243, 134, 109, 55; $[\alpha]_D$ = -124° (c = 1.08, $CHCl_3$).

(1'*R*,2'*S*,3'*R*)-9-(2',3'-Dihydroxy-4'-ethyl-4'-cyclopentenyl)adenine (2g). Ketal 10g was hydrolyzed as described in the synthesis of 1c: mp 212-214 °C; ^{1}H-NMR (500 MHz; DMSO-d_6 + D_2O) δ 1.05 (t, J = 7 Hz, 3 H), 2.15 (m, 2 H), 4.25 (dd, J = 5, 5, 1 H), 4.40 (d, J = 5, 1 H), 5.30 (s, 1 H), 5.55 (s, 1 H), 8.05 (s, 1 H), 8.10 (s, 1 H); ^{13}C-NMR (125 MHz; DMSO-d_6 + D_2O) δ 11.6, 22.2, 64.9, 74.6, 76.8, 119.3, 123.1, 140.1, 149.9, 151.6, 152.8, 156.1; IR (KBr) 3360, 3320, 3140 (br), 2960, 1650, 1605, 1570, 1480, 1410, 1325, 1305, 1245, 1115, 1050, 860, 800, 690 cm^{-1}; MS (EI) *m/e* calcd for (M + 1) of $C_{12}H_{15}N_5O_2$ 262.1304, found 262.1300; 261 (M^+), 233, 214, 186, 149, 136, 108. Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

Determination of AdoHcy Hydrolase Inhibition Constants. AdoHcy hydrolase was isolated and purified from bovine liver as previously reported[37] except that Q Sepharose (Pharmacia) was used instead of DE-52 cellulose, and the CM-Sephadex column was not applied. The enzyme activity was determined by incubating 20 nM AdoHcy hydrolase with 0.2 mM adenosine and 5 mM homocysteine for 5 min at 37 °C in 150 mM phosphate buffer (pH 7.6) containing 1 mM EDTA and assaying the AdoHcy produced by HPLC after the reaction was stopped by addition of perchloric acid (final concentration: 0.5 N). Volume of 100 μL of supernatant obtained by centrifugation of the reaction mixture was injected into an HPLC column (C-18 reverse-phase column, Econosphere, Alltech, 25 cm × 4.6 mm) and analyzed with a two-step gradient program at flow rate of 1.0 mL/min [solvent A, acetonitrile; solvent B, 50 mM sodium phosphate (pH 3.2) containing 10 mM heptanesulfonic acid; Program, 5-20% A for 15 min, 20-25% A for 10 min]. The peak area of AdoHcy was monitored at 254 nm to quantitate the AdoHcy. For the determination of inhibition constants, AdoHcy hydrolase was preincubated with various concentrations of inhibitors for various amounts of time, and the remaining enzyme activity was measured. The pseudo-first-order rate of inactivation (k_{obs}) was determined from a plot of the remaining activity versus preincubation time. K_I and k_2 were obtained from a plot of $1/k_{obs}$ versus 1/[inhibitor] using the equation $1/k_{obs} = K_I/(k_2[I]) + 1/k_2$.

(37) Narayanan, S. R.; Borchardt, R. T. Purification of Bovine Liver *S*-Adenosylhomocysteine Hydrolase by Affinity Chromatography on Blue Dextran-agarose. *Biochim. Biophys. Acta* 1988, *965*, 22-28.

Acknowledgment. We thank Dr. David Vander Velde here at the University of Kansas for assistance with the NOE experiments on intermediate 7f. This work was supported by a National Institute of Health Predoctoral Training Grant (GM-07775; M.S.W.), a National Science Foundation Graduate Fellowship (W.J.B.), a United States Public Health Service Grant (GM-29332), and Glaxo, Inc.

3-(2-Carboxyindol-3-yl)propionic Acid-Based Antagonists of the *N*-Methyl-D-aspartic Acid Receptor Associated Glycine Binding Site

Francesco G. Salituro,* Boyd L. Harrison, Bruce M. Baron, Philip L. Nyce, Kenneth T. Stewart, John H. Kehne, H. Steven White,† and Ian A. McDonald

Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215, and Anticonvulsant Drug Development Program, Department of Pharmacology & Toxicology, University of Utah, Salt Lake City, Utah 84108.
Received November 14, 1991

A series of substituted 3-(2-carboxyindol-3-yl)propionic acids was synthesized and tested as antagonists for the strychnine-insensitive glycine binding site of the NMDA receptor. Chlorine, and other small electron-withdrawing substituents in the 4- and 6-positions of the indole ring, greatly enhanced binding and selectivity for the glycine site over the glutamate site of the NMDA receptor; one of the most potent compounds is 3-(4,6-dichloro-2-carboxyindol-3-yl)propionic acid (IC_{50} = 170 nM; >2100-fold selective for glycine). The importance of a heteroatom NH and the enhancing effect of the propionic acid side chain were demonstrated and are consistent with previous results which suggest the presence of a pocket on the receptor which can accept an acidic side chain. Substitution of a sulfur at C3 led to the most potent compound 3-[(carboxymethyl)thio]-2-carboxy-4,6-dichloroindole (IC_{50} = 100 nM).

Introduction

The role which the acidic amino acids glutamic acid and aspartic acid play as neurotransmitters in the mammalian central nervous system (CNS) has been intensely studied over the past several years. Several distinct receptor complexes have been defined by the ligands kainic acid (kainate), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and *N*-methyl-D-aspartic acid (NMDA). From a potential therapeutic point of view, the NMDA receptor complex has attracted considerable interest as there is increasing evidence that abnormal stimulation of the receptor may play a role in the neuropathology of disease states such as epilepsy, Huntington's disease, and Alzheimer's disease.[1-3] Furthermore, it is thought that the neurodegeneration which occurs following events of cerbral ischemia is, in part, a result of an overstimulation of the NMDA receptor.

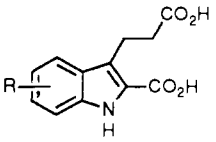
The NMDA receptor complex possesses several allosteric binding sites which alter the cellular response to glutamic acid.[4] This response is presumably initiated by the influx

† University of Utah.

(1) Robinson, M. B.; Coyle, J. T. Glutamate and Related Acyclic Excitatory Neurotransmitters: From Basic Science to Clinical Application. *FASEB J.* 1987, *1*, 446-455.
(2) Meldrum, B. *Neurology and Neurobiology*; Hicks, T. P., Lodge, D., McLennan, H., Eds.; Alan R. Liss, Inc.: New York, 1987; Vol. 24, pp 189-196.
(3) (a) Olney, J. W. Excitatory Amino Acids and Neuropsychiatric Disorders. *Biol. Psychiatry* 1989, *26*, 505-525. (b) Lodge, D. Modulation of *N*-Methyl Aspartate Receptor-Channel Complexes. *Drugs Today* 1989, *25*, 395-411. (c) Wood, P. L.; Rao, T. S.; Iyengar, S.; Lanthorn, T.; Monahan, J.; Cordi, A.; Sun, E.; Vazquez, M.; Gray, N.; Contreras, P. A Review of the *In Vitro* and *In Vivo* Neurochemical Characterization of the NMDA/PCP/Glycine/Ion Channel Receptor Macrocomplex. *Neurochem. Res.* 1990, *15*, 217-230.
(4) Lodge, D.; Jones, M.; Fletcher, E. *The NMDA Receptor*; Watkins, J. C., Collingridge, G. L., Eds.; Oxford University: Oxford, 1989; Chapter 3.

