Pyrrolidine-2,4-diones with Affinity to the *N*-Methyl-D-aspartate (Glycine Site) Receptor, Part II [1]

5-Arylidene-pyrrolidine-2,3,4-trione 3-Oximes as NMDA Receptor Antagonists

Hermann Poschenrieder, Georg Höfner, and Hans-Dietrich Stachel*

Institut für Pharmazie/Zentrum für Pharmaforschung der Universität München, Butenandtstraße 7, D-81377 München, Germany

Key Words: *3-Hydroxyiminopyrrolidine-2,4-diones; 3-hydroxyiminopyridine-2,4-diones; nitrosation; NMDA (glycine site) receptor affinity; [^{3}H]MDL 105,519 displacement*

Summary

A series of oximes deriving from 5-arylidene-pyrrolidine-2,3,4-triones and pyridine-2,3,4-triones has been prepared. The presence of the tautomeric nitrosoenol was proven in solutions of α-ketooxime **7a**. The binding affinity of the new oximes toward the *N*-methyl-D-aspartate (glycine site) receptor has been measured as a basis for more detailed structure-activity relationship studies. Oxime **13b** showed the highest binding potency acting as glycine antagonist in nanomolar concentration.

Introduction

The function of L-glutamate as the primary excitatory transmitter in the mammalian central nervous system has been generally accepted since the 1980s. It is now established that excessive stimulation by excitatory amino acids like glutamate causes degeneration and death of neurons and that *N*-methyl-D-aspartate (NMDA) receptors, a subtype of ionotropic glutamate receptors, plays a major role in this excitotoxicity of glutamate [2]. It is also well known that glycine is an obligatory co-agonist for NMDA receptor activation [3, 4]. Therefore the glycine site on NMDA receptors represents an interesting target in the development of potential neuroprotective drugs.

A number of compounds from different classes have been recognized as functional antagonists at the glycine site such as kynurenic acid derivatives [5], certain 1,2,3,4-tetrahydroquinoline-2-carboxylic acids [6], 4-hydroxyquinoline-2(1H)ones **1** [7], quinazoline-2,4-diones [8], and aryl-substituted 3-arylidene tetramic acids **2** [1]. These compounds have in common certain steric and structural aspects including the presence of an acid group, i.e. a carboxyl or β-dicarbonyl function [9], and a sufficient lipophilicity [7]. An account of aspects of structure-activity relationships of compounds active at the glycine site of the NMDA receptor and possible therapeutic implications has been given by Dannhardt and Kohl [10].

In a recent paper the high affinity of 1,2,3,4-tetrahydroquinoline-2,3,4-trione 3-oximes **3** for the NMDA receptor glycine site has been stated [11]. These compounds, dubbed QTOs, seem to fit into the binding model proposed for this receptor [8, 9]. We have synthesized and tested a series of oximes of the general formula **4**. These pyrrolidine-2,3,4-trione 3-oximes, abbreviated as PTOs, bear a resemblance with the quinolone oximes **3** just mentioned, as is likewise the case for the pair of active compounds **1** and **2**.

Chemistry

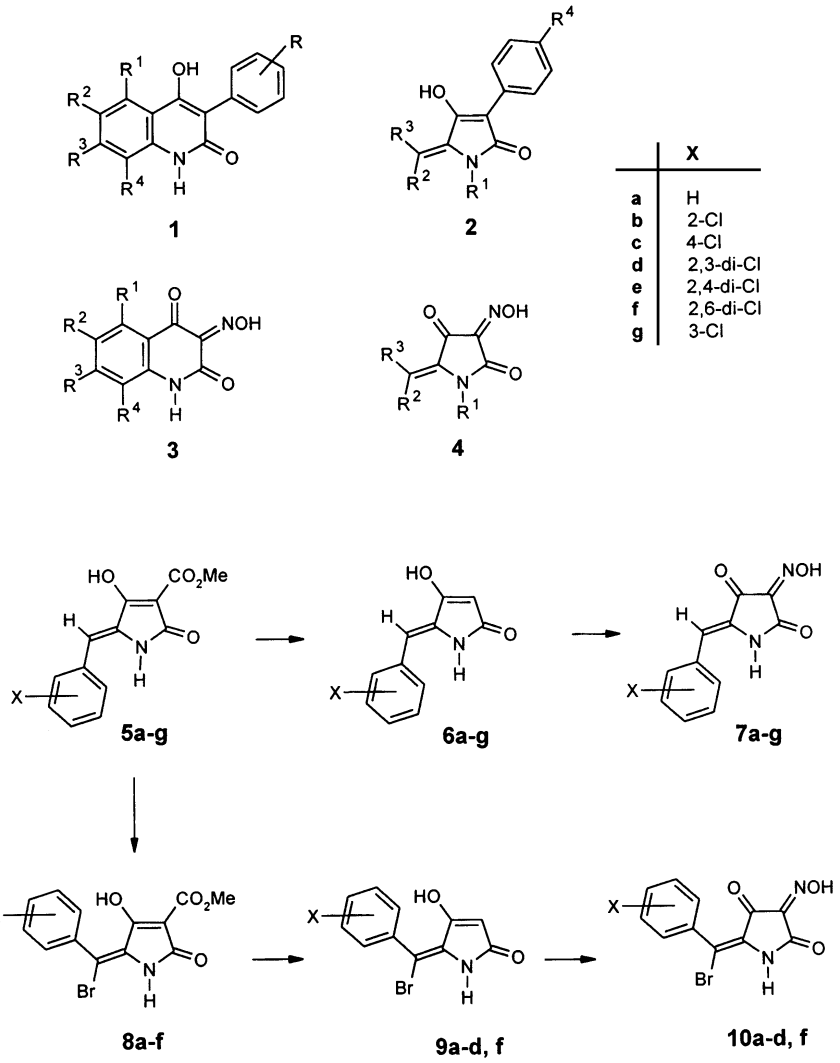
The oximes **7** (Scheme 1) are derivatives of the known tetramic acids **6** [1, 12] and were formed by nitrosation of the CH-acidic precursors **6** with nitrous acid. The NMR spectra of the well crystallizing substances **7** showed duplication of the *NH*- as well as the vinylic proton signals indicating the existence of isomers, possibly the *syn*/*anti* isomers, in a ratio of 2:1 in DMSO solution.

