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# Synthesis and Anticonvulsant Activity of Novel and Potent 1-Aryl-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-ones†

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**Abstract**—The synthesis and anticonvulsant activity of 1-aryl-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-(thi)ones (**4a–d**) and their 3-*N*-alkylcarbamoyl derivatives (**4e–h**) are reported. The new compounds possess marked anticonvulsant properties, comparable to those of the dehydro analogues **3** and higher than that of GYKI 52466 (**1**). Noteworthy, compound **4c** shows a longer-lasting anticonvulsant activity. Electrophysiological experiments show that derivative **4c** is less effective than **1** and **3c** to reduce the KA-evoked currents in cerebellar granule neurons. © 2001 Elsevier Science Ltd. All rights reserved.

Discovery of GYKI 52466 (**1**, Fig. 1), the prototype of noncompetitive AMPA receptor antagonists endowed with anticonvulsant and neuroprotective properties,[1] induced growing interest focused on 2,3-benzodiazepines. Highly active analogues of GYKI 52466 have been found among its 3,4-dihydro-3-*N*-alkylcarbamoyl and 3,4-dihydro-3-acyl derivatives (**2a–2c**, Fig. 1).[2] In particular, the 4*R* enantiomer of GYKI 53773 (**2b**) was chosen as drug candidate and is now in clinical investigation as LY 300164 (talampanel).[3]

We have recently investigated[4,5] a new series of 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3-benzodiazepin-4-(thi)ones (**3a–d**) and their 3-*N*-alkylcarbamoyl derivatives (**3e–h**) which have been shown to possess remarkable anticonvulsant properties acting as noncompetitive antag-onists at the AMPA receptor complex.

Compared to GYKI 52466, they are endowed with a higher potency and a better protective index. In this context we noticed that the nature of the substituent appended at the N-3 position as well as the presence of the iminohydrazide moiety of the diazepine nucleus affect the anticonvulsant activity.

As an extension of our studies on the structure–activity relationships of this set of derivatives, we synthesized and screened for anticonvulsant activity new 1-aryl-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-(thi)ones (**4a–d**) and their 3-*N*-alkylcarbamoyl derivatives (**4e–h**), in order to check if the 1,2-azomethine moiety of the diazepine nucleus constitutes an essential requisite for a productive interaction with the AMPA receptor complex.

2,3-Benzodiazepines **3a–c** and **3e–h**, prepared as previously reported,[4,5] were treated with sodium cyanoborohydride in methanol at room temperature to produce the corresponding new tetrahydro derivatives **4a–c** and **4e–h** (Scheme 1) in high yields (88–94%). Compound **4c** was converted into the corresponding thiocarbonyl derivative **4d** (78%) by treatment with Lawesson's reagent at reflux in toluene. Both analytical and spectral data ($^1$H and $^{13}$C NMR) of all the synthesized compounds are in full agreement with the proposed structures.[6]

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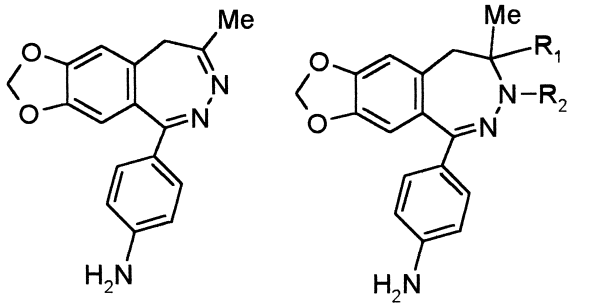
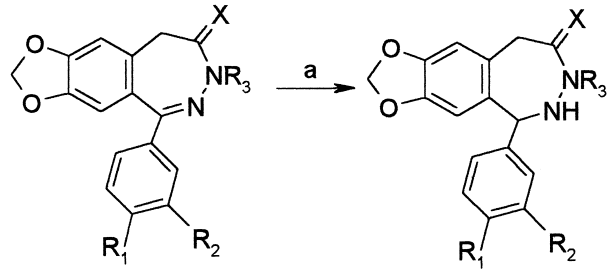