0022-2623/92/1835-1791$03.00/0

Table I. Binding Affinity and Physical Properties



4

compd	R	method[a]	% yield[b]	mp, °C[c]	formula[d]	IC_{50} (μM) [^{3}H]Gly[e]	IC_{50} (μM) [^{3}H]CPP[f]	ratio CPP/GLY
4a	H	A, B	40	184–86	$C_{12}H_{11}NO_4$	27	34	1
4b	4-Cl	A, B	14	255–56	$C_{12}H_{10}ClNO_4$	6	60	10
4c	5-Cl	A, B	54	249–50	$C_{12}H_{10}ClNO_4$	23	25	2
4d	6-Cl	A, B	8	234–36	$C_{12}H_{10}ClNO_4$	2	283	140
4e	7-Cl	A, B	14	255–56	$C_{12}H_{10}ClNO_4$	>100	>1000	>1
4f	4,6-Cl	C	40	273–80	$C_{12}H_9Cl_2NO_4$	0.17	358	2100
4g	4,6-Cl, NCH_3	C	81[g]	233–36	$C_{13}H_{11}Cl_2NO_4$	45	>100	>2
4h	4-F	A, B	12	245–46	$C_{12}H_{10}FNO_4$	17	51	3
4i	6-F	A, B	43	220–21	$C_{12}H_{10}FNO_4$	5		
4j	4,6-F	A, B	8	233–35	$C_{12}H_9F_2NO_4$	4		
4k	6-OCH_3	A, B	21	235–37	$C_{13}H_{13}NO_5$	13	141	11
4l	4,6-OCH_3	A, B	40	234–35	$C_{14}H_{15}NO_6$	>100		
4m	4-NO_2	C	5	290(d)	$C_{12}H_{10}N_2O_6$	75		
4n	6-NO_2	C	15	265–66	$C_{12}H_{10}N_2O_6$	2.5		
4o	4-CF_3	A, B	24	260(d)	$C_{13}H_{10}F_3NO_4$	13		
4p	6-CF_3	A, B	27	230–31	$C_{13}H_{10}F_3NO_4$	8		
4q	6-OPh	A, B	44	202–05	$C_{18}H_{15}NO_5$	>1000		
4r	6-NH_2	C						
4s	6-$NHSO_2CH_3$	C	40[h]	264–65	$C_{13}H_{14}N_2O_6S$	>100		
4t	benzo[e]	A, B	56	230–30.5	$C_{16}H_{13}NO_4$	>100		
4u	benzo[g]	A, B	60	228–29	$C_{16}H_{13}NO_4 \cdot 0.5H_2O$	>100		
8		C	85[i]	238–39	$C_9H_5Cl_2NO_2$	6	>1000	>150
19a		C	10[j]	194–95	$C_{11}H_9NO_4S$	12	16	1.6
19b		C	20[j]	152–53	$C_{11}H_7Cl_2NO_4S$	0.10	88	880
indole-2-carboxylic acid (20)		D				106	4200	40
kynurenic acid (1)		D				16	71	4
5,7-dichlorokynurenic acid (21)		D				0.143	12	80
2		D				25	710	28
11		C	50[k]	195–200	$C_{11}H_9NO_3$	>100	>100	
15		C	16[k]	230–36[l]	$C_{13}H_{11}NO_4 \cdot 0.3H_2O$	>100	>100	

[a] A and B, see general methods; C, refer to Experimental Section for preparation; D, literature compound. [b] Yields were overall yields starting from the corresponding aniline unless otherwise noted. [c] All compounds are recrystallized from ethyl acetate/hexane unless otherwise noted. [d] All compounds were analyzed for C, H, N and are within ±0.40% of the theoretical value. [e] Competition assay vs [^{3}H]glycine for rat cortical and hippocampal membrane binding sites $n \geq 2$ (standard errors are ±10–30% of the reported mean value). [f] Competition assay against [^{3}H]CCP for rat cortical and hippocampal membrane glutamate binding sites $n \geq 2$ (standard errors are ±10–30% of the reported mean value). [g] Yield is from 7f. [h] Yield is from 7n. [i] Yield is from (3,5-dichlorophenyl)hydrazine hydrochloride. [j] Yield is from the corresponding 2-carbethoxyindole. [k] Yield is from methyl kynurenate. [l] Compound was filtered directly from acidic H_2O.

of calcium ions through an associated magnesium gated ion channel. With distinct domains which bind glutamic acid,[4] phencyclidine[4] (PCP), glycine[4], zinc ion,[4] polyamines,[5] and spider/wasp toxins[6] (such as philanthotoxin), the NMDA receptor has proven a fruitful area of research since potent, selective antagonists for these sites have been described.[7] From protein isolation studies and ligand binding site stoichiometry, a hypothetical model for the configuration of protein subunits has been proposed which include $\alpha_2\beta_2\gamma\delta$ subunits. Two identical subunits are proposed to bind glutamic acid while two other identical subunits of approximately 42KDa bind the simplest amino acid glycine to a strychnine-insensitive binding site.[7] It has been established that glycine is a cotransmitter with glutamic acid and is obligatory for receptor activation.[8-12] Therefore, potent glycine antagonists, like competitive NMDA antagonists, should have potential clinical applications in the above-mentioned disorders. In fact, recent

(5) Ranson, R. W.; Stec, N. L. Cooperative Modulation of [^{3}H] MK-801 Binding to the NMDA Receptor-Ion Channel Complex by L-Glutamate, Glycine and Polyamines. *J. Neurochem.* **1988**, *51*, 830–836.

(6) Johnson, G. Recent Advances in Excitatory Amino Acid Research. *Annual Reports in Medicinal Chemistry*, Vol. 24; Allen, R. C., Ed.; Academic Press Inc.: New York, 1989; Chapter 5, pp 40–41.

(7) Johnson, G.; Bigge, C. F. Recent Advances in Excitatory Amino Acid Research. *Annual Reports in Medicinal Chemistry*, Vol. 26; Bristol, J. A., Ed.; Academic Press Inc.: New York, 1991; Chapter 2, pp 11–22, and references therein.

(8) Johnson, J. W.; Ascher, P. Glycine Potentiates the NMDA Response in Cultured Mouse Brain Neurons. *Nature* **1987**, *325*, 529–531.

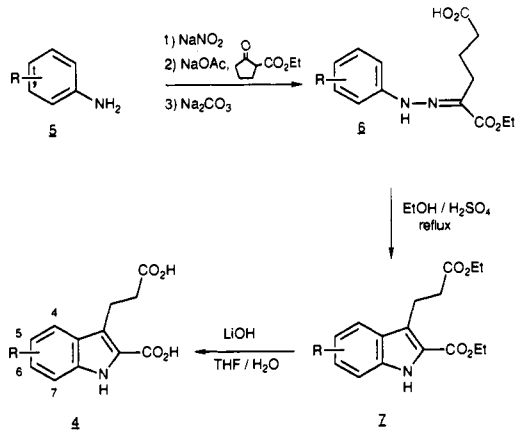
(9) Baron, B. M.; Harrison, B. L.; Miller, F. P.; McDonald, I. A.; Salituro, F. G.; Schmidt, C. J.; Sorensen, S. M.; White, H. S.; Palfreyman, M. G. Activity of 5,7-Dichlorokynurenic Acid, a Potent Antagonist at the *N*-methyl-D-aspartate Receptor-Associated Glycine Binding Site. *Mol. Pharmacol.* **1990**, *38*, 554–561.

