Bioorganic & Medicinal Chemistry Letters, Vol.3, No.1, pp. 65-70, 1993
Printed in Great Britain

0960-894X/93 $5.00 + .00

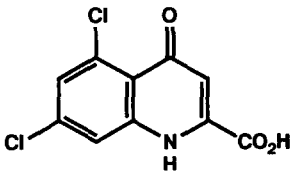
ANTICONVULSANT ACTIVITY OF GLYCINE-SITE NMDA ANTAGONISTS. 2. *TRANS* 2-CARBOXY-4-SUBSTITUTED TETRAHYDROQUINOLINES.

Robert W. Carling, Paul D. Leeson,* Angela M. Moseley, Julian D. Smith, Kay Saywell, Mark D. Tricklebank, John A. Kemp, George R. Marshall, Alan C.Foster and Sarah Grimwood

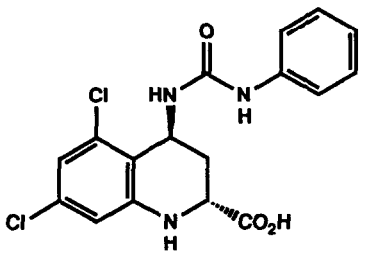
Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K.

Abstract: Anticonvulsant activity has been optimized in a series of glycine-site NMDA antagonists based on 2-carboxy tetrahydroquinoline, leading to the benzylamine **7** (L-690,590), its methyl ester prodrug **13** (L-691,470) and the phenylalanine **8** (L-696,833) which have ED_{50} values of 39, 31.5 and 29 mg/kg (i.p.) respectively in the DBA/2 mouse audiogenic seizure model. Correlations between *in vivo* and *in vitro* activities suggest that systemic anticonvulsant action of glycine antagonists depends on both brain penetration as well as 'access' to receptors within the brain.

Antagonists acting at the glycine modulatory site of the NMDA receptor complex are potential anticonvulsant and neuroprotective agents, and although compounds have been identified with good *in vitro* affinity, they generally lack systemic CNS-mediated activity.[1,2,3] We have shown that the *in vivo* anticonvulsant activity of the kynurenic acid derivative (**1**) can be improved by the preparation of 2-carboxyl prodrug esters.[2] 2-Carboxy-4-substituted tetrahydroquinolines (eg **2**) have much higher affinity than derivatives of **1**[4] and offer wider scope for structural modification[5,6] in pursuit of *in vivo* activity.



