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STRUCTURE-ACTIVITY RELATIONSHIPS OF TRICYCLIC QUINOXALINEDIONES AS POTENT ANTAGONISTS FOR THE GLYCINE BINDING SITE OF THE NMDA RECEPTOR 1.

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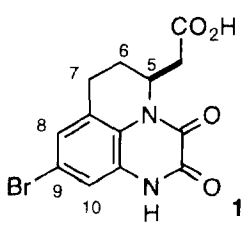
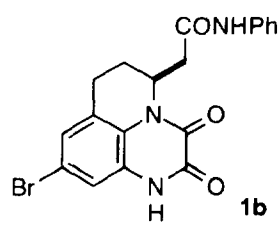
Abstract: All possible methyl substituted isomers of tricyclic quinoxalinediones **20a** - **28a** and **20b** - **27b** were synthesized and evaluated for their affinity for the glycine binding site of the NMDA receptor. Trans 6-methyl derivatives **26a** and **26b** showed comparable activity to the parent compounds **1a** and **1b**, respectively.

There is increasing evidence that over-excitation of the NMDA receptor plays an important role in neuronal cell death during ischemic or hypoxic conditions such as stroke.[1] Several binding sites on the NMDA receptor including glutamate, glycine, and channel blocker binding sites, have been identified and these sites offer a target of the drug for treatment of not only stroke but other neurodegenerative disorders such as Alzheimer's and Hungtington's diseases.[2] However, an antagonist acting at the glycine binding site should be therapeutically more beneficial than at other sites, since a glycine antagonist appears to have less adverse side effects such as the behavioral and autonomic effects which are often seen in animals treated with a certain channel blocker.[3]

We have recently synthesized a series of tricyclic quinoxalinediones as potent antagonists for the glycine site of the NMDA receptor.[4] Among them, six membered ring fused tricyclic quinoxalinediones **1a** (Ki = 9.9 nM) and **1b** (Ki = 2.6 nM) showed extremely high affinity for the glycine site, as determined by radio ligand binding assay using [^{3}H] 5,7-dichlorokynurenic acid. First, it is clear that hydrophobic ring system attached to the northern region is well-tolerated and that, therefore, there would be a hydrophobic pocket in the same region on the receptor-side. Second, the six membered ring of the northern part could fix the C5 side chain to an axial conformation and this would result in giving high affinity to the molecules. To learn more about the receptor pharmacophore and especially to know the size of the particular hydrophobic pocket, we synthesized all possible methyl substituted isomers **20a** - **28a** and **20b** - **27b** where a methyl group was introduced onto the C5 side chain and the six membered ring of the northern part of the molecule. The molecular modeling analysis using the AMBER force field predicted that the carboxymethyl and the phenyl carbamoylmethyl group at the C5 side chain are always in an axial position in all cases. Therefore, the measured activity of the molecule would solely depend on the potency of the interaction between the introduced methyl group and the hydrophobic pocket of the receptor.