**1**, GYKI 52466

**2a**, GYKI 53655 $R_1$=H, $R_2$=CONHMe
**2b**, GYKI 53773 $R_1$=H, $R_2$=COMe
**2c**, EGIS 8332 $R_1$=CN, $R_2$=COMe

**Figure 1.**




**3** **4**

| | $R_1$ | $R_2$ | $R_3$ | X |
|---|---|---|---|---|
| **a** | H | H | H | O |
| **b** | H | $NH_2$ | H | O |
| **c** | $NH_2$ | H | H | O |
| **d** | $NH_2$ | H | H | S |
| **e** | $NH_2$ | H | NHCOMe | O |
| **f** | $NH_2$ | H | NHCOEt | O |
| **g** | $NH_2$ | H | NHCOPr | O |
| **h** | $NH_2$ | H | NHCOBu | O |

**Scheme 1.** (a) $NaBH_3CN$, MeOH/2 N HCl, rt, 1 h; (b) Lawesson's reagent, toluene, reflux, 2 h.

The anticonvulsant activity of derivatives **4a–h** against audiogenic seizures was evaluated in vivo on DBA/2 mice following a previously described protocol.[7] The results are compared with those previously reported[4,5] for reference compounds **1** and **3** (Table 1).

All new compounds possess a remarkable anticonvulsant activity which, with the exception of derivative **4h**, is higher than that displayed by **1**.

A survey of the results reported in Table 1 reveals that the azomethine moiety is not an essential requisite for anticonvulsant activity. As a matter of fact, tetrahydroderivatives **4** display a potency comparable to that of the corresponding dehydro analogues **3** (e.g., 18.6 μmol/kg for **4b** vs 18.0 μmol/kg for **3b**).

The unsubstituted 4-phenyl derivative **4a** is equipotent to **1** at variance of **3a**. The presence of an amino group (**4b–c**), in position 3′ or 4′, produced an enhancement in the anticonvulsant activity (e.g., $ED_{50}$ 11.4 μmol/kg for **4c** vs $ED_{50}$ 32.3 μmol/kg for **4a**), as previously observed in the dihydrobenzodiazepine series (**3**).

In this series of compounds, either the replacement of the carbonyl group with a thiocarbonyl moiety (**4d**) and the introduction of an alkylcarbamoyl moiety (**4e–h**) appended at the N-3 position of the heterocyclic nucleus did not produce any increase in the anticonvulsant activity. A different trend was previously observed in the series of 2,3-benzodiazepines analogues (**3d–h**).

The anticonvulsant activity of compounds **4** was effective at doses which did not cause sedation and ataxia, in analogy with other series of noncompetitive AMPA antagonists.[8] It is noteworthy that the present compounds **4** possess a protective index (PI) roughly 2-fold higher than that of **1** (Table 1).

Due to its potent anticonvulsant activity in the audiogenic seizure model, derivative **4c** was further tested in other experimental models of epilepsy. As shown in Table 2, the tonic extension and the clonic phase of the seizures induced by MES[9] and PTZ,[10] respectively, were significantly reduced at 45 min after ip administration of compound **4c**. This compound is nearly as potent as its analogue **3c** and **1** in both tests.

To investigate the relationship between the anticonvulsant activity of **4c** and its activity in non-NMDA receptors, additional tests were performed (Table 2). Compound **4c** produced a dose-dependent protection against AMPA- and KA-induced seizures.[11] The $ED_{50}$ values are higher than those needed to block audiogenic seizures (Table 1) and lower than or similar to those capable of protecting the animals against hind limb extension in the MES test.

The influence of aniracetam, a potentiator of the AMPA receptors,[12] on the anticonvulsant activity of derivative **4c** in DBA/2 mice was also tested. Administration of aniracetam 60 min before the injection of **4c** reversed its anticonvulsant effects and shifted the dose–response curves to the right with a pattern of activity similar to that of **1** and **3c**.

The time course of the anticonvulsant activity of compound **4c** was also studied and compared to that of its analogue **3c** and of model compound **1**. Compounds **3c** and **1** displayed their peak effect at 15 min from ip administration and a return to the control seizure response at 90 min, at variance of **4c** which showed an initial latency and a longer-lasting activity. At 15 min **4c** did not evidence any protection against seizures; its peak effect was detected at 30 min and a significant anticonvulsant activity was observed even at 120 min from administration (Table 3).

The activity of **4c** was also evaluated with electrophysiological experiments by measuring the KA-evoked currents with the patch clamp technique in cerebellar granule neurons grown in primary cultures.[13] At variance of the AMPA-response which is fast desensitizing,[14] KA elicits an inward non-desensitizing current that is mediated by the activation of both AMPA and KA receptors. The KA-evoked current is weakly reduced by the application of **4c** (100 μM) and to a bigger extent by **1** and **3c** (100 μM) (Table 4).

