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Structural Analogues of Some Highly Active Non-Competitive AMPA Antagonists

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Abstract—Some 5-methyl analogues (**14a–e**) of the non-competitive AMPA antagonists 3-acylated 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-3*H*-2,3-benzodiazepines (**2**,**3**) have been synthesized. Generally they show diminished or low biological activity but two derivatives (**14a,b**) reveal effects comparable to those of GYKI 52466 (**1**), the prototype non competitive AMPA antagonist.

The discovery of GYKI 52466 (**1**, Fig. 1) as the prototype of the non-competitive AMPA antagonists (also called negative allosteric modulators of the AMPA receptor) induced wide-ranging research activities around the 2,3-benzodiazepines.[1] We have found highly active analogues (**2**,**3**) among the 3-acylated 1-(4-aminophenyl)-7,8-methylenedioxy-4,5-dihydro-3*H*-2,3-benzodiazepines.[2] An enantioselective synthesis[3] elaborated by us rendered access to the pure isomers and the enantiomer (−)**2** (GYKI 53773) with an 4R configuration was chosen as drug candidate, which is now in clinical investigation as LY 300164.[4]

A thorough investigation by us revealed several structural features which are important for the non-competitive AMPA antagonist activity of the original molecules **1**–**3**.[5] Additionally, it has been found that the 3-acyl functionality of **2** and **3** can be substituted by a 2-pyridyl moiety, obviously through the similarity of the two substituents concerning the H-bond acceptor capabilities of the carbonyl oxygen and the ring nitrogen atom of similar orientation.[6] Several substituted 4*H*-2,3-benzodiazepin-4-ones also have been found to be novel non competitive AMPA antagonists.[7] Some 1,2-dihydrophthalazines such as **4** and **5** have been found to have activity similar to that of GYKI 52466 (**1**).[8] Compound **4** can be seen as derivative of **3** by deleting the methylene group from **3**.

During our structure–activity studies we have investigated some compounds where the 4-methyl group of **2** has been moved to the 5-position. Herein we report the chemistry and biological activity of these compounds.

Chemistry

A known transformation of 1-aryl-1-propenes to 2-arylpropionaldehydes by treatment with iodine in the presence of silver(I)oxide in aqueous solution[9] was applied to isosafrol **6**, providing **7** in 80% yield (Scheme 1). Reduction of the latter with sodium borohydride in ethanol gave phenylethanol derivative **8**. Reaction of **8** with 4-nitrobenzaldehyde in benzene at rt using concd HCl gave a nearly 7:3 *cis*/*trans* mixture of isochromane **9** (60%), which was converted to hemiacetal **10** (93%) as a 1:1 mixture of stereoisomers by air oxidation in the presence of 50% NaOH in a mixture of DMF and DMSO (4:1). Reactions of **10** with different acylhydrazides were performed in isopropanol in the presence of catalyst amount of concd HCl, providing hydrazones **11a–d,f** in good yields (84–93%) as mixtures of *Z*/*E* isomers. In the case of **11e** (86%), slightly more than 1 equiv of HCl was used. Mesilations of **11a–f** in dichloromethane gave **12a–f** generally as solid foams, which were in turn further reacted in methanol with a 50% solution of NaOH in water to give **13a–e** with variable yields (33–76%). Under these reaction conditions the isonicotinoyl group of **12f** was hydrolyzed to give **13g** (47%) as the main product and only trace amount of **13f** was isolated (7%). **13a–e** and **13g** were