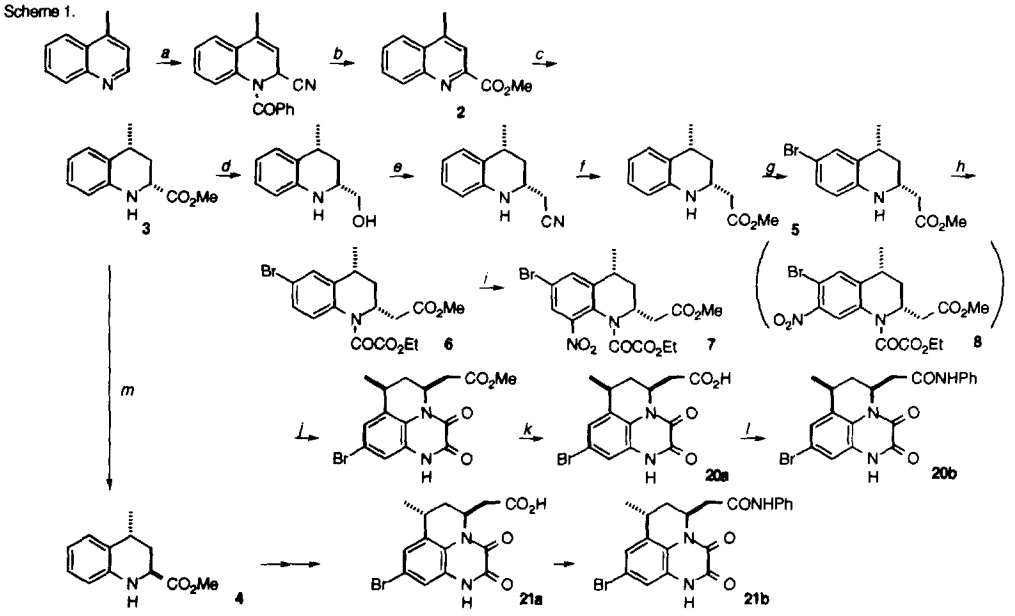
1a



1b

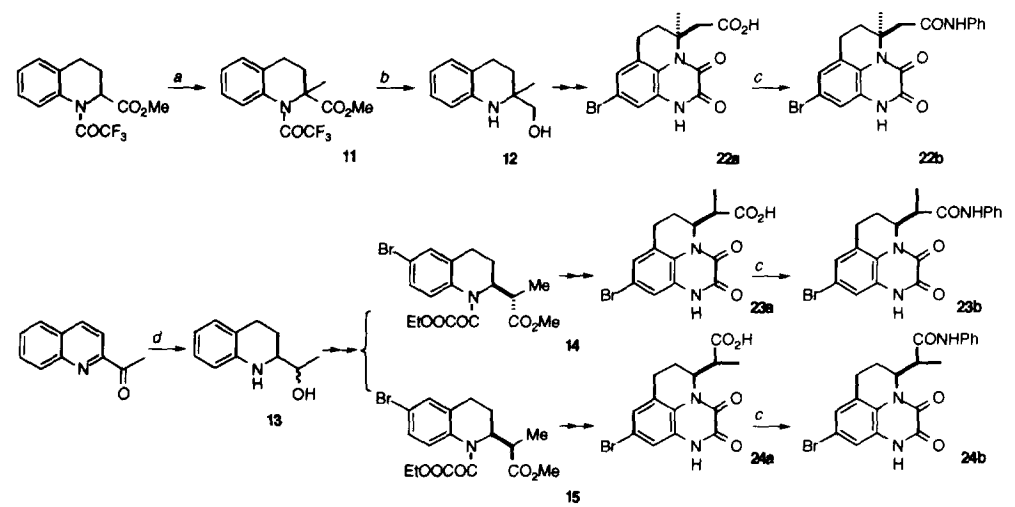
4-Methylquinoline was transformed to 2-methoxycarbonyl-4-methylquinoline (**2**) by Reissert reaction with sodium cyanide and benzoyl chloride[5] followed by acid hydrolysis and methylation using thionyl chloride in methanol. Reduction of **2** with platinum oxide in acetic acid under hydrogen or more favorably, with sodium borohydride in the presence of a catalytic amount of nickel chloride in methanol gave cis 2-methoxycarbonyl-3-methyl-1,2,3,4-tetrahydroquinoline (**3**) as a major product, together with a small amount of the trans isomer **4** (**3** : **4** ≅ 10 : 1). Treatment of **3** with 5 mol% sodium methoxide in methanol at room temperature followed by methylation using thionyl chloride in methanol led to isomerization to give a ca. 1 : 1 mixture of **3** and the trans isomer **4**. After chromatography on a silica gel column, pure **4** as well as **3** was obtained. According to the procedure described previously,[4] methyl ester **3** was converted into the corresponding methoxycarbonylmethyl derivative **5** without epimerization via alcohol, iodide, cyanide, and carboxylic acid. Compound **5** was transformed to the corresponding tricyclic quinoxalinediones **20a**[8] and **20b** according to the procedure described previously[4] (Scheme 1). When isopropyl nitrate in conc. sulfuric acid was employed as a nitration agent in the conversion of **6** to **7**, the desired product **7** was obtained in only 6% yield and instead, the isomer **8** was formed in 51% yield. Interestingly, the use of nitronium tetrafluoroborate in dichloromethane drastically changed the selectivity and the desired product **7** was obtained as a major product (61%) concomitantly with **8** (7%). Similarly, trans 2-methoxycarbonyl-4-methyl-1,2,3,4-tetrahydroquinoline (**4**) was converted into the corresponding tricyclic quinoxalinediones **21a**[8] and **21b** (Scheme 1). Cis 2-methoxycarbonyl-3-methyl-1,2,3,4-tetrahydroquinoline (**9**) and the trans isomer **10** obtained starting from 3-methylquinoline were also transformed into the tricyclic quinoxalinediones **25a,b** and **26a,b** (Scheme 3). *N*-trifluoroacetyl-2-methoxycarbonyl-1,2,3,4-tetrahydroquinoline was treated with potassium hexamethyldisilazide at - 78 °C and then methyl iodide to give *N*-trifluoroacetyl-2-methyl-2-methoxycarbonyl-1,2,3,4-tetrahydroquinoline (**11**). Reduction of **11** with $LiAlH_4$ directly afforded alcohol **12** which was similarly converted to the corresponding tricyclic quinoxalinediones **22a** and **22b**. Hydrogenation of 2-acetylquinoline[7] over platinum oxide in acetic acid provided a diastereomixture of 2-(1-hydroxyethyl)tetrahydroquinoline (**13**) which was led to the ethyl oxalate derivative as a mixture of **14** and **15** by a similar procedure (Scheme 2). Fortunately, the mixture of **14** and **15** could be separated as a pure isomer at this stage by using silica gel column chromatography, although their relative configurations were not determined. Carboxylic acids **23a** and **24a** could be obtained with more than 90% of a diastereomeric purity, respectively, as determined by ^1H nmr spectroscopy. The relative configurations of **23a** and **24a** were rigorously determined by using the NOE and the decoupling technique. For example, in the case of **23a**, the coupling constant between C5 proton and α proton of the side chain was 9.6 Hz, implying that these protons were in an antiperiplanar position with each other and the NOE was observed between the methyl group of the side chain and C6 equatorial proton. As a result, **23a** was concluded to be syn. Similarly, the relative configurations of **24a**, **23b**, and **24b** were confirmed to be anti, syn, and anti, respectively. In all cases except for **22a** and **22b**, the C5 carboxymethyl and phenylcarbamoylmethyl groups were confirmed to be in axial positions, as judged from the coupling constant (J[H5-H6axial] = ca. 4 and J[H5-H6equatrial] = ca. 5). However, the result was opposite in the case of **22a** and **22b**. Unexpectedly, the NOEs between the C5 methyl group and C6 equatrial proton and between the C5 methyl group and C7 axial proton were observed. As a result, both the C5 carboxymethyl of **22a** and the C5 phenylcarbamoylmethyl of **22b** were in equatrial positions although the molecular modeling predicted the reverse (Figure).

Scheme 1.



[a]PhCOCl, KCN/CH_2Cl_2-water, 77%; [b]1) conc.HCl, 100 °C, 2) $SOCl_2$/MeOH, 85%; [c]NaBH4-cat.$NiCl_2$/MeOH, 56%; [d]$LiAlH_4$/THF, 96%; [e]1) I_2-PPh_3-imidazole/toluene-acetonitrile, 2) NaCN/DMF, 53%; [f]1) conc.HCl, 100 °C, 2) $SOCl_2$/MeOH, 80%; [g]NBS/DMF, 97%; [h]$ClCOCO_2Et$-NEt_3/CH_2Cl_2, 91%; [i]$NO_2^+BF_4^-$/CH_2Cl_2, 61%; [j]aqueous $TiCl_3$/acetone, 64%; [k]1N NaOH/THF-MeOH, 100%; [l]aniline,WSC-HOBt/DMF, 93%; [m]1)5% NaOMe/MeOH, 2) $SOCl_2$/MeOH.

