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Synthesis and pharmacological properties of novel glycine antagonists

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Abstract

The NMDA receptor is an ionotropic receptor complex widely distributed in the central nervous system and its activation, particularly in hypoxic conditions such as stroke, traumatic head injury and hypoglycemia, results in a massive influx of calcium ions into the post-synaptic neurones, leading to cell death through the activation of several neurotoxic cascades. The NMDA receptor is a unique ionotropic receptor complex because its activation requires the simultaneous binding of glutamate and glycine and selective antagonists at the glycine binding site are endowed with a better side-effect profile than competitive NMDA antagonists. Then, considerable efforts have been devoted to find potent and selective ligands, resulting in the identification of several classes of glycine antagonists. The research at Glaxo Wellcome has been aimed at the identification of novel in vivo active glycine antagonists, and led to the synthesis and pharmacological characterization of a number of novel, potent and systemically active compounds belonging to different chemical classes.

Keywords: NMDA; Glycine antagonists; Stroke; Indole-2-carboxylates; Benzoazepines; Pyrido[2,3-*b*]pyrazines

1. Introduction

In the recent past, the role of excitatory amino acids (EAAs) in the physiology and pathophysiology of the central nervous system has been widely investigated and elucidated (Meldrum and Garthwaite, 1990; McCullogh, 1992) and since the discovery of the neurotoxic properties of glutamate, the most important excitatory neurotransmitter in mammalian brain, a significant body of evidence have associated, among others, the glutamatergic hypothesis of acute neurodegeneration with stroke (Choi, 1988). Glutamate acts through the activation of either the ionotropic (NMDA, AMPA and kainic acid subtypes) or the metabotropic receptors (Hollman and Hwinemann, 1994). A great attention has been concentrated on the activation of NMDA receptors as a key event in triggering excitotoxicity and therefore in studying their role in major brain physiological and pathological conditions, such as stroke, traumatic head injury and hypoglycemia. Stroke is generally defined as an abrupt onset of neurological functions caused by a sudden reduction of cerebral blood flow, which is due in turn to either an ischaemic occlusion or a haemorrhagic episode. This event is one of the leading causes of death worldwide and the first cause of long-term disability with a high health-care cost for society. Although it is possible to reduce the factors of risk for stroke, no effective neuroprotective agents, able to block the progression of the cerebral damage, are currently available. Therefore, neuroprotection after stroke is clearly an area of unmet need in current medicine.

In ischaemic conditions, the reduction of the oxygen-energy supply to the cells causes a significant increase of glutamate within the synaptic clefts (Cotman et al., 1981; Watchins and Evans, 1981; Choi and Rothman, 1991). This event is responsible for the overstimulation of the glutamatergic receptor (Fig. 1). In particular, the activation of the ion channel associated with the NMDA receptor results in a massive influx of Ca^{2+} into the post-synaptic neurones, leading to cell death through the activation of different neurotoxic cascades (excitotoxicity) (Di Fabio et al., 1998a). Therefore, a pharmacological intervention with competitive and non-competitive NMDA antagonists, blocking the abnormal influx of Ca^{2+} within the post-synaptic neurones, has been suggested to have a potential therapeutic benefit (Meldrum, 1991; Collingridge and Watkins, 1994). The molecular structure of the NMDA receptor is not fully elucidated yet. By analogy with other receptor gated ion channels (e.g., nicotinic receptor), it is believed that NMDA receptor is formed (Fig. 2) by the assembly of five different subunits named NR1 and $NR2_{A\text{-}B\text{-}C\text{-}D}$, respectively (Nakanishi, 1992; Mori and

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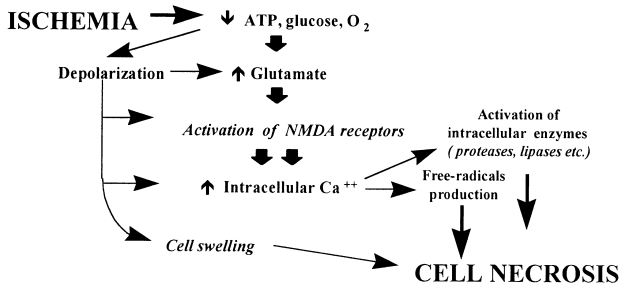


Fig. 1. Involvement of NMDA receptors in the excitotoxic cascade.