Another set of oximes was obtained starting with the known tetramic acids **5** [1, 12]. As reported earlier, compound **5a** is easily transformed into lactam **9a** [13] by bromination to **8a** [14] followed by saponification and decarboxylation. We have ramified this reaction sequence in preparing the brominated lactams **8b–f** and **9b–d**, **f** from compounds **5b–f**. All new tetramic acids **9** formed mixtures of the respective keto-enol tautomers in a ratio of 1:1 in DMSO as judged from their ^{1}H NMR spectra. Finally nitrosation of the tetramic acids **9** afforded the oximes **10**. According to their NMR spectra, all these oximes formed equilibria of two isomers in a ratio of 2:1 when dissolved in DMSO.

It is known that the brominated tetramic acid **8a** is capable of nucleophilic displacement of the vinylic halogen [13]. With sodium methoxide mainly the methoxylated tetramic acid derivative **11a** was formed, accompanied by the pyridone **14a** as a by-product (Scheme 2). Analogously the treatment of brominated tetramic acids **8b–f** with sodium methoxide led to the substituted tetramic acids **11b–f** as well as the pyridones **14b**, **c**, **f** with yields of the ring enlargement products differring in a wide range. So in the reaction of the bromotetramic acid **8f** the pyridone **14f** was found to be the chief component. In general, using potassium methoxide instead of the sodium salt enhanced the amount of the ring enlargement products **14** at the expense of the substitution products **11**. However, in the reaction of **8a** with potassium ethoxide or propoxide we observed only substitution of the halogen and no ring enlargement at all. Since this reaction was accompanied by transesterification, the corresponding tetramic acids **17** or **18** were isolated as the sole products.

The tetramic acids **11**, **17**, and **18** were subjected to dealkoxycarbonylation forming the new tetramic acids **12**, **19**, and **20** all of which showed keto-enol tautomerism in a ratio of 3:1 in DMSO. Compound **12a** has been prepared previously in this way [13]. In comparison it was less simple to accom-

  0365-6233/99/0909/0309 $17.50 +.50/0



Scheme 1

plish the removal of the ester group of pyridones **14** by saponification and decarboxylation leading to the 3-unsubstituted pyridones **15.** Compound **15a** has been prepared in this way already [13]. According to the ^{1}H NMR spectra pyridones **15** exist predominantly as enols in DMSO as solvent.

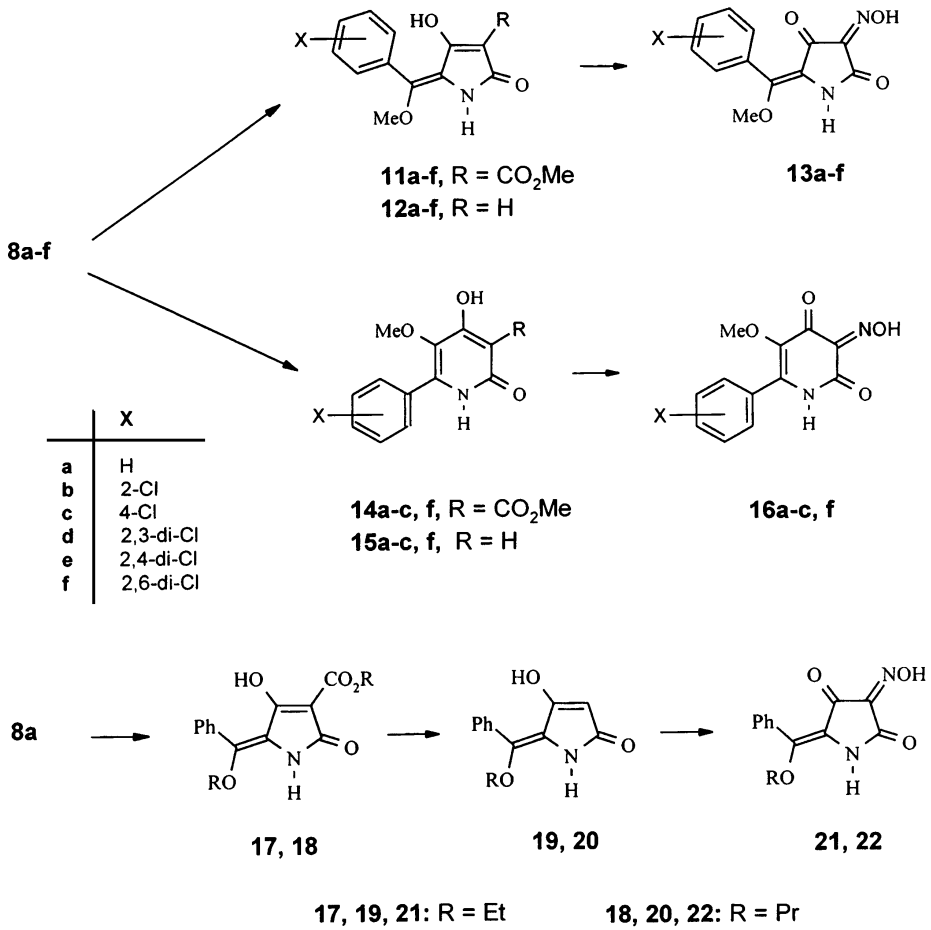
Nitrosation of these new compounds **12**, **15**, **19**, and **20** led to the usually yellow or orange colored pyrrolidinetrione oximes **13a–f**, and **21/22** and to the yellow pyridinetrione oximes **16a–c, f**, respectively. Judging from the NMR spectra, all but oxime **16** were 1:2 mixtures of two isomers.

These findings led to the question whether the observed isomers of the tetramic acid oximes **4** are the assumed *syn*/*anti* isomers or the isomers resulting from an α-ketooxime-nitrosoenol tautomerism. We have examined this question in the case of oxime **7a** which may also exist as nitrosotetramic acid **23**. Informative was the synthesis of the oxime derivative **25** by reaction of the hitherto unknown pyrrolidinetrione **26** and *O*-methylhydroxylamine (Scheme 3) and the comparison of the ^{13}C NMR spectra of **25** and **7a**. We observed nearly congruent signals of the corresponding C atoms. In both cases the C=O signals were doubled. Evidently, **7a** indeed was a mixture of the *syn*- and *anti*-oximes just as the oxime ether **25** is a mixture of *syn*/*anti* isomers.

