Bioorganic & Medicinal Chemistry Letters, Vol.3, No.1, pp. 81-84, 1993
Printed in Great Britain

0960-894X/93 $5.00 + .00

STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF GLYCINE ANTAGONISTS RELATED TO 5,7-DICHLOROKYNURENIC ACID AND 3-(2-CARBOXY-6-CHLOROINDOL-3-YL)ACETIC ACID

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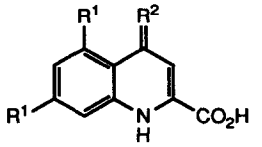
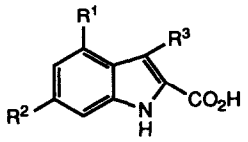
Abstract: A series of 3-substituted acetic and propanoic acid derivatives of 5,7-dichlorokynurenic acid were prepared and evaluated as antagonists of the glycine site on the NMDA receptor complex. They were found to possess reduced affinity for the glycine site relative to 5,7-dichlorokynurenic acid and the analogous 2-carboxyindole-3-acetic acid series of glycine antagonists.

The involvement of *N*-methyl-D-aspartate (NMDA) receptor activation in a variety of neurodegenerative and cognitive processes has been well established. Recent studies have lent good pharmacological evidence for the therapeutic potential of modulators of the NMDA receptor which act via the glycine site. For example, activation of the glycine receptor is required for induction of LTP in the neonatal hippocampus.[1] D-Cycloserine, a glycine partial agonist, has been shown to enhance task learning in rats.[2] Another partial agonist, 1-aminocyclopropane-1-carboxylic acid (ACPC) effectively blocked NMDA-induced convulsions without eliciting any overt behavioral effects of its own.[3] Furthermore, glycine antagonists have proven active in a variety of seizure models.[4-6] Other reports have shown glycine antagonists protect against NMDA induced neurotoxicity in vitro and neurodegeneration in vivo.[7-9]

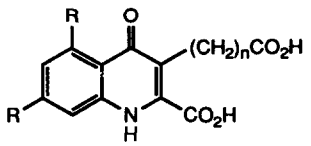
5,7-Dichlorokynurenic acid (**1b**)[8,10] and 2-(2-carboxy-6-chloroindol-3-yl)acetic acid[11] (**2c**) are two potent and selective antagonists of the glycine site. Recently, McDonald and coworkers[12] have reported on the increased potency of the 4,6-dichloro substituted 3-(2-carboxyindol-3-yl)propanoic acid **2e** relative to **2c**. Additionally, they have described a kynurenic acid analogue **1c** which



1a $R^1 = H$; $R^2 = O$
1b $R^1 = Cl$; $R^2 = O$
1c $R^1 = Cl$; $R^2 = NCH_2CO_2H$



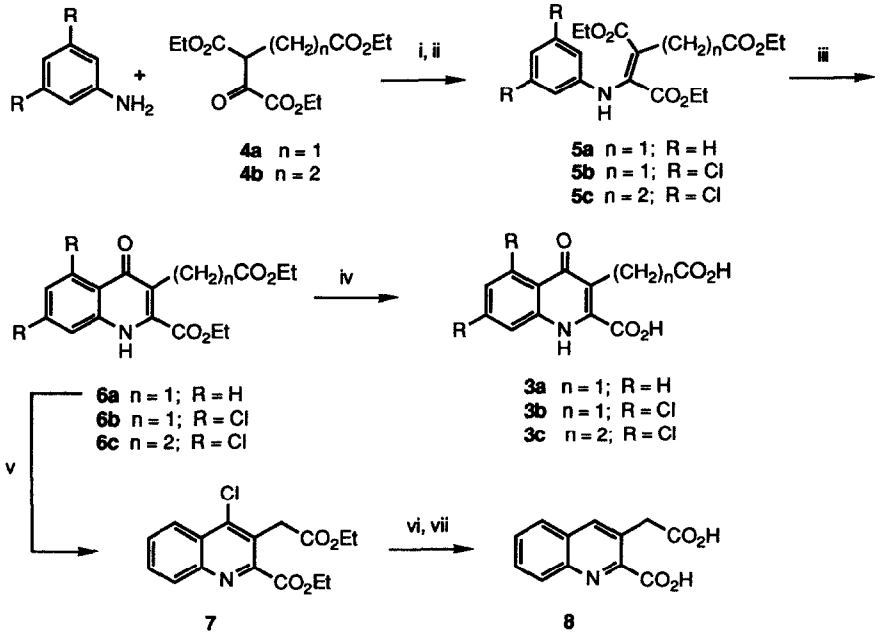
2a $R^1 = R^2 = Cl$; $R^3 = H$
2b $R^1 = R^2 = H$; $R^3 = CH_2CO_2H$
2c $R^1 = H$; $R^2 = Cl$; $R^3 = CH_2CO_2H$
2d $R^1 = R^2 = Cl$; $R^3 = CH_2CO_2H$
2e $R^1 = R^2 = Cl$; $R^3 = CH_2CH_2CO_2H$



3a R = H; n = 1
3b R = Cl; n = 1
3c R = Cl; n = 2

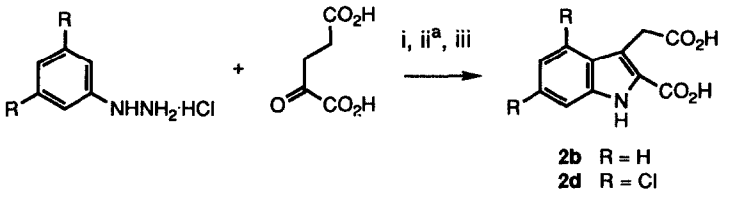
possesses a heteroatom-linked acetic acid group at C-4.[13] In an effort to further explore the structural commonality between these series, we have synthesized and evaluated kynurenic acid derivatives with an acetic or propanoic acid moiety appended to the 3-position as represented by **3a-c**.

