Substituted Indole-2-carboxylates as *in Vivo* Potent Antagonists Acting as the Strychnine-Insensitive Glycine Binding Site

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A series of indole-2-carboxylates bearing suitable chains at the C-3 position of the indole nucleus was synthesized and evaluated in terms of *in vitro* affinity using [^{3}H]glycine binding assay and *in vivo* potency by inhibition of convulsions induced by *N*-methyl-D-aspartate (NMDA) in mice. 3-[2-[(Phenylamino)carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic acid (**8**) was an antagonist at the strychnine-insensitive glycine binding site (noncompetitive inhibition of the binding of [^{3}H]TCP, pA_2 = 8.1) displaying nanomolar affinity for the glycine binding site (pK_i = 8.5), coupled with high glutamate receptor selectivity (>1000-fold relative to the affinity at the NMDA, AMPA, and kainate binding sites). This indole derivative inhibited convulsions induced by NMDA in mice, when administered by both iv and po routes (ED_{50} = 0.06 and 6 mg/kg, respectively). The effect of the substituents on the terminal phenyl ring of the C-3 side chain was investigated. QSAR analysis suggested that the pK_i value decreases with lipophilicity and steric bulk of substituents and increases with the electron donor resonance effect of the groups present in the *para* position of the terminal phenyl ring. According to these results the terminal phenyl ring of the C-3 side chain should lie in a nonhydrophobic pocket of limited size, refining the proposed pharmacophore model of the glycine binding site associated with the NMDA receptor.

Introduction

In the last decade the role of excitatory amino acid receptors in several disorders of the central nervous system (CNS) has been extensively investigated. It is now generally agreed that in ischaemic or hypoxic conditions[1] such as stroke, hypoglycemia, and traumatic head and spinal cord injuries, the increased amount of glutamate,[2] released into the synaptic clefts, causes the overstimulation of the ionotropic receptor complex responding to the exogenous agonist *N*-methyl-D-aspartate (NMDA). This key event results in a massive influx of Ca^{2+} into the postsynaptic neurons leading ultimately to cell death[3] through the activation of several neurotoxic cascades. Intervention with competitive and noncompetitive NMDA antagonists,[4] to block the opening of the ion channel associated with the NMDA receptor,[5] might be of therapeutic benefit. Among the endogenous modulators of the NMDA receptor, glycine has gained considerable interest in view of both its functional role in the activation of the ionotropic receptor complex as co-agonist of glutamate[6] and the greater therapeutic index seen for one of the first glycine antagonists identified, the 7-chlorokynurenic acid (7-Cl KA, **1**) depicted in Figure 1, compared to different series of NMDA antagonists.[7] Therefore, in the last few years the glycinergic site associated with the NMDA receptor complex was perceived as a unique target for medicinal chemistry, and considerable effort has been devoted to find potent and selective ligands, resulting in the identification of several classes of glycine antagonists.[8] A selected number of these derivatives is shown in Figure 1. These templates have been reported to be endowed with nanomolar *in vitro* affinity at the strychnine-insensitive glycine binding site, but with the exception of compounds **5** and **6**, poor *in vivo* activity was claimed in a variety of animal models, probably due to their insufficient CNS bioavailability.[9]

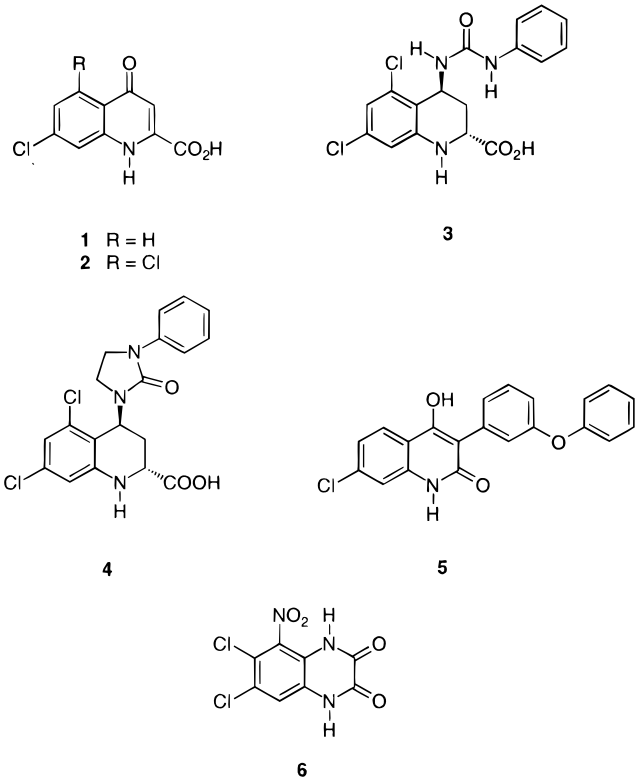

Figure 1. Structure of various glycine antagonists.

The indole-2-carboxylates of general structure **7**, depicted in Figure 2, represent a different class of

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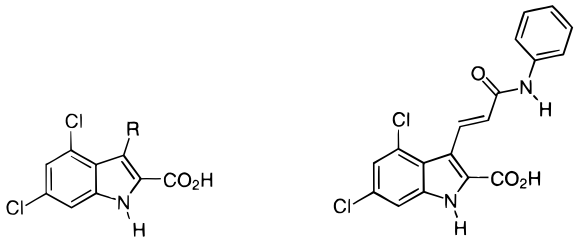


Figure 2. Indole-2-carboxylates.

glycine antagonists widely investigated during the last years by several research groups,[10] as a consequence of the preliminary evidence that the modulation at the C-3 position of the indole nucleus by different substituents could considerably enhance the affinity of this template at the glycine binding site.

The present paper deals with the synthesis and the pharmacological characterization of a novel series of indole-2-carboxylates substituted at the C-3 position with suitable α,β-unsaturated side chains. This class of glycine antagonists showed nanomolar affinity for the glycine binding site coupled with high receptor selectivity and high *in vivo* potency in the NMDA-induced convulsions model in mice, both by iv and po route. In particular compound **8**, GV150526A, shown in Figure 2, represents one of the most promising antagonists acting at the strychnine-insensitive glycine binding site identified to date.

Synthesis

Compounds **14** have been prepared starting from the known 3-unsubstituted indole derivative **9**[10b] following both synthetic routes A and B shown in Scheme 1. Formylation at position C-3 of the indole nucleus, performed according to the Vilsmaier−Haack procedure, afforded the intermediate **10** in high yield. This compound was submitted to the Wittig-type olefination reaction using a series of phosphorane derivatives, widely substituted at the terminal phenyl ring, to give compounds **11** with high regiocontrol in the formation of the E double bond (route A). Alternatively, **10** was transformed into the α,β-unsaturated acid intermediate **13**, easily obtained from intermediate **12** by the chemoselective deprotection of the *tert*-butyl ester group (route B). Amidation was then performed using different synthetic methods.[11] In particular, it is worth noting that, in this case, the activation of the carboxyl group through the formation of the corresponding 2-pyridyl thioester, generated *in situ* by the mild "oxidation−reduction" condensation reaction[12] in the presence of 2,2′-dipyridyl disulfide and PPh_3, was found to be particularly efficient also in the case of poorly nucleophilic aromatic amines, affording the amide derivatives **11** in high yield (either from the isolated 2-pyridyl thioester intermediate or from the acid **13** using a one-pot procedure). Finally, the basic hydrolysis of the 2-ethyl ester protecting group gave quantitative yields of the target compounds **14**.

Biology

The biological evaluation of the new chemical entities (NCE) was performed according to the following screening sequence: (a) binding assay to evaluate the affinity for the glycine site and selectivity for glutamate receptors; (b) "*in vitro*" functional antagonism studies to evaluate potency and activity; (c) "*in vivo*" anticonvulsant activity.

