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# DESIGN, SYNTHESIS AND MOLECULAR MODELING OF 3-ACYLAMINO-2-CARBOXYINDOLE NMDA RECEPTOR GLYCINE-SITE ANTAGONISTS

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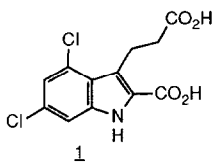
**ABSTRACT:** *The syntheses and molecular modeling of several 3-acylamino-2-carboxyindole antagonists of the glycine binding site associated with the NMDA receptor complex is described. Tertiary amide 8 is a potent antagonist; this result is rationalized by a preferred cis amide bond conformation. The potency of the trans secondary oxalamide 10 is explained by binding to the receptor in its enolic form.*

The reports that overstimulation of the NMDA receptor complex plays a role in the neuropathology of epilepsy, stroke, Huntington's disease, and Alzheimer's disease,[1,2] has prompted a great deal of research aimed at developing antagonists of the various modulatory binding sites of this complex. Recently, several groups[3,4] in addition to our own have described potent antagonists of the strychnine-insensitive glycine binding site of the NMDA receptor complex. In particular, we have reported that 3-(2-carboxy-4,6-dichloroindol-3-yl)propionic acid (1), like 5,7-dichlorokynurenic acid (2), is a potent antagonist ($IC_{50}$ = 140 nM versus [$^3$H]glycine) of this modulatory site.[6]



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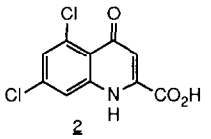

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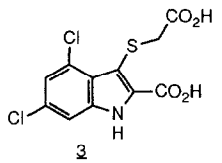

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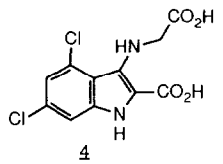
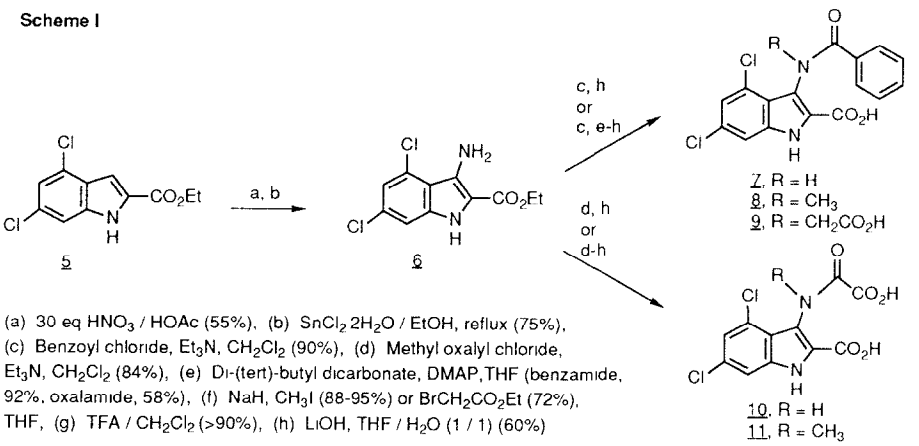


4

When considering these antagonists as potential therapeutic agents, of considerable importance will be their ability to cross various membranes such as those encountered in passing from the gut into the blood stream and the blood-brain barrier. The highly polar nature of these bis-carboxylic acids, however, poses a potential problem in this regard. Therefore, we embarked on a project to design equipotent and selective antagonists possessing a single carboxylic acid group by synthesizing amides of 3-amino-substituted indole-2-carboxylic acid derivatives.

**Scheme I**




(a) 30 eq $HNO_3$ / HOAc (55%), (b) $SnCl_2 2H_2O$ / EtOH, reflux (75%), (c) Benzoyl chloride, $Et_3N$, $CH_2Cl_2$ (90%), (d) Methyl oxalyl chloride, $Et_3N$, $CH_2Cl_2$ (84%), (e) Di-(tert)-butyl dicarbonate, DMAP, THF (benzamide, 92%, oxalamide, 58%), (f) NaH, $CH_3I$ (88-95%) or $BrCH_2CO_2Et$ (72%), THF, (g) TFA / $CH_2Cl_2$ (>90%), (h) LiOH, THF / $H_2O$ (1 / 1) (60%)