Mishina, 1995) which are encoded by separate genes and several splice variants for each gene have been described (Danysz and Parsons, 1998). On the basis of morphological studies, it has been shown that NMDA receptors are probably heteromeric assemblies of at least four subunits, with their composition depending from the regional expression in different brain areas (Danysz and Parsons, 1998). Such a conclusion is also supported by electrophysiological and pharmacological studies showing that the pharmacology of NMDA receptor ligands (agonist and antagonists) is strongly influenced by the receptor composition. The pathophysiological implication of these differences is still unknown, but this remains a fascinating area of research interest.

In addition to the molecular/structural complexity described above, the NMDA receptor is also characterized by a higher level of complexity which is given by the different regulatory sites which control its function. Among the different binding sites present within the NMDA receptor, a special interest has been attracted by the strychnine-insensitive glycine site (glycine receptor). The conductance through the channel associated with the NMDA receptor is indeed strongly influenced by the presence of glycine. Some years ago, it has been reported (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988) that in cultured cerebral neurones, the frequency of the opening of this ion channel was markedly affected by the presence of glycine. This event was so relevant that in the absence of glycine, glutamate failed to activate the NMDA receptor. Based on these results, glycine was defined as *co-agonist* of glutamate. In other words, glutamate and glycine co-operate together to promote the transition of the NMDA ion channel associated from the closed to the open state (Corsi et al., 1996; Danysz and Parsons, 1998).

Therefore, the glycinergic site associated to the NMDA receptor has gained considerable interest and, during the last decade, it was perceived as a unique target for medicinal chemistry in terms of potential therapeutic intervention (Iversen and Kemp, 1994; Leeson and Iversen, 1994; Di Fabio et al., 1996), since the observation that one of the first glycine antagonists identified, the 7-chloro kynurenic acid (7-Cl KA) (**1**) depicted in Fig. 3, was endowed with a greater therapeutic index compared to different series of competitive and non-competitive NMDA antagonists (Chiamulera et al., 1990). Then, considerable efforts have been devoted to find potent and selective ligands, resulting in the identification of several classes of glycine antagonists (e.g., tetrahydroquinolines, indoles, quinoxalines, etc.) as exemplified in Fig. 3.

However, despite their high in vitro affinity at the glycine binding site, many of the aforementioned compounds showed poor in vivo activity in a variety of animal models, probably because of their insufficient brain penetration (Carling et al., 1993; Moore et al., 1993). As a result of our efforts aimed at the identification of novel in vivo active glycine antagonists as potential antistroke agents, a number of new templates were selected for further exploration, and, among others, we focused our attention on pyrido[2,3-*b*]pyrazines (**7**) (Micheli et al., 1996, 1997) indole-2-carboxylates (**8**) (see for example, Di Fabio et al., 1998a) and benzoazepines (**9**) (Di Fabio et al., 1999a) (Fig. 4). The synthesis and pharmacological characterization of both indoles and benzoazepines will be described in detail in the following sections. Even though the

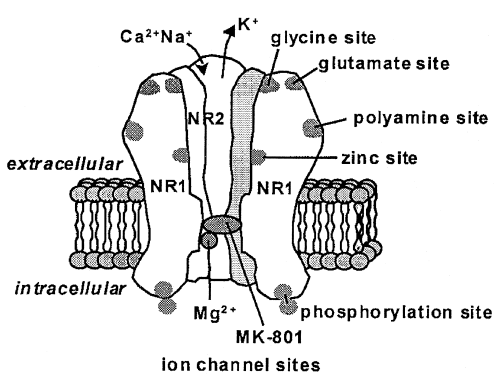


Fig. 2. Schematic representation of the NMDA receptor.

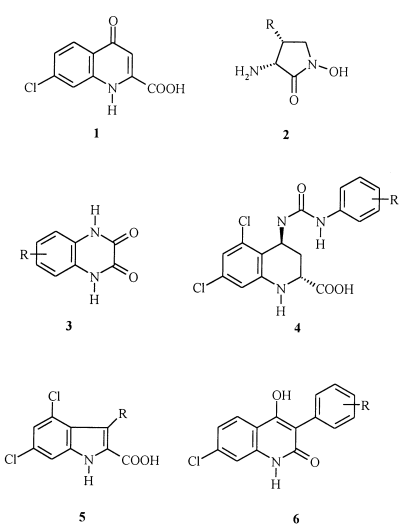
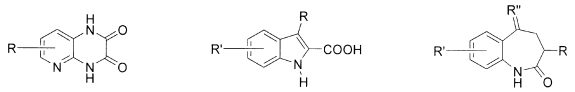


Fig. 3. Classes of glycine antagonists.



