Novel Indole-2-carboxylates as Ligands for the Strychnine-Insensitive *N*-Methyl-D-aspartate-Linked Glycine Receptor

Nancy M. Gray,*,† Michael S. Dappen, Brian K. Cheng, Alexis A. Cordi, John P. Biesterfeldt, William F. Hood, and Joseph B. Monahan

Central Nervous System Diseases Research, G. D. Searle & Company, Mail Zone AA51, 700 Chesterfield Village Parkway, St. Louis, Missouri 63198. *Received April 11, 1990*

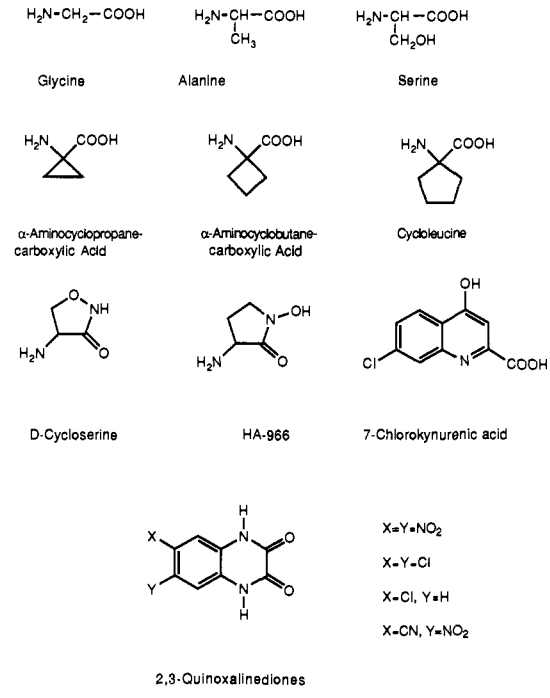
A series of indole-2-carboxylates were prepared and evaluated for their ability to inhibit the binding at the strychnine-insensitive glycine receptor that is associated with the NMDA–PCP–glycine receptor complex. All of the compounds were selective for the glycine site relative to other sites on the receptor macrocomplex and several of the compounds in this series were found to have submicromolar affinity for this receptor. The lead compound, 2-carboxy-6-chloro-3-indoleacetic acid (K_i = 1.6 μM vs [^{3}H]glycine), was also found to noncompetitively inhibit the binding of MK-801, a ligand for the phencyclidine site on the receptor macrocomplex. These latter data suggest that the compound functions as an antagonist at the strychnine-insensitive glycine receptor. The structural activity relationships within this series of indole-2-carboxylates is discussed and several key pharmacophores are identified for this series of glycine ligands. In general, the most potent compounds were the C-3 acetamides, with *N*-propyl-2-carboxy-6-chloro-3-indoleacetamide having the highest receptor affinity.

Introduction

Glycine, alanine, and serine (Chart I) have been shown to enhance the electrophysiological response induced by the excitatory amino acid, *N*-methyl-D-aspartate (NMDA), in cortical neurons from primary cell culture.[1] This potentiation was shown to be independent of the strychnine-sensitive glycine receptor.[2] A strychnine-insensitive, sodium-independent [^{3}H]glycine recognition site in rat brain has been reported[3] and autoradiographic studies have shown a good correlation between the regional distribution of [^{3}H]glycine and NMDA-sensitive L-[^{3}H]glutamate binding sites.[4] Additional evidence for the interaction of glycine with the NMDA subclass of excitatory amino acid receptors comes from neurochemical studies of the phencyclidine (PCP) receptor. Neurochemical, electrophysiological, and behavioral studies of the PCP receptor have provided substantial evidence that a complex may exist which includes the NMDA receptor, PCP receptor, and an associated cation channel.[5] Glycine and other neutral amino acids have been shown to enhance the binding of [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine (TCP)[6,7] and [^{3}H]MK-801[8,9] to the PCP receptor in a strychnine-insensitive manner. This enhancement is blocked by the competitive NMDA receptor antagonists 2-amino-5-phosphonopentanoate (AP5), 2-amino-7-phosphonoheptanoate (AP7), and 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonate (CPP). In addition, D-serine has been found to antagonize the phencyclidine-induced stereotyped behaviors in rats.[10] All of these studies indicate that a glycine site exists in rat brain which is insensitive to strychnine, has a similar anatomical distribution as the NMDA receptor, and is functionally linked to both the NMDA and PCP receptors.

Compounds which interact with the NMDA–PCP–glycine receptor complex can have a variety of therapeutic applications. Compounds which positively modulate the receptor complex through interaction with the glycine site have been demonstrated to enhance the performance of learning tasks in rats, suggesting the use of glycine agonists as cognitive enhancers.[11,12] These compounds may also function as antipsychotic agents.[13] Both competitive and noncompetitive antagonists of the NMDA receptor have been shown to possess anticonvulsant, muscle relaxant, and anxiolytic properties.[14] Antagonists of the receptor complex also have potential utility as neuroprotective agents.[15]

Chart I. Structures of Various Glycine Ligands



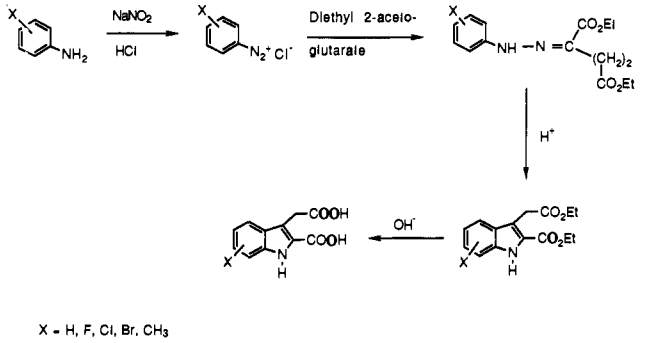
Compounds from many different chemical classes have been found to interact with the glycine site (Chart I). In

† Current address: Searle R&D, 4901 Searle Parkway, Skokie, IL 60077.

(1) Johnson, J. W.; Ascher, P. *Nature* 1987, *325*, 529.
(2) Graham, D.; Pfeiffer, F.; Simler, R.; Betz, H. *Biochemistry* 1985, *24*, 990.
(3) Kishimoto, H.; Simon, J. R.; Aprison, M. H. *J. Neurochem.* 1981, *37*, 1010.
(4) Nguyen, L.; Monaghan, D. T.; Cotman, C. W. *Abstr. Soc. Neurosci.* 1987, *13*, 759.
(5) Contreras, P. C.; Monahan, J. B.; Lanthorn, T. H.; Pullan, L. M.; DiMaggio, D. A.; Handelmann, G. E.; Gray, N. M.; O'Donohue, T. L. *Mol. Neurobiol.* 1987, *1*, 191.
(6) Thomas, J. W.; Hood, W. F.; Monahan, J. B.; Contreras, P. C.; O'Donohue, T. L. *Brain Res.* 1988, *442*, 396.
(7) Bonhaus, D. W.; Burge, B. C.; McNamara, J. O. *Eur. J. Pharmacol.* 1987, *142*, 489.
(8) Wong, E. H. F.; Knight, A. R.; Ransom, R. *Eur. J. Pharmacol.* 1987, *142*, 487.
(9) Reynolds, I. J.; Murphy, S. N.; Miller, R. J. *Proc. Natl. Acad. Sci. U.S.A.* 1987, *84*, 7744.
(10) Contreras, P. C. *Neuropharmacology* 1990, *29*, 291.
(11) Monahan, J. B.; Handelmann, G. E.; Hood, W. F.; Cordi, A. A. *Pharmacol. Biochem. Behav.* 1989, *34*, 649.

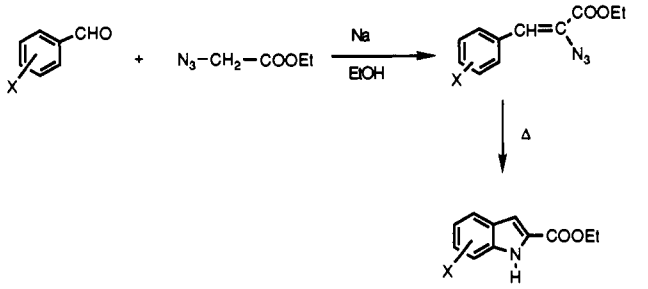
0022-2623/91/1834-1283$02.50/0

Scheme I



X = H, F, Cl, Br, CH_3

Scheme II



X = Cl, $-OCH_2Ph$

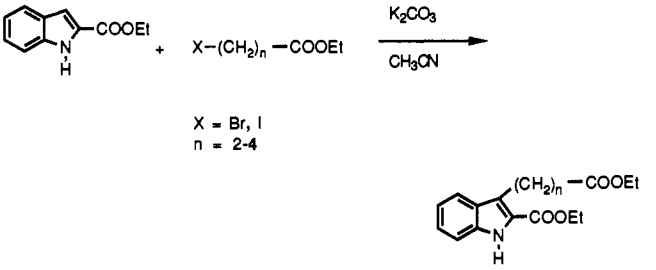
addition to the naturally occurring neutral amino acids, which function as agonists at this site, several cyclic amino acids have been found to bind to the glycine site.[16-18] The cyclic amino acids range in activity from potent agonists to weak antagonists, depending on ring size and the pharmacological system used for evaluation. Nonclassical amino acid ligands include the partial agonist D-cycloserine[11] and HA966,[19] 7-chlorokynurenic acid,[20] and several 2,3-quinoxalinediones[21] as antagonists. In a recent report, a series of indole-2-carboxylates have been reported to have very weak affinity for the glycine site.[22] In this study, a series of indole derivatives, most containing a carboxyl group at the 2-position, was prepared and evaluated for their receptor affinity.

Chemistry

Compounds in Tables I and II which contain the indole-3-acetate function, as well as the lactones **55–57**, were obtained by using variations of the Fischer indole synthesis (Scheme I). In general, the halogen-substituted compounds in this group required the use of sulfuric acid to effect cyclization, while the remaining compounds were efficiently prepared with ethanolic hydrogen chloride. Lactones **56** and **57** were prepared by a known method,[23] but their separation had not been achieved previously. Ethyl 4-(trifluoromethyl)indole-2-carboxylate, which was prepared by a similar method,[24] was used as an intermediate for the preparation of compound **20**. An alternate method for the preparation of the indole ring was employed for compounds **7** and **53**. This procedure involved the thermal cyclization of an intermediate azide as shown in Scheme II. Compound **54** was prepared by the hydrogenation of compound **53**.