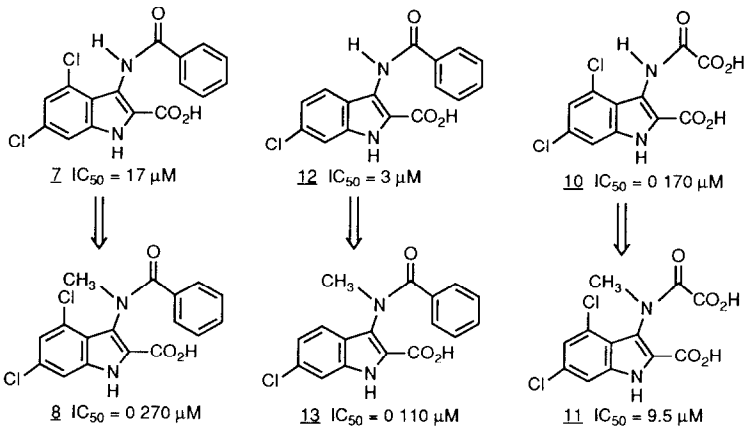
3-Amino derivatives were chosen as synthetic targets as a result of previous studies in the quinoline-2-carboxylic acid series which indicated that increasing the basicity of the heterocyclic amine (i.e., the indole nitrogen in the present case) led to more potent antagonists[3,5,7]. In the propionic acid series, replacement of carbon by sulfur led to the most potent indole-based antagonist 3 ($IC_{50}$ = 100 nM)[7]. At the outset of this study, we had intended to prepare non-acylated 3-amino derivatives, such as 4, but found these compounds to be unstable. The corresponding amides, however, are quite stable. The amino group was introduced into the 3-position of the indole-2-carboxylic acid derivatives via nitration, followed by reduction (Scheme I); subsequent amide formation and deprotection was straightforward. In the initial part of this study, both the benzamide 7 and the oxalamide 10 were prepared as a part of the overall structure-activity study. Methylation of the intermediate protected compounds necessitated prior protection of the indole nitrogen with the BOC group. These molecules were evaluated for their ability to compete with [$^3$H]glycine for the strychnine-insensitive binding sites on rat cortical and hippocampal membranes according to published procedures[8]; the results are presented in the Table I.

**TABLE I**

| Compound | m.p.[a] | $IC_{50}$[$^3$H]gly[b](nM) |
|---|---|---|
| 2[c] | -- | 140 |
| 3[d] | -- | 100 |
| 7 | 270 | 17,000 |
| 8 | 275 | 270 |
| 9 | 235-237 | 400 |
| 12 | 205-210 | 3,000 |
| 13 | 250-255 | 110 |
| 10 | 220-223 | 170 |
| 11 | 230-234 | 9,500 |

[a]All final compounds analyzed for C, H, N (±0.4%), 300 MHz NMR, MS, IR. Recrystalization was from ethyl acetate/hexane. [b]See Ref. 8. Standard errors are ± 10-30% of the mean ($n \geq 2$). [c]See Ref. 6. [d]See Ref. 7

**Scheme II**




These results, although initially surprising, have enabled us to develop a pharmacophore which, in turn, has led to a better understanding of the topology of the glycine binding site. The most unexpected result was that whereas the benzamide 7 had very poor affinity ($IC_{50}$ = 17 μM) for the glycine binding site, simple methylation on nitrogen led to 8 ($IC_{50}$ = 270 nM) which was almost as potent as the bis-carboxylic acid lead compound 1. In contrast, in the oxalic acid series the relationship was exactly the reverse; methylation of 10 ($IC_{50}$ = 170 nM) afforded the weakly active analogue 11 ($IC_{50}$ = 9.5 μM). When the mono-chloro analogues in the benzamide series, prepared utilizing the same synthetic methods[9], were evaluated, the relationship of these molecules was similar to that of 7 and 8 (Scheme II). The 6-chlorobenzamide derivative 12 was a weak antagonist ($IC_{50}$ = 3 μM) whereas the N-methyl analogue 13 was quite potent ($IC_{50}$ = 110 nM); in fact, 13 is 2-fold more potent than the dichloro analogue 8. This represents the first example, in quinoline- or indole-based antagonists, where the monochloro analogue is actually the more potent antagonist. Currently, the explanation for these observations is unclear since molecular modeling has unveiled only slight differences in the minimum energy conformations of these two compounds.

