Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2127–2143

BIOORGANIC & MEDICINAL CHEMISTRY

New Non Competitive AMPA Antagonists

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Received 13 April 2000; accepted 24 May 2000

Abstract—New halogen atom substituted 2,3-benzodiazepine derivatives condensed with an azole ring on the seven membered part of the ring system of type **3** and **4** as well as **5** and **6** were synthesized. It was found that chloro-, dichloro- and bromo-substitutions in the benzene ring and additionally imidazole ring condensation on the diazepine ring can successfully substitute the methylenedioxy group in the well known molecules GYKI 52466 (**1**) and GYKI 53773 (**2**) and the 3-acetyl-4-methyl structural feature in **2**, respectively, preserving the highly active AMPA antagonist characteristic of the original molecules. From the most active compounds (**3b**,**i**) **3b** (GYKI 47261) was chosen for detailed investigations. **3b** revealed an excellent, broad spectrum anticonvulsant activity against seizures evoked by electroshock and different chemoconvulsive agents indicating a possible antiepileptic efficacy. **3b** was found to be highly active in a transient model of focal ischemia predictive of a therapeutic value in human stroke. **3b** also reversed the dopamine depleting effect of MPTP and antagonized the oxotremorine induced tremor in mice indicating a potential antiparkinson activity.

Introduction

Excessive activation of the glutamate receptors may be a major factor in the pathologenesis of several acute and chronic neurological disorders.[1] Attention has been focused to identify selective antagonists according to the types of the ionotropic glutamate receptors classified according to their exogenous ligands *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-methylisoxazol-4-yl)propionic acid (AMPA) and kainic acid.[1c] Selective AMPA antagonists have gained special importance recently.[2] Among them the noncompetitive antagonists exerting their effect at an allosteric site of the receptor are of particular interest because of the theoretical possibility that they are effective even at extraordinarily high levels of the natural transmitter glutamate, as well.[2] Some of the potential therapeutic targets of these antagonists may be epilepsy, spasticity, pain, and neurodegenerative disorders.[3]

The 2,3-benzodiazepine derivative GYKI 52466 (**1**) was the prototype of the noncompetitive AMPA antagonists[4] (Fig. 1). From our structure–activity relationship study GYKI 53773 (**2**, LY300164, talampanel), a highly active AMPA antagonist emerged from the 3-acylated 3,4-dihydro analogues of **1**,[5] which is now one of the clinically most advanced agents among the non competive AMPA antagonists.[6] Further structure–activity relationship studies revealed several structural features which are important to maintain the potent AMPA antagonistic character of the original molecules **1** and **2**.[7] We have found that halogen atoms in the benzene ring can successfully substitute the dioxolane ring in **1** and **2**[7d] and the biological activity was also retained when the 3-acyl-4-methyl substitution pattern in **2** was replaced by some nitrogen containing heterocycles attached to the 3,4-positions of the 2,3-benzodiazepine ring system.[7b] In this paper we report the synthesis and preliminary pharmacological studies of compounds **3**–**6**, where halogen atom(s) and condensed nitrogen containing five membered heterocycles were simultaneously applied to the 2,3-benzodiazepine ring system.[7e]

Chemistry Results

One of the key steps in the synthesis of new 2,3-benzodiazepines with halogen atoms in the benzene ring is the

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PII: S0968-0896(00)00133-4

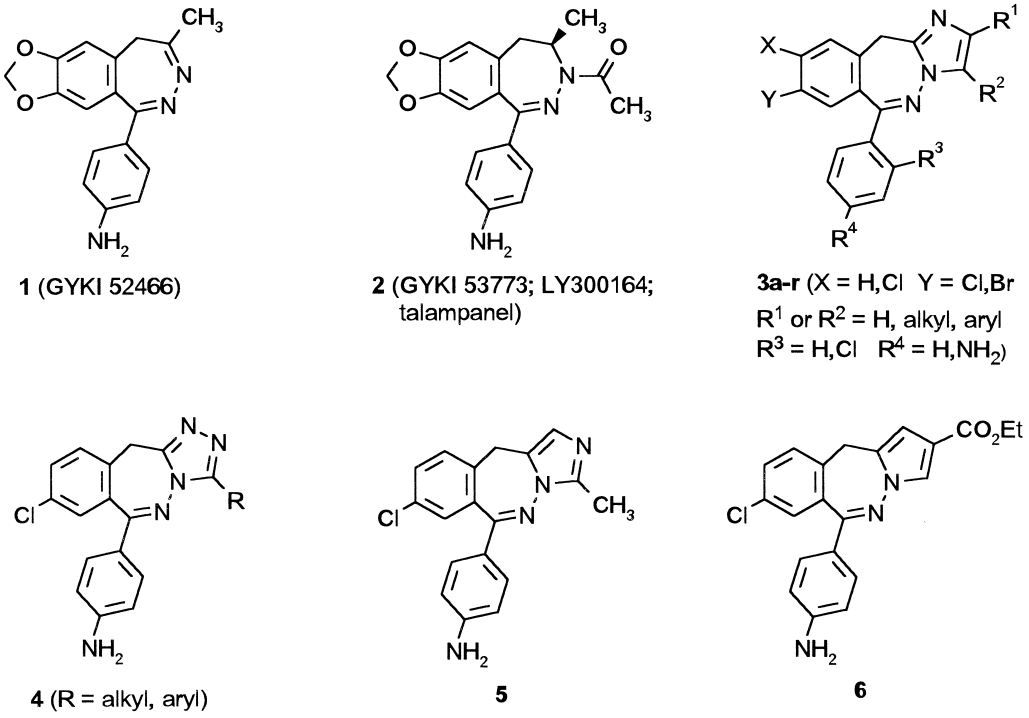


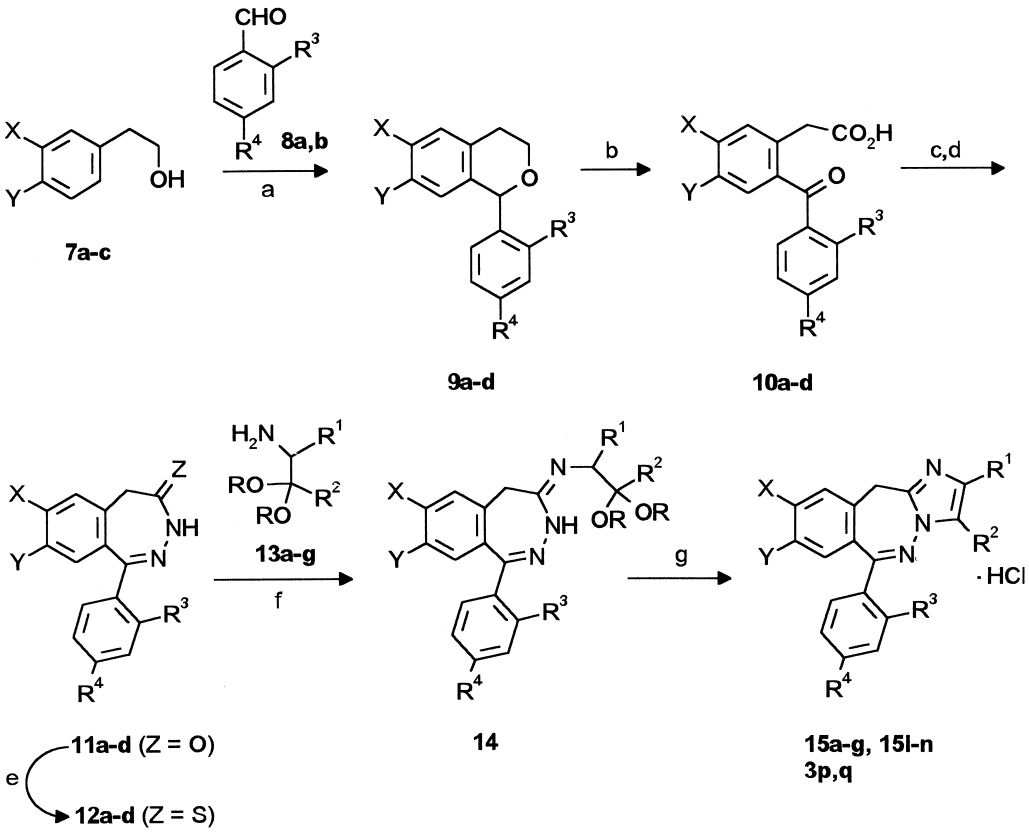
Figure 1.

formation of isochromanes **9a–d** from the corresponding alcohols and an aldehyde (Scheme 1). While the same reaction can be easily performed in the case of alcohols with electron donating groups, like, e.g. with 2-(3,4-dimethoxyphenyl)- or 2-(3,4-methylenedioxyphenyl)ethanol or the corresponding isopropanols and 4-nitrobenzaldehyde using only slightly more than one equivalent of concd hydrochloric acid,[8] the analogous reactions with **7a–c** need anhydrous conditions in benzene using freshly molten zinc chloride and dry hydrogen chloride gas and even then the yields are moderate. But on the other hand the enhanced electrophilicity of the reacting benzaldehyde derivative must contribute to the success of the reaction as well, since, e.g. under the same reaction conditions only negligible reaction could be noticed between **7a** and unsubstituted benzaldehyde. It was also found e.g. with the less electrophilic **8b** that apart from the unknown steric contribution of the *ortho* substituent to the reaction the corresponding isochromane **9d** formed with significantly lower yield than the analogous **9b** in the reaction between **7b** and **8a**.

The reaction of isochromanes **9a–d** with Jones reagent in acetone provided ketocarboxylic acids **10a–d**. The latter were reacted with excess hydrazine hydrate in ethanol to give the intermediate hydrazones which were treated in turn with dicyclohexylcarbodiimide to achieve ring closure. Another possibility to induce ring closing reaction was the treatment of the intermediate hydrazone with excess hydrochloric acid for longer time to provide the 4-oxo-2,3-benzodiazepine derivative, e.g. **11a**.

In order to enhance the reactivity of the 4-oxo compounds **11a–d** towards condensation reactions to build up the expected imidazolo- or triazolo-2,3-benzodiazepines, we have chosen the corresponding thiooxo derivatives **12a–d**, which were prepared from **11a–d** by phosphorous pentasulfide in dry pyridine with acceptable yields.[9] The latter were reacted further with the known aminoacetals **13a–g**. This condensation step was most successful when the reactants were heated in 2-methoxyethanol using red mercury oxide as the sulfur binding agent. The resulting intermediates of type **14** were generally purified by column chromatography and then further reacted with hydrochloric acid to give the imidazolo compounds **15a–g** and **15i–n** as well as **3p,q**, respectively. The nitro groups in benzodiazepines **15a–n** were reduced by a standard method using hydrazine hydrate and RaNi to give products **3a–n**.[10] Because of solubility considerations the reductions were carried out generally in a mixture of methanol and dichloromethane. As imidazolo-benzodiazepines with unsubstituted phenyl ring can hardly be synthesized by the above route, because of the difficulties concerning the corresponding isochromane formation, this type of compound was prepared by removal of the amino group, e.g. in **3c** by reaction with isoamyl nitrite and 5n hydrochloric acid to provide **3r**.[11] In one instance the aromatic amino group of **3b** was acetylated to give **3o** as one of the potential metabolites. The prepared imidazolo compounds are listed in Table 1.

When **3b** as one of the biologically most interesting compounds had to be prepared in multigram scale we became interested to avoid some disadvantages of the synthetic route outlined in Scheme 1. These were the 5 step synthesis of the acetal **13b** from alanine and the optional but useful column chromatography of the intermediates of type **14**. It was attractive to make use of a relatively scarcely used 4 + 1 cyclization method to prepare the imidazole ring, where an acylamidoketone is



7	a	b	c
X	H	H	Cl
Y	Cl	Br	Cl

8	a	b
R^3	H	Cl
R^4	NO_2	H

9-12	a	b	c	d
X	H	H	Cl	H
Y	Cl	Br	Cl	Br
R^3	H	H	H	Cl
R^4	NO_2	NO_2	NO_2	H

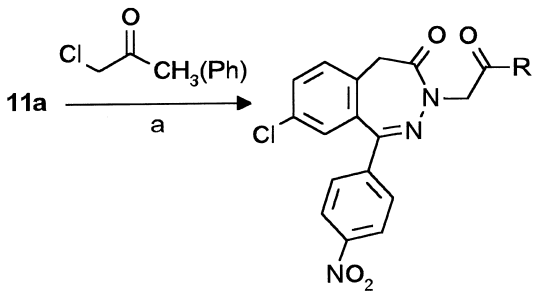
13	a	b	c	d	e	f	g
R^1	H	Me	H	Me	Et	H	H
R^2	H	H	Me	Me	H	4-NO_2-C_6H_4-	4-pyridyl
R+R	CH_2CH_2	CH_2CH_2	CH_2CH_2	CH_2CH_2	CH_2CH_2	Et, Et	Et, Et

3/15	a	b	c	d	e	f	g
X	H	H	H	H	H	H	H
Y	Cl	Cl	Cl	Cl	Cl	Cl	Cl
R^1	H	Me	H	Me	Et	H	H
R^2	H	H	Me	Me	H	4-NH_2-C_6H_4-/4-NO_2-C_6H_4-	4-pyridyl
R^3	H	H	H	H	H	H	H
R^4	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2

3/15	i	j	k	l	m	n
X	Cl	Cl	H	H	H	H
Y	Cl	Cl	Br	Br	Br	Br
R^1	Me	H	Me	H	Me	H
R^2	H	Me	H	Me	Me	4-pyridyl
R^3	H	H	H	H	H	H
R^4	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2	NH_2/NO_2

3	o	p	q	r
X	H	H	H	H
Y	Cl	Br	Br	Cl
R^1	Me	Me	H	H
R^2	H	H	Me	Me
R^3	H	Cl	Cl	H
R^4	NHCOMe	H	H	H

Scheme 1. Route I. (a) benzene, $ZnCl_2$, HCl(g); (b) acetone, CrO_3/H_2SO_4; (c) EtOH, $H_2NNH_2{\cdot}H_2O$, Δ; (d) DCC or HCl; (e) pyridine, P_2S_5, 80 °C; (f) 2-methoxyethanol, red HgO, Δ; (g) HCl, Δ; (h) MeOH-CH_2CL_2, RaNi, $H_2NNH_2{\cdot}H_2O$; (i) DMF, isoamylnitrite, 65 °C; (j) pyridine/AcCl, 5 °C.