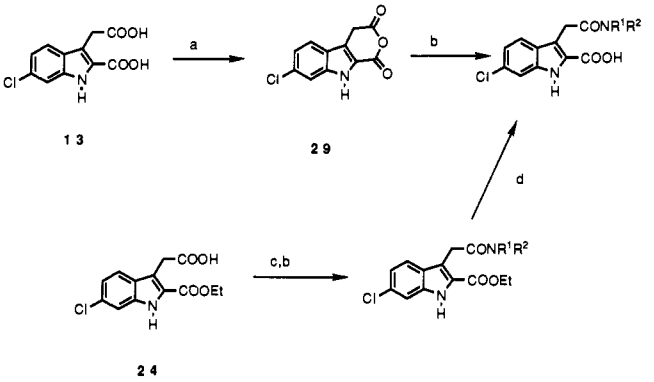
Compounds **16–19** and **41** were prepared by the reaction of ethyl indole-2-carboxylate with an appropriate electrophile in the presence of base (Scheme III), whereas a similar procedure was employed for the preparation of compound **9**. Partial esterification of diacid **13** in alcoholic HCl provided compounds **23** and **26–28**, while partial hydrolysis of diester **22** provided compound **24**. The mixed

Scheme III



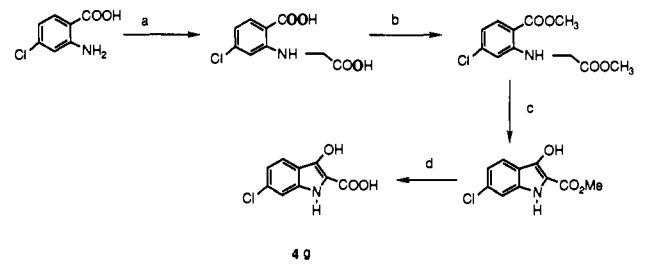
X = Br, I
n = 2-4

Scheme IV[a]



[a] Reagents and conditions: (a) dicyclohexylcarbodiimide, THF, room temperature; (b) HNR^1R^2; (c) isobutyl chloroformate, *N*-methylmorpholine, THF, −10 °C; (d) 2.5 N NaOH, EtOH/H_2O, room temperature.

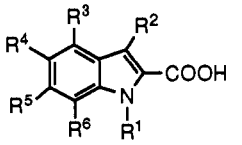
Scheme V[a]



[a] Reagents and conditions: (a) $ClCH_2COOH$, K_2CO_3, H_2O, reflux; (b) CH_3OH, H_2SO_4, reflux; (c) tBuO$^-$K$^+$, THF, reflux; (d) NaOH, EtOH, reflux.

(12) Handelmann, G. E.; Nevins, M. E.; Mueller, L. L.; Arnolde, S. M.; Cordi, A. A. *Pharmacol. Biochem. Behav.* **1989**, *34*, 823.
(13) Deutsch, S. I.; Mastrapaolo, J.; Schwartz, B. L.; Rosse, R. B.; Morihisa, J. M. *Clin. Neuropharmacol.* **1989**, *12*, 1.
(14) Cavalheiro, E. A.; Lehmann, J.; Turski, L. *Frontiers in Excitatory Amino Acids*; Alan R. Liss: New York, 1988.
(15) Choi, D. W. *Trends Neurosci.* **1988**, *11*, 465.
(16) Snell, L. L.; Johnson, K. M. *Eur. J. Pharmacol.* **1988**, *151*, 165.
(17) Hood, W. F.; Sun, E. T.; Compton, R. P.; Monahan, J. B. *Eur. J. Pharmacol.* **1989**, *161*, 281.
(18) Dappen, M. S.; Markos, C. S.; Gresk, C. J.; Cordi, A. A. *J. Labelled Compd. Radiopharm.* **1990**, *28*, 841.
(19) Foster, A. C.; Kemp, J. A. *J. Neurosci.* **1989**, *9*, 2191.
(20) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestly, T.; Tridgett, R.; Iversen, L. L.; Woodruff, G. N. *Proc. Natl. Acad. Sci. U. S.A.* **1988**, *85*, 6547.
(21) Kessler, M.; Terramani, T.; Lynch, G.; Baudry, M. *J. Neurochem.* **1989**, *52*, 1319.
(22) Huettner, J. E. *Science* **1989**, *243*, 1611.
(23) Lehmann, J.; Ghoneim, K. M.; El-Fattah, B. A.; El-Gendy, A. A. *Arch. Pharm. (Weinheim)* **1987**, *320*, 22.
(24) Bornstein, J.; Leone, S. A.; Sullivan, W. F.; Bennett, O. O. *J. Am. Chem. Soc.* **1957**, *79*, 1745.

Table I. Indoledicarboxylate Derivatives[a]

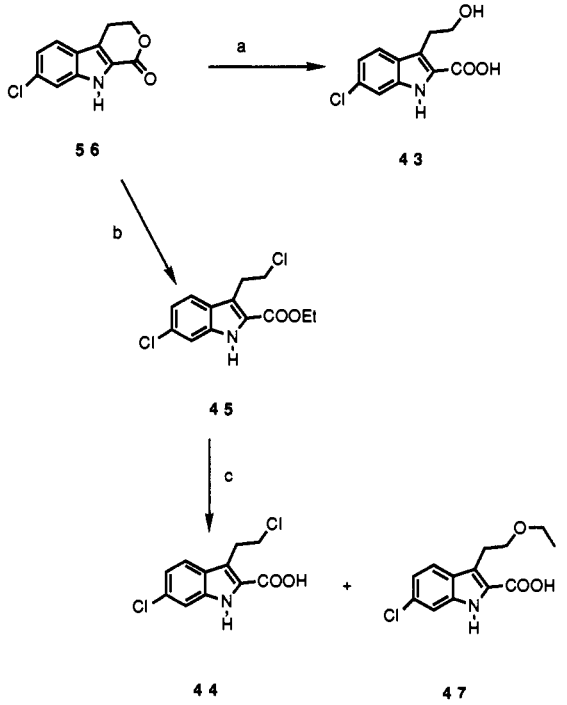


1-20

compd	R^1	R^2	R^3	R^4	R^5	R^6	formula[b]	mp, °C	K_i,[c] μM, vs [^{3}H]Gly
1	H	H	H	H	H	H	[d]	205–208	>100
2	H	H	H	Cl	H	H	[d]	286–287	>100
3	H	H	H	F	H	H	[d]	258–259	91.0
4	H	H	H	Br	H	H	[d]	280–281	>100
5	H	H	H	CH_3	H	H	[d]	246–248	>100
6	H	H	H	OCH_3	H	H	[d]	199–201	>100
7	H	H	H	H	Cl	H	$C_9H_6ClNO_2 \cdot 0.1H_2O$	257–259	10.0
8	H	CH_2COOH	H	H	H	H	$C_{11}H_9NO_4 \cdot 0.1H_2O$	225–227	24.8
9	CH_3	CH_2COOH	H	H	H	H	$C_{12}H_{11}NO_4$	236–238	>100
10	H	CH_2COOH	H	Cl	H	H	$C_{11}H_8ClNO_4 \cdot 0.8C_3H_6O$	274–279	10.9
11	H	CH_2COOH	H	Br	H	H	$C_{11}H_8BrNO_4 \cdot 0.9C_3H_6O$	269–271	29.2
12	H	CH_2COOH	H	F	H	H	$C_{11}H_8FNO_4$	252–254	14.1
13	H	CH_2COOH	H	H	Cl	H	$C_{11}H_8ClNO_4$	257–259	1.61
14	H	CH_2COOH	H	CH_3	Cl	H	$C_{12}H_{10}ClNO_4 \cdot 0.4H_2O$	265–269	>30
15	H	CH_2COOH	H	H	H	Cl	$C_{11}H_8ClNO_4 \cdot 0.8H_2O$	262–264	>30
16	H	CH_2CH_2COOH	H	H	H	H	$C_{12}H_{11}NO_4 \cdot 0.3H_2O$	218–220	>100
17	H	$(CH_2)_3COOH$	H	H	H	H	$C_{13}H_{13}NO_4 \cdot 0.2H_2O$	184–186	>100
18	H	$(CH_2)_4COOH$	H	H	H	H	$C_{14}H_{15}NO_4 \cdot 0.1H_2O$	144–145	>100
19	H	$CH(C_2H_5)COOH$	H	H	H	H	$C_{13}H_{13}NO_4$	174–177	>100
20	H	H	COOH	H	H	H	$C_{10}H_7NO_4 \cdot 0.5H_2O$	324–328	>100

[a] All compounds exhibited NMR consistent with assigned structure. [b] C, H, and N analyses were within 0.4% of the theoretical values for the formulae given unless otherwise noted. [c] All K_i values represent a mean value (n = 3). The standard error values were all less than 20% of the mean. [d] Commercially available.

Scheme VI[a]

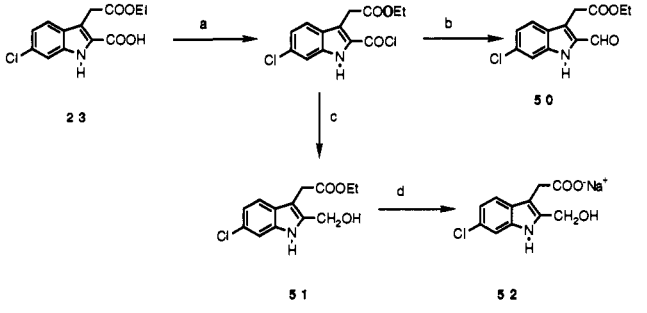


[a] Reagents and conditions: (a) LiOH, MeOH, H_2O, room temperature; (b) EtOH, HCl, reflux; (c) NaOH, EtOH, H_2O, room temperature.

diester **25** was prepared from monoester **23** via the intermediate acid chloride.

Anhydride **29** was prepared by dehydration of compound **13** with dicyclohexylcarbodiimide. This anhydride was then used to prepare amides **31**, **32**, and **35–37** (Table III) by reaction with the appropriate amine (Scheme IV). Compound **31** was esterified to provide compound **38**. Compounds **30**, **33**, and **34** were prepared from monoester **24** by using a mixed anhydride method, followed by ester hydrolysis (Scheme IV). Diester **22** was directly converted to diamide **39** by heating in the presence of the amine.