(7) (8) (9)

Fig. 4. General structures of pyrido[2,3-*b*]pyrazines (**7**), indole-2-carboxylates (**8**) and benzoazepines (**9**).

following discussion will be centered on the discovery and characterization of novel glycine antagonists as potential antistroke agents, it is worth mentioning that increasing evidences support the hypothesis that the NMDA receptors play a pivotal role in the development and maintenance of pain hypersensitivity (Woolf, 1983; Haley et al., 1990; Woolf, 1995; Harris et al., 1996). Following such a hypothesis, glycine antagonists, blocking the overactivation of the NMDA receptor and restoring the baseline level of nociceptive transmission should be effective for the treatment of the chronic pain. The identification of novel glycine antagonists as potential and effective antihyperalgesic agents was therefore considered an attractive and further objective for our project. The results obtained in this field will however be reported and discussed in detail elsewhere (Di Fabio et al., 1998b; Quartaroli et al., 1999).

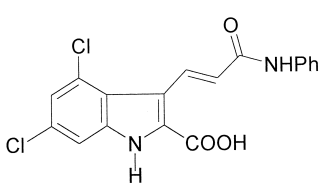
2. Results and discussion

2.1. Biological evaluation

All the new chemical entities (NCEs) were evaluated, both in vitro and in vivo, according to the following screening cascade: (a) binding assay to evaluate the affinity for the glycine site (Kishimoto et al., 1981) and selectivity for glutamate receptors (Honoré et al., 1986; Giberti et al., 1991; Van Amsterdam et al., 1992); (b) in vitro functional antagonism studies to evaluate potency and activity (Kloog et al., 1988; Ratti et al., 1990); (c) in vivo anticonvulsant activity (Chiamulera et al., 1990); and (d) the most interesting compounds in the NMDA-induced convulsions model, were then submitted for in vivo models of stroke in rats (MCAo) both pre- and post-ischaemia (Tamura et al., 1981).

2.2. Indole-2-carboxylates

Indole-2-carboxylates of general structure (**5**) represent a class of glycine antagonists widely explored in the last years by a number of different research groups (see for example, Huettner, 1989; Salituro et al., 1990; Rowley et al., 1992) following the preliminary evidence that the substituent at the C-3 position of the indole nucleus could modulate the affinity of these compounds for the strychnine-insensitive glycine binding site. However, glycine antagonists identified within this class were devoid of significant in vivo activity probably because of their poor ability to penetrate the brain. Therefore, we became interested in the synthesis of novel indole-2-carboxylates exhibiting the desired in vivo profile. This activity led to the synthesis of indole-2-carboxylates substituted at the C-3 position with suitable α,β-unsaturated side chains, which showed nanomolar affinity for the glycine binding site coupled with both high receptor selectivity and high in vivo potency in the NMDA-induced convulsions model in mice (i.v. an p.o. route). Among the compound initially synthesized, GV150526 (gavestinel) (**10**), currently undergoing clinical studies in man, is the most promising glycine antagonist identified to date (Di Fabio et al., 1997; Di Fabio et al., 1998a). The most relevant characteristics of GV150526 are summarized in the Table 1. The ability of GV150526 to cross the blood–brain barrier results in a substantial neuroprotective effect in a rat model of Middle Cerebral Artery occlusion (MCAo) when given both pre-ischaemia (70% of maximal protection at 3 mg/kg i.v.) and post-ischaemia up to 6 h from the induction of the cerebral damage. As shown in Fig. 5, GV150526, given 6 h after the occlusion, was able to completely stop the damage progression.



(10)
GV150526 (gavestinel)

These results support the view that the blockade of NMDA receptor could be of therapeutic benefit, and, in this respect, glycine antagonists appear to be extremely effective. Furthermore, GV150526 lacks the adverse behavioral effects in rats observed for both competitive NMDA antagonists and NMDA channel blockers: neither

Table 1
Pharmacological profile of GV150526

Type of study	Assay	Results	Notes
Affinity	[^{3}H]-glycine binding	$PK_i = 8.49 \pm 0.02$	Rat cortical membranes
Selectivity	Receptogram (40 brain receptors)	No displacement at 10 μM	
Systemic activity	NMDA induced convulsion in mice, i.v.	$ED_{50} = 0.06$ mg/kg	Reversed by glycine agonist (D-serine)
MCAo model	Rat, i.v.	$ED_{50} = 0.8$ mg/kg pre-ischaemia	
		$ED_{50} = 3.0$ mg/kg post-ischaemia	Single fully protective dose given 6 h after occlusion