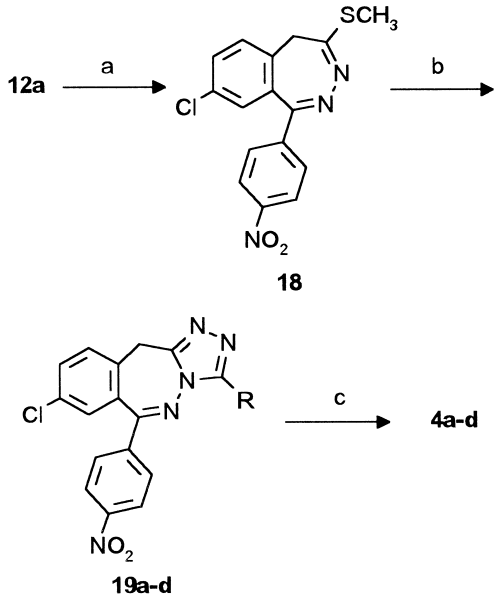
15	b	h
X	H	H
Y	Cl	Cl
R^1	Me	Ph
R^2	H	H
R^3	H	H
R^4	NO_2	NO_2

Scheme 2. Route II. (a) DMF, K_2CO_3; (b) NH_4OAc, AcOH, Δ.

reacted with ammonium acetate as nitrogen atom donor in acetic acid.[12] The necessary amidoketone **16** was prepared in very good yield by alkylation of **11a** with chloroacetone (Scheme 2). We have found that condensation of **16** to give the corresponding imidazolo-benzodiazepine **15b** needs a large excess (>50 equivalents) of ammonium acetate in acetic acid and the yield is modest (40%). The use of other ammonium salts like formate or carbonate as well as solvents, e.g. formamide, propionic acid, trifluoroacetic acid or microwave techniques instead of conventional heating did not give significant improvement. Despite the lower yield of the imidazole forming step, **3b** can be produced by this route without any laborious synthetic steps. We attribute the lower yield of this condensation step to the sensitive nature of the acetone side chain in **16** towards reactions, since with the phenacyl derivative **17** the same condensation could be performed with a significantly better yield (82%). (Scheme 2.)

For the formation of the triazolo ring in compounds **4a–d** acylhydrazides were used (Scheme 3).[13] Better results could be achieved in the condensation steps when instead of thione **12a** the corresponding 4-methylthio-2,3-benzodiazepine **18** was used and additionally a catalytic amount of hydrochloric acid was applied, as well. The nitro groups in compounds **19a–d** were reduced by the standard RaNi-hydrazine hydrate method and the resulting compounds **4a–d** are shown in Table 2.

To build up the 11*H*-imidazolo[3,4-c][2,3]benzodiazepine ring system in **5** we started from **23** which was synthesized on the analogy to well established methods (Scheme 4).[14] The methyl group in **23** was oxidized by selenium dioxide to form the corresponding aldehyde **24**, which was then reduced to the alcohol **25** by sodium borohydride. The



4/19	a	b	c	d
R	Me	4-pyridyl	4-NH_2-C_6H_4- /4-NO_2-C_6H_4-	CH_3O-CH_2-

Scheme 3. (a) acetone, MeJ, K_2CO_3; (b) DMF, acylhydrazine, cat. HCl; (c) MeOH-CH_2Cl_2, RaNi, $H_2NNH_2{\cdot}H_2O$.

Table 1. 11H-Imidazolo[1,2-c][2,3]benzodiazepine derivatives (**3a–r**)

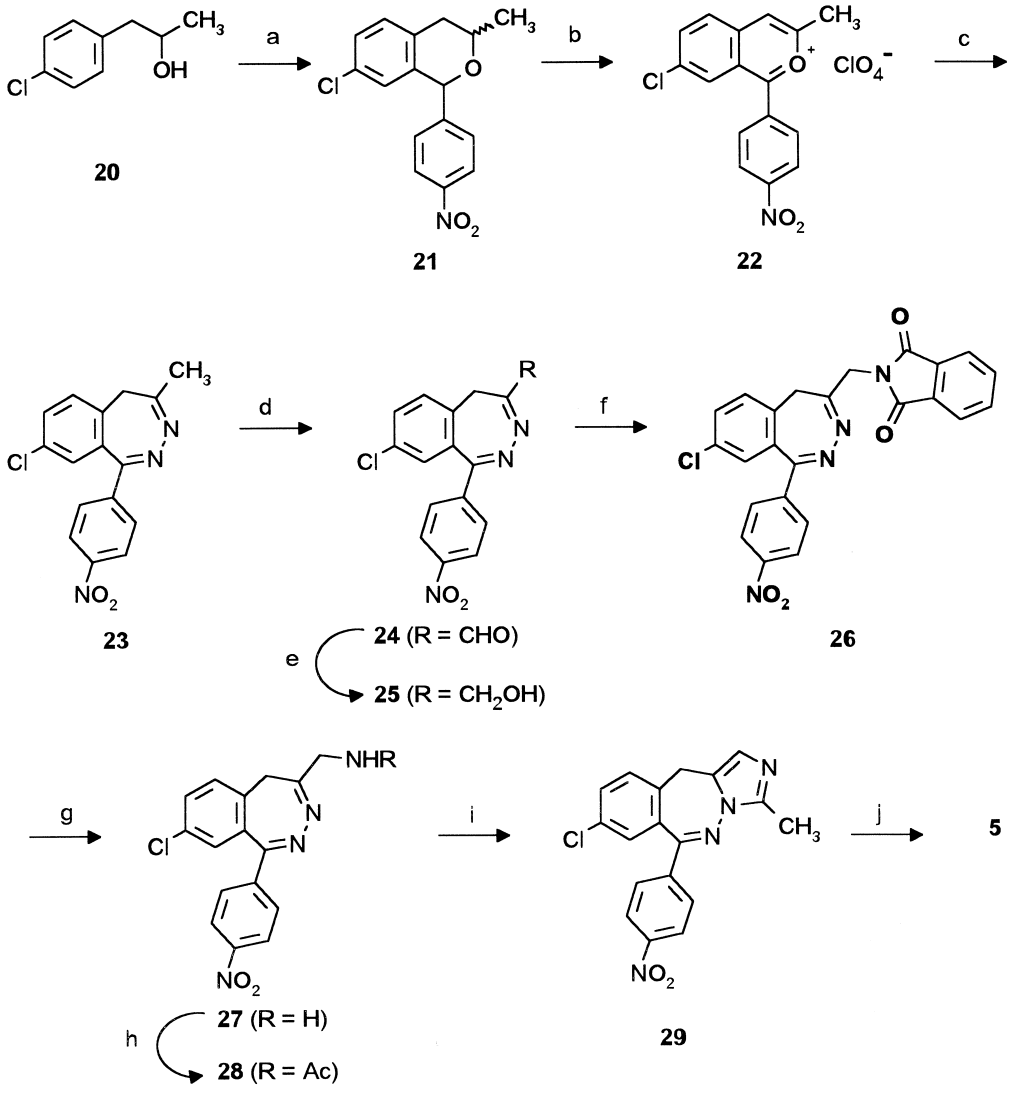
Compound	X	Y	R^1	R^2	R^3	R^4	mp (°C)	Yield (%)[a]
3a	H	Cl	H	H	H	NH_2	210–214	67
3b	H	Cl	Me	H	H	NH_2	229–230	79
3c	H	Cl	H	Me	H	NH_2	267–270	71
3d	H	Cl	Me	Me	H	NH_2	274–278	83
3e	H	Cl	Et	H	H	NH_2	247–250	72
3f	H	Cl	H	4-NH_2-C_6H_4—	H	NH_2	250–253	84
3g	H	Cl	H	4-pyridyl	H	NH_2	293–294[b]	68
3h	H	Cl	Ph	H	H	NH_2	223–226	81
3i	Cl	Cl	Me	H	H	NH_2	254–255	40
3j	Cl	Cl	H	Me	H	NH_2	284–286	71
3k	H	Br	Me	H	H	NH_2	248–251	64
3l	H	Br	H	Me	H	NH_2	263–268	77
3m	H	Br	Me	Me	H	NH_2	272–275	84
3n	H	Br	H	4-pyridyl	H	NH_2	295–300[b]	41
3o	H	Cl	Me	H	H	NHCOMe	265–266	63
3p	H	Br	Me	H	Cl	H	132–140[c]	21
3q	H	Br	H	Me	Cl	H	210–215[c]	48
3r	H	Cl	H	Me	H	H	166–169	39

[a]Yields refer to the last step of the synthesis.
[b]Decomposition.
[c]Hydrochloride salts.

Table 2. 6-(4-Aminophenyl)-8-chloro-11H-1,2,4-triazolo[4,5-c][2,3] benzodiazepine derivatives (**4a–d**)

Compound	R	mp (°C)	Yield (%)[a]
4a	Me	228–231	91
4b	4-pyridyl	284–288	92
4c	4-NH_2-C_6H_4—	191–193	85
4d	CH_3OCH_2—	195–197	83

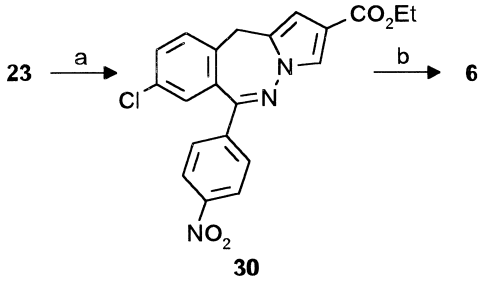
[a]Yields refer to the last step of the synthesis.



Scheme 4. (a) 4-nitrobenzaldehyde, benzene, $ZnCl_2$, HCl(g); (b) acetone, CrO_3/H_2SO_4, then 70% $HClO_4$; (c) DMF, $H_2NNH_2{\cdot}H_2O$; (d) dioxane, SeO_2; (e) THF-H_2O, $NaBH_4$; (f) THF, Ph_3P, phthalimid, DEAD; (g) MeOH, $H_2NNH_2{\cdot}H_2O$; (h) Ac_2O; (i) $ClCH_2CH_2Cl$, $POCl_3$, Δ; (j) MeOH-CH_2Cl_2, RaNi, $H_2NNH_2{\cdot}H_2O$.

latter was transformed under Mitsunobu conditions[15] with phthalimide into **26** then hydrazinolysis and acetylation gave **27** and **28,** respectively. Cyclization of **28** with phosphorus oxychloride gave **29** which was reduced by RaNi-hydrazine hydrate to give **5**.

The pyrrolo derivative **6** was prepared from **23** by using a combined alkylation-condensation procedure[16] with ethyl bromopyruvate to give the nitro compound **30** which on reduction provided **6** (Scheme 5).



Scheme 5. (a) Br-CH_2-C(O)-CO_2Et, EtOH, Δ; (b) MeOH-CH_2Cl_2, RaNi, $H_2NNH_2{\cdot}H_2O$.

Biological Results and Discussion

Primary pharmacological testing and structure–activity relationships

Table 3 contains primary in vitro and in vivo biological data with the newly synthesized compounds in comparison with the standard molecules **1** (GYKI 52466) and **2** (GYKI 53773, LY300164, talampanel).