Scheme VII[a]

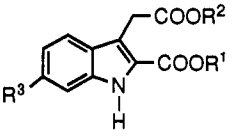


[a] Reagents and conditions: (a) oxalyl chloride, DMF, room temperature; (b) $LiAlH(O\text{-}tBu)_3$, DME, −78 °C; (c) $NaBH_4$, THF, room temperature; (d) NaOH, EtOH, room temperature.

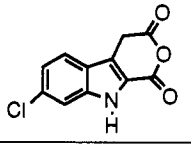
Compounds in Table IV were prepared using a variety of reaction schemes. Compound **40** was prepared from N-(2-carboxy-4-chlorophenyl)glycine[25] according to the methods outlined in Scheme V. Lactone **56** was hydrolyzed to provide 3-(hydroxyethyl)indole **43**. Ethanolysis of lactone **56** in the presence of HCl provided 6-chloro-3-(2-chloroethyl)-2-indolecarboxylate ester **45**. Basic hydrolysis of **45** in the presence of ethanol provided a separable mixture of 6-chloro-3-(2-chloroethyl)-2-indolecarboxylic acid (**44**) and 6-chloro-3-(2-ethoxyethyl)-2-indolecarboxylic acid (**47**) (Scheme VI). The analogous

(25) Holt, S. J.; Sadler, P. W. *Proc. R. Soc. Chem. B* **1958**, *148*, 481.

Table II. Indolecarboxylic Acid Ester Derivatives[a]

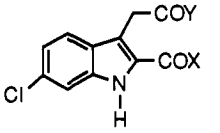


21-28

compd	R_1	R_2	R_3	formula[b]	mp, °C	K_i,[c] μM vs [^{3}H]Gly
23	H	C_2H_5	Cl	$C_{13}H_{12}ClNO_4$	243–245	0.68
24	C_2H_5	H	Cl	$C_{13}H_{12}ClNO_4$	237–245	20.9
25	$(CH_2)_3N(CH_3)_2$	C_2H_5	Cl	$C_{18}H_{23}ClN_2O_4$	105–106	>100
26	H	$CH(CH_3)_2$	Cl	$C_{14}H_{14}ClNO_4 \cdot 1.1H_2O$	225–233	3.85
27	H	CH_3	Cl	$C_{12}H_{10}ClNO_4 \cdot 0.15H_2O$	230–232	1.56
28	H	$CH_2CH_2CH_3$	Cl	$C_{14}H_{14}ClNO_4 \cdot 0.4H_2O$	206–212	6.80
29				$C_{11}H_6ClNO_3$	209–220	75.1

[a-c] Same as in Table I.

Table III. Indolecarboxylic Acid Amide Derivatives[a]



30-39

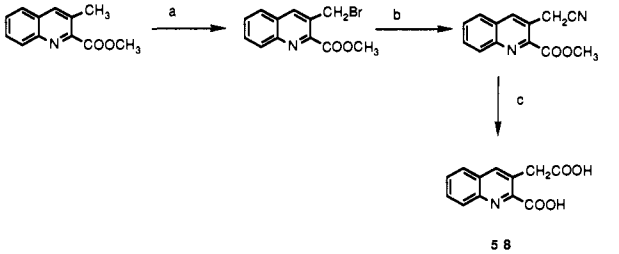
compd	X	Y	formula[b]	mp, °C	K_i,[c] μM, vs [^{3}H]Gly
30	OH	$N(CH_3)_2$	$C_{13}H_{13}ClN_2O_3 \cdot 0.35H_2O$	268–272	>30
31	OH	NH_2	$C_{11}H_9ClN_2O_3$	257–275	1.18
32	OH	$NHCH_3$	$C_{12}H_{11}ClN_2O_3 \cdot 1.2H_2O$	230–233	0.92
33	OH	$NHCH_2CH_2OH$	$C_{13}H_{13}ClN_2O_4 \cdot 1.9H_2O$	241–247	10.3
34	OH	$NH(CH_2)_3N(CH_3)_2$	[d]	130–140	>30
35	OH	$NHCH_2CH_3$	$C_{13}H_{13}ClN_2O_3$	230–231	0.70
36	OH	$NHCH_2CH_2CH_3$	$C_{14}H_{15}ClN_2O_3$	232–233	0.47
37	OH	$NHCH_2CH_2Ph$	$C_{19}H_{17}ClN_2O_3 \cdot 1.9H_2O$	187–191	0.63
39	$NHCH_2CH_2N(CH_3)_2$	$NHCH_2CH_2N(CH_3)_2$	$C_{19}H_{28}ClN_5O_2$	166–168	>30

[a-c] Same as in Table I. [d] Insufficient material for microanalysis. Mass spectral analysis (FAB) m/z 338 (M + H, 100), 293, 249, 208, 190.

scheme was also demonstrated beginning with lactone **55** to provide the corresponding 4-chloro analogues. Compound **23** was converted to its acid chloride and reduced with tri-*tert*-butoxyaluminohydride to provide compound **50** or with sodium borohydride to provide compound **51** (Scheme VII). Compound **52** was obtained by the basic hydrolysis of compound **51**. Compound **48** was prepared by the basic hydrolysis of ethyl-3-formylindole-2-carboxylate,[26] whereas compound **49** was similarly obtained from 2-(ethoxycarbonyl)-3-(2-oxopropyl)indole.[27]

Table V contains a series of non-indole compounds that were also prepared. Compound **58** was prepared from 2-carbomethoxy-3-methylquinoline[28] as outlined in Scheme VIII. Compounds **59** and **60** were prepared from methyl 2-(methoxycarbonyl)coumaran-3-acetate by previously described procedures.[29]

Scheme VIII[a]

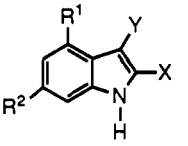


58

[a] Reagents and conditions: (a) *N*-bromosuccinimide, dibenzoyl peroxide, CCl_4, reflux; (b) KCN, MeOH, room temperature, then reflux; (c) 6 N HCl, reflux.

(26) Monge Vega, A.; Aldana, I.; Fernandez-Alverez, E. *Eur. J. Med. Chem.* **1978**, *13*, 573.
(27) Monge Vega, A.; Martinez, M. T.; Palop, J. A.; Mateo, J. M.; Fernandez-Alverez, E. *J. Heterocycl. Chem.* **1981**, *18*, 889.
(28) Danishefsky, S.; Bryson, T. A.; Puthenpurayil, J. *J. Org. Chem.* **1975**, *40*, 796.
(29) Chatterjea, J. N.; Sahai, R. P. *J. Indian Chem. Soc.* **1980**, *57*, 633.

Table IV. Other Indole Derivatives[a]

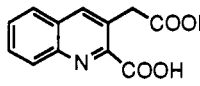
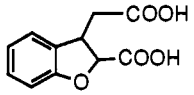
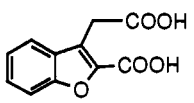
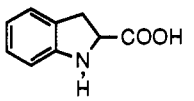


40-54

compd	X	Y	R^1	R^2	formula[b]	mp, °C	K_i,[c] μM, vs [^{3}H]Gly
40	COOH	OH	H	Cl	$C_9H_6ClNO_3 \cdot 0.42H_2O$	232–234	>100
41	COOH	CH_2OH	H	H	$C_{10}H_9NO_3$	226–230	>100
42	COOH	CH_2CH_2OH	H	H	$C_{11}H_{11}NO_3$	196–198	>30
43	COOH	CH_2CH_2OH	H	Cl	$C_{11}H_{10}ClNO_3 \cdot H_2O$	175–176	11.4
44	COOH	CH_2CH_2Cl	H	Cl	[d]	128–129	13.2
47	COOH	$CH_2CH_2OC_2H_5$	H	Cl	[d]	142–142.5	8.25
48	COOH	CHO	H	H	$C_{10}H_7NO_3$	245–247	11.2
49	COOH	CH_2COCH_3	H	H	$C_{12}H_{11}NO_3$	166–168	29.6
50	CHO	$CH_2COOC_2H_5$	H	Cl	[e]	121–123	>30
51	CH_2OH	$CH_2COOC_2H_5$	H	Cl	$C_{13}H_{14}ClNO_3$	118–120	>30
52	CH_2OH	$CH_2COO^-Na^+$	H	Cl	$C_{11}H_9ClNO_3 + 0.65NaOH$	215–235	>30
53	COOH	H	OCH_2Ph	H	$C_{16}H_{13}NO_3$	245–249	>30
54	COOH	H	OH	H	$C_9H_7NO_3 \cdot 0.1H_2O$	255–258	>30

[a-c] Same as in Table I. [d] Insufficient material for Microanalysis. Mass spectral data given in the Experimental Section. [e] Material air sensitive. Microanalysis unavailable. Mass spectral analysis given in the Experimental Section.

Table V. Non-Indole Derivatives[a]

compd	structure	formula[b]	mp, °C	K_i,[c] μM, vs [^{3}H]Gly
58		$C_{12}H_9NO_4 \cdot 0.6H_2O$	225–229	>100
59		$C_{11}H_{10}O_5$	172–175	>30
60		$C_{11}H_8O_5 \cdot 0.11H_2O$	237–242	>100
61		[d]		>30

[a-d] Same as in Table I.