**Table 1.** Anticonvulsant activity of compounds **1, 3a–h** and **4a–h** against audiogenic seizures in DBA/2 mice[a] and $TD_{50}$ values on locomotion assessed by rotarod test

| Compds | $ED_{50}$, μmol/kg[b] | | $TD_{50}$, μmol/kg[b] locomotor deficit | PI[c] $TD_{50}/ED_{50}$ |
|---|---|---|---|---|
| | Clonic phase | Tonic phase | | |
| **1** | 35.8 (24.4–52.4) | 25.3 (16.0–40.0) | 76.1 (47.5–122) | 2.1 |
| **3a** | 43.3 (34.4–54.6) | 40.6 (30.1–54.9) | 159 (82.6–306) | 3.7 |
| **4a** | 32.3 (27.6–37.9) | 28.2 (19.7–40.3) | 113 (76.4–167) | 3.5 |
| **3b** | 18.0 (10.0–32.5) | 12.7 (6.13–26.2) | 101 (52.0–194) | 5.6 |
| **4b** | 18.6 (11.1–31.4) | 16.0 (10.3–24.8) | 67.3 (39.9–113) | 3.6 |
| **3c** | 15.4 (10.1–23.5) | 10.9 (4.60–24.6) | 99.1 (72.4–135) | 4.5 |
| **4c** | 11.4 (5.93–22.1) | 6.47 (4.20–9.98) | 41.2 (27.8–61.1) | 3.6 |
| **3d** | 11.8 (6.14–22.5) | 5.09 (2.14–12.1) | 398 (25.0–63.3) | 3.4 |
| **4d** | 22.4 (8.87–56.4) | 14.2 (6.07–33.1) | 673 (35.1–129) | 4.7 |
| **3e** | 12.4 (6.44–23.8) | 8.70 (4.61–16.4) | 486 (31.4–54.6) | 3.9 |
| **4e** | 14.7 (8.56–25.3) | 12.4 (7.93–19.5) | 599 (44.6–80.6) | 4.1 |
| **3f** | 35.0 (18.5–66.3) | 23.8 (13.4–42.1) | 134 (70.7–255) | 3.8 |
| **4f** | 25.4 (16.2–39.7) | 22.0 (15.9–30.3) | 721 (48.6–106) | 2.8 |
| **3g** | 69.4 (36.2–133) | 49.2 (25.9–93.4) | 182 (95.1–350) | 2.6 |
| **4g** | 24.6 (13.9–43.7) | 18.1 (12.2–26.8) | 86.1 (62.8–118) | 3.5 |
| **3h** | 38.7 (21.2–70.8) | 32.6 (18.2–58.4) | 108 (82.2–143) | 2.8 |
| **4h** | 39.2 (24.9–61.7) | 32.7 (21.3–50.2) | 113 (64.4–199) | 2.9 |

[a]All compounds were given ip (at doses spanning the range 3.3–200 μmol/kg) 30 min before auditory stimulation.
[b]All data were calculated according to the method of Litchfield and Wilcoxon;[15] 95% confidence limits are given in parentheses. At least 32 animals were used to calculate each $ED_{50}$ and $TD_{50}$ value.
[c]PI, protective index, represents the ratio between $TD_{50}$ and $ED_{50}$ (from the clonic phase of the audiogenic seizures).

**Table 2.** $ED_{50}$ values of **1**, **3c** and **4c** against MES-, PTZ-, AMPA- and KA-induced seizures and against audiogenic seizures after pretreatment with aniracetam

