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Synthesis and anticonvulsant activity of N-3 substituted 2,3-benzodiazepines

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Abstract

A series of new 3-alkylcarbamoyl-1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones was synthesized starting from the corresponding 3-N-unsubstituted derivatives, previously described as noncompetitive AMPA-type glutamate receptor antagonists. The new compounds proved to protect against seizures induced by means of auditory stimulation in DBA/2 mice and some of them showed anticonvulsant properties comparable or better than those of GYKI 52466, the prototype of 2,3-benzodiazepine noncompetitive AMPA receptor antagonists.

Keywords: AMPA receptor antagonists; 3-alkylcarbamoyl-1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones; Anticonvulsant agents

1. Introduction

L-Glutamate (Glu), the most important excitatory neurotransmitter in vertebrate brain, interacts with two different classes of receptors, ionotropic receptors (iGluRs) and metabotropic receptors (mGluRs). iGluRs consist of three primary families: N-methyl-D-aspartate (NMDA), kainate (KA) and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate (AMPA) receptors involved in fundamental physiological processes such as neuronal development, learning and memory. Nevertheless their excessive activation is held responsible for the destruction of neural tissue during ischemic states and epileptic seizures and may be involved in a variety of other neurological diseases [1-4].

Consequently, several antagonists of AMPA receptor (AMPAR) type of iGluRs have been reported in the literature and show promise in terms of their therapeutic potential for the prevention and treatment of epilepsy [5-8]. In fact, a selective and noncompetitive blockade of AMPAR was shown by 1-(4'-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466) and 3-N-acetyl-1-(4'-aminophenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-2,3-benzodiazepine (Talampanel), which possess potent anticonvulsant properties [9-11].

In our previous publications [12-13] we reported chemical and biological studies of 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones, which have shown marked antiepileptic properties in various seizure models interacting in a noncompetitive fashion with AMPAR as confirmed by electrophysiological experiments.

In recent years a wide series of 2,3-benzodiazepines [14-17] has been reported by various research groups and structure-activity relationship (SAR) studies on these derivatives have pointed out some structural features required for AMPAR interaction. Furthermore molecular modelling studies described the main common features of these negative allosteric modulators, such as a hydrogen acceptor group, two hydrophobic regions and aromatic ring function [18].

In order to further explore SAR studies on this class of compounds, we planned the synthesis of a series of novel 2,3-benzodiazepines by the introduction of an alkylcarbamoyl chain on the nitrogen atom in 3 position of the heptatomic ring considering that this function increased pharmacological properties both of 2,3-benzodiazepine and phthalazine derivatives as reported in literature [8,17].

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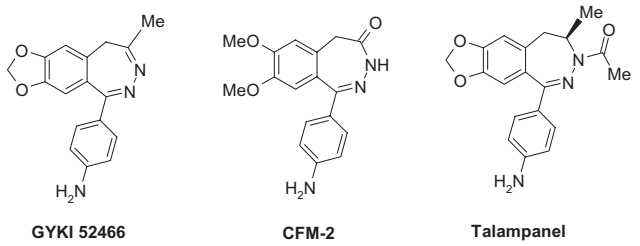


Fig. 1.

Herein we report the chemical and structural features of 3-alkylcarbamoyl-1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones synthesized. Their anticonvulsant properties were tested and compared with those of GYKI 52466, Talampanel and 1-(4'aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (CFM-2 lab. code) used as our lead compound (Figure 1).

2. Experimental

2.1. 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; 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.

*2.1.1. General procedure for the synthesis of 3-alkylcarbamoyl-1-aryl-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-ones (**2-5**)*

To a solution of 2,3-benzodiazepin-4-one derivative (**1**) in CH_2Cl_2 were added triethylamine (14.4 mmol) and the suitable isocyanate (7.7 mmol). The reaction mixture was stirred at room temperature for 24-48h and then concentrated in vacuo. The crude was crystallized by adding a small amount of suitable solvent.

*2.1.1.1. 3,5-Dihydro-7,8-dimethoxy-3-methylcarbamoyl-1-phenyl-4H-2,3-benzodiazepin-4-one (**2a**).* Mp 152-154 °C. Yield 68% (MeOH). ^{1}H-NMR: 2.92 (d, 3H, J=4.7, -$CONHCH_3$), 3.57 (s, 2H, CH_2-5), 3.74 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.70 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.42-7.51 (m, 3H, Ar), 7.75-7.78 (m, 2H, Ar), 8.62 (m, 1H, -CONH). Anal. ($C_{19}H_{19}N_3O_4$) C, H, N.