1



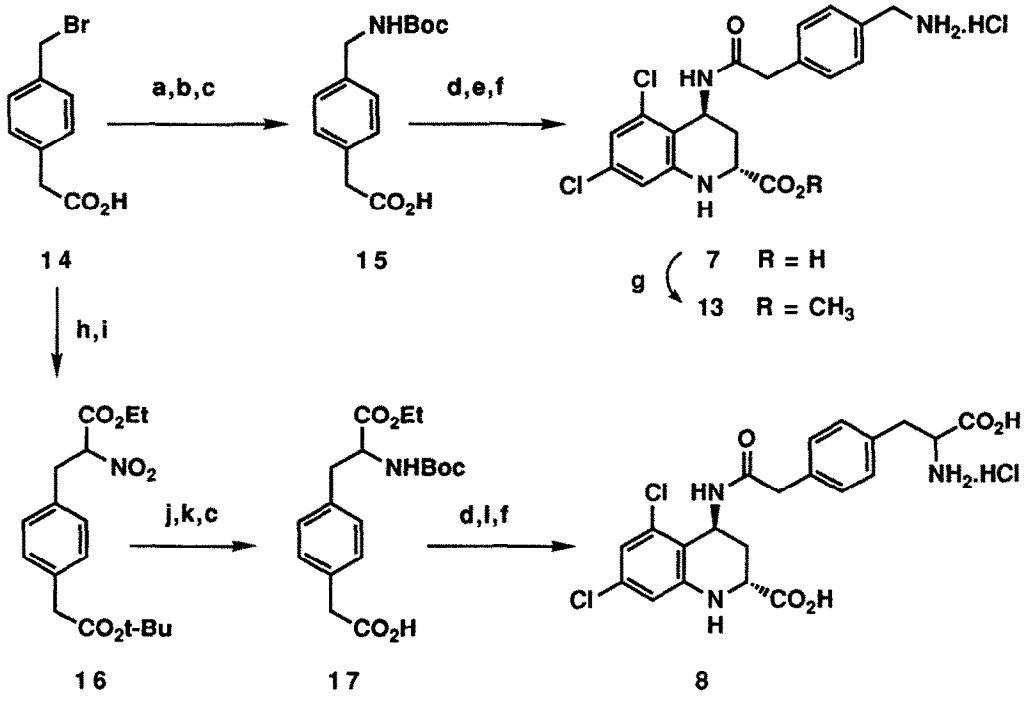
2

Herein we report the anticonvulsant properties of a series of glycine antagonists derived from **2** (compounds **2-8**, see Table) in the DBA/2 mouse audiogenic seizure model. To help establish which factors influence overall *in vivo* activity, anticonvulsant potencies (ED_{50} values) have been measured following both systemic (i.p.)[7] and intracerebroventricular (i.c.v.)[8] administration and

correlated with *in vitro* activity (K_b for blockade of NMDA-induced depolarisation in the rat cortical slice). The quinoxalinedione MNQX (**10**), a glycine-site NMDA receptor antagonist reported to possess anticonvulsant activity,[9] was also tested. Kynurenic acids (**1** and **9**),[1] the competitive NMDA receptor antagonist, CGP 37856 (**11**),[10] and the non-competitive NMDA receptor ion channel blocker, MK-801 (**12**),[11] were included in the study as reference compounds.

Compounds **2**-**5** have been described.[5,6] The *p*-aminomethyl derivative (**7**) was synthesized *via* coupling of the phenylacetic acid **15** with the previously described 4-amino tetrahydroquinoline precursor,[3,6] followed by appropriate deprotections (Scheme). The α-amino acid (**8**) was prepared

Scheme



Reagents: a) Hexamethylenetetramine, $CHCl_3$, reflux; b) 6N HCl; c) $(Me_3CO)_3O$, DMF, THF, Et_3N; d) *trans*-4-amino-5,7-dichloro-2-methoxycarbonyl-1,2,3,4-tetrahydroquinoline[3,5], $Me_2N(CH_2)_3N{=}C{=}NEt.HCl$, HOBT, Et_3N, THF; e) NaOH, H_2O, MeOH; f) HCl, EtOAc; g) HCl, MeOH; h) $O_2NCH_2CO_2Et$, NaH, DMF; i) $Me_2C{=}CH_2$, CH_2Cl_2, H_2SO_4; j) Raney Ni, H_2, EtOH, $(Me_3CO)_2O$; k) CF_3CO_2H; l) LiOH, THF, H_2O.

as a 1:1 mixture of diastereoisomers. Reaction of the anion of ethyl nitroacetate with **14** followed by treatment with isobutylene under acidic conditions gave the differentially protected diester **16**.

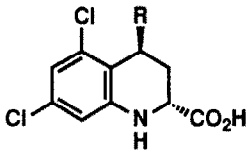
This was hydrogenated over Raney nickel in the presence of di-*tert*-butyl dicarbonate and the product was converted to the phenylacetic acid **17** by treatment with trifluoroacetic acid followed by reprotection. Standard amide coupling of **17** and subsequent deprotection gave **8**. The N-methyl urea (**6**) was synthesised via a 3-step procedure from the 4-amino tetrahydroquinoline precursor involving N-methylation (methyl iodide, triethylamine), reaction with phenylisocyanate and saponification.

The tetrahydroquinoline derivatives **2-8** are potent glycine-site NMDA receptor antagonists *in vitro*, as shown by their high affinities (IC_{50} values) for the glycine recognition site of the NMDA receptor labelled by [^{3}H]-L-689,560 ([^{3}H]-**2**)[12] or by [^{3}H]-glycine,[1] and by their blockade of NMDA responses in the rat cortical slice preparation (K_b values, see Table). We have previously shown that substitution within the amide and ureide groups of **2** and **3** is generally not detrimental,[6] and the retention of affinity in the benzylamine **7** and the phenylalanine **8** shows that ionic substitutents are also allowed. The *in vivo* anticonvulsant activities of compounds **1-10**, following systemic (i.p.) administration, are clearly very weak and bear no overall relationship to their *in vitro* affinities. For example, the urea **2**, the most potent compound *in vitro*, lacks *in vivo* activity at a dose of 100 mg/kg i.p. Replacement of the 4-amino group with methylene (**4**) and N-methylation (**6**) offered no improvement, but replacement of the aniline amino group by methylene results in a systemically active compound (**3**, ED_{50} 46 mg/kg). The reduced hydrogen bonding capacity of **3** relative to **2** is consistent with increased brain penetration,[13] but the octanol-water partition coefficients of **2** and **3** (log P 1.08 and 0.55 respectively at pH 7.4) are clearly not predictive of *in vivo* activity. In view of these results, the absence of systemic activity found with the highly polar dicarboxylic acid (**5**) is not surprising.

