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Enantiomerically Pure Tetrahydroquinoline Derivatives as In Vivo Potent Antagonists of the Glycine Binding Site Associated to the NMDA Receptor

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Abstract—To identify neuroprotective agents after stroke, new substituted tetrahydroquinoline derivatives were designed as antagonists of the glycine binding site associated to the NMDA receptor, satisfying the key pharmacophoric requirements. In particular, the racemate **3c** exhibited outstanding in vivo activity in the MCAo model in rats, when given iv both pre- and post-ischemia. Pure enantiomers **3c**-(+) and **3c**-(−) have been prepared following an original synthetic route. Despite the significant difference of activity observed in vitro, they shown similar neuroprotective profile in the MCAo model in rats.

The control of the excitotoxic cascade triggered by the activation of the NMDA receptor[1] is a promising strategy to discover effective neuroprotective agents able to prevent cerebral tissues degeneration after stroke.[2]

The extensive exploration performed by former Glaxo-Wellcome on non-competitive NMDA antagonists acting at the glycine binding site,[3–5] gave rise to the identification of the glycine antagonists,[6–15] of type **1** and **2** shown in Figure 1.

Notably, some of these compounds exhibited outstanding neuroprotective activity in animal model of cerebral ischemia, after permanent occlusion of the middle cerebral artery (MCA),[16] without relevant side effects in rodents. As part of a wide program aimed toward the identification of new glycine antagonists, the tetrahydroquinoline (THQ) derivatives of general structure **3**, bearing the *exo* α,β-unsaturated *N*-aryl amide moiety at the C-4 position, were identified as a class of compounds fulfilling the necessary pharmacophore requirements.[15]

In particular, after the preparation of **3a** (R = H), the first member of this series, the compound was characterized both in terms of in vitro affinity to the glycine binding site[19] affinity and in vivo in the NMDA induced convulsions model in mice, after iv administration.[20] High in vitro affinity was observed ($pK_i = 8.18$ vs 8.52 for compound **3a** and **1**, respectively) but poor in vivo anticonvulsant activity ($pIC_{50} = 1$ mg/kg vs 0.06 mg/kg[15] for compound **3a** and **1**, respectively). Therefore, the substitution of the *para* position of the terminal aromatic moiety was explored in detail to maximize both the in vitro affinity and to optimize the in vivo profile. To this event, the pool of compounds shown in Table 1 was prepared (Scheme 1) starting from the free carboxyl derivative **4**, intermediate synthesized in large scale as previously reported.[8,17] This compound was transformed in high yield into the corresponding *N*-phenyl amide derivatives **6a–p** in two steps, by sequential activation of the carboxyl group via the corresponding 2-pyridyl thiolester,[18] to afford intermediate **5** in 67% yield after purification by flash chromatography, and reaction with substituted anilines in toluene at reflux for 2 h. Finally,

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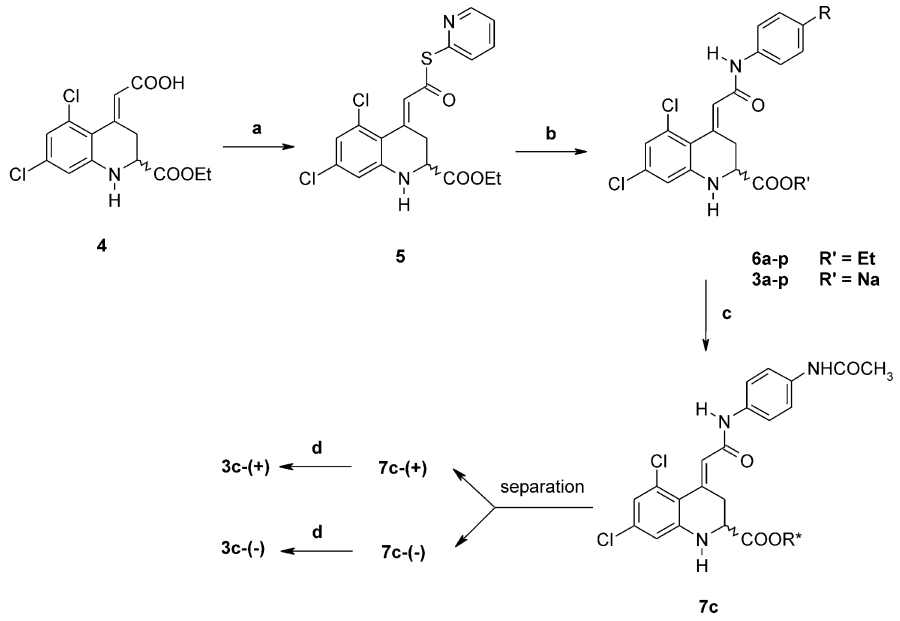