(10) Kleckner, N. W.; Dingledine, R. Requirement for Glycine in Activation of NMDA Receptors Expressed in Xenopus Oocytes. *Science* **1989**, *241*, 835–837.

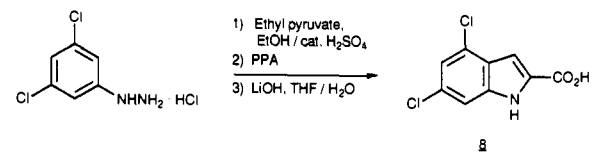
(11) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestly, T.; Tridgett, R.; Iversen, L. L. 7-Chlorokynurenic Acid is a Selective Antagonist at the Glycine Modulatory site of the *N*-methyl-D-aspartate Receptor Complex. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6547–6550.

(12) Birch, P. J.; Grossman, C. J.; Hayes, A. G. Kynurenate and F69041 have Both Competitive and Non-competitive Antagonist Actions at Excitatory Amino Acid Receptors. *Eur. J. Pharmacol.* **1988**, *151*, 313–315.

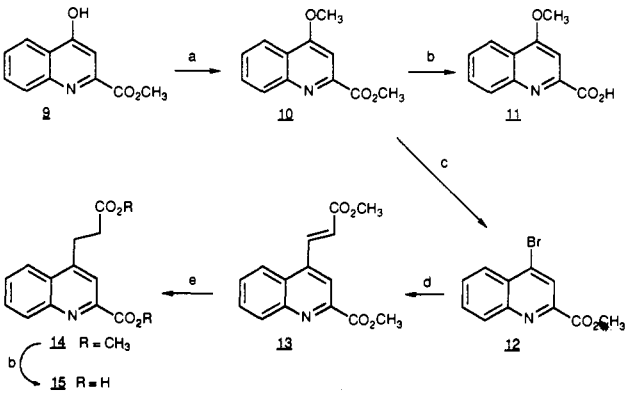
Scheme I



Scheme II



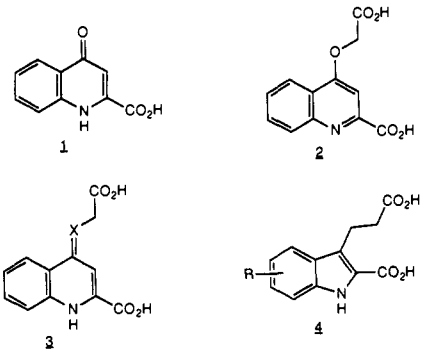
Scheme III[a]



[a] (a) NaH, CH_3I, DMF; (b) LiOH, THF/H_2O; (c) PBr_3, DMF, 70 °C; (d) methyl acrylate, $Pd_2(DBA)_3$, PPh_3, $Pd^{II}(PPh_3)_2Cl_2$, CH_3CN, Et_3N; (e) Mg turnings, methanol.

data have suggested that glycine-site antagonists, useful as anticonvulsants or anxiolytics, may lack some of the liabilities, such as impaired learning or muscle relaxation, observed with glutamate antagonists.[13,14]

Kynurenic acid (1), a tryptophan metabolite, was one of the earliest reported NMDA antagonists with micromolar affinities for both the glycine and glutamic acid sites[15,16] (see Table I). 4-[(Carboxymethyl)oxy]-quinoline-2-carboxylic acid (2), which possesses key structural features of both kynurenic acid and glutamic acid, is a good antagonist at the glycine site (IC_{50} = 25 μM) but a poor antagonist at the glutamic acid site (IC_{50} = 710 μM).[17] Modeling and structure–activity relationship (SAR) studies of these and related compounds suggested that the minimum structural requirements for good selective affinity at the glycine site were encompassed in the generic structure **3**.[17] We hypothesized that indole-2-carboxylic acid derivatives, substituted in the 3 position with a propionic acid side chain, also approximated this pharmacophore and, therefore, synthesized a series of 3-(2-carboxyindol-3-yl)propionic acid derivatives (**4**) as potential competitive glycine site antagonists. We have recently communicated that 3-(2-carboxy-4,6-dichloroindol-3-yl)propionic acid (**4f**)[18] and 3-[(carboxymethyl)-thio]-2-carboxy-4,6-dichloroindole (**19b**)[19] are potent, selective antagonists. The synthesis, properties, and structure–activity relationships of these indole-based glycine antagonists are fully explored in this paper.



Results and Discussion

Chemistry. The indoles **4a–u** in Table I were synthesized from substituted anilines or naphthylamines (**4t, 4u**) and 2-(ethoxycarbonyl)cyclopentanone under Japp–Klingemann reaction conditions (Scheme I).[20] In general, the initial hydrazones **6** were cyclized without purification to yield the corresponding indole diesters **7a–u**, which were purified by flash chromatography and recrystallization. In examples starting with meta-substituted anilines, both the 4- and 6-substituted indoles were formed except for the 6-methoxy compound (**4k**) where none of the 4-substituted regioisomer was detected. In general, the regioisomers could easily be separated by flash chromatography at the diester stage. One exception was the 6-phenoxy derivative

(13) Chiamulera, C.; Costa, S.; Reggiani, A. Effect of NMDA- and strychnine-insensitive Glycine Site Antagonists on NMDA-Mediated Convulsions and Learning. *Psychopharmacology* **1990**, *102*, 551–552.

(14) Kehne, J. H.; McCloskey, T. C.; Baron, B. M.; Chi, E. M.; Harrison, B. L.; Whitten, J. P.; Palfreyman, M. G. NMDA Receptor Complex Antagonists Have Potential Anxiolytic Effects as Measured with Separation-induced Ultrasonic Vocalizations. *Eur. J. Pharmacol.* **1991**, *193*, 283–292.

(15) Kessler, M.; Terramani, T.; Lynch, G.; Baudry, M. A Glycine Site Associated with *N*-methyl-D-aspartic Acid Receptors: Characterization and Identification of a New Class of Antagonists. *J. Neurochem.* **1989**, *52*, 1319–1328.

(16) Birch, P. J.; Grossman, C. J.; Hayes, A. G. Kynurenic Acid Antagonizes Responses to NMDA Via an Action at the Strychnine-insensitive Glycine Receptor. *Eur. J. Pharmacol.* **1988**, *154*, 85–87.