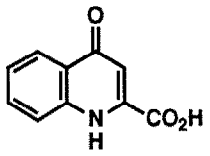
The *p*-substituted benzyl derivatives **7** (L-690,590, ED_{50} 39 mg/kg) and **8** (L-696,833, ED_{50} 29 mg/kg) retain the systemic activity of compound **3**. The benzylamine **7** can exist as a zwitterionic species, and it was envisaged that the lack of an overall charge would assist brain penetration. However, 5 minutes after a single i.p. injection of 30 mg/kg (the ED_{50} dose) to rats, plasma levels of **7** were 15 μg/mL (plasma $t_{1/2}$ 110 minutes) but CSF levels were at or below the limit of detection of **7** by HPLC (0.4 μg/mL, 0.90 μM),[14] showing poor brain penetration. Since the K_b of **7** is 0.23 μM, brain levels below the detection limit should be sufficient to account for its anticonvulsant activity. The phenylalanine derivative **8**, which was synthesised in an attempt to exploit amino acid transport[15] to increase brain levels, has similar systemic potency to **7**. Compounds **3**, **7** and **8** are the most potent anticonvulsants identified from an extensive series of 2-carboxytetrahydroquinolines that were made.[5,6] The quinoxalinedione MNQX (**10**) lacked anticonvulsant activity at a dose of 10 mg/kg. This result contrasts with the reported activity of **10** in DBA/2 mice (ED_{50} 0.1 mg/kg i.p.).[9] The reasons for this discrepancy are not clear.

Each of the glycine antagonists **1-9** were anticonvulsant after i.c.v. dosing (Table). ED_{50} values (nmol/mouse) of the tetrahydroquinolines **2-7** are reasonably well correlated with *in vitro* K_b, as shown by the comparable values of the corresponding ED_{50}/K_b ratios. The most potent

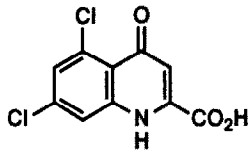
Table



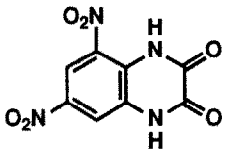
2-8



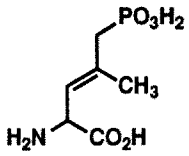
9



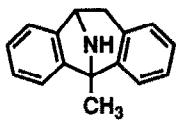
1



10



11



12

No[a]	R (Compounds 2-8)[b]	IC_{50} (μM)[c] [^{3}H]L-689,560	K_b (μM)[d] (NMDA)	ED_{50} DBA/2 mouse[e] i.p. (mg/kg)[f]	i.c.v. (nmol/mouse)[g]	ED_{50} (i.c.v.)/K_b
2	NHCONHPh	0.004	0.13	>100 (0/8)	1.5	12
3	$NHCOCH_2Ph$	0.014	0.53	46	5	9
4	$CH_2CONHPh$	0.019	1.08	>50 (0/8)	30	28
5	CH_2CO_2H	0.074	1.20	>100 (1/8)	35	29
6	$N(CH_3)CONHPh$	0.009	0.29	>100 (0/8)	8	28
7	$NHCOCH_2Ph(CH_2NH_2)$-4	0.019	0.24	39	3.5	15
8	$NHCOCH_2Ph(CH_2CH.NH_2(CO_2H))$-4	0.032	0.84	29	0.5	0.6
9	kynurenic acid	13.8	154	>250 (0/8)	20	0.1
1	5,7-dichlorokynurenic acid	0.064	3.0	>250 (0/8)	7.5	2.5
10	MNQX	0.17	1.23	>10 (0/8)	>20	>16
11	CGP 37849	-	0.20	0.8	0.02	0.1
12	MK-801	-	0.08	0.2	1.8	23

a Compounds **2-8** are racemic.