Scheme 2.



[a]1) KN(TMS)$_2$/THF, -70 °C, 2) MeI, -70 °C ~ r.t., 98%; [b]$LiAlH_4$/THF, 60°C, 95%; [c]aniline, BOP-Cl/CH_2Cl_2, 73%, [d]PtO_2/AcOH, 78%.

Scheme 3.

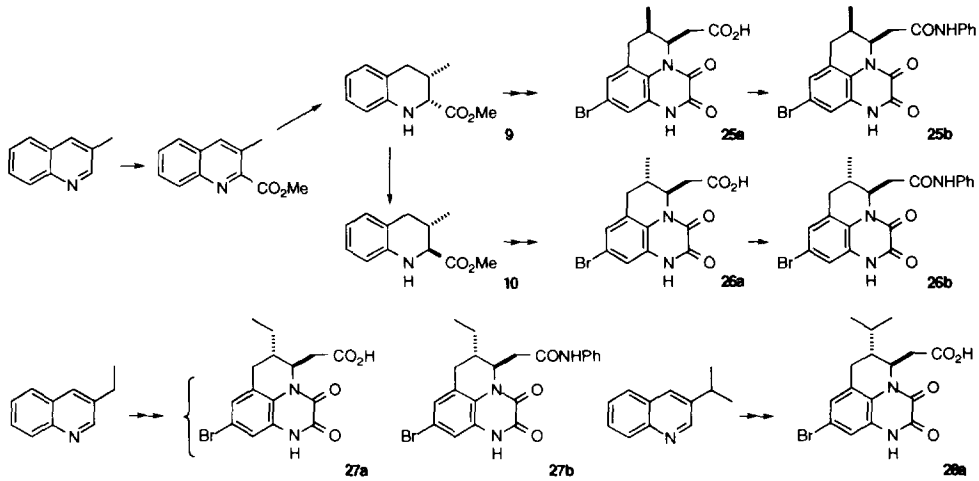


Table. The affinity for the glycine binding site[a]

compound	R =	Ki (nM) vs [^{3}H] DCKA[b]	compound	R =	Ki (nM) vs [^{3}H] DCKA[b]
1a	H	9.9[c]	**1b**	H	2.6[c]
20a	*cis* -7-methyl	42.0	**20b**	*cis* -7-methyl	17.1
21a	*trans*-7-methyl	38.3	**21b**	*trans*-7-methyl	7.4
22a	5-methyl	451	**22b**	5-methyl	760
23a	C5-α-methyl (syn)	241	**23b**	C5-α-methyl (syn)	665
24a	C5-α-methyl (anti)	300	**24b**	C5-α-methyl (anti)	590
25a	*cis*-6-methyl	29.6	**25b**	*cis*-6-methyl	21.0
26a	*trans*-6-methyl	3.3	**26b**	*trans*-6-methyl	3.0
27a	*trans*-6-ethyl	1.4	**27b**	*trans*-6-ethyl	3.8
28a	*trans*-6-isopropyl	8.4			

[a]See refs. 4 and 9. [b]DCKA: 5,7-dichlorokynurenic acid. [c]See ref. 4.

The affinity of the compounds was measured by radio ligand binding assay using [^{3}H] 5,7-dichlorokynurenic acid[9] and listed in Table. Both C7-cis (or C7 axial) methyl derivatives **20a** and **20b** and C7-trans (or C7 equatrial) methyl derivatives **21a** and **21b** showed 3 ~ 7-fold lower affinity than the parent tricyclic quinoxalinediones **1a** and **1b**, respectively. The affinity of C6-cis (or C6 equatrial) methyl derivatives