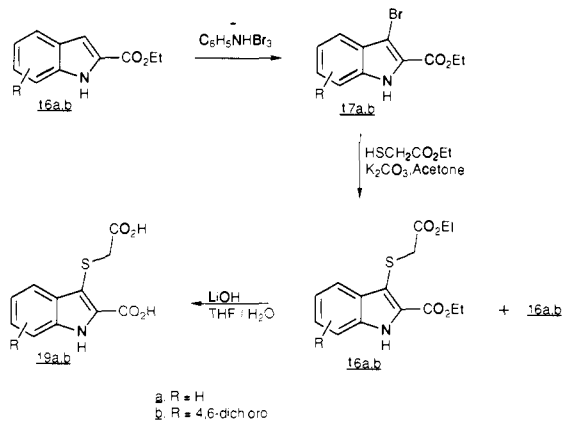
(17) Harrison, B. L.; Baron, B. M.; Cousino, D. M.; McDonald, I. A. 4-[(Carboxymethyl)oxy]- and 4-[(Carboxymethyl)amino]-5,7-dichloroquinolin-2-carboxylic Acid: New Antagonists of the Strychnine-Insensitive Glycine Binding Site on the *N*-Methyl-D-Aspartate Receptor Complex. *J. Med. Chem.* **1990**, *33*, 3130–3132.

(18) Salituro, F. G.; Harrison, B. L.; Baron, B. M.; Nyce, P. L.; Stewart, K. T.; McDonald, I. A. 3-(2-Carboxyindol-3-yl)-propionic Acid Derivatives: Antagonists of the Strychnine-Insensitive Glycine Receptor Associated with the *N*-Methyl-D-Aspartate Receptor Complex. *J. Med. Chem.* **1990**, *33*, 2944–2946.

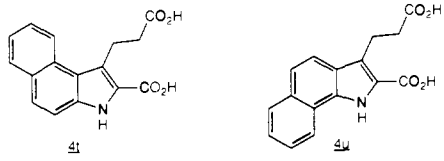
(19) McDonald, I. A.; Harrison, B. L.; Salituro, F. G.; Baron, B. M.; Stemerick, D. M.; Tomlinson, R. C. Selective Antagonists of the NMDA Receptor-associated Glycine Binding Site. *Abstracts of Papers*, 201st National Meeting of the American Chemical Society, Atlanta, GA, 1991; American Chemical Society: Washington, DC, 1991; Division of Medicinal Chemistry, Abstract No. 8.

(20) Nagasaka, T.; Ohki, S. Indoles V. Syntheses and Reactions of Cycloalkanone [c,d] Indole Derivatives. *Chem. Pharm. Bull.* **1977**, *25*, 3023–3033, and references therein.

Scheme IV



(7q) which was formed with the 4-phenoxy isomer in about a 1:1 ratio (300-MHz NMR). Due to a difficult separation problem, only the 6-isomer could be obtained in pure form. In some cases, cyclization was sluggish under standard conditions (refluxing ethanol/H_2SO_4). For example, the (dichlorophenyl)hydrazone leading to **7f** cyclized poorly, affording the hydrazone diester. However, this compound could be isolated and cyclized in high yield by treatment with *p*-toluenesulfonic acid in refluxing toluene. The isomeric nitro derivatives **7m** and **7n** were prepared in similar fashion. The 6-amino derivative (**7r**) and the sulfonamide (**7s**) were prepared from the corresponding nitro analogue (**7n**). Thus, reduction of **7n** with tin(II) chloride dihydrate in refluxing ethanol[21] afforded **7r**, which was converted to **7s** under standard conditions. The benzoindole analogues (**7t** and **7u**) were prepared from β-naphthylamine and α-naphthylamine, respectively.[22] Saponification of esters **7a–u** was achieved using LiOH in aqueous tetrahydrofuran, whereupon the diacids **4a–u** were obtained in good yields following recrystallization. These conditions failed in the case of the amino indole **7r**, which led to unidentifiable mixtures.



4,6-Dichloroindole-2-carboxylic acid (**8**), required for comparative purposes, was prepared from (3,5-dichlorophenyl)hydrazine. Formation of the hydrazone with ethyl pyruvate followed by polyphosphoric acid-induced cyclization and saponification afforded **8** in high yield (Scheme II).

The quinoline derivatives **11** and **15** played key roles in important structure–activity relationship studies. These compounds were synthesized as outlined in Scheme III. Thus, treatment of methyl kynurenate with sodium hydride and methyl iodide in DMF afforded **10** in good yield; saponification afforded **11**. Proceeding toward compound **15**, the methoxy derivative **10** could be cleanly converted to the 4-bromo compound **12** by treatment with PBr_3 in DMF. A palladium-catalyzed coupling between **12** and an excess of methyl acrylate afforded (*E*)-isomer **13** in high yield.[23] Conditions for this reaction were highly specific, requiring the presence of both a Pd^0 and a Pd^{II} species. Whereas the use of either $Pd_2(DBA)_3$ or in situ generated $Pd(PPh_3)_4$ was unsuccessful, product was formed in high yield when $Pd(PPh_3)_2Cl_2$ was added to these reaction mixtures. Similarly, initiating the reaction with $Pd(PPh_3)_2Cl_2$ alone failed to catalyze the reaction, but with subsequent addition of a Pd^0 catalyst, **13** was formed in good yield. Reduction of the double bond (Mg/MeOH)[24] followed by saponification afforded **15** in 16% overall yield from methyl kynurenate.

Table II. Binding Affinities for the AMPA and Kainic Acid Sites

compd	IC_{50} (μM)[c] AMPA[a]	kainate[b]
4f	273	418
19b	>100	>100

[a] Competition assays against [^{3}H]AMPA.[27] [b] Competition assays against [^{3}H]kainate.[26] [c] Values shown are means ± SEM of 2 or more equivalents. Standard errors are 20–30% of the mean values.

The side chain-substituted sulfur anologues **19a** and **19b** were prepared according to Scheme IV from the corresponding 2-carbethoxyindoles. Bromination with pyridinium perbromide afforded the 3-bromoindoles **17a** and **17b** in high yields. Treatment of **17a** or **17b** with K_2CO_3 and ethyl 2-mercaptoacetate in acetone afforded **18a** and **18b**, respectively, in low to moderate yields accompanied by the reduced starting indoles **16a** and **16b**, which presumably arose from nucleophilic attack of sulfur on bromine. Base hydrolysis of **18a** and **18b** led to the desired diacids in good yields.

All compounds, unless specifically mentioned, were analyzed by 300-MHz NMR, mass spectrometry, IR, and elemental analysis.

Receptor Binding. The compounds in Table I were evaluated for their ability to compete with [^{3}H]glycine for strychnine-insensitive binding sites on rat cortical and hippocampal membranes according to published procedures.[25] The IC_{50} values reported, representing the concentration of compound required to reduce glycine binding by 50%, were mean values from two or more experiments; standard errors in all cases were within 10–30% of the mean. The most potent compounds **4f** and **19b**, were established as antagonists by their ability to inhibit NMDA-stimulated accumulation of cyclic GMP.[25] Similar competition assays were run with [^{3}H]CPP (3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) in order to evaluate the affinity of the compounds for the glutamic acid binding site of the NMDA receptor complex.[25] In the case of **4f** and **19b**, the two most potent compounds in the series, competition assays were extended to include the kainic acid and AMPA binding sites using [^{3}H]kainic acid and [^{3}H]AMPA, respectively[26,27] (Table II).

