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STRUCTURE-ACTIVITY RELATIONSHIPS OF 2,3-BENZODIAZEPINE COMPOUNDS WITH GLUTAMATE ANTAGONISTIC ACTION

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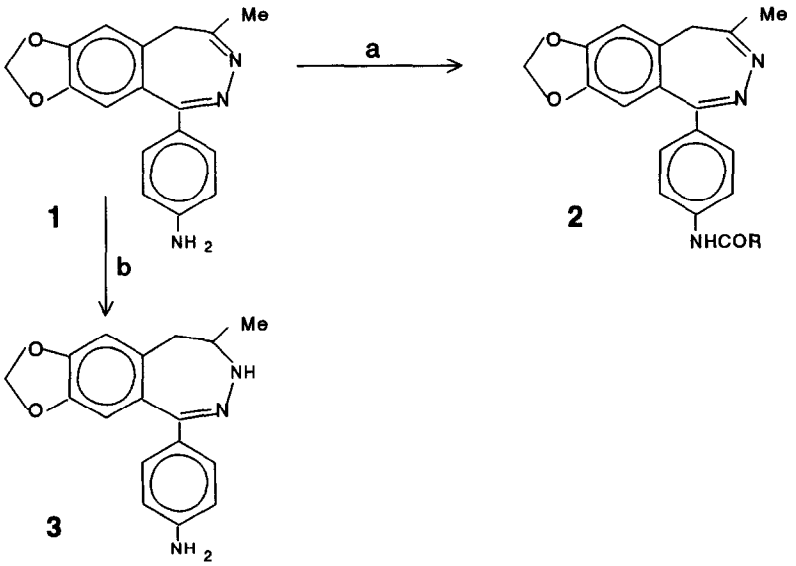
Abstract: A series of N-substituted 1-(4'-aminophenyl)-4-methyl-3,4-dihydro-7,8-methylene-dioxy-5H-2,3-benzodiazepines, structural analogues of the selective non-NMDA antagonist GYKI 52466, has been synthesized and tested for biological activity, in vivo and in vitro.

GYKI 52466 (**1**) is a 2,3-benzodiazepine compound, with muscle relaxant and anticonvulsant potencies[1-3]. It has been reported that this drug inhibits neuronal responses mediated by the non-NMDA type

Scheme 1

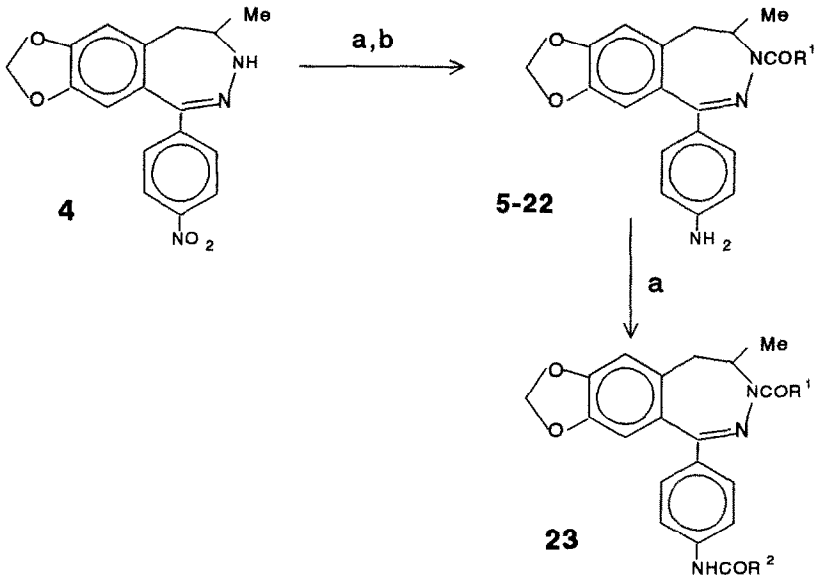


a[11]: acylation, b: $NaBH_4$,/pyridine

glutamate receptors in neocortical neurons in vitro[4], in abducens motoneurons in vivo[5], and in various seizure models[3,6]. Non-NMDA receptors seem to be involved in certain neurodegenerative disorders,

suggesting a possible therapeutic application for their antagonists as neuroprotective drugs[7]. GYKI 52466 has been shown to protect animals from ischaemic brain damage[8,9] and hippocampal neurons from the toxic action of the non-NMDA agonist kainate applied in local injection[10] as well.

Scheme 2



a[11]: acylation, b: $N_2H_4.H_2O$, RaNi, MeOH

In the present study several structural analogues of GYKI 52466 have been prepared and evaluated in order to develop new compounds with similar, but stronger pharmacological actions. The structure-activity relationships were investigated in vivo and in vitro as well. The first results of this study are presented here.