Affinity for the glycine binding site associated with the NMDA receptor channel complex was measured by inhibition of the binding of [^{3}H]glycine to crude synaptic membranes, prepared from adult rat cerebral cortex as described by Kishimoto *et al.*[13] The incubation (20 min, 4 °C) was carried out in Tris/citrate (50 mM, pH 7.10) using 20 nM [^{3}H]glycine. Data of displacement experiments, to determinate the inhibition constants (K_i) of displacer ligands, were analyzed using a nonlinear curve-fitting program LIGAND.[14] K_i values were measured from at least six-point inhibition curves and are the geometric mean of at least three different experiments. The standard error associated with the mean was less than 0.05.

The selectivity of NCE toward the glutamate binding site of the NMDA receptor channel complex, AMPA, and kainate ionotropic glutamate receptors was assessed by inhibition of the binding to rat cortical membranes of [^{3}H]CPP, [^{3}H]AMPA, and [^{3}H]kainic acid, respectively. In this case experiments were performed as reported by Van Amsterdam *et al.*,[15] Giberti *et al.*,[16] and Honoré *et al.*[17]

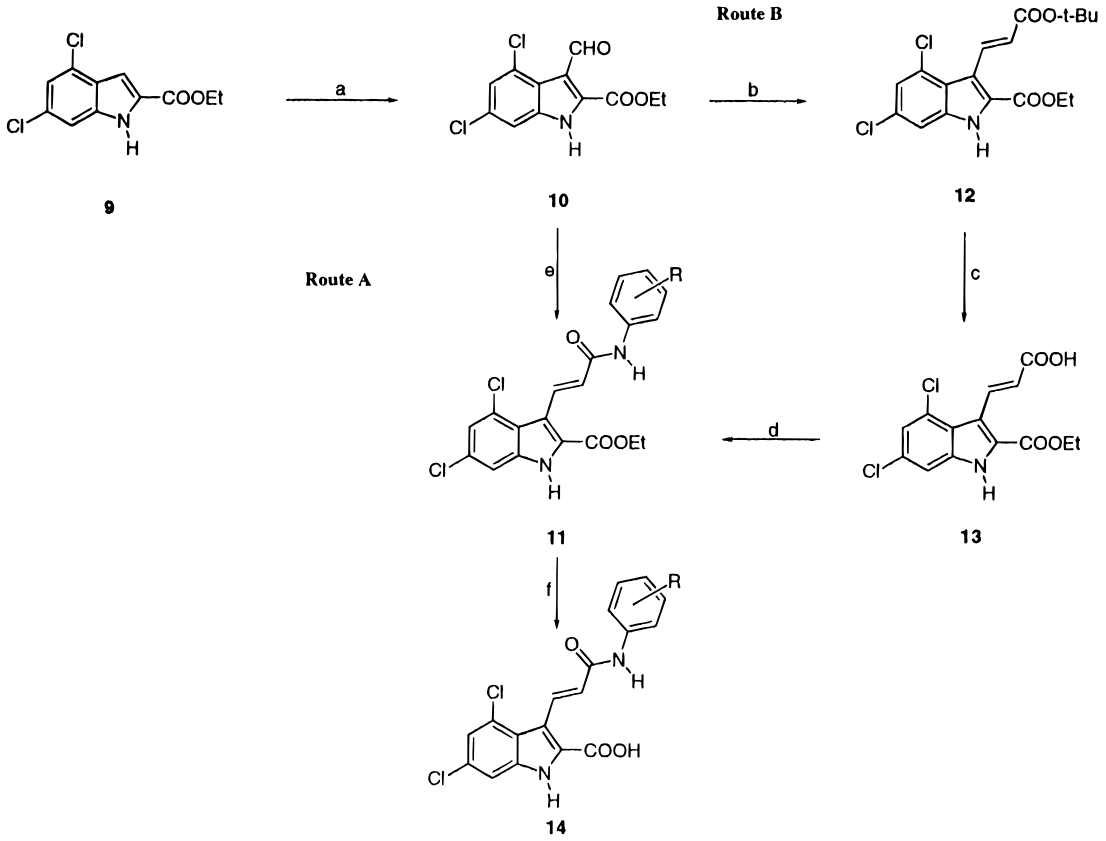
Functional antagonism at the glycine binding site was demonstrated by the ability of NCE to antagonise the glycine-induced enhancement of the binding of the channel-blocking agent [^{3}H]TCP[18] in a glycine-sensitive extensively washed rat cortical preparation.[19] Binding of [^{3}H]TCP was carried out (2 h, 30 °C) in Tris/HCl (5 mM, pH 7.7) and in the presence of glutamic acid (1 μM). Nonspecific binding was determined by addition of (+)-MK801 (30 μM). In the presence of increasing concentrations of NCE, parallel rightward shifts of the glycine concentration−response curves could be observed, with no depression of the maximum response. The antagonist potency (pA_2) was calculated according to the method of Arunlakshana and Schild.[20]

NCE were evaluated *in vivo* assessing their anticonvulsant effect in male CD-1 mice (18-29 g), both by iv and po administration.[7c] Convulsions were induced by icv injection of *N*-methyl-D-aspartate (1 nM/mouse) 1 min and 1 h after the iv and the po administration of the NCE, respectively. Animals were observed for the occurrence of generalized seizures during the first 30 min after NMDA treatment and considered protected if convulsions did not occur within this period. The percentage of animals showing convulsions in each treatment group was recorded and ED_{50} values, i.e. protecting dose of NCE providing 50% anticonvulsant effect, with their 95% confidence limits were estimated.

Results and Discussion

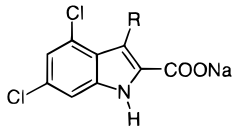
Our interest in the indole-2-carboxylate derivatives originated with the observation that the 3-unsubstituted-4,6-dichloro derivative **7** (R = H) shown in Figure 2 was endowed with good glutamate receptor selectivity.[10b] However, its *in vitro* affinity for the glycine binding site was low ($pK_i = 5.7$) compared to the most potent glycine antagonist hitherto identified, due to the lack of the necessary hydrogen bond accepting group as predicted by the proposed 3D-pharmacophore model of the glycine binding site.[21]

Scheme 1[a]



[a] Reagents and conditions: (a) PhN(Me)CHO, $POCl_3$, $ClCH_2CH_2Cl$; (b) $Ph_3P{=}CHCOO$-*t*-Bu, THF; (c) HCO_2H; (d) 2-Aldrithiol, PPh_3, $ArNH_2$, THF; (e) $R_3P{=}CHCONHAr$; (f) NaOH, EtOH, H_2O.

Table 1. Substitution at Position C-3



no.	R	pK_i[a]	ED_{50} (mg/kg)[b] iv	po
7	H	5.7		
8	CH=CHCONHPh	8.5	0.06 (0.005 − 0.42)	6 (4.40−7.94)
15	CH_2CH_2COOH	7.4	>10	
16	$CH_2CH_2CONHPh$	7.6	3.70 (1.51 − 12.12)	
17	CH=CHCOOH	7.7	17.21 (15.31 − 19.73)	>200
18	CH=CHCOO-*t*-Bu	6.3		
19	$CH{=}CHSO_2NHPh$	6.1		
20	$CH{=}CHCONHCH_2Ph$	6.9	>30	>200
3		8.2	0.21 (0.14 − 0.31)	
5		9.0	0.78 (0.45 − 1.63)	
6		8.1	0.21 (0.10 − 0.57)	

[a] Inhibition of binding of [^{3}H]Gly (ref 13). [b] NMDA-induced convulsion model (ref 7c).

