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2,3-Dihydro-6,7-dichloro-pyrido[2,3-*b*]pyrazine-8-oxide as Selective Glycine Antagonist with In Vivo Activity

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Abstract—2,3-Dihydro-6,7-dichloro-pyrido[2,3-*b*]pyrazine-8-oxide was synthesized and evaluated for in vitro/in vivo antagonistic activity at the strychnine insensitive glycine binding site on the NMDA receptor revealing it to be a useful tool to evaluate the effectiveness of glycine antagonists in vivo. © 1997 Elsevier Science Ltd.

Introduction

Over-activation of the *N*-methyl-D-aspartate (NMDA) receptor has been implicated in several neurodegenerative disorders including epilepsy, stroke and Alzheimer disease;[1] actually the over-stimulation of this receptor leads to a massive influx of calcium ions into post-synaptic neurons.[2]

The stimulatory action of glycine on the NMDA receptor was reported by Johnson and Ascher in 1987.[3] Among the endogenous modulators of the NMDA receptor, glycine created a great interest as potential therapeutic site of intervention because of its action as coagonist of glutamate. A large number of glycine antagonists have been reported, among them it is worth mentioning kynurenic acid derivatives (**1**, Fig. 1),[4] tetrahydroquinolines (**2**, Fig. 1),[5] 2-carboxy indoles (**3**, Fig. 1), [6] and quinoxaline derivatives (**4**, Fig. 1).[7]

Quinoxaline-2,3-diones like CNQX (**5**, R = CN, Fig. 1) and DNQX (**5**, R = NO_2, Fig. 1) were discovered first as an antagonist of the AMPA-subtype non-NMDA excitatory amino acids receptor and were then shown to have comparable affinity for the glycine site on the NMDA receptor. A large synthetic effort was produced in order to increase the glycine vs. AMPA selectivity and to make these molecules active in vivo. Two recently discovered derivatives, ACEA 1021[8] (**6**, Fig. 2) and the tricyclic quinoxaline dione **7**[9] (Fig. 2) showed interesting results in both the above mentioned tasks.

We have recently reported[10] the newly synthesized classes of pyrido[2,3-*b*]pyrazines (**8**, Fig. 2) and pyrido[2,3-*b*]pyrazines *N*-oxide (**8a**, Fig. 2) as selective glycine antagonists endowed with in vivo activity in animal models of ischemia.

In this paper we report the synthesis and the biological evaluation of 2,3-dihydro-6,7-dichloro-pyrido[2,3-*b*]-pyrazine-8-oxide **13** as the most interesting derivative belonging to the series mentioned above.

Chemistry

Commercially available 2-amino-3-nitro-6-chloropyridine **9** was treated with gaseous Cl_2 in anhydrous EtOH at 0 °C obtaining **10** as a yellow solid after filtration in 76% yield, as depicted in Scheme 1. Subsequent reduction with stannous chloride in concd hydrochloric acid at 80 °C for 1.5 h produced the diamino derivative **11** in 73% yield. This product was cyclised with diethyl oxalate at 150 °C for 3 h giving the compound **12** after cooling and dilution with diethyl ether in 40% yield. Finally, the product **13** was obtained in 84% yield after treatment of **12** with hydrogen peroxide in acetic acid for 3 h at 45 °C.

Biology

The product **13** was tested in vitro for its affinity for the strychnine insensitive glycine binding site associated to the NMDA receptor according to the method by Kishimoto et al.,[11] showing a pK_i of 7.0; moreover **13** was found to be more selective for the glycine binding site than towards the other ionotropic glutamate receptors (AMPA, NMDA, Kainate) by 100 times. Activity in vivo was then assessed and compared to 7-chlorokynurenic acid and MK-801.

Good activity was shown in a NMDA-induced convulsion model in mice (Table 1) when **13** was administered

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Key words: glycine, NMDA, pyrido[2,3-*b*]pyrazine, stroke, MCAo.

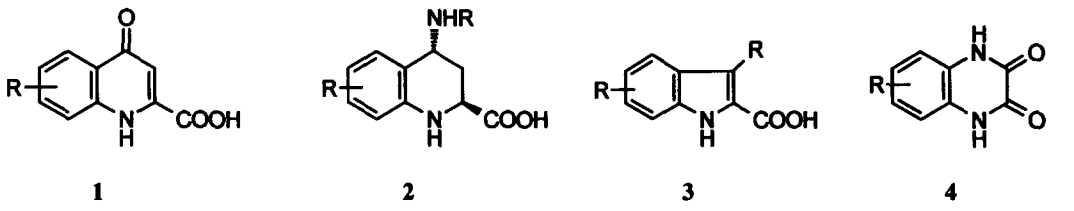


Figure 1.