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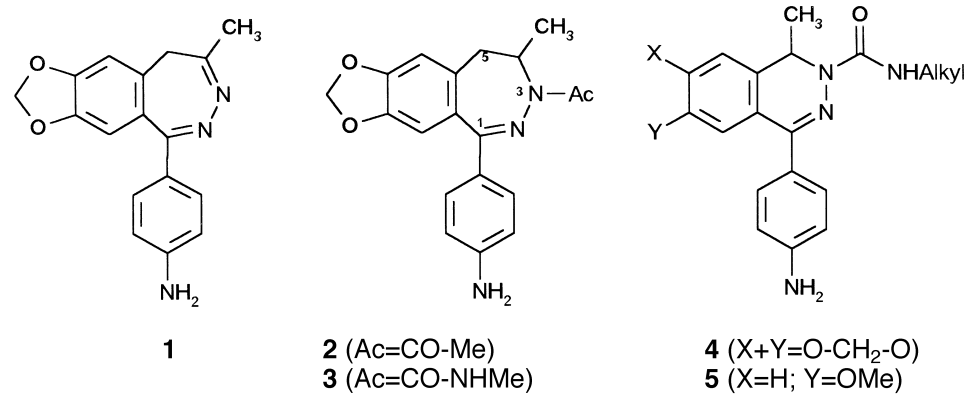
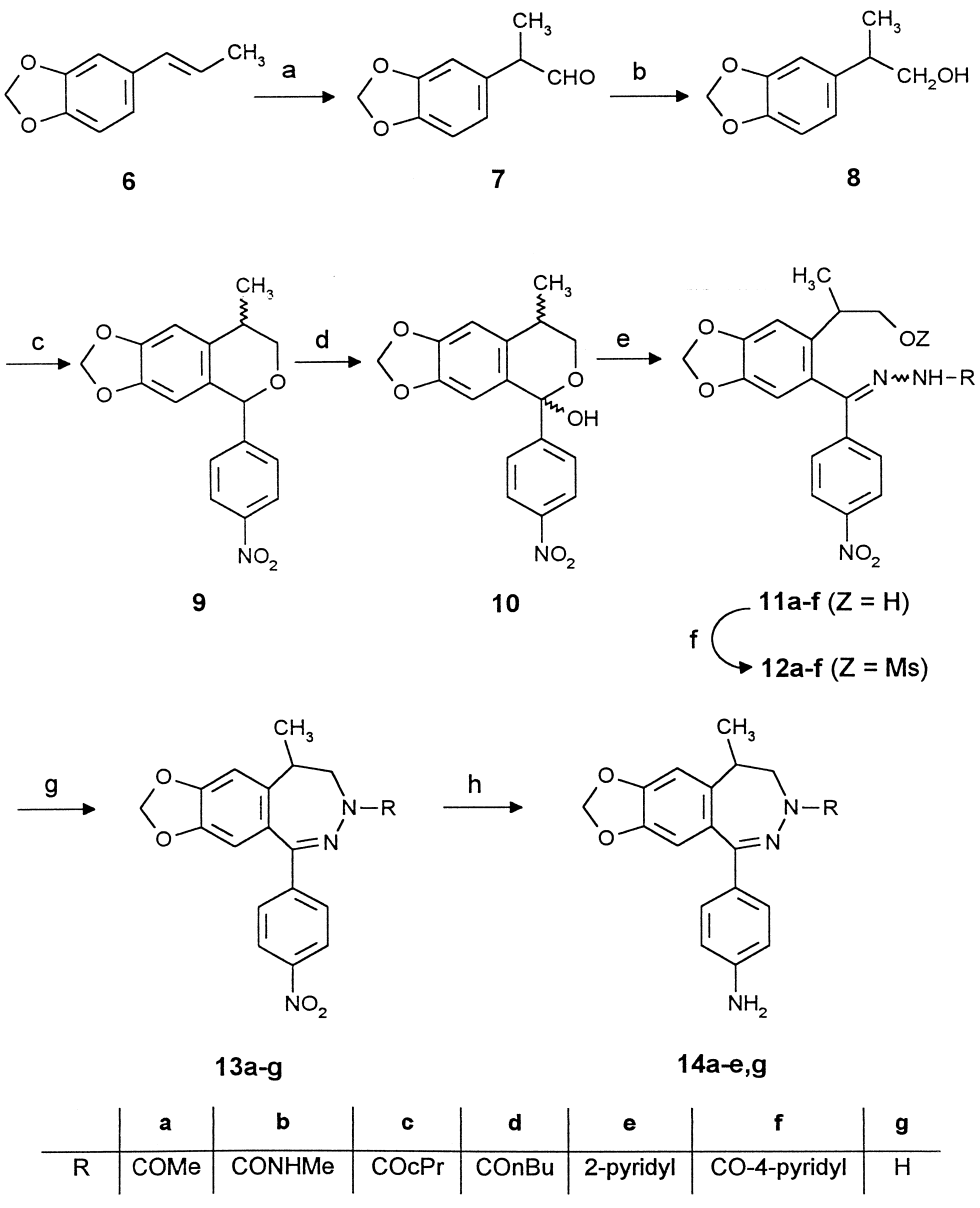