Suitable C-3 side chains, bearing different H-bond accepting groups and terminal lypophilic substituents, were designed with the aim of further increasing the affinity of the indole-2-carboxylate template for the glycinergic site, giving rise to a short series of saturated and unsaturated indole derivatives, as reported in Table 1. All of the proposed compounds, in addition to the known derivatives **7** and **15**,[10b] were synthesized and then characterized in terms of *in vitro* affinity at the glycine binding site in rat cortical membrane preparations as previously described. On the basis of the results summarized in Table 1, the following observations can be addressed: (a) all of the compounds tested showed an affinity for the glycine binding site which was found to be greater than the C-3 unsubstituted analogue **7** (R = H), confirming the validity of the proposed 3D-pharmacophore model and in particular the need of a suitable H-bond acceptor group on the C-3 side chain; (b) the affinity of ligands possessing an α,β-unsaturated substituent directly conjugated with the indole nucleus tends to be higher with respect to the corresponding saturated system (compounds **8** *vs* **16** and **17** *vs* **15**). This effect could be due to the greater conformational entropy of compounds **8** and **17** *vs* **16** and **15**, respectively, and both to the higher electron density on the oxygen atom of the carbonyl group and the improved H-bond donor character of the indole NH group; (c) the observed 270-fold lower affinity at the glycine binding site of the sulfonamide derivative **19** with respect to the amide analogue **8** confirms the importance of a correct spatial orientation of the H-bond accepting group and the reduced H-bond accepting character of the sulfonamide group with respect to the corresponding amide carbonyl group; (d) the homologation of the terminal amide (entry **20** *vs* entry **8**) resulted in a large reduction of affinity as a consequence of both the increased steric bulk of the terminal substituent and/or the presence of a phenyl ring not directly conjugated with the nitrogen of the amide group; (e) the

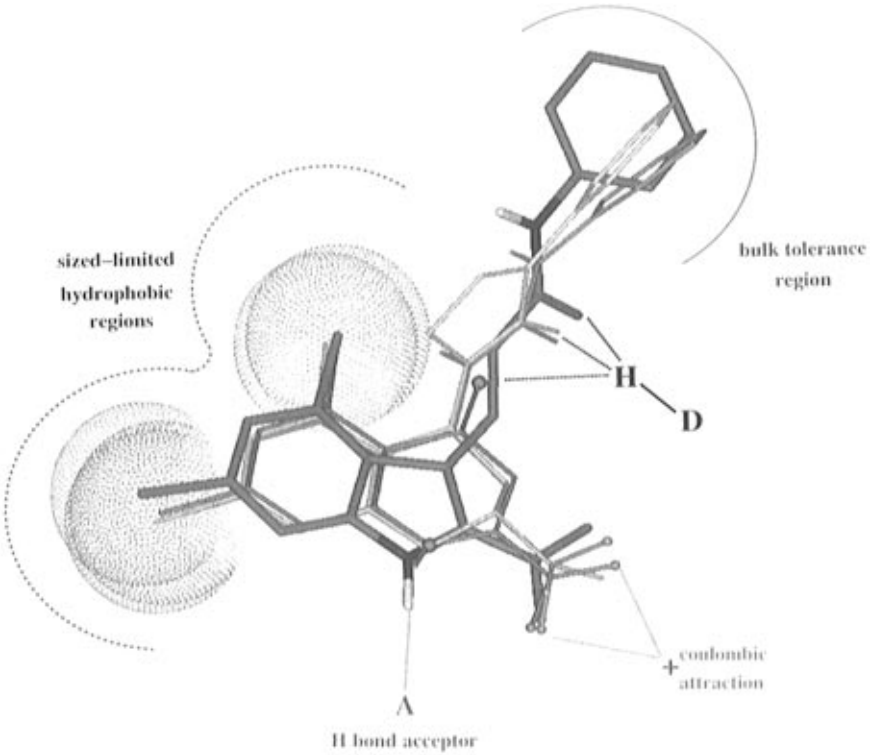


Figure 3. Superimposition of compound **8** with the pharmacophore model of the glycine binding site.

tert-butyl ester derivative **18** showed a significant reduction in affinity with respect to compound **17** in view of both the different H-bond accepting character of the carboxyl group and the steric bulk associated with the terminal lipophilic substituent.

In conclusion, the presence of an α,*β*-unsaturated side chain in which both the carbonyl group and the terminal phenyl ring play a key role in recognition seems to be crucial to maximize the affinity of these indole templates for the glycinergic site associated with the NMDA receptor. In Figure 3 is shown the superimposition of compound **8** with the known pharmacophore of the glycine binding site,[21] further confirming the validity of the proposed model. This model was built up in house using the active analog approach (AAA)[22] implemented within Sybyl 6.0[23a] on the tetrahydroquinoline derivatives **3** and **4** shown in Figure 1. As depicted in Figure 3 the indole derivative **8** as well as 5.7-dichlorokynurenic acid **2**, depicted in Figures 1 and 2, respectively, were superimposed to this model satisfying the observed pharmacophore arrangement.[23b]

Compound **8**, the indole derivative endowed with the highest affinity for the glycine binding site ($pK_i = 8.5$, as reported in Table 1), showed more than 1000-fold receptor selectivity for the strychnine-insensitive glycine binding site with respect to other glutamate binding sites ($pK_i = 5.0$, 5.1, and 5.4 for the NMDA, AMPA, and kainate binding site, respectively). Moreover, this compound was found to noncompetitively inhibit the binding of [^{3}H]TCP in extensively washed cortical membranes preparation, according to the procedure described above (this binding value can be considered as an index of the functional activation of the NMDA-gated ion channel). This study suggested that **8** is a noncompetitive antagonist acting at the glycine binding site with an estimated pA_2 of 8.1 nM, in good agreement with the affinity for the glycine recognition site ($pK_i = 8.5$) assessed, as previously reported,[18] by inhibition of the binding of [^{3}H]glycine. Moreover, no evidence of agonist activity was detected.

As reported in Table 1, the most potent *in vitro* indole derivatives of this series were tested as antagonists of NMDA-induced convulsions in mice both by iv and by po routes. Compound **8**, the glycine antagonist endowed with the highest *in vitro* affinity for the glycine binding site, was also found to be the most potent compound *in vivo*, showing considerable anticonvulsant activity when tested in the range of doses between 0.001−3 mg/kg iv and 1−100 mg/kg po, according to the general procedure described above. In both cases, dose-dependent inhibition of convulsions reached 80% at 3 mg/kg iv and 90% at 100 mg/kg po, respectively. The estimated ED_{50} by iv route was 0.06 mg/kg (0.005−0.42 mg/kg), whereas the ED_{50} by oral route was 6 mg/kg (4.4−7.94 mg/kg). As shown in Table 1, reference compounds **3**, **5**, and **6** were found to be slightly less active than **8**.[24]

The excellent *in vivo* activity of **8** in mice was confirmed by performing the same studies in rats by the iv route. The compound was tested in the range of doses between 0.03 and 3 mg/kg and a dose-dependent inhibition of NMDA-induced convulsions (up to 90% blockade) was observed, with an estimated ED_{50} of 0.38 mg/kg (0.03−1.34 mg/kg).

Substitution of the Terminal Phenyl Ring. After the identification of the indole derivative **8**, the effect of the substitution on the aromatic ring of the C-3 side chain was investigated with the aim of further improving the affinity of this class of derivatives for the glycine binding site associated with the NMDA receptor.

