# Substituted Analogues of GV150526 as Potent Glycine Binding Site Antagonists in Animal Models of Cerebral Ischemia

Romano Di Fabio,* Nadia Conti, Elisabetta De Magistris, Aldo Feriani, Stefano Provera, Fabio Maria Sabbatini, Angelo Reggiani, Luca Rovatti, and Robert J. Barnaby

*Medicines Research Centre, Glaxo Wellcome S.p.A., Via Fleming 4, 37100 Verona, Italy*

*Received October 9, 1998*

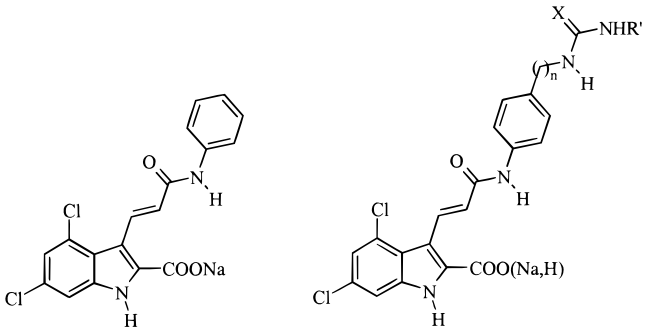
A series of analogues of the indole-2-carboxylate GV150526, currently in clinical trials as a potential neuroprotective agent for the control of the cerebral damage after stroke onset, was designed based on previous studies dealing with the electronic features of the north-east region of the glycine binding site associated with the NMDA receptor. In particular, the substitution of the *para* position of the terminal phenyl ring of GV150526 with suitable hydrophilic groups resulted in the identification of a new class of glycine antagonists. These compounds exhibited nanomolar in vitro affinity to the glycine binding site, high receptor selectivity, and outstanding in vivo potency. In particular, 3-[(*E*)-2-[(4-ureidomethylphenyl)aminocarbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic acid was found to be highly effective in the middle cerebral artery occlusion (MCAo) model in the rat, an animal model of focal ischemia, when given both prior to and after the occlusion of the middle cerebral artery. Notably, a significant neuroprotective effect was seen in this model postischamia, when the administration of this compound was delayed up to 6 h from the occlusion of the middle cerebral artery, further confirming the wide therapeutic window seen for GV150526A.

## Introduction

During stroke[1] the overstimulation of the NMDA receptor[2] elicited by the increased release of glutamate[3] results in a massive influx of $Ca^{2+}$ into the postsynaptic neurons, leading ultimately to cell death through the activation of several neurotoxic cascades. No efficacious treatments are available to preserve neuronal integrity during stroke to date,[4] and the identification of suitable neuroprotective agents is expected to give substantial improvement over the existing vascular therapy. As the endogenous glutamate can activate the NMDA receptor only in the presence of glycine as coagonist,[5] in the past decade the glycine binding site associated with the NMDA receptor became an attractive target[6] for the discovery of effective neuroprotective agents able to control the progression of brain damage after stroke onset. Both competitive and noncompetitive NMDA antagonists[7] showed high neuroprotective efficacy in animal models of stroke.[8] In particular, GV150526[9] (**1**), depicted in Chart 1, currently undergoing clinical studies in man, has been identified as a potential neuroprotectant drug. In animal models of stroke this indole-2-carboxylate exhibited high efficacy and a long post-ischemia window, up to 6 h from occlusion of the middle cerebral artery (MCA). Moreover, this indole derivative lacks the adverse behavioral effects[9] in rats (memory disturbances, motor impairments, and psychotomimetic effects) observed for both competitive NMDA antagonists and NMDA channel blockers.[10]

On the basis of the considerable body of experience gained with this class of glycine antagonists, and with the aim of identifying novel series of indole derivatives structurally related to GV150526, compounds of general

**Chart 1**




structure **2**, shown in Chart 1 (R′ = alkyl, aryl, heterocycle; X = O, S), in which hydrophilic groups were introduced in the *para* position of the phenyl ring, belonging to the α,*β*-unsaturated side chain, were prepared. These compounds showed nanomolar affinity to the glycine binding site associated with the NMDA receptor, coupled to an outstanding neuroprotective activity in the MCAo model in rats when given both pre- and postischemia.

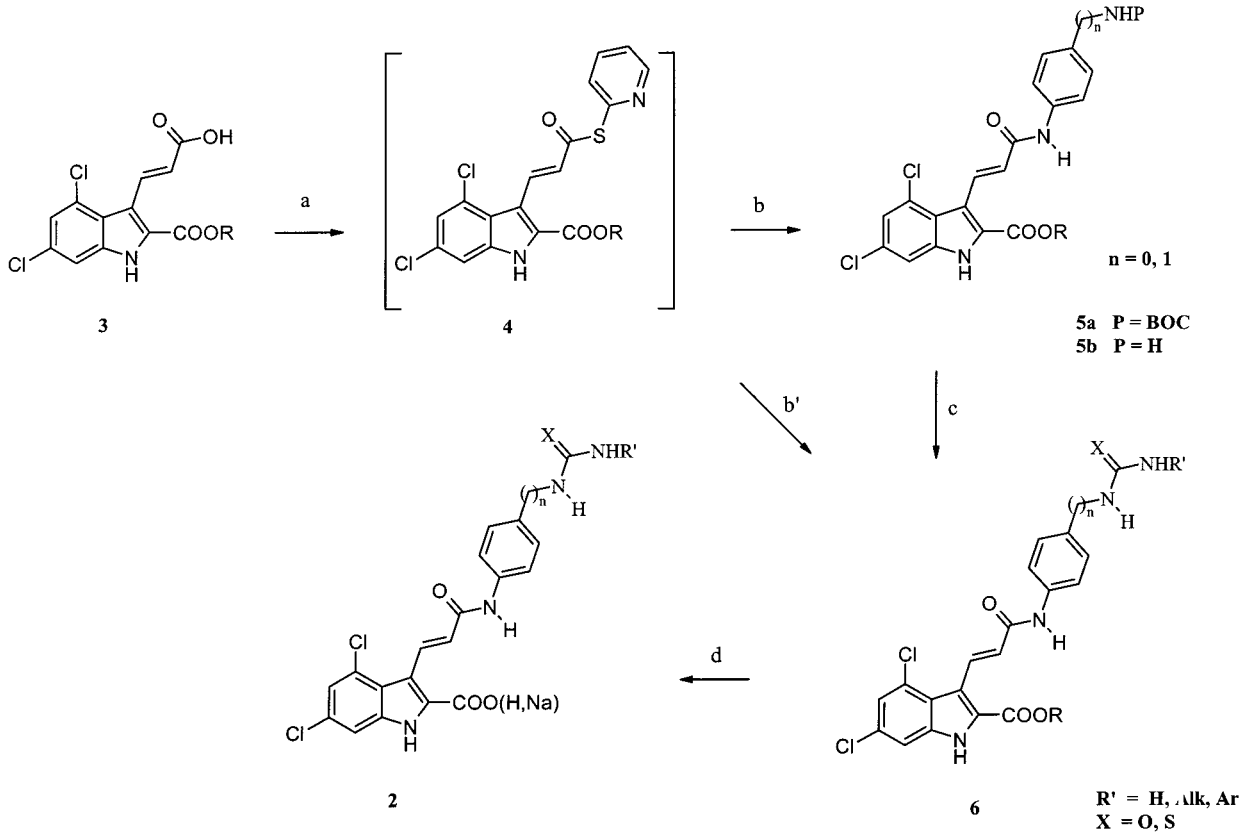
## Synthesis

As shown in Scheme 1, the compounds belonging to this class of glycine antagonists were prepared starting from the known indole-2-carboxylate derivative **3**, available in large scale as previously described.[9b] This intermediate was transformed into the amido derivatives **5a** using different methods of activation of the carboxyl group. The best results were obtained via the formation of the corresponding 2-pyridyl thioester **4**, according to the known "oxidation−reduction" procedure,[11] using a stoichiometric amount of 2,2′-dipyridyl

* Corresponding author. Fax: 39-45-9218196. E-mail: rdf26781@GlaxoWellcome.co.uk.