b All compounds had satisfactory elemental analyses and displayed spectral properties (^{1}H NMR and MS) consistent with their proposed structures

c Concentration giving 50% inhibition of [^{3}H]-L-689,560 binding to rat cortical membranes (ref 12); the values obtained are the means of 3 determinations.

d Antagonist potencies for blockade of NMDA-induced depolarisations in rat cortical slice (ref 1); the values obtained are the means from at least 3 experiments.

e Audiogenic seizure model in DBA/2 mice (21-23 days old, weighing 5-9g); the end point was tonic seizure within 30 seconds (ref. 7).

f ED_{50} values were measured 30 minutes after intraperitoneal (i.p.) administration of compounds dissolved in sodium hydrogen carbonate solution. Figures in brackets are numbers of mice protected from convulsions/numbers of mice tested at the dose indicated.

g ED_{50} values were measured15 minutes after intracerebroventicular (i.c.v.) administration (ref 8). Compounds were dissolved in1N NaOH solution and the pH adjusted to 8.0 with1N HCl.

tetrahydroquinoline following i.c.v. administration is the amino acid **8** (ED_{50} 0.5 nmol/mouse), which has an ED_{50}/K_b ratio 15-48 fold lower than derivatives **2-7**. A number of factors will be expected to influence antagonist potency following i.c.v. dosing, including aqueous and lipid solubility, access to receptors and retention of the compound within the brain. Differences in aqueous solubility do not appear to be important since the sodium salts of each of the tetrahydroquinolines studied were sufficiently soluble at pH 8.0 to allow i.c.v. dosing. If poor brain penetration is, at least in part, responsible for the weak systemic activity found, it seems unlikely that rapid exit of these compounds from the brain would occur after the i.c.v. administration. Assuming that distribution of **8** within the brain compartment (volume 0.5 mL) occurs readily during the time course of the anticonvulsant experiment (15 minutes), then the brain concentration of **8** at the ED_{50} dose will be approximately 1.0 μM, a value consistent with the functional antagonist potency of **8** *in vitro* (K_b 0.84 μM). In contrast, the predicted brain concentrations of **2-7** at their ED_{50} doses are 20-60 fold above the corresponding K_b values.

Kynurenic acid (**9**) is considerably more potent following i.c.v. dosing than expected from its K_b for NMDA antagonism alone (ED_{50}/K_b 0.1). It is possible that the comparable potency of **9** in blocking the AMPA subtype of excitatory amino acid receptor[1] contributes to *in vivo* anticonvulsant activity,[16] since 5,7-dichlorokynurenic acid (**1**) is a more selective glycine-site NMDA antagonist[1] and has a much increased ED_{50}/K_b ratio. MNQX (**10**) also shows AMPA antagonist activity (K_b for AMPA-mediated responses on the rat cortical slice, 2.0 μM), but unexpectedly, **10** lacked *in vivo* activity following i.c.v. administration at a dose of 20 nmol/mouse. The competitive NMDA antagonist CGP 37849 (**11**) is highly potent following i.c.v. dosing and has the lowest ED_{50}/K_b ratio (0.1) of the compounds studied. This result supports the view that α-amino acid structures, such as **8** and **11**, gain good access to NMDA receptors within the brain following i.c.v. dosing. The weaker i.c.v. potency of MK-801 (**12**) relative to **8** and **11** contrasts with its superior i.p. activity. These results are readily explained by the unimpeded brain uptake (and exit) of **12**, and slow or limited passage of **8** and **11** across the blood-brain barrier.

The methyl ester prodrug[2] (**13**, L-691,470, ED_{50} 31.5 mg/kg) was not markedly more potent than the parent compound **7** (ED_{50} 39 mg/kg). However the high aqueous solubility of **13** allowed testing at a wide range of doses and it was found that **13** lacked the behavioural stimulation observed[7] with **12**, supporting increasing evidence[2,17,18,19] showing *in vivo* differences between glycine-site and channel blocking NMDA receptor antagonists.

The results indicate general difficulties in extrapolating *in vitro* affinity to *in vivo* activity, and show that factors influencing *in vivo-in vitro* correlations are likely to differ within each class of glycine-site NMDA antagonist. Amongst the tetrahydroquinolines, it is suggested that, in addition to brain entry, further processes affecting 'access' to receptors in the brain may play a significant role in determining *in vivo* activity.

Acknowledgements. We thank Dr R. Herbert for NMR, mass spectra and log P measurements and A. Watt for HPLC.