According to the Hansch approach, substituents are described in terms of their electronic and steric effects and lipophilicity. Compounds were chosen to ensure maximum variance and minimum collinearity between parameters characterizing the physicochemical properties of the substituents. The set of traditional descriptors[25−27] shown in Table 2 was considered, and MRA

Table 2. Physicochemical Parameters Utilized for MRA[a]

no.	R_o	R_m	R_p	MR_{omp}	MR_o	MR_{mp}	π_{omp}	π_o	π_{mp}	σ_{meta}	σ_{para}	F_{mp}	F_{omp}	R_{mp}	R_{omp}
8	H	H	H	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	H	H	$NHCONH_2$	1.24	0.00	1.24	−1.30	0.00	−1.30	0.00	−0.24	0.04	0.06	−0.28	−0.28
22	H	NH_2	H	0.37	0.00	0.37	−1.23	0.00	−1.23	−0.16	0.00	0.02	0.02	−0.24	−0.24
23	NH_2	H	H	0.37	0.37	0.00	−1.23	−1.23	0.00	0.00	0.00	0.00	0.03	0.00	−0.59
24	H	H	NH_2	0.37	0.00	0.37	−1.23	0.00	−1.23	0.00	−0.66	0.02	0.02	−0.68	−0.68
25	H	H	OH	0.15	0.00	0.15	−0.67	0.00	−0.67	0.00	−0.37	0.29	0.29	−0.64	−0.64
26	H	OH	H	0.15	0.00	0.15	−0.67	0.00	−0.67	0.12	0.00	0.28	0.28	−0.22	−0.22
27	OH	H	H	0.15	0.15	0.00	−0.44	−0.44	0.00	0.00	0.00	0.00	0.36	0.00	−0.55
28	NO_2	H	H	0.72	0.73	0.00	−0.37	−0.37	0.00	0.00	0.00	0.00	0.84	0.00	0.14
29	H	OMe	OMe	1.23	0.00	1.23	−0.35	0.00	−0.35	0.12	−0.27	0.52	0.52	−0.69	−0.69
30	Me	H	OMe	1.08	0.46	0.61	−0.19	−0.26	0.08	0.00	−0.27	0.26	0.21	−0.51	−0.62
31	NO_2	H	F	0.74	0.73	0.01	−0.15	−0.37	0.40	0.00	0.06	0.43	1.27	−0.34	−0.20
32	F	NO_2	H	0.74	0.02	0.73	−0.09	−0.20	0.30	0.71	0.00	0.66	1.19	0.06	−0.24
33	F	H	F	0.03	0.02	0.01	0.02	−0.20	0.40	0.00	0.06	0.43	0.97	−0.34	−0.63
34	H	H	COOH	0.65	0.00	0.65	0.04	0.00	0.04	0.00	0.45	0.33	0.33	0.15	0.15
35	OH	NO_2	H	0.88	0.15	0.73	0.11	−0.44	0.30	0.71	0.00	0.66	1.02	0.06	−0.50
36	H	H	$N(CH_3)_2$	1.29	0.00	1.29	0.20	0.00	0.19	0.00	−0.83	0.10	0.10	−0.92	−0.92
37	H	H	NO_2	0.72	0.00	0.72	0.30	0.00	0.30	0.00	0.78	0.67	0.67	0.16	0.16
38	H	H	F	0.01	0.00	0.01	0.40	0.00	0.40	0.00	0.06	0.43	0.43	−0.34	−0.34
39	H	H	OEt	1.08	0.00	1.08	0.61	0.00	0.61	0.00	−0.24	0.22	0.22	−0.44	−0.44
40	i-Pr	H	H	1.39	1.39	0.00	0.67	0.67	0.00	0.00	0.00	0.00	−0.06	0.00	−0.09
41	H	NO_2	Cl	1.21	0.00	0.74	0.89	0.00	0.89	0.71	0.23	1.07	1.07	−0.09	−0.09
42	H	H	$N(Et_2)$	2.22	0.00	2.22	1.09	0.00	1.09	0.00	−0.90	0.01	0.01	−0.91	−0.91
43	H	H	CF_3	0.51	0.00	0.51	1.33	0.00	1.33	0.00	0.54	0.38	0.38	0.19	0.19
44	H	H	NHPh	2.88	0.00	2.88	1.48	0.00	1.48	0.00	−0.40	−0.02	−0.02	−0.38	−0.38

[a] MR_{omp} = sum of molar refractivities of *ortho, meta, para* substituents; $*MR_o$ = molar refractivity of *ortho* substituent; MR_{mp} = sum of molar refractivities of *meta, para* substituents; π_{omp} = sum of lipophilicity of *ortho, meta, para* substituents; $*\pi_o$ = lipophilicity of *ortho* substituent; π_{mp} = sum of lipophilicity of *meta, para* substituents; σ_{meta} = sigma meta of *meta* substituent;[25] σ_{para} = sigma para of *para* substituent;[27] $**F_{omp}$ = global Swain and Lupton F[25] for *ortho, meta, para* positions; F_{mp} = global Swain and Lupton F[25] for *meta, para* positions; R_{omp} = global Swain and Lupton R[25] for *ortho, meta, para* positions; R_{mp} = global Swain and Lupton R[25] for *meta, para* positions. *Molar refractivities and lipophilicity were calculate with Daylight v4.41[31] program. **Combinations of Swain and Lupton's F and R were calculated according to ref 26.

(multiple regression analysis)[28] implemented with RS/1[29] was employed to analyse the data. Data handling and parametrization were performed with the programs TSAR v2.2[30] and Daylight v4.41.[31]

The pK_i values (observed affinity for the glycine binding site) for compounds shown in Table 2 are reported in Table 3. The following statistically significant equation was derived from the analysis of these results:

$$pK_i = -0.53MR_{omp} - 0.39\pi_{omp} - 0.82\sigma_{para} + 8.23 \quad (1)$$

$$n = 25 \qquad R^2 = 0.84 \qquad s = 0.28 \qquad F = 37$$

$$p < 0.0001 \qquad R^2_{cv} = 0.76 \text{ (ref 32)}$$

where MR_{omp} represents the total bulk of *ortho, meta,* and *para*[25] substituents, π_{omp} is the global lipophilicity of *ortho, meta,* and *para*[25] substituents, and finally σ_{para} corresponds to the Hammett's σ_{para}.[27] The calculated *vs* observed pK_i values for eq 1 are shown in Figure 4, whereas the correlation matrix[33] for variables in eq 1 is reported in Table 4.

As shown in eq 1, the affinity for the glycine receptor increases as total bulk (MR_{omp}) and lipophilicity (π_{omp}) of substituents decrease. Furthermore, pK_i increases as σ_{para} decreases, i.e. as the electron donor resonance effect of the *para* substituent increases.

According to these results *the terminal aromatic ring of the α,β-unsaturated C-3 side chain should lie in a nonhydrophobic pocket of limited size* (negative coefficient of π_{omp} and MR_{omp}), refining the previous pharmacophore model[21] of the glycine binding site associated with the NMDA receptor. The negative linear relation between lipophilicity and pK_i could be the descending portion of a parabolic or bilinear[34] trend; this aspect could be verified by exploring a wider range of negative values of lipophilicity).

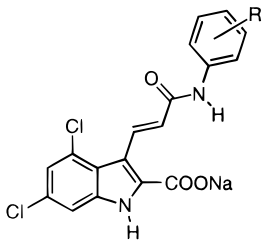
The electronic donor term is more difficult to interpret in terms of drug−receptor interactions. It can be speculated that this effect improves the hydrogen bonding acceptor ability of the carbonyl present within the C-3 α,β-unsaturated side chain.