*2.1.1.2. 3,5-Dihydro-7,8-dimethoxy-1-(4'-fluorophenyl)-3-methylcarbamoyl-4H-2,3-benzodiazepin-4-one (**2b**).* Mp 174-176°C. Yield 69% (MeOH). ^{1}H-NMR ($CDCl_3$): 2.92 (d, 3H, J=4.7, -$CONHCH_3$), 3.55-3.73 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.67 (s, 1H, H-6), 6.88 (dd, 2H, J_{HF}=8.5 and J_{HH}=8.8, H-3',5'), 7.75-7.80 (dd, 2H, J_{HF}=5.5 and J_{HH}=8.8, H-2', 6'), 8.64 (m, 1H, -CONH). Anal. ($C_{19}H_{18}FN_3O_4$) C, H, N.

*2.1.1.3. 3,5-Dihydro-7,8-dimethoxy-3-methylcarbamoyl-1-(4'-nitrophenyl)-4H-2,3-benzodiazepin-4-one (**2c**).* Mp 271-273 °C. Yield 60% (MeOH). ^{1}H-NMR ($CDCl_3$): 2.94 (d, 3H, J=4.7, -$CONHCH_3$), 3.55-3.61 (m, 2H, CH_2-5), 3.79 (s, 3H, OCH_3-8), 3.99 (s, 3H, OCH_3-7), 6.60 (s, 1H, H-6), 6.91 (s, 1H, H-9), 7.98 (d, 2H, J=8.7, H-2', 6'), 8.29 (d, 2H, J=8.7, H-3', 5'), 8.64 (m, 1H, -CONH). Anal. ($C_{19}H_{18}N_4O_6$) C, H, N.

*2.1.1.4. 3,5-Dihydro-7,8-dimethoxy-3-methylcarbamoyl-1-(3'-nitrophenyl)-4H-2,3-benzodiazepin-4-one (**2d**).* Mp >300°C. Yield 63% (MeOH). ^{1}H-NMR ($CDCl_3$): 2.94 (d, 3H, J=4.7, -$CONHCH_3$), 3.55-3.62 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 4.00 (s, 3H, OCH_3-7), 6.63 (s, 1H, H-6), 6.92 (s, 1H, H-9), 7.65-7.71 (t, 1H, J=8, H-5'), 8.36-8.41 (m, 3H, Ar), 8.63 (m, 1H, -CONH). Anal. ($C_{19}H_{18}N_4O_6$) C, H, N.

*2.1.1.5. 3,5-Dihydro-7,8-dimethoxy-3-ethylcarbamoyl-1-phenyl-4H-2,3-benzodiazepin-4-one (**3a**).* Mp 150-152 °C. Yield 62% (MeOH). ^{1}H-NMR ($CDCl_3$): 1.19 (t, 3H, J=7.3, -$CONHCH_2CH_3$), 3.36 (m, 2H, -$CONHCH_2CH_3$), 3.55 (s, 2H, CH_2-5), 3.72 (s, 3H, OCH_3-8), 3.96 (s, 3H, OCH_3-7), 6.68 (s, 1H, H-6), 6.87 (s, 1H, H-9), 7.40-7.75 (m, 5H, Ar), 8.67 (m, 1H, -CONH). Anal. ($C_{20}H_{21}N_3O_4$) C, H, N.

*2.1.1.6. 3,5-Dihydro-7,8-dimethoxy-3-ethylcarbamoyl-1-(4'-fluorophenyl)-4H-2,3-benzodiazepin-4-one (**3b**).* Mp 178-180 °C. Yield 51% (MeOH). ^{1}H-NMR ($CDCl_3$): 1.20 (t, 3H, J=7.3, -$CONHCH_2CH_3$), 3.36 (m, 2H, -$CONHCH_2CH_3$), 3.56 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.68 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.12 (dd, 2H, J_{HF}=8.5 and J_{HH}=8.8, H-3',5'), 7.75-7.80 (dd, 2H, J_{HF}=5.5 and J_{HH}=8.8, H-2', 6'), 8.72 (m, 1H, -CONH). Anal. ($C_{20}H_{20}FN_3O_4$) C, H, N.

