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New 7,8-ethylenedioxy-2,3-benzodiazepines as noncompetitive AMPA receptor antagonists

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Abstract—A series of 1-aryl-3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-ones **2a**–**f**, were synthesized and screened as anticonvulsant agents in DBA/2 mice against sound-induced seizures. The new compounds display anticonvulsant properties although the ED_{50} values are higher than those of prototypes 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**1**) and GYKI 52466, well-known noncompetitive AMPA receptor antagonists. Functional tests were performed to evaluate the antagonistic activity at the AMPA and kainate receptors.

There is increasing evidence of the potential therapeutic utility of AMPA receptor antagonists in the treatment of several neurodegenerative disorders, including stroke and epilepsy. The discovery of GYKI 52466 as the prototype of noncompetitive AMPA antagonists endowed with anticonvulsant and neuroprotective properties, induced wide-ranging research activities focused on 2,3-benzodiazepines.[1]

Highly active analogs of GYKI 52466 (Fig. 1) have been found among its 3,4-dihydro-3-*N*-methylcarbamoyl (GYKI 53655) and 3,4-dihydro-3-acetyl (GYKI 53405) derivatives.[2] In particular, the 4-*R* enantiomer of GYKI 53405 was chosen as drug candidate and is now in clinical investigation as LY 300164 (talampanel).[3]

We have previously investigated[4] a new series of 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-ones, e.g., **1a**, and their 3-*N*-alkylcarbamoyl derivatives, e.g., **1b**–**1c**, which have been shown to possess remarkable anticonvulsant properties and are endowed with a higher potency compared to GYKI 52466.

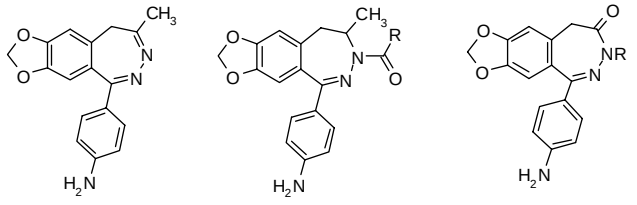

Figure 1.

In this context we noticed that substitution of the iminohydrazone portion of the diazepine nucleus of GYKI 52466 by the iminohydrazide moiety as well as the presence of the substituent appended at the *N*-3-position positively affect the anticonvulsant activity. Furthermore, we demonstrated that these derivatives compete with GYKI 52466 for the same allosteric binding site of the AMPA receptors.[5]

As an extension of our studies on the structure–activity relationships of this set of derivatives, we designed and screened for anticonvulsant activity new 3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-ones **2a**–**c**, in order to check how the substitution of the dioxole

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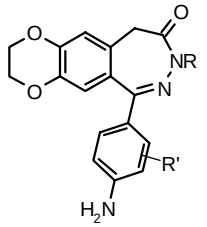
nucleus by the dioxane homologue affects the anticonvulsant activity.

Moreover, we synthesized derivatives **2d**–**2f** since it has been reported[6] that, within a new set of 2,3- benzodiazepines, the introduction of an additional substituent at the 1-phenyl moiety, e.g., 3-methyl-, markedly lengthened the time course of AMPA receptor blocking action.

The involvement of AMPA and KA receptors has been assessed by means of functional tests.