| | $ED_{50}$, μmol/kg[a] (±95% confidence limits) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MES | PTZ | AMPA[b] | | KA[c] | Pretreatment with aniracetam[d] | |
| Compds | tonic phase (Swiss mice) | clonic phase (Swiss mice) | clonic phase (DBA/**2** mice) | tonic phase (DBA/**2** mice) | (Swiss mice) | clonic phase (DBA/**2** mice) | tonic phase (DBA/**2** mice) |
| **1** | 35.7 (29.3–43.4) | 68.3 (56.2–83.1) | 57.5 (43.5–76.0) | 40.5 (26.3–60.8) | 27.8 (18.8–40.9) | 134* (88.8–203) | 100* (63.4–158) |
| **3c** | 32.1 (23.2–44.3) | 71.8 (53.2–96.9) | 37.6 (26.4–53.6) | 27.0 (18.4–40.3) | 19.6 (7.74–49.6) | 62.6* (44.7–87.7) | 39.6* (22.9–68.7) |
| **4c** | 35.7 (21.5–59.4) | 59.7 (27.0–131) | 24.6 (16.9–35.9) | 17.5 (11.9–25.7) | 15.9 (10.6–23.7) | 52.3* (30.4–89.7) | 33.1* (17.9–61.1) |

[a]All data were calculated according to the method of Litchfield and Wilcoxon.[15] At least 32 animals were used to calculate each $ED_{50}$ value.
[b]AMPA was administered icv at the $CD_{97}$ for either clonus (9.7 nmol) or forelimb tonic extension (11.7 nmol) 30 min after ip injection of tested compounds.
[c]KA was administered sc at the $CD_{97}$ (32 mg/kg) 15 min after ip injection of tested compounds.
[d]Significant differences between $ED_{50}$ values of the group treated with aniracetam + 2,3-benzodiazepine and the group treated with 2,3-benzodiazepine alone (Table 1) are denoted: $*p < 0.01$.

**Table 3.** $ED_{50}$ values at various times following ip administration of compounds **1**, **3c** and **4c**

| | $ED_{50}$, μmol/kg (±95% confidence limits),[a] clonic phase | | | | | |
|---|---|---|---|---|---|---|
| Compd | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min |
| **1** | 10.8 (7.11–16.4) | 35.8 (24.4–62.4) | 37.3 (27.2–52–1) | 39.5 (29.6–52.7) | >50 | >50 |
| **3c** | 7.55 (4.08–14.0) | 15.4** (10.1–23.5) | 18.7** (11.3–31.0) | 21.3* (14.2–31.9) | >50 | >50 |
| **4c** | >50 | 11.4** (5.93–22.1) | 18.7** (12.3–28.6) | 23.4* (14.3–38.4) | 37.1* (27.9–49.4) | 39.5* (29.4.53.0) |

[a]Significant differences among compounds **3c** and **4c** with respect to **1**, were evaluated at the corresponding times and denoted as $*p < 0.05$ and $**p < 0.01$ using the method of Litchfield and Wilcoxon.[15]

By considering either the lower ability of **4c** to reduce the KA-evoked currents with respect to **1** and **3c**, and the profile of its in vivo anticonvulsant activity which is characterized by an initial latency and a longer-lasting effect, we assume, as a working hypothesis, the biotransformation of **4c** into its dehydro derivative **3c** through an oxidative process. Pharmacokinetic investigations have been planned in order to check our hypothesis.

In conclusion, the present results indicate that, within this series of compounds, the 1,2-azomethine moiety of the diazepine nucleus is not an essential requisite for the in vivo anticonvulsant activity. In fact, all the novel 1-aryl-7,8-methylenedioxy-1,2,3,5-tetrahydro-4*H*-2,3-benzodiazepin-4-ones (**4**), similarly to their analogues 1-aryl-3,5-dihydro-7,8-methylenedioxy-4*H*-2,3- benzodiazepin-4-ones (**3**) possess a remarkable anticonvulsant activity coupled with a toxicity lower than that of compound **1**.

**Table 4.** Reduction of KA-evoked current induced by **1**, **3c** and **4c**[a]

| Compds | % Reduction[b] (mean ± SE) |
|---|---|
| **1** | 73 ± 3 |
| **3c** | 79 ± 4 |
| **4c** | 38 ± 4 |

[a]KA and compounds **1**, **3c** and **4c** were tested at 100 μM.
[b]Each value is the mean ± SE of at least 10 cells.

As a consequence derivative **4c** could become the lead compound of new anticonvulsants characterized by a different pharmacological profile. Nevertheless the mode of action of **4c** needs to be fully clarified; experiments on this line are underway. Furthermore, the profile of **4c** will be further defined through the preparation of its enantiomers and the subsequent pharmacological characterization.