10.1021/jm980576n CCC: $18.00 
Published on Web 08/21/1999

**Scheme 1**[a]




[a] (a) 2-Aldrithiol, $PPh_3$, THF or DMF; (b) $PNH(CH_2)_nC_6H_4NH_2$, DMF; (b) $R'HNCONH(CH_2)_nC_6H_4NH_2$, DMF; (c) R′NCX, THF; (d) NaOH or LiOH, $EtOH-H_2O$.

disulfide and triphenylphosphine. Notably, the 2-pyridyl thioester derivative was found to be stable enough to be purified by standard chromatographic methods, and then it was reacted with the desired arylamines. Alternatively, a "one-pot" procedure was used.[12] Chemoselective removal of the N-Boc protecting group afforded the free amino derivative **5b** in high yield. This intermediate was smoothly transformed into the corresponding urea derivatives **6** in high yield by reaction with suitable isocyanates. Alternatively, the arylurea derivatives were preformed and reacted with the activate intermediate **4** to give compounds **6**. Finally, the basic hydrolysis of the C-2 ester group gave in quantitative yields the target derivatives **2**.

## Biology

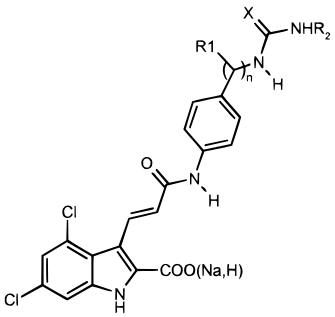
The biological evaluation of the new chemical entities (NCEs) was performed following the screening cascade described in the Experimental Section: (a) binding assay to evaluate the affinity for the glycine site[13]/functional activity;[14,15] (b) selectivity for the glutamate receptors[16−18] (NMDA/AMPA/KA); (c) in vivo anticonvulsant activity in the NMDA-induced convulsions model[19] in CD-1 mice (iv and po) to assess preliminary pharmacological profile; (d) in vivo evaluation of the neuroprotective activity in the middle cerebral artery occlusion (MCAo) model in male Sprague−Dawley rats as described by Tamura.[8]

As far as the NMDA-induced convulsions model is concerned, being a medium-throughput test, it was useful to gather rapid information on pharmacological profile/brain penetration of the NCEs in an early phase of the screening cascade and to select the best compound to be evaluated in the following low-throughput MCAo model. This first in vivo test can be considered as a surrogate model of stroke, assuming that the overactivation of the NMDA receptors is the key event in neurodegeneration following cerebral ischemia. The ability of NCEs given systemically, both iv and po, of inhibiting convulsions induced by the preemptive icv administration of the glycine binding site agonist NMDA was used as an end point of this model. The selected compounds identified following the screening sequence described above were then evaluated in terms of neuroprotective activity in an animal model of focal ischemia, both pre- and postischemia.

## Discussion

After the identification of GV150526A**,** the further exploration[9b,c] of the north-east region of the glycine binding site resulted in a more detailed understanding of the 3D-pharmacophore model, giving additional information on the electronic and steric requirements necessary to design novel classes of glycine antagonists within the indole-2-carboxylate series. In particular, it was proposed that the region of the receptor surrounding the *para* position of the terminal phenyl ring belonging to the C-3 side chain could be able to accept hydrophilic substituents endowed with limited steric bulk. To identify novel series of indole derivatives, a preliminary limited exploration of this potential modulating site was attempted. In particular, the urea derivative **6** and the corresponding homologues **7** and **8**, shown in Table 1, in which a spacer was introduced

**Table 1.** Preliminary Pharmacological Profile




| | | | | | ED$_{50}$ (mg/kg)[b] | |
|---|---|---|---|---|---|---|
| no. | $R_1$ | $R_2$ | *n*, X | p$K_i$[a] | iv | po |
| **1** | | | | 8.49 ± 0.02 | 0.06 (0.005−0.4) | 6 (4.4−7.9) |
| **6** | H | H | 0, O | 8.80 ± 0.02 | 0.55 (0.05−0.30) | 9.6 (6.9−13.5) |
| **7** | H | H | 1, O | 8.67 ± 0.03 | 0.07 (0.03−0.21) | 2 (0.9−3.8) |
| **8** | H | H | 2, O | 8.20 ± 0.01 | 0.14 (0.5−0.30) | 17.3 (9.1−49.4) |

[a] Displacement of [$^3$H]glycine. [b] NMDA-induced convulsions model in mice.

**Table 2.** Substituted Analogues of Compound **7**

| no. | $R_1$ | $R_2$ | *n*, X | p$K_i$[a] |
|---|---|---|---|---|
| **9** | H | $CH_2CH_3$ | 1, O | 8.62 ± 0.02 |
| **10** | H | $CH_2CH_3$ | 1, S | 7.91 ± 0.01 |
| **11** | H | $CH_2COOH$ | 1, O | 8.57 ± 0.02 |
| **12** | H | *c*-$C_3H_7$ | 1, O | 8.25 ± 0.02 |
| **13** | H | 4-THP | 1, O | 8.15 ± 0.03 |
| **14** | H | $C_6H_5$ | 1, O | 7.70 ± 0.03 |
| **15** | H | 4-$OCH_3$-$C_6H_5$ | 1, O | 7.69 ± 0.04 |
| **16** | H | 3-$C_5H_5N$ | 1, O | 7.83 ± 0.02 |
| **17** | *R*-($CH_3$) | H | 1, O | 8.20 ± 0.01 |
| **18** | *S*-($CH_3$) | H | 1, O | 7.93 ± 0.02 |

[a] Displacement of [$^3$H]glycine.

between the urea group and the aromatic ring, were synthesized and their in vitro affinity to the glycine binding site was evaluated. The results observed seem to confirm the presence of a hydrophilic pocket of limited size within the north-east region of the receptor. Compounds **6**−**8** were characterized in vivo in the NMDA-induced convulsions model in mice. As reported in Table 1, the urea derivative **7** resulted as the most potent derivative, when given by both by iv and po routes. In particular, when tested in the range of doses between 0.001−3 mg/kg, iv, and 1−100 mg/kg, po, according to the general procedure described above, a dose-dependent inhibition of convulsions was observed. The estimated ED$_{50}$ by iv route was 0.07 mg/kg, whereas the ED$_{50}$ by po route was 2 mg/kg (ED$_{50}$ = 0.06 and 6 mg/kg by iv and po administration, respectively, for GV150526A).

Following these preliminary positive observations, a series of analogues to compound **7** was prepared. On the basis of the results shown in Table 2, the following comments can be addressed:

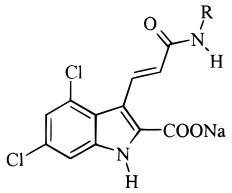
(a) As expected, the affinity at the glycine binding site of this new series of indole-2-carboxylate derivatives varies with the steric bulk and the hydrophilicity of the substituent $R_2$. In particular, the presence of groups $R_2$ of limited size and/or more hydrophilic (compounds **7**, **9**, and **11**) seems crucial to maximize the in vitro affinity of this series of indole-2-carboxylates. (b) The replacement of the urea with the corresponding thiourea group (compound **10**) resulted in a significant reduction of the in vitro potency. At present, it is difficult to speculate whether this detrimental effect, compared to the affinity of the parent compound **9**, could depend on the reduced H-bond acceptor character of the thiocarbonyl group and/or reduced H-bond donor character of the NH groups. (c) The introduction of aryl groups (compounds **14**−**16**) caused a 10 times reduction of the affinity, further confirming the limited space available within this region of the receptor. A slight improvement was observed in the case of the 4-tetrahydropyranyl (THP) group (compound **13** vs **14**) due probably to the greater conformational adaptability of the saturated ring to the receptor with respect to the aromatic moiety and/or the lack of the detrimental electonic effects of the aryl substituent. (d) The improvement of the steric bulk at the benzylic position ($R_1$ substituent) resulted in a partial reduction of affinity. This effect, slightly more relevant for the (*R*)-enantiomer compared to the (*S*)-enantiomer, could give as an additional insight on the shape and asymmetry of the hydrophilic pocket in which the terminal portion of the α,*β*-unsaturated side chain of the indole nucleus should lie.