(21) Bellamy, F. D.; Ou, K. Selective Reduction of Aromatic Nitro Compounds with Stannous Chloride in Non-acidic and Non-aqueous Medium. *Tetrahedron Lett.* **1984**, *25*, 839–842.
(22) Both α- and β-naphthylamine are OSHA-regulated carcinogens and should be handled with extreme caution.
(23) Ziegler, C. B.; Heck, R. F. Palladium-catalyzed Vinylic Substitution with Highly Activated Aryl Halides. *J. Org. Chem.* **1978**, *43*, 2941–2946.
(24) Hudlicky, T.; Sinai-Zingde, G.; Natchus, M. G. Selective Reduction of α,β-Unsaturated Esters in the Presence of Olefins. *Tetrahedron Lett.* **1987**, *28*, 5287–5290.
(25) Baron, B. M.; Dudley, M. W.; McCarty, D. R.; Miller, F. P.; Reynolds, I. J.; Schmidt, C. I. Guanine Nucleosides are Competitive Inhibitors of *N*-Methyl-D-Aspartate at its Receptor Site Both *In Vitro* and *In Vivo*. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 162–169.
(26) London, E. D.; Coyle, J. T. Specific Binding of [^{3}H]Kainic Acid to Receptor Sites in Rat Brain. *Mol. Pharmacol.* **1979**, *15*, 492–505.

Structure–Activity Relationships. (a) Aromatic Ring Substitution. The substitution of various functional groups on the indole nucleus has offered some insight into the topography of the glycine binding site. The lead compound **4a** (IC_{50} = 27 μM) was found to be approximately equipotent with the endogenous antagonist kynurenic acid (IC_{50} = 16 μM). Since it is known that chlorine substitution of kynurenic acid results in enhanced binding affinity,[9,11,17] compounds **4a–f** were synthesized and tested to determine whether similar effects were operable in the indole series. This was indeed the case; for example incorporation of chlorine into the 4, 5, or 6 positions results in enhanced potency; the 4 and 6 positions having the most dramatic effects. The 4 and 7 positions appear to be the most sensitive toward bulky substituents; see compounds **4m** and **4e** for example. Addition of a chlorine atom in the 7 position (**4e**, IC_{50} > 100 μM) or substituting the 6 and 7 position as the benzoindole (**4u**, IC_{50} > 100 μM) leads to complete loss of binding affinity. This was consistent with results obtained in the quinoline series where substitution at the 8 position dramatically increased the IC_{50} value.[28] Substitution at the 4 position appears to be under steric as well as electronic control. Small electron-withdrawing groups at this position (**4b**, **4h**, **4o**) increases affinity over **4a** from 2- to 5-fold, whereas introduction of larger electron withdrawing groups significantly lowers affinity for the receptor. The 6-fold increase in the IC_{50} value observed when substituting 4-CF_3 for the more bulky 4-NO_2 group (both electron withdrawing), and the complete inactivity of the fused benzo derivative **4t**, are illustrative of the restricted steric demands in this region of the receptor pocket. Introduction of an electron-donating group in the 4 and 6 positions (see **4l**, IC_{50} > 100 μM) greatly reduces binding potency compared to other compounds bearing an electron-withdrawing group in this position. Unfortunately, with only a limited number of compounds with electron-donating substituents having been prepared, any SAR conclusions would be tenuous at best.

Substitution at the 6 position is also critical for potent binding affinity; however, this site seemed to have different requirements from the 4-position in that both lipophilic and electronic effects are important. Although introduction of either electron donating (**4k**) or withdrawing groups (**4d**, **4i**, **4n**, **4p**) enhances binding over unsubstituted **4a**, there is a trend which suggests that electron-withdrawing groups have the greater effect on binding potency. Nevertheless, while small electron-withdrawing groups may be preferred, small electron-donating groups were also beneficial in some cases (e.g., **4k**), perhaps due to an increase in lipophilicity. A dramatic increase in the size of the 6-substituent (**4q** and **4s**) results in complete loss of activity.

Only one analogue with a 5 position substituent was prepared. The 5-chloro analogue **4c** shows a modest enhancement in binding consistent with results obtained in other systems.[29]

Introduction of chlorine into both the 4 and 6 positions (**4f** and **19b**) of the indole ring has led to the most potent compounds of this series (170 and 100 nM, respectively) which are 120–160-fold more potent than the parent compounds (**19a** and **4a**), and equipotent with dichlorokynurenic acid (**21**). For reasons which are not immediately obvious, there is an apparent synergistic effect of the two chlorine atoms, since **4f** is considerably more potent than would be predicted from the binding results on the monochloro analogues **4b** and **4d** alone. However, in view of recent modeling studies in the kynurenic acid series,[30] together with our QSAR efforts, which suggest that several critical physical properties (such as total volume, lipophilicity, and dipole) act in concert to determine the binding properties of these molecules, this is not surprising. Furthermore, this may explain why the difluoro analogue **4j** is significantly weaker than **4f**, as it may lack the total volume and lipophilicity of **4f**.

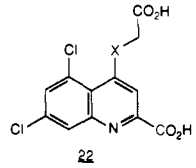
A second important consequence of aromatic ring substitution is apparent from the results in Table I. Whereas unsubstituted **4a** is equipotent at the glycine and glutamic acid sites, substitution with a 6-chlorine atom (**4d**) dramatically decreases binding potency at the glutamic acid site; similar trends were observed for **4b**. As a result, **4f** and **19b** are highly selective antagonists for the glycine site (2100- and 880-fold, respectively). In retrospect, the modest activity of **4a** for the glutamate site is not surprising since there is superficial structural similarity between **4a** and the α-amino dicarboxylic acids. It is interesting to note that the high degree of selectivity observed in the indole series results more from increased affinity for the glycine site (160-fold) than from changes at the glutamatic acid site (only about 10-fold). Also, chlorine substitution on indole **4a** results in a 10-fold increase in IC_{50} value at the glutamate site (**4a**, IC_{50} = 34 μM, [^{3}H]-CPP; **4f**, IC_{50} = 358 μM, [^{3}H]CPP), chlorine substitution on kynurenic acid results in a 6-fold decrease (1, IC_{50} = 71 μM, [^{3}H]CPP; **21**, IC_{50} = 12 μM, [^{3}H]CPP).[28] Consequently, the indole antagonists are significantly more selective than 5,7-dichlorokynurenic acid (1) (2100-fold vs 80-fold).

Although we have not extensively studied the SAR of this series of compounds at other excitatory amino acid sites, we established that **4f** and **19b** are also highly selective for glycine over both kainic acid and AMPA binding sites (see Table II).