However, oxime **7a** smoothly reacted with diazomethane forming a compound different from both the *syn*- or *anti*-oxime ethers **25** in nearly quantitative yield. The ^{1}H NMR spectrum revealed that the new isomer of **25** was uniform and contained one methoxy group, the signal of which at 4.3 ppm is in agreement with the expectation for the nitrosotetramic acid derivative **24**. The structure of **24** was finally established by the ^{13}C NMR spectrum which was very similar to that of the nitrotetramic acid **29** with two signals each for the carbonyl-C and the β-C atom between 172 and 150 ppm. The nitro derivative was obtained by oxidation of **7a** or by nitration of oxime **6a**.

Compound **24** may be regarded as a formal ester of a tetramic acid and was therefore expected to react with amines forming tetramic acid amides. In this way, reaction of **24** with benzylamine afforded compound **27**, which also might be seen as a vinylogous nitrosamine. We shall report on the synthesis of other compounds of this type as well as on the results of their pharmacological testing in the near future.

From the reaction of **7a** with diazomethane it is apparent that in ethereal solution the oxime is in equilibrium with the nitrosotetramic acid **23** to a small extent. The disfavored enol is intercepted by diazomethane thus continually disturbing



Scheme 2

the certainly swift equilibrium. Most likely, all the oximes mentioned above will react with diazomethane in the same way. The scope of this reaction is currently under investigation. It is worth mentioning that a similar behavior as in the case of oxime **7a** with respect to tautomerism and the reaction with diazomethane was observed earlier [15] with the formyltetramic acid **28**.

All the oximes are acidic compounds. The pK_a values measured by potentiometric titration of selected PTOs in aqueous dioxane (Table 1) showed a marked dependence on the substituents in the benzene ring as well as in the side chain. The values range from 6.25 to 4.74. As to be expected, the nitrotetramic acid **29** showed a considerable higher acidity. Compounds **7a** and **27–29** may form mesomeric anions of the same type as depicted in general formula **30**. Therefore we see reason to include componds **27–29** and their derivatives in further testing.

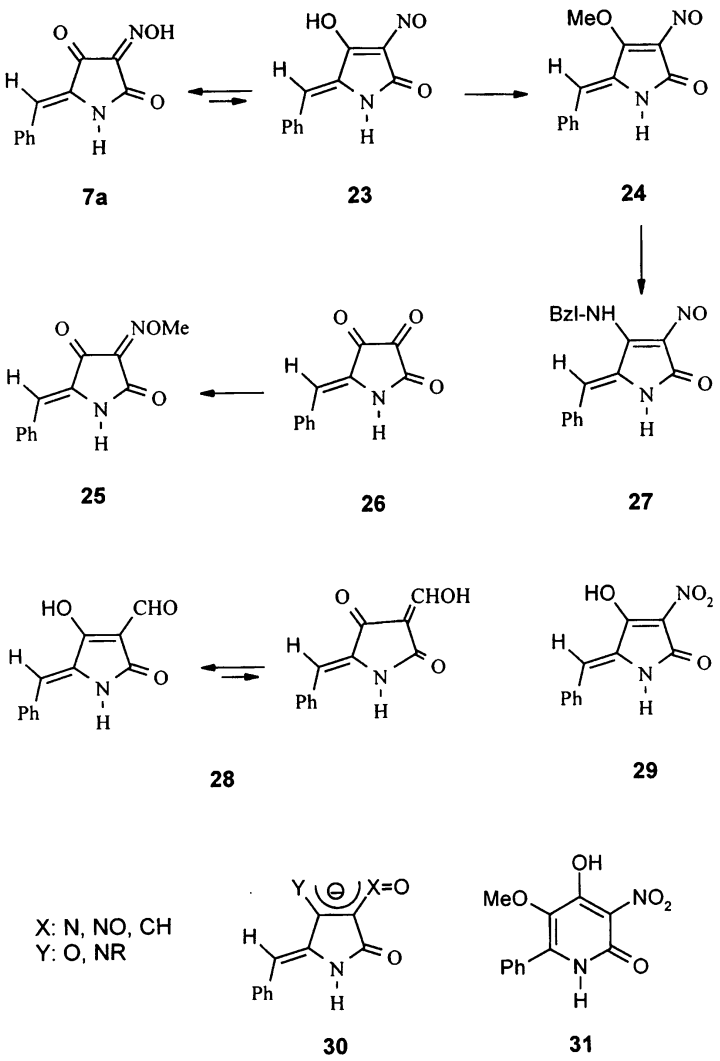
Table 1: pK_a values of selected PTOs and **29** in dioxane-water (1:5).

Compd.	pK_a	Compd.	pK_a
7a	5.75	**13a**	6.25
7b	4.75	**16a**	4.20
10a	4.75	**29**	2.75

As mentioned before, the pyridinetrione oxime **16a** exists as a single isomer as judged from the ^{1}H NMR spectrum. It had to be the oxime because the ^{13}C NMR spectrum is dissimilar to that of the corresponding nitro derivative **31** which was obtained by oxidation of **16a** or directly by nitration of **15a**. Oxime **16a** was found to be a markedly stronger acid than the oxime **7a** derived from a tetramic acid as shown by the respective pK_a values of 4.20 and 5.75.

Pharmacological Results and Discussion

The tetramic acid derived oximes (PTOs) **7a–g, 10a–d,f, 13a–f, and 21/22** as well as the pyridinetrione oximes **16a–c,f** have been evaluated for their potency to inhibit [^{3}H]MDL 105,519 binding at the NMDA receptor associated glycine site in pig cortical brain membranes. The results are summarized in Table 2.



Scheme 3

Table 2: Affinity of new ligands at the NMDA (glycine site) receptor.

Compd. (X)	K_i values (μM). 7	10	13	16	21	22
a (H)	19.4 ± 1.9	0.257 ± 0.033	0.280 ± 0.017	19	0.603 ± 0.053	2.9 ± 0.28
b (2-Cl)	42.8 ± 8.8	0.265 ± 0.081	0.0935 ± 0.0132	>100		
c (4-Cl)	36.2 ± 3.7	10.95	13	36.6		
d (2,3-Cl)	>100	1.372 ± 0.349	0.215 ± 0.013			
e (2,4-Cl)	88.9	13.9 ± 1.45	>100			
f (2,6-Cl)	>100	0.481 ± 0.016	0.318 ± 0.039			
g (3-Cl)	>100					

The PTOs **7a** as well as oxime **16a** inhibited [^{3}H]MDL 105,519 binding in the same order of magnitude in the micromolar range. Their halogenated derivatives showed reduced potency. A higher binding affinity in the nanomolar range was observed with the PTOs **10a** and **13a**. Again, halogenated derivatives of **10a** were less potent with the sole exception of **10b**. On the other hand, halogenated derivatives of **13a** partly showed improved binding. The highest potency of the compounds under investigation was found for **13b** bearing a 2-chlorophenyl group.