Compound 8 (GV150526A): Further Biological Characterization. In view of its promising pharmacological profile, compound **8** (GV150526A) was selected among the number of indole derivatives identified for further evaluation in an experimental model of focal cerebral ischaemia in rats (MCAo).[35] Compound **8** showed an excellent neuroprotective activity (significant reduction of the infarct volume with respect to control groups) after iv and po administration, both pre- and postischaemia.[36] Moreover this indole derivative was characterized in terms of safety and general pharmacology: neither ataxic effects (rotarod) nor impairment of performance (passive avoidance) were observed in mice up to a dose of 30 mg/kg administered iv (500-fold the ED_{50} observed in inhibition of NMDA-induced convulsions), confirming the wider *therapeutic ratio* observed with glycine antagonists compared to competitive NMDA antagonists and NMDA receptor ion channel blockers.[7]

Conclusions

A novel class of indole-2-carboxylates was explored with the aim of identifying potent *in vivo* glycine antagonists. The substitution of the C-3 position of the indole nucleus with a suitable α,β-unsaturated side chain gave potent antagonists endowed with nanomolar affinity at the glycine binding site. These compounds

Table 3. Substitution of the Terminal Phenyl Ring of the C-3 Side Chain: *in Vitro* Pharmacological Characterization



no.	synthetic method	R_o	R_m	R_p	pK_i[a]
8	B	H	H	H	8.5
21	A	H	H	$NHCONH_2$	8.8
22	A	H	NH_2	H	8.3
23	A	NH_2	H	H	8.5
24	A	H	H	NH_2	8.9
25	B	H	H	OH	8.7
26	B	H	OH	H	8.4
27	B	OH	H	H	8.4
28	B	NO_2	H	H	7.6
29[b]	B	H	OCH_3	OCH_3	8.1
30	B	CH_3	H	OCH_3	7.7
31	B	NO_2	H	F	7.5
32[b]	B	F	NO_2	H	8.3
33	B	F	H	F	8.3
34	A	H	H	COOH	7.2
35	B	OH	NO_2	H	8.0
36	A	H	H	$N(CH_3)_2$	7.9
37	B	H	H	NO_2	7.0
38	A	H	H	F	8.2
39	B	H	H	OEt	8.3
40	B	i-Pr	H	H	7.3
41	B	H	NO_2	Cl	6.9
42	A	H	H	$N(C_2H_5)_2$	7.0
43	B	H	H	CF_3	6.8
44	A	H	H	NHC_6H_5	6.7

[a] Inhibition of binding of [^{3}H]Gly (ref 13). [b] Isolated and characterized as acid derivative.

showed high receptor selectivity, coupled with an excellent *in vivo* activity after systemic administration inhibiting the convulsions induced by NMDA in mice and in the MCAo model in rats.

In conclusion, the identification of this class of *in vivo* potent and selective glycine antagonists will be useful to further clarify the involvement of the glycine binding site associated with the NMDA receptor antagonists in different pathological conditions.

Experimental Section

Infrared spectra were recorded on a Bruker IFS 48 spectrometer. ^{1}H NMR spectra were recorded on a Varian Unity 400 (400 MHz); the data ar reported as follows: chemical shift in ppm from the internal standard Me_4Si on δ scale, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constants (hertz).

Chromatography was carried out with the use of Merck silica gel 60 (230−400 mesh) as described by Still *et al.*[37] Mass spectra were recorded on a VG-4 triple quadrupole Fison instrument in FAB mode. Elemental analyses were performed by our own analytical group on Carlo Erba elemental analyzer. Melting points were determined on a Büchi 530 apparatus (scale 0−250 °C) and are uncorrected. All reactions were carried out under a controlled atmosphere in oven-dried glassware. Anhydrous DMF was purchased from Aldrich; THF was used after distillation over K/benzophenone; CH_2Cl_2 and CH_3CN were used after distillation from P_2O_5. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates (0.25 mm).

Ethyl 3-Formyl-4,6-dichloroindole-2-carboxylate (10). A solution of *N*-methylformanilide (5.19 g, 38.4 mmol) and phosporous oxychloride (5.53 g, 36.1 mmol) was stirred at 23 °C for 15 min under nitrogen atmosphere. Dry 1,2-dichloroethane (60 mL) and ethyl 4,6-dichloroindole-2-carboxylate (6 g, 23.3 mmol) were added, and the suspension was stirred at 80 °C for 6 h. The reaction mixture was poured into a 50% aqueous solution of NaOAc (300 mL) to give, after filtration, 4.1 g (62%) of the title compound as a yellow solid: mp 220−222 °C; IR (Nujol) ν_{max} 2725−2669, 1726, 1663, 1556 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.15 (s, 1H), 10.60 (s, 1H), 7.54 (d, 1H), 7.40 (d, 1H), 4.43 (q, 2H), 1.36 (t, 3H).

(*E*)-3-[2′-[(*tert*-Butylcarbonyl)oxy]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Ethyl Ester (12). A magnetically stirred solution of [(*tert*-butoxycarbonyl)ethenyl]triphenylphosphorane (5.2 g, 15 mmol) and ethyl 3-formyl-4,6-dichloroindole-2-carboxylate (3.3 g, 11.6 mmol) in a 1:1 mixture of CH_3CN/dioxane (60 mL) was heated at 70 °C for 7 h under nitrogen atmosphere. The solvent was evaporated under reduced pressure and the crude residue purified by flash chromatography (cyclohexane/ethyl acetate, 1:1) to give 3.3 g of pure compound **12** (75%): mp 157−158 °C; IR (Nujol) ν_{max} 3302, 1703, 1674 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.20 (bs, 1H), 8.32 (d, 1H, 16 Hz), 7.33 (d, 1H), 7.19 (d, 1H), 6.48 (d, 1H, 16 Hz), 4.43 (q, 2H), 1.56 (s, 9H), 1.42 (t, 3H).

(*E*)-3-(2′-Carboxyethenyl)-4,6-dichloroindole-2-carboxylic Acid (13). (*E*)-Ethyl 3-[2-(*tert*-butoxycarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylate (0.5 g, 1.3 mmol) was dissolved in 100% HCOOH (60 mL), and the suspension was stirred at 23 °C for 2 h. The solvent was evaporated *in vacuo* to give 0.408 g of 3-(2-carboxyethenyl)-4,6-dichloroindole-2-carboxylate **13** as a white solid (96%): mp >250 °C; IR (Nujol) ν_{max} 3246−3128, 1699−1670, 1634 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.6 (bs, 2H), 8.28 (d, 1H, 16.2 Hz), 7.51 (d, 1H), 7.32 (d, 1H), 6.44 (d, 1H, 16.2 Hz), 4.37 (q, 2H), 1.35 (t, 3H).

General Procedure for the Synthesis of Aryl Amides: Procedure A. To a solution of ethyl 3-(2′-carboxyethenyl)-4,6-dichloroindole-2-carboxylate (**13**) (280 mg, 0.85 mmol) dissolved in dry DMF (18 mL) were added portionwise 2,2′-dipyridyl disulfide (267 mg, 1.2 mmol) and PPh_3 (317 mg, 1.2 mmol) at room temperature under nitrogen atmosphere. The solution was stirred for 3 h, and then the chosen arylamine derivative (1.2 mmol) was added. The reaction mixture was heated at 80 °C and stirred for additional 20 h. Resultant precipitate was filtered to give pure title compounds in 65−83% yields.

General Procedure for the Synthesis of Aryl Amides: Procedures B. A magnetically stirred solution of the chosen triphenylphosphorane (15 mmol) and intermediate **10** (11.6 mmol) in a 1:1 mixture CH_3CN/dioxane (60 mL) was stirred at 70 °C for 7 h under nitrogen atmosphere. The solvent was evaporated *in vacuo*, and the crude products were purified by flash chromatography to give the final compounds (70−88%).

General Procedure for the Basic Hydrolysis of the Ethyl Esters. To an aliquot of indole-2-carboxylic acid ethyl ester (0.25 mmol) dissolved in isopropyl alcohol (20 mL) was added NaOH (20 mg, 0.5 mmol). The solution was heated at 60 °C for 11 h and then diluted with water (30 mL). The aqueous solution was concentrated *in vacuo*, and the precipitate was filtered and washed with water to give pure sodium salt derivative in quantitative yield.

3-[2′-[(Phenylamino)carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (8). Prepared from compound **10** following the general procedure B: $C_{18}H_{11}Cl_2N_2O_3Na$; IR (Nujol) ν_{max} 3404−3126, 1624, 1616, 1600 cm^{-1}; MS *m/e* 397 (M + 1); ^{1}H NMR (DMSO-d_6) δ 11.8 (bs, 1H), 10.06 (s, 1H), 8.59 (d, 1H, 15.5 Hz), 7.75 (d, 2H), 7.44 (d, 1H), 7.27 (t, 2H), 7.21 (d, 1H, J = 15.5 Hz), 7.10 (d, 1H), 6.98 (t, 1H).