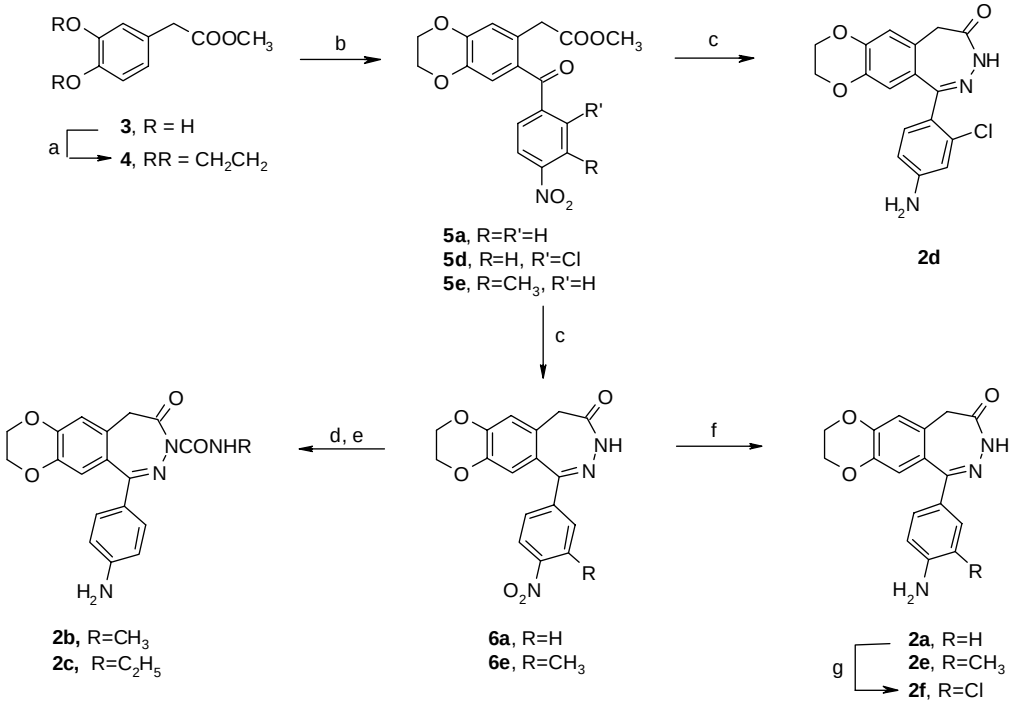
a, R = R' = H
b, R = CONHCH$_3$, R' = H
c, R = CONHC$_2$H$_5$, R' = H
d, R = H, R' = 2-Cl
e, R = H, R' = 3-CH$_3$
f, R = H, R' = 3-Cl

2a-f

The synthesis of 1-(4-aminophenyl)-3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-ones (**2a**, **2d**–**2f**) and their 3-*N*-alkylcarbamoyl derivatives (**2b**–**c**) was accomplished according to the reaction sequence reported in Scheme 1.

Methyl 3,4-dihydroxyphenylacetate (**3**), obtained according to a reported procedure,[7] was reacted with dibromoethane in the presence of potassium carbonate in DMF to afford methyl 3,4-ethylenedioxyphenylacetate **4**. Ketoesters (**5a**, **5d**, **5e**) were prepared by acylation of derivative **4** with the appropriate 4-nitrobenzoic acid in the presence of an excess of phosphorous pentoxide. The subsequent treatment of **5a** and **5e** with hydrazine afforded 1-aryl-3,5-dihydro-7,8-ethylenedioxy-4*H*-2,3-benzodiazepin-4-ones **6a** and **6e** in satisfactory yields. Conversely, ketoester **5d** in the same reaction conditions afforded directly 1-(4-amino-2-chlorophenyl)-2,3-benzodiazepine **2d** through a cyclization and reduction of the nitro group. Compounds **6a** and **6e** were then converted into the corresponding aminoderivatives **2a** and **2e** by reduction with ammonium formate in the presence of Ni/Raney. Compound **2a** was reacted with *N*-chlorosuccinimide (NCS) to give **2f**. 1-(4-Nitrophenyl)-2,3-benzodiazepine (**6a**) was also treated with an excess of an methyl or ethyl isocyanate in the presence of triethylamine to give the corresponding 3-*N*-methyl or ethylcarbamoyl derivatives which afforded compounds **2b**–**c** by catalytic hydrogenation with 5% Pd/C. Analytical and spectral data of all the synthesized compounds are in full agreement with the proposed structures.[8]

The anticonvulsant activity of derivatives **2a**–**f** against audiogenic seizures was evaluated 30 min after intraperitoneal administration into DBA/2 mice, a strain genetically susceptible to sound-induced seizures. This test has been considered the best animal model for generalized epilepsy and for screening new anticonvulsant drugs.[9] The results are compared with those previously reported for the set of derivatives **1** and reference compound GYKI 52466 (Table 1). [4]