Table 3: Inhibition of [^{3}H]MK-801 binding.

Compd.	IC_{50} values (μM)	Compd.	IC_{50} values (μM)
10a	5.31 ± 1.36	**13a**	4.52 ± 1.00
10b	3.62 ± 0.97	**13b**	1.82 ± 0.40

Tentatively, two conclusions can be drawn from these results in the [^{3}H]MDL 105,519 displacement assay. Firstly, that the acid strength of the ligand molecules is playing only a modest role, as shown by the same receptor affinity (260 nM) of oximes **10a** and **13a** despite a considerable difference in acidity (pK_a = 4.75 and 6.25, repectively). And secondly, that the electronic properties of the substituted benzene ring and/or sidechain have little effect on the potency, as illustrated by the same receptor affinity of oximes **10a** and **13a** in spite of the different character of their substituents. The sole exception in this line is 2-chloro-**13a**. The increase in binding potency in comparison with the parent substance may be attributed to the difference in lipophilicity. On the other hand, the stereochemistry of the compounds investigated appear to be highly significant. This assumption is supported, for instance, by the decrease in activity observed after replacement of the side-chain methoxy group in **13a** by an ethoxy- or propoxy group (**20**, **21**). The binding affinity of **21**, however, still is higher than the binding of the parent compound **7a**, both of which differ in the position of the sidechain aryl substituent [1].

Ligands acting as agonists or antagonists at the NMDA receptor associated glycine site can be recorded by enhancement or inhibition of [^{3}H]MK-801 binding performed under non-equilibrium conditions [16, 17]. Compounds **10a**, **10b**, **13a**, and **13b** reduced [^{3}H]MK-801 binding to the level of non-specific binding and can therefore be classified as glycine antagonists. The potencies in reduction of [^{3}H]MK-801 binding (Table 3) correlated with the results observed in [^{3}H]MDL 105,519 displacement assays.

Experimental Section

General

All melting points were determined with a Dr. Tottoli melting point apparatus (Büchi) and are uncorrected. Infrared spectra were measured as potassium bromide plates using IR Spectrometer FT-IR 1600 Series (Perkin Elmer). Ultraviolet spectra were determined in methanolic solutions on Uvicon 810 Anacomp 220 (Kontron Analytik). ^{1}H NMR spectra were recorded using tetramethylsilane as internal standard on spectrometer A 60 A, EM 360 A (Varian) and JEOL GSX 400 (Jeol).

All new compounds gave correct elemental analyses which were carried out applying an Analysator CHN-O-Rapid (Heraeus) or were done by I. Beetz, Mikroanalytisches Laboratorium, Kronach, Germany. The melting points and spectroscopical data of the new compounds are listed in Table 4.

Chemistry

General Procedure for the Synthesis of Oximes **7**, **10**, **13**, **16**

An aqueous solution of sodium nitrite (0.075 g, 1.1 mmol) was added dropwise to a stirred and cooled suspension of the corresponding tetramic acids **6** (2 mmol) [1, 12], **9a** [13], **9b–d**, **f**, **12a** [13], and **12b–f** or the pyridones **15a** [13] and **15b**, **c**, **f** in 5 ml acetic acid. The resulting solution was allowed to remain at room temp. for 0.5 h and than, irrespective of a new precipitation, brought to dryness in vacuo. The residue was purified by crystallization from ethanol. Yellow or orange crystals.

5-Benzylidene-3-hydroxyimino-pyrrolidine-2,4-dione (**7a**): ^{13}C NMR ([D$_6$]DMSO): δ = 182.3, 175.0, 161.8, 156.7, 140.0, 132.8 – 128.3 (split signals), 107.3, 106.9.

General Procedure for the Synthesis of Tetramic acids **8b–f**

Bromine (1.1 ml, 22 mmol) was added to a suspension of compounds **5b–f** (20 mmol) [1, 12], respectively, in acetic acid (50 ml). The resulting solution was allowed to stand at room temp. for 1 h and than brought to dryness in vacuo. The yellow residue was crystallized from methanol.

General Procedure of the Synthesis of Tetramic acids **9b–d**, **f**, **12b–f**, **19**, **20**

A solution of the corresponding tetramic acids **8**, **11**, **17**, **18** (2 mmol) in ethyl acetate (50 ml) was refluxed for 1 h in presence of 1 ml of water. The solution was dried over Na_2SO_4 and concentrated under reduced pressure to afford the pure product.

General Procedure of the Synthesis of Tetramic Acids **11b–f**

A solution of the corresponding tetramic acid **8** (10 mmol) and sodium methoxide (1.6 g, 30 mmol) or potassium methoxide (2.1 g, 30 mmol) in methanol (70 ml) was heated to 150 °C for 1.5 h in a sealed tube. The solution was than brought to dryness in vacuo and the residue dissolved in water. After acidification the solution was extracted with dichloromethane, the organic phase evaporated and the dry residue extracted twice with ethyl acetate. The product remained undissolved and was crystallized from methanol if necessary. The solution was retained for isolation of the respective compounds **14**. The use of potassium methoxide usually changed the ratio of compounds **11** and **14** in favor of the pyridones **14**.

General Procedure for the Synthesis of Pyridones **14b**, **c**, **f**

The mother lie obtained in the preparation of the corresponding tetramic acids **11** was refluxed after addition of a small amount of water. The product separated on cooling.

General Procedure for the Synthesis of Pyridones **15b**, **c**, **f**

The corresponding pyridones **14** (2 mmol) were refluxed with 3 N KOH solution (30 ml). The product precipitated upon acidification.

*Ethyl 5-(α-ethoxybenzylidene)-2-oxo-3-pyrroline-3-carboxylate (***17***)*

The compound was prepared from **8a** (3.2 g, 10 mmol) [13] and potassium ethoxide (2.5 g, 30 mmol) in ethanol (70 ml) analogously to the synthesis of compounds **11**.

*Propyl 5-(α-propoxybenzylidene)-2-oxo-3-pyrroline-3-carboxylate (***18***)*

The compound was prepared from **8a** (3.2 g, 10 mmol) [13] and potassium propoxide (3.0 g, 30 mmol) in propanol (70 ml) analogously to the synthesis of compounds **11**.