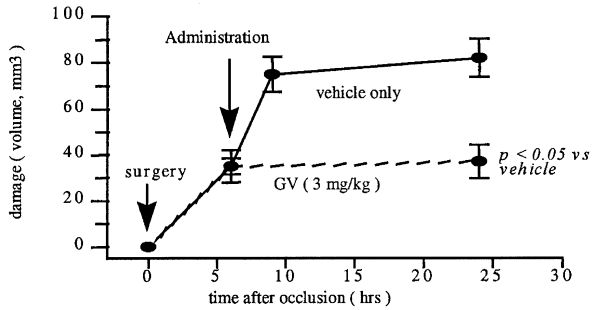
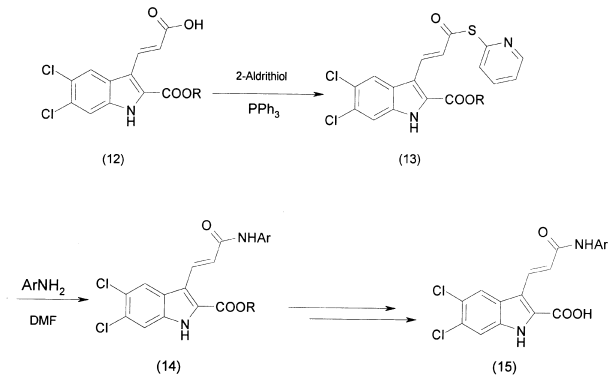
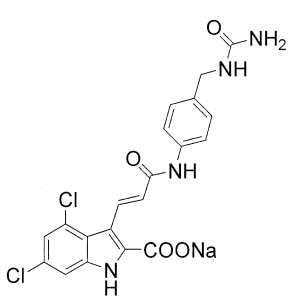


Fig. 5. Neuroprotective effect of GV150526A after post-ischaemia administration (6 h).



Scheme 1. Synthesis of substituted indole-2-carboxylates.

ataxic effects (rotarod) nor impairment of performance (passive avoidance) were observed in mice up to a dose of 30 mg/kg administered i.v. (500-fold the ED_{50} observed in inhibition of NMDA-induced convulsions), confirming the wider therapeutic window observed with glycine antagonists (Di Fabio et al., 1997; Di Fabio et al., 1998a and references cited therein). These results also confirm the view that our indole 2-carboxylate template could be useful to generate novel and effective compounds, strengthening the need of continuing the chemical exploration of such a class (Di Fabio et al., 1998a). This further chemical exploration allowed identification of a number of analogues of GV150526, and among them, GV228869 (**11**) exhibited high in vitro affinity to the glycine binding site (Table 2) and promising physico-chemical and pharmacological profile (Di Fabio et al., 1999b). In particular, it was found to be effective in blocking NMDA induced convulsions and it showed a significantly better neuroprotective activity in the MCAo model with respect to GV150526 when given post-ischaemia up to 6 h after occlusion of the middle cerebral artery.



(11) GV228869

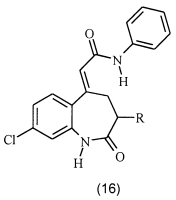
The aforementioned indoles and their analogues can be prepared according to different reported methods (Di Fabio et al., 1997, 1999b; Giacobbe et al., 1999 and references cited therein), and in particular, following the general synthetic route described in Scheme 1 (Di Fabio et al., 1999b and references cited therein). The key intermediate (**12**), which is now available in large-scale as previously described (Di Fabio et al., 1997) was transformed into the amido derivative (**14**) by using different methods of activation of the carboxyl group. The best results were obtained via the formation of the corresponding 2-pyridyl thioester (**13**) by using a stoichiometric amount of 2,2′-dipyridyldisulphide and triphenylphosphine. In general, (**13**) is stable enough to be purified by classical chromatographic procedures; then, it can be reacted with the proper arylamines to give the amides (**14**) (the same products can be also obtained by a ''one-pot'' procedure, without the isolation of (**13**)). The amides (**14**) can then be transformed into the final indole-2-carboxylates (**15**) in fairly good overall yields by removal of the various protecting groups according to standard methods.