Figure 1.



	a	b	c	d	e	f	g
R	COMe	CONHMe	COcPr	COnBu	2-pyridyl	CO-4-pyridyl	H

Scheme 1. (a) I_2, Ag_2O; (b) $NaBH_4$; (c) p-NO_2-C_6H_4-CHO, HCl, benzene; (d) O_2, OH^-; (e) R-$NHNH_2$, cat. HCl; (f) MsCl, Et_3N; (g) OH^-; (h) RaNi, H_2NNH_2.

reduced in a mixture of methanol and dichloromethane by the RaNi/hydrazine methodology[10] to give final products **14a**–**e** (yields vary between 52–81%) and **14g** (68%) respectively.[11]

Biological evaluation and results

The structure–activity relationships of the new compounds were investigated in vivo and in vitro. The compounds were examined in vitro for inhibition of kainate (5 μM) and/or AMPA (5 μM) evoked spreading depression in isolated retina prepared from young chicken.[12] Selected compounds (**14a** and **14b**) were also investigated for inhibition of AMPA (5 μM) induced whole-cell currents in freshly isolated cerebellar Purkinje cells.[13] Table 1 shows that **14a** and **14b** had comparable potencies to **1** (GYKI 52466). Compound **14b** (20 μM) was further tested for inhibition of whole cell currents induced by 1, 10 and 20 μM AMPA and no difference was seen in its inhibitory potency compared with that of 5 μM AMPA, indicating a non competitive mode of action (data not shown).

The molecules were tested for anticonvulsant activity using the maximal electroshock seizure (MES) model[14] and the maximal metrazole seizure model (MMS)[15] and for muscle relaxant activity, using the inclined screen test in mice.[16] The gross behavioural changes were evaluated in mice according to Irwin.[17]

The drugs were administered orally in the seizure models 60 min before testing the electric shock or metrazole, and intraperitonally (ip) 30 min before testing in the muscle relaxation model. For the Irwin test they were administered in doses of 100 and 200 mg/kg ip and po, respectively. The ED_{50} values were determined by the Lichtfield–Wilcoxon method.[18] As shown in Table 1, the efficacies of **14b** in all in vivo assays were similar to those of **1**. This compound was selected for testing in a transient middle cerebral artery occlusion (MCAO) model in rats.[19,20] The middle cerebral artery was occluded for 60 min by use of an intraluminal suture technique, with reperfusion for 24 h following removal of the occluding filament. Neuronal damage was determined by measurement of the area of necrotic tissue following 2,3,5-triphenyl-tetrazolium chloride (TTC) staining of sections taken from the ischemic region. Figure 2 shows that **14b** provided significant neuroprotection in the MCAO model in rats. In independent experiments, compounds **1** and (−)**2** also had protective effect in the dose of 1 and 2 mg/kg iv, respectively.