Scheme 1. Effect of the substituents in the *para* position of the terminal aromatic moiety; (a) 2,2′dipyridyl disulphide, PPh_3, THF, rt, 3 h, 67%; (b) (i) $ArNH_2$, toluene, 2 h, reflux, 78–85%; (ii) NaOH, EtOH/MeOH 95:5, rt, 30 min, 90%; (c) DEAD, PPh_3, R*OH, THF, rt, 30 min, 0–100%; (d) (i) LiOH, THF/H_2O, 3:1, rt, 1 h; (ii) Me_3SiONa, THF, rt, 1 h, 85–90% (two steps).

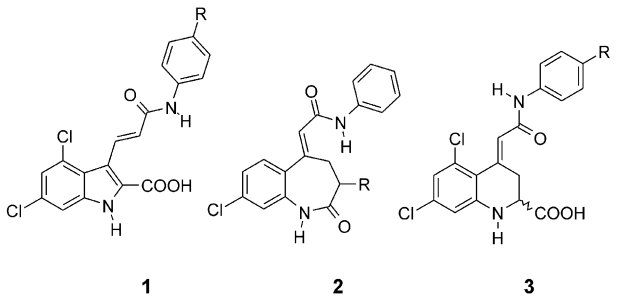

Figure 1. New series of glycine antagonists.

the hydrolysis of the ethyl ester with NaOH in EtOH/MeOH 95:5, gave title racemates **3a–p** quantitatively.[21]

As reported in Table 1, all the compounds prepared exhibited high in vitro affinity, confirming the presence in the north-east part of the receptor of a non-hydrophobic pocket of limited size.[15]

Table 1. In vitro affinity at the glycine binding site

Entry	R	pK_i[a]
1	H	8.52
3a	H	8.18
3b	NH_2	7.76
3c	$NHCOCH_3$	8.52
3d	$NHCOCH_2OCH_3$	8.18
3e	$NHSO_2CH_3$	8.18
3f	$NHSO_2NH_2$	8.17
3g	CH_2CONH_2	8.18
3h	CH_2CN	8.15
3i	CH_2COOH	7.72
3l	$CH_2NHCONH_2$	8.38
3m	$CH_2NHCOCH_3$	8.32
3n	CH_2NHCO-*i*-C_3H_7	7.78
3o	$CH{=}CHCONH_2$	8.15
3p	$CH{=}CHCN$	7.73

[a]Inhibition of binding of [^{3}H] glycine. [19]

The most active compound of the series **3c** was found to be also the most potent in vivo in the NMDA induced convulsions model in mice, after iv administration ($ED_{50} = 0.2$ mg/kg).

Based on this preliminary profile, this compound was characterized in the MCAo model in rats, both pre- and post-ischemia. When **3c** was given iv, 5 min prior occlusion of the MCA (pre-ischemia), at the doses of 0.01, 0.1, 1 and 10 mg/kg, respectively, as shown in Table 2, a significant reduction of the size of gross brain damage was observed (brain damage volume $73.7 \pm 8.5\,mm^3$ ($n = 10$) for the vehicle treated group). At the same doses the infarct volumes were $64.3 \pm 12.7\,mm^3$ ($n = 8$), $47.8 \pm 7.1\,mm^3$ ($n = 8$; $p < 0.05$), $41.8 \pm 6.2\,mm^3$ ($n = 8$; $p < 0.01$) and $39.7 \pm 7.6\,mm^3$ ($n = 8$; $p < 0.01$), respectively [$ED_{50} = 0.02$ mg/kg (0.01-0.12)] for the group of rats treated with **3c**.