2.3. Benzoazepines

To discover novel non-indole classes of glycine antagonists as potential back-up to GV150526A, new templates were designed by receptor mapping techniques based on the pharmacophore model of the glycine binding site (Di

Table 2
Summary of the pharmacological profile of GV228869 compared to GV150526

Type of study	GV150526	GV228869
Affinity K_i	3 nM	2 nM
Receptor selectivity IC_{50}	> 10 μM (70 receptors)	> 10 μM (70 receptors)
NMDA-induced convulsions ED_{50}	0.06 mg/kg (mice, i.v.)	0.07 mg/kg (mice, i.v.)
MCAo model pre-ischaemia (rat, i.v.)	ED_{50} = 0.8 mg/kg	ED_{50} = 0.2 mg/kg
Max. protection	70% at 3 mg/kg i.v.	70% at 3 mg/kg i.v.



(16)

Fig. 6. Benzoazepine derivatives.

Fabio et al., 1997 and references cited therein). Among others, a novel series of benzoazepine derivatives of general structure (**16**), bearing the same α,β-unsaturated side chain as GV150526 at the position C-5 (Fig. 6) and different substituent at C-3 was proposed. A number of different compounds was then prepared and evaluated and results are reported in Table 3 (Di Fabio et al., 1999a).

As expected, the unsubstituted derivative was devoid of any in vitro activity, while the most active compound of such a series was the 3-carboxylic derivative GV224029 (Table 3, entry 3). Also, 3-carboxyamides (entries 4 and 5) have shown an interesting affinity, comparable to GV224029. The last one was further characterized from a pharmacological point of view and results are reported in Table 4 below. In particular, it is worth underlying that in spite of its 10-fold lower affinity for the glycine binding site, GV224029 exhibited a similar neuroprotective activity with respect to GV150526 in the MCAo test both pre- and post-ischaemia. The synthesis of benzoazepine derivatives was performed following different synthetic routes, according to the nature of the substituent at C-3. For the sake of simplicity, only the synthesis of the C-3 carboxylic derivatives GV224029 is reported (Scheme 2). Thus, 4-chloro-2-nitro-iodobenzene (**18**) was obtained via Sandmeyer reaction from 4-chloro-2-nitroaniline (**17**), then reduced to the corresponding aniline derivative (**19**) in high yield using Fe in AcOH/EtOH. Reaction with *p*-methoxybenzyl chloride and sodium iodide in DMF, gave the protected aniline (**20**), which was reacted with methyl malonylchloride to give the amide (**21**) in high yield after proper deprotection and esterification with *t*-butyl alcohol (the protection as *t*-butyl ester in place of the methyl ester was required to avoid the double bond isomerization during deprotections in the last steps). When the malonamide (**21**) was reacted with 3-bromo-*N*-phenylacrylamide under basic conditions, we obtained the open intermediate (**22**) which was then cyclized, in the presence of palladium tetrakis(triphenylphosphine) to give the protected benzoazepine (**23**). The final removal of both the N-1 and C-3 carboxylic protecting groups was smoothly accomplished treating (**23**) first with anisole in H_2SO_4/TFA and then with base to give the final compound GV224029 (**24**).

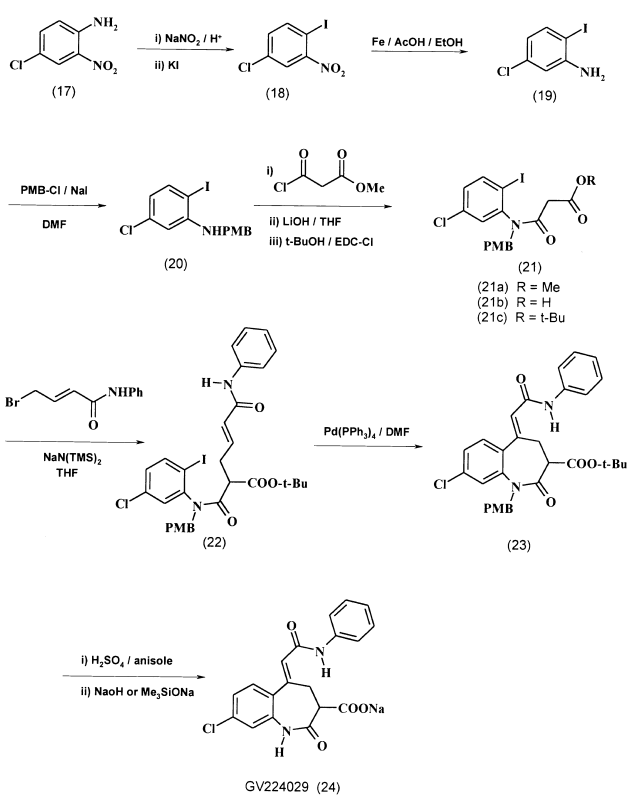
Table 3
Affinity of benzoazepine derivatives for the strychnine-insensitive binding site

Entry	*R*	K_i (nM)[a]
1	H	$>10^5$
2	OH	1288
3 (GV224029)	COOH	32
4	$CONH_2$	39
5	CONHPh	72
6	$COOCH_3$	916
7	CN	339

[a][^{3}H]-glycine displacement.