*2.1.1.7. 3,5-Dihydro-7,8-dimethoxy-3-ethylcarbamoyl-1-(4'-nitrophenyl)-4H-2,3-benzodiazepin-4-one (**3c**).* Mp 260-262 °C. Yield 55% (MeOH). ^{1}H-NMR ($CDCl_3$): 1.21 (t, 3H, J=7.3, -$CONHCH_2CH_3$), 3.38 (m, 2H, -$CONHCH_2CH_3$), 3.61 (m, 2H, CH_2-5), 3.74 (s, 3H, OCH_3-8), 3.99 (s, 3H, OCH_3-7), 6.61 (s, 1H, H-6), 6.91 (s, 1H, H-9), 7.97 (d, 2H, J=8.5, H-2', 6'), 8.28 (d, 2H, J=8.5, H-3', 5'), 8.70 (t, 1H, J=5.3, -CONH). Anal. ($C_{20}H_{20}N_4O_6$) C, H, N.

*2.1.1.8. 3,5-Dihydro-7,8-dimethoxy-3-ethylcarbamoyl-1-(3'-nitrophenyl)-4H-2,3-benzodiazepin-4-one (**3d**).* Mp >300°C. Yield 63% (MeOH). ^{1}H-NMR ($CDCl_3$): 1.21 (t, 3H, J=7.3, -$CONHCH_2CH_3$), 3.39 (m, 2H, -$CONHCH_2CH_3$), 3.58 (m, 2H, CH_2-5), 3.74 (s, 3H, OCH_3-8), 4.00 (s, 3H, OCH_3-7), 6.64 (s, 1H, H-6), 6.92 (s, 1H, H-9),

7.67 (t, 1H, J=8, H-5'), 8.35-8.41 (m, 3H, Ar), 8.70 (m, 1H, -CONH). Anal. ($C_{20}H_{20}N_4O_6$) C, H, N.

*2.1.1.9. 3,5-Dihydro-7,8-dimethoxy-1-phenyl-3-propylcarbamoyl-4H-2,3-benzodiazepin-4-one (**4a**).* Mp 153-155°C. Yield 65% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.94 (t, 3H, J=7.5, -$CONHCH_2CH_2CH_3$), 1.57-1.64 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.28-3.30 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.57 (s, 2H, CH_2-5), 3.74 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.70 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.41-7.78 (m, 5H, Ar), 8.78 (m, 1H, -CONH). Anal. ($C_{21}H_{23}N_3O_4$) C, H, N.

*2.1.1.10. 3,5-Dihydro-7,8-dimethoxy-1-(4'-fluorophenyl)-3-propylcarbamoyl-4H-2,3-benzodiazepin-4-one (**4b**).* Mp 160-162°C. Yield 70% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.94 (t, 3H, J=7.5, -$CONHCH_2CH_2CH_3$), 1.56-1.61 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.30 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.56 (s, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.68 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.10-7.15 (m, 2H, H-3',5'), 7.75-7.80 (m, 2H, H-2',6'), 8.78 (m, 1H, -CONH). Anal. ($C_{21}H_{22}FN_3O_4$) C, H, N.

*2.1.1.11. 3,5-Dihydro-7,8-dimethoxy-1-(4'-nitrophenyl)-3-propylcarbamoyl-4H-2,3-benzodiazepin-4-one (**4c**).* Mp 261-262°C. Yield 62% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.95 (t, 3H, J=7.5, -$CONHCH_2CH_2CH_3$), 1.58.1.65 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.32 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.54-3.61 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 4.00 (s, 3H, OCH_3-7), 6.61 (s, 1H, H-6), 6.91 (s, 1H, H-9), 7.97 (d, 2H, J=8.4, H-2', 6'), 8.29 (d, 2H, J=8.4, H-3', 5'), 8.78 (m, 1H, -CONH). Anal. ($C_{21}H_{22}N_4O_6$) C, H, N.

*2.1.1.12. 3,5-Dihydro-7,8-dimethoxy-1-(3'-nitrophenyl)-3-propylcarbamoyl-4H-2,3-benzodiazepin-4-one (**4d**).* Mp >300°C. Yield 53% (MeOH). ^{1}H-NMR($CDCl_3$): 0.95 (t, 3H, J=7.5, -$CONHCH_2CH_2CH_3$), 1.58-1.65 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.30-3.34 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.55-3.62 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 4.01 (s, 3H, OCH_3-7), 6.64 (s, 1H, H-6), 6.93 (s, 1H, H-9), 7.68 (t, 1H, J=8, H-5'), 8.37-8.40 (m, 3H, Ar), 8.77 (m, 1H, -CONH). Anal. ($C_{21}H_{22}N_4O_6$) C, H, N.