Post-ischemia, when **3c** was given both at 0.3 and 1 mg/kg, iv, 6 h after MCA occlusion, a significant neuroprotective effect was also observed. In this case, the infarct volume was, $36.6 \pm 5.9\,mm^3$ ($n = 8$; $p < 0.05$) and $29.9 \pm 3.6\,mm^3$ ($n = 8$; $p < 0.01$) at 0.3 and 1.0 mg/kg dose, respectively (brain damage volume $73.6 \pm 6.5\,mm^3$ ($n = 10$) for the vehicle treated group). Considering the outstanding neuroprotective activity observed, both pre- and post-ischemia, a suitable synthetic strategy was put in place to prepare sufficient amount of the single enantiomers. In this event, as shown in Scheme 1, a 'library' of diastereoisomers was prepared reacting in parallel the racemic carboxylic acid derivative **3c** with a series of commercially available alcohols (R*OH) belonging to the chiral pool. Several classic esterification methods were attempted, using a variety of carboxyl activating agents (CDI; mixed anhydride; DCC; EDC; Mitsunobu reaction). Among those, the Mitsunobu reaction was proven to be the most effective

Table 2. Neuroprotective activity of racemate **3c** in the MCAo model in rats

Type of study	Dose (mg/kg, iv)	Infarct volume ($mm^3 \pm SEM$)	Damage reduction (%)
Pre-ischaemia	0.01	64.3±12.7	12.7
	0.1	47.8±7.1[b]	35.1
	1	41.8±6.2[c]	43.3
	10	39.7±7.6[c]	46.1
	Vehicle	73.7±8.5	
Post-ischaemia[a]	0.3	36.6±5.9[c]	50.3
	1	29.9±3.6[c]	59.4
	Vehicle	73.6±6.5	

[a]Single dose given 6 h after occlusion.
[b]$p < 0.05$ vs vehicle.
[c]$p < 0.01$ vs vehicle.

method. Accordingly, when **3c** was treated with 'diverse' primary and secondary chiral alcohols in THF at room temperature, in the presence of PPh_3 and DEAD, the corresponding ester derivatives were obtained in variable conversion (0–100% by HPLC analysis).

Total conversion was observed with (*S*)-(+)-1-indanol, methyl-(*S*)-(+)-mandelate, methyl (*R*)-(−)-lactate, *tert*-butyl (*R*)-(+)-lactate, methyl (*R*)-(−)-3-hydroxy-2-methylpropionate and (*R*)-(−)-2-butanol; no reaction occurred with (+)-menthol. The *tert*-butyl (*R*)-(+)-lactate ester derivative **7c** was chosen based on the efficiency of the separation of the two diastereoisomers both by tlc. (AcOEt/cyclohexanes 7:3, $R_f = 0.40$ and 0.47 respectively) and HPLC (Supelcosil LC-CN, THF–hexane 30:70, flow = 0.8 mL/min, $\lambda = 260$ nm: retention time = 12.56 and 15.60 min, respectively).

After large scale preparation of diastereoisomers **7c**-(+) and **7c**-(−) (performed both by flash chromatography or preparative HPLC), the next issue was the identification of mild hydrolysis conditions able to prevent both the racemization of the α-amino acid type stereogenic centre and to minimize the formation of lactic acid during the final removal the ester group. To that end, when the reaction was performed in the presence of NaOH in EtOH/MeOH 95:5, at room temperature for 3 h, a partial conversion of the ester into the corresponding carboxyl acid was observed, due to the competitive hydrolysis of the *tert*-butyl lactate ester and formation of significant amount of lactic acid. Conversely, when the basic hydrolysis was attempted with LiOH in THF/H_2O 3:1 at room temperature for 30 min, the reaction was chemoselective and the single enantiomers **3c**-(+) and **3c**-(−) were isolated in high yield after acid work up, and then quantitatively transformed into the corresponding Na^+ salts in the presence of $(CH_3)_3SiONa$ in THF.[22]

The two enantiomers were then evaluated in the binding assay in vitro. A significant difference of potency was observed [$pK_i = 8.79$ and 7.30, for **3c**-(+) and **3c**-(−), respectively vs 8.52 for the racemate **3c**].