Inhibition of AMPA-, or kainate-triggered spreading depression in isolated chicken retina. Excitation of isolated chicken retinas by glutamate receptor agonists provokes spreading depression (SD), which is accompanied by a characteristic change of the light scattering properties of the preparation. The phenomenon is easily visible by unaided eye, thus it is a convenient method for drug

Table 3. Screening results of the condensed 2,3-benzodiazepine derivatives **3–6**[a]

Compond	Behavioural changes (100 mg/kg ip; 200 mg/kg po)	Retinal spreading depr. IC_{50} (μM)	MES ED_{50}, mg/kg po	Inc. screen ED_{50}, mg/kg ip
1	Loss of righting reflex	A: 6.3 K: 9.5	37.4	47.1
2	Loss of righting reflex	A: 1.7 K: 2.6	8.6	13.4
3a	Short loss of righting reflex	A: 6.5 K: 3.8	61.6	47.1
3b	Loss of righting reflex	A: 7.3 K: 2.5	24.0	36.5
3c	Ataxia, muscle relaxation, po: loss of righting reflex	A: 4.1 K: 0.5 (flat curve)	61.3	100-125
3d	Ataxia	A: 11.8 K: 1.5	~100	> 200
3e	Loss of righting reflex, ataxia, SMA↓	A: > 20 K: > 20	44.6	> 150
3f	SMA↓, ataxia	A: 4.3 K: 7.1	> 100	~200
3g	SMA↓, ataxia	A: 3.1 K: n.t.	> 100	> 200
3h	∅	A: > 20 K: n.t.	> 100	> 200
3i	Loss of righting reflex	A: 3.1 K: 1.9	24.0	47.2
3j	po: SMA↓, ataxia	A: > 20 (0.6–10 μM: max. 47% inhibition) K: 1.1	50–100	> 200
3k	Loss of righting reflex	A: 9.3 K: 2.2	40.3	68.4
3l	SMA↓, ataxia, weak muscle relaxation	A: 4.0 K: 1.3	~100	~200
3m	SMA↓, ataxia	A: > 20 K: 7.4	~100	> 200
3n	po: SMA↓	A: 3.2 K: > 20	> 100	> 200
3o	SMA↓, ataxia	A: > 20 K: > 20	56.0	> 200
3p	ip: Weak SMA↓	A: > 20 K: > 20	> 100	> 200
3q	ip: SMA↓	A: > 20 K: > 20	> 100	~200
3r	ip: Weak SMA↓	A: > 20 K: > 20	> 100	> 200
4a	Ataxia, weak muscle relaxation	A: 2.9 K: 4.1	~100	~100
4b	∅	A: > 20 K: ~20	> 100	> 200
4c	∅	A: > 20 K: 1.0	> 100	> 200
4d	SMA↓, ataxia	A: 9.2 K: 5–10	> 100	~150
5	ip: SMA↓, ataxia	A: > 20 K: n.t.	~100	> 200
6	100 ip.: ∅	A: > 20 K: n.t.	> 100	~200

[a]SMA↓: decrease of spontaneous motor activity; A: AMPA; K: kainate; n.t.: not tested.

testing. Retinal SD can be blocked by glutamate antagonists.[17] In our study the majority of the tested compounds were effective in the retinal spreading depression (SD) test, i.e. blocked either AMPA- or kainate induced SD with an IC_{50} lower than 10 μM. The IC_{50} values of the most potent compounds were similar to those of **1** or **2** in the AMPA test, and lower in the kainate test. Compounds **3b,d,j,k,l,m** seemed to show some selectivity towards kainate responses, i.e. their IC_{50} values were more than 2-fold higher against AMPA than against kainate. The concentration-response curves of **3b** against the two agonists are shown in Fig 2. Interestingly, some compounds such as **3e**, **3m**, or **4d** caused only partial inhibition of kainate-triggered SD (maximal inhibition: 40–60% at 20 μM; not shown), or had a flat dose-response curve (**3a**, **3c**, or **3i**; not illustrated). The significance of the differences between AMPA and kainate antagonistic actions in the case of some compounds is difficult to tell, as the role of the specific kainate receptors in triggering SD in this preparation has not been characterized yet. It is to be noted here that the competitive AMPA antagonist 1,2,3,4-terahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) also had a flat dose-response curve against kainate in this model.[17] Regarding the dose-response curves in the AMPA-induced spreading depression test, the curves were usually steeper than in the kainate test, except for **3j** which only partially depressed AMPA response (maximum inhibition: 45% at 2.5 μM).

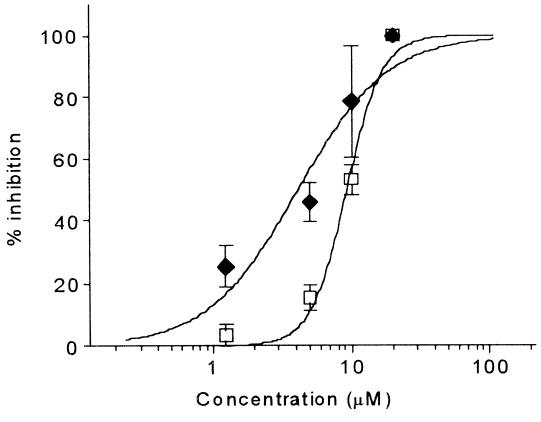


Fig. 2. Inhibition of kainate- and AMPA induced spreading depression in isolated chick retina by **3b**. □ 5 μm AMPA; ◆ 5 μm kainate.

Behavioral changes, effects of the compounds in the maximal electroshock (MES) test, and in the inclined screen test. Most compounds elicited some gross behavioral effects after ip (100 mg/kg) or po (200 mg/kg) application, indicating a reasonable absorption and penetration through the blood brain barrier. These included a decrease in motility, ataxia, muscle weakness and even a loss of righting reflex with the most effective substances. These behavioral symptoms have also been observed with other 2,3-benzodiazepine compounds[5a] and can be regarded as the consequence of the blockade of central AMPA receptors function.

AMPA receptor blockade also results in an anticonvulsant action.[2,5a] MES test has been found a suitable method for establishing in vivo structure–activity relationships among another group of 2,3-benzodiazepine

AMPA antagonists.[5a] The two most effective compounds of the present series were **3b** and **3i**, both with an ED_{50} of 24 mg/kg po, which is between the ED_{50} values of **1** and **2**.

The ataxia inducing or muscle relaxant action was quantified in the inclined screen assay. The ED_{50} values of the compounds in this test (30 min ip pretreatment) were in general slightly higher than in the MES test (60 min oral pretreatment). A bigger gap between the anticonvulsant and motor impairment inducing doses would be expected if identical treatment schedules were used. This MES preference does not hold true in respect of **3a**, which was more potent in the muscle relaxant assay. A possible explanation for this discrepancy is that the compound seems to have an extremely short duration of action (probably due to a rapid metabolism in mice), and 60 min after the treatment its blood concentration decreases to a level that can not protect the animal from MES. Another possible explanation is that—in contrast to most of the 2,3-benzodiazepines[3]—**3a** has a poor absorption from the intestines.

Correlating the in vitro and in vivo pharmacological data with the structural features of the new compounds the following relationships can be established. Applying chloro-, dichloro- or bromo-substitution instead of the methylendioxy group of molecules **1** and **2** and substituting simultaneously the hydrogen bond acceptor type acetyl group of **2** by a condensed nitrogen containing five membered heterocycle preserve the high AMPA antagonist character of the original molecules. Even among the bromo compounds we found a compound (**3k**) with significant activities.

Concerning the condensed heterocycles optimum efficacy was found among the imidazolo[1,2-c][2,3]benzodiazepines of type **3**. Although most of the triazolo-derivatives **4a**–**d** showed acceptable in vitro efficacy, the additional hydrogen bond acceptor atom in the condensed ring may have prevented a proper absorption resulting in an in vivo inactivity. With pyrrolo-derivative **6**, where no hydrogen bond possibility exists in a similar way as it may occur for molecules **3**, **4** and **2** or where the acceptor nitrogen atom is probably at a wrong place (e.g. imidazolo[3,4-c][3,4]benzodiazepine derivative **5**) neither in vitro nor in vivo effects were found.

Within the imidazolo-derivatives **3** the optimum efficacy was found among the compounds substituted with little aliphatic groups (**3b,c,e,i,k**). Bigger aryl substitutions resulted in inactive or nearly inactive compounds, e.g. **3f,g,h,n**, whereas methyl substitution in position 3 seems to enhance kainate selectivity, e.g. **3c,d,j,l**. But double methyl substitution, e.g. in **3d** and **3m**, resulted in loss of in vivo activity, which may be explained by a worsened ADME profile of these derivatives. It is worth mentioning that **3e**, a homologue of one of the most active substances (**3b**), is inactive in vitro but still has a reasonable anticonvulsant activity. Formation of biologically active metabolites may underlie this discrepancy, which is supported by the observation that the compound was ineffective in the inclined screen assay, where a shorter ip pretreatment was applied. Similar metabolic activation was described among other 2,3-benzodiazepine AMPA antagonists.[5a]

Similarly to earlier 2,3-benzodiazepine type AMPA antagonists[5a,7] the efficacy of the present compounds is also generally restricted to the 4-aminophenyl derivatives (see e.g. the dezamino compounds **3p,q,r)**, and even an acetylation of the amino group (e.g. in **3o**) decreases biological activity.

On the basis of the screening results **3b** and in certain cases additionally **3i** were chosen for more detailed and comparative investigations.

Inhibition of AMPA-, or kainate-induced whole-cell currents by 3b in isolated neurons. The AMPA receptor antagonistic effect of **3b** in freshly isolated cerebellar Purkinje cells is shown in Fig. 3. In this preparation **3b** is nearly equiactive with **2** and more potent than **1**. Its IC_{50} against AMPA (2.3 μM) was somewhat lower than against kainate (4.5 μM, not shown). However, under our experimental conditions most current elicited by kainate was probably mediated by AMPA receptors. Thus these data are not suitable for analyzing a possible subtype selectivity of the compound. The current blocking action of **3b** (10 μM) against 5 μM AMPA developed slowly and the recovery from the blockade took several seconds (τ_{on} and τ_{off} of the current trajectories were 4.2±0.8 and 5.2±0.7 sec, respectively), suggesting considerably slower binding and unbinding kinetics than those of **1** and the racemate of **2** (τ_{on} and τ_{off} values below 1 sec).[18] To assess the biological significance of this feature of **3b** warrants further studies. Just as **1** or **2**,[18,19] **3b** acted in a non-competitive way, as its current blocking action was not dependent on the agonist concentration (not shown).

Anticonvulsant profile of selected 2,3-benzodiazepines. The broad-spectrum anticonvulsant activity of **1** and **2** has already been described.[3,5a] Table 4 illustrates that the anticonvulsant profile of **3b** and **3i** is similar to the former compounds. Their potencies were usually between those of **1** and **2**. It is notable, however, that both newly synthesized compounds protected mice from

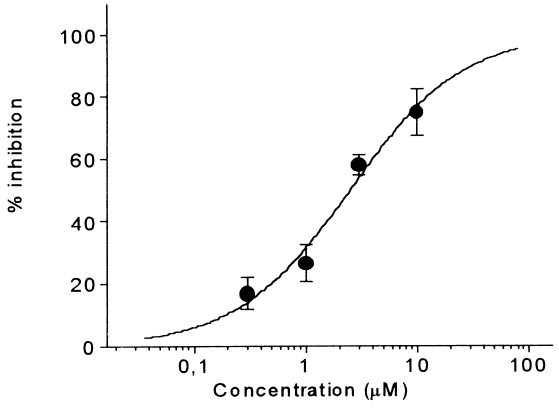


Fig. 3. Inhibition of AMPA induced whole cell current in freshly isolated cerebellar Purkinje cells by **3b**.

Table 4. Anticonvulsive effects of selected compounds (**3b** and **3i**) against MES and various chemical convulsants

	ED_{50} mg/kg po			
	1	**2**	**3b**	**3i**
MES	37.4 (29.2–47.5)	8.6 (7.0–10.6)	24.0 (17.9–32.1)	24.0 (17.9–32.1)
Metrazole	119.8 (108.5–132.3)	16.8 (10.2–27.6)	53.8 (43.7–66.2)	62.2 (44.1–87.6)
Strychnine	86.7 (71.7–104.9)	17.4 (10.6–28.4)	67.4 (43.7–66.2)	44.2 (38.5–50.7)
Bemegride	71.9 (62.0–83.4)	23.9 (16.0–35.7)	40.7(21.9–56.9)	39.9 (26.5–60.1)
Bicuculline	35.0 (28.5–43.1)	14.6 (5.8–36.7)	22.8 (16.7–31.2)	22.5 (13.4–37.7)
Nicotine	71.8 (49.8–103.7)	22.7 (16.2–31.9)	13.1 (8.1–21.2)	18.3 (14.2–23.8)
4-aminopyridine	43.0 (39.9–59.8)	8.4 (5.6–12.5)	55.5 (47.1–65.4)	6.7 (3.5–12.7)
3-mercapto-propionic acid	47.0 (32.9–67.2)	17.1 (9.7–30.1)	38.1 (28.7–50.5)	66.8 (43.0–103.1)

nicotine-induced seizures and death more potently than **2** did, which may indicate a potential antiparkinsonian efficacy. Further, **3i** was the most effective of the four compounds in preventing 4-aminopiridine (4-AP)-induced seizures.

Muscle relaxant effect of selected 2,3-benzodiazepines. A common feature among 2,3-benzodiazepine AMPA antagonists is the inhibition of motor functions with a primarily spinal site of action.[3,20] The ataxia/muscle relaxation inducing ED_{50} values of **3b** and **3i** are listed and compared to those of **1** and **2** in the inclined screen and rotarod tests (Table 5).

Antiischemic effect of 3b in a transient focal ischemia model, in rats. The antiischemic property of AMPA antagonists renders them promising drug candidates in various acute and chronic neurodegenerative disorders such as stroke.[1–3] Compound **1** has been shown to protect animals from ischemic damage in various models.[21] The transient focal ischemia model is a relevant stroke model.[22,23] Table 6 shows that **3b**, applied at 2.5–5-fold lower doses, had a considerably more potent effect on infarct size than **1**.

The antiparkinsonian effects of 3b

Oxotremorine induced tremor. The cholinergic agonist oxotremorine, applied systemically, provokes tremor with a central action. Table 7 shows that the 2,3-benzodiazepine compounds **1**, **2** and **3b** potently mitigated the tremor induced by oxotremorine. Salivation, a peripheral effect of oxotremorine, was not affected by these drugs. Since Parkinson's disease is characterized by a disturbed balance between cholinergic and dopaminergic neurotransmission in the basal ganglia, an indirect central anticholinergic effect of compounds **1**, **2** and **3b** may be indicative for a therapeutically suitable antiparkinsonian effect.