Biochemical Results and Discussion

The compound structures and results of the radioreceptor assays are summarized in Tables I–V. Compounds **8–61** were also evaluated for their selectivity for the glycine site by evaluating their ability to interact with the glutamate receptor sites labeled by [^{3}H]glutamate, [^{3}H]kainate, and [^{3}H]-α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA).[30,31] Concentrations up to 100 μM were used in these assays, but only compound **8** had any significant affinity for the glutamate site (K_i = 56 μM vs [^{3}H]glutamate), while none of the compounds showed any significant affinity for the sites labeled by [^{3}H]kainate or [^{3}H]AMPA. Table I contains six commercial reference standards, five of which have been reported to have weak affinity for the strychnine-insensitive glycine site.[22] The indole-2-carboxylates bear a slight structural similarity to kynurenic acid, which has a moderate affinity (IC_{50} = 41 μM)[20] for the same glycine site. An analogue of kynurenic acid, 7-chlorokynurenic acid, has an increased affinity for the glycine site, with a K_i = 0.94 μM in our laboratory (lit.[22] IC_{50} = 0.56 μM). Compound **7** in Table I was prepared to evaluate the effect of a comparable chloro substitution in the indole series. As can be seen from the binding data, a chloro group in the C-6 position of the indole greatly increased the potency in this series. 7-Chlorokynurenic acid also contains an acidic phenolic group in the C-4 position. Substitution of an acetic acid moiety in the C-3 position of the indole series also enhanced receptor affinity relative to the compounds without an acidic function (compound **8** vs **1**, compound **10** vs **2**, compound **11** vs **4**, compound **12** vs **3**, and compound **13** vs **7**). The combination of a C-6 chloro and C-3 acetic acid substitution in the indole series led to the most potent compound in the diacid series presented in Table I. Overlapping a 6,7-dichloro analogue of compound **13** with 7-chlorokynurenic acid (Figure 1a) such that the nitrogen atoms and the carboxyl groups are directly superimposed suggests that the 7-chloro compound might also maintain a high affinity for the glycine receptor. However, compound **15** proved to be significantly less potent than compound **13**, suggesting that the orientation of the acetic acid moiety shown in Figure 1b, in which the benzo rings are overlapping, may be the closer approximation of the active conformation. Increasing the distance between the carboxylic acid group and the indole ring led to compounds with little or no affinity for the glycine site (compounds **16–18**). Moving the acidic function to the C-4 position of the indole (compound **20**) or placing an alkyl group on the carbon between the carboxyl and the indole ring (compound **19**) also diminished activity.

The ability of compound **13** to inhibit the binding of [^{3}H]MK-801 was also evaluated.[32] This assay has often been used to assess the ability of a compound to function as an antagonist of the receptor macrocomplex. The IC_{50} for the inhibition of [^{3}H]MK-801 binding by compound **13** was 6.8 ± 0.9 μM, which is in good agreement with its K_i value for the inhibition of [^{3}H]glycine binding (K_i = 1.61 μM). Higher concentrations of compound **13** were able to

(30) Monahan, J. B.; Michel, J. *J. Neurochem.* **1987**, *48*, 1699.
(31) Pullan, L. M.; Olney, J. W.; Price, M. T.; Compton, R. P.; Hood, W. F.; Michel, J.; Monahan, J. B. *J. Neurochem.* **1987**, *49*, 1301.
(32) Monahan, J. B.; Biesterfeldt, J. P.; Hood, W. F.; Compton, R. P.; Cordi, A. A.; Vazquez, M. L.; Lanthorn, T. H.; Wood, P. L. *Mol. Pharmacol.* **1990**, *37*, 780.

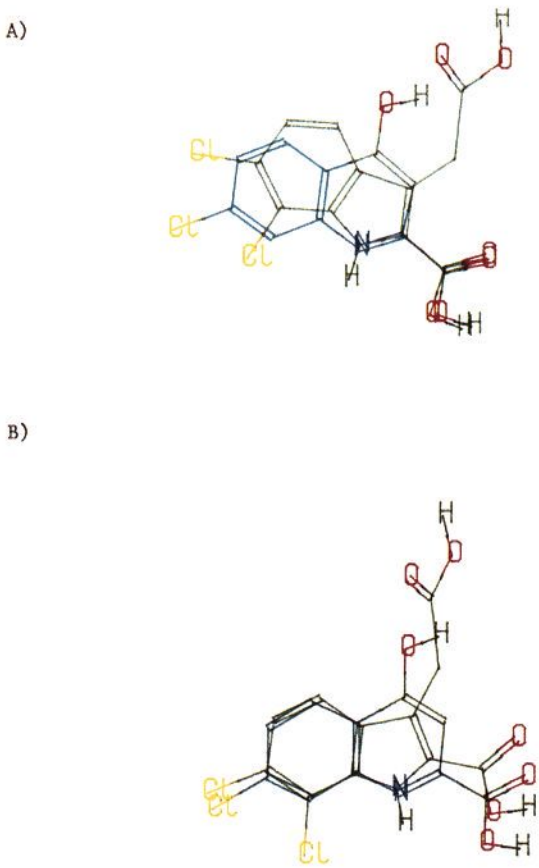


Figure 1. Superimposition of 6,7-dichloro-2-carboxy-3-indoleacetic acid onto 7-chlorokynurenic acid: (A) overlap with respect to carboxyls and nitrogens, (B) overlap with respect to phenyl ring.

inhibit 100% of the [^{3}H]glycine binding. Compounds **8**, **12**, **23**, **24**, **31**, **32**, and **43**, at high concentrations, also inhibited 90–100% of the [^{3}H]glycine binding. These data suggest that the indole compounds act as full antagonists at the glycine recognition site.

A series of ester derivatives of compound **13** were also prepared (Table II) and evaluated for their receptor affinity. Masking both acidic functional groups (compound **25**) greatly decreased affinity, as did masking the C-2 carboxyl group (compound **24**). However, the C-3 monoesters maintained high affinity for the glycine site, with the ethyl ester **23** being the most potent. A similar trend was seen in the amide series (Table III), where masking of the C-3 acid group led to compounds which maintained high affinity for the site. Several of the ester and amide compounds were slightly more potent than the parent diacid (compounds **35**–**37**), suggesting that a hydrogen-bonding interaction with the receptor may be occurring rather that an electrostatic interaction that could potentially occur with the carboxylate anion. By analogy to 7-chlorokynurenic acid, these data may also suggest that the preferred pharmacophore in the case of the kynurenates may be the tautomeric C-4 ketone. This latter tautomer also provides for the existence of a nonbasic N-H function analogous to that of the indoles. The addition of a second polar group on the amide function (compounds **33** and **34**) was detrimental to activity.

The compounds in Table IV were prepared to evaluate the nature of the potential hydrogen-bonding interaction between the receptor and the C-3 acetate moiety. Compounds **40**–**43** all had significantly less affinity for the receptor than their corresponding acetate analogues, indicating that a hydroxyl group is not a good substitute for the acetate regardless of its position within the molecule. The analogous ether and chloro compounds also proved to have less affinity (compounds **44** and **47**). However, substituents containing an aldehyde (compound **48**) or ketone (compound **49**) maintained their activity for the receptor site relative to the parent acetate **8**, suggesting that the receptor prefers to interact with a carbonyl oxygen. Compounds **50**–**52** indicate, as in the case of diester **25** and C-2 monoester **24** described above, that the presence of the C-2 carboxyl group is required for this series. Compound **54** contains a phenolic group which, in the orientation shown in Figure 1a, should mimic the phenolic group in 7-chlorokynurenic acid. However, its lack of significant receptor affinity also suggests that the orientation shown in Figure 1b more closely represents the active conformation.

To evaluate the importance of the nonbasic indole nitrogen, the compounds shown in Table V were prepared. As can be seen from the binding results for compounds **58**–**60**, the receptor appears to prefer compounds containing a functional group which can serve as a hydrogen-bond donor. This assumption is also supported by the lack of activity of *N*-methyl compound **9**. The results for compound **61** reinforces the assumption that a basic nitrogen is unnecessary for receptor affinity and may indicate that molecules which are relatively planar are preferred for interaction with this site. This class of indole-2-carboxylates has provided a novel structural lead for the development of highly potent receptor ligands that might serve to clarify the physiological role of the strychnine-insensitive glycine receptor that is associated with the NMDA–PCP–glycine receptor complex.

Experimental Section

^{1}H NMR spectra were taken on a Varian XL-300 spectrometer and were consistent with assigned structures. Mass spectral analyses were performed on either a VG40-250T (FAB) or a Finnigan 4500 (CI) mass spectrometer. All distillations were done on a Kugelrohr apparatus. Melting points are uncorrected. Microanalyses were performed for the stated elements and were within 0.4% of the theoretical values for the stated empirical formulas, unless otherwise indicated. The structures for Figure 1 were prepared with MacroModel (Columbia University, 1988) to minimize the conformational energy for the two compounds. The conformations of 7-chlorokynurenic acid and 2-carboxy-6,7-dichloro-3-indoleacetic acid were independently derived with semiempirical calculations of MM2 energies for each molecule in a vacuum using the same MacroModel program. The two structures were then overlaid by simple atom matching. For Figure 1a, the 2-carboxyl groups, nitrogens, and C-2's for each structure were aligned, while for Figure 1b, the nitrogen, C-7, and C-8a of the indole were aligned with the analogous nitrogen, C-8, and C-9a of the quinoline.

Synthesis. 2-Carboxy-3-indoleacetic Acid (8). Compound **21** was prepared by a modification of the method described by Findlay and Dougherty.[33] A diazonium chloride solution prepared from aniline (50 g, 0.54 mol) in 56 mL of H_2O was treated with an ice-cold solution of diethyl 2-acetoglutarate (123.9 g, 0.54 mol) and NaOH (120.4 g, 3.0 mol) in H_2O (490 mL). The resulting mixture was stirred in an ice bath for 30 min. The mixture was acidified with concentrated HCl and extracted with ether (5 × 500 mL). The combined ether extracts were washed with 1 N HCl (3 × 200 mL) and H_2O (3 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting hydrazone, isolated as a crude red oil, was treated with HCl-saturated absolute ethanol (350 mL) and heated to reflux for 1 h. The mixture was allowed to cool to room temperature and treated with H_2O (500 mL). The mixture was extracted with CH_2Cl_2 (5 × 400 mL), and the com-

(33) Findlay, S. P.; Dougherty, G. *J. Org. Chem.* **1948**, *13*, 560.

bined organic layers were washed with 5% $NaHCO_3$ (3 × 200 mL) and H_2O (3 × 200 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting crude material was warmed under high vacuum to remove volatile impurities (80–90 °C, 0.2 mmHg) and the residue was recrystallized from ethanol/H_2O to provide diester **21** (27 g, 18%). An analytically pure sample of **21** was obtained by sublimation of the crude material (95 °C, 0.15 mmHg). mp 75–78 °C; ^1H NMR ($CDCl_3$) δ 8.90 (br s, 1 H), 7.15–7.70 (m, 4 H), 4.40 (q, 2 H), 4.17 (m, 4 H), 1.38 (t, 3 H), 1.27 (t, 3 H). Anal. ($C_{15}H_{17}NO_4$) C, H, N.