Scheme 1. Reagents and conditions: (a) $(CH_2Br)_2$, K_2CO_3, acetone, Δ, 42 h, 64% (**4**); (b) ArCOOH, P_2O_5, $(CH_2Cl)_2$, rt, 16 h, 54% (**5a**), 60% (**5d**), 41% (**5e**); (c) $NH_2NH_2{\cdot}H_2O$, *n*-butanol, Δ, 20 h, 28% (**2d**), 64% (**6a**), 47% (**6e**); (d) CH_3NCO or C_2H_5NCO, CH_2Cl_2, Et_3N, rt, 24 h; (e) H_2/5% Pd-C, $CHCl_3$, rt, 3 h; for the two-step sequence: 18% (**2b**), 20% (**2c**); (f) Ni-Raney, $HCOONH_4$, EtOH, Δ, 2 h, 92% (**2a**), 100% (**2e**); (g) NCS, DMF, rt, 20 h, 33% (**2f**).

Table 1. Anticonvulsant activity of compounds **1**, **2** and GYKI 52466 against audiogenic seizures in DBA/2 mice and TD_{50} values on locomotion assessed by rotarod test[a]

Compounds	ED_{50} (μmol/kg)		TD_{50} (μmol/kg locomotor deficit)	PI,[b] TD_{50}/ED_{50}
	Clonic phase	Tonic phase		
1a	15.4 (10.1–23.5)	10.9 (4.60–24.6)	99.1 (72.4–135)	4.5
1b	12.4 (6.44–23.8)	8.70 (4.61–16.4)	48.6 (31.4–54.6)	3.9
1c	35.0 (18.5–66.3)	23.8 (13.4–42.1)	134 (70.7–255)	3.8
2a	61.3 (41.2–91.1)	44.8 (36.2–58.8)	102 (80.6–130)	1.7
2b	45.5 (36.1–57.3)	32.1 (22.0–46.8)	96.4 (62.7–148)	2.1
2c	48.1 (32.6–70.9)	41.7 (28.3–61.4)	95.3 (64.5–140)	2.0
2d	>100	>100	ND	ND
2e	48.2 (32.2–72.3)	24.6 (12.1–50.2)	115 (82.1–163)	2.4
2f	70.8 (42.7–117)	27.2 (15.1–49.0)	99.1 (68.4–143)	1.4
GYKI 52466	35.8 (24.4–52.4)	25.3 (16.0–40.0)	76.1 (47.5–122)	2.1

nd, not detectable.

[a] All compounds were given ip (at doses spanning the range 3.3–200 μmol/kg) 30 min before auditory stimulation. All data were calculated according to the method of Litchfield and Wilcoxon.[12] Ninety-five percent confidence limits are given in parentheses. At least 32 animals were used to calculate each ED_{50} and TD_{50} value.

[b] PI, protective index, represents the ratio between TD_{50} and ED_{50} (from the clonic phase of the audiogenic seizures).

As shown in Table 1, the new compounds possess anticonvulsant properties lower than those of prototypes **1** as well as of lead compound GYKI 52466. In particular, a comparison among the activity of **2a**–**c** with that of the corresponding derivatives **1a**–**1c** suggests that the replacement of the methylenedioxy moiety with the ethylenedioxy homologue is detrimental to the anticonvulsant activity.

The introduction of a methyl group in the 3′-position slightly enhanced the anticonvulsant activity (ED_{50} 48.2 μmol/kg for **2e** vs ED_{50} 61.3 μmol/kg for **2a**); this result parallels that previously noticed in the GYKI 52466 series.[6] The presence of a chlorine atom (**2d**, **2f**) in the 4-aminophenyl group negatively influences the activity.

Compounds **2a**–**b** were tested for their ability to inhibit the kainate-induced increase of the $[Ca^{2+}]_i$ in a primary culture of rat cerebellar granule cells (CGC) which express AMPA receptors; GYKI 52466 was used as the control (Table 2). [10]

Noteworthy, compounds **2a** and **2b** showed a concentration-dependent inhibition of the calcium influx in CGC test and were more potent than the corresponding derivatives **1a** and **1b** (IC_{50} 2.9 μM for **2a** and 5.4 μM for **2b** vs IC_{50} 12 μM for **1a** and 11 μM for **1b**) and GYKI 52466 (IC_{50} 22 μM). Furthermore, the insertion of a chlorine or a methyl group at C-3′ of the phenyl substituent (**2e** and **2f**) does substantially affect the antagonistic potency of these derivatives, as confirmed by binding assay.[10]