Nearly 100 substances have been synthesized with the method outlined in **Scheme 1** and **Scheme 2.** They were tested in mice for anticonvulsant activity, using the maximal electroshock (MES)[12], and the maximal metrazole seizure (MMS)[13] models, and for muscle relaxant activity, using the inclined screen (IS)[14] test, respectively. Drugs were applied orally, at a 100 mg/kg dose, usually 60 min prior to the electric shock, or i.p. application of 130 mg/kg metrazole, (MES and MMS, respectively) and in a dose of 200 mg/kg i.p., 30 min prior to testing for muscle relaxation (IS). With the most effective substances, the time of pretreatment was varied in order to study the time-course of the protective action, and the effect of

intravenous application was also investigated. Substances were investigated in groups of 5 animals in each test, at the screening doses mentioned above. ED_{50} values were determined with the substances that had a reasonable effect in screening doses.

25 substances were selected for in vitro testing in rat hippocampal slice preparations[15]. The Schaffer collateral - commissural pathway was stimulated and field responses were recorded with extracellular electrodes, positioned into the CA1 pyramidal layer. Non-NMDA type glutamate receptors have been shown to be involved in mediation of neuronal excitation underlying field potential responses in this area, and these potentials can be inhibited by antagonists of the non-NMDA receptors, like the quinoxaline derivative CNQX[16,17]. Thus, we used inhibition of field potentials as a relevant bioassay to evaluate glutamate (non-NMDA) antagonistic activity. Compounds were dissolved in the incubating medium and perfused for 30 or 60 min.

All derivatives that had biological activity displayed an in vivo pharmacological profile very similar to that of GYKI 52466. Substances all seem to be rapidly and completely absorbed from the intestines. In vitro, the inhibitory action of the active compounds developed very slowly, even a 30 min perfusion application seemed insufficient to reach the maximum effect. The action was reversible, but the recovery took a long time. According to the general structure **2**, where R includes the R^1 groups listed in Table 1., except for the one in **16**, plus $C_{15}H_{31}$, $(CH_2)_3COOH$, CH_2Cl, $(CH_2)_3NH_2$, and $CH_2N(C_2H_5)_2$, 22 N-acylated derivatives of GYKI 52466 have been synthesized and tested in vivo. The derivatives belonging to this group usually had a weaker in vivo effect than that of GYKI 52466. Relative potencies to GYKI 52466 varied between 0.00 and 1.18, the glycyl and methoxyacetyl derivatives being the most active. Two compounds belonging to this group, the acetyle and glycyl derivatives, have been tested in vitro and none of them had any noticeable effect. It is interesting to note that the acetyl derivative was totally ineffective also in vivo, in contrast to the acetyl derivative of **5** (see later).

Saturation of the double bond between position 3 and 4 in the benzodiazepine ring (**3**) weakened the anticonvulsant activity and almost abolished the inhibition of population spikes in vitro. Introducing various substituents into position 3 of the ring, however, sometimes resulted in considerably more potent compounds than GYKI 52466. As it is listed in Table 1., among the compounds where R^1 is an alkyl group, the acetyl (**6**) and propionyl (**7**) derivatives proved to be the most effective ones in vivo and in vitro, respectively. The cyclopropanecarbonyl derivative (**9**) was also effective, while the longer acyl (**8,10**), benzoyl (**11**) or phenylacetyl (**12**) ones were without any effect in vivo, although compound **8** had some effect in vitro. The methyl-carbamoyl analogue, GYKI 53655 (**15**), was superior to all the other compounds discussed in this study. Its oral ED_{50} value was 8.4 mg/kg in the MES test, while it had an IC_{50} of 12.0 μM. The corresponding values of GYKI 52466 were 38 mg/kg and 29.5 μM, respectively. The other alkylcarbamoyls, or arylcarbamoyls (**16,17**) were less potent, while the aminoacyl and cyanacetyl substituents (**18-22**) practically eliminated both in vivo and in vitro activities. One possible explanation of

TABLE 1.: In vivo and in vitro potencies of the derivatives with substitutions at the position 3 of the reduced benzodiazepine ring

Compound No.	R^1	Relative Potency# Compared to GYKI 52466	
		in vivo	in vitro
(5)	H	1.72	0.44
(6)	CH_3	2.74	1.12
(7)	C_2H_5	2.55	1.55
(8)	n-C_4H_9	0.00	0.22
(9)	cyclopropyl	1.66	1.48
(10)	$C(CH_3)_3$	0.00	not tested
(11)	C_6H_5	0.00	not tested
(12)	$CH_2C_6H_5$	0.00	not tested
(13)	CH_2OCH_3	1.02	not tested
(14)	CF_3	0.00	not tested
(15)	$NHCH_3$	4.39	2.46
(16)	NHC_4H_9	0.35	0.65
(17)	NHC_6H_5	0.13	not tested
(18)	CH_2NH_2	0.00	0.10
(19)	CH_2NHCH_3	0.00	0.02
(20)	$CH_2N(CH_3)_2$	0.00	not tested
(21)	CH_2CN	0.21	not tested
(22)	$CH(CH_3)NH_2$	0.08	0.00