The diester (0.7 g 2.5 mmol) was dissolved in absolute ethanol (9 mL) and treated with 50% NaOH solution (1 mL). The resulting mixture was heated to reflux 15 min, then poured into H_2O (50 mL). The aqueous solution was washed with ether (2 × 30 mL), made acidic with concentrated HCl, and chilled in an ice bath. Diacid **8** was filtered and dried in vacuo over P_2O_5 overnight (429 mg, 78%): mp 225–227 °C; ^1H NMR (DMSO) δ 12.62 (br s, 2 H) 11.57 (s, 1 H), 7.63 (d, 1 H), 7.41 (d, 1 H), 7.27 (t, 1 H), 7.07 (t, 1 H), 4.04 (s, 2 H). Anal. ($C_{11}H_9NO_4 \cdot H_2O$) C, H, N.

2-Carboxy-6-chloro-3-indoleacetic Acid (13). Crude hydrazone was prepared as described for compound **8** by starting from 3-chloroaniline (3.19 g, 25 mmol) and diethyl 2-acetoglutarate (5.76 g, 25 mmol). The crude hydrazone was distilled on a Kugelrohr apparatus (160 °C, 0.06 mmHg) and dissolved in 40 mL of absolute ethanol. The solution was treated with concentrated H_2SO_4 (10 mL) and heated to reflux for 6 h. The solution was poured onto ice (100 g) and the resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic solutions were washed with 5% $NaHCO_3$ (2 × 50 mL) and H_2O (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The resulting crude solid was recrystallized from ethyl acetate/hexane to provide diester **22** (1.5 g, 19%): mp 144–145 °C; ^1H NMR ($CDCl_3$) δ 8.95 (br s, 1 H), 7.58 (d, 1 H), 7.36 (d, 1 H), 7.13 (dd, 1 H), 4.40 (q, 2 H), 4.16 (q, 2 H), 4.12 (s, 2 H), 1.41 (t, 3 H), 1.24 (t, 3 H). Anal. ($C_{15}H_{16}ClNO_4$) C, H, N. Hydrolysis of **22** (400 mg, 1.3 mmol) to provide diacid **13** was as described for compound **8**. The crude diacid was recrystallized from acetone (280 mg, 85%): mp 274–279 °C; ^1H NMR (D_2O) δ 7.54 (d, 1 H), 7.49 (d, 1 H), 7.12 (dd, 1 H), 4.06 (s, 2 H). Anal. ($C_{11}H_8ClNO_4 \cdot 0.8$acetone) C, H, N.

2-Carboxy-1-methyl-3-indoleacetic Acid (9). Diester **21** (1 g, 3.6 mmol) and K_2CO_3 (2 g) in acetonitrile (50 mL) were treated, with stirring, with dimethyl sulfate (0.5 g, 4 mmol). After the addition, the mixture was heated to reflux for 20 h, filtered, and the solvent removed in vacuo. The residue was suspended in ether (150 mL) and washed with 5% HCl (3 × 50 mL), 5% $NaHCO_3$ (2 × 50 mL), and H_2O (2 × 50 mL). The ether solution was dried ($MgSO_4$) and the solvent removed in vacuo. The resulting crude diester was hydrolyzed as described for compound **8** to provide diacid **9** (437 mg, 52%): mp 236–238 °C; ^1H NMR (D_2O) δ 7.69 (d, 1 H), 7.59 (d, 1 H), 7.38 (t, 1 H), 7.16 (t, 1 H), 4.03 (s, 2 H), 4.00 (s, 3 H). Anal. ($C_{12}H_{11}NO_4$) C, H, N.

2-Carboxy-3-indolepropanoic Acid (16). Ethyl 2-indolecarboxylate (3 g, 15.9 mmol), ethyl 3-iodopropanoate (5.4 g, 22.3 mmol), K_2CO_3 (5 g), and acetonitrile (50 mL) were combined and heated to reflux for 48 h. The mixture was poured into H_2O (50 mL) and the aqueous mixture was extracted with ether (3 × 75 mL). The combined ether solutions were washed with H_2O (3 × 30 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting crude diester was hyrolyzed to diacid **16** (2.37 g, 64%) as described for compound **8**: mp 218–220 °C; ^1H NMR (DMSO) δ 12.7 (br s, 2 H), 7.75–7.05 (m, 5 H), 4.77 (t, 2 H), 2.69 (t, 2 H). Anal. ($C_{12}H_{11}NO_4 \cdot 0.3H_2O$) C, H, N.

2,4-Indoledicarboxylic Acid (20). The crude diacid was prepared following a previously described method[25] and was purified on a 200-mL column of Amberlite strong anion-exchange resin (acetate form) using a gradient of 0–0.5 M triethylammonium acetate buffer (pH = 7.4) as the eluant. The fractions containing product, as detected by UV monitoring of the eluant at 226 nm, were pooled and acidified with concentrated HCl. The H_2O was removed in vacuo and the residue was dissolved in H_2O and lyophilized to provide diacid **20** (17%): mp 324–328 °C (lit.[25] mp 299–300 °C); ^1H NMR (DMSO) δ 12.95 (br s, 2 H), 12.25 (s, 1 H), 7.78 (d, 1 H), 7.69 (d, 1 H), 7.57 (s, 1 H) 7.35 (t, 1 H). Anal. ($C_{10}H_7NO_4 \cdot 0.5H_2O$) C, H, N.

Ethyl 2-Carboxy-6-chloro-3-indoleacetate (23). Ethanol (2 mL) was saturated with HCl gas and diluted to 20 mL with additional ethanol. The solution was poured onto solid diacid **13** (1.10 g, 4.34 mmol) and the mixture was stirred at room temperature for 1 h. The mixture was made basic by rapid addition of a 1 M aqueous ammonia solution and cooled in an ice bath and the pH adjusted to 2.8–3.0 with 6 M H_3PO_4. The resulting mixture was extracted with ethyl acetate (2 × 100 mL), and the combined extracts were dried (Na_2SO_4) and concentrated in vacuo to give a residue which was recrystallized from acetone/hexane to yield C-3 monoester **23** (1.11 g, 91%): mp 243–245 °C; ^1H NMR (acetone) δ 10.85 (br s, 1 H), 7.71 (d, 1 H), 7.55 (d, 1 H), 7.12 (dd, 1 H), 4.20 (s, 2 H), 4.11 (q, 2 H), 1.20 (t, 3 H). Anal. ($C_{13}H_{12}ClNO_4$) C, H, N.

2-(Ethoxycarbonyl)-6-chloro-3-indoleacetic Acid (24). A solution of diester **22** (1.042 g, 3.36 mmol) in CH_2Cl_2 (15 mL) was treated with iodotrimethylsilane (740 mg, 3.70 mmol) and the resulting mixture was heated in a sealed tube at 60 °C for 72 h. The mixture was cooled to room temperature and poured into 0.5 N HCl (40 mL). The mixture was extracted with ethyl acetate (2 × 25 mL) and the combined organic extracts were extracted with saturated aqueous $NaHCO_3$ (2 × 25 mL). The organic layer was concentrated in vacuo to give the recovered diester (390 mg). The combined aqueous extracts were carefully acidified with concentrated HCl and extracted with ethyl acetate (2 × 30 mL). The organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give a residue which was recrystallized from acetone/hexane to provide C-2 monoester **24** (298 mg, 61% based on consumed diester): mp 237–245 °C; ^1H NMR (acetone) δ 10.95 (br s, 1 H), 7.74 (d, 1 H), 7.54 (d, 1 H), 7.12 (dd, 1 H), 4.38 (q, 2 H), 4.18 (s, 2 H), 2.82 (br s, 1 H), 1.37 (t, 3 H). Anal. ($C_{13}H_{12}ClNO_4$) C, H, N.

2-Carboxy-6-chloro-3-indoleacetic Acid Anhydride (29). A solution of diacid **13** (155 mg, 0.611 mmol) in dry THF (5 mL) was treated with dicyclohexylcarbodiimide (132 mg, 0.642 mmol) at room temperature. After stirring for 1.5 h, the mixture was filtered to remove precipitated dicyclohexylurea and the filtrate was washed with additional dry THF (5 mL). The combined THF solutions were concentrated in vacuo to leave a residue (172 mg) which was suspended in ether (25 mL) and stirred for 20 min. The solution was filtered to remove additional precipitated dicyclohexylurea and concentrated in vacuo to give a residue (110 mg) which was recrystallized from acetone/hexane to yield the anhydride **29** (59 mg, 41%) as brown needles: mp 209–220 °C; ^1H NMR (acetone) δ 7.81 (d, 1 H), 7.63 (d, 1 H), 7.23 (dd, 1 H), 4.42 (s, 2 H). Anal. ($C_{11}H_6ClNO_3$) C, H, N.

Ethyl 2-[[[3-(Dimethylamino)propyl]oxy]carbonyl]-6-chloro-3-indoleacetate (25). A mixture of monoacid **23** (177 mg, 0.628 mmol) in oxalyl chloride (2 mL) was treated with one drop of dimethylformamide. After stirring for 30 min, the residual oxalyl chloride was removed in vacuo to give a solid acid chloride which was dissolved in dimethoxyethane (3 mL). The solution was cooled to 0 °C and treated dropwise with a solution of 3-(dimethylamino)propanol (97 mg, 0.91 mmol) in dimethoxyethane (5 mL). The mixture was warmed to room temperature and stirred for 1 h before diluting with ethyl acetate (25 mL). The organic solution was extracted with 0.5 N HCl (25 mL) and the aqueous layer was made basic with 2.5 N NaOH. The resulting aqueous phase was extracted with ethyl acetate (2 × 25 mL), and the combined extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a residue (176 mg) which was recrystallized from ethyl acetate/hexane to yield diester **25** (112 mg, 49%): mp 105–106 °C; ^1H NMR (acetone) δ 10.95 (br s, 1 H), 7.72 (d, 1 H), 7.53 (d, 1 H), 7.12 (dd, 1 H), 4.18 (s, 2 H), 4.11 (q, 2 H), 2.40 (t, 2 H), 2.19 (s, 6 H), 1.91 (m, 2 H), 1.21 (t, 3 H). Anal. ($C_{18}H_{23}ClN_2O_4$) C, H, N.