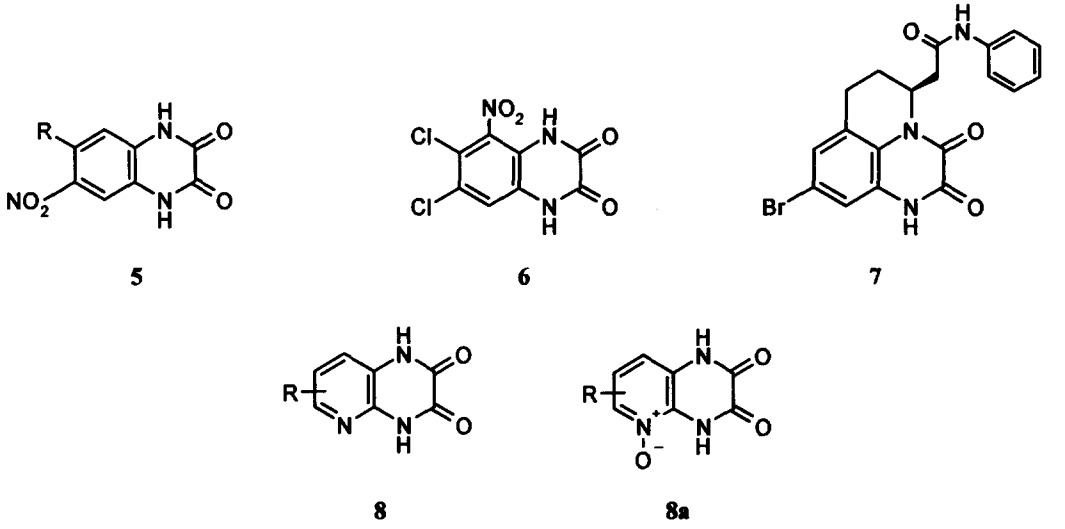


Figure 2.

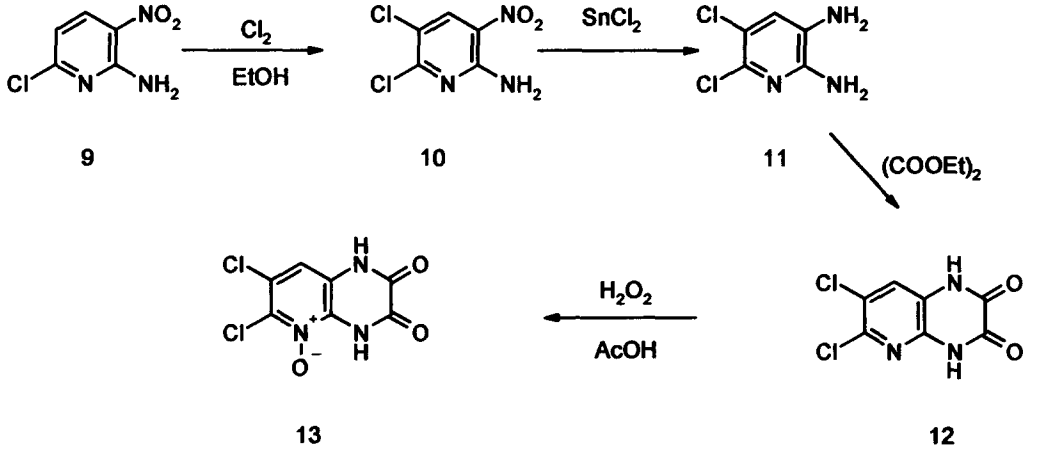
both intracerebroventricularly (icv) and intraperitoneally (ip) showing that the compound was able to penetrate to some extent into the brain, in spite of its high calculated log *P* value (−1.7).

Moreover, we tested **13** in the permanent middle cerebral artery occlusion (MCAo)[12] model in mice, a model of focal ischemia. It is worth noting that when given pre-ischemia at the dose of 20 mg/kg ip the compound **13** was able to reduce the infarcted area by 50%; moreover it is remarkable that when **13** was given post-ischemia (3 h after surgery) at the dose of 10 mg/kg ip, a 44% reduction of neuronal damage was observed as shown in Figure 3.

Possible side-effects of this molecule were carefully evaluated up to 50 mg/kg ip, but **13** gave no amnesic effect (passive avoidance in mice), no stereotyped behavior (Digiscan analysis in rats), no locomotor excitation (Digiscan analysis in rats and mice) and finally no motor incoordination (rota rod in mice).

Conclusion

2,3-Dihydro-6,7-dichloro-pyrido[2,3-*b*]pyrazine-8-oxide **13** was revealed to be a very useful tool in testing the efficacy of a glycine antagonist as a potential neuroprotective drug after an ischemic insult, leading us to explore chemically different classes, which will be the object of future communications.



Scheme 1.