*5-Benzylidene-4-methoxy-3-nitroso-pyrrolin-2-one (***24***)*

A suspension of compound **7a** (0.42 g, 2 mmol) in methanol (5 ml) was treated with an excess of an ethereal solution of diazomethane. After the vigorous evolution of N_2 had ceased the solution was evaporated to dryness in vacuo. Orange crystals from methanol.– ^{13}C NMR ([D$_6$]DMSO): δ = 166.6, 147.4, 133.8, 130.1, 129.6, 129.2, 128.7, 128.5, 127.6, 107.6, 103.8, 56.1.

*5-Benzylidene-3-methoxyimino-pyrrolidine-2,4-dione (***25***)*

A solution of trione **26** (0.30 g, 1.5 mmol) and of *O*-methylhydroxylamine hydrochloride (0.17 g, 2 mmol) in methanol (15 ml) was refluxed in the presence of 1 ml triethylamine for 2 h. The product precipitated in pure state. Orange crystals.– ^{13}C NMR ([D$_6$]DMSO): δ = 181.3, 175.1, 160.7, 156.3, 140.5, 132.7 – 128.4 (split signals), 107.7, 107.5, 65.9, 65.8.

Table 4. Chemical and physical data.

Compd.	Analysis Mol. mass	Mp (°C) Yield (%)	UV (methanol, nm): λ_{max} (log ε)	IR (KBr, cm^{-1}): ν	^{1}H NMR ([D$_6$]DMSO): δ
7a	$C_{11}H_8N_2O_3$ 216.19	205 (dec.) 65	295 (4.489)	3435, 3200, 1738, 1715, 1635	14.66 (s, 1H), 11.25 (s, 0.66H), 11.18 (s, 0.33H), 7.64–7.31 (m, 5H), 6.42 (s, 0.33H), 6.36 (s, 0.66H)
7b	$C_{11}H_7ClN_2O_3$ 250.64	176 60	305 (4.267)	3478, 1745, 1718, 1642	11.40 (s, 0.66H), 11.34 (s, 0.33H), 7.46–7.21 (m, 4H), 6.52 (s, 0.33H), 6.47 (s, 0.66H)
7c	$C_{11}H_7ClN_2O_3$ 250.64	190 50	301 (4.354)	3230, 1739, 1718, 1639, 1587	11,35 (s, 0.66H), 11,28 (s, 0.33H), 7.67–7.64 (m, 2H), 7,45–7.35 (m, 2H), 6.40 (s, 0.33H), 6.35 (s, 0.66H).
7d	$C_{11}H_6Cl_2N_2O_3$ 285.09	180 55	221 (4.157), 290 (4.292)	3433, 3181, 1743, 1721, 1638	11.43 (s, 0.66H), 11.37 (s, 0.33H), 7.63–7.37 (m, 3H), 6.50 (s, 0.33H), 6.45 (s, 0.66H)
7e	$C_{11}H_6Cl_2N_2O_3$ 285.09	176 45	300 (4.297)	3435, 1745, 1723, 1646	11.38 (s, 0.66H), 11.32 (s, 0.33H), 7.68–7.65 (m, 2H), 7.45–7.43 (m, 1H), 6.42 (s, 0.33H), 6.36 (s, 0.66H)
7f	$C_{11}H_6Cl_2N_2O_3$ 285.09	232 65	221 (4.277), 267 (4.392)	3230, 1742, 1710, 1662	11.12 (s, 0.66H), 11.08 (s, 0.33H), 7.50–7.48 (m, 2H), 7.39–7.35 (m, 1H), 6.27 (s, 0.33H), 6.22 (s, 0.66H)
7g	$C_{11}H_7ClN_2O_3$ 250.64	185 50	293 (4.369)	3473, 1735, 1709, 1638	14.69 (s, 0.66H), 14.53 (s, 0.33H), 11.47 (s, 0.66H), 11.40 (s, 0.33H), 7.72–7.20 (m, 4H), 6.38 (s, 0.33H), 6.33 (s, 0.66H).
8b	$C_{13}H_9BrClNO_4$ 358.57	215 (dec) 60	297 (4.222)	3190, 1724, 1673, 1659, 1600	9.79 (s, 1H), 7.54–7.38 (m, 4H), 3.61 (s, 3H)
8c	$C_{13}H_9BrClNO_4$ 358.57	195 55	225 (4.204), 313 (4.239)	3170, 1720, 1656, 1598	9.67 (s, 1H), 7.49–7.46 (m, 4H), 3.64 (s, 3H)
8d	$C_{13}H_8BrCl_2NO_4$ 393.02	220 70	299 (4.177)	3420, 1724, 1659, 1597	9.82 (s, 1H), 7.70–7.44 (m, 3H), 3.63 (s, 3H)
8e	$C_{13}H_8BrCl_2NO_4$ 393.02	220 (dec.) 85	298 (4.250)	3187, 1716, 1673, 1658, 1598, 1581	9.79 (s, 1H), 7.78–7.51 (m, 3H), 3.65 (s, 3H)
8f	$C_{13}H_8BrCl_2NO_4$ 393.02	224	289 (4.049)	3432, 3184, 1718, 1657, 1602	9.65 (s, 1H), 7.59–7.45 (m, 3H), 3.60 (s, 3H)