#The way of calculation of in vivo relative potencies was that the mean of the ED_{50} values determined in the MES, MMS and IS tests for a given substance was compared to the mean of the corresponding values for GYKI 52466. The ED_{50} values for GYKI 52466 were as follows (mg/kg, 95% confidence limit): MES: 38.0 (30.6 - 47.1); MMS: 115.0 (106.9 - 123.6) IS: 47.0 (45.1 - 49.0). If the substance did not show any activity at the first screen doses, in any of the tests, a relative potency of 0.00 was assigned. When calculating in vitro relative potencies, the percentage inhibition caused by a given drug (determined after 30 min perfusion application) was compared to the inhibition caused by GYKI 52466 in the same concentrations. When IC_{50} values were available, these were used for calculating relative potencies. The IC_{50} of GYKI 52466 after 30 min perfusion application was 29.5 μM[18].

the fact that compounds **8** and **16** have some in vitro activity but they are not effective in vivo is that a too big alkyl substituent may influence the bioavailability of the compounds. In addition to the N-acyl compounds, listed in Table 1., the N-methyl and N-ethyl derivatives[19] have also been synthesized and tested. They had some effect, but weaker than that of GYKI 52466.

The third group of compounds examined consists of disubstituted reduced analogues of GYKI 52466 (**23**). They can be regarded as derivatives of the corresponding free aromatic amines listed in Table 1. R^2 means substituents that were mentioned in relation to the former group of compounds. In a particular analogue R^2 could be the same, but it was not necessarily the same as R^1. 52 such substances have been prepared and tested. These analogues displayed a considerably weaker in vitro potency than the corresponding free amines (**5-22**). However, the in vivo potency was sometimes retained or even improved, especially if acetyl, propionyl, trifluoroacetyl, or glycyl groups[20] were introduced. These substances were considerably longer acting than the corresponding monoacyl compounds. None of the inactive amino compounds (**8,10-12,14,18-20,22**) gained biological activity with such a substitution. The decrease or disappearance of in vitro activity suggests that a metabolic activation might take place in vivo. According to this hypothesis acetyl, propionyl, trifluoroacetyl and glycyl substituents are the best ligands for the deacylating enzyme(s), which are responsible for converting these compounds to an active one. As it was noted earlier, for the compounds characterized by the general formula **2**, an acetyl group was not a good substitution in respect of biological activity. It must also be noted that the acetyl or glycyl derivatives of compound **6** had some weak in vitro potency suggesting a limited intrinsic activity of their own.

Comparing the in vivo and in vitro relative potencies of the compounds listed in Table 1., the orders of potencies are more-or-less in accordance with each other (correlation coefficient: 0.755[21]).

Summarizing our results, introducing short acyl or alkylcarbamoyl substituents at position 3 of the reduced benzodiazepine ring resulted in an increase of both in vitro and in vivo potencies. Further substitution at the aromatic amine of these analogues was sometimes advantageous in respect of in vivo potency, but always lowered in vitro activity. The most effective analogue (**15**) is about 2.5 times and 4.4 times more effective than GYKI 52466 in vitro and in vivo, respectively.

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15. The experimental procedure used during our brain slice studies is described elsewhere (Tarnawa, I.; Molnár, P.; Gaál, L.; Andrási, F. *Acta Physiol. Hung.* **1992**, *79*, 169.) Briefly: transverse hippocampal slices (450 μm thick) were prepared, transferred into an interface-type recording chamber and stimulated continuously at 0.1 Hz, with an intensity that gave a near-maximum response. Responses were continuously monitored on a digital oscilloscope, or with the aid of a computer program and the decrease of the amplitude of the population spike measured after 30 or 60 min drug application was compared to the pre-drug (control) amplitude. Ineffective or weakly effective substances were studied on 2 or 3 slices at 40 or 80 μM, while IC_{50} values were determined with the effective substances.
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18. It must be noted, that under our experimental conditions, in vivo ED_{50} values were determined not only by its intrinsic potency but also by its duration of action. Similarly, the inhibitory effect measured in vitro was influenced not only by the potency, but for some extent also by the diffusibility of the substance in the brain tissue.
19. For description of their synthesis, see HU Patent Appl. No. 8397/90.
20. In vivo relative potencies of these compounds vary between 1.96 and 3.31. It is worth mentioning that the trifluoroacetyl and glycyl groups are not preferable substituents at position 3 of the ring(see Table 1.).
21. The correlation coefficient was calculated from the data listed in Table 1. In general, in vivo relative potency values are bigger than the corresponding in vitro values. This can be explained by two factors. First, GYKI 52466 has a relatively short duration of in vivo action compared to most of its analogues. After oral application, GYKI 52466 reaches its peak effect at the 10th-15th min, then the effect starts to decline gradually, probably due to a rapid decrease of the brain concentration of the drug. Thus, measurements based on pretreatment as long as 60 min leads to an underestimation of the intrinsic potency of the drug, resulting in a bigger relative potency for the compounds that are compared to. Second, diffusibility in brain tissue of GYKI 52466 seems to be somewhat better than that of the substituted analogues. Thus, after a 30 min perfusion application, when the inhibition of field potentials is determined, its concentration is closer to the supposed plateau concentration than in the cases of the other compounds. In some experiments drugs were perfused for 60 min instead of 30. Based on comparisons of the data of these studies, relative potency values for compounds **6** and **15** were 1.48 and 3.06, respectively, and these ones are closer to the corresponding in vivo relative potency values.