As far as the in vivo activity of this series of substituted urea derivatives is concerned, despite the somehow higher in vitro affinity seen for some compounds, none of them shown considerable advantages over **7** in terms of inhibition of the convulsions induced by the preemptive icv injection of NMDA in mice.

Therefore, compound **7** was selected and further characterized following the screening cascade described above. As expected on the base of the profile of GV150526, **7** was found to inhibit noncompetitively the binding of [$^3$H]TCP in extensively washed cortical membranes preparation, according to the known procedure,[14,15] confirming the noncompetitive antagonism of this series of glycine antagonists. Moreover, this indole derivative was highly selective for the strychnine-insensitive glycine binding site with respect to glutamate, AMPA, and kainate binding sites (p$K_i$ = 3.48 ± 0.03, 3.98± 0.03, and 4.68 ± 0.02 for the NMDA, AMPA, and kainate binding sites, respectively). Finally **7** was found to be devoid of any significant affinity for 70 different CNS receptors up to the maximum tested concentration (10 M, p$K_i$ < 5).

**Plasma Protein Binding Studies.** Indole-2-carboxylates are known to be highly bound to plasma proteins;[20] nevertheless, GV150526 was found to be highly neuroprotective in vivo in an animal model of cerebral ischemia, raising, once again, doubts on the unresolved dependence between pharmacological activity and plasma protein binding. To understand whether the introduction of suitable hydrophilic and/or the presence of nonaromatic groups in the terminal position of the α,*β*-unsaturated side chain could modify the amount of the free fraction of these compounds in plasma, the relative serum protein binding of several compounds, carefully selected based on their CHI value,[24] was assessed comparing their HPLC retention

**Table 3.** Estimation of the Relative Rat Serum Protein Binding




| entry | R | RSA[d] $t_R$ (min) | CHI[e] |
|---|---|---|---|
| **24**[c] | adamantyl | 141.2 | 4.07 |
| **1**[a] | $C_6H_5$ | 60.1 | 3.13 |
| **23**[c] | *c*-$C_6H_{11}$ | 25.7 | 3.03 |
| **22**[a] | $CH_2C_6H_5$ | 66.9 | 2.83 |
| **10** | $C_6H_4$-*p*-$CH_2NHCONHC_2H_5$ | 34.1 | 2.13 |
| **21** | $C_6H_4$-*p*-$NHCOCH_3$ | 31.6 | 2.10 |
| **13** | $C_6H_4$-*p*-$CH_2NHCONH$-4-$C_5H_9O$ | 29.6 | 2.00 |
| **8** | $C_6H_4$-*p*-$CH_2CH_2NHCONH_2$ | 33.9 | 1.87 |
| **7** | $C_6H_4$-*p*-$CH_2NHCONH_2$ | 40.7 | 1.76 |
| **6** | $C_6H_4$-*p*-$NHCONH_2$ | 28.3 | 1.74 |
| **19** | *m*-$C_6H_4N$ | 26.2 | 1.15 |
| **20**[a] | $C_6H_4$-*p*-$NH_2$ | 23.2 | 1.13 |
| **25**[b] | ACEA-1021 | 12.1 | 0.95 |

[a] Ref 9b. [b] Ref 23. [c] Ref 9c. [d] Column: Shandon hypersyl rat albumin (30 cm × 4.6 mm; particle size 7 $\mu$m). Analysis conditions: 0.1 M $Na_2HPO_4/NaH_2PO_4$ buffer (pH = 7.4)/$CH_3CN$, 88−12. Flow: 0.8 mL/min. [e] CHI (chromatographic hydrophobicity index), see ref 24.

times assessed by a column of rat serum albumin (RSA) immobilized on silica gel. The results observed are shown in Table 3. These figures are known to be related to the amount of bound fraction in rat plasma.[21] From this preliminary analysis, the indole-2-carboxylates evaluated seem to be significantly different in terms of plasma protein binding with the HPLC retention time ranging from 141.2 min for the bulky and lipophilic adamantyl derivative **24** to 23.2 min for **20**, the *p*-amino analogue of GV150526. In particular, as far as the aromatic series is concerned, the relative retention time was reduced by introducing hydrophilic groups in the *para* position of the terminal phenyl ring. At this moment in time, it is difficult to understand if this effect could be related to the general hydrophilicity of these compounds and/or the inhibition of specific weak interactions within the binding site of the albumin. To validate these preliminary results, the absolute unbound fraction in rat plasma of both compound **7** and GV150526 was assessed using the ultracentrifugation method.[22] These studies, performed at a fixed concentration of compound (100 $\mu$M), resulted in 20 and 14 $\mu$g/mL, respectively, of free compound in rat plasma, corresponding to 99.80% and 99.86% of bound compound. Therefore, despite the significant differences seen by the HPLC method (40.7 vs 60.1 min for **7** and GV150526, respectively) both compounds exhibited a similar absolute protein binding in rat plasma, raising doubts, at least for this series of indole derivatives, on the validity of the HPLC method, previously described, as a filter to select the most appropriate compounds to progress throughout the screening cascade. Moreover, as far as the neuroprotective profiles in the MCAo model in rats of compound **7** and GV150526 are concerned, these compounds were found to be both highly potent (see the following paragraph) despite their limited amount of free fraction in rat plasma, raising once again the complex and unsolved issue of the relationship between brain penetration/pharmacological effect and plasma protein binding.

**Pharmacological Characterization of Compound 7.** On the basis of the preliminary pharmacological profile descibed above, compound **7** (GV228869) was selected among the different compounds prepared and evaluated in terms of neuroprotective profile in the permanent MCAo model in rats, a model of focal ischemia.

A summary of the results achieved is reported in Table 4. A significant neuroprotective effect was observed after both pre- and postischemia administration. In the preischemia protocol (compound administered 5 min prior to occlusion), as shown in Figure 1, compound **7** was found to be slightly more potent with respect to GV150526A, MK-801, and ACEA1021.[23] In particular, a dose-dependent neuroprotective effect was seen in the range between 0.1 and 3 mg/kg, iv, with a maximal protection of 66% observed at 3 mg/kg. Postischemia, a significant neuroprotective effect was seen when the administration of compound **7** was delayed up to 6 h from occlusion. In these conditions a single 1 mg/kg, iv, bolus significantly reduced the brain infarct volume by 33%, with respect to the control animals. The apparent decline with respect to the effect seen preischemia at the same dose (52%) was only reflecting the rate of damage progression, considering that, in control animals, 6 h after the occlusion the amount of salvageable neurons was significantly reduced. Therefore, compound **7** was able to block the progression of the cerebral damage even at delayed times from occlusion, further confirming the results achieved with GV150526A. As far as the reference compounds MK801 and ACEA1021 are concerned, at 3 mg/kg, iv, they were found to be active only up to 3 h after the occlusion. Therefore, these results confirm that indole-2-carboxylates are potential neuroprotective agents able to halt the progression of brain damage after stroke onset, with a therapeutic window of opportunity significantly wider in comparison with other classes of NMDA and glycine antagonists.

Finally, compound **7** was tested in the Irwin test in rats up to 30 mg/kg, iv, and complete absence of adverse effects (ataxia) has been observed, further confirming the lack of adverse behavioral effects observed in animal models for this class of glycine antagonists with respect to competitive and noncompetitive NMDA antagonists.

