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# SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRIDO[2,3-b]PYRAZINE AND PYRIDO[2,3-b]PYRAZINE -N-OXIDE AS SELECTIVE GLYCINE ANTAGONISTS.

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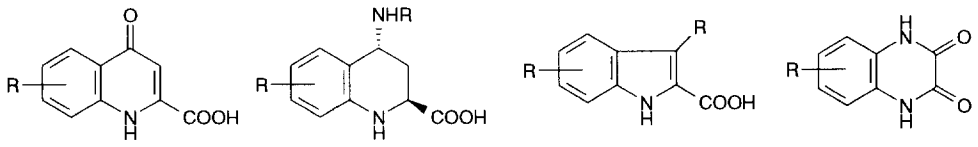
**Abstract:**

Pyrido[2,3-b]pyrazines and pyrido[2,3-b]pyrazines-N-oxides have been synthesized and evaluated for *in vitro/in vivo* antagonistic activity at the glycine site on the NMDA receptor. 

Overactivation of the N-methyl-D-aspartate (NMDA) receptor has been implicated in several neurodegenerative disorders including epilepsy, stroke and Alzheimer's disease[1]; actually over stimulation of this receptor leads to a massive influx of Calcium ions into post-synaptic neurons.
The resulting cell swelling, together with the activation of a huge number of neurotoxic cascades, leads to cell death[2].

The stimulatory action of glycine on the NMDA receptor was discovered in 1987 by Johnson and Ascher[3]. Among the endogenous modulators of the NMDA receptor, glycine gained a huge interest as a therapeutic site of intervention because of its action as co-agonist of the glutamate. Since then a large number of glycine antagonists have been developed; among them kynurenic acid derivatives (**1**, Fig. 1)[4], tetrahydroquinolines (**2**, Fig. 1)[5], 2-carboxyindoles (**3**, Fig. 1)[6] and quinoxalines derivatives (**4**, Fig. 1)[7] are worth of particular consideration.

Fig.1




**1** **2** **3** **4**

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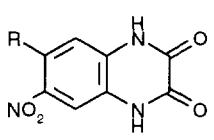
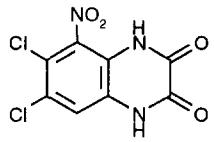
Quinoxaline-2,3-diones like CNQX (**5**, R = CN, Fig. 2) and DNQX (**5**, R = $NO_2$, Fig. 2) were introduced firstly as antagonists of the AMPA-subtype non-NMDA excitatory amino acids receptor and were subsequently shown to have comparable affinities for the glycine site.

Efforts to improve the glycine vs. AMPA selectivity in this series have focussed on both aromatic substitution and on modification of the heterocyclic ring[8].

Fig. 2




**5**

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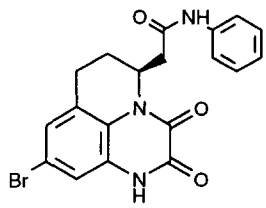



**7**

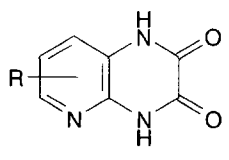
Moreover two recently disclosed derivatives, ACEA 1021[9] (**6**, Fig. 2) and the tricyclic quinoxaline dione[10] (**7**, Fig.2), showed interesting results both from the affinity and the selectivity point of view.

As a part of our research aimed at modulating the selectivity of the glycine vs. AMPA binding, we evaluated the replacement of the phenyl ring of the quinoxaline diones with different heteroaromatic rings.

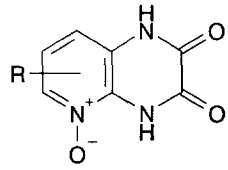
Among the different derivatives we produced, we particularly focused on pyrido[2,3-b]pyrazine and their correspondent N-oxides.

In this article we report the synthesis of these two new[11] classes of selective glycine antagonists in which the phenyl ring of the aromatic moiety was replaced by a pyridine (**8**) or a pyridine N-oxide (**9**) ring as depicted in Fig. 3.

Fig. 3



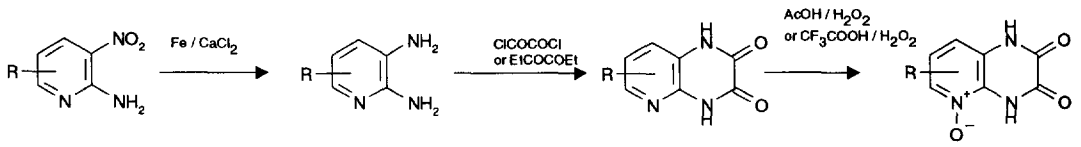

**8**




**9**

The general synthetic route used for the preparation of **8** and **9** is outlined in Scheme 1.