The 2-(2-carboxy-4-hydroxyquinolin-3-yl)acetic acids **3a** and **3b** were prepared as shown in **Scheme I.** The appropriate aniline was condensed with diethyl ethoxalylsuccinate[14] (**4a**) in the presence of 10 mol% glacial acetic acid to give the triethyl 1-anilino-2-carboxy-1,2-dihydrosuccinates **5a** and **5b.** The water which formed was azetroped off with refluxing benzene using a Dean and Stark trap. The intermediates **5a** and **5b** were then purified by flash chromatography before cyclizing in refluxing diphenyl ether to afford **6a** and **6b**, respectively. Saponification gave the corresponding



Scheme I. (i) AcOH, r.t., 7 days; (ii) benzene, reflux; (iii) Ph_2O, 260°; (iv) 1. aq. NaOH, 2. HCl; (v) $POCl_3$, reflux; (vi) H_2, Pd/C, EtOH, Et_3N; (vii) 1. aq. NaOH, 2. HCl

diacids **3a** and **3b**. The 3-propanoic acid derivative **3c** was prepared analogously except that triethyl 2-oxalylglutarate[15] (**4b**) was used in the initial condensation step. Compound **8**[11] which lacks a 4-hydroxy substituent was obtained by refluxing **6a** in $POCl_3$ to yield **7** followed by hydrogenolysis and saponification. The 2-carboxyindole-3-acetic acid derivatives **2b** and **2d** were synthesized using standard methodology[16] as summarized in **Scheme II.** In the case of **2d**, the hydrazone



Scheme II. (i) HCl, EtOH, reflux; (ii) *p*-Toluenesulfonic acid, benzene, reflux; (iii) 1. aq. NaOH, reflux, 2. HCl
[a] Used only in preparation of **2d**

intermediate was isolated prior to cyclization with *p*-toluenesulfonic acid in refluxing benzene. All compounds exhibited physical chemical data and elemental analyses consistent with their assigned structures.

The compounds were evaluated for their ability to displace [^{3}H]-glycine at a screening dose of 10 μM. K_i values were determined for those compounds which showed greater than 50% displacement at 10 μM (**Table 1**.) Compounds possessing affinity for the glycine site were shown to be antagonists by virtue of their ability to inhibit [^{3}H]-MK801 binding.[17] As previously reported,[11] we found 2-(2-carboxyindol-3-yl)acetic acid (**2b**) possessed affinity for the glycine site approximately equal to that of kynurenic acid (**1a**) while quinoline **8**, a ring expanded version of **2b**, lacked appreciable affinity for the glycine site. Addition of the 3-acetic acid side chain to kynurenic acid (**1a**) to give **3a** led to a modest loss of affinity for the glycine site, while substitution of 5,7-dichlorokynurenic acid (**1b**)

Table 1. Glycine Antagonist Affinity

Compound	[^{3}H]-Glycine[a] K_i (μM) ± S.E.M.	[^{3}H]-MK801[b] IC_{50} (μM) ± S.E.M.
1a	5.4 ± 0.05	40.25 ± 16.4
1b	0.04 ± 0.04	0.86 ± 0.19
2a[c]	0.98 ± 0.14	5.91 ± 1.2
2b	5.24 ± 2.7	45.55 ± 35.1
2d	0.34 ± 0.07	3.33 ± 0.79
2e	0.14[d]	NT
3a	14.8 ± 2.9	83.43 ± 71.1
3b	0.65 ± 0.1	3.54 ± 0.66
3c	7.04 ± 0.59	102.9 ± 72.3
8	> 100	> 100

[a] [^{3}H]-Glycine binding was performed on rat cortical membranes prepared by the freeze/thaw Triton extraction procedure developed for GABA-receptor binding with minor modifications.[18] Samples were incubated in the presence of 10 nmol [^{3}H]-glycine and 250 μg tissue on ice for 1 h, and terminated by single manifold rapid filtration through Whatman GF/B filters. Non-specific binding was defined as that remaining in the presence of 100 μM D-serine. [b] [^{3}H]-MK801 binding was performed in well washed rat cortical membranes[17] with an added freeze/thaw procedure. The effect of compounds on [^{3}H]-MK801 binding (2.5 nM) was determined in the presence of glutamate (1μM), and glycine (0.2 μM.) Samples were incubated for 2 h at 27° and terminated by filtration. Nonspecific [^{3}H]-MK801 binding was defined as that remaining in the presence of 0.5 μM MK-801. [c] Synthesis described in reference 12. [d] IC_{50} Data from reference 12. NT = Not tested.

with the 3-acetic acid side chain to give **3b** resulted in a marked decrease in affinity for the glycine site. Further homologation of the 3-acetic side chain to give the 3-propanoic acid derivative **3c** led to a further loss of affinity for the receptor. This is in contrast to the 4,6-dichloro substituted indole series where a modest increase in affinity is associated with the introduction of the 3-acetic acid moiety **2d** or the 3-propanoic acid moiety **2e**.[12] Gray et al.[11] showed a similar increase in potency upon introduction of the 3-acetic acid moiety to the 6-chloroindole series. These results suggest that the 3-acetic acid moiety appended to 5,7-dichlorokynurenic acid is unable to reach the same distal hydrogen bonding site that has been postulated[11,13] for the favorable interaction of the indole 3-acetic acid group.

Acknowledgment. The authors gratefully acknowledge Dr. William B. Lacefield for providing compound **2b** and the Physical Chemistry department for spectral and analytical data.

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