***N*-Ethyl-2-carboxy-6-chloro-3-indoleacetamide (35).** A solution of diacid **13** (0.42 g, 1.65 mmol) in dry THF (10 mL) was treated with dicyclohexylcarbodiimide (3.58 mL, 1.73 mmol) at room temperature. After stirring for 1.5 h, the mixture was filtered to remove precipitated dicyclohexylurea which was washed with additional dry THF. The combined THF filtrates were cooled to 0 °C and treated with 70% ethylamine in water (0.68 mL, 8.3 mmol). After stirring for 2 h at 0 °C, the THF was removed in vacuo and the residue was dissolved in saturated aqueous $NaHCO_3$ (25 mL). The aqueous solution was washed with ether (2 × 25

mL) and acidified with 6 N HCl. The resulting precipitate was collected by filtration and this crude product was recrystallized from acetone/hexane to give amide **35** (300 mg, 65%): mp 230–231 °C; ^{1}H NMR (acetone) δ 10.85 (br s, 1 H), 7.78 (d, 1 H), 7.63 (br s, 1 H), 7.55 (d, 1 H), 7.12 (dd, 1 H), 4.02 (s, 2 H), 3.21 (m, 2 H), 2.70 (br s, 1 H), 1.06 (t, 3 H). Anal. ($C_{13}H_{13}ClN_2O_3$) C, H, N.

***N,N*-Dimethyl-2-carboxy-6-chloro-3-indoleacetamide (30).** A solution of C-2 monoester **24** (176 mg, 0.625 mmol) in THF (3 mL) was cooled to −10 °C and treated in succession with *N*-methylmorpholine (70 mg, 0.69 mmol) and isobutyl chloroformate (94 mg, 0.69 mmol). After stirring at −10 °C for 4 min, dimethylamine gas was bubbled into the solution for 3 min. The mixture was stirred at −10 °C for 30 min, then warmed to room temperature for 30 min, before diluting with ethyl acetate (25 mL). The solution was washed with 0.5 N HCl (25 mL), H_2O (25 mL), and saturated aqueous $NaHCO_3$ (25 mL), dried (Na_2SO_4), and concentrated in vacuo to give the intermediate amide/ester (159 mg, 82%).

The ester (91 mg, 0.25 mmol) was dissolved in ethanol (2 mL) and treated at 0 °C with 2.5 N NaOH (0.14 mL, 0.35 mmol). The mixture was warmed to room temperature and water (0.5 mL) was added until the solution was homogeneous. After stirring for 24 h, the solvent was removed in vacuo and the residue was dissolved in H_2O (25 mL). The aqueous phase was washed with ethyl acetate (3 × 25 mL) and acidified with 6 N HCl. The mixture was extracted with ethyl acetate (2 × 25 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to provide a residue which was recrystallized from acetone to provide the dimethylamide **30** (35 mg, 42%): mp-268–272 °C; ^{1}H NMR (acetone) δ 10.80 (br s, 1 H), 7.71 (d, 1 H), 7.55 (d, 1 H), 7.20 (dd, 1 H), 4.23 (s, 2 H), 3.28 (s, 3 H), 2.91 (s, 3 H). Anal. ($C_{13}H_{13}ClN_2O_3 \cdot 0.35H_2O$) C, H, N.

***N*-[3-(Dimethylamino)propyl]-2-carboxy-6-chloro-3-indoleacetamide (34)** was prepared according to this same general procedure except for the workup. After acidification of the aqueous extract, the aqueous layer was washed with ethyl acetate (2 × 25 mL) and concentrated in vacuo to give a residue which was purified by ion-exchange chromatography (strongly acidic resin, Dowex 50-8 X100) with 1 N aqueous pyridine as the eluant (yield 30%): mp 130–140 °C; ^{1}H NMR (D_2O) δ 7.43 (d, 1 H), 7.32 (s, 1 H), 7.08 (d, 1 H), 3.89 (s, 2 H), 3.25 (t, 2 H), 3.01 (t, 2 H), 2.72 (s, 6 H), 1.87 (m, 2 H); MS (FAB) *m/z* 338 (M + H, 100), 293, 249, 208, 190.

2-Carbethoxy-6-chloro-3-indoleacetamide (38). A solution of ethanol (10 mL) saturated with HCl gas was added to amide/acid **31** (144 mg, 0.57 mmol) and the mixture was stirred at room temperature for 22 h. The solvent was removed in vacuo and the residue was diluted with H_2O (25 mL) and extracted with ethyl acetate (2 × 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (25 mL) and H_2O (25 mL), dried (Na_2SO_4), and concentrated in vacuo to leave a solid residue. The solid was recrystallized from ethyl acetate/hexane to yield ester **38** (60 mg, 38%): mp 229–230 °C; ^{1}H NMR (acetone) δ 10.95 (br s, 1 H), 7.74 (d, 1 H), 7.51 (d, 1 H), 7.12 (dd, 1 H), 6.61 (br s, 1 H), 6.17 (br s, 1 H), 4.39 (q, 2 H), 4.02 (s, 2 H), 1.38 (t, 3 H). Anal. ($C_{13}H_{13}ClN_2O_3$) C, H, N.

2-Carboxy-6-chloro-3-indoleacetic Acid Bis[2-(dimethylamino)ethyl]amide (39). A solution of diester **22** (441 mg, 1.42 mmol) in *N,N*-dimethylethylenediamine (3 mL) was heated to 90–100 °C for 72 h. The mixture was cooled to room temperature and the diamine was removed in vacuo. The residue was dissolved in ether (25 mL) and extracted with 0.5 N HCl (30 mL). The acidic extract was made basic with 2.5 N NaOH and extracted with ether (2 × 30 mL). The combined ether extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a residue (401 mg) which was recrystallized from ether/hexane to yield diamide **39** (256 mg, 45%): mp 166–168 °C; ^{1}H NMR (acetone) δ 7.72 (d, 1 H), 7.52 (d, 1 H), 7.07 (dd, 1 H), 3.91 (s, 2 H), 3.51 (t, 2 H), 3.28 (t, 2 H), 2.81 br s, 1 H), 2.50 (t, 2 H), 2.34 (t, 2 H), 2.23 (s, 6 H), 2.16 (s, 6 H). Anal. ($C_{19}H_{28}ClN_5O_2$) C, H, N.

6-Chloro-3-hydroxy-2-indolecarboxylic Acid (40). 2-Amino-5-chlorobenzoic acid (5 g, 29.1 mmol) and chloroacetic acid (2.75 g, 29.1 mmol) were mixed and slowly neutralized with careful addition of 1 N K_2CO_3 solution. The resulting mixture was heated to reflux and the mixture treated periodically with 1 N K_2CO_3 solution to keep the mixture slightly basic. Reflux was continued until the solution remained slightly basic for 45 min after the addition of the K_2CO_3 solution. The mixture was cooled to room temperature and filtered through charcoal, and the filtrate acidified with 1 N HCl. The precipitate was filtered and dried under vacuum for 48 h to provide *N*-(2-carboxy-4-chlorophenyl)glycine [4.7 g, mp 208–209 °C (lit.[26] mp 210° C)].

Crude *N*-(2-carboxy-5-chlorophenyl)glycine was esterified by dissolving the acid in methanol (50 mL) and treating the solution with concentrated H_2SO_4 (6 mL). The solution was heated to reflux for 20 h and slowly poured onto ice (500 g) and the resulting solid was filtered. The solid was washed with saturated aqueous $NaHCO_3$ (75 mL) and H_2O (75 mL) and dried under vacuum for 24 h to provide the crude diester 3.8 g). The diester (2.3 g) was dissolved in dry THF (20 mL) and added dropwise to a mixture of potassium *tert*-butoxide (1.9 g) in dry THF (30 mL). The resulting mixture was heated to reflux for 2 h, cooled in an ice bath, then poured onto ice (325 g). The aqueous mixture was acidified with acetic acid and the resulting precipitate filtered, washed with water, and air-dried. This crude methyl ester was hydrolyzed as described for compound **8** to provide the acid **40** as a crude black solid. The product was purified with a DEAE Sepharose ion-exchange column with a linear gradient of 0–0.5 N $NaHCO_3$. The appropriate fractions, as detected by UV monitoring of the eluant at 226 nm, were combined. The solution was acidified with concentrated HCl and extracted with ether (3 × 100 mL), and the combined ether solutions were dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in ethyl acetate and purified further by preparative centrifugally accelerated radial thin-layer chromatography on silica gel using ethyl acetate as the eluant to provide acid **40** (368 mg, 17%): mp 232–234 °C; ^{1}H NMR (D_2O) δ 7.70 (d, 1 H), 6.90 (s, 1 H), 6.80 (d, 1 H). Anal. ($C_9H_6ClNO_3 \cdot 0.42H_2O$) C, H, N; N: calcd, 6.39; found, 7.40.

3-(Hydroxymethyl)-2-indolecarboxylic Acid (41). Ethyl indole-2-carboxylate (5.87 g, 31 mmol), K_2CO_3 (4.28 g, 31 mmol), 37% formaldehyde solution (2.6 g, 31 mmol), and ethanol (85 mL) were combined and heated to reflux for 4 days. The mixture was cooled to room temperature, and the solvents were removed on a rotary evaporator. The residue was dissolved in H_2O (100 mL) and the aqueous solution was washed with ether (2 × 50 mL) and made acidic with 3.6 N H_2SO_4, and the latter mixture was extracted with CH_2Cl_2 (2 × 50 mL) and ether (2 × 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in acetone and purified by preparative centrifugally accelerated radial thin-layer chromatography on silica gel using 10% methanol in methylene chloride as the eluant to provide acid **41** (1.05 g, 18%): mp 226–230 °C; ^{1}H NMR (D_2O) δ 7.22 (d, 2 H), 6.95 (t, 1 H), 6.68 (t, 1 H), 4.95 (s, 2 H). Anal. ($C_{10}H_9NO_3$) C, H, N.