## Conclusions

Neuroprotection after stroke is clearly an area of unmet need in current medicine. No efficacious drugs are available to treat this disease in humans. Although in principle different pharmacological neuroprotective mechanisms are being pursued, great hope has been placed in the excitatory amino acid approach in view of the increasing body of experience gained in the glutamate-induced neuronal damage after cerebral ischemia. Indole-2-carboxylates substituted at the C-3 position with suitable $\alpha,\beta$-unsaturated side chains have been identified as potent, selective, and systemically active glycine antagonists. In particular, after the discovery of GV150526 (**1**), the careful exploration of the *para* position of the terminal phenyl ring, belonging to the C-3 side chain, allowed to understand better the space available in this region of the receptor and the

**Table 4.** Summary of the Pre- and Postischemia Effect of Compound **7** in the MCAo Model in Rats

| type of study | dose (mg/kg, iv) | infarct volume ($mm^3 \pm SEM$)[a] | damage reduction (%) | notes |
|---|---|---|---|---|
| preischemia | vehicle | 82.0 ± 10.8 | | |
| | 0.1 | 61.9 ± 12.0 | 24 | |
| | 0.3 | 36.4 ± 10.1[b] | 41 | $ED_{50}$ = 0.2 (0.25−1.51) |
| | 1 | 38.9 ± 5.8[b] | 52 | |
| | 3 | 27.8 ± 10.3[b] | 66 | |
| postischemia | vehicle | 82.0 ± 10.8 | | |
| | 1[c] | 54.7 ± 11.2* | 33 | |

[a] Measured 24 h after occlusion ($n$ = 8). [b] $P < 0.05$ *vs* vehicle. [c] Single dose given 6 h after occlusion.

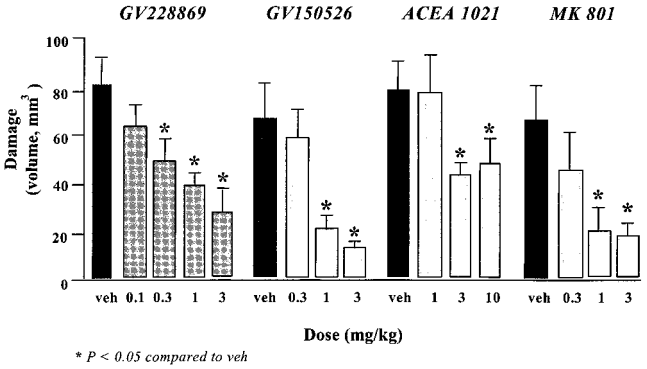



**Figure 1.** Neuroprotective profile of GV228869 in the MCAo model in rats, preischemia, with respect to reference compounds.

nature of the substituents allowed; accordingly, a series of substituted analogues of compound **1** was prepared.[25] In particular the urea derivative **7** exhibited an excellent neuroprotective profile in the MCAo model in rats, when administered both pre- and postischemia with a wide window of therapeutic intervention, further confirming the potential of this class of glycine antagonists as antistroke agents.

## Experimental Section

Infrared spectra were recorded on a Bruker IFS 48 spectrometer. $^1$H NMR spectra were recorded on a Varian Unity 400 spectrometer (400 MHz); the data are reported as follows: chemical shift in ppm from the internal standard $Me_4$-Si on $\delta$ scale, multiplicity ($b$ = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant (Hz).

Mass spectra were recorded on a VG-4 triple quadrupole Fison instrument in FAB mode. Elemental analyses were performed by our own analytical group on a Carlo Erba elemental analyzer. Melting points were determined on a Büchi 530 apparatus (scale 0−250 °C) and are uncorrected. All reactions were carried out under a controlled atmosphere in oven-dried glassware. Anhydrous toluene was purchased from Aldrich; THF was used after distillation over K/benzophenone. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates (0.25 mm).

**General Procedure for the Synthesis of Arylamides: Procedure A.** 2,2′-Dipyridyl disulfide (1.4 equiv) and triphenylphosphine (1.4 equiv) were added to a suspension of ethyl (*E*)-3-(2′-carboxyethenyl)-4,6-dichloro-1*H*-indole-2-carboxylate (**3**) (12.19 mmol, 1 equiv) in dry THF (100 mL), and the solution was stirred at room temperature for 2.5 h affording the intermediate **4**. The chosen BOC-protected arylamine (1.1 equiv), easily prepared by reaction at room temperature in ethyl acetate of 1 equiv of the corresponding commercially available amine with 1 equiv of di-*tert*-butyl dicarbonate, was then added, and the reaction mixture was refluxed for 2 h. After cooling to room temperature the precipitate obtained was filtered giving compound **5a** which was suspended in dichloromethane (55 mL) and treated with trifluoroacetic acid (22 mL). The reaction mixture was stirred at room temperature for 1 h; then the solvent was removed under reduced pressure. The oil obtained was repeatedly treated with diethyl ether and dried on the rotary evaporator to remove any residue of trifluoroacetic acid. Trituration with ethyl acetate and filtration of the solid obtained gave the trifluoroacetate salt which was stirred at room temperature for 10 min in a 0.5 N solution of sodium hydroxide to give, after filtration, the title compounds **5b** in 64−71% yields.

**(*E*)-3-[2-[(4-Aminophenyl)carbamoyl]vinyl]-4,6-dichloro-1*H*-indole-2-carboxylic Acid Ethyl Ester (5b, *n* = 0).** Prepared from the commercially available 4-(*tert*-butoxycarbonylamino)aniline according to the general procedure A: IR (Nujol) $\nu_{max}$ 3308, 1678, 1659, 1620 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.73 (bs, 1H), 8.18 (d, 1H, 15.5 Hz), 7.46 (d, 1H), 7.36 (d, 2H), 7.22 (bs, 1H), 6.64 (d, 1H, 15.5 Hz), 6.50 (d, 2H), 4.9 (bs, 2H), 4.3 (q, 2H), 1.3 (t, 3H).

**(*E*)-3-[2-[(4-Aminomethylphenyl)carbamoyl]vinyl]-4,6-dichloro-1*H*-indole-2-carboxylic Acid Ethyl Ester (5b, *n* = 1).** Prepared from 4-amino-*tert*-butoxycarbonylbenzylamine according to the general procedure A: IR (Nujol) $\nu_{max}$ 3425, 3337, 1704, 1664, 1607 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.02 (bs, 1H), 8.32 (d, 1H, 15.5 Hz), 7.63 (d, 2H), 7.44 (d, 1H), 7.24 (d, 2H), 7.19 (d, 1H), 6.69 (d, 1H, 15.5 Hz), 4.33 (q, 2H), 3.65 (s, 2H), 1.32 (t, 3H).

**(*E*)-3-[2-[(4-Aminoethylphenyl)carbamoyl]vinyl]-4,6-dichloro-1*H*-indole-2-carboxylic Acid Ethyl Ester (5b, *n* = 2).** Prepared from 2-(4-aminophenyl)-*tert*-butoxycarbonylethylamine according to the general procedure A: IR (Nujol) $\nu_{max}$ 3302, 3194, 1676 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.13 (s, 1H), 8.28 (d, 1H), 7.65 (d, 2H), 7.52 (d, 1H), 7.31 (d, 1H), 7.17 (d, 2H), 6.77 (d, 1H), 4.39 (q, 2H), 2.83 (t, 2H), 2.66 (t, 2H), 1.37 (t, 3H).

**General Procedure for the Synthesis of Isocyanates.** Triethylamine (1 equiv) and diphenyl phosphorazidate (1 equiv) were added to a solution of the chosen carboxylic acid (1.54 mmol, 1 equiv) in dry toluene (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2.5 h, heated at 80 °C over a range of 1.5−3.5 h, and then allowed to cool to room temperature. The isocyanates formed were used without any purification for the next reaction.