Table 2. Binding and functional assays for compounds **1,2** and GYKI 52466

Compounds	[^{3}H]CP-526,427 IC_{50} (μM)	KA-$[Ca^{2+}]$i IC_{50} (μM)
1a	32	12
1e	12	11
2a		2.9
2b		5.4
2c	9.3	
2d	>20	
2e	1.8	
2f	2.0	
GYKI52466	12.6	22

For compound **2a**, another functional test was carried out in rat HEK293 cells expressing GluR5, a kainate receptor subtype, stimulated by domoic acid; SYM 2081 was used as the control.[11] Compound **2a** did not inhibit responses in the GluR5 cell line (IC_{50} > 10 μM).

To sum up, the in vivo results on DBA/2 mice reported in this study suggest that the replacement of the dioxole fragment with the dioxane moiety led to a reduction in the anticonvulsant activity even if the in vitro data from functional $[Ca^{2+}]_i$ measurements clearly indicate for compounds **2** a selective antagonistic activity at the AMPA receptor higher than that displayed by **1** and GYKI 52466.

Acknowledgments

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References and notes

1. (a) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609; (b) Zappalà, M.; Grasso, S.; Micale, N.; Polimeni, S.; De Micheli, C. *Mini Rev. Med. Chem.* **2001**, *1*, 243; (c) Sólyom, S.; Tarnawa, I. *Curr. Pharm. Design* **2002**, *8*, 913.
2. Szabados, T.; Gigler, G.; Gacsalyi, I.; Gyertan, I.; Levay, G. *Brain Res. Bull.* **2001**, *55*, 387.
3. (a) 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; (b) Lagan, Y.

