticonvulsant effects. In particular we observed that: (a) the increase of lipophilicity is favorable to pharmacological profile; (b) the cyclofunctionalization of the diazepine nucleus influences the anticonvulsant potency depending on the nature of the fused heterocyclic ring; (c) the unsubstituted (thio)lactam moiety plays a pivotal role in the case of both bicyclic and annelated 2,3-benzodiazepines; and (d) the phthalazine derivatives are less potent than the corresponding CFM-benzodiazepines.

Acknowledgements

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