Table 4
Summary of the pharmacological profile of GV224029 compared to GV150526

Type of study	GV150526	GV224029
Affinity K_i	3 nM	32 nM
Receptor selectivity IC_{50}	>10 μM (70 receptors)	>10 μM (70 receptors)
NMDA-induced convulsions ED_{50}	0.06 mg/kg (mice, i.v.)	0.07 mg/kg (mice, i.v.)
MCAo model		
Pre-ischaemia (rat, i.v.)	$ED_{50} = 0.8$ mg/kg	$ED_{50} = 0.6$ mg/kg
Post-ischaemia (rat, i.v.)	$ED_{50} = 3$ mg/kg	$ED_{50} = 2.5$ mg/kg



Scheme 2. Synthesis of GV224029.

3. Conclusions

In spite of great efforts by many research groups, there are still no effective drugs available to treat stroke in humans. Among the number of different approaches that are currently under investigation, the search for novel and effective antagonists at the strychnine-insensitive binding site remains probably the most exciting in the field of neuroprotection. Our search in this context has provided new and further evidences of the validity of such an approach and the discovery of new templates has represented a clear step forward. The synthesis and pharmacological characterization of novel indole-2-carboxylates bearing a suitable α,β-unsaturated side chain at position C-3 led to the identification of a number of potent, selective and systemically active glycine antagonists and a member of this class, GV150526 (gavestinel) is currently in phase III clinical trials. In this respect, the importance of the α,β-unsaturated side chain in the interaction with the glycine binding site has been recently supported with the synthesis and the pharmacological characterization of pyrrole-2-carboxylic acids bearing the same side chain as GV150526 and analogues (Tarzia et al., 1998; Tarzia et al., 1999). At the same time, the identification of novel antagonists belonging to the benzoazepine series has allowed us to further elaborate the existing pharmacophoric model concerning the interaction with the glycine binding site and GV224029 represents a valid prototype for a further progression of the relevant studies.

Acknowledgements

I wish to thank all my colleagues who gave a strong enthusiastic contribution to the very important results reported above; their names are reported in the references below.

References

Carling, R.W., Leeson, P.D., Moseley, A.M., Smith, J.D., Saywell, K., Tricklebank, M.D., Kemp, J.A., Marshall, J.R., Foster, A.C., Grimwood, S., 1993. Anticonvulsant activity of glycine-site NMDA antagonists: 2. Trans 2-carboxy-4-substituted tetrahydroquinolines. Bioorg. Med. Chem. Lett. 3, 65–70.

Chiamulera, C., Costa, S., Reggiani, A., 1990. Effect of NMDA and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning. Psychopharmacology 102, 551.

Choi, D.W., 1988. Neuron 1, 623.

Choi, D.W., Rothman, S.M., 1991. The role of glutamate neurotoxicity in hypoxic–ischemic neuronal death. Annu. Rev. Pharmacol. Toxicol. 31, 171–182.

Collingridge, G.L., Watkins, J.C., 1994. The NMDA Receptor, 2nd edn. IRL Press, Oxford.

Corsi, M., Fina, P., Trist, D.G., 1996. Co-agonism in drug receptor activation: illustrated by the NMDA receptor. Trends Pharmacol. Sci. 17, 220–222.

Cotman, C.W., Foster, A.C., Lanthorn, T.H., 1981. An overview of glutamate as a neurotransmitter. Adv. Biochem. Biopharmacol. 27, 1–27.

Danysz, W., Parsons, C.G., 1998. Glycine and NMDA receptors—physiological significance and possible therapeutic applications. Pharmacol. Rev. 50, 597–664.

Di Fabio, R., Gaviraghi, G., Reggiani, A., 1996. Strychnine-insensitive glycine binding site and the NMDA receptor. La Chimica e l'Industria 78, 283–289.

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., 1997. Substituted indole-2-carboxylates as in vivo potent antagonists acting at the strychnine-insensitive glycine binding site. J. Med. Chem. 40, 841–850.