*2.1.1.13. 3-Butylcarbamoyl-3,5-dihydro-7,8-dimethoxy-1-phenyl-4H-2,3-benzodiazepin-4-one (**5a**).* Mp 150-152°C. Yield 56% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.92 (t, 3H, J=7.2, -$CONHCH_2CH_2CH_2CH_3$), 1.33-1.41 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 1.54-1.64 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.32-3.36 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.56 (s, 2H, CH_2-5), 3.74 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.70 (s, 1H, H-6), 6.88 (s, 1H, H-9), 7.41-7.77 (m, 5H, Ar), 8.72 (m, 1H, -CONH). Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

*2.1.1.14. 3-Butylcarbamoyl-3,5-dihydro-7,8-dimethoxy-1-(4'-fluorophenyl)-4H-2,3-benzodiazepin-4-one (**5b**).* Mp 172-174°C. Yield 68% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.92 (t, 3H, J=7.2, -$CONHCH_2CH_2CH_2CH_3$), 1.33-1.40 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 1.54-1.59 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.31-3.36 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.56 (s, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.98 (s, 3H, OCH_3-7), 6.67 (s, 1H, H-6), 6.89 (s, 1H, H-9), 7.13 (dd, 2H, J_{HF}=8.1 and J_{HH}=7.3, H-3', 5'), 7.76-7.80 (dd, 2H, J_{HH}=7.3 and J_{HF}=5.5 H-2', 6'), 8.76 (m, 1H, -CONH). Anal. ($C_{22}H_{24}FN_3O_4$) C, H, N.

*2.1.1.15. 3-Butylcarbamoyl-3,5-dihydro-7,8-dimethoxy-1-(4'-nitrophenyl)-4H-2,3-benzodiazepin-4-one (**5c**).* Mp 268-268°C. Yield 65% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.92 (t, 3H, J=7.2, -$CONHCH_2CH_2CH_2CH_3$), 1.34-1.41 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 1.55 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.35 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.54-3.60 (m, 2H, CH_2-5), 3.74 (s, 3H, OCH_3-8), 3.99 (s, 3H, OCH_3-7), 6.61 (s, 1H, H-6), 6.91 (s, 1H, H-9), 7.97 (d, 2H, J=8.2, H-2', 6'), 8.28 (d, 2H, J=8.2, H-3', 5'), 8.73 (m, 1H, -CONH). Anal. ($C_{22}H_{24}N_4O_6$) C, H, N.

*2.1.1.16. 3-Butylcarbamoyl-3,5-dihydro-7,8-dimethoxy-1-(3'-nitrophenyl)-4H-2,3-benzodiazepin-4-one (**5d**).* Mp >300°C. Yield 53% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.92 (t, 3H, J=7.2, -$CONHCH_2CH_2CH_2CH_3$), 1.34-1.41 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 1.55-1.60 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.35 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.55-3.61 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 4.00 (s, 3H, OCH_3-7), 6.64 (s, 1H, H-6), 6.92 (s, 1H, H-9), 7.67 (t, 1H, J=8, H-5'), 8.35-8.41 (m, 3H, Ar), 8.73 (m, 1H, -CONH). Anal. ($C_{22}H_{24}N_4O_6$) C, H, N.

*2.1.2. General procedure for the synthesis of aminophenyl derivatives (**2 e-f** and **5 e-f**)*

The nitro derivatives (**2 c-d** and **5 c-d**) (0.36 mmol) were hydrogenated in vacuo by adding 5% Pd/C as catalyst to a methanol solution (30 ml) and stirred at room temperature for 1h. The Pd/C was filtered out and the solvent was removed under vacuo. The resulting residue was crystallized from the suitable solvent.

*2.1.2.1. 1-(4'-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-methylcarbamoyl-4H-2,3-benzodiazepin-4-one (**2e**).* Mp 125-127°C. Yield 54% (AcOEt). ^{1}H-NMR ($CDCl_3$): 2.95 (d, 3H, J=4.7, -$CONHCH_3$), 3.55 (s, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.96 (s, 3H, OCH_3-7), 6.68 (d, 2H, J=8.5, H-2',6'), 6.75 (s, 1H, H-6), 6.86 (s, 1H, H-9), 7.57 (d, 2H, J=8.5, H-3',5'), 8.62-8.63 (m, 1H, -CONH). Anal. ($C_{19}H_{20}N_4O_4$) C, H, N.