Table 6. Effect of **3b** on the size of ischemic damage in rats after transient occlusion of the medial cerebral artery

Comp.	Doses (mg/kg iv)	Infarct size scores (mean ± S.E.)	Decrease (%)
Veh.[a]		48920±4012	
1	6×5	32218±6325*	34.1
Veh.[a]		40518±5928	
3b	6×2	13229±2313*	67.4
Veh.[a]		42795±7513	
3b	6×1	16122±4368*	62.3

[a]Vehicle. * $P < 0.01$ Significance was calculated by one-way ANOVA followed by the Duncan test. Treatment schedule: first injection 30 min after occlusion and repeated 5 times in every 30 min.

Table 7. Antagonism of oxotremorine induced tremor in mice

Oxotremorine antagonism ED_{50} mg/kg po		
1	**2**	**3b**
20.5 (14.9–28.3)	5.6 (3.6–8.5)	16.8 (12.0–23.6)

***N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine·HCl (MPTP) neurotoxicity.** Degeneration of dopaminergic neurons in Parkinsons disease is well known.[24] Recent findings indicate that impairment of striatal dopaminergic neurotransmission results in an overactivity of glutamatergic neurons in the subthalamic nucleus.[25] This glutamatergic overactivity is thought to play an important role in the expression of some parkinsonian symptoms, such as hypokinesia and rigidity as well as in development of neuronal damage.[26] Therefore we tested the effects of **3b** against MPTP-induced neurotoxicity in mice, a relevant rodent model of Parkinsons disease, in comparison with those of **1** and of the competitive AMPA antagonist NBQX.

Table 5. Muscle relaxant effects of selected compounds (**3b** and **3i**) in the inclined screen and rotarod tests, in mice

	ED_{50} mg/kg ip			
Method	**1**	**2**	**3b**	**3i**
Inclined screen	47.1 (44.2–50.2)	13.4 (11.2–16.0)	36.5 (29.4–45.2)	47.2 (41.9–53.3)
Rotarod	24.0 (22.0–26.2)	2.3 (1.6–3.4)	15.8 (13.0–19.3)	13.7 (12.4–15.1)

Table 8. Effects of **3b** on MPTP induced depletion of dopamine and its metabolites in mouse striatum[a]

Treatment					Concentration (μg/g) of		
1 0 min.	2 30 min.	3 150 min.	4 270 min.	5 390 min.	DA	DOPAC	HVA
Vehicle	Vehicle	Vehicle	Vehicle	Vehicle	11.14±0.43	0.54±0.03	0.87±0.05
MPTP	Vehicle	Vehicle	Vehicle	Vehicle	1.07±0.17[b]	0.10±0.04[b]	0.33±0.06[b]
MPTP	**3b**	**3b**	Vehicle	**3b**	4.38±0.89[c]	0.21±0.05	0.45±0.06
MPTP	**1**	**1**	**1**	Vehicle	3.60±0.37[c]	0.27±0.03[d]	0.36±0.03
MPTP	NBQX	NBQX	NBQX	Vehicle	2.74±0.52[d]	0.16±0.04	0.37±0.03

[a]Significance was calculated by one-way ANOVA followed by the Duncan's test.
[b] < 0.01 versus saline treated control group.
[c] $P < 0.01$ versus MPTP treated group.
[d] $P < 0.05$ versus MPTP treated group.

Table 9. Determination of acute toxicity in mice

Compound	LD_{50} mg/kg	
	ip	po
1	302.1 (273.9–333.3)	296.9 (258.6–340.8)
3b	259.4 (205.8–327.1)	372.9 (322.5–431.1)

The effects of 3×20 mg/kg (ip dose) of **3b**, **1** and NBQX on MPTP-induced dopamine (DA) depletion in mouse striatum are shown in Table 8. MPTP injection reduced DA concentration in the striatum by 90% and the levels of DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid, (HVA) were also decreased significantly (Table 8). MPTP administration did not affect the concentrations of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) in the striatum indicating the specific toxic effect of MPTP on dopaminergic neurons (data not shown). **3b** and **1**, when they were injected repeatedly, reversed the MPTP-induced decrease in striatal concentrations and NBQX exhibited similar effect (Table 8). The neuroprotective effects of 2,3-benzodiazepines against MPTP neurotoxicity suggest that non-competitive AMPA receptor antagonists may have a beneficial role in the treatment of Parkinson's disease.[25,26]

Acute toxicity. Compound **3b** caused lethality in mice only at doses several fold higher than its ED_{50} values in the pharmacological assays. Its toxicity is similar to that of **1**. The LD_{50} values of **3b** and **1** were almost the same after ip and oral administrations suggesting a good gastro-intestinal absorption (Table 9).

Conclusion

Among the newly synthesized, with heterocycle condensed and halogen substituted 2,3-benzodiazepine derivatives several non-competitive AMPA antagonists were identified with potencies that approached or exceeded those of the reference compounds **1** or **2**. Some of them also had potent in vivo activity. **3b**, the compound selected for detailed studies, displayed a pharmacological profile largely similar to that of the formerly described 2,3-benzodiazepine AMPA antagonists,[3] but a potent anti-parkinsonian activity was also revealed as an additional feature. The compound showed an excellent, broad spectrum anticonvulsant activity against seizures evoked by electroshock and different chemoconvulsive agents indicating a possible antiepileptic efficacy. **3b** (GYKI 47261) was also found to be highly active in a transient model of focal ischemia predictive of a therapeutic value in human stroke. A significant effect antagonizing the MCAO induced infarct size in rat brain was found in a five times lower dose than for GYKI 52466 (**1**), the representative of the non competitive AMPA antagonists. In addition **3b** reversed the dopamine depleting effect of MPTP and antagonized the oxotremorine induced tremor in mice. These findings indicate that the non-competitive AMPA antagonist **3b** may have therapeutical potential not only in acute but chronic neurodegenerative disorders as well.[27]

Experimental

Chemistry

Melting points were measured on a Boëtius hotstage microscope and are uncorrected. IR (KBr): Bruker IFS-85 spectrophotometer. ^{1}HNMR ($CDCl_3$, internal standard TMS, T = 298°K): Bruker AC 250, other solvents are indicated. Mass spectra: Finnigan MAT 8430 mass spectrometer. Operating conditions: electron ionization, E_{el} = 70eV, I_{el} = 0.5 mA, U_{acc} = 3 kV, R = 1250. FAB: ION-TECH atom gun with Xe, in *m*-nitrobenzyl alcohol matrix. Column chromatography: silica gel, Kieselgel 60, Merck. Satisfactory elemental analyses (±0.4 %) for *C*, *H*, *N* and *S* were obtained for all new described compounds except for **25**–**29** which were not measured this way. The following compounds were prepared according to the literature: **7a**,[28] **7b**,[28] **7c**,[29] **13a**,[30] **13b**,[31] **13c**,[30] **13d**,[30] **13e**,[31] **13f**,[32] **13g**.[32]

General procedure for the synthesis of isochromanes 9a–d. To a stirred solution of the phenylethanol derivative (**7a**–**c**) (0.1 mol) and the corresponding benzaldehyde **8a,b** (0.1 mol) in dry benzene (300 mL) freshly molten and ground zinc chloride (13.6 g, 0.1 mol) was given. Dry hydrogen chloride gas was introduced into the suspension over 4 h and the mixture was stirred overnight. The resulting mixture was then washed with water and a 5% sodium-hydrogensulfit solution. The organic phase was dried and after filtration the solvent evaporated. The residue was recrystallized to give the title products.

7-Chloro-1-(4-nitrophenyl)isochromane (9a). Yield: 56%. Mp 98–101 °C (ethanol). $C_{15}H_{12}ClNO_3$ (289.7). ^{1}H NMR: δ 2.79 (ddd, J_1 = 16.5 Hz, J_2 = J_3 = 3.6 Hz, 4-H) and 3.12 (ddd, J_1 = 16.5 Hz, J_2 = 9.7 Hz, J_3 = 5.6 Hz, 4-H), 3.92 (ddd, J_1 = 11.5 Hz, J_2 = 9.7 Hz, J_3 = 3.6 Hz, 3-H) and 4.21 (ddd, J_1 = 16.5 Hz, J_2 = 5.6 Hz, J_3 = 3.6 Hz, 3-H), 5.75 (s, 1H, 1-H), 6.65 (d, J = 1.9 Hz, 1H, 8-H), 7.13 (d, J = 8.3 Hz, 1H, 5-H), 7.17 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H, 6-H), 7.50 (d, J = 8.8 Hz, 2H) and 8.25 (d, J = 8.8 Hz, 2H, nitrophenyl).

7-Bromo-1-(4-nitrophenyl)isochromane (9b). Yield: 62%. Mp 104–107 °C (ethyl acetate). $C_{15}H_{12}BrNO_3$ (334.2). ^{1}H NMR: δ 2.78 (ddd, J_1 = 16.2 Hz, J_2 = J_3 = 3.6 Hz, 4-H) and 3.12 (ddd, J_1 = 16.2 Hz, J_2 = 9.9 Hz, J_3 = 5.8 Hz, 4-H), 3.93 (ddd, J_1 = 13.5 Hz, J_2 = 9.9 Hz, J_3 = 3.6 Hz, 3-H) and 4.21 (ddd, J_1 = 13.5 Hz, J_2 = 5.8 Hz, J_3 = 3.6 Hz, 3-H), 5.78 (s, 1-H), 6.82 (d, J = 1.9 Hz, 8-H), 7.10 (d, J = 8.2 Hz, 5-H), 7.35 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 6-H), 7.55 (d, J = 8.8 Hz, 2H) and 8.22 (d, J = 8.8 Hz, 2H, nitrophenyl).

7,8-Dichloro-1-(4-nitrophenyl)isochromane (9c). Yield: 30%. Mp 130–132 °C (ethanol). $C_{15}H_{11}Cl_2NO_3$ (324.1). ^{1}H NMR: δ 2.80 (ddd, J_1 = 16.9 Hz, J_2 = J_3 = 3.9 Hz, 4-H) and 3.13 (ddd, J_1 = 16.9 Hz, J_2 = 10.0 Hz, J_3 = 5.9 Hz, 4-H), 3.91 (ddd, J_1 = 11.5 Hz, J_2 = 10.0 Hz, J_3 = 3.9 Hz, 3-H) and 4.20 (ddd, J_1 = 11.5 Hz, J_2 = 5.9 Hz, J_3 = 3.9 Hz, 3-H), 5.72 (s, 1H, 1-H), 6.76 (s, 1H, 8-H), 7.30 (s, 1H, 5-H), 7.48 (d, J = 8.7 Hz, 1H) and 8.23 (d, J = 8.7 Hz, 1H, nitrophenyl).

7-Bromo-1-(2-chlorophenyl)isochromane (9d). Yield: 40%. Mp 62–65 °C (ethanol). $C_{15}H_{12}BrClO$ (323.6). ^{1}H NMR: δ 2.75 (ddd, J_1 = 16.4 Hz, J_2 = J_3 = 3.6 Hz, 4-H) and 3.09 (ddd, J_1 = 16.4 Hz, J_2 = 9.7 Hz, J_3 = 5.7 Hz, 4-H), 3.93 (ddd, J_1 = 13.6 Hz, J_2 = 9.7 Hz, J_3 = 3.6 Hz, 3-H) and 4.21 (ddd, J_1 = 13.6 Hz, J_2 = 5.7 Hz, J_3 = 3.6 Hz, 3-H), 6.88 (d, J = 1.7 Hz, 1H, 8-H), 7.05 (d, J = 8.0 Hz, 1H, 5-H), 7.14–7.34 (m, 4H, 2-clorophenyl), 7.45 (dd, J_1 = 8.0 Hz, J_2 = 1.7 Hz, 1H, 5-H).

General procedure for the synthesis of phenylacetic acid derivatives 10a–d. To a solution of the corresponding isochromane (**9a**–**d**) (90 mmol) in acetone (360 mL) Jones reagent (260 mL) was gradually given and the mixture was stirred for 16 h. The separated chromium sulfate was filtered and the filtrate evaporated to dryness. The residue was treated with excess 10% sodium carbonate solution and dichloromethane. After separation the aqueous layer was acidified with concd hydrochloric acid and the separated crystals were collected by filtration.

4-Chloro-2-(4-nitrobenzoyl)phenylacetic acid (10a). Yield: 78%. Mp 150–152 °C (ethyl acetate). $C_{15}H_{10}ClNO_5$ (319.7). ^{1}H NMR: (DMSO-d_6): δ 3.82 (s, 2H, C<u>H</u>$_2$), 7.42 (d, J = 1.8 Hz, 1H, 3-H), 7.48 (d, J = 8.2 Hz, 1H, 6-H), 7.62 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H, 5-H), 7.90 (d, J = 8.7 Hz, 2H) and 8.32 (d, J = 8.7 Hz, 2H, nitrobenzoyl).