Di Fabio, R., Cugola, A., Donati, D., Feriani, A., Gaviraghi, G., Ratti, E., Trist, D.G., Reggiani, A., 1998a. Identification and pharmacological characterization of GV150526, a novel glycine antagonist as a potent neuroprotective agent. Drugs Future 23, 61–69.

Di Fabio, R., Corsi, M., Donati, D., Gaviraghi, G., Quartaroli, M., Ratti, E., Reggiani, A., Trist, D.G., 1998. Glycine antagonists as potent analgesic compounds, XVth EFMC International Symposium on Medicinal Chemistry. Book of Abstract, D3.

Di Fabio, R., Antolini, M., Bertani, B., Conti, N., Feriani, A., Messeri, T., Missio, A., Pasquarello, A., Pentassuglia, G., Quaglia, A.M., Reggiani, A., Sabbatini, F.M., 1999. SAR and neuroprotective activity of a novel class of glycine antagonists. Book of Abstracts 217th ACS National Meeting, Anaheim, USA, no. 278.

Di Fabio, R., Barnaby, R.J., Conti, N., De Magistris, E., Feriani, A., Provera, S., Sabbatini, F.M., Reggiani, A., Rovatti, L., 1999b. Substituted analogues of GV150526 as potent glycine binding site antagonists in animal models of cerebral ischaemia. J. Med. Chem. 42, in press.

Giacobbe, S., Di Fabio, R., Baraldi, D., Cugola, A., Donati, D., 1999. Synthesis of substituted indole-2-carboxylates: versatile introduction of a carbamoyl moiety at the C-position. Synth. Commun. 29, 3125–3135.

Giberti, A., Ratti, E., Gaviraghi, G., van Amsterdam, F.Th.M., 1991. Binding of DL-[^{3}H]-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) to rat cortex membranes reveals two sites or affinity states. J. Recept. Res. 11 (5), 727–741.

Haley, J.E., Sullivam, A.F., Dickenson, A.H., 1990. Evidence for spinal *N*-methy-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Res. 518, 218–226.

Harris, J.A., Corsi, M., Quartaroli, M., Arban, R., Bentivoglio, M., 1996. Up-regulation of spinal glutamate receptors in chronic pain. Neuroscience 74, 7–12.

Hollman, M., Hwinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Honoré, T., Drejer, J., Nielsen, M., 1986. Calcium discriminates two [^{3}H]-kainite binding sites with different molecular targets sizes in rat cortex. Neurosci. Lett. 65, 47–52.

Huettner, J.E., 1989. Indole-2-carboxylic acid: a competitive antagonist of potentiation by glycine at the NMDA receptor. Science 243, 1611–1613.

Iversen, L.L., Kemp, J.A., 1994. Noncompetitive NMDA antagonists as drug. In: Collingridge, G.L., Watkins, J.C. (Eds.), The NMDA Receptor, 2nd edn. IRL Press, Oxford, pp. 469–486.

Johnson, J.W., Ascher, P., 1987. Glycine potentiates the NMDA receptor reponse in cultured brain mouse neurones. Nature 325, 529–531.

Kishimoto, H., Simon, J.R., Aprison, M.H., 1981. Determination of the equilibrium dissociation constant at a number of glycine binding site in several areas of the rat central nervous system, using a Na-independent system. J. Neurochem. 37, 1015–1024.

Kleckner, N.W., Dingledine, R., 1988. Requirements for glycine in activation of NMDA receptors expressed in xenopus oocytes. Science 241, 835–837.

Kloog, Y., Haring, R., Sokolovsky, M., 1988. Kinetic characterization of

the phenylcyclidine-*N*-methyl-D-aspartate receptor interaction: evidence for steric blockade of the channel. Biochemistry 27, 843–848.

Leeson, P.D., Iversen, L.L., 1994. The glycine site on the NMDA receptor: structure–activity relationships and therapeutic potential. J. Med. Chem. 37, 4053–4067.

McCullogh, J., 1992. Excitatory amino acid antagonists and their potential for the treatment of ischemic brain damage in man. Br. J. Clin. Pharmacol. 34, 106–114.

Meldrum, B., 1991. Excitatory amino acid antagonists. Blackwell, Oxford.

Meldrum, B., Garthwaite, J., 1990. Excitatory amino acid neurotoxicity and neurodegenerative diseases. Trends Pharmacol. 11, 379–387.