**General Procedure for the Synthesis of Arylureas: Procedure B.** The chosen isocyanate (2−4 equiv), prepared as described above, was added to a solution of arylamide **5b** (0.33 mmol, 1 equiv) in dry THF (4 mL). The reaction mixture was stirred at room temperature overnight, then diluted with water, and extracted with ethyl acetate. The organic extracts were dried ($Na_2SO_4$) and evaporated to a small volume under reduced pressure. The solid suspended was filtered to give the title compounds **6** in 70−99% yields with the exception of the 3-pyridino derivative (26%).

**General Procedure for the Synthesis of Arylamides: Procedure C.** 2,2′-Dipyridyl disulfide (1.4 equiv) and triphenylphosphine (1.4 equiv) were added to a suspension of ethyl (*E*)-3-(2′-carboxyethenyl)-4,6-dichloro-1*H*-indole-2-carboxylate (**3**) (0.78 mmol, 1 equiv) in dry THF (4 mL), and the solution was stirred at room temperature for 2 h affording the intermediate **4**. A solution in dry THF (4 mL) of 1-(4-

aminophenyl)ethylurea (1.2 equiv), easily prepared by reaction of the commercially available α-methyl-4-nitrobenzylamine hydrochloride with trimethylsilyl isocyanate followed by hydrogenation with palladium on carbon, was then added, and the reaction mixture was refluxed for 5 h. After cooling to room temperature the precipitate obtained was filtered giving the title compounds **5b** in 52% yield.

**General Procedure for the Basic Hydrolysis of Ethyl Esters: Procedure D.** A solution of ester (0.14 mmol, 1 equiv) and lithium hydroxide monohydrate (3−4 equiv) in ethanol (2 mL) was stirred at 50 °C over a range of 2.5−4 h. After cooling to room temperature the solution was acidified with a 2 N solution of hydrochloric acid until a solid precipitated. The precipitate was filtered to give carboxylic acids in 58−84% yields with the exception of the 3-pyridino derivative **16** (26%) and the ethylthio derivative **10** (27%). The sodium salts were obtained in quantitative yields by freeze-drying the carboxylic acids with 1 equiv of sodium hydroxide (0.1 N).

**(*E*)-4,6-Dichloro-3-[2-[(4-ureidophenyl)carbamoyl]vinyl]-1*H*-indole-2-carboxylic Acid Sodium Salt (6).** Prepared starting from the commercially available trimethylsilyl isocyanate according to general procedures A, B, and D: mp >250 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.86 (bs, 1H), 9.96 (bs, 1H), 9.77 (s, 1H), 8.76 (d, 1H), 7.63 (d, 2H), 7.51 (d, 2H), 7.41 (d, 1H), 7.13 (d, 1H), 7.00 (d, 1H), 6.36 (bs, 2H); MS *m/e* 455. Anal. ($C_{19}H_{13}Cl_2N_4O_4Na$) C, H, N.

**(*E*)-4,6-Dichloro-3-[2-[(4-methylureidophenyl)carbamoyl]vinyl]-1*H*-indole-2-carboxylic Acid Sodium Salt (7).** Prepared starting from the commercially available trimethylsilyl isocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3408, 3360, 3192, 1645, 1620 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.8 (b, 1H), 9.97 (s, 1H), 8.58 (d, 1H, 15.2 Hz), 7.68 (d, 2H), 7.39 (d, 1H), 7.19 (d, 1H, 15.2 Hz), 7.15 (d, 2H), 7.10 (d, 1H), 6.32 (t, 1H), 5.47 (bs, 2H), 4.10 (d, 2H); MS *m/e* 469. Anal. ($C_{20}H_{15}Cl_2N_4O_4Na\cdot0.5H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-[2-[(4-ethylureidophenyl)carbamoyl]vinyl]-1*H*-indole-2-carboxylic Acid Sodium Salt (8).** Prepared starting from the commercially available trimethylsilyl isocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3325, 1657, 1609 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.75 (bs, 1H), 9.94 (s, 1H), 8.57 (d, 1H), 7.66 (d, 2H), 7.39 (d, 1H), 7.20 (d, 1H), 7.10 (d, 2H), 7.09 (d, 1H), 5.88 (bt, 1H), 5.41 (bs, 2H), 3.14 (m, 2H), 2.60 (m, 2H); MS *m/e* 483. Anal. ($C_{21}H_{17}Cl_2N_4O_4Na\cdot1.5H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureidoethyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid Sodium Salt (9).** Prepared starting from the commercially available ethyl isocyanate according to the general procedures A, B, and D: mp 220 °C; IR (Nujol) $\nu_{max}$ 3315, 1599 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.77 (bs, 1H), 9.97 (bs, 1H), 8.58 (d, 1H), 7.67 (m, 2H), 7.39 (d, 1H), 7.19 (d, 1H), 7.14 (d, 2H), 7.09 (d, 1H), 6.21 (t, 1H), 5.83 (t, 1H), 4.12 (d, 2H), 3.02 (m, 2H), 0.98 (t, 3H); MS *m/e* 497. Anal. ($C_{22}H_{19}Cl_2N_4O_4Na$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylthioureidoethyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid (10).** Prepared starting from the commercially available ethyl isothiocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3290, 3196, 1712, 1664 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.37 (bs, 1H), 12.54 (bs, 1H), 10.18 (bs, 1H), 8.26 (d, 1H, 15.8 Hz), 7.65 (d, 2H), 7.48 (d, 1H), 7.31 (d, 1H), 7.23 (d, 2H), 7.78−7.4 (m, 2H), 6.77 (d, 1H, 15.8 Hz), 4.58 (m, 2H), 3.32 (m, 2H), 1.06 (t, 3H); MS *m/e* 491. Anal. ($C_{22}H_{20}Cl_2N_4O_3S\cdot H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureidomethylcarboxyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid (11).** Prepared starting from the commercially available ethyl isocyanoacetate according to the general procedures A, B, and D: mp 190 °C; IR (Nujol) $\nu_{max}$ 3400−2500, 1661, 1610 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.8 (b, 2H), 12.54 (bs, 1H), 10.18 (bs, 1H), 8.25 (d, 1H), 7.64 (d, 2H), 7.48 (d, 1H), 7.30 (d, 1H), 7.18 (m, 2H), 6.77 (d, 1H), 6.59 (bs, 1H), 6.16 (bs, 1H), 4.15 (s, 2H), 3.71 (s, 2H); MS *m/e* 505. Anal. ($C_{22}H_{18}Cl_2N_4O_6\cdot1.5H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureidocyclopropyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid Sodium Salt (12).** Prepared starting from cyclopropyl isocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3327, 1700, 1661, 1601 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.80 (bs, 1H), 9.99 (bs, 1H), 8.57 (d, 1H), 7.67 (d, 2H), 7.40 (d, 1H), 7.18 (d, 1H), 7.14 (d, 2H), 7.09 (d, 1H), 6.30 (bt, 1H), 6.18 (bd, 1H), 4.13 (d, 2H), 2.41 (m, 1H), 0.55 (m, 2H), 0.33 (m, 2H); MS *m/e* 509. Anal. ($C_{23}H_{19}Cl_2N_4O_4Na$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureido(tetrahydropyran-4-yl))phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid Sodium Salt (13).** Prepared starting from 4-tetrahydropyranyl isocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3312, 1622 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.75 (b, 1H), 9.94 (s, 1H), 8.59 (d, 1H), 7.67 (d, 2H), 7.37 (d, 1H), 7.18 (d, 1H), 7.14 (d, 2H), 7.09 (bs, 1H), 6.14 (t, 1H), 5.92 (d, 1H), 4.12 (d, 2H), 3.77 (m, 2H), 3.58 (m, 1H), 3.3 (m, 2H), 1.72 (m, 2H), 1.29 (m, 2H); MS *m/e* 553. Anal. ($C_{25}H_{23}Cl_2N_4O_5Na$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureidophenyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid Sodium Salt (14).** Prepared starting from the commercially available phenyl isocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3500−2500, 1657, 1590 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.81 (bs, 1H), 9.99 (bs, 1H), 8.61 (bs, 1H), 8.57 (d, 1H), 7.70 (d, 2H), 7.40 (m, 3H), 7.26−7.14 (m, 5H), 7.11 (d, 1H), 6.87 (m, 1H), 6.66 (bt, 1H), 4.23 (d, 2H); MS *m/e* 545. Anal. ($C_{26}H_{19}Cl_2N_4O_4Na\cdot1.5H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureido(4-methoxyphenyl))phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid (15).** Prepared starting from the commercially available 4-methoxyphenyl isocyanate according to the general procedures A, B, and D: mp 220 °C; IR (Nujol) $\nu_{max}$ 3400−3200, 1650 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.73 (bs, 1H), 12.55 (s, 1H), 10.19 (s, 1H), 8.32 (s, 1H), 8.26 (d, 1H), 7.67 (d, 2H), 7.48 (m, 1H), 7.31 (m, 1H), 7.29 (d, 2H), 7.24 (d, 2H), 6.80 (d, 2H), 6.78 (d, 1H), 6.44 (t, 1H), 4.23 (d, 2H), 3.68 (s, 3H); MS *m/e* 553. Anal. ($C_{27}H_{22}Cl_2N_4O_5\cdot H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(methylureido(3-pyridinyl))phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid (16).** Prepared starting from 3-pyridinyl isocyanate according to the general procedures A, B, and D: mp >250 °C; IR (Nujol) $\nu_{max}$ 3206, 1696, 1653, 1609 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.73 (bs, 1H), 12.51 (s, 1H), 10.17 (s, 1H), 8.73 (s, 1H), 8.52 (d, 1H), 8.26 (d, 1H, 15.6 Hz), 8.10 (dd, 1H), 7.89 (ddd, 1H), 7.66 (d, 2H), 7.47 (d, 1H), 7.29 (d, 1H), 7.24 (d, 2H), 6.79 (d, 1H, 15.6 Hz), 6.73 (t, 1H), 4.25 (d, 2H); MS *m/e* 524. Anal. ($C_{25}H_{19}Cl_2N_5O_4\cdot H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(*R*)-(1-ureidoethyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid Sodium Salt (17).** Prepared starting from (*R*)-1-(4-aminophenyl)ethylurea according to the general procedures C and D: mp >250 °C; $[\alpha]_D = +23.8$ ($c = 0.30$ in DMSO); IR (Nujol) $\nu_{max}$ 3369, 3184, 1657, 1599 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.84 (bs, 1H), 9.99 (bs, 1H), 8.61 (d, 1H), 7.70 (d, 2H), 7.43 (d, 1H), 7.22 (d, 1H), 7.21 (d, 2H), 7.11 (d, 1H), 6.35 (d, 1H), 5.41 (s, 2H), 4.67 (m, 1H), 1.31 (d, 3H); MS *m/e* 483. Anal. ($C_{21}H_{17}Cl_2N_4O_4Na$) C, H, N.