*2.1.2.2. 1-(3'-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-methylcarbamoyl-4H-2,3-benzodiazepin-4-one (**2f**).* Mp 122-123°C. Yield 52% (AcOEt). ^{1}H-NMR ($CDCl_3$): 2.94 (d, 3H, J=4.7, -$CONHCH_3$), 3.55 (s, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.97 (s, 3H, OCH_3-7), 6.73 (s, 1H, H-6), 6.86 (s, 1H, H-9), 6.80-7.30 (m, 4H, Ar), 8.61-8.70 (m, 1H, -CONH). Anal. ($C_{19}H_{20}N_4O_4$) C, H, N.

*2.1.2.3. 1-(4'-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-ethylcarbamoyl-4H-2,3-benzodiazepin-4-one (**3e**).* Mp 168-170°C. Yield 58% (MeOH). ^{1}H-NMR ($CDCl_3$): 1.19 (t, 3H,

J=7.3, -$CONHCH_2CH_3$), 3.11 (bs, 2H, NH_2), 3.36 (m, 2H, -$CONHCH_2CH_3$), 3.55 (m, 2H, CH_2-5), 3.76 (s, 3H, OCH_3-8), 3.97 (s, 3H, OCH_3-7), 6.76 (s, 1H, H-6), 6.87 (s, 1H, H-9), 6.72 (d, 2H, J=8.5, H-2', 6'), 7.58 (d, 2H, J=8.5, H-3', 5'), 8.68 (t, 1H, J=5.3, 1H, -CONH). Anal. ($C_{20}H_{22}N_4O_4$) C, H, N.

*2.1.2.4. 1-(3'-Aminophenyl)-3,5-dihydro-3-ethylcarbamoyl-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one (**3f**).* Mp 178-180°C. Yield 50% (EtOH). ^{1}H-NMR ($CDCl_3$): 1.20 (t, 3H, J=7.3, -$CONHCH_2CH_3$), 3.36 (m, 2H, -$CONHCH_2CH_3$), 3.55 (m, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.81 (bs, 2H, NH_2), 3.97 (s, 3H, OCH_3-7), 6.73 (s, 1H, H-6), 6.79-6.85 (m, 2H, H-4', 6'), 6.86 (s, 1H, H-9), 7.17 (t, 1H, J=8, H-5'), 7.3 (s, 1H, H-2'), 8.73 (m, 1H, -CONH). Anal. ($C_{20}H_{22}N_4O_4$) C, H, N.

*2.1.2.5. 1-(4'-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-propylcarbamoyl-4H-2,3-benzodiazepin-4-one (**4e**).* Mp 173-175°C. Yield 60% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.93 (t, 3H, J=7.5, -$CONHCH_2CH_2CH_3$), 1.56-1.63 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.26-3.31(m, 2H, -$CONHCH_2CH_2CH_3$), 3.54-3.56 (m, 2H, CH_2-5), 3.76 (s, 3H, OCH_3-8), 3.97 (s, 3H, OCH_3-7), 3.99 (bs, 2H, NH_2), 6.68 (d, 2H, J=8.1, H-2',6'), 6.76 (s, 1H, H-6), 6.86 (s, 1H, H-9), 7.58 (d, 2H, J=8.1, H-3', 5'), 8.77 (m, 1H, -CONH). Anal. ($C_{21}H_{24}N_4O_4$) C, H, N.

*2.1.2.6. 1-(3'-Aminophenyl)-3,5-dihydro-7,8-dimethoxy-3-propylcarbamoyl-4H-2,3-benzodiazepin-4-one (**4f**).* Mp 113-115°C. Yield 52% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.93 (t, 3H, J=7.5, -$CONHCH_2CH_2CH_3$), 1.56-1.66 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.29-3.31 (m, 2H, -$CONHCH_2CH_2CH_3$), 3.55 (s, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.81 (bs, 2H, NH_2), 3.97 (s, 3H, OCH_3-7), 6.73 (s, 1H, H-6), 6.80-6.85 (m, 3H, H-4', 6'), 6.87 (s, 1H, H-9), 7.17 (t, 1H, J=7.8, H-5'), 7.30 (s, 1H, H-2'), 8.78 (m, 1H, -CONH). Anal. ($C_{21}H_{24}N_4O_4$) C, H, N.