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## References and Notes

1. (a) Chapman, A. G.; Smith, S. E.; Meldrum, B. S. *Epilepsy Res*. **1991**, *9*, 92. (b) Smith, S. E.; Meldrum, B. S. *Stroke* **1992**, *23*, 861.
2. Tarnawa, I.; Berzsenyi, P.; Andrasi, F.; Botka, P.; Hamori, T.; Ling, I.; Körösi, J. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 99.
3. (a) Ling, I.; Podányi, B.; Hamori, T.; Sólyom, S. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1423. (b) Anderson, B. A.; Harn, N. K.; Hansen, M. M.; Harkness, A. R.; Lodge, D.; Leander, J. D. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1953.
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) De Sarro, A.; De Sarro, G.; Gitto, R.; Grasso, S.; Micale, N.; Zappalà, M. *Farmaco* **1999**, *54*, 179.
5. Grasso, S.; De Sarro, G.; De Sarro, A.; Micale, N.; Zappalà, M.; Puia, G.; Baraldi, M.; De Micheli, C. *J. Med. Chem*. **1999**, *42*, 4414.
6. Selected data for compound **3c**: mp 213–215 °C. $^1$H NMR (300 MHz, $CDCl_3$) 3.70 and 4.26 (dd, 2H, $J$ = 14.2, $CH_2$-5), 3.80 (bs, 2H, $NH_2$), 4.01 (bs, 1H, NH-2), 5.17 (s, 1H, H-1), 5.88 (s, 2H, $OCH_2O$), 6.31 (s, 1H, H-9), 6.62 (d, 2H, $J$ = 8.2, H-3′,5′), 6.64 (s, 1H, H-6), 6.83 (bs, 1H, NH-3), 6.96 (d, 2H, $J$ = 8.2, H-2′,6′); $^{13}$C NMR (75 MHz, $CDCl_3$) 41.54 (C-5), 66.99 (C-1), 101.13 ($OCH_2O$), 109.77 (C-9), 110.58 (C-6), 115.08 (C-3′,5′), 123.96 (C-5a), 129.93 (C-2′,6′), 131.19 (C-9a), 132.13 (C-1′), 146.34 (C-4′), 146.51 (C-7), 146.98 (C-8), 176.56 (C-4).
7. (a) Collins, R. L. In *Experimental Models of Epilepsy;* Purpura, P., Penry, J. K., Tower, D., Woodbury, D. M., Walter, R., Eds.; Raven Press: New York, 1972; pp 347–372. (b) De Sarro, G. B.; Croucher, M. J.; Meldrum, B. S. *Neuropharmacology* **1984**, *23*, 526.
8. (a) Smith, S. E.; Durmuller, N.; Meldrum, B. S. *Eur. J. Pharmacol*. **1991**, *201*, 179. (b) Donevan, S. D.; Yamaguchi, S. I.; Rogawski, M. A. *J. Pharmacol. Exp. Ther*. **1994**, *271*, 25.
9. Swinyard, E. A.; Brown, W. C.; Goodman, L. S. *J. Pharmacol. Exp. Ther*. **1952**, *106*, 319.
10. Swinyard, E. A.; Woodhead, J. M. In *Epileptic Drugs;* Woodbury, D. M., Penry, J. K., Pippenger, C. E., Eds.; Raven Press: New York, 1982; pp 111–126.
11. De Sarro, G. B.; Ongini, E.; Bertorelli, R.; Aguglia, U.; De Sarro, A. *Eur. J. Pharmacol*. **1994**, *262*, 11.
12. Ito, I.; Tanabe, S.; Kohda, A.; Sugiyama, H. *J. Physiol. (London)* **1990**, *424*, 533.
13. (a) Gallo, V.; Ciotti, M. T.; Coletti, A.; Aloisi, F.; Levi, G. *Proc. Natl. Acad. Sci. U.S.A*. **1982**, *79*, 7919. (b) Hamil, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflugers Arch*. **1981**, *414*, 85. (c) Murase, K.; Ryu, P. D.; Randic, M. *Neurosci. Lett*. **1989**, *103*, 56.
14. (a) Patneau, D. K.; Mayer, M. L. *Neuron* **1991**, *6*, 785. (b) Donevan, S. D.; Rogawski, M. A. *Neuron* **1993**, *10*, 51. (c) Wilding, T. J.; Huettner, J. E. *Mol. Pharmacol*. **1995**, *47*, 582.
15. Litchfield, J.; Wilcoxon, F. *J. Pharmacol. Exp. Ther*. **1949**, *96*, 99.