(b) Importance of a NH. Results obtained with both the indole and quinoline antagonists suggest that a heterocyclic nitrogen bearing a proton is critical for tight binding to the glycine site. The dichloro analogue (**4f**), when methylated (**4g**), shows a 260-fold decrease in binding affinity. A similar example has been reported in an indole-3-acetic acid series.[31] Although direct steric hindrance cannot be ruled out, these results suggest the requirement for a free NH. Further evidence comes from the quinoline series. From literature[32] and our own calculations,[33] it has been shown that substituted kynurenates prefer the quinolone form by about 12 kcal/mol. In the case where this tautomeric form is not attainable, such as

(27) Honore, T.; Drejer, J. Chaotropic Ions Affect the Conformation of Quisqualate Receptors in Rat Cortical Membranes. *J. Neurochem.* **1988**, *51*, 457–461.
(28) Harrison, B. L.; Baron, B. M. Unpublished results.
(29) Heuttner, J. E. Indole-2-carboxylic acid: A Competitive Antagonist of Potentiation by Glycine at the NMDA receptor. *Science* **1989**, *243*, 1611–1613.
(30) Weintraub, H. J. R.; Demeter, D. A.; et al. Unpublished results.
(31) Gray, N. M.; Dappen, M. S.; Cheng, B. K.; Cordi, A. A.; Biesterfeldt, J. P.; Hood, W. F.; Monahan, J. B. Novel Indole-2-carboxylates as Ligands for Strychnine-Insensitive *N*-Methyl-D-Aspartate-Linked Glycine Receptor. *J. Med. Chem.* **1991**, *34*, 1283–1292.
(32) Tucker, G. F.; Irvin, J. L. Apparent Ionization Exponents of 4-Hydroxyquinoline, 4-Methoxyquinoline and *N*-Methylquinoline; Evaluation of Lactam-Lactim Tautomerization. *J. Am. Chem. Soc.* **1951**, *73*, 1923–1929.
(33) The calculations were performed with AMPAC on the carboxylate anion of kynurenic acid derivatives. In the protonated form, only minimal energy differences were observed between tautomeric forms.

the quinoline-2-carboxylic acids 11 and 15, no binding to the glycine site could be detected. Similar results were obtained with 5,7-dichlorokynurenic acid upon N- or O-methylation.[34] Additionally, other published work has shown that 4-aminoquinolines, such as **22a**, which can tautomerize to an imino form, were dramatically more potent than the related oxy compound **22b**.[17]



22

a, X = NH
b, X = O

(c) Importance of the Side Chain. The results of the present study demonstrate the importance of the propionic acid side chain and suggest the existence of a binding pocket within the receptor which can interact with a carboxylic acid group. For instance, **4a** has about 3-fold better affinity for the glycine binding site than indole-2-carboxylic acid (**20**). Furthermore, adding this propionic acid side chain to 4,6-dichloroindole-2-carboxylic acid (to give **4f**) has an even more dramatic effect, increasing affinity by over 40-fold. As would be expected, the side chain is also important in the quinoline series. As previously mentioned, 4-methoxyquinolines-2-carboxylic acid (**11**) has no affinity for the glycine site (IC_{50} > 100 μM); however, the 4-(carboxymethyl)oxy derivative (**2**) has significant affinity (IC_{50} = 25 μM). We are currently studying this side-chain interaction in more detail utilizing molecular modeling techniques and will report our findings in forthcoming publications.

(d) C3-Heteroatom Substitution. The propionic side chain functionality can be substituted with heteroatoms. Incorporation of a sulfur atom at C-3 of the indole nucleus led to the most potent antagonist in the indole series (**19b**, IC_{50} = 100 nM) which suggests that electron-rich heteroatoms, although not essential, are preferred in this region. In the quinoline series we prepared **15**, a carbon analogue of **2**; the complete inactivity of this analogue demonstrates that a heteroatom plays a significant role in binding. Clearly, neither **2** nor **15** can exist in the critical NH tautomeric form. While this would appear to indicate that the distal carboxylic acid group has a less important role in the quinoline series, the situation is more complex. We believe that the second carboxyl group in **2** is essential for activity but equally important is the abiliy of the side-chain oxygen to increase the basicity of the quinoline nitrogen atom. Therefore, removal of either the distal carboxylic acid or the oxygen atom leads to inactive molecules.

In Vivo Activity. Compounds **4f** and **19b** were evaluated for in vivo activity in two different seizure models: mouse quinolinic acid induced seizures (QA),[35] and DBA/2J audiogenic seizure susceptible mouse model (DBA).[36] As shown in Table III, both **4f** and **19b** have good anticonvulsant properties against quinolinic acid induced seizures when administered icv; however, systemic administration of either compound showed weak anticonvulsant properties. Both compounds, however, show significantly greater activity (100–150 mg/kg, ip) in the DBA/2J audiogenic seizure model. Although this weak activity may indicate that the highly polar nature of these compounds prevent them from crossing the blood–brain barrier, preliminary data indicate that rapid excretion may be the primary reason. Whereas no anticonvulsant activity was observed upon iv administration of **4f**, pretreatment with probenecid, which blocks the transport of carboxylic acids,[37] resulted in a significant lowering of the ED_{50} (50 mg/kg). Similarly, the ED_{50} of **19b** in the audiogenic seizure model is lowered 3-fold upon probenecid administration. Current studies focus on determining a full anticonvulsant profile on these compounds using a variety of seizure models.

Table III. Anticonvulsant Activity of 4f and 19b

seizure model[a]	route[b]	pretreat time[c]	ED_{50} 4f	ED_{50} 19b
quinolinic acid	icv	5 min	1.5 μg	1.1 μg
quinolinic acid	ip	2 h	256 mg/kg	>256 mg/kg
quinolinic acid	iv	5 min	>50 mg/kg	>50 mg/kg
quinolinic acid + probenecid[d]	iv	5 min	50 mg/kg	
audiogenic seizure	ip	2 h	100 mg/kg	140 mg/kg
audiogenic seizure + probenecid[e]	ip	1 h		45 mg/kg

[a] See ref 35–37 for protocols. [b] icv, intracerebroventricularly; ip, intraperitoneally; iv, intravenously. [c] Drug pretreatment time prior to administration of seizure stimulus. [d] Probenecid (200 mg/kg) was administered (ip) 30 min prior to test compound dosing. [e] Probenecid (100 mg/kg) was coadministered (ip) with test compound.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and were uncorrected. ^1H NMR spectra were obtained on a Varian VXR-300 spectrometer; chemical shifts were reported in parts per million relative to a tetramethylsilane internal standard. IR spectra were recorded on a Perkin-Elmer 1800 spectrometer.

Analytical thin-layer chromatography was performed using 0.25-mm silica gel glass-backed plates. Flash chromatography was performed on 230–400-mesh silica gel from E. Merck.

General Procedure A: Japp-Klingemann Reaction To Form Indole Diethyl Esters. Aniline (1 equiv) was dissolved in concentrated HCl (3 equiv) diluted with H_2O (~2 mL/mmol of aniline), and cooled to 0 °C in a three-neck round-bottom flask. Sodium nitrite (2.5 M, 1 equiv) in H_2O was added dropwise such that the temperature remained below 5 °C. When the addition was complete, sodium acetate (4.5 M, 5.5 equiv) in H_2O was added followed by neat ethyl 2-oxocyclopentanecarboxylate (1 equiv). The reaction was stirred at 0 °C for 15 min and then allowed to warm to room temperature over a period of approximately 1 h. Chloroform (~100 mL/mmol of aniline) was added, and the layers were separated. The chloroform layer was dried and concentrated to leave a dark oil which was added to a boiling solution of sodium carbonate (~0.7 M aq, 1.1 equiv) and stirred for 5 min. The mixture was cooled to room temperature and carefully acidified

(34) Leeson, P. D.; Baker, R.; Carling, R. W.; Curtis, N. R.; Moore, K. W.; Williams, B. J.; Foster, A. C.; Donald, A. E.; Kemp, J. A.; Marshall, G. R. Kynurenic Acid Derivatives. Structure–Activity Relationships for Excitatory Amino Acid Antagonism and Identification of Potent and Selective Antagonists at the Glycine Site on the *N*-Methyl-D-Aspartate Receptor. *J. Med. Chem.* **1991**, *34*, 1243–1252.