The potencies of 8, 10, and 13 suggest that the receptor binding pocket has sites to accommodate both a carboxylic acid side chain and a phenyl group. To test this hypothesis, the hybrid molecule 9 was prepared according to Scheme I. When tested in the binding assay, the potency of 9 ($IC_{50}$ = 400 nM) was disappointing, a result we now believe is due to the inability of both functional groups to correctly occupy the respective binding pockets simultaneously.

With these results in hand, we turned to molecular modeling in order to understand why 8 is more potent than 7 and to develop a pharmacophore model from which we could design even more effective molecules. Low energy conformations of these molecules were sought by a dihedral driver calculation[10] in which the highlighted bond (Figure 1) was rotated in 10° increments. Compounds 7 and 8, initially in the cis amide bond form (Figure 1) were subjected to these minimizations, during the course of which, the amide bond flipped from

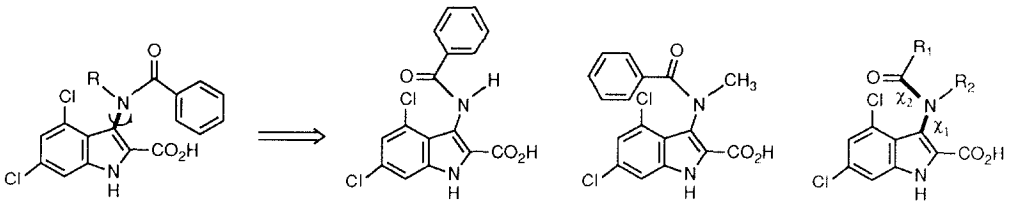



**Figure 1** Highlighted bond was rotated in 10° increments and minimized according to ref 10 Preferred amide bond orientations of 7 and 8 are shown Torsion angles were calculated for the highlighted bonds in the generic structure 14 see Table II

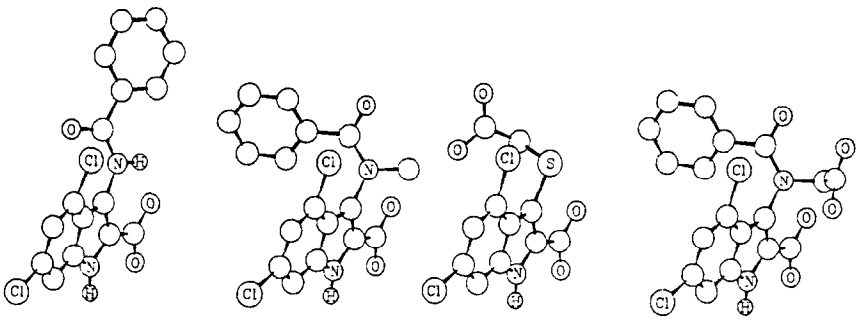
cis to trans. Results (Table II) showed that the more congested tertiary amide 8 favored the cis amide orientation while 7 favored the trans geometry. Subsequently, compounds 9-13 were also subjected to the described modeling conditions; the results are shown in Table II. It is clear from these results that the N-methylbenzamides prefer to be in the

**TABLE II**

Calculated minumum energies (Kcal/mol) and torsion angles for cis and trans amide bond forms.

| Compound | cis | trans | Δ | favored | $X_1$ | $X_2$ |
|---|---|---|---|---|---|---|
| 7 | 131.1 | 126.7 | 4.4 | trans | -30 | 175 |
| 8 | 144.5 | 148.3 | 3.8 | cis | -60 | -14 |
| 9 | 146.7 | 156.4 | 9.7 | cis | -70 | -9 |
| 10 | 104.3 | 98.4 | 5.9 | trans | 40 | -177 |
| 11 | 118.3 | 116.6 | 1.7 | trans | 60 | -176 |
| 12 | 128.4 | 122.3 | 6.1 | trans | -20 | 176 |
| 13 | 143.6 | 148.0 | 4.4 | cis | -50 | -20 |

cis configuration which would indicate that the glycine receptor also accepts the antagonist in this conformation. This suggests that the binding region in the receptor pocket that accepts the phenyl group is located in a region above the plane of the indole nucleus overlapping the 4-chlorine atom (see Figure 2). It seems clear, therefore, that the addition of a methyl group to 7 leads to the potent antagonist 8 by virtue of a conformational change which allows the phenyl group of the benzamide to interact with a lipophilic binding pocket within the glycine receptor.