9b	$C_{11}H_7BrClNO_2$ 300.54	178 70	288 (4.170)	3197, 1764, 1714, 1640	11.48, 10.90, 9.38 (3s, 1.5H), 7.49–7.35 (m, 4H), 4.96 (d, 0.5H, J = 2Hz), 3.34 (s, 1H)
9c	$C_{11}H_7BrClNO_2$ 300.54	187 75	228 (4.174), 306 (4.166)	3147, 1755, 1712, 1624, 1589	10.75, 9.29 (2s, 1H), 7.42–7.40 (m, 4H), 5.01 (d, 0.5H, J = 2Hz), 3.24 (s, 1H)
9d	$C_{11}H_6BrCl_2NO_2$ 334.99	193 90	288 (4.351)	3178, 1760, 1722, 1637	11.56, 10.97, 9.46 (3s, 1.5H), 7.65–7.34 (m, 3H), 4.96 (d, 0.5H, J=2Hz), 3.27 (s, 1H)
9f	$C_{11}H_6BrCl_2NO_2$ 334.98	235 (dec) 80	283 (4.038)	3134, 1762, 1724, 1647	11.60, 11.07 (2s, 1H), 7.54–7.34 (m, 3H), 4.95 (d, 0.5H, J=2Hz), 3.37 (s, 1H)
10a	$C_{11}H_7BrNO_3$ 295.09	158 65	266 (4.250)	3430, 3221, 2866, 1743, 1725, 1616	14.46 (s, 1H), 11.06 (s, 0. 70H), 11.00 (s, 0.30H), 7.40–7.26 (m, 5H)
10b	$C_{11}H_6BrClN_2O_3$ 329.54	215 60	263 (4.276)	3177, 2887, 1747, 1726, 1646	1195 (s, 1H), 11.26 (s, 0.66H), 11.20 (s, 0.33H), 7.52–7.30 (m, 4H)
10c	$C_{11}H_6BrClN_2O_3$ 329.54	208 40	265 (4.297)	3208, 1750, 1725, 1608	11.10 (s, 0.66H), 11.04 (s, 0.33H), 7.45–7.44 (m, 4H)
10d	$C_{11}H_5BrCl_2N_2O_3$ 363.98	188 45	262 (4.320)	3179, 2892, 1747, 1728, 1649	11.98 (s, 1H), 11.36 (s, 0.66H), 11.30 (s, 0.33H), 7.69–7.66 (m, 1H), 7.40–7.38 (m, 2H)
10f	$C_{11}H_5BrCl_2N_2O_3$ 363.98	195 60	264 (4.324)	3545, 3180, 1741, 1718, 1643	11.49 (s, 0.66H), 11.43 (s, 0.33H), 7.56–7.43 (m, 3H)
11b	$C_{14}H_{12}ClNO_5$ 309.70	183 (dec.) 40	234 (4.009), 325 (4.258)	3357, 1713, 1674, 1624, 1584	9.58 (s, 1H), 8.30 (s, 1H), 7.58–7.50 (m, 4H), 3.63 (s, 3H), 3.40 (s, 3H)
11c	$C_{14}H_{12}ClNO_5$ 309.70	192 30	236 (4.133), 332 (4.286)	3352, 1708, 1167, 1622, 1578	9.88 (s, 1H), 9.61 (s, 1H), 7.64–7.44 (m, 4H), 3.66 (s, 3H), 3.44 (s, 3H)
11d	$C_{14}H_{11}Cl_2NO_5$ 314.15	179 80	326 (4.162)	3380, 1705, 1675, 1640, 1586	9.63 (s, 1H), 7.79–7.46 (m, 3H), 3.62 (s, 3H), 3.42 (s, 3H)
11e	$C_{14}H_{11}Cl_2NO_5$ 344.15	208 (dec.) 60	325 (4.273)	3342, 1709, 1672, 1623, 1578	9.72 (s, 1H), 7.90–7.62 (m, 3H), 3.71 (s, 3H), 3.51 (s, 3H)
11f	$C_{14}H_{11}Cl_2NO_5$ 344.15	202 15	307 (4.103)	3430, 1734, 1675, 1644	12.18 (s, 1H), 9.66 (s, 1H), 7.65–7.57 (m, 3H), 3.62 (s, 3H), 3.45 (s, 3H)
12b	$C_{12}H_{10}ClNO_3$ 251.67	181 75	306 (4.156)	3434, 3176, 1706, 1680, 1639, 1608	10.37, 8.99 (2s, 1H), 7.54–7.38 (m, 4H), 4.74 (d, 0.25 H, J = 2Hz), 3.34 (s, 3H), 3.05 (s, 1.5 H)
12c	$C_{12}H_{10}ClNO_3$ 251.67	200 65	239 (4.043), 319 (4.149)	3212, 1707, 1642, 1552	10.98, 10.38, 9.04 (3s, 1.25H), 7.49–7.40 (m, 4H), 4.87 (d, 0.25H, J=2Hz), 3.41 (s, 3H), 3.08 (s, 1.5H)
12d	$C_{12}H_9Cl_2NO_3$ 286.11	195 50	305 (4.108)	3124, 1740, 1707, 1638	10.87, 10.43, 9.07 (3s, 1.25H), 7.76–7.38 (m, 3H), 4.76 (d, 0.25H, J=2Hz), 3.38 (s, 3H), 3.07 (s, 1.5H)
12e	$C_{12}H_9Cl_2NO_3$ 286.11	168 60	308 (4.114)	3428, 1691, 1633, 1610	10.85, 10.43, 9.08 (3s, 1.25H), 7.53–7.42 (m, 3H), 4.77 (d, J=2Hz, 0.25H), 3.38 (s, 3H), 3.08 (s, 1.5H)