4-(Benzyloxy)-2-indolecarboxylic Acid (53). A solution of sodium (0.7 g) in ethanol (20 mL) was cooled in an ice/salt bath and treated over 1 h with a solution of *o*-(benzyloxy)benzaldehyde (1.58 g, 7.4 mmol) and ethyl azidoacetate (5 mL) in ethanol (2 mL). The resulting mixture was stirred 2 h at −10 °C, warmed to room temperature, and poured into H_2O (150 mL). The resulting solid was filtered, washed with H_2O (50 mL) and dried in vacuo 24 h (1.13 g). The crude azide was dissolved in toluene (25 mL) and heated to reflux for 3 h. The solvent was removed in vacuo and the residue recrystallized from hexane/chloroform to provide the ester (0.46 g, 21%). Acid **53** was obtained by hydrolysis of the ester by the method outlined for compound **8** (329 mg, 79%): mp 245–249 °C; ^{1}H NMR (DMSO) δ 12.85 (br s, 1 H), 11.79 (s, 1 H), 7.55–7.00 (m, 9 H), 6.62 (d, 1 H), 5.25 (s, 2 H). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

4-Hydroxy-2-indolecarboxylic Acid (54). A solution of 4-(benzyloxy)-2-indolecarboxylic acid (**53**; 0.35 g, 1.3 mmol) in 40% ethyl acetate in ethanol (35 mL) was hydrogenated over 10% palladium on carbon at 50 psi for 3 h at room temperature. The mixture was filtered through Celite and the solvent removed in vacuo. The residue was recrystallized from H_2O to provide acid **54** (135 mg, 58%): mp 255–258 °C; ^{1}H NMR (DMSO) δ 12.75 (br s, 1 H), 11.58 (s, 1 H), 9.63 (s, 1 H), 7.14 (d, 1 H), 7.02 (t, 1 H), 6.87 (d, 1 H), 6.48 (d, 1 H). Anal. ($C_9H_7NO_3 \cdot 0.1H_2O$) C, H, N.

7-Chloro-4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one (56) and 5-Chloro-4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one (57). The

mixture of the two lactones, **56** and **57**, were prepared as a 2:1 mixture by a previously described method.[24] The mixture was resolved by silica gel chromatography (7 × 45 cm column, elution with ethyl acetate/hexane, 15:85). From a 2-g mixture of lactones, 400 mg of **57** and 900 mg of **56** were isolated in the pure form. An additional 550 mg of the lactone mixture was recovered. For compound **57**: mp 240–241 °C; ^{1}H NMR ($CDCl_3$) δ 9.21 (s, 1 H), 7.36 (d, 1 H), 7.27 (t, 1 H), 7.16 (d, 1 H), 4.71 (t, 2 H), 3.48 (t, 2 H). Anal. ($C_{11}H_8ClNO_2$) C, H, N. For compound **56**; mp 228–230 °C; ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1 H), 7.54 (d, 1 H), 7.52 (d, 1 H), 7.16 (dd, 1 H), 4.73 (t, 2 H), 3.16 (t, 2 H). Anal. ($C_{11}H_8ClNO_2$) C, H, N.

6-Chloro-3-(2-hydroxyethyl)indole-2-carboxylic Acid (43). A mixture of **56** (600 mg, 2.1 mmol), LiOH (100 mg, 2.3 mmol), and methanol (40 mL) was stirred overnight at room temperature. After evaporation of the methanol, the solid residue was dissolved in water (30 mL) and extracted with ether (3 × 20 mL). The aqueous phase was made acidic by the addition of concentrated HCl and the resulting white solid was filtered and recrystallized from methanol/H_2O (450 mg, 70%): mp 175–176 °C; ^{1}H NMR ($CDCl_3$ + DMSO) δ 11.12 (s, 1 H), 7.60 (d, 1 H), 7.44 (s, 1 H), 7.05 (d, 1 H), 3.87 (t, 2 H), 3.34 (t, 2 H). Anal. ($C_{11}H_{10}ClNO_3 \cdot 1.0H_2O$) C, H, N.

Ethyl 6-Chloro-3-(2-chloroethyl)indole-2-carboxylate (45). Lactone **56** (2.3 mmol, 500 mg) was dissolved in HCl-saturated ethanol (30 mL) and the solution heated to reflux overnight. The solvent was removed in vacuo and the yellow solid was placed on a silica gel column. The column was eluted with a mixture of hexane/ethyl acetate (9:1). The white solid, obtained after evaporation of the solvent, was crystallized from ethanol to give **45** (290 mg, 44%): mp 140–141 °C; ^{1}H NMR ($CDCl_3$) δ 8.81 (s, 1 H), 7.63 (d, 1 H), 7.38 (s, 1 H), 7.14 (dd, 1 H), 4.44 (q, 2 H), 3.76 (t, 2 H), 3.52 (t, 2 H), 1.44 (t, 3 H). Anal. ($C_{13}H_{13}Cl_2NO_2$) C, H, N.

6-Chloro-3-(2-ethoxyethyl)indole-2-carboxylic Acid (47) and 6-Chloro-3-(2-chloroethyl)indole-2-carboxylic Acid (44). A solution of **45** (210 mg, 0.73 mmol) in H_2O (3 mL) and ethanol (10 mL) was stirred overnight in the presence of NaOH (40 mg, 1 mmol). Water (20 mL) was added and the suspension was extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was filtered and acidified by the addition of concentrated HCl. The resulting solid was filtered and the two components were separated by silica gel chromatography with a mixture of CH_2Cl_2/EtOH/AcOH (95:4.5:0.5) as the eluant. Compound **47** was isolated (1.5 mg, 1%): mp 142–142.5 °C; ^{1}H NMR ($CDCl_3$ + DMSO) δ 10.19 (s, 1 H), 7.60 (d, 1 H), 7.44 (s, 1 H), 7.06 (d, 1 H), 3.65 (t, 2 H), 3.52 (q, 2 H), 3.36 (t, 2 H), 1.19 (t, 3 H); MS (FAB), 267 (M^+), 268 (MH^+), 221 ($MH^+ - C_2H_5OH$). Compound **44** was also isolated (2 mg, 1%); mp 128–129 °C; ^{1}H NMR ($CDCl_3$ + DMSO) δ 10.27 (s, 1 H), 7.60 (d, 1 H), 7.44 (s, 1 H), 7.06 (d, 1 H), 3.77 (t, 2 H), 3.52 (t, 2 H); MS (Cl), 257 (MH^+), 221 ($MH^+ - HCl$), 177 ($MH^+ - HCl - CO_2$).

Ethyl 2-(Hydroxymethyl)-6-chloro-3-indoleacetate (51). To a mixture of monoacid **23** (1.72 g, 6.11 mmol) in oxalyl chloride (13 mL) was added three drops of dimethylformamide. After stirring for 2.5 h at room temperature, the residual oxalyl chloride was removed in vacuo to give a solid acid chloride. To a solution of this acid chloride in THF (40 mL) was added sodium borohydride (0.92 g, 24 mmol) and the mixture was stirred at room temperature for 40 h. Ethanol (5 mL) was carefully added and the mixture was stirred for 1 h before the dropwise addition of 1 N HCl (100 mL) at 0 °C. The mixture was poured into water (200 mL) and extracted with ethyl acetate (2 × 200 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a residue (1.53 g) which was purified by silica gel chromatography (20 cm × 50 mm, eluted with 2:1 hexane/ethyl acetate) to yield alcohol **51** (0.75 g, 46%): mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 8.51 (br s, 1 H), 7.47 (d, 1 H), 7.31 (d, 1 H), 7.09 (dd, 1 H), 4.76 (s, 2 H), 4.15 (q, 2 H), 3.73 (s, 2 H), 3.04 (br s, 1 H), 1.27 (t, 3 H). Anal. ($C_{13}H_{14}ClNO_3$) C, H, N.

Ethyl 2-Formyl-6-chloro-3-indoleacetate (50). To a mixture of monoacid **23** (339 mg, 1.20 mmol) in oxalyl chloride (3 mL) was added one drop of dimethylformamide. After stirring for 1 h at room temperature, the residual oxalyl chloride was removed in vacuo to give a solid acid chloride. To a solution of this acid chloride in dimethoxyethane (5 mL) was added a solution of lithium tri-*tert*-butoxyaluminohydride (612 mg, 2.41 mmol) in dimethoxyethane (3 mL) dropwise at −78 °C. The mixture was allowed to warm to 0 °C over 1 h and then poured onto a mixture of ice and 1 N HCl (15 mL). The resulting mixture was extracted with ether (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a residue (750 mg) which was purified by silica gel chromatography (20 cm × 30 mm, eluted with 5% methanol in CH_2Cl_2) to yield aldehyde **50** (160 mg, 50%): mp 121–123 °C; ^{1}H NMR (acetone) δ 10.15 (s, 1 H), 7.72 (d, 1 H), 7.57 (d, 1 H), 7.16 (dd, 1 H), 4.22 (s, 2 H), 4.13 (q, 2 H), 2.81 (br s, 1 H), 1.21 (t, 3 H); MS (CI), 266 (MH^+).

Sodium 2-(Hydroxymethyl)-6-chloro-3-indoleacetate (52). A solution of ester/alcohol **51** (210 mg, 0.784 mmol) in ethanol (3 mL) was treated with 0.5 N NaOH (1.49 mL, 0.745 mmol) at 0 °C. The mixture was warmed to room temperature and stirred for 72 h. The ethanol was removed in vacuo and the residue was dissolved in H_2O (25 mL) and washed with ether (2 × 25 mL). The aqueous layer was lyophilized to provide the white solid sodium salt **52** (206 mg, 100%): mp 215–235 °C; ^{1}H NMR (D_2O) δ 7.49 (d, 1 H), 7.45 (d, 1 H), 7.09 (dd, 1 H), 4.70 (s, 2 H), 3.61 (s, 2 H). Anal. ($C_{11}H_9ClNO_3 \cdot 0.65NaOH$) C, H, N.

3-Formylindole-2-carboxylic Acid (48). Ethyl 3-formylindole-2-carboxylate (250 mg, 1.16 mmol), prepared by a previously described method,[27] was dissolved in ethanol (15 mL) and treated with $LiOH \cdot 2H_2O$ (52 mg, 1.2 mmol). The mixture was stirred for 2 h at room temperature, treated with additional LiOH (30 mg) in H_2O (5 mL), and stirred for 1 h. The solution was concentrated in vacuo and the residue was dissolved in H_2O (30 mL). The aqueous solution was acidified with 6 N HCl and extracted with ether (3 × 30 mL). The combined ether layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was recrystallized from ethyl acetate to provide product **48** (165 mg, 76%): mp 245–247 °C; ^{1}H NMR (D_2O) δ 9.58 (s, 1 H), 7.92 (m, 1 H), 7.47 (m, 1 H), 7.11 (m, 2 H). Anal. ($C_{10}H_7NO_3 \cdot 0.1EtOAc$) C, H, N.