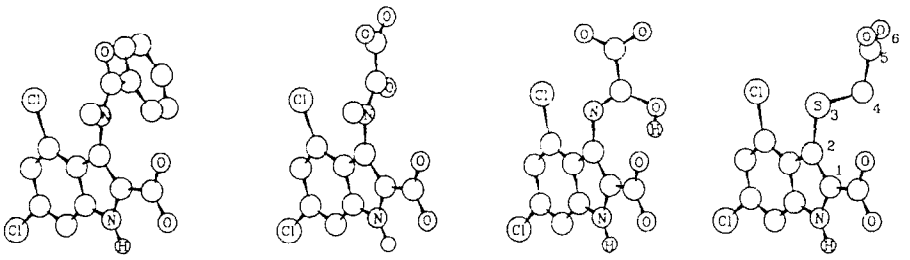
7 8 3 9

Figure 2. Global minimum energy conformations. Only heteroatom hydrogens are displayed.

When the hybrid molecule 9, was subjected to a similar molecular modeling study, the phenyl group once again was located above the plane of the indole, very closely aligned with that of 8. The acetic acid group was forced into a position on the opposite side of the indole ring (Figure 2). An explanation why 9 apparently does not interact strongly with both sites (which should have led to an exceedingly potent molecule) is that the hydrophilic and hydrophobic groups need to be on the same side of the planar indole ring. When the sulfide 3 was examined in the same way, the acetic acid side chain was located in a position distinct from the above-mentioned hydrophobic pocket (Figure 2). Conceivably, this could be located on the same side of the molecule.

Surprisingly, both the oxalamide analogues 10 and 11 preferred the trans geometry (Table II). It is difficult, therefore, to account for the differences in potencies of these two molecules, 10 being the more potent. In fact, it is not possible to fit 10 to the pharmacophore that was developed from the other potent compounds. Both 10 and 11, when fitted to the pharmacophore, place their polar amide carbonyl groups in the hydrophobic pocket which accepts the phenyl group (8) and are unable to overlay the side chain carboxyl group with that of 3. An explanation for these observations is based upon the assumption that 10 binds to the receptor in its enolic form[11]. Modeled in this way (Figure 3), the secondary amide 10 superimposed one of its carboxylic acid oxygen atoms onto the corresponding atom in the very potent sulfide 3[12] and at the same time moved the enolic hydroxyl group away from the phenyl pocket.

Several important topological features of the glycine binding site have thus been defined. Further testing of the model with newly designed molecules should provide further insight.



8 11 10 enol 3

Figure 3. Global minimum energy conformations of 8, 11, and 10 enol. Torsion angles of the global minimum of 3 were rotated.[12]

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9. 3-Amino-2-carboxymethyl-6-chloroindole was prepared from 5-chloro-2-cyanoaniline by the Method of Unangst, P.C. J. Heterocyclic Chem. 1983, 20, 495.
10. Using Insight 2.60 and Discover 2.60 (Biosym Technologies, Inc., La Jolla, CA), employing the CVFF forcefield torsion forcing calculations were performed on 7-13. The bond between indole C-3 and the amide nitrogen was sampled in 10°increments, with a forcing constant of 10,000 kcal/radian$^2$. Each conformation was minimized using no cross terms, no Morse potential, with a VA09A minimizer until the maximum derivative was less than 0.001 Kcal/mol·Å. The dielectric used was 2.0*R and the simulation was set to add-automatic bond, torsion, valence and out of plane parameters. Default settings were used for all other variables. Two complete rotations assured that both cis to trans and trans to cis barriers would be observed.
11. Reaction of 10 under conditions e (Scheme I) produced ~30% yield of bis-boc protected compound suggesting a significant increase in acidity over the benzamide 7 NH.
12. Side chain torsion angles of the global minimum of 3 were rotated to overlap one of the carboxyl oxygens with 10. Torsion angle 1-2-3-4 = 73°, 2-3-4-5 = 169°, 3-4-5-6 = 52°.