(35) (a) Haley, T. J.; McCormick, W. G. Pharmacological Effects Produced by Intracerebral Injection of Drugs in the Conscious Mouse. *Br. J. Pharmacol.* **1957**, *12*, 12. (b) Turski, L.; Meldrum, B. S.; Collins, J. F. Anticonvulsant Action of β-Kainic Acid in Mice. Is β-Kainic Acid an *N*-Methyl-D-Aspartate Antagonist? *Brain Res.* **1985**, *336*, 162.

(36) Swinyard, E. A.; Woodhead, J. H.; White, H. S.; Franklin, M. R. Experimental Selection, Quantification, and Evaluation of Anticonvulsants. *Antiepileptic Drugs*, 3rd ed.; Levy, R., Marrison, R., Meldrum, B., Perry, J. K., Dreifuss, F. G., Eds.; Raven Press: New York, 1989.

(37) Weiner, I. M.; Mudge, G. H. *Inhibitors of Tubular Transport of Organic Compounds in the Pharmacological Basis of Therapeutics*, 7th ed.; Goodman, A. G., Goodman, L. S., Rall, T. W., Murad, F., Eds.; Macmillan Publishing Company: New York, 1985; pp 920–925.

with 6 N HCl. The precipitate formed was extracted into chloroform, dried ($MgSO_4$), and evaporated. The residue was then dissolved in ethanol (1 mL/mmol of aniline) containing concentrated H_2SO_4 (~0.1 mL) and refluxed overnight.

The reaction was then cooled, diluted with H_2O, and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated. The residue, in general, could be dissolved in a minimum amount of methylene chloride and applied to a silica gel flash column eluting with 25% ethyl acetate/hexane. Pooled fractions were concentrated, and the residue was recrystallized from hot ethyl acetate/hexane.

General Procedure B: Saponification of Ethyl Esters. The diester **7** was dissolved in THF and diluted with an equal volume of H_2O and then treated with $LiOH \cdot H_2O$ (3 equiv). The reaction mixture was stirred at room temperature overnight, diluted with H_2O, acidified, and washed with EtOAc. The organic layer was dried ($MgSO_4$) and concentrated. The product could be recrystallized from hot ethyl acetate/hexane.

Representative Examples of Procedures A and B. Ethyl 3-(2-carbethoxy-4-chloroindol-3-yl)propionate (**7b**) and ethyl 3-[2-(carboxyethyl)-6-chloroindol-3-yl]propionate (**4**). Compounds **7b** and **7d** were prepared in about a 1:1 ratio by general procedure A, starting with *m*-chloroaniline. Compounds **7b** and **7d** were easily separable by flash silica gel chromatography (ethyl acetate/hexane). Recrystallization from ethyl acetate/hexane afforded products **7b** (16%) and **7d** (16%). **7b**: mp 116–116.5 °C; NMR ($CDCl_3$) δ 1.26 (t, 3 H, J = 8 Hz), 1.45 (t, 3 H, J = 8 Hz), 2.70 (m, 2 H), 3.72 (m, 2 H), 4.18 (q, 2 H, J = 8 Hz), 4.45 (q, 2 H, J = 8 Hz), 7.10 (d, 1 H, J = 7 Hz), 7.18 (m, 1 H), 7.29 (d, 1 H, J = 7 Hz), 9.05 (b, 1 H). Anal. ($C_{16}H_{18}ClNO_4$) C, H, N. **7d**: mp 120–121 °C; NMR ($CDCl_3$) δ 1.20 (t, 3 H, J = 7 Hz), 1.42 (t, 3 H, J = 7 Hz), 1.42 (t, 3 H, J = 7 Hz), 2.68 (t, 2 H, J = 8 Hz), 3.39 (t, 2 H, J = 8 Hz), 4.10 (q, 2 H, J = 7 Hz), 4.42 (q, 2 H, J = 7 Hz), 7.10 (dd, 1 H, J = 9 Hz, 3 Hz), 7.37 (d, 1 H, J = 3 Hz), 7.63 (d, 1 H, J = 9 Hz), 8.95 (b, 1 H). Anal. ($C_{16}H_{18}ClNO_4$) C, H, N.

3-(2-Carboxy-4-chloroindol-3-yl)propionic Acid (4b). The title compound was prepared using procedure B from **7b**. Recrystallization from ethyl acetate/hexane afforded product as a colorless solid (91%): mp 255–256 °C; NMR (DMSO-d_6) δ 2.50 (m, 2 H), 3.55 (m, 2 H), 7.10 (d, 1 H, J = 6 Hz), 7.20 (m, 1 H), 7.40 (d, 1 H, J = 6 Hz), 11.85 (s, 1 H) 12.70 (b, 2 H). Anal. ($C_{12}H_{10}ClNO_4$) C, H, N.

3-(2-Carboxy-6-chloroindol-3-yl)propionic Acid (4d). The title compound was prepared from **7d** using procedure B. Recrystallization (ethyl acetate/hexane) afforded product as a colorless solid (50%): mp 234–236 °C; NMR (DMSO-d_6) δ 2.50 (m, 2 H), 2.25 (m, 2 H), 7.08 (d, 1 H, J = 7 Hz), 7.40 (s, 1 H), 7.70 (d, 1 H, J = 7 Hz), 11.64 (s, 1 H), 12.60 (b, 2 H). Anal. ($C_{12}H_{10}ClNO_4$) C, H, N.

Ethyl 3-(2-Carbethoxy-4,6-dichloroindol-3-yl)propionate (7f). Subjecting 4,6-dichloroaniline to conditions described in procedure A afforded the title compound in a poor yield (<20%, 24 h), the major product being the hydrazone diethyl ester. The hydrazone was purified by flash silica gel chromatography (ethyl acetate/hexane) and treated with dry *p*-toluenesulfonic acid (1.5 equiv) in refluxing toluene for 2 h. The reaction mixture was concentrated and applied to a silica gel flash chromatography 25% (ethyl acetate/hexane). Recrystallization (ethyl acetate/hexane) afforded product as fluffy colorless needles (73%): mp 156–157 °C; NMR ($CDCl_3$) δ 1.23 (t, 3 H, J = 7 Hz), 1.43 (t, 3 H, J = 7 Hz), 2.69 (m, 2 H), 3.67 (m, 2 H), 4.17 (q, 2 H, J = 7 Hz), 4.44 (q, 2 H, J = 7 Hz), 7.12 (s, 1 H), 7.25 (s, 1 H), 9.00 (b, 1 H). Anal. ($C_{16}H_{17}Cl_2NO_4$) C, H, N.

3-(2-Carboxy-4,6-dichloroindol-3-yl)propionic Acid (4f). The title compound was prepared from **7f** using procedure B. Recrystallization (ethyl acetate/hexane) afforded product as a colorless powder (84%): mp 273–280 °C dec; NMR (DMSO-d_6) δ 2.45 (m, 2 H), 3.55 (m, 2 H), 7.19 (s, 1 H), 7.40 (s, 1 H), 12.0 (s, 1 H), 12.75 (b, 2 H). Anal. ($C_{12}H_9Cl_2NO_4$) C, H, N.