2-Carboxy-3-(2-oxopropyl)indole (49). 2-(Ethoxycarbonyl)-3-(2-oxopropyl)indole was prepared according to previously described procedures.[28] The ester (1 g, 4.0 mmol) was hydrolyzed by heating with 50% aqueous NaOH (0.5 mL) in ethanol (10 mL) and H_2O (2 mL) for 10 min. The solution was poured into H_2O (50 mL) and washed with ethyl acetate (2 × 25 mL). The aqueous layer was acidified with concentrated HCl and extracted with ethyl acetate (3 × 30 mL). The combined organic solutions were dried ($MgSO_4$) and concentrated in vacuo. The residue was recrystallized from ethyl acetate/hexane to provide product **49** (570 mg, 66%): mp 166–168 °C; ^{1}H NMR (DMSO) δ 13.03 (br s, 1 H), 11.60 (s, 1 H), 7.59 (d, 1 H), 7.41 (d, 1 H), 7.25 (t, 1 H), 7.05 (t, 1 H), 4.19 (s, 2 H), 2.12 (s, 3 H). Anal. ($C_{12}H_{11}NO_3$) C, H, N.

2-Carboxy-3-quinolineacetic Acid (58). A solution of 2-carbomethoxy-3-methylquinoline[29] (5 g, 24.8 mmol) in CCl_4 (15 mL) was added to a mixture of *N*-bromosuccinimide (4.4 g, 24.7 mmol) and dibenzoyl peroxide (291 mg, 1.2 mmol) in CCl_4 (50 mL). The mixture was slowly heated to reflux over 1 h, and reflux was continued for 20 h. The reaction mixture was cooled to room temperature and filtered, and the filtrate was washed with H_2O (2 × 30 mL), 5% NaOH solution (2 × 30 mL), and H_2O (2 × 30 mL). The organic layer was dried ($MgSO_4$) and the solvent removed in vacuo. The crude bromide was purified on a silica gel column using 25% CCl_4 in CH_2Cl_2 as the eluant to provide 2-carbomethoxy-3-(bromomethyl)quinoline (1.54 g, 22%). The bromide (0.78 g, 2.8 mmol) was dissolved in methanol (25 mL) and treated with KCN (0.272 g, 4.2 mmol). The mixture was stirred at room temperature for 15 h, then heated to reflux for 2 h. The resulting solution was poured into H_2O (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with H_2O (30 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by preparative centrifugally accelerated radial thin-layer chromatography to provide the intermediate nitrile (420 mg, 66%). The nitrile (200 mg, 0.88 mmol) was placed in 6 N HCl (70 mL) and heated to reflux for 4 h. The solvent was removed in vacuo and the residue was suspended in H_2O (25 mL) and reconcentrated (two repetitions). The residue was triturated with H_2O (10 mL), filtered, and placed in a Soxhlet extractor and extracted with 50% acetonitrile in H_2O. The aqueous solution was cooled and the resulting precipitate was collected by filtration and dried in vacuo to give **58** (92 mg,

45%): mp 225-229 °C, ^{1}H NMR (DMSO) δ 8.37 (s, 1 H), 8.11 (d, 1 H), 8.01 (d, 1 H), 7.83 (t, 1 H), 7.71 (t, 1 H), 4.06 (s, 2 H). Anal. ($C_{12}H_9NO_4 \cdot 0.6H_2O$) C, H, N.

2-Carboxycoumaran-3-acetic Acid (59). Methyl 2-(methoxycarbonyl)coumaran-3-acetate was prepared by a previously described procedure.[30] The diester (1 g, 4.0 mmol) was saponified as described for compound 8 to yield the diacid as a colorless oil. Product **59** was crystallized from acetone/cyclohexane (850 mg, 96%): mp 172-175 °C; ^{1}H NMR (DMSO) δ 12.84 (br s, 2 H), 7.22 (m, 2 H), 6.87 (m, 2 H), 4.91 (d, 1 H), 3.85 (m, 1 H), 2.70 (m, 2 H). Anal. ($C_{11}H_{10}O_5$) C, H.

2-Carboxybenzofuran-3-acetic Acid (60). Diacid **60** was prepared by a previously described procedure,[30] but the crude product was obtained directly upon acidification of a basic extract, without isolation of the reported contaminating brown oils. The crude diacid was recrystallized from acetone/cyclohexane to provide **60** (53% yield): mp 237-242 °C (lit.[30] mp 230 °C dec); ^{1}H NMR (DMSO) δ 12.80 (br s, 2 H), 7.80 (d, 1 H), 7.68 (d, 1 H), 7.51 (t, 1 H), 7.35 (t, 1 H), 4.10 (s, 2 H). Anal. ($C_{11}H_8O_5$) C, H.

Radioreceptor Assays. Inhibition of [^{3}H]Glycine Binding. Strychnine-insensitive [^{3}H]glycine binding to the NMDA receptor-associated recognition site was performed with Triton X-100-washed synaptic plasma membranes (SPM) prepared from rat forebrain (30-45 day old, male Sprague-Dawley; Sasco, St. Charles, MO) as described previously.[34] The assay was initiated by the addition of 0.2-0.4 mg of SPM to an incubation containing 10 nM [^{3}H]glycine (49.0 Ci/mmol; New England Nuclear, Boston, MA), and various concentrations, in triplicate, of the appropriate test compounds in a total volume of 1 mL, with all additions made in 50 mM of Tris/acetate, pH 7.4. Following a 10-min incubation at 2 °C, the bound radioactivity was separated from the free by either centrifugation (12000g for 15 min at 4 °C) or vacuum filtration through Whatman GF/B filters using a Brandel MB-18 Harvester. The radioactivity associated with the SPM was quantitated using liquid scintillation spectrometry. Nonspecific binding was defined in the presence of 100 μM glycine. K_i values were determined from logit-log transformations of the binding data.

Inhibition of [^{3}H]Glutamate, [^{3}H]Kainate, and [^{3}H]AMPA Binding. The [^{3}H]glutamate, [^{3}H]kainate, and [^{3}H]AMPA radioreceptor assays were carried out by using the methods described previously.[30,31] K_i values were determined from logit-log transformations of the binding data.

Modulation of [^{3}H]MK-801 Binding. The modulation of [^{3}H]MK-801 binding was performed as described earlier.[32] Briefly, the synaptic plasma membranes (SPM) were treated with Triton X-100 (0.04% v/v) and then extensively washed with 50 mM Tris acetate, pH 7.4. The assay incubation was initiated by the addition of SPM (0.2-0.4 mg) to 5 nM [^{3}H]MK-801, 10 nM L-glutamate, and the appropriate concentration of the test compound in 50 mM Tris acetate, pH 7.4. After 30 min at 25 °C, the samples were filtered through polyethylenimine treated (0.05% v/v) Whatman GF/B filters and then washed four times with 2 mL of cold buffer. Radioactivity associated with the filter was determined by liquid scintillation spectrometry and nonspecific binding defined with 60 μM MK-801.

(34) Monahan, J. B.; Corpus, V. M., Hood, W. F.; Thomas, J. W.; Compton, R. P. *J. Neurochem.* **1989**, *53*, 370.

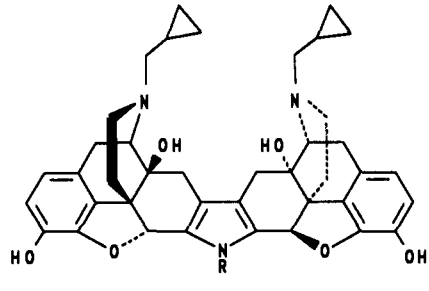
Role of the Spacer in Conferring κ Opioid Receptor Selectivity to Bivalent Ligands Related to Norbinaltorphimine

P. S. Portoghese,* A. Garzon-Aburbeh, H. Nagase, C.-E. Lin, and A. E. Takemori†

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455. *Received May 10, 1990*

The thiophene **2** and pyran **3** analogues of the κ-selective opioid antagonist norbinaltorphimine (**1a**, norBNI) were synthesized and tested in an effort to determine the contribution of the spacer to the interaction of bivalent ligands at different opioid receptor types. Both **2** and **3** were found to be selective κ opioid receptor antagonists in smooth muscle preparations, and they bound selectively to κ-recognition sites. The thiophene analogue **2** displayed binding selectivity that was of the same order of magnitude as that of **1a**, while **3** was considerably less selective for κ site. This is consistent with the fact that the second pharmacophore in **1a** and **2** displayed a greater degree of superposition than **1a** and **3**. The results of this study suggest that the pyrrole moiety of norBNI functions primarily as an inert spacer to rigidly hold the basic nitrogen in the second pharmacophore at an "address" subsite that is unique for the κ opioid receptor.

Norbinaltorphimine (**1a**, norBNI) and binaltorphimine (**1b**, BNI) are bivalent ligands that are highly selective for κ opioid receptors.[1-3] They contain two naltrexone-derived



1a, R = H(norBNI)

1b, R = Me(BNI)

pharmacophores linked through a pyrrole spacer which is fused with ring C of the morphinan structure. The structure-activity relationship of related compounds has revealed that only one pharmacophore is required for κ antagonist selectivity.[4,5] We have suggested that the κ selectivity arises as a consequence of the pyrrole moiety holding the second half of the molecule in a specific, rigid orientation with respect to the first half, thereby facilitating its interaction with a subsite (the "address") of the κ receptor recognition locus.[5,6]

In an effort to determine whether the pyrrole moiety functions merely as a spacer or is a requirement for κ opioid

† Department of Pharmacology.

(1) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. *J. Med. Chem.* **1987**, *30*, 238.
(2) Takemori, A. E.; Ho, B. Y.; Naeseth, J. S.; Portoghese, P. S. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 255.
(3) Birch, P. J.; Hayes, A. G.; Sheehan, M. J.; Tyers, M. B. *Eur. J. Pharmacol.* **1987**, *144*, 405.
(4) Portoghese, P. S.; Nagase, H.; Lipkowski, A. W.; Larson, D. L.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 836.
(5) Portoghese, P. S.; Nagase, H.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 1344.
(6) Portoghese, P. S. *Trends Pharmacol. Sci.* **1989**, *10*, 230.

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