Micheli, F., Cugola, A., Donati, D., Guarneri, M., Missio, A., Pecunioso, A., Reggiani, A., Tarzia, G., Zanirato, V., 1996. Synthesis and biological evaluation of pyrido[2,3-*b*]pyrazines and pyrido[2,3-*b*]pyrazine-*N*-oxide as selective glycine antagonists. Bioorg. Med. Chem. Lett. 6, 2749–2754.

Micheli, F., Cugola, A., Donati, D., Missio, A., Pecunioso, A., Reggiani, A., Tarzia, G., 1997. 2,3-Dihydro-6,7-dichloro-pyrido[2,3-*b*]pyrazine-8-oxide as selective glycine antagonist with in vivo activity. Bioorg. Med. Chem. 5, 2129–2132.

Moore, K.W., Leeson, P.D., Carling, R.W., Tricklebank, M.D., Singh, L., 1993. Anticonvulsant activity of glycine-site NMDA antagonists: 1. 2-Carboxyl prodrugs of 5,7-dichlorokynurenic acid. Bioorg. Med. Chem. Lett. 3, 61–64.

Mori, H., Mishina, M., 1995. Structure and function of the NMDA receptor channel. Neuropharmacology 34, 1219–1237.

Nakanishi, S., 1992. Molecular diversity of glutamate receptors and implications for brain function. Science 258, 597–603.

Quartaroli, M., Carignani, C., Dal Forno, G., Mugnaini, M., Ugolini, A., Arban, R., Bettelini, L., Maraia, G., Belardetti, F., Reggiani, A., Trist, D.G., Ratti, E., Di Fabio, R., Corsi, M., 1999. Potent antihyperalgesic activity without tolerance produced by glycine site antagonist of *N*-methyl-D-aspartate receptor GV196771A. J. Pharmacol. Exp. Ther. 290, 158–169.

Ratti, E., Tacconi, S., Graziani, F., Gaviraghi, G., 1990. Requirement of the glycine for the glutamate activity at the NMDA receptor site. Eur. J. Pharmacol. 183, 1665.

Rowley, M., Leeson, P.D., Grimwood, S., Foster, A., Saywell, K., 1992. 2-Carboxy-indolines and indoles as potential glycine/NMDA antagonists: effect of five-membered ring conformation of affinity. Bioorg. Med. Chem. Lett. 2, 1627–1630.

Salituro, F.G., Harrison, B.L., Baron, B.M., Nyce, P.M., Stewart, K.T., McDonald, I.A., 1990. Indole derivatives: antagonists of the stychnine-insensitive glycine receptor associated with the *N*-methyl-D-aspartate receptor complex. J. Med. Chem. 33, 2944–2946.

Tamura, A., Graham, D.I., McCullogh, J., Teasdale, G.M., 1981. Focal cerebral ischaemia in rat: 1. Description of techniques and early neuropathological consequences. J. Cereb. Blood Flow Metab. 1, 53–60.

Tarzia, G., Balsamini, C., Bedini, A., Diamantini, G., Spadoni, G., Tontini, A., Di Fabio, R., Feriani, A., Reggiani, A., Tedesco, G., Valigi, R., 1998. (*E*)-3-(2-(*N*-Phenylcarbamoyl)vinyl)pyrrole-2-carboxylic acid derivatives: a novel class of glycine antagonists. J. Med. Chem. 41, 808–820.

Tarzia, G., Balsamini, C., Bedini, A., Diamantini, G., Spadoni, G., Tontini, A., Di Fabio, R., Donati, D., 1999. 3-(2-Carbamoylvinyl)-4,5-dimethylpyrrole-2-carboxylic acids as ligands at the NMDA glycine-binding site: a study on the 2-carbamoylvinyl chain modification. Il Farmaco 54, 101–111.

Van Amsterdam, F.Th.M., Giberti, A., Mugnaini, M., Ratti, E., 1992. 3-[(+)-2-Carboxypiperazin-4-yl]propyl-1-phosphonic acid recognizes two *N*-methyl-D-aspartate binding sites in rat cerebral cortex membranes. J. Neurochem. 59, 1850–1855.

Watchins, J.C., Evans, R.H., 1981. Excitatory amino acids transmitters. Annu. Rev. Pharmacol. Toxicol. 21, 165–204.

Woolf, C.J., 1983. Evidence for a central component of post-injury hypersensitivity. Nature 306, 686–688.

Woolf, C.J., 1995. An overview of the mechanism of hyperalgesia. Pulm. Pharmacol. 8, 161–167.