Based on these results, it can be stated that the receptor binding site of the glycine antagonists shows a preferential stereospecific recognition of the enantiomers of type R.[23] The same kind of difference between the two enantiomers was observed in vivo in the NMDA-induced convulsion model in mice, being **3c**-(+) significantly more potent than **3c**-(−) [$ED_{50} = 0.31$ mg/kg and 1.52 for **3c**-(+) and **3c**-(−), respectively, vs 0.2 mg/kg for **3c**].

Table 3. Neuroprotective activity of single enantiomers **3c**-(+) and **3c**-(−) post-ischemia in the MCAo model in rats (single dose given 6 h after occlusion)

Compd	Dose (mg/kg, iv)	Infarct volume ($mm^3 \pm SEM$)[b]	Damage reduction
3c-(+)	0.3	55.6±9.5	26.3%
	1	44.4±6.3[a]	41.2%
3c-(−)	0.3	58.7±10.1	22.2%
	1	42.9±7.7[a]	43.2%
Vehicle		75.5±8.5	

[a]$p < 0.05$ vs vehicle.
[b]$p < 0.01$ vs vehicle.

In the MCAo model in rat, when **3c**-(+) was given iv, 6 h post-ischemia, the compound exhibited a significant neuroprotective activity at 1 mg/kg (Table 3: 44.4±6.3 mm^3 in treated animals; vehicle treated rats 75.5±8.5 mm^3; $p < 0.05$; $n = 8$) but it was found inactive at the lower dose (0.3 mg/kg, 55.6±9.5 mm^3 in treated animals; vehicle treated rats 75.5±8.5 mm^3; $n = 8$).

This result was explained in terms of unbalanced PK/PD profile in rat of the two enantiomers, being the most potent enantiomer **3c**-(+) was also the less abundant compound in plasma (mean $AUC_{0-inf} = 3.44$, 19.8 and 11.5 μgh/mL for **3c**-(+), **3c**-(−) and **3c**, respectively). In fact, when the less in vitro active enantiomer **3c**-(−) was evaluated in the MCAo model, as in the case of **3c**-(+), despite the significant difference of in vitro potency, a similar neuroprotective profile was observed (Table 2: 1 mg/kg, 42.9±7.7 mm^3 in treated animals; vehicle treated rats 75.5±8.5 mm^3; $p < 0.05$; $n = 8$).

In conclusion, enantiomerically pure THQ derivatives **3c**-(+) and **3c**-(−) have been identified as in vitro and in vivo potent glycine antagonists. Based on their outstanding pharmacological profile observed in animal models of cerebral ischemia, both enatiomers can be considered as promising and effective neuroprotective agents.

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21. Compounds **3a**–**p**: C, H and N analyses were within 0.4% of the theoretical values; mp were over 250 °C.
22. **3c-**(+): $[\alpha]_D = +16°$ (*c* 0.25, DMSO); **3c-**(−): $[\alpha]_D = -16°$ (*c* 0.25, DMSO).
23. After the completion of this study, the enantiomer **3c-**(+) was re-synthesized (R. Di Fabio and G. Alvaro, unpublished results) following the same kind of asymmetric synthesis recently published for the preparation of some analogue THQ derivatives: Di Fabio, R.; Alvaro, G.; Bertani, B.; Donati, D.; Giacobbe, S.; Marchioro, C.; Palma, C.; Lynn, S. M. *JOC*, **2002**, *67*, 7319. By this approach, it was clearly proven that the absolute configuration of the C-2 stereogenic center of the enantiomer **3c-**(+) was of type R.