4-Bromo-2-(4-nitrobenzoyl)phenylacetic acid (10b). Yield: 62 %. Mp 128–130 °C. $C_{15}H_{10}BrNO_5$ (364.2). ^{1}H NMR: (DMSO-d_6): δ 3.80 (s, 2H, C<u>H</u>$_2$), 7.46 (d, J = 8.2 Hz, 1H, 6-H), 7.57 (d, J = 2.0 Hz, 1H, 3-H), 7.81 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 5-H), 7.95 (d, J = 8.9 Hz, 2H) and 8.38 (d, J = 8.9 Hz, 2H, nitrobenzoyl), 12.1–12.7 (1H).

4,5-Dichloro-2-(4-nitrobenzoyl)phenylacetic acid (10c). The crude product prepared by the evaporation of its solution in acetone was recrystallized from 96% acetic acid and further purified by column chromatography. Eluent: chloroform:methanol (9:1). Yield: 54 %. Mp 187–190 °C. $C_{15}H_9Cl_2NO_5$ (354.1). ^{1}H NMR: (DMSO-d_6): δ 3.90 (s, 2H, C<u>H</u>$_2$), 7.68 (s, 1H), 7.85 (s, 1H), 7.97 (d, J = 8.7 Hz, 2H) and 8.38 (d, J = 8.7 Hz, 2H, nitrobenzoyl).

4-Bromo-2-(2-chlorobenzoyl)phenylacetic acid (10d). Yield: 47%. Mp 135–138 °C. $C_{15}H_{10}BrClO_3$ (353.6). ^{1}H NMR: (DMSO-d_6): 3.90 (s, 2H, C<u>H</u>$_2$), 7.37 (d, J = 2.2 Hz, 1H, 3-H), 7.43 (d, J = 8.1 Hz, 1H, 6-H), 7.78 (dd, J_1 = 8.1 Hz, J_2 = 2.2 Hz, 1H, 5-H), 7.47 (m, 2H) and 7.60 (m, 2H, chlorophenyl), 12.40 (br. s).

8-Chloro-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (11a). Method A. A solution of **10a** (17.6 g, 55.0 mmol) and 85% hydrazine hydrate (8 mL) in ethanol (340 mL) was heated at reflux for 4 h. The mixture was then chilled, treated with 1N hydrochloric acid (115 mL) and the solvent was evaporated. The residue was treated with water (50 mL) and the precipitate filtered and dried. This intermediate hydrazone was dissolved in dichloromethane (300 mL) and treated with a solution of dicyclohexylcarbodiimide (13.4 g 65.0 mmol) in dichloromethane (210 mL). The mixture was stirred overnight at rt and the resulting precipitate was filtered and washed with dichloromethane to give **11a** (12.5 g). Yield: 72 %. Mp 275–278 °C.

Method B. The reaction was performed as in Method A, but isopropanol was used as solvent and after reflux it was treated with 1N hydrochloric acid (125 mL) and stirred overnight at rt The resulting precipitate was filtered and the crude product was recrystallized from 2-methoxyethanol. Yield: 88%. Mp 275–277 °C. $C_{15}H_{10}ClN_3O_3$ (315.7). ^{1}H NMR: (DMSO-d_6): δ 3.62 (s, 2H, 5-H), 7.20 (d, J = 2.0 Hz, 1H, 9-H), 7.58 (d, J = 8.2 Hz, 1H, 6-H), 7.70 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 7-H), 7.80 (d, J = 8.7 Hz, 2H) and 8.30 (d, J = 8.7 Hz, 2H, nitrophenyl).

8-Bromo-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (11b). Starting from **10b** (12.7 g, 35.0 mmol) Method A was essentially followed as described for **11a**. Yield: 65% (8.19 g). Mp 264–267 °C. $C_{15}H_{10}BrN_3O_3$ (360.2). ^{1}H NMR: (DMSO-d_6): δ 3.60 (br. s, 2H, 5-H), 7.29 (d, J = 1.8 Hz, 1H, 9-H), 7.52 (d, J = 8.3 Hz, 1H, 6-H), 7.77 (overlapping, 7-H), 7.78 (d, J = 8.7 Hz) and 8.32 (d, J = 8.7 Hz, 2H, nitrophenyl), 11.41 (s, 1H, N-<u>H</u>).

7,8-Dichloro-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (11c). A solution of **10c** (6.1 g, 17.2 mmol) and 85% hydrazine hydrate (6 mL) in isopropanol (300 mL) was treated at reflux for 6 h. The solvent was evaporated and the residue was dissolved in a mixture of 40% acetic acid (40 mL) and dichloromethane (400 mL). After separation the organic layer was washed with water, dried and treated with dicyclohexylcarbodiimide (3.60 g 17.5 mmol). The reaction mixture was stirred

overnight and the precipitate filtered. The filtrate was evaporated to dryness and the residue was boiled with methanol (120 mL) and filtered while hot to give the product (4.40 g). Yield: 73%. Mp 268–270 °C. $C_{15}H_9Cl_2N_3O_3$ (350.1). ^{1}H NMR: (DMSO-d_6): δ 3.76 (s, 2H, 5-H), 7.48 (s, 1H, 6-H), 7.90 (d, J = 8.8 Hz, 2H) and 8.39 (d, J = 8.8 Hz, 2H, nitrophenyl), 8.01 (s, 1H, 9-H).

8-Bromo-1-(2-chlorophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (11d). A solution of **10d** (4.28 g, 12.1 mmol) and 98% hydrazine hydrate (1.8 mL) in ethanol (45 mL) was refluxed for 5 h. The mixture was evaporated to dryness and the residue was dissolved in dichhorometane (120 mL) and washed with water (3×20 mL). The organic layer was dried and evaporated. The crude product was recystallized from ethanol to give the product (2.26 g). Yield: 53%. Mp 255–258 °C. $C_{15}H_{10}BrClN_2O$ (349.6). ^{1}H NMR: (DMSO-d_6): δ 3.62 (s, 2H, 5-H), 7.02 (d, J = 1.8 Hz, 1H, 9-H), 7.52 (d, J = 8.1 Hz, 1H, 6-H), 7.79 (dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz, 1H, 7-H), 7.55–7.75 (m, 4H, 2-chlorophenyl), 11.35 (br. s, 1H, 3-H).

General method for the synthesis of 3,5-dihydro-4*H*-2,3-benzodiazepine-4-thione derivatives 12a–d. To a solution of the corresponding 4-oxo-2,3-benzodiazepine derivative (**11a**–**d**) (38.0 mmol) in dry pyridine (150 mL) phosphorous pentasulfide (13.3 g, 60.0 mmol) was given and the mixture was kept at 80 °C for 2–3 h. The reaction mixture was then cooled to rt and poured onto ice (1 kg). The precipitate was collected by filtration and washed with water. The crude products were recrystallized from 2-methoxyethanol.

8-Chloro-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepine-4-thione (12a). Yield: 71%. Mp 231–234 °C. $C_{15}H_{10}ClN_3O_2S$ (331.8). ^{1}H NMR: (DMSO-d_6): δ 4.00 (br. s, 2H, 5-H), 7.48 (d, J = 8.2 Hz, 1H, 6-H), 7.15 (d, J = 1.9 Hz, 1H, 9-H), 7.72 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 1H, 7-H), 7.80 (d, J = 8.7 Hz, 2H) and 8.28 (d, J = 8.7 Hz, 2H, nitrophenyl).

8-Bromo-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepine-4-thione (12b). Yield: 67%. Mp 220–223 °C. $C_{15}H_{10}BrN_3O_2S$ (376.8). ^{1}H NMR: (DMSO-d_6): δ 4.05 (br. s, 2H, 5-H), 7.35 (d, J = 1.8 Hz, 1H, 9-H), 7.48 (d, J = 8.3 Hz, 1H, 6-H), 7.88 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1H, 7-H), 7.84 (d, J = 8.8 Hz, 2H) and 8.36 (d, J = 8.8 Hz, 2H, nitrophenyl), 13.1 (s, 1H, N-H).

7,8-Dichloro-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepine-4-thione (12c). Yield: 61%. Mp 210–213 °C. $C_{15}H_9Cl_2N_3O_2S$ (366.2). MS(EI): m/z: M: 366/368.

8-Bromo-1-(2-chlorophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepine-4-thione (12d). Yield: 79%. Mp 198–20 °C. $C_{15}H_{10}BrClN_2S$ (365.7). MS(EI): m/z: M: 365/367.

General procedure for the synthesis of 11*H*-imidazolo[1,2-c][2,3]-benzodiazepine derivatives (15a–g, 15i–n, 3p, q). A mixture of the appropriate 2,3-benzodiazepine-4-thione derivative (**12a**–**d**) (10.0 mmol), the corresponding aminoacetal (**13a**–**g**) (20.0 mmol) and red mercury oxide (10.0 mmol) in 2-methoxyethanol was stirred and heated at reflux for 1–10 h. After filtration the solvent was removed and the residue was purified by chromatography, eluent was chloroform:methanol (98:2). The fractions containing the condensation product of type 14 were evaporated to dryness and the residue was treated with a 1:1 mixture of concd hydrochloric acid and ethanol and heated at reflux for 1–2 h. Evaporation gave the title products as hydrochloride salts. In a few cases (15f,g,n) the intermediate condensation product was reacted with methanesulfonic acid at rt for 1–2 h to induce the ring closure reaction. In these cases the reaction mixture was diluted with water and made alkaline with 5N sodium hydroxide. The products were collected by filtration. The yields, physical and spectroscopic date are collected in Table 10.

8-Chloro-1-(4-nitrophenyl)-3-(2-oxopropyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (16). To a solution of **11a** (36.3 g, 115 mmol) in DMF (250 mL) potassium carbonate (19.9 g, 144 mmol) was given and the mixture was heated at 80 °C for 10 min. Then chloroacetone (11 mL, 138 mmol) was added and the mixture was heated at 80 °C for 2 h. The reaction mixture was then cooled to rt and poured onto ice (1.2 kg). The precipitate was collected by filtration and washed with water. The crude product was recrystallized from acetic acid to give **16** (39.3 g). Yield: 92%. Mp 222–224 °C. $C_{18}H_{14}ClN_3O_4$ (371.8). ^{1}H NMR: (DMSO-d_6): δ 2.12 (s, 3H, CH$_3$), 3.75 (br. s, 2H, 5-H), 4.72 (s, 2H, CH$_2$), 7.23 (d, J = 2.1 Hz, 1H, 9-H), 7.63 (d, J = 8.3 Hz, 1H, 6-H), 7.74 (dd, J_1 = 8.3 Hz, J_2 = 2.1 Hz, 1H, 7-H), 7.83 (d, J = 8.8 Hz, 2H) and 8.33 (d, J = 8.8 Hz, 2H, nitrophenyl).

8-Chloro-1-(4-nitrophenyl)-3-phenacyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (17). The compound was synthesized from **11a** according to the procedure as given above by using 2-chloroacetophenone. Yield: 83%. Mp 223–225 °C. $C_{23}H_{16}ClN_3O_4$ (433.8). ^{1}H NMR: (DMSO-d_6): δ 3.8 (br. s, 2H, 5-H), 5.4 (br. s, 2H, phenacyl), 7.22 (d, J = 1.9 Hz, 1H, 9-H), 7.64 (d, J = 8.3 Hz, 1H, 6-H), 7.74 (dd, J_1 = 8,3 Hz, J_2 = 1.9 Hz, 1H, 7-H), 7.54 (dd, J_1 = J_2 = 7.5 Hz, 2H, phenyl), 7.66 (dd, J_1 = J_2 = 7.5 Hz, 1H, phenyl), 7.98 (d, J = 7.5 Hz, 2H, phenyl), 7.83 (d, J = 8.8 Hz, 2H) and 8.32 (d, J = 8.8 Hz, 2H, nitrophenyl).

8-Chloro-2-methyl-6-(4-nitrophenyl)-11*H*-imidazolo[1,2-c][2,3]benzodiazepine hydrochloride (15b). (Alternative route.) To a solution of ammonium acetate (180 g) in acetic acid (180 mL) **16** (9.0 g, 24.2 mmol) was added and the mixture was heated at 140 °C for 3 h. The reaction mixture was then cooled to rt and poured onto ice (0.9 kg). The precipitate was collected by filtration and washed with water. The crude product was recrystallized twice from DMF to give the product as base (3.42 g). Yield: 40%. Mp 246–250 °C. A sample was converted to the hydrochloride with mp 230–231 °C which was identical with the substance prepared by the general method described earlier, for further data see Table 10.

8-Chloro-6-(4-nitrophenyl)-2-phenyl-11*H*-imidazolo[1,2-c][2,3]benzodiazepine (15h). The compound was prepared from **17** according to the procedure as described above for **15b**. (For yield, physical and spectroscopic data see Table 10.)