*2.1.2.7. 1-(4'-Aminophenyl)-3-butylcarbamoyl-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one (**5e**).* Mp 185-187°C. Yield 60% (MeOH). ^{1}H-NMR ($CDCl_3$): 0.91 (t, 3H, J=7.2, -$CONHCH_2CH_2CH_2CH_3$), 1.32-1.40 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 1.50-1.59 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.30-3.34 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.49-3.56 (m, 2H, CH_2-5), 3.76 (s, 3H, OCH_3-8), 3.97 (s, 3H, OCH_3-7), 4.00 (bs, 2H, NH_2), 6.68 (d, 2H, J=8.5, H-2', 6'), 6.76 (s, 1H, H-6), 6.86 (s, 1H, H-9), 7.58 (d, 2H, J=8.5, H-3', 5'), 8.75 (m, 1H, -CONH). Anal. ($C_{22}H_{26}N_4O_4$) C, H, N.

*2.1.2.8. 1-(3'-Aminophenyl)-3-butylcarbamoyl-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one (**5f**).* Mp 112-113°C. Yield 55% (AcOEt). ^{1}H-NMR ($CDCl_3$): 0.92 (t, 3H, J=7.2, -$CONHCH_2CH_2CH_2CH_3$), 1.25-1.42 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 1.56-1.60 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.36 (m, 2H, -$CONHCH_2CH_2CH_2CH_3$), 3.55 (s, 2H, CH_2-5), 3.75 (s, 3H, OCH_3-8), 3.97 (s, 3H, OCH_3-7), 6.68 (s, 1H, H-6), 6.86 (s, 1H, H-9), 6.80-7.28 (m, 4H, Ar), 8.73 (m, 1H, -CONH). Anal. ($C_{22}H_{26}N_4O_4$) 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 [19]. DBA/2 mice (8-12 g; 22-25-days-old) were purchased from Harlan Italy (Corezzano, Italy). Groups of 10 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 ip (0.1 mL/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 [20].

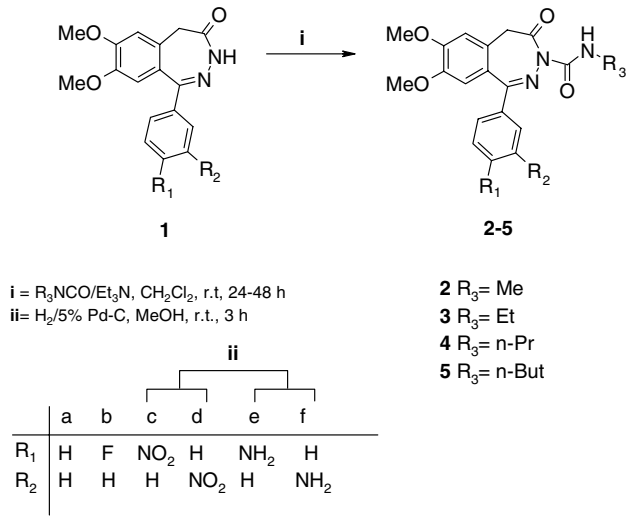
The experimental protocol and all the procedures involving animals and their care were conducted in conformity with the institutional guidelines and the European Council Directive of laws and policies.

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 [21].

3. Results and Discussion

The anticonvulsant properties of 3-alkylcarbamoyl-1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-ones (**2-5**) (Scheme 1) were evaluated 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 [22]. The results were compared with those observed for the parent compound CFM-2, GYKI 52466 and Talampanel. The structure-activity relationships was examined by two types of structural changes consisting of the introduction of different substituents i) on the phenyl ring at C-1 and ii) at N atom at 3 position of heptatomic ring.



Scheme 1.

The effects of these modifications introduced in influencing the anticonvulsant activity of compounds under study are shown in Table 1. As can be seen from the data reported, derivatives **2-5** possess anticonvulsant activity sensitive to the nature of the substituent on the C-1 phenyl ring but in particular to the N-3 substitution. In fact, the biological data show that there are some highly active compounds only among the N-3 ethylcarbamoyl derivatives (**3**). The replacement of the ethyl group by CH_3 or longer alkyl group results in compounds with diminished anticonvulsant activity.