M.; Lucas, R.; Jewell, H.; Toublane, N.; Schaefer, H.; Sander, J. W.; Patsalos, P. N. *Epilepsia* **2003**, *44*, 46.
4. (a) De Sarro, A.; De Sarro, G.; Gitto, R.; Grasso, S.; Micale, N.; Quartarone, S.; Zappalà, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 971; (b) 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.
5. Grasso, S.; Micale, N.; Zappalà, M.; Galli, A.; Costagli, C.; Menniti, F. S.; De Micheli, C. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 443.
6. (a) Szenasi, G.; Harsing, L. G. *Drug Discov. Today* **2004**, *1*, 69; (b) Sólyom, S.; Ábrahám, G.; Hámori, T.; Berzsenyi, P.; Andrási, F.; Kurucz, I.; U.S. Patent 152693, 2004; *Chem. Abstr*. **2004**, 141, 174197.
7. Cabedo, N.; Andreu, I.; Ramirez de Arellano, M. C.; Chagraoui, A.; Serrano, A.; Bermejo, A.; Protais, P.; Cortes, D. *J. Med. Chem.* **2001**, *44*, 1794.
8. Compound **2a**: mp 222–224 °C; $R_f = 0.50$ (EtOAc/cyclohexane 90/10); ^{1}H NMR (300 MHz, $CDCl_3$): 3.43 (s, 2H, CH_2-5), 3.92 (br s, 2H, NH_2), 4.24–4.31 (m, 4H, OCH_2CH_2O), 6.68 (d, 2H, $J_{AA'XX'} = 8.5$ Hz, H-3′ and H-5′), 6.80 (s, 1H, H-9), 6.86 (s, 1H, H-6), 7.42 (d, 2H, $J_{AA'XX'} = 8.5$ Hz, H-2′ and H-6′), 8.24 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 65.88; H, 4.96; N, 13.74.
Compound **2b**: mp 193–195 °C; $R_f = 0.34$ ($CHCl_3$/EtOAc 50/50) ^{1}H NMR (300 MHz, $CDCl_3$): 2.90 (d, 3H, $J = 4.7$ Hz, CH_3), 3.50 (s, 2H, CH_2), 4.19–4.31 (m, 6H, OCH_2CH_2O and NH_2), 6.68 (d, 2H, $J_{AA'XX'} = 8.8$ Hz, H-2′ and H-6′), 6.82 (s, 1H, H-9), 6.89 (s, 1H, H-6), 7.58 (d, 2H, $J_{AA'XX'} = 8.8$ Hz, H-3′ and H-5′), 8.63 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29. Found: C, 62.43; H, 4.80; N, 15.17.
Compound **2c**: mp 185–187 °C; $R_f = 0.37$ ($CHCl_3$/EtOAc 50/50) ^{1}H NMR (300 MHz, $CDCl_3$): 1.19 (t, 3H, $J = 7.1$ Hz, CH_3), 3.35 (m, 2H, CH_2), 3.50 (m, 2H, CH_2), 3.96 (br s, 2H, NH_2), 4.24–4.31 (m, 4H, OCH_2CH_2O), 6.67 (d, 2H, $J_{AA'XX'} = 8.5$ Hz, H-3′ and H-5′), 6.82 (s, 1H, H-9), 6.89 (s, 1H, H-6), 7.57 (d, 2H, $J_{AA'XX'} = 8.5$ Hz, H-2′ and H-6′), 8.70 (br s, 1H, NH). Anal. Calcd for $C_{20}H_{20}N_4O_4$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.28; H, 5.39; N, 14.80.
Compound **2d**: mp 256–258 °C; $R_f = 0.40$ ($CHCl_3$/EtOAc 50/50); ^{1}H NMR (300 MHz, $CDCl_3$): 3.53 (s, 2H, CH_2-5), 3.91 (s, 2H, NH_2), 4.22–4.29 (m, 4H, OCH_2CH_2O), 6.55 (s, H, H-9), 6.63 (dd, 1H, $J_0 = 8.0$ Hz, $J_m = 2.0$ Hz, H-5′), 6.66 (d, 1H, $J_m = 2.0$ Hz, H-3′), 6.83 (s, 1H, H-6), 7.35 (d, 1H,$J_0 = 8.0$ Hz, H-6′), 8.64 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{14}ClN_3O_3$: C, 59.40; H, 4.10; N, 12.22. Found: C, 59.56; H, 4.21; N, 12.09.
Compound **2e**: mp 203–205 °C; $R_f = 0.48$ (EtOAc/cyclohexane 90/10); ^{1}H NMR (300 MHz, $CDCl_3$): 2.19 (s, 3H, CH_3), 3.43 (s, 2H, CH_2-5), 3.85 (br s, 2H, NH_2), 4.23–4.32 (m, 4H, OCH_2CH_2O), 6.66 (d, 1H, $J_0 = 8.2$ Hz, H-5′), 6.80 (s, 1H, H-9), 6.85 (s, 1H, H-6), 7.24 (dd, 1H, $J_0 = 8.2$ Hz, $J_m = 2.2$ Hz, H-6′), 7.37 (d, 1H, $J_m = 2.2$ Hz, H-2′), 8.20 (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, 66.71; H, 5.18; N, 13.19.
Compound **2f**: mp 256–258 °C; $R_f = 0.60$ (EtOAc/cyclohexane, 70/30); ^{1}H NMR (300 MHz, $CDCl_3$): 3.43 (s, 2H, CH_2-5), 4.25–4.30 (m, 6H, OCH_2CH_2O and NH_2), 6.76 (d, 1H, $J_0 = 8.2$ Hz, H-5′), 6.78 (s, 1H, H-9), 6.86 (s, 1H, H-6), 7.33 (dd, 1H, $J_0 = 8.2$ Hz, $J_m = 2.0$ Hz, H-6′), 7.55 (d, 1H, $J_m = 2.0$ Hz, H-2′), 8.26 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{14}ClN_3O_3$: C, 59.40; H, 4.10; N, 12.22. Found: C, 59.54; H, 4.22; N, 12.05.
9. (a) Chapman, A. G.; Croucher, M. J.; Meldrum, B. S. *Arzneim. Forsch.* **1984**, *34*, 1261; (b) Engstrom, F. L.; Woodbury, D. M. *Epilepsia* **1988**, *29*, 389.
10. 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.
11. Parks, T. N.; Artman, L. D.; Alasti, N.; Nemeth, E. F. *Brain Res.* **1991**, *552*, 13.
12. Litchfield, J.; Wilcoxon, F. J. *Pharmacol. Exp. Ther.* **1949**, *96*, 99.