Table 10. Physical and spectroscopic data of the 11*H*-imidazolo[1,2-c][2,3]benzodiazepine derivatives **15a–n**, **3p–q**

Compound[a]	mp (°C)	Yield (%)	Molecular formula (Mol. mass)	Spectroscopic data: ^{1}H NMR (in DMSO-d_6; δ) or MS
15 a	210–215	48	$C_{17}H_{12}Cl_2N_4O_2$ (375.2)	4.55 (s, 2H, 11-H), 7.22 (d, J = 1.8 Hz, 1H, 7-H), 7.65 (d, J = 8.2 Hz, 1H, 10-H), 7.84 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H, 9-H), 7.62 (overlapping) and 7.95 (2-H and 3-H), 8.00 (d, J = 8.5 Hz, 2H) and 8.43 (d, J = 8.5 Hz, 2H, nitrophenyl).
15 b	229–230	79	$C_{18}H_{14}Cl_2N_4O_2$(389.2)	2.25 (d, J = 1.0 Hz, 3H, 2-CH_3), 4.52 (s, 2H, 11-H), 7.23 (d, J = 1.8 Hz, 1H, 7-H), 7.63 (d, J = 8.2 Hz, 1H, 10-H), 7.72 (q, J = 1.0 Hz, 1H, 3-H), 7.82 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H, 9-H). 7.96 (d, J = 8.6 Hz) and 8.44 d, J = 8.6 Hz, nitrophenyl).
15 c	205–208	41	$C_{18}H_{14}Cl_2N_4O_2$(389.2)	2.40 (d, J = 1.0 Hz 3H, 3-CH_3), 7.22 (d, J = 1. Hz, 1H, 7-H), 7.42 (q, J = 1.0 Hz, 1H, 2-H), 7.62 (d, J = 8.2 Hz, 1H, 10-H), 7.86 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H, 9-H), 8.05 (d, J = 8.6 Hz) and 8.40 (d, J = 8.6 Hz, nitrophenyl).
15 d	207–210	46	$C_{19}H_{16}Cl_2N_4O_2$(403.3)	2.22 (s, 3H), 2.35 (s, 3H), 4.49 (s, 2H, 11-H), 7.22 (d, J = 1.9 Hz, 1H, 7-H), 7.63 (d, J = 8.2 Hz, 1H, 10-H), 7.86 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 1H, 9-H), 8.02 (d, J = 8.7 Hz) and 8.40 (d, J = 8.7 Hz, nitrophenyl).
15 e	150–153	20	$C_{19}H_{16}Cl_2N_4O_2$(403.3)	MS(EI): m/z: M: 403/405.
15 f[b]	270–272	30	$C_{23}H_{14}ClN_5O_4$(459.8)	4.25 (br. s, 2H, 11-H), 7.26 (d, J = 1.8 Hz, 1H, 7-H), 7.55 (s, 1H, 2-H), 7.72 (d, J = 8.3 Hz, 1H, 10-H), 7.76 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1H, 9-H), 7.95 (d, J = 8.9 Hz, 2H), 8.04 (d, J = 8.9 Hz, 2H) and 8.36 (d, J = 8.9 Hz, 2H), 8.41 (d, J = 8.9 Hz, nitrophenyls).
15 g[b]	250–252	30	$C_{22}H_{14}ClN_5O$(415.8)	4.10 (br. s, 2H, 11-H), 7.22 (d, J = 1.9 Hz, 1H, 7-H), 7.35 (s, 1H, 2-H), 7.47 (d, J = 8.3 Hz, 1H, 10-H), 7.56 (d, J = 6.2 Hz, 2H, pyridyl), 7.60 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H, 9-H), 7.92 (d, J = 8.9 Hz, 2H) and 8.37 (d, J = 8.9 Hz, 2H, nitrophenyl), 8.72 (d, J = 6.2 Hz, 2H, pyridyl).
15 h[b]	239–232	82	$C_{23}H_{15}ClN_4O_2$414.8	4.25 (br. s, 2H, 11-H), 7.21 (br. s, 1H, 7-H), 7.72 (br. s, 2H, 9-H and 10-H), 7.12–7.26 (1H, phenyl, overlapping), 7.37 (dd, J_1 = 1_2 = 7.3 Hz, 2H, phenyl), 7.78 (d, J = 7.3 Hz, 2H, phenyl), 8.00 (d, J = 8.8 Hz, 2H, nitrophenyl), 8.10 (s, 1H, 3-H), 8.42 (d, J = 8.8 Hz, nitrophenyl).
15 i	221–224	13	$C_{18}H_{13}Cl_3N_4O_2$(423.7)	MS (EI): *m*/*z* (%): M 386/388 (100/68), 385/387 (45/30), 339/341 (16/12).
15 j	240–244	41	$C_{18}H_{13}Cl_3N_4O_2$(423.7)	MS (EI): *m*/*z* (%): M 386/388 (73/45), 385/387 (100/68), 339/341 (20/14).
15 k	215–220	28	$C_{18}H_{14}BrClN_4O_2$(433.8)	2.25 (d, J = 1.0 Hz, 3H, 2-CH_3), 4.43 (br. s, 2H, 11-H), 7.35 (d, J = 1.9 Hz, 1H, 7-H), 7.58 (d, J = 8.3 Hz, 1H, 10-H), 7.71 (q, J = 1.0 Hz, 1H, 3-H), 7.96 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H, 9-H), 7.98 (d, J = 8.82 Hz) and 8.43 (d, J = 8.8 Hz, nitrophenyl).
15 l	194–202	50	$C_{18}H_{14}BrClN_4O_2$(433.8)	2.42 (d, J = 1.0 Hz, 3H, 3-CH_3), 4.50 (s, 2H, 11-H), 7.32 (d, J = 1.8 Hz, 1H, 7-H), 7.42 (q, J = 1.0 Hz, 1H, 2-H), 7.55 (d, J = 8.1 Hz, 1H, 10-H), 7.97 (dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz, 1H, 9-H), 8.05 (d, J = 8.7 Hz) and 8.42 (d, J = 8.7 Hz, nitrophenyl).
15 m	212–219	42	$C_{19}H_{16}BrClN_4O_2$(447.8)	2.22 (s, 3H), 2.34 (s, 3H), 4.45 (s, 2H, 11-H), 7.42 (d, J = 1.9 Hz, 1H, 7-H), 7.58 (d, J = 8.1 Hz, 1H, 10-H), 7.95 (dd, J_1 = 8.1 Hz, J_2 = 1.9 Hz, 1H, 9-H), 8.01 (d, J = 8.8 Hz) and 8.42 (d, J = 8.8 Hz, nitrophenyl).
15 n[b]	Foam	60	$C_{22}H_{14}BrN_5O$(460.3)	MS(EI): m/z: M: 460/462.
3 p[c,d]	—	—	$C_{18}H_{14}BrCl_2N_3$(423.2)	
3 q[c,d]	—	—	$C_{18}H_{14}BrCl_2N_3$(423.2)	

[a]Hydrochlorides.
[b]Data refer to the base.
[c]For Mp and yield see Table 1.
[d]For spectroscopic data see Table 12.

8-Chloro-4-methylthio-1-(4-nitrophenyl)-5*H*-2,3-benzodiazepine (18). Thione **12a** (3.32 g 10.0 mmol) was dissolved in acetone (200 mL) and potassium carbonate (2.76 g 20.0 mmol) and methyliodide (1.87 mL, 30.0 mmol) were added. The mixture was stirred at rt for 3 h, then the product was filtered, washed with water and recrystallized from DMF to give the title product (2.84 g). Yield: 82%. Mp 249–252 °C. $C_{16}H_{12}ClN_3O_2S$ (345.8). ^{1}H NMR: δ 2.42 (s, 3H, S-CH_3), 3.35 (d, J = 13.0 Hz, 5-H), and 3.45 (d, J = 13.0 Hz), 7.20 (d, J = 2.0 Hz,1H, 9-H), 7.25 (d, J = 8.2 Hz, 1H, 6-H), 7.55 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 7-H), 7.87 (d, J = 8.8 Hz) and 8.38 (d, J = 8.8 Hz, nitrophenyl).

General procedure for the synthesis of 3-substitued 8-chloro-6-(4-nitrophenyl)-11*H*-1,2,4-triazolo[4,5-c][2,3]benzodiazepine derivatives (19a–d). 18 (3.45 g 10.0 mmol) was dissolved in DMF (120 mL) then the corresponding acylhydrazide (25.0 mmol) and concd HCl (0.5 mL) were added and the mixture was stirred and heated at 120–130 °C for 9–15 h. The reaction mixture was poured onto ice and the product filtered. The crude products were recrystallized. The yields, physical and analytical data are collected in Table 11.

7-Chloro-3-methyl-1-(4-nitrophenyl)isochromane (21). Prepared from 1-(4-chlorophenyl)-2-propanol[33] according to the general method as described for **9a–d**. Yield: 32%. Mp 120–123 °C. $C_{16}H_{14}ClNO_3$ (303.8). ^{1}H NMR: δ 1.42 (d, J = 6.4 Hz, 3H, 3-CH_3), 2.7–2.95 (m, 2H, 4-H), 4.02 (m, 1H, 3-H), 5.80 (s, 1H, 1-H), 6.60 (d, J = 2.0 Hz, 1H, 8-H), 7.10 (d, J = 8.2 Hz, 1H, 5-H), 7.16 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 6-H), 7.53 (d, J = 8.8 Hz, 2H) and 8.24 (d, J = 8.8 Hz, 2H, nitrophenyl).

7-Chloro-3-methyl-1-(4-nitrophenyl)-2-benzopyrilium perchlorate (22). To an ice cold solution of **21** (6.8 g, 22.4 mmol) in acetone (70 mL) Jones reagent (29 mL, 78 mmol) was added dropwise during 1h and the mixture was then stirred at rt for 4 h. The separated chromium salt was filtered and the solution evaporated to dryness. The residue was suspended with water (25 mL) and filtered. The precipitate was then dissolved in hot acetic acid (76 mL) and 70% perchloric acid (1.48 mL) was

Table 11. Physical and spectroscopic data of the 3-substitued 8-chloro-6-(4-nitrophenyl)-11*H*-1,2,4-triazolo[4,5-c][2,3]benzodiazepine derivatives **19a–d**

Compound	mp (°C)	Yield (%)	Molecular formula (Mol. mass)	Spectroscopic data: ^{1}H NMR or MS
19 a	271–274	72	$C_{17}H_{12}ClN_5O_2$ (353.8)	2.58 (s,3H) 4.16 (s, 2H), 7.12 (d, J = 1.9 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.55 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H), 7.90 (d, J = 8.7 Hz) and 8.35 (d, J = 8.7 Hz, nitrophenyl).
19 b	287–289	86	$C_{21}H_{13}ClN_6O_2$ (416.8)	(in DMSO-d_6) 4.46 (br. s, 2H), 7.24 (br. s, 1H), 7.78 (br. s, 2H), 7.98 (d, J = 8.8 Hz, 2H) and 8.42 (d, J = 8.8 Hz, 2H, nitrophenyl), 8.18 (d, J = 5.5 Hz, 2H) and 8.83 (d, J = 5.5 Hz, 2H, pyridyl).
19 c	287–290	68	$C_{22}H_{13}ClN_6O_4$(460.8)	MS(EI):m/z: M:460/462.
19 d	266–268	88	$C_{18}H_{14}ClN_5O_3$(383.8)	MS(EI): m/z: M: 383/385.

added. A product separated which was filtered after cooling and washed with acetic acid to give the title product. Yield: 42% (3.73 g). Mp 247–255 °C. $C_{16}H_{11}Cl_2NO_7$ (400.2). IR: 1096 cm^{-1} (ClO_4^-).

8-Chloro-4-methyl-1-(4-nitrophenyl)-5*H*-2,3-benzodiazepine (23). Compound **22** (4.1 g, 10.2 mmol) was added to a solution of 98% hydrazine hydrate (1.5 mL, 70.7 mmol) in DMF (20 mL) at 10–15 °C. The reaction mixture was then stirred for 1.5 h at rt Water (25 mL) was added and the separated precipitate was collected by filtration and washed with water. The crude product was recrystallized from isopropanol. Yield: 87% (2.82 g). Mp 199–203 °C. $C_{16}H_{12}ClN_3O_2$ (313.7). ^{1}H NMR: (DMSO-d_6): δ 2.11 (s, 3H, CH_3), 2.93 and 3.65 (d, J = 12.2 Hz, 2H, 5-H), 7.33 (d, J = 2.0 Hz, 1H, 9-H), 7.61 (d, J = 8.2 Hz, 1H, 6-H), 7.73 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 7-H), 7.82 (d, J = 8.7 Hz, 2H), 8.32 (d, J = 8.7 Hz, 2H).

8-Chloro-4-formyl-1-(4-nitrophenyl)-5*H*-2,3-benzodiazepine (24). To a solution of **23** (9.17 g, 29.0 mmol) in dioxane (120 mL) powdered selenium dioxide (2.27 g, 20.5 mmol) was given and the mixture was stirred at 90 °C for 40 min. Charcoal was added to the mixture and it was filtered. The clear solution was poured into water (1.5 L) and the separated crystalls were filtered and washed with water. Column chromatography with benzene as eluent gave the aldehyde. Yield: 29% (2.8 g). Mp 208–210 °C. $C_{16}H_{10}ClN_3O_3$ (327.7). ^{1}H NMR: (DMSO-d_6): 2.98 (d, J = 13.1 Hz) and 4.13 (d, J = 13.1 Hz, 2H, 5-H), 7.42 (d, J = 2.2 Hz, 1H, 9-H), 7.50 (d, J = 8.3 Hz, 1H, 6-H), 7.73 (dd, J_1 = 8.3 Hz, J_2 = 2.2 Hz, 1H, 7-H), 7.92 (d, J = 8.9 Hz, 2H) and 8.36 (d, J = 8.9 Hz, 2H, nitrophenyl).