25a and **25b** was ca. 3- and 8-fold weaker than that of **1a** and **1b**, respectively. Methyl derivatives at the C5 side chain **23-24a** and **23-24b** were also less potent than **1a** and **1b**, respectively, in one to two orders of magnitude. These findings demonstrate that the corresponding receptor pocket should not be able to have a positive interaction with the methyl group introduced to these positions. However, the C6-trans (or C6 axial) methyl derivative **26a** (Ki = 3.3 nM) was more potent than **1a** (Ki = 9.9 nM) and the anilide **26b** (Ki = 3.0 nM) was as potent as **1b** (Ki = 2.6 nM). We prepared 6-trans ethyl and isopropyl derivatives **27a,b** and **28a** starting from 3-ethyl and 3-isopropyl quinoline, respectively, by a similar sequence as described above. We found that these derivatives were again more potent than **1a** in a carboxylic acid series and as potent as **1b** in an anilide series. This suggests that the receptor pocket would be expanded to the opposite side to the C5 side chain (Figure). In addition, 6-trans alkyl derivatives **26-28a** and **26-27b** were clearly a class of the most potent NMDA-glycine antagonists so far reported.[10] Both C5 methyl derivatives **22a** and **22b** showed much less potency than **1a** and **1b**, respectively, but the situation was different from the others. The reduced activity would not be caused by negative steric interaction of the C5 methyl with the hydrophobic receptor pocket, but caused by incorrect charge-charge interaction or hydrogen bonding interaction of the C5 carboxymethyl or phenylcarbamoylmethyl group with the receptor,[4] since these groups are expected to be unfavorably directed to equatrial orientation, judged by the NOE experiment.

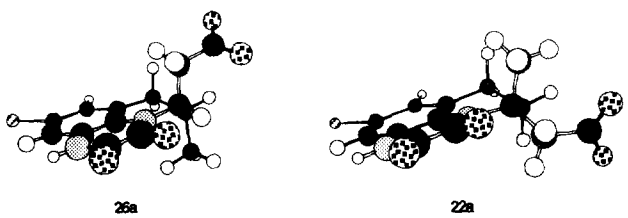


Figure. The global energy minimized conformation of **26a** (left) and the observed conformation of **22a** (right).

In the following paper, we will report the additional informations on the hydrophobic pocket of the glycine binding site of the NMDA receptor. The detailed analysis of the receptor pocket using the CoMFA method is in progress and will be reported elsewhere.

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(8) **20a**: mp 287.5 ~ 288 °C (dec); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.46 (bs, 1 H), 12.06 (bs, 1 H), 7.27 (d, 1 H, J = 2.3 Hz), 7.15 (d, 1 H, J = 2.3 Hz), 4.92 ~ 5.02 (m, 1 H), 3.00 ~ 3.16 (m, 1 H), 2.75 (dd, 1 H, J = 16.2, 4.6 Hz), 2.51 (dd, 1 H, J = 16.2, 9.9 Hz), 2.18 (dt, 1 H, J = 14.7, 5.6 Hz, H6eq), 2.03 (dt, 1 H, J = 14.7, 2 Hz, H6ax), 1.40 (d, 3 H, J = 7.3 Hz); Anal. calcd for ($C_{14}H_{13}N_2O_4Br•H_2O$): C 45.30, H 4.07, N 7.55; found, C 45.13, H 3.88, N 7.43. **21a**: mp 287 ~ 291 °C (dec); ^{1}H NMR (270 MHz, DMSO-d_6) δ 11.20 (bs, 1 H), 7.34 (s, 1 H), 7.22 (s, 1 H), 5.21 ~ 5.31 (m, 1 H), 3.15 (ddq, 1 H, J = 12.2, 4.6, 6.6 Hz), 2.75 (dd, 1 H, J = 15.5, 4.3 Hz), 2.61 (dd, 1 H, J = 15.5, 9.9 Hz), 2.31 (ddd, 1 H, J = 14.2, 4.6, 2.3 Hz, H6eq), 1.77 (ddd, 1 H, J = 14.2, 12.2, 4.9 Hz, H6ax), 1.41 (d, 3 H, J = 6.6 Hz); Anal. calcd for ($C_{14}H_{13}N_2O_4Br•1/2H_2O$): C 46.43, H 3.90, N 7.73; found, C 46.39, H 3.75, N 7.63.

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