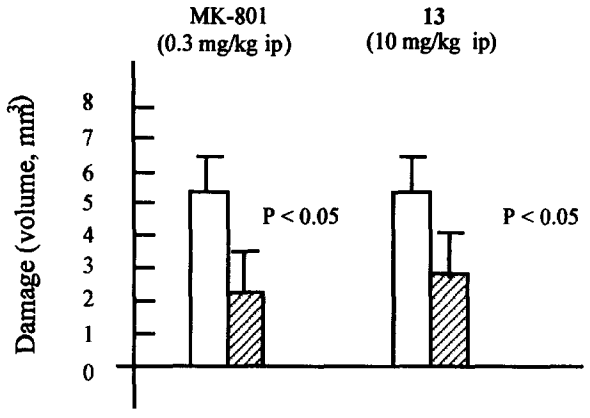


Figure 3. Protective effect of **13** and MK-801 given 3 h after the occlusion (volume of damage).

Experimental[13]

4,5-Dichloro-3-nitro-2-aminopyridine (10). A stirred suspension of 25 g (144 mmol) of 5-chloro-3-nitro-2-aminopyridine in 1200 mL of dry ethyl alcohol was cooled at 0 °C and a stream of chlorine was carefully bubbled through. After 40 min the temperature was raised to ca. 25 °C and a stream of nitrogen was bubbled into the resulting orange suspension to remove the excess of chlorine. The yellow solid was then recovered by filtration and dried under vacuum to give 22.5 g (76%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 8.50 (s, 1H). IR (nujol) 3433–3285, 1622, 1558, 1315. Anal. calcd for $C_5H_3C_{12}N_3O_2$: C, 28.87; H, 1.45; N, 20.20; Cl, 34.09; found: C, 28.73; H, 1.39; N, 20.24; Cl, 33.28.

4,5-Dichloro-2,3-diaminopyridine (11). To a suspension of 4,5-dichloro-3-nitro-2-aminopyridine (20 g, 96.1 mmol) in 500 mL of a ethanol:water 9:1 (v/v) solution were added, under vigorous stirring, 4.5 g (40 mmol) of $CaCl_2$ and 42 g (752 mmol) of Fe powder. The suspension was heated at reflux for ca. 1 h then cooled down to room temperature and filtered over Celite to remove the residual iron powder. The organic solution was concentrated under vacuum and the resulting dark-brown solid was purified by chromatography over a short silica gel column eluting with a dichloromethane:methanol (95:5 v/v) solvent mixture. The solvent was evaporated under vacuum to give 12.7 g (71.1 mmol) of the 4,5-dichloro-2,3-diaminopyridine as a gray solid (mp 165/166 °C uncorrected). ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 1H), 3.33 (bs, 2H), 3.28 (bs, 2H). IR ($CHCl_3$) 3464–3381, 1620. Anal. calcd for $C_5H_5C_{12}N_3$: C, 33.73; H, 2.83; N, 23.60; Cl, 39.83; found: C, 33.43; H, 2.72; N, 23.30; Cl, 38.82.

6,7-Dichloro-2,3-dihydroxypirido[2,3-*b*]pyrazine (12). A solution of 4,5-dichloro-2,3-diaminopyridine (5.1 g, 28.6 mmol) in diethyl oxalate (20 mL) was heated at reflux for 5 h. The resulting suspension was cooled to room temperature, diluted with ethyl acetate (60 mL) and filtered. The solid was washed with 3 × 15 mL of ethyl acetate and dried in vacuum overnight to give 4.7 g (71% yield) of an off-white solid. ^{1}H NMR (DMSO) δ 12.6–12.0 (bs+bs, 2H), 7.55 (s, 1H). IR (nujol) 1717, 1690. Anal. calcd for $C_7H_3Cl_2N_3O_2$: C, 36.23; H, 1.30; N, 18.11; Cl, 30.56; found: C, 35.92 ;H, 1.23; N, 18.09; Cl 30.59.

6,7-Dichloro-2,3-dihydroxypirido[2,3-*b*]pyrazine-5-oxide (13). To a solution of 3 g (13 mmol) of 6,7-dichloro-2,3-dihydroxypirido[2,3-*b*]pyrazine in 45 mL of acetic acid were dropwise added 20 mL of 30% H_2O_2. The solution was heated at 45 °C for 3 h, cooled down to rt and successively diluted with 100 mL of water and 200 mL of methanol. The obtained precipitate was collected by filtration, washed with 3 × 20 mL of methanol and dried under vacuum to give 2.7 g (84% yield) of a white solid showing: ^{1}H NMR (DMSO) δ 12.8–12.2 (bs+bs, 2H), 7.19 (s, 1H). IR (nujol) 1705. Anal. calcd for $C_7H_3C_{12}N_3O_3$: C, 33.90; H, 1.22; N, 16.94 ; Cl 28.59; found: C, 33.62; H, 1.16; N, 16.81; Cl, 28.82.

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