6-(4-Aminophenyl)-8-chloro-3-methyl-11*H*-imidazolo[3,4-c][2,3]benzodiazepine (5). The solution of **24** (2.15 g, 6.60 mmol) in a 1:1 mixture of THF:water (88 mL) was chilled with icewater and sodium borohydride (0.12 g 3.30 mmol) was given in portions. After stirring at rt for 40 min the solution was diluted with water (90 mL) to give a precipitate which was filtered and after drying chromatographed with the eluent of benzene:ethyl acetate (1:1) to give pure 4-(hydroxymethyl)-8-chloro-1-(4-nitrophenyl)-5*H*-2,3-benzodiazepine (**25**; 1.62 g). Mp > 163 °C (decomposition). (MS(FAB): M: 329/331.)

The intermediate **25** (1.62 g, 4.9 mmol), triphenylphosphine (2.54 g, 9.7 mmol) and phthalimide (1.42 g, 9.7 mmol) were dissolved in dry THF (72 mL) and a solution of diethyl azodicarboxylate (1.52 mL, 9.7 mmol) in THF (11 mL) was added dropwise. After stirring for 3 h at rt the solvent was removed and the residue recrystallized from ethanol to give **26** (1.34 g). Mp 254–256 °C (dec.). (MS(FAB): M: 458/460.)

The solution of the intermediate **26** (1.34 g, 2.9 mmol) and 98% hydrazine hydrate (1.09 mL, 21.7 mmol) in methanol (134 mL) was heated at boiling for 4 h. After evaporation of the solvent the residue was treated with methanol (50 mL) and the resulting precipitate was filtered. The filtrate was then evaporated and the residue suspended with water to give **27** (0.97 g, mp 105–107 °C (dec.)). This crude intermediate was taken up with acetic anhydride (8 mL) and stirred for 2 h. Dilution with water (40 mL) gave a solid substance which was collected by filtration. Column chromatography of this product with the eluent ethyl acetate:benzene (4:1) gave the acetylamino derivative **28** (0.60 g). Mp 216–218 °C. (MS(FAB): M: 370/372.)

A solution of **28** (0.59 g, 1.6 mmol) and $POCl_3$ (0.73 mL, 7.95 mmol) in 1,2-dichloroethane (30 mL) was heated at reflux for 3 h. The solution was then chilled with ice and treated with sodium hydrogencarbonate solution. After separation the organic phase was washed with water, dried and evaporated. The residual oil was chromatographed with an eluent of ethyl acetate:benzene (4:1) to give 8-chloro-3-methyl-6-(4-nitrophenyl)-11*H*-imidazolo[3,4-c][2,3]benzodiazepine (**29**), which was then reduced according to the general procedure as described for compounds **3a**–**n**. The crude product was boiled with ethanol to remove impurities. By the above sequence 0.12 g of pure **5** could be prepared. Mp 256–258 °C (dec.). $C_{18}H_{15}ClN_4$ (322.8). MS(EI): *m*/*z* (%): M: 322/324 (100/33), 321/323 (26/9), 108.5 (22), 65 (11), 219 (10), 106 (10), 218 (8), 217 (8).

6-(4-Aminophenyl)-8-chloro-2-ethoxycarbonyl-11*H*-pyrrolo[1,2-c][2,3]benzodiazepine (6). The solution of **23** (0.50 g, 1.60 mmol) and ethyl bromopyruvate (0.27 mL, 2.20 mmol) in ethanol (20 mL) was heated at reflux for 12 h. After evaporation of the solvent the residue was chromatographed with benzene as eluent to give **30** (0.29 g) which was reduced without further purification according to the general procedure as described for **3a**–**n.** It was recrystallized from ethanol (0.11 g). Mp 247–249 °C. $C_{21}H_{18}ClN_3O_2$ (379.8). MS (EI): *m*/*z* (%): M: 379/381 (100/35), 378/380 (41/14), 350/352 (35/14), 306/308 (35/12), 65 (23).

General procedure for the reduction of the nitro compounds 15a–n, 19a–d, 29 and 30. (Preparation of 3a–n, 4a–d, 5 and 6). To a stirred solution of the corresponding nitro compound (2 mmol) in a 1:1 mixture of methanol and dichloromethane hydrazine hydrate (4–5 equiv) and RaNi (0.1–2 g) were added. After stirring at 20–40 °C for 1–5 h the catalyst was filtered and the solvents evaporated. The residues were recrystallized from ethanol. Mp-s and yields of the aminophenyl compounds **3a–n** and **4a–d** are shown in Table 1 and 2 respectively. The composition and spectroscopic data of **3a–3r** and **4a–d** are collected in Table 12.

6-(4-Acetylaminophenyl)-8-chloro-2-methyl-11*H*-imidazolo[1,2-c][2,3]benzodiazepine (3o). Prepared from **3b** (0.46 g, 1.42 mmol) in pyridine (8 mL) by reacting with acetylchloride (0.20 mL) at 5–10 °C for 1.5 h. Dilution with ice water gave a solid precipitate which was recrystallized from ethanol. For yield and mp see Table 1, spectroscopic data are shown in Table 12.

8-Chloro-3-methyl-6-phenyl-11*H*-imidazolo[1,2-c][2,3]benzodiazepine (3r). To a stirred solution of **3c** (1.10 g, 3.20 mmol) in DMF (12 mL) at 65 °C isoamyl nitrite (0.80 mL) was added droppwise over 8 min. Heating was continued for 30 min and the cooled mixture was then treated with 5N HCl. The product was extracted with ether and purified by column chromatography. Eluent: chloroform:methanol (98:2). For yield and mp see Table 1, spectroscopic data are shown in Table 12.

Biology, in vitro methods

Retinal spreading depression (S.D.) test. Experiments were performed according to Sheardown.[17] The posterior chamber of each eye of 1–5-day-old chickens was dissected and placed in a Petri dish containing physiological saline of the following composition: NaCl (100 mM), KCl (6 mM), $CaCl_2$ (1 mM), $MgSO_4$ (1 mM), $NaHCO_3$ (30 mM), NaH_2PO_4 (1 mM). The solution was saturated with 95% O_2 and 5% CO_2 and maintained at 26 °C. The eyes were initially incubated in normal saline for 30 min and then transferred to a solution containing AMPA (5 μM) or kainate (5 μM). These solutions triggered S.D., which could be easily observed by eye. The latency of S.D. was determined. The presence of a white area (0.5 mm in diameter) was taken as the onset of S.D. The eye cups were then returned to normal saline. After a further 15 min recovery period the eyes were incubated in a solution containing the test compounds and incubated for 15 min. Thereafter the eyes were transferred to a glutamate agonist containing solution which also contained the test compound and the latency of S.D. was determined again. 60 min following this measurement, the whole procedure was repeated, with another test substance/concentration. Each drug concentration was tested in 6 retina preparations. An increase in the latency of 30 s or more was considered to be 100% inhibition of S.D. (cut-off time was 1 min).[17] The drug effects therefore are expressed as the percentage maximum inhibition obtained for a given concentration. Dose-response curves were constructed from 3–6 concentration points and IC_{50}±S.E.M. values were calculated by sigmoidal curve fitting using the MICROCAL ORIGIN 4.1 computer program.

Electrophysiological studies. Electrophysiological experiments were carried out on acutely isolated cerebellar Purkinje cells according to Bleakman et al.[19] Briefly: cells were isolated from the cerebellum of 6–9-day-old rats using enzymatic treatment and mechanical trituration, then plated to poly-L-lysine coated glass coverslips. Cells were kept alive in a tissue culture medium, in a CO_2 thermostat, and used for electrophysiological experiments on the day of isolation. Purkinje cells (identified by their bigger size) were patched with glass electrodes (4–6 MΩ). The membrane potential was fixed at −70 mV. Experiments were performed at room temperature. The internal recording solution contained NaCl (140 mM), $MgCl_2$ (1 mM), HEPES (10 mM), glucose (10 mM), EGTA (0.1 mM), pH = 7.2. The cells were continuously perfused with a solution composed of NaCl (138 mM), KCl (5 mM), $CaCl_2$ (5 mM), $MgCl_2$ (1 mM), HEPES (10 mM), glucose (10 mM), pH = 7.35. Excitatory amino acids (kainate 100 μM or S-AMPA 5 μM) were applied alone, or in combination with various concentrations of **1**, **2** or **3b**. The percentage decrease of the plateau current was evaluated. IC_{50}±S.E.M. values were calculated from 4–6 concentrations (5–6 cells for each concentration), using the MICROCAL ORIGIN 4.1 computer program for sigmoidal curve fitting.

In vivo experiments

Animals were purchased from Charles River Hungary Ltd. (Budapest, Hungary) and were housed for a minimum of six days prior to experiments with free access to standard laboratory diet and tap water and maintained on a 12–12 h light-dark cycle (light from 6.00 am to 6.00 p.m.). All compounds under study were suspended in saline (ip administration) or distilled water (oral administration) containing 1–2% Tween-80. Control animals received vehicle under the same conditions. ED_{50} and LD_{50} values were calculated by the Litchfield–Wilcoxon method.[34] The volumes of administration were 0.1 mL/10 g and 0.5 mL/100 g body weight for mice and rats, respectively, unless otherwise stated.

Behavioural changes in mice. 5 male CD1 mice (21–26 g) were starved for 16 h and treated with test compounds in doses of 100 and 200 mg/kg ip and po, respectively. The gross behavioural changes were evaluated continuously for 5 h according to Irwin.[35]

Seizure assays. 10 male CD1 mice (21–26 g) per group, after 16 h starvation, were used. Animals were treated orally with test compounds 60 min before maximal electroshock (MES)[36] or chemical convulsants (metrazole 130 mg/kg ip, strychnine 3 mg/kg ip, bemegride 50 mg/kg ip, bicuculline 1 mg/kg iv, nicotine 3.5 mg/kg iv, 4-aminopyridine 12.5 mg/kg ip and 3-mercapto-propionic acid 110 mg/kg iv).[37] Prevention of tonic convulsion (MES) and/or death (convulsive agents) served as measure of anticonvulsive potency.

Muscle relaxation in mice. (Inclined screen and rotarod tests). For determination of muscle relaxant activity the inclined screen test[38] and rotarod test[39] were used. 10 male CD1 mice (21–25 g) per group were examined.

Table 12. Composition and spectroscopic data of compounds **3a**–**r** and **4a**–**d**