3-[2′-[(Phenylamino)carbonyl]ethyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (16). An aliquot of 3-(2′-carboxyethyl)-4,6-dichloroindole-2-carboxylic acid ethyl ester (*Chem. Pharm. Bull.* **1977**, *25* (11), 3023) was treated with equimolar amount of CDI and aniline in anhydrous DMF at 60 °C for 6 h. The reaction mixture was poured in H_2O and extracted with AcOEt. The organic layer was separated, dried over Na_2SO_4, and evaporated *in vacuo*. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to give the final compound (40%). The hydrolysis of the 2-carboxyethyl ester group was performed according to the general

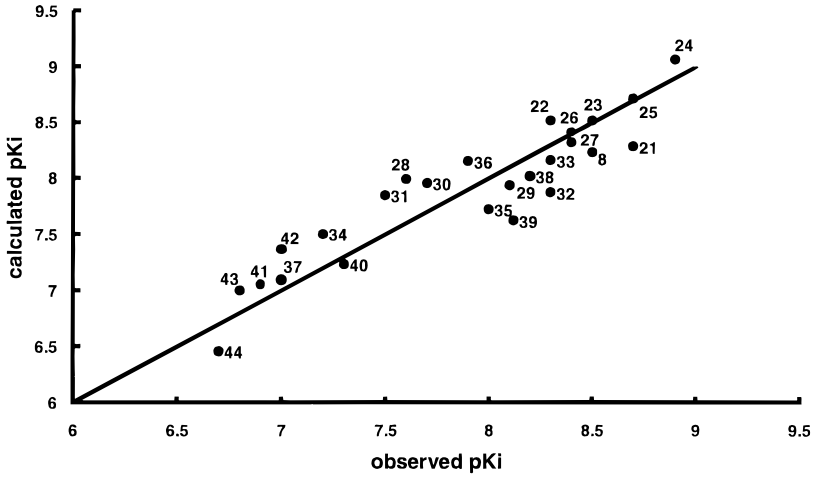


Figure 4. Calculated *vs* observed pK_i values for compounds in eq 1.

Table 4. Correlation Matrix for variables in Eq 1

	MR_{omp}	π_{omp}	σ_{omp}
MR_{omp}	1	0.536	−0.415
π_{omp}		1	0.106
σ_{omp}			1

Table 5. Physical Data of New Compounds

no.	R_o	R_m	R_p	formula[a]	mp, °C[b]	MS (*m*/*z*)
8	H	H	H	$C_{18}H_{11}Cl_2N_2O_3Na$	a	397
16				$C_{18}H_{13}Cl_2N_2O_3Na$	a	399
17				$C_{12}H_5Cl_2NO_4Na_2$	a	344
18				$C_{16}H_{14}Cl_2NO_4Na$	a	402
19				$C_{17}H_{12}Cl_2N_2O_4SNa$	a	434
20				$C_{19}H_{13}Cl_2N_2O_3Na$	a	411
21	H	H	$NHCONH_2$	$C_{19}H_{13}Cl_2N_4O_4Na\cdot H_2O$	a	455
22	H	NH_2	H	$C_{18}H_{12}Cl_2N_3O_3Na\cdot H_2O$	a	412
23	NH_2	H	H	$C_{18}H_{12}Cl_2N_3O_3Na\cdot 5H_2O$	a	412
24	H	H	NH_2	$C_{18}H_{12}Cl_2N_3O_3Na\cdot 0.1H_2O$	a	412
25	H	H	OH	$C_{18}H_{10}Cl_2N_2O_4Na_2\cdot H_2O$	a	437
26	H	OH	H	$C_{18}H_{10}Cl_2N_2O_4Na_2\cdot 3H_2O$	a	437
27	OH	H	H	$C_{18}H_{10}Cl_2N_2O_4Na_2\cdot H_2O$	a	437
28	NO_2	H	H	$C_{18}H_{10}Cl_2N_3O_5Na\cdot H_2O$	a	442
29	H	OCH_3	OCH_3	$C_{20}H_{16}Cl_2N_2O_5\cdot 0.5H_2O$	243	435
30	CH_3	H	OCH_3	$C_{20}H_{15}Cl_2N_2O_4Na\cdot 0.1H_2O$	a	442
31	NO_2	H	F	$C_{18}H_9Cl_2FN_3O_5Na\cdot H_2O$	a	460
32	F	NO_2	H	$C_{18}H_{10}Cl_2FO_5N_3$	>250	438
33	F	H	F	$C_{18}H_9Cl_2F_2N_2O_3Na\cdot H_2O$	a	434
34	H	H	COOH	$C_{19}H_{10}Cl_2N_2O_5Na_2\cdot 2H_2O$	a	463
35	OH	NO_2	H	$C_{18}H_9Cl_2N_3O_6Na_2\cdot H_2O$	a	480
36	H	H	$N(CH_3)_2$	$C_{20}H_{16}Cl_2N_3O_3Na$	a	439
37	H	H	NO_2	$C_{18}H_{10}Cl_2N_3O_5Na\cdot H_2O$	a	442
38	H	H	F	$C_{18}H_{10}Cl_2N_2O_3FNa$	a	415
39	H	H	OEt	$C_{20}H_{15}Cl_2N_2O_4Na\cdot 0.5H_2O$	a	441
40	i-Pr	H	H	$C_{21}H_{17}Cl_2N_2O_3Na$	a	439
41	H	NO_2	Cl	$C_{18}H_9Cl_3N_3O_5Na\cdot H_2O$	a	476
42	H	H	$N(C_2H_5)_2$	$C_{22}H_{20}Cl_2N_3O_3Na\cdot 0.5H_2O$	a	468
43	H	H	CF_3	$C_{19}H_{11}Cl_2F_3N_2O_3Na\cdot 0.5H_2O$	a	466
44	H	H	NHC_6H_5	$C_{24}H_{16}Cl_2N_3O_3Na\cdot H_2O$	a	488

[a] C, H, N analyses were within 0.4% of the theoretical values for the formulae given unless otherwise noted. [b] a: sodium salt derivatives melting over 250 °C.

procedure described above to give title compound **16** in quantitative yield: $C_{18}H_{13}Cl_2N_2O_3Na$; IR (Nujol) ν_{max} 1661 cm^{-1}; ^1H NMR (DMSO-d_6) 13.4 (bs, 1H), 11.9 (s, 1H), 9.93 (bs, 1H), 7.57 (d, 2H), 7.38 (s, 1H), 7.26 (t, 2H), 7.15 (s, 1H), 7.0 (t, 1H), 3.62 (t, 2H), 3.57 (t, 2H).

3-(2′-Carboxyethenyl)-4,6-dichloroindole-2-carboxylic Acid Disodium Salt (17). Prepared from compound **13** according to the general procedure described above for basic hydrolysis: $C_{12}H_5Cl_2NO_4Na_2$; IR (Nujol) ν_{max} 1684, 1636, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) 12.26 (bs, 1H), 8.34 (d, 1H), 7.49 (d, 1H), 7.32 (d, 1H), 6.50 (d, 1H).

3-[2′-(*tert*-Butoxycarbonyl)ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (18). Prepared from compound **12** according to the general procedure above described for basic hydrolysis: $C_{16}H_{14}Cl_2NO_4Na$; IR (Nujol) ν_{max} 3339, 1700, 1653, 1612 cm^{-1}; ^1H NMR (DMSO-d_6) 12.0 (bs, 1H), 8.61 (d, 1H), 7.41 (m, 1H), 7.11 (m, 1H), 7.07 (d, 1H), 1.45 (bs, 1H).