The potency of the novel ethylcarbamoyl 2,3-benzodiazepines **3** could be correlated to their pharmacokinetic properties and in particular to their relative lipophilicity which is intermediate between compounds **1-2** and compounds **4-5**, less or too lipophilic respectively.

Analogously to nearly all biologically active series of 2,3-benzodiazepines, the compound **3e**, containing a 4'-amino group on the phenyl ring, has a strong anticonvulsant effect. But, as reported in a recent molecular modeling study [18], the amino substituent is not essential for the efficacy; in fact the unsubstituted derivative **3a** shows activity comparable to that of **3e**. Considering also the other substituents on the phenyl ring, the presence of a nitro group reduces the activity whereas the fluorine atom is the best tolerated, compound **3b** being the most active compound of the series. These results provide further evidence that the presence of electron acceptor substituents too might be useful to optimize the pharmacological profile of anticonvulsant agents acting as non-competitive AMPAR antagonists [16,23-25]. It is noteworthy to observe that compounds **3a-b** and **3e** are more potent than GYKI 52466 in audiogenic seizure test. This

Table 1
Anticonvulsant activity of **CFM-2, Talampanel, GYKI 52466**, and compounds **2-5** against audiogenic seizures in DBA/2 mice

				ED_{50}, μmol/kg[a](±95% confidence limits)	
compd	**R_1**	**R_2**	**R_3**	clonic phase	tonic phase
2a	**H**	**H**	**Me**	88.4 (61.8-126)	77.8 (58.3-103)
2b	**F**	**H**	**Me**	87.9 (53.9-143)	77.3 (59.0-101)
2c	**NO_2**	**H**	**Me**	>100	>100
2d	**H**	**NO_2**	**Me**	>100	>100
2e	**NH_2**	**H**	**Me**	>100	80.3 (47.9-134)
2f	**H**	**NH_2**	**Me**	>100	19.1 (46.7-7.83)
3a	**H**	**H**	**Et**	31.2 (19.2-50.6)	22.6 (13.0-39.6)
3b	**F**	**H**	**Et**	22.4 (11.5-43.6)	10.8 (6.60-17.3)
3c	**NO_2**	**H**	**Et**	72.6 (43.5-121)	66.1 (40.4-108)
3d	**H**	**NO_2**	**Et**	>100	>100
3e	**NH_2**	**H**	**Et**	27.7 (15.7-49.7)	19.4 (9.90-37.8)
3f	**H**	**NH_2**	**Et**	62.9 (42.7-92.7)	47.9 (27.0-34.8)
4a	**H**	**H**	**n-Pr**	48.1 (27.5-83.6)	28.6 (21.8-37.5)
4b	**F**	**H**	**n-Pr**	>100	>100
4c	**NO_2**	**H**	**n-Pr**	>100	84.5 (55.1-129)
4d	**H**	**NO_2**	**n-Pr**	>100	>100
4e	**NH_2**	**H**	**n-Pr**	70.1 (56.6-87.8)	53.9 (39.8-80.5)
4f	**H**	**NH_2**	**n-Pr**	>100	>100
5a	**H**	**H**	**n-But**	60.2 (35.1-103)	51.9 (34.1-77.8)
5b	**F**	**H**	**n-But**	80.5 (56.0-115)	67.8 (44.7-102)
5c	**NO_2**	**H**	**n-But**	>100	>100
5d	**H**	**NO_2**	**n-But**	>100	>100
5e	**NH_2**	**H**	**n-But**	90.7 (72.3-113)	78.0 (45.5-132)
5f	**H**	**NH_2**	**n-But**	>100	64.1 (48.1-85.3)
CFM-2				15.0 (9.01-24.0)	12.6 (8.01-19.0)
Talampanel				13.4 (10.1-17.8)	9.70 (7.00-13.4)
GYKI 52466				35.8 (24.4-52.4)	25.3 (16.0-40.0)

[a] All data were calculated according to the method of Litchfield and Wilcoxon. 95% confidence limits are given in parentheses. At least 32 animals were used to calculate each ED_{50} [21].

behaviour could be due to the introduction of the carbamoyl moiety which might interact as an additional hydrogen-bond acceptor in the binding site. These results match previous findings in other series of 2,3-benzodiazepine derivatives [17].

In conclusion, new 2,3-benzodiazepine derivatives were synthesized and some of them N-ethylcarbamoyl substituted (**3**) proved to be anticonvulsant agents more potent than GYKI 52466.

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