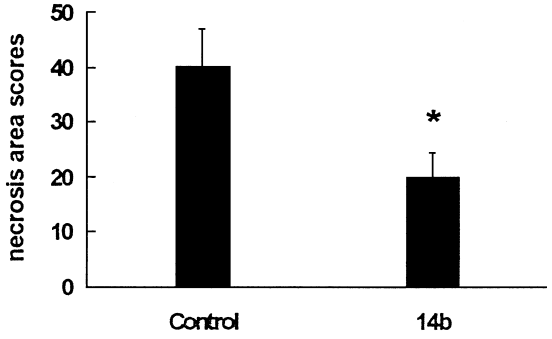


Figure 2. Neuroprotective effect of **14b** against MCAO in rats. **14b** was administered 6 times in every 30 min after occlusion in a dose of 2 mg/kg iv $*p < 0.01$, significance was calculated by unpaired Student's test.

The pharmacological results revealed that 3-acylated 1-(4-aminophenyl)-5-methyl-7,8-methylenedioxy-4,5-dihydro-3*H*-2,3-benzodiazepines had a somewhat lower biological activity than the corresponding 4-methyl analogues,[2] but some of these compounds retained reasonable in vitro and in vivo AMPA antagonistic potencies. **14a** Inhibited kainate response at lower concentration than AMPA response in the retinal spreading

Table 1. Screening results with (±)-5-methyl-7,8-methylenedioxy-4,5-dihydro-3*H*-2,3-benzodiazepines[a]

Compd no.	Behav. changes (100 mg/kg ip; 200 mg/kg po)	Retinal spreading depr. test IC_{50} (μM)[b]	Whole-cell current inhibition 5 μM AMPA	MES test ED_{50}, mg/kg, po	MMS test ED_{50}, mg/kg, po	Incl. screen test ED_{50}, mg/kg ip
1	Loss of righting reflex	A: 6.3[b] K: 9.5	IC_{50} = 9.9 μM	37.4 (29.2–47.5)	119.8 (108.5–132.3)	47.1 (44.2–50.2)
(−)2	Loss of righting reflex	A: 1.7 K: 2.6	IC_{50} = 2.5 (μM)	8.6 (7.0–10.6)	16.8 (10.2–27.6)	13.4 (11.2–16.0)
14a	Adynamia	A: ∼20 K: 3.4	5 μM: 54.0 (%)	69.3 (49.3–97.4)	135.3 (105.3–173.8)	95.3 (76.2–119.1)
14b	Loss of righting reflex	A: 15.6 K: ∼20	5 μM: 52.7 (%)	48.8 (41.0–57.9)	67.6 (55.6–82.2)	55.9 (46.9–66.7)
14c	Ø	A: > 20	NT	>100	NT	>200
14d	Ø	A: > 20	NT	>100	NT	>200
14e	SMA↓[c], ataxy, mild muscle relaxation	A: >20	NT	>100	NT	>200
14g	SMA↑, ataxy, stereotypia, licking, muscle rigidity	NT[d]	NT	47.1 (40.5–54.7)	NT	100–200

[a]The **14g** derivative, without acyl group in position 3, caused behavioural excitation, therefore it was not examined in retinal spreading depression test. This feature resembled that of another subclass of 2,3-benzodiazepines, such as GYKI 52895,[21] which had DA uptake inhibitory property but had no AMPAantagonistic potency (IC_{50} >200 μM).
[b]A, AMPA K, kainate.
[c]SMA↓: decrease of spontaneous motor activity; SMA↑, increase of spontaneous motor activity.
[d]NT, not tested.

depression test, suggesting a preferential action to kainate receptors. However, the compound inhibited AMPA induced whole cell currents in cerebellar Purkinje cells at 5 μM, thus the clarification of the issue of kainate receptor specificity needs further experiment. **14a** and **14b** showed a considerable activity in the in vitro tests. However, they were relatively less effective in the in vivo tests, compared with (−)**2**. This discrepancy may reflect the slightly different pharmacokinetic profiles of the compounds.

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11. Target compounds were characterized by IR and NMR as well as by MS methods. Melting points (°C) are as follows: **14a** (201–203), **14b** (185–187), **14c** (231–233), **14d** (176–178), **14e** (195–197), **14g** (108–110).
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