3-[2′-[(Phenylamino)sulfonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (19). An aliquot of 4,6-dichloroindole-2-carboxylic acid ethyl ester (*J. Med. Chem.* **1990**, *33*, 2244) was treated with 2 equiv of *N*-phenylethenesulfonamide (RN 3192-10-7), 1 equiv of $PdCl_2$, and 4 equiv of $Cu(OAc)_2$ in CH_3CN. The solution was refluxed for 16 h under argon atmosphere. The reaction mixture was poured in H_2O and extracted with AcOEt. The organic layer was separated, dried over Na_2SO_4, and evaporated *in vacuo*. The crude residue was purified by flash chromatography (cyclohexane/AcOEt, 7:3) to give the final compound (20%). The final hydrolysis of the 2-carboxyethyl ester group was carried out according to the general procedure described above, to give title compound **19** in quantitative yield: $C_{17}H_{12}Cl_2N_2O_4SNa$; IR (Nujol) ν_{max} 3288, 1691, 1603, 1315, 1138 cm^{-1}; ^1H NMR (DMSO-d_6) 12.7 (s, 1H), 10.02 (s, 1H), 8.20 (d, 1H), 7.45 (d, 1H), 7.27 (d, 1H), 7.30−7.16 (m, 5H), 7.0 (m, 1H).

3-[2′-[(Benzylamino)carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (20). Prepared according to the general procedure A: $C_{19}H_{13}Cl_2N_2O_3Na$; IR (Nujol) ν_{max} 3400, 1649, 1609 cm^{-1}; ^1H NMR (DMSO-d_6) 11.70 (bs, 1H), 8.45 (d, 1H), 8.44 (t, 1H), 7.38 (d, 1H), 7.35−7.25 (m, 4H), 7.20 (t, 1H), 7.07 (d, 1H), 6.99 (d, 1H), 4.34 (d, 2H).

3-[2′-[[(4-Ureidophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (21). Prepared according to the general procedure A: $C_{19}H_{13}Cl_2N_4O_4Na\cdot H_2O$; IR (Nujol) ν_{max} 3268−3190, 1684, 1650 cm^{-1}; ^1H-NMR (DMSO-d_6) 11.86 (bs, 1H), 9.96 (bs, 1H), 9.77 (s, 1H), 8.76 (d, 1H), 7.63 (d, 2H), 7.51 (d, 2H), 7.41 (d, 1H), 7.13 (d, 1H), 7.00 (d, 1H), 6.36 (bs, 2H).

3-[2′-[[(2-Aminophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (22). Prepared according to the general procedure A: $C_{18}H_{12}Cl_2N_3O_3Na\cdot H_2O$; IR (Nujol) ν_{max} 3500−3100, 1647, 1607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.85 (bs, 1H), 9.32 (bs, 1H), 8.56 (d, 1H, 15.6 Hz), 7.43 (d, 1H), 7.32 (d, 1H), 7.22 (d, 1H, 15.6 Hz), 7.09 (d, 1H), 6.86 (m, 1H), 6.71 (dd, 1H), 6.54 (m, 1H), 4.94 (bs, 2H).

3-[2′-[[(3-Aminophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (23). Prepared according to the general procedure A: $C_{18}H_{12}Cl_2N_3O_3Na\cdot 5H_2O$; IR (Nujol) ν_{max} 3188, 1601 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.8 (bs), 10.05 (s), 9.72 (s), 8.62 (d), 8.55 (d), 7.43 (d), 7.12 (m), 7.11 (d), 6.91 (t), 6.84 (d), 6.23 (d), 5.00 (bs).

3-[2′-[[(4-Aminophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (24). Prepared according to the general procedure A: $C_{18}H_{12}Cl_2N_3O_3Na\cdot 0.1H_2O$; IR (Nujol) ν_{max} 3389−3196, 1607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.80 (bs, 1H), 9.61 (bs, 1H), 8.47 (d, 1H), 7.40 (d), 7.38 (d), 7.08 (d), 7.05 (d), 6.48 (d, 2H), 4.79 (bs, 2H).

3-[2′-[[(4-Hydroxyphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (25). Pre-

pared according to the general procedure B: $C_{18}H_{10}Cl_2N_2O_4Na_2 \cdot H_2O$; IR (Nujol) ν_{max} 3437−2670, 1684 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.7 (bs, 1H), 12.47 (bs, 1H), 9.94 (bs, 1H), 9.20 (bs, 1H), 8.21 (d, 1H), 7.49 (d), 7.46 (d), 7.28 (6.73, 1H), 6.69 (d).

3-[2′-[[(3-Hydroxyphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (26). Prepared according to the general procedure B; $C_{18}H_{10}Cl_2N_2O_4Na_2 \cdot 3H_2O$; IR (Nujol) ν_{max} 3098, 1705, 1676, 1607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.70 (bs, 1H), 12.53 (s, 1H), 10.05 (s, 1H), 9.38 (s, 1H), 8.23 (d, 1H, 15.9 Hz), 7.47 (d, 1H), 7.30 (d, 2H), 7.06 (m, 2H), 6.74 (d, 1H, 15.9 Hz), 6.44 (dt, 1H).

3-[2′-[[(2-Hydroxyphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (27). Prepared according to the general procedure B: $C_{18}H_{10}Cl_2N_2O_4Na_2 \cdot H_2O$; IR (Nujol) ν_{max} 3150, 1736, 1656, 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 14.2−13.2 (bs, 1H), 12.56 (s, 1H), 9.97 (bs, 1H), 9.7 (s, 1H), 8.24 (s, 1H), 7.8 (d, 1H), 7.49 (d, 1H), 7.30 (d, 1H), 6.96 (d, 1H), 6.96 (dt), 6.88 (dd), 6.79 (td).

3-[2′-[[(2-Nitrophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (28). Prepared according to the general procedure B: $C_{18}H_{10}Cl_2N_3O_5Na \cdot H_2O$; IR (Nujol) ν_{max} 3234, 1684, 1636, 1609 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.20 (bs, 1H), 10.51 (bs, 1H), 8.59 (d, 1H), 7.95 (dd, 1H), 7.81 (dd, 1H), 7.69 (m, 1H), 7.48 (d, 1H), 7.38−7.28 (m, 2H), 7.20 (d, 1H).

3-[2′-[[(3,4-Dimethoxyphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid (29). Prepared according to the general procedure B: $C_{20}H_{16}Cl_2N_2O_5 \cdot 0.5H_2O$; IR (Nujol) ν_{max} 3420, 3281, 1690, 1680, 1620, 1607 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.8−13.6 (bs, 1H), 12.53 (bs, 1H), 10.08 (bs, 1H), 8.23 (d, 1H, 16 Hz), 7.47 (m, 2H), 7.29 (d, 1H), 7.2 (dd, 1H), 6.89 (d, 1H), 6.74 (d, 1H, 16 Hz), 3.73 (s, 3H), 3.7 (s, 3H).

3-[2′-[[(2-Methyl-4-methoxyphenyl)amino]carbonyl]-ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (30). Prepared according to the general procedure B: $C_{20}H_{15}Cl_2N_2O_4Na \cdot 0.1H_2O$; IR (Nujol) ν_{max} 3279, 1703, 1661, 1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.41 (bs, 1H), 9.39 (bs, 1H), 8.26 (d, 1H), 7.48 (d), 7.36 (d), 7.27 (d), 6.90 (d), 6.80 (d), 6.75 (dd), 3.73 (s, 3H), 2.19 (s, 3H).

3-[2′-[[(4-Fluoro-2-nitrophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (31). Prepared according to the general procedure B: $C_{18}H_9Cl_2FN_3O_5Na \cdot H_2O$; IR (Nujol) ν_{max} 3312, 1653, 1634 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.00 (bs, 1H), 10.44 (bs, 1H), 8.65 (d, 1H), 7.88 (dd), 7.79 (dd), 7.62 (m, 1H), 7.45 (d), 7.45 (d), 7.15 (d, 1H).