**(*E*)-4,6-Dichloro-3-{2-[[4-(*S*)-(1-ureidoethyl)phenyl]carbamoyl]vinyl}-1*H*-indole-2-carboxylic Acid Sodium Salt (18).** Prepared starting from (*S*)-1-(4-aminophenyl)ethylurea according to the general procedures C and D: mp >250 °C; $[\alpha]_D = -25.6$ ($c = 0.38$ in DMSO); IR (Nujol) $\nu_{max}$ 3323, 1657, 1609 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.83 (bs, 1H), 9.99 (bs, 1H), 8.62 (d, 1H), 7.70 (d, 2H), 7.43 (d, 1H), 7.22 (d, 1H), 7.21 (d, 2H), 7.11 (d, 1H), 6.35 (d, 1H), 5.40 (bs, 2H), 4.67 (m, 1H), 1.31 (d, 3H); MS *m/e* 483. Anal. ($C_{21}H_{17}Cl_2N_4O_4Na$) C, H, N.

**(*E*)-4,6-Dichloro-[3-(2-pyridin-3-ylcarbamoyl)vinyl]-1*H*-indole-2-carboxylic Acid Sodium Salt (19).** Prepared reacting 3-aminopyridine with intermediate **4** following the general procedure previously described:[10b] mp >250 °C; IR (Nujol) $\nu_{max}$ 3229, 1624 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.03 (bs, 1H), 10.33 (bs, 1H), 8.89 (d, 1H), 8.66 (d, 1H), 8.19 (m, 2H),

7.48 (m, 1H), 7.31 (m, 2H), 7.12 (d, 1H); MS *m/e* 398. Anal. ($C_{17}H_{10}Cl_2N_3O_3Na \cdot H_2O$) C, H, N.

**(*E*)-4,6-Dichloro-3-[2-[(4-acetylamidophenyl)aminocarbonyl]vinyl]-1*H*-indole-2-carboxylic Acid Sodium Salt (21).** Prepared according to the general procedure B: mp >250 °C; IR (Nujol) $\nu_{max}$ 1616 cm $^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.86 (bs, 1H), 9.98 (bs, 1H), 9.85 (bs, 1H), 8.57 (d, 1H), 7.65 (d, 2H), 7.46 (d, 2H), 7.42 (d, 1H), 7.17 (d, 1H), 7.09 (d, 1H), 2.00 (s, 3H); MS *m/e* 454. Anal. ($C_{20}H_{14}Cl_2N_3O_4Na \cdot H_2O$) C, H, N.

**Pharmacology**. The research complied with national legislation and with company policy on the *Care of Use of Animals* and with related codes of practice.

**Binding Assay.** NCEs were dissolved at a 5 mM concentration in 100% DMSO and tested at seven different concentrations in duplicate in the [$^3$H]glycine displacement experiments and, in the other binding assays, at five concentrations (from 10 nM to 100 $\mu$M). A reference compound was always included as internal control.

Affinity to the glycine binding site was measured by inhibition of the binding of [$^3$H]glycine to crude synaptic membranes prepared from adult rat cerebral cortex, as described by Kishimoto et al.[13] Incubation (20 min at 4 °C) was carried out in 50 mM Tris/citrate (pH 7.10) using 20 nM [$^3$H]glycine. Data for displacement experiments, performed to determine the inhibition constants ($K_i$) of displacer ligands, were analyzed using the nonlinear curve fitting software LIGAND.[26] $K_i$ values were measured from at least six-point inhibition curves and are geometric means of at least three different experiments. Inhibition of the binding of [$^3$H]CPP, [$^3$H]AMPA, and [$^3$H]kainate experiments were performed according to the methods by van Amsterdam et al.,[14] Giberti et al.,[15] and Honoré.[16] Enhancement of the binding of the channel blocking agent [$^3$H]TPC[17,18] was expressed by the ability to open the NMDA receptor-associated ion channel allowing more [$^3$H]TPC to bind to its specific site within the ion channel. Binding of [$^3$H]TPC was carried out (2 h, 30 °C) in Tris/5 mM HCl (pH 7.7) and in the presence of 1 $\mu$M glutamic acid. Nonspecific binding was determined by 30 M (+)-MK801. In the presence of increasing concentration of the NCE, parallel rightward shifts of the glycine concentration−response curves could be observed, with no depression of the maximum response.

**Anticonvulsant Activity.** NCEs were evaluated in vivo by assessing their anticonvulsant effect[19] when convulsions were induced in male CD-1 mice (18−29 g) by icv injection of NMDA (1 nmol/mouse) 1 min and 1 h after the iv and po administration of the NCE, respectively. Animals were observed for the occurrence of generalized seizures during the first 30 min after the treatment with NMDA and were considered protected if convulsions did not occur within this period. The percentage of animals showing anticonvulsant activity in each treatment group was recorded, and $ED_{50}$ values were estimated along with their 95% confidence limits.