Compound	Molecular formula (Mol. mass)	Spectroscopic data: ^{1}H NMR (in DMSO-d_6;) or MS
3a	$C_{17}H_{13}ClN_4$ (308.8)	4.00 (br. s, 2H, 11-H), 5.85 (br. s, NH_2), 6.84 and 7.38 (d, J = 1.5 Hz, 2-H and 3-H), 6.63 (d, J = 8.7 Hz, 2H) and 7.36 (d, J = 8.7 Hz, 2H, aminophenyl), 7.21 (d, J = 2.0 Hz, 1H, 7-H), 7.58 (d, J = 8.3 Hz, 1H, 10-H), 7.63 (dd, J_1 = 8.3 Hz, J_2 = 2.0 Hz, 1H, 9-H).
3b	$C_{18}H_{15}ClN_4$(322.8)	2.04 (d, J = 1.1 Hz, 1H, 2-CH_3), 3.91 (br. s, 2H, 11-H), 5.80 (br. s, 2H, NH_2), 6.63 (d, J = 8.7 Hz, 2H) and 7.37 (d, J = 8.7 Hz, 2H, aminophenyl), 7.07 (q, J = 1.1 Hz, 1H, 3-H), 7.19 (d, J = 2.1 Hz, 1H, 7-H), 7.56 (d, J = 8.3 Hz, 2H, 10-H), 7.64 (dd, J_1 = 8.3 Hz, J_2 = 2.1 Hz, 1H, 9-H).
3c	$C_{18}H_{15}ClN_4$(322.8)	(in $CDCl_3$) 2.32 (d, J = 1.0 Hz, 3H, 3-CH_3), 3.92 (br. s, 2H, 11-H), 4.08 (br. s, 2H, NH_2), 6.63 (q, J = 1.0 Hz, 1H, 2-H), 6.75 (d, J = 8.5 Hz, 2H) and 7.55 (d, J = 8.5 Hz, 2H, aminophenyl), 7.29 (d, J = 1.9 Hz, 1H, 7-H), 7.36 (d, J = 8.1 Hz, 1H, 10-H), 7.44 (dd, J_1 = 8.1 Hz, J_2 = 1.9 Hz, 1H, 9-H).
3d	$C_{19}H_{17}ClN_4$(336.8)	2.00 and 2.18 (s, 3H, CH_3), 3.90 (br. s, 2H, 11-H), 5.82 (br. s, 2H, NH_2), 6.68 (d, J = 8.8 Hz, 2H) and 7.42 (d, J = 8.8 Hz, 2H, aminophenyl), 7.20 (d, J = 1.9 Hz, 1H, 7-H), 7.54 (d, J = 8.3 Hz, 1H, 10-H), 7.64 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H, 9-H).
3e	$C_{19}H_{17}ClN_4$(336.8)	(in $CDCl_3$) 1.26 (t, J = 7.3 Hz, 3H), 2.6 (q, J = 7.3 Hz, 2H), 3.94 (br. s, 2H, 11-H), 6.72 (d, J = 8.6 Hz, 2H) and 7.52 (d, J = 8.6 Hz, 2H, aminophenyl), 6.97 (s, 1H, 3-H), 7.27 (d, J = 2.0 Hz, 1H, 7-H), 7.34 (d, J = 8.2 Hz, 1H, 10-H), 7.45 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 9-H).
3f	$C_{23}H_{18}ClN_5$(399.9)	3.6-4.3 (2H, 11-H), 5.25 (br. s, 2H) and 5.86 (br. s, 2H, NH_2), 6.64 (d, J = 8.8 Hz, 2H), 6.66 (d, J = 8.8 Hz, 2H) and 7.27 (d, J = 8.8 Hz, 2H), 7.35 (d, J = 8.8 Hz, 2H, aminophenyls), 7.30 (d, J = 1.9 Hz, 1H, 7-H), 7.60 (d, J = 8.3 Hz, 1H, 10-H), 7.65 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H, 9-H).
3g	$C_{22}H_{16}ClN_5$(385.9)	3.8-4.3 (2H, 11-H), 7.30 (d, J = 2.0 Hz, 1H, 7-H), 7.46 (s, 1H, 2-H), 7.62 (d, J = 8.2 Hz, 1H, 10-H), 7.67 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 9-H), 6.68 (d, J = 8.6 Hz, 2H) and 7.40 (d, J = 8.6 Hz, 2H, aminophenyl), 7.68 (d, J = 5.6 Hz, 2H) and 8.62 (d, J = 5.6 Hz, 2H, pyridyl).
3h	$C_{23}H_{17}ClN_4$(384.9)	MS (EI): m/z (%): M: 384/386 (100/33).
3i	$C_{18}H_{14}Cl_2N_4$(357.2)	(in $CDCl_3$) 2.22 (d, J = 1.0 Hz, 3H, 2-CH_3), 3.96 (br. s, 2H, 11-H), 4.08 (br. s, 2H, NH_2), 6.98 (q, J = 1.0 Hz, 1H, 3-H), 6.65 (d, J = 8.0 Hz, 2H) and 7.53 (d, J = 8.6 Hz, 2H, aminophenyl), 7.30 (s, 1H), 7.39 (s, 1H).
3j	$C_{18}H_{14}Cl_2N_4$(357.2)	2.21 (d, J = 1.0 Hz, 3H, 3-CH_3), 4.00 (br. s, 2H, 11-H), 6.60 (q, J = 1.0 Hz, 1H, 2-H), 6.68 (d, J = 8.6 Hz, 2H) and 7.45 (d, J = 8.6 Hz, 2H, aminophenyl), 7.40 (s, 1H), 7.93 (s, 1H).
3k	$C_{18}H_{15}BrN_4$(367.3)	2.02 (d, J = 1.2 Hz, 3H, 2-CH_3), 3.90 (br. s, 2H, 11-H), 5.90 (br. s, 2H, NH_2), 7.07 (q, J = 1.2 Hz, 1H, 3-H), 6.62 (d, J = 8.5 Hz, 2H) and 7.35 (d, J = 8.5 Hz, 2H, aminophenyl), 7.32 (d, J = 2.2 Hz, 1H, 7-H), 7.47 (d, J = 8.3 Hz, 1H, 10-H), 7.75 (dd, J_1 = 8.2 Hz, J_2 = 2.2 Hz, 1H, 9-H).
3l	$C_{18}H_{15}BrN_4$(367.3)	2.22 (d, J = 1.0 Hz, 3H, 3-CH_3), 3.92 (br. s, 2H, 11-H), 5.82 (br. s, 2H, NH_2), 6.55 (q, J = 1.0 Hz, 1H, 2-H), 6.65 (d, J = 8.6 Hz, 2H) and 7.42 (d, J = 8.6 Hz, 2H, aminophenyl), 7.32 (d, J = 1.8 Hz, 1H, 7-H), 7.50 (d, J = 8.0 Hz, 1H, 10-H), 7.72 (dd, J_1 = 8.0 Hz, J_2 = 1.8 Hz, 1H, 9-H).
3m	$C_{19}H_{17}BrN_4$(381.3)	1.98 (s, 3H) and 2.16 (s, 3H, CH_3), 3.85 (br. s, 2H, 11-H), 5.80 (s, 2H, NH_2), 6.65 (d, J = 8.6 Hz, 2H) and 7.34 (d, J = 8.6 Hz, 2H, aminophenyl), 7.31 (d, J = 1.9 Hz, 1H, 7-H), 7.45 (d, J = 8.2 Hz, 1H, 10-H), 7.75 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 1H, 9-H).
3n	$C_{22}H_{16}BrN_5$(430.3)	3.7–4.3 (br, 2H, 11-H), 5.95 (br. s, 2H, NH_2), 6.67 (d, J = 8.6 Hz, 2H) and 7.40 (d, J = 8.6 Hz, 2H, aminophenyl), 7.47 (s, 3H, 2-H), 7.45 (d, J = 2.0 Hz, 1H, 7-H), 7.57 (d, J = 8.2 Hz, 1H, 10-H), 7.78 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, 9-H), 7.71 (d, J = 5.6 Hz, 2H) and 8.63 (d, J = 5.6 Hz, 2H, pyridyl).
3o	$C_{20}H_{17}ClN_4O$(364.8)	(in $CDCl_3$) 2.18 (d, J = 1.0 Hz, 3H, 2-CH_3), 2.23 (s, 3H, acetyl), 3.94 (br. s, 2H, 11-H), 6.98 (q, J = 1.0 Hz, 1H, 3-H), 7.18 (d, J = 2.2 Hz, 1H, 7-H), 7.31 (d, J = 8.2 Hz, 1H, 10-H), 7.46 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 1H, 9-H), 7.63 and 7.68 (d, J = 8.8 Hz, acetylaminophenyl), 8.13 (br. s, 1H, NH-Ac).
3p [a]	$C_{18}H_{14}BrCl_2N_3$(423.1)	2.32 (d, J = 1.0 Hz, 3H, 2-CH_3), 4.48 (br. s, 2H, 11-H), 7.12 (q, J = 1.0 Hz, 1H, 3-H), 7.55–7.80 (m, 5H), 7.90–8.00 (m, 2H).
3q [a]	$C_{18}H_{14}BrCl_2N_3$(423.1)	2.35 (d, J = 1.0 Hz, 3H, 3-CH_3), 4.45 (br. s, 2H, 11-H), 7.08 (d, J = 2.1 Hz, 1H, 7-H), 7.43 (q, J = 1.0 Hz, 1H, 2-H), 7.58 (d, J = 8.3 Hz, 1H, 10-H), 7.60–7.73 (m, 4H), 7.95 (dd, J_1 = 8.3 Hz, J_2 = 2.1 Hz, 1H, 9-H).
3r	$C_{18}H_{14}ClN_3$(307.8)	2.30 (br. s, 3H, CH_3), 4.05 (br. s, 2H, 11-H), 6.63 (br. s, 1H, 2-H), 7.12 (d, J = 1.9 Hz, 1H, 7-H), 7.50–7.85 (m, 7H).
4a	$C_{17}H_{14}ClN_5$(323.8)	2.40 (br. s, 3H, 3-CH_3), 4.13 (br, 2H, 11-H), 5.94 (br, 2H, NH_2), 6.64 (d, J = 8.6 Hz, 2H) and 7.43 (d, J = 8.6 Hz, 2H, aminophenyl), 7.22 (br. s, 1H, 7-H), 7.65 (br. s, 2H, 9-H and 10-H).
4b	$C_{21}H_{15}ClN_6$(386.8)	3.95–4.6 (br, 2H, 11-H), 6.67 (d, J = 8.6 Hz, 2H) and 7.42 (d, J = 8.6 Hz, 2H, aminophenyl), 7.28 (br. s, 1H, 7-H), 7.68 (br. s, 2H, 9-H and 10-H), 8.02 (br. s, 2H, pyridyl), 8.82 (br. s, 2H, pyridyl).
4c	$C_{22}H_{17}ClN_6$(400.9)	(in $CDCl_3$) 4.12 (br, 2H, 11-H), 6.72 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H) and 7.54 (d, J = 8.6 Hz, 2H), 7.84 (d, J = 8.6 Hz, 2H, aminophenyls), 7.32 (d, J = 2.1 Hz, 1H, 7-H), 7.40 (d, J = 8.2 Hz, 1H, 10-H), 7.50 (dd, J_1 = 8.2 Hz, J_2 = 2.1 Hz, 1H, 9-H)
4d	$C_{18}H_{16}ClN_5O$(353.8)	3.35 (s, 3H, OCH_3), 4.20 (br. s, 2H, 11-H), 4.62 (s, 2H, O-CH_2), 5.98 (br. s, 2H, NH_2), 6.63 (d, J = 8.7 Hz, 2H) and 7.42 (d, J = 8.7 Hz, 2H, aminophenyl), 7.22 (br. s, 1H, 7-H), 7.62 (br. s, 2H, 9-H and 10-H).

[a]Hydrochloride salts.

They were treated ip 30 min before testing. In the inclined screen test the untrained mice were placed on a screen inclining 60°. The number of falling mice was noted within 60 s test period and scored as positive. In the rotarod test mice were trained to do coordinated motor movements for 120 s on the rod (3.5 cm diameter) rotating at 15 rpm. The impairment of coordinated motor movements was defined as inability of selected mice to remain on the rotating rod for 120 s test period.

Transient occlusion of the middle cerebral artery (MCA) in rats. Male CD RB rats, weighing 370–410 g, were

used. The anaesthesia was induced by 5% and maintained by 2% halothane in a mixture of 30% N_2O and 70% O_2. The rectal body temperature was controlled and kept at 37 °C throughout the experimental period using a thermostatically controlled blanket.

The occlusion of the MCA was achieved as follows:[22,23] the left common carotid artery (CCA), the extracranial external carotid artery (ECA) and internal carotid artery (ICA) were exposed through a midline incision. The branches of the ECA were then isolated and coagulated. The pterygopalatine artery, this posteriorly directed extracranial branch of the ICA, was occluded by a microvascular clip. Next a silk suture was tied loosely around the mobilised ECA stump which was cut at its first branching (leaving a stump about 2–3 mm) and used to introduce the embolus. The CCA was occluded by microvascular clip and a 5 cm length of 4–0 monofilament nylon suture, its tip rounded by heating near a flame, was introduced into the ECA lumen (22 mm distal to the carotid bifurcation). This was secured in place by tightening the silk ligature. At this point the intraluminal suture has blocked the origin of the MCA occluding of blood flow from the ICA, anterior and posterior cerebral arteries.

60 min after embolization a second short and light anaesthesia was induced by face mask. The nylon suture and the microvascular clip on the CCA were carefully removed, so the blood-flow from the CCA into the ICA was restored. 24 h after reperfusion the rats were decapitated. Their brains were rapidly removed 5 mm from the frontal pole 3 coronal sections (2 mm thick) were cut out from the cerebrum, immersed in a 2% solution of TTC for vital staining and placed in a 37 °C water-bath for 10 minutes and fixed in 10% formalin solution. TTC stained the intact areas of brain to deep red colour, but did not stain the infarcted tissue.

From each animal three brain slices were obtained with 2-fold magnification by using a copy machine. The areas of ischaemic damages were measured from these black-white pictures by a computerised scanning method (ARTEC SCAN A400).

Antagonism of oxotremorine-induced tremor in mice. 10 male CD1 mice (21–25 g) per group were starved for 16 h, then treated orally with test compounds or vehicle. 60 min later they received oxotremorine 10 mg/kg ip[40] The intensity of tremor caused by oxotremorine was scored (0–0.5–1–2–3) and noted in every 5 min for 30 min. The scores were summed individually, means and SEM were calculated.

MPTP induced dopaminergic neurotoxicity. Male C57 black mice, weighing 23–30 g were used. Three days after a single ip injection of MPTP (30 mg/kg) and four consecutive injections of the test compound (see Table 8 for the treatment schedule for each compound) mice were killed by decapitation. Both striata were dissected and kept at −80 °C until assayed. Striata were weighed and then sonicated in 200 µl of distilled water. Aliquots (80 µl) of the homogenates were resonicated in 80 µl of HPLC mobile phase containing α-methyl-dopamine as internal standard. The samples were centrifuged (10,000×*g* for 15 min at 4 °C) and a 5 µl aliquots were injected into the HPLC system. Calculated values are expressed as µg/g wet tissue.

The concentrations of dopamine, DOPAC, HVA, 5-HT and 5-HIAA in the samples were determined by HPLC/electrochemistry according to Patthy and Gyenge.[41] The HPLC system used in this study consisted of a Knauer 64 isocratic pump with a pulse dampener, a BAS Unijet microbore injector equipped with a 5 µl sample loop, a BAS microbore ODS 3 µm (100×1 mm) analytical column. For electrochemical detection, glassy carbon electrodes (BAS LC-4C) set at +0.75 V against an Ag/AgCl reference electrode were used. The mobile phase composition was aqueous buffer:acetonitrile (95:5) ($^{v}/_{v}$). The aqueous buffer consisted of 3 mM HFBA (heptafluorobutyric acid) as pairing ion, 100 mM sodium hydroxide, 0.1 g/l Na_2EDTA, and the pH was adjusted to 4.3 with 42.5% ($^{w}/_{v}$) phosphoric acid. All other chemicals used were of analytical reagent grade. One-way analysis of variance (ANOVA) followed by the Duncan's multiple range test was used for statistical analysis.

Acute toxicity in mice. The LD_{50} values were calculated from lethality within 14 days after ip and po administration of the drug.[42]

Acknowledgements

The authors wish to express their thanks to the Hungarian National Committee for Technological Development (OMFB) for the generous financial support of the project.

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