3-[2′-[[(2-fluoro-3-nitrophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid (32). Prepared according to the general procedure B: $C_{18}H_{10}Cl_2FO_5N_3$; IR (Nujol) ν_{max} 3414−3100, 1684−1614, 1539−1348 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.82 (bs), 12.60 (bs), 10.46 (bs), 9.21 (dd), 8.41 (d), 8.05 (m), 7.59 (t), 7.50 (d), 7.33 (d), 7.06 (d).

3-[2′-[[(2,4-Difluorophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodiumn Salt (33). Prepared according to the general procedure B: $C_{18}H_9Cl_2F_2N_2O_3Na \cdot H_2O$; IR (Nujol) ν_{max} 3431−3233, 1707, 1678, 1612 cm^{-1}; ^1H NMR (DMSO-d_6) δ 14.0−13.6 (bs, 1H), 12.54 (bs, 1H), 9.99 (bs, 1H), 8.29 (d, 1H, 15.6 Hz), 7.97 (m, 1H), 7.48 (d, 1H), 7.30 (m, 1H), 7.29 (d, 1H), 7.07 (m, 1H), 6.90 (d, 1H, 15.6 Hz).

3-[2′-[[(4-Carboxyphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (34). Prepared according to the general procedure A: $C_{19}H_{10}Cl_2N_2O_5Na_2 \cdot 2H_2O$; IR (Nujol) ν_{max} 3406, 1734 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.94 (bs, 1H), 8.57 (d, 1H, 15.6 Hz), 7.72 (d, 2H), 7.59 (d, 2H), 7.37 (d, 1H), 7.17 (d, 1H, 15.6 Hz), 7.09 (d, 1H).

3-[2′-[[(2-Hydroxy-3-nitrophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (35). Prepared according to the general procedure B: $C_{18}H_9Cl_2N_3O_6Na_2 \cdot H_2O$; IR (Nujol) ν_{max} 3383, 3298, 1700, 1530, 1377 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.53 (s, 1H), 9.74 (s, 1H), 9.10 (d, 1H), 8.30 (d, 1H), 7.90 (dd, 1H), 7.48 (d, 1H), 7.29 (d, 1H), 7.06 (d, 1H), 7.02 (d, 1H).

3-[2′-[[[4-(Dimethylamino)phenyl]amino]carbonyl]-ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (36). Prepared according to the general procedure A: $C_{20}H_{16}Cl_2N_3O_3Na$; IR (Nujol) ν_{max} 3184, 2670, 1700−1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.70 (bs, 1H), 12.42 (bs, 1H), 9.82 (bs, 1H), 8.21 (d, 1H), 7.53 (d), 7.46 (d), 7.26 (d, 1H), 6.75 (d), 7.69 (d), 2.84 (s, 6H).

3-[2′-[[(4-Nitrophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (37). Prepared according to the general procedure B: $C_{18}H_{10}Cl_2N_3O_5Na \cdot H_2O$; IR (Nujol) ν_{max} 3346 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.20 (bs, 1H), 10.89 (bs, 1H), 8.74 (d, 1H), 8.19 (m, 2H), 8.05 (m, 2H), 7.52 (d, 2H), 7.45 (d, 1H), 7.13 (d, 1H).

3-[2′-[[(4-Fluorophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (38). Prepared according to the general procedure A: $C_{18}H_{10}Cl_2N_2O_3FNa$; IR (Nujol) ν_{max} 3206, 1628−1609 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.08 (bs, 1H), 10.14 (bs, 1H), 8.62 (d, 1H, 15.6 Hz), 7.79 (m, 1H), 7.45 (d, 1H), 7.25 (d, 1H, 15.6 Hz), 7.11 (m), 7.11 (d).

3-[2′-[[(4-Ethoxyphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (39). Prepared according to the general procedure B: $C_{20}H_{15}Cl_2N_2O_4Na \cdot 0.5H_2O$; IR (Nujol) ν_{max} 3248, 1663, 1632, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.70 (bs, 1H), 12.50 (bs, 1H), 10.04 (bs, 1H), 8.22 (d, 1H, 15.6 Hz), 7.61 (d, 2H), 7.47 (d, 1H), 7.29 (d, 1H), 6.86 (d, 2H), 6.74 (d, 1H, 15.6 Hz), 3.97 (q, 2H), 1.29 (t, 3H).

3-[2′-[[(2-Isopropylphenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (40). Prepared according to the general procedure B: $C_{21}H_{17}Cl_2N_2O_3Na$; IR (Nujol) ν_{max} 3261, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.10 (bs, 1H), 9.39 (bs, 1H), 8.57 (d, 1H), 7.57 (bs, 1H), 7.38−7.28 (m), 7.28−7.10 (m), 3.25 (m, 1H), 1.15 (d, 6H).

3-[2′-[[(4-Chloro-3-nitrophenyl)amino]carbonyl]ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (41). Prepared according to the general procedure B: $C_{18}H_9Cl_3N_3O_5Na \cdot H_2O$; IR (Nujol) ν_{max} 3383−3182, 1610, 1587 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.18 (bs, 1H), 10.87 (bs, 1H), 8.71 (d, 1H, 15.6 Hz), 7.67 (d, 1H), 8.00 (dd, 1H), 7.69 (d, 1H), 7.53 (d, 1H), 7.48 (d, 1H, 15.6 Hz), 7.13 (d, 1H).

3-[2′-[[[4-(diethylamino)phenyl]amino]carbonyl]-ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (42). Prepared according to the general procedure A: $C_{22}H_{20}Cl_2N_3O_3Na \cdot 0.5H_2O$; IR (Nujol) ν_{max} 3248, 3192, 1649, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.80 (bs, 1H), 9.67 (s, 1H), 8.47 (d, 1H, 15.6 Hz), 7.51 (d, 2H), 7.39 (d, 1H), 7.08 (d, 1H), 7.08 (d, 1H, 15.6 Hz), 6.59 (d, 2H), 3.4 (q, 4H), 1.05 (t, 6H).

3-[2′-[[[4-(Trifluoromethyl)phenyl]amino]carbonyl]-ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (43). Prepared according to the general procedure A: $C_{19}H_{11}Cl_2F_3N_2O_3Na \cdot 0.5H_2O$; IR (Nujol) ν_{max} 3430, 3000, 1700, 1678, 1636, 1614 cm^{-1}; ^1H NMR (DMSO-d_6) δ 14.00−13.50 (bs, 1H), 12.55 (bs, 1H), 10.54 (bs, 1H), 8.37 (d, 1H, 15.8 Hz), 7.91 (d, 2H), 7.67 (d, 2H), 7.48 (d, 1H), 7.30 (d, 1H), 6.86 (d, 1H, 15.8 Hz).

3-[2′-[[[4-(Phenylamino)phenyl]amino]carbonyl]-ethenyl]-4,6-dichloroindole-2-carboxylic Acid Sodium Salt (44). Prepared according to the general procedure A: $C_{24}H_{16}Cl_2N_3O_3Na \cdot H_2O$; IR (Nujol) ν_{max} 3389, 1595, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.88 (bs, 1H), 8.55 (d, 1H, 15.7 Hz), 8.00 (s, 1H), 7.63 (d, 2H), 7.40 (d, 1H), 7.17 (t, 2H), 7.16 (d, 1H, 15.7 Hz), 7.10 (d, 1H), 7.02 (d, 2H), 6.98 (d, 2H), 6.73 (t, 1H).

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(24) Although moderately, compound **16** was found to be active (ED_{50} = 3.7 mg/kg) in our study in contrast to what published by Rowley *et al.* (ref 10f). This discrepancy could be due to the different model used (DBA/2 model in mice).

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