Table 4. *Continued.*

Compd.	Analysis Mol. mass	Mp (°C) Yield (%)	UV (methanol, nm): λ_{max} (log ε)	IR (KBr, cm^{-1}): ν	^{1}H NMR ([D$_6$]DMSO): δ
12f	$C_{12}H_9Cl_2NO_3$ 286.11	206 55	304 (4.054)	3434, 1712, 1648, 1559	10.81, 10.47, 9.72 (3s, 1.25H), 7.61–7.53 (m, 3H), 4.72 (d, 0.25H, J=2Hz), 3.56 (s, 3H), 3.11 (s, 1.5H)
13a	$C_{12}H_{10}N_2O_4$ 246.22	174 60	278 (4.269)	3433, 1734, 1714, 1627	11.95 (s, 1H), 10.74 (s, 0.66H), 10.71 (s, 0.33H), 7.46–7.42 (m, 5H), 3.42 (s, 1H), 3.41 (s, 2H)
13b	$C_{12}H_9ClN_2O_4$ 280.67	171 55	270 (4.239)	3202, 2941, 1738, 1717, 1642	10.83 (s, 0.66H), 10.79 (s, 0.33H), 7.60–7.43 (m, 4H), 3.45 (s, 1H), 3.40 (s, 2H)
13c	$C_{12}H_9ClN_2O_4$ 280.67	193 60	289 (4.181)	3244, 1733, 1716, 1622	10.80 (s, 0.66H), 10.76 (s, 0.33H), 7.51–7.43 (m, 4H), 3.44 (s, 1H), 3.43 (s, 2H)
13d	$C_{12}H_8Cl_2N_2O_4$ 315.11	152 40	267 (4.221)	3205, 2944, 1738, 1720, 1645	10.87 (s, 0.66H), 10.84 (s, 0.33H), 7.79–7.43 (m, 3H), 3.42 (t, 3H)
13e	$C_{12}H_8Cl_2N_2O_4$ 315.11	150 (dec.) 35	267 (4.251)	3186, 2940, 1738, 1715, 1634, 1586	10.78 (s, 0.66H), 10.74 (s, 0.33H), 7.79–7.78 (m, 1H), 7.53–7.45 (m, 2H), 3.42 (s, 1H), 3.41 (s, 2H)
13f	$C_{12}H_8Cl_2N_2O_4$ 315.11	151 45	269 (4.174)	3210, 2944, 1737, 1713, 1639	10.95 (s, 0.66H), 10.91 (s, 0.33H), 7.65–7.56 (m, 3H), 3.57 (s, 1H), 3.46 (s, 2H)
14b	$C_{14}H_{12}ClNO_5$ 309.70	209 15	228 (4.214), 329 (3.960)	2952, 2771, 1644, 1562	11.54 (s, 1H), 7.60–7.47 (m, 4H), 3.82 (s, 3H), 3.40 (s, 3H)
14c	$C_{14}H_{12}ClNO_5$ 309.70	218 (dec) 25	233 (4.247), 338 (4.091)	3058, 2953, 1668, 1642, 1497	7.65–7.52 (m, 4H), 3.82 (s, 3H), 3.44 (s, 3H)
14f	$C_{14}H_{11}Cl_2NO_5$ 344.15	303 15	219 (4.333),) 328 (3.923	2950, 2771, 1656, 1560	11.60 (s, 1H), 7.62–7.53 (m, 3H), 3.81 (s, 3H), 3.47 (s, 3H)
15b	$C_{12}H_{10}ClNO_3$ 251.67	240 (dec) 90	298 (3.832)	2927, 1646, 1614, 1587	11.00 (s, 1H), 7.56–7.42 (m, 4H), 5.72 (s, 1H), 3.39 (s, 3H)
15c	$C_{12}H_{10}ClNO_3$ 251.67	280 (dec) 75	312 (4.009)	2933, 1644, 1602, 1561	10.81 (s, 1H), 7.64–7.49 (m, 4H), 5.76 (s, 1H), 3.44 (s, 3H)
15f	$C_{12}H_9Cl_2NO_3$ 286.11	256 70	297 (3.853)	2930, 1648, 1613, 1562	10.94 (s, 1H), 7.58–7.50 (m, 3H), 5.76 (s, 1H), 3.46 (s, 3H)
16a	$C_{12}H_{10}N_2O_4$ 246.22	192 70	280 (3.995)	3437,1700, 1684, 1602, 1524	10.92 (s, 1H), 7.56–7.50 (m, 5H), 3.48 (s, 3H)
16b	$C_{12}H_9ClN_2O_4$ 280.67	204 (dec) 60	342 (3.832)	3422, 1647, 1582	9.62 ((s, 1H), 8.30 (s, 1H), 7.54–7.34 (m, 4H), 3.55 (s, 3H)
16c	$C_{12}H_9ClN_2O_4$ 280.67	230 (dec) 50	300 (3.948)	3077, 2934, 1698, 1644, 1600	10.94 (s, 1H), 7.64–7.49 (m, 4H), 3.49 (s, 3H)
16f	$C_{12}H_8Cl_2N_2O_4$ 315.11	222 45	343 (3.924)	3425, 1648, 1585	7.55–7.43 (m, 3H), 3.47 (s, 3H)
17	$C_{16}H_{17}NO_5$ 303.31	216 40	236 (4.010), 338 (4.295)	3174, 1687, 1646, 1566	9.55 (s, 1H), 7.51–7.46 (m, 5H), 4.18 (q, 2H), 3.67 (q, 2H), 1.23–1.14 (2t, 6H)
18	$C_{18}H_{21}NO_5$ 331.37	169 52	235 (4.043), 338 (4.259)	3178, 2967, 1682, 1563	9.50 (s, 1H), 7.49–7.46 (m, 5H), 4.08 (t, 2H), 3.55 (t, 2H), 1.64–1.57 (sext. 4H), 0.90–0.81 (2t, 6H)
19	$C_{13}H_{13}NO_3$ 231.25	129 45	236 (3.952), 318 (4.088)	3176, 1706, 1651, 1616, 1559	10.29 and 8.95 (2s, 1H), 7.40–7.38 (m, 5H), 4.85 (d, 0.25H, J=2Hz), 3.63 and 3.60 (2q, 2H), 3.09 (s, 1.5H), 1.16 and 1.14 (2t, 3H)
20	$C_{14}H_{15}NO_3$ 245.28	127 55	237 (3.925), 316 (4.130)	3170, 2963, 1740, 1700, 1631	10.81, 10.25, 8.88 (3s, 1,25H), 7.42–7.37 (m, 5H), 4.83 (d, 0.25H, J=2Hz), 3.52–3.45 (2t, 2H), 3.07 (s, 1.5H), 1.61–1.54 (sext, 2H), 0.83–0.80 (2t, 3H)
21	$C_{13}H_{12}N_2O_4$ 260.25	142 55	282 (4.199)	3433, 1739, 1717, 1634	10.70 (s, 0.66H), 10.66 (s, 0.33H), 7.44–7.41 (m, 5H), 3.67–3.62 (2q, 2H), 1.16 (t, 3H)
22	$C_{14}H_{14}N_2O_4$ 274.27	147 45	281 (4.098)	3244, 1739, 1715, 1623	10.72 (s, 0.66H), 10.68 (s, 0.33H), 7.65–7.35 (m, 5H), 3.50 (2t, 2H), 1.64–1.48 (1 sext., 2H), 0.83 (t, 3H)
24	$C_{12}H_{10}N_2O_3$ 230.22	172 90	322 (4.51)	3190, 1718, 1697, 1636	11.16 (s, 1H), 7.66–7.33 (m, 5H), 6.42 (s, 1H), 4.34 (s, 3H)
25	$C_{12}H_{10}N_2O_3$ 230.22	230 (dec) 45	292 (4.392), 382 (3.300)	3207, 1741, 1720, 1639	11.33 (s, 1H), 7.64–7.31 (m, 5H), 6.44 (s, 0.25H), 6.38 (s, 0.75H), 4.25 (s, 2.25H), 4.24 (s, 0.75H)
26	$C_{11}H_7NO_3$ 201.18	140 (dec) 80	259 (3.998), 330 (4.066)	3336, 1755, 1718, 1636	11.10 (s, 1H), 7.63–7.34 (m, 5H), 6.40 (s, 1H)
27	$C_{18}H_{15}N_3O_2$ 305.34	220 (dec) 60	304 (4.402), 390 (4.223), 542 (3.921)	3194, 1696, 1618	10.06 (s, 1H), 8.09 (s, 1H), 7.57–7.23 (m, 10H), 6.09 (s, 1H), 4.06 (s, 2H)
29	$C_{12}H_8N_2O_4$ 232.19	250(dec) 60	232 (4.010), 324 (4.484)	3361, 1704, 1650	9.30 (s, 1H), 7.54–7.18 (m, 5H), 6.14 (s, 1H)
31	$C_{12}H_{10}N_2O_5$ 262.22	238 (dec) 46	319 (3.759), 377 (3.777)	3065, 2953, 1676	11.89 (s, 1H), 7.61–7.50 (m, 5H), 3.32 (s, 3H)