Scheme 1

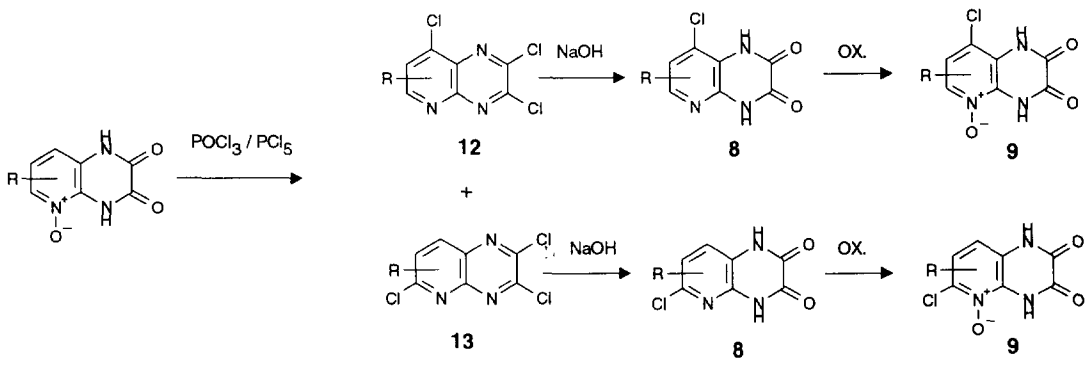



**10** **11** **8** **9**

Some of the starting substituted nitro amino pyridines (**10**) were commercially available, otherwise they were prepared according to classical standard synthetic procedures. The substituted diamino pyridines (**11**), obtained by reduction of the corresponding nitro-derivatives (**10**), were subsequently reacted with either diethyl oxalate or oxalyl chloride to give the desired pyrido[2,3-b]pyrazines (**8**). The corresponding N-oxides (**9**) were generally prepared by oxidation of (**8**) with a $CF_3COOH/ H_2O_2$ mixture. In few cases the milder AcOH / $H_2O_2$ mixture was used to avoid decomposition of the pyrido[2,3-b]pyridine (**8**).

The preparation of some chlorinated derivatives of the classes **8** and **9** that could not be easily obtained with the previously described route, was accomplished by direct halogenation of the pyrido[2,3-b]pyrazine system followed by the selective hydrolysis of the chlorines on the pyrazine ring as outlined in Scheme 2.

Scheme 2



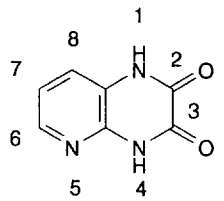

All these derivatives were tested *in vitro* to measure their affinity for the glycine binding site as shown in the tables below[12].

Notably both classes are endowed with a good affinity for the glycine binding site and show more than 100-fold selectivity vs. the AMPA receptor although the oxidised (**9**) appear to be slightly more potent and selective. We also discovered a good *in vivo* activity of our products either when intraperitoneally (i.p.) or intravenously (i.v.) administered, in a NMDA induced convulsion model in rats.

Among these structures, compound **9c** (Table 2), was shown to be extremely effective in reducing neuronal damage in the Middle Cerebral Artery occlusion model[13] (MCAo) not only with "pre-ischaemial" administration, but also with "post-ischaemial" administration following a multiple dose regimen.

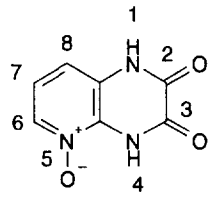
This compound revealed to be a very useful tool in testing the efficacy of a glycine antagonist as a neuroprotective drug after an ischaemic insult, leading us to explore chemically different classes which will be the object of future communications.

Table 1




| Entry | 8 | 7 | 6 | pKi | $ED_{50}$ i.p mg/kg. | $ED_{50}$ i.v. mg/kg |
|---|---|---|---|---|---|---|
| **8a** | Cl | Cl | Cl | 6.96 | | |
| **8b** | | Br | Cl | 6.84 | 100 | 20 |
| **8c** | | Cl | Cl | 6.73 | 41 | 0.7 |
| **8d** | Me | Cl | Cl | 6.72 | | |
| **8e** | | I | Cl | 6.69 | | |
| **8f** | Me | Br | Cl | 6.55 | | 10 |
| **8g** | | $CF_3$ | Cl | 6.37 | | 15 |
| **8h** | | I | Me | 6.33 | | |
| **8i** | | $CF_3$ | | 6.3 | 27 | 13 |
| **8l** | Me | Cl | Me | 6.09 | | |
| **8m** | | Cl | Pr | 5.82 | | |
| **8n** | Me | Br | | 5.8 | | |
| **8o** | Cl | $CF_3$ | | 5.8 | | |
| **8p** | Me | I | | 5.75 | | |
| **8q** | | Br | Cl | 5.72 | | |
| **8r** | Me | Cl | Pr | 5.56 | | |
| **8s** | Me | Cl | | 5.54 | 20 | |
| **8t** | | | Cl | 5.21 | | |

Table 2




| Entry | 8 | 7 | 6 | pKi | $ED_{50}$ i.p. mg/kg | $ED_{50}$ i.v. mg/kg |
|---|---|---|---|---|---|---|
| **9b** | | Br | Cl | 6.95 | 45 | 18 |
| **9c** | | Cl | Cl | 6.95 | 24 | 14 |
| **9d** | Me | Cl | Cl | 5.5 | 30 | |
| **9e** | | I | Cl | 6.68 | | |
| **9f** | Me | Br | Cl | 5.52 | | |
| **9g** | | $CF_3$ | Cl | 6.29 | 60 | 30 |
| **9h** | | I | Me | 6.65 | | |
| **9i** | | $CF_3$ | | 6.37 | | 15 |
| **9m** | | Cl | Pr | 6.34 | | |
| **9o** | Cl | $CF_3$ | | 5.43 | 50 | |
| **9p** | Me | I | | 6.04 | 30 | |
| **9s** | Me | Cl | | 6.43 | 7 | 3 |
| **9t** | | | Cl | 5.33 | | |
| **9u** | | Cl | | 6.29 | | |
| **9v** | Me | Br | | 6.27 | 9 | 5 |
| **9z** | | I | | 6.13 | 21 | |

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11) During the preparation of this work two patents were published, (JP 90-305656 901109) and (WO 9518616 A2 , claiming activity for derivatives belonging to class (**8**) and (**9**).

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