**Neuroprotective Activity. MCAo (distal MCA occlusion):** The experimental procedure was performed according to Tamura et al.,[8] with minor modification. Male Sprague−Dawley rats (280−350 g) were anesthetized with chloral hydrate (400 mg/kg, ip). The animals were maintained normothermic by means of a heating pad and placed under an operating microscope. A skin incision was made, the temporalis muscle was reflected, and a craniectomy was performed by drilling at the junction between the medial wall and the roof of the infero-temporal fossa. The dura was opened, and the MCA was permanently occluded by electrocoagulation proximal to the frontal branch. The muscles and the skin were reported to the original position, and the skin incision was closed. Rats were allowed to recover for 24 h before the evaluation of the ischaemic damage.

**Histology:** Animals were sacrificed 24 h after MCAo; the brain was removed carefully and sectioned coronally at 0.5-mm intervals by using a motorized vibroslice. The brain slices were immersed in a solution of 2% triphenyltetrazolium chloride (TTC) at 37 °C for 20 min and then stored in 4% neutral buffered formalin. The TTC staining clearly distinguishes between the normal tissue (stained in red) and the ischaemic area (white, not stained). The area of cerebral damage in each coronal section was assessed by using an image analyzer (Imaging Research Inc., Canada). The infarct volumes were calculated by using the trapezoidal rule method.

**Drugs and statistical analysis:** Compounds were dissolved in DMSO and then diluted to a final 3% DMSO concentration with distilled water. Solutions were prepared fresh on the day of the experiment. Values are expressed as mean ± SEM. Statistical differences between groups were analyzed using the Student's *t*-test for unpaired data on GBStat 5.3 program (Dynamic Microsystem).

**Acknowledgment.** The authors thank Dr. C. Marchioro and co-workers for $^1$H NMR spectra, Dr. M. Handam for mass spectral data, and Dr. A. Garofalo for elemental analysis. Thanks are also due to Dr. C. Pietra, Mr. S. Costa, and Mr. E. Valerio for the in vivo characterization in the NMDA-induced convulsions model in mice and in the MCAo model in rats.

## References

(1) For a recent review on several neuroprotective approaches potentially useful after stroke onset, see: Zivin, J. A. Neuroprotective Therapies in Stroke. *Drugs* **1997**, *54*, 83−89.

(2) Collingridge, G. L., Watkins, J. C., Eds. *The NMDA Receptor*, 2nd ed.; IRL Press: Oxford, England, 1994.

(3) (a) Graham, S. H.; Chen, J.; Sharp, F. R.; Simon, P. R. Limiting Ischemic Injury by Inhibition of Excitatory Amino Acids Release. *J. Cerebr. Blood Flow Metab.* **1993**, *13*, 88−97. (b) McCullogh, J. Excitatory Amino Acid Antagonists and Their Potential for the Treatment of Ischaemic Brain Damage in Man. *Br. J. Clin. Pharmacol.* **1992**, *34*, 106−114. (c) Choi, D. W.; Rothman, S. M. The Role of Glutamate Neurotoxicity in Hypoxic-Ischemic Neuronal Death. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 171−182. (d) Choi, D. W. Methods for Antagonizing Glutamate Neurotoxicity. *Cerebrovasc. Brain Metab. Rev.* **1990**, *2*, 105−147. (e) Meldrum, B.; Garthwaite, J. Excitatory Amino Acid Neurotoxicity and Neurodegenerative Diseases. *Trends Pharmacol.* **1990**, *11*, 379−387. (f) Choi, D. W. Cerebral Hypoxia: Some New Approaches and Unaswered Questions. *J. Neurosci.* **1990**, *10*, 2493−2501. (g) Meldrum, B. Protection Against Ischaemic Neuronal Damage by Drugs Acting on Excitatory Neurotransmission. *Cerebrovasc. Brain Metab. Rev.* **1990**, *2*, 27−57.

(4) Bath, P. *Stroke Therapy: Advances and Commercial Opportunities*, 1st ed.; CONNECT Pharma Ltd.: Oxford, England, 1995.

(5) (a) Johnson, J. W.; Asher, P. Glycine Potentiates the NMDA Receptor Response in Cultured Brain Mouse Neurones. *Nature* **1987**, 325, 529−531. (b) Kleckner, N. W.; Dingledine, R. Requirements for Glycine in Activation of NMDA Receptor Expressed in Xenopus Oocytes. *Science* **1988**, *241*, 835−837. Reviews: (c) Huettner, J. E. Competitive Antagonism of Glycine at hte *N*-Methyl-D-Aspartate (NMDA) Receptor. *Biochem. Pharmacol.* **1991**, *41*, 9−16. (d) Thomson, A. M. Glycine is a Coagonist at the NMDA Receptor/ Ion Channel Complex. *Prog. Neurobiol.* **1990**, *35*, 53−74. Wong, E. H. F.; Kemp, J. A. Site for Antagonism on the *N*-methyl-D-aspartate Receptor Channel Complex. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 401−425.

(6) Reviews: (a) Palfreyman, M. G.; Baron, M. B. Noncompetitive NMDA Antagonists Acting at the Glycine Site. In *Excitatory Amino Acid Antagonists*; Meldrum, B., Ed.; Blackwell: Oxford, England, 1991; Chapter 6, pp 101−129. (b) Carter, A. J. Glycine Antagonists: Regulation of the NMDA Receptor-Complex by the Strychnine -insensitive Glycine Site. *Drugs Future* **1992**, *17*, 595−613. (c) Kemp, J. A.; Leeson, P. D. The Glycine Site of the NMDA Receptor − Five Years On. *TiPS* **1993**, *14*, 20−25. (d) Leeson, P. D.; Iversen, L. L. The Glycine Site on the NMDA Receptor: Structure−Activity Relationships and Therapeutic Potential. *J. Med. Chem.* **1994**, *37*, 4053−4067. (e) Iversen, L. L.; Kemp, J. A. Non Competitive NMDA Antagonists as Drugs. In *The NMDA Receptor*, 2nd ed.; Collingridge, G. L., Watkins, J. C., Eds.; IRL Press: Oxford, England, 1994; Chapter 20, pp 469−486. (f) Di Fabio, R.; Gaviraghi, G.; Reggiani, A. The Strychnine-insensitive Glycine Binding Site and the NMDA Receptor. *Chim. l'Indust.* **1996**, *78*, 283−289.

(7) (a) Bielemberg, G. W.; Beck, T. The Effect of Dizocilpine (MK-801), Phenylcyclidine, and Nemodipine on Infart Size 48 h After Middle Cerebral Artery Occlusion in the Rat. *Brain Res.* **1991**, *552*, 338−342. (b) Park, C. K.; Nehls, D. G.; Graham, D. I.; Teasdale, G. M.; McCullogh, J. The Glutamate Antagonist MK-801 Reduced Focal Ischemic Brain Damage in the Rat. *Ann. Neurol.* **1988**, *24*, 543−551. (c) Warner, D. S.; Martin, H.;

Ludwig, P.; McAllister, A.; Keana, J. F. W.; Weber, E. In Vivo Models of Cerebral Ischemia: Effects on Parenterally Administered NMDA Receptor Glycine Site Antagonists. *J. Cerebr. Blood Flow Metab.* **1995**, *15*, 188−196.

(8) Tamura, A.; Graham, D. I.; McCullogh, J.; Teasdale, G. M. Focal Cerebral Ischaemia in the Rat: I. Description of techniques and Early Neuropathological Consequences Following Middle Cerebral Artery Occlusion. *J. Cerebr. Blood Flow Metab.* **1981**, *1*, 53−60.