REFERENCES AND NOTES

1. Leeson, P.D.; Baker, R.; Carling, R.W.; Curtis, N.R.; Moore, K.W.; Williams, B.J.; Foster, A.C.; Donald, A.E.; Kemp, J.A.; Marshall, G.R. *J. Med. Chem.* **1991**, *34*, 1243.
2. Moore, K.W.; Leeson, P.D.; Carling, R.W.; Tricklebank, M.D.; Singh, L. *Bioorg. Med. Chem. Lett.*, preceding paper.
3. Salituro, F.G.; Harrison, B.L.; Baron, B.M.; Nyce, P.L.; Stewart, K.T.; Kehne, J.H.; White, H.S.; McDonald, I.A. *J. Med. Chem.* **1992**, *35*, 1791.
4. Foster, A.C.; Kemp, J.A.; Leeson, P.D.; Grimwood, S.; Donald, A.E.; Marshall, G.R.; Priestley, T.; Smith, J.D.; Carling, R.W. *Mol. Pharmacol.*, **1992**, *41*, 914.
5. Carling, R.W.; Leeson, P.D.; Moseley, A.M.; Baker, R.; Foster, A.C.; Grimwood, S.; Kemp, J.A.; Marshall, G.R. *J. Med. Chem.* **1992**, *35*, 1942.
6. Leeson, P.D.; Carling, R.W.; Moore, K.W.; Moseley, A.M.; Smith, J.D.; Stevenson, G.; Chan, T.; Baker, R.; Foster, A.C.; Grimwood, S.; Kemp, J.A.; Marshall, G.R.; Hoogsteen, K. *J. Med. Chem.* **1992**, *35*, 1954.
7. Tricklebank, M.D.; Singh, L.; Oles, R.J.; Preston, C.; Iversen, S.D. *Eur. J. Pharmacol.* **1989**, *167*, 127.
8. Singh, L.; Oles, R.J.; Tricklebank, M.D. *Br. J. Pharmacol.* **1990**, *99*, 285.
9. Sheardown, M.J.; Drejer, J.; Jensen, L.H.; Stidsen, C.E.; Honore, T. *Eur. J. Pharmacol.* **1989**, *74*, 197.
10. Fagg, G.E.; Olpe. H.-R.; Pozza, M.F.; Baud, J.; Steinmann, M.; Schmutz, M.; Portet, C.; Baumann, P.; Thedinga, K.; Bittiger, H.; Allgeier, A, Heckendorn, R.; Angst, C.; Brundish, D.; Dingwall, J.G. *Br. J. Pharmacol.* **1990**, *97*, 791.
11. Kemp, J.A.; Foster, A.C.; Wong, E.H.F. *Trends Neurosci.* **1987**,*10*, 294.
12. Grimwood, S.; Moseley, A.M.; Carling, R.W.; Leeson, P.D.; Foster, A.C. *Mol. Pharmacol.* **1992**, *41*, 923.
13. Young, R.C.; Mitchell, R.C.; Brown, T.H.; Ganellin, C.R.; Griffiths, R.; Jones, M.; Rana, K.K.; Saunders, D.; Smith, I.R.; Sore, N.E.; Wilks, T.J. *J. Med. Chem.* **1988**, *31*, 656.
14. A.C. Foster, A. Watt and C.L. Willis, unpublished results.
15. Miller, L.P.; Pardridge, W.M.; Braun, L.D.; Oldendorf, W.H. *J. Neurochem.* **1985**, *45*, 1427.
16. Chapman, A.G.; Smith, S.E.; Meldrum, B.S. *Epilepsy Res.* **1991**, *9*, 92.
17. Koek, W.; Colpaert, F.C. *J. Pharm. Exp. Ther.* **1990**, *252*, 349.
18. Kehne, J.H.; McCloskey, T.C.; Baron, B.M.; Chi, E.M.; Harrison, B.L.; Whitten, J.P.; Palfreyman, M.G. *Eur. J. Pharmacol.* **1991**, *193*, 283.
19. (a) Bourson, A.; Tricklebank, M.D. *Fundam. Clin. Pharmacol.* **1991**, *5*, 443. (b) Hargreaves, R.J.; Rigby, M.; Kemp, J.A.; Smith, D.; Foster, A.; Hurley, C.J.; Hill, R. *J. Cereb. Blood Flow Metab.*, **1991**, *11 (Suppl. 2)*, S301.