Ethyl 3-(2-Carbethoxy-*N*-methyl-4,6-dichloroindol-3-yl)propionate (7g). A solution of **7f** (800 mg, 2.23 mmol) in dry THF (15 mL) was added to a suspension of NaH (1 equiv) in THF (5 mL) under an atmosphere of nitrogen. After stirring for 0.5 h at room temperature, methyl iodide (1 equiv) was added. The reaction was stirred at room temperature for 24 h, quenched with H_2O, and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel eluting with 10% ethyl acetate/hexane. The pure product (**7g**) was isolated as a viscous oil which crystallized upon standing (750 mg, 90%): mp 88–90 °C; NMR ($CDCl_3$) δ 1.25 (t, 3 H, J = 8 Hz), 1.43 (t, 3 H, J = 8 Hz), 2.65 (m, 2 H), 3.11 (m, 2 H), 3.92 (s, 3 H), 4.16 (q, 2 H, J = 8 Hz), 4.43 (q, 2 H, J = 8 Hz), 7.11 (s, 1 H), 7.27 (s, 1 H). Anal. ($C_{17}H_{19}Cl_2NO_4$) C, H, N.

3-(2-Carboxy-*N*-methyl-4,6-dichloroindol-3-yl)propionic Acid (4g). The title compound was prepared from **7g** using procedure B. Recrystallization afforded **4g** as colorless crystals (90%): mp 233–236 °C; NMR (DMSO-d_6) δ 2.40–2.60 (m, 2 H), 3.45 (m, 2 H), 3.91 (s, 3 H), 7.21 (s, 1 H), 7.72 (s, 1 H), 12.8 (b, 2 H). Anal. ($C_{13}H_{11}Cl_2NO_4$) C, H, N.

Ethyl 3-(2-Carbethoxy-4-nitroindol-3-yl)propionate (7m) and Ethyl 3-(2-Carbethoxy-6-nitroindol-3-yl)propionate (7n). Reacting 3-nitroaniline under the conditions of procedure A resulted only in the formation of the hydrazone diester. This compound could be cyclized to a 1:1 mixture of **7m** and **7n** using dry *p*-toluenesulfonic acid (1.5 equiv) in refluxing toluene for 1 h. The isomers were separated by silica gel chromatography and recrystallized to analytically pure products. **7m** (24%): mp 124–125 °C; NMR ($CDCl_3$) δ 1.24 (t, 3 H, J = 8 Hz), 1.45 (t, 3 H, J = 8 Hz) 2.72 (t, 2 H, J = 7 Hz), 3.47 (t, 2 H, J = 7 Hz), 4.13 (q, 2 H, J = 8 Hz), 4.48 (q, 2 H, J = 8 Hz), 7.32 (m, 1 H), 7.66 (d, 1 H, J = 9 Hz), 7.77 (d, 1 H, J = 9 Hz), 9.63 (b, 1 H). Anal. ($C_{16}H_{18}N_2O_6$) C, H, N. **7n** (25%): mp 141–142 °C; NMR ($CDCl_3$) δ 1.23 (t, 3 H, J = 7 Hz), 1.47 (t, 3 H, J = 7 Hz), 2.73 (t, 2 H, J = 7 Hz), 3.43 (t, 2 H, J = 7 Hz), 4.13 (q, 2 H, J = 7 Hz), 4.50 (q, 2 H, J = 7 Hz), 7.81 (d, 1 H, J = 9 Hz), 8.01 (d, 1 H, J = 9 Hz), 8.33 (s, 1 H), 9.58 (b, 1 H). Anal. ($C_{16}H_{18}N_2O_6$) C, H, N.

3-(2-Carboxy-4-nitroindol-3-yl)propionic Acid (4m). The title compound was prepared from **7m** using procedure B. Recrystallization (ethyl acetate/hexane) afforded **4m** as a yellow solid (20%): mp 290 °C dec; NMR (DMSO-d_6) δ 2.45 (m, 2 H), 3.25 (m, 2 H), 7.40 (m, 1 H), 7.79 (m, 2 H), 12.40 (s, 1 H), 12.80 (b, 2 H). Anal. ($C_{12}H_{10}N_2O_6$) C, H, N.

3-(2-Carboxy-6-nitroindol-3-yl)propionic Acid (4n). The title compound was prepared from **7n** using procedure B. Recrystallization afforded **4n** as a yellow solid (62%): mp 265–266 °C; NMR (DMSO-d_6) δ 2.55 (m, 2 H), 3.30 (m, 2 H), 7.90 (m, 2 H), 8.30 (s, 1 H), 12.26 (s, 1 H), 12.40–13.20 (b, 2 H). Anal. ($C_{12}H_{10}N_2O_6$) C, H, N.

Ethyl 3-(2-Carbethoxy-6-aminoindol-3-yl)propionate (7r). 6-Nitro compound **7n** (3.1 g, 9.3 mmol) was treated with $SnCl_2 \cdot 2H_2O$ (10.5 g, 46.5 mmol) in refluxing ethanol under an N_2 atmosphere for 4 h. The mixture was poured into ice water made slightly basic with $NaHCO_3$, and extracted with EtOAc. Chromatography (1/1 ethyl acetate/hexane) followed by recrystallization (ethyl acetate/hexane) afforded **7r** as maroon needles (2.3 g, 70%): mp 102–104 °C. Anal. ($C_{16}N_2ON_2O_4$) C, H, N.

Ethyl 3-[2-Carbethoxy-6-[(methylsulfonyl)amino]indol-3-yl]propionate (7s). **7r** (1.28 g, 4.2 mmol) dissolved in methylene chloride (10 mL) was treated with Et_3N (641 μL, 4.6 mmol) and methanesulfonyl chloride (35 μL, 4.6 mmol). After 1 h, the reaction was diluted with ethyl acetate (100 mL) and washed with 1 N HCl, saturated $NaHCO_3$, and saturated NaCl. The organic layer was dried and concentrated. Recrystallization (ethyl acetate/hexane) afforded product (1.37 g, 85%): mp 179–183 °C. Anal. ($C_{17}H_{22}N_2SO_6$) C, H, N.

2-Carbomethoxy-4-methoxyquinoline (10). To a suspension of NaH (60% dispersion in mineral oil, 1.87 g, 46.7 mmol) in dry DMF (40 mL) under N_2 at room temperature was carefully added a suspension of 2-(carboxymethoxy)-4-hydroxyquinoline (9.5 g, 46.7 mmol) in DMF (50 mL). The resulting yellow solution was stirred at room temperature for 0.5 h. Methyl iodide (2.23 mL, 46.7 mmol) in DMF (10 mL) was then added via syringe. The reaction was stirred at room temperature overnight, then quenched with H_2O (300 mL), and extracted ethyl acetate (500 mL). The organic layer was washed several times with H_2O, dried, and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with 50% acetone in hexane. Isolated material was recrystallized from 25% acetone/hexane, affording **10** as colorless crystals (70%): mp 148–150 °C.

2-Carbomethoxy-4-bromoquinoline (12). Compound **10