(9) (a) Di Fabio, R.; Cugola, A.; Donati, D.; Feriani, A.; Gaviraghi, G.; Ratti, E.; Trist, D. G.; Reggiani, A. Identification and Pharmacological Characterization of GV150526A, a Novel Glycine Antagonist as a Potent Neuroprotective Agent. *Drugs Future* **1998**, *23*, 1, 61−69. (b) Di Fabio, R.; Capelli, A. M.; Conti, N.; Cugola, A.; Donati, D.; Feriani, A.; Gastaldi, P.; Gaviraghi, G.; Hewkin, C. T.; Micheli, F.; Missio, A.; Mugnaini, M.; Pecunioso, A.; Quaglia, A. M.; Ratti, E.; Rossi, L.; Tedesco, G.; Trist, D. G.; Reggiani, A. Substituted Indole-2-carboxylates as in Vivo Potent Antagonists Acting at the Strychnine-Insensitive Glycine Binding Site. *J. Med. Chem.* **1997**, *40*, 841−850. (c) Micheli, F.; Di Fabio, R.; Capelli, A. M.; Cugola, A.; Curcuruto, O.; Feriani, A.; Gastaldi, P.; Gaviraghi, G.; Marchioro, C.; Orlandi, A.; Pozzan, A.; Quaglia, A. M.; Reggiani, A.; van Amsterdam, F. Cycloalkyl Indole-2-Carboxylates as Useful Tools for Mapping the "North-Eastern" Region of the Glycine Binding Site Associated with the NMDA Receptor. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 73−80. (d) The Glycine Antagonist GV150526 Protects Somatosensory Evoked Potentials and Reduces the Infarct Area in the MCAo Model of Focal Ischaemia in the Rat. *Exp. Neurol.* **1997**, *145*, 425−433. (e) Di Fabio, R.; Cugola, A.; Donati, D.; Feriani, A.; Gaviraghi, G.; Ratti, E.; Trist, D. Novel Substituted Indole-2-Carboxylates as Potent Glycine Antagonists. *ACS Book of Abstracts*; 211th ACS National Meeting, New Orleans, LA, March 24−28, 1996; Abstract 107. (f) Bordi, F.; Pietra, C.; Ziviani, L.; Reggiani, A. Neuroprotection of GV150526A. Comparison with Reference Compounds. *Stroke* **1996**, *27*, 26. (g) Gaviraghi, G.; Cugola, A.; Giacobbe, S. Eur. Patent 0568136 A1, 1993.

(10) (a) Meldrum, B. S.; Chapman, A. G. Competitive NMDA Antagonists as Drug. In *The NMDA Receptor,* 2nd ed.; Collingridge, G. L., Watkins, J. C., Eds.; IRL Press: Oxford, England, 1994; Chapter 19, pp 457−468. (b) Iversen, L. L.; Kemp, J. A. Non competitive NMDA Antagonists as Drug. In *The NMDA Receptor*, 2nd ed.; Collingridge, G. L., Watkins, J. C., Eds.; IRL Press: Oxford, England, 1994; Chapter 20, pp 469−486.

(11) Mukaiyama, T.; Matsueda, R.; Suzuky, M. Peptide Synthesis via the Oxidation−Reduction Condensation by Use of 2,2′-Dipyridyl disulfide as an Oxidant. *Tetrahedron Lett.* **1970**, *22*, 1901−1904.

(12) Di Fabio, R.; Summa, V.; Rossi, T.; Synthesis of Amides: An Efficient and Chemoselective Method for the Preparation of *β*-lactam Derivatives Related to HLE Inhibitors. *Tetrahedron* **1993**, *49*, 2300−2306.

(13) Kishimoto, H.; Simon, J. R.; Aprison, M. H. Determination of the Equilibrium Dissociation Constants at a Number of Glycine Binding Sites in Several Areas of the Rat Central Nervous System, Using a Na-Independent System. *J. Neurochem.* **1981**, *37*, 1015−1024.

(14) Kloog, Y.; Haring, R.; Sokolovsky, M. Kinetic Characterization of the Phenylcyclidine-*N*-Methyl-D-Aspartate Receptor Interaction: Evidence for Steric Blockade of the Channel. *Biochemistry-US* **1988**, *27*, 843−848.

(15) Ratti, E.; Tacconi, S.; Graziani, F.; Gaviraghi, G. Requirement of the Glycine for the GlutamateActivity at the NMDA Receptor Site. *Eur. J. Pharmacol.* **1990**, *183*, 1665.

(16) van Amsterdam, F. Th. M.; Giberti, A.; Mugnaini, M.; Ratti, E. 3-[(±)-2-Carboxypiperazin-4-yl]propyl-1-Phosphonic Acid Recognizes Two *N*-Methyl-D-Aspartate Binding Sites in Rat Cerebral Cortex Membranes. *J. Neurochem.* **1992**, *59*, 1850−1855.

(17) Giberti, A.; Ratti, E.; Gaviraghi, G.; van Amsterdam, F. Th. M. Binding of DL-[$^3$H]-a-Amino-3-Hydroxy-5-Methyl-Isoxazole-4-Propionic Acid (AMPA) to Rat Cortex Membranes Reveals Two Sites or Affinity States. *J. Recept. Res.* **1991**, *11*, 727−741.

(18) Honoré, T.; Drejer, J.; Nielsen, M. Calcium Discriminates Two [$^3$H]Kainate Binding Sites with Different Molecular Target Sizes in Rat Cortex. *Neurosci. Lett.* **1986**, *65*, 47−52.

(19) Chiamulera, C.; Costa, S.; Reggiani, A. Effect of NMDA and Strychnine-Insensitive Glycine Site Antagonists on NMDA-Mediated Convulsions and Learning. *Psycopharmacology* **1990**, *112*, 551−552.

(20) Rowley, M.; Kulagowsky, J. J.; Watt, A. P.; Rathbone, D.; Stevenson, G. I.; Carling, R. W.; Baker, R.; Marshall, G. R.; Kemp, J. A.; Foster, A. C.; Grimwood, S.; Hargreaves, R.; Hurley, C.; Saywell, K. L.; Trickleback, M. D.; Leeson, P. D. Effect of Plasma Protein Binding on in vivo Activity and Brain Penetration of Glycine/NMDA Receptor Antagonists. *J. Med. Chem.* **1997**, *40*, 4053−4068.

(21) Domenici, E.; Bertucci, E.; Salvatori, P.; Motellier, S.; Wainer, I. W. Immobilized Serum Albumin: Rapid HPLC Probe of Steroselective Protein-Binding Interactions. *Chirality* **1990**, *2*, 263−268.

(22) Pacifici, G. M.; Viani, A. Methods of Determining Plasma and Tissue Protein Binding: Pharmacokinetic Consequences. *Clin. Pharmacokinet.* **1992**, *23*, 449−468.

(23) (a) Keana, J. F. W.; Kher, S. M.; Sui, X.; Dinsmore, C. M.; Glenn, A. G.; Guastella, J.; Huang, Jin-Cheng; Ilyin, V.; Lu, Y. 1,4-Dihydroquinoxaline-2,3-diones: Antagonists of *N*-methyl-D-aspartate (NMDA) Receptor Glycine Site and Non-NMDA Glutamate Receptor. *J. Med. Chem.* **1995**, *38*, 4367−4379. (b) Woodward, R. M.; Huettner, J. E.; Guastella, J.; Keana, J. F. W.; Weber, E. In vitro Pharmacology of ACEA-1021 and ACEA-1031: Sistemically Active Quinoxalidines with High Affinity and Selectivity for the *N*-methyl-D-aspartate Receptor Glycine Site. *Mol. Pharmacol.* **1995**, *47*, 568−581.

(24) Valko, K.; Bevan, C.; Reynolds, D. Chromatographic Hydrophobicity Index by Fast-Gradient RP-HPLC: A high-Throughput Alternative to LogP/LogD. *Anal. Chem.* **1997**, *11*, 6669.

(25) Di Fabio, R.; Conti, N.; De Magistris, E.; Feriani, A. WO 9627588 A1.

(26) Munson, P. D.; Rodbard, D. LIGAND: A Versatile Computerized Approach for Characterization of Ligand-binding Data. *Anal. Biochem.* **1980**, *107*, 220−239.

JM980576N