*5-Benzylidene-pyrrolidine-2,3,4-trione (***26***)*

A solution of 5-benzylidene-3,4-dihydroxy-3-pyrrolin-2-one (0.40 g, 2 mmol) [18] in dioxane/water (1:4) was titrated with 0.1 N iodine solution in the presence of soluble starch until a faint blue color appeared. The solution was extracted twice with ethyl acetate, the organic phase dried over Na_2SO_4. The volatile was removed in vacuo and the residue purified. Colorless crystals (from dichloromethane).

*4-Benzylamino-5-benzylidene-3-nitroso-3-pyrroline-2-one (***27***)*

A solution of compound **24** (0.11 g, 0.5 mmol) in methanol (50 ml) was heated with 0.25 ml benzylamine for 2 min. The red-violet precipitate was crystallized from methanol.

*5-Benzylidene-4-hydroxy-3-nitro-3-pyrroline-2-one (***29***)*

To a suspension of tetramic acid **6a** (0.18 g, 1 mmol) in acetic acid (2 ml) was added 0.5 ml of concentrated nitric acid. After a few min the precipitation of the product was complete. Yellow crystals.– ^{13}C NMR ([D_6]DMSO): δ = 172.0, 165.0, 134.4, 131.9, 129.2, 128.7, 127.3, 113.5, 104.4.

*4-Hydroxy-5-methoxy-3-nitro-6-phenyl-pyridine-2-one (***31***)*

A suspension of pyridone **15a** (0.11 g, 0.5 mmol) in acetic acid (2 ml) was treated with 0.2 ml of concentrated nitric acid. The mixture was evaporated to dryness irrespective of a precipitation and crystallized from acetic acid. Yellow crystals.

Pharmacology

[^{3}H]MDL 105,519 and [^{3}H]MK-801 binding to pig cortical brain membranes were performed as previously described [16]. Test compounds insoluble in water were dissolved by addition of DMSO. The total amount of DMSO in the assay did not exceed 1 % and inhibited binding only negligibly.

IC_{50} values for test compounds were calculated from experiments with at least six concentrations of test compounds using InPlot 4.0 (GraphPad Software, San Diego, CA). The K_D value for [^{3}H]MDL 105,519 used in the Cheng-Prusoff equation [19] to calculate Ki values was determined in saturation experiments as 3.73 ± 0.43 nM for [^{3}H]MDL 105,519. If not stated otherwise, data are expressed as means ± SEM of three experiments, each carried out in triplicate.

References

[1] Part I: H. Poschenrieder, G. Höfner, H.-D. Stachel, *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 389–394.

[2] S. M. Rothman, J. W. Olney, *Trends Neurosci.* **1995**, *18*, 57–58.

[3] J. W. Johnson, P. Ascher, *Nature (London)* **1987**, *325*, 529–531.

[4] B. S. Meldrum, *Excitatory Amino Acid Antagonists*, Blackwell Scientific Publications, Oxford 1991.

[5] P. D. Leeson, R. Baker, R. W. Carling, N. R. Curtis, K. W. Moore, B. J. Williams, A. C. Foster, A. E. Donald, J. A. Kemp, G. R. Marshall, *J. Med. Chem.* **1991**, *34*, 1243–1252.

[6] P. D. Leeson, R. W. Carling, K. W. Moore, A. M. Moseley, J .D. Smith, G. I. Stevenson. T. Chan, R. Baker, A.C. Foster, S. Grimwood, J. A. Kemp, G. R. Marshall, K. J. Hoogsteen, *J. Med. Chem.* ***1992***, *35*, 1954–1968.

[7] M. Rowley, J. J. Kulagowski, A. P. Watt, D. Rathbone, G. I. Stevenson, R.W. Carling, Baker, G. B. Marshall, J. A. Kemp, A.C. Foster, S. Grimwood, R. Hargreaves,. C. Hurley, K. L. Saywell, M. D. Tricklebank, P. D. Leeson, *J. Med. Chem.* **1997**, *40*, 4053–4068.

[8] V. Carlotta, D. Catarzi, F. Varano, L. Cecchi, G. Filacchioni, A. Galli, C. Costagli, *Arch. Pharm. Pharm. Med. Chem.*, **1997**, *330*, 129–134.

[9] I. M. Mawer, J. J. Kulagowski, P. D. Leeson, S. Grimwood, G. R. Marshall, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2643–2648.

[10] G. Dannhardt, B. K. Kohl, *Current Med. Chem.*, **1998**, *5*, 253–263.

[11] S. X. Cai, Z.-L. Zhou, J.-C. Huang, E. R. Whittemore, Z. O. Egbuwoku, Y. Lu, J. E. Hawkinson, R. M. Woodward, E. Weber, J. F. W. Keana, *J. Med. Chem.* **1996**, *39*, 3248–3255.

[12] H.-D. Stachel, K. K. Harigel, H. Poschenrieder, H. Burghard, *J. Heterocycl. Chem.* **1980**, *17*, 1195–1199.

[13] H.-D. Stachel, H. Poschenrieder, *Liebigs Ann. Chem.*, **1985**, 1692–1696.

[14] H.-D. Stachel, H. Poschenrieder, *Arch. Pharm. (Weinheim)*, **1985**, *318*, 311–318.

[15] H. Burghard, *PhD. Thesis*, Universität München 1972

[16] G. Höfner, K.Th. Wanner, Neurosci. *Lett.* **1997**, *226*, 79–82.

[17] Y. Yoneda, T. Suzuki, K. Ogita, D. Han, *J. Neurochem.* **1993**, *60*, 634–645.

[18] H.-D. Stachel, H. Poschenrieder, H. Burghard, *Z. Naturforsch.* **1980**, *35b*, 724–726.

[19] Y.-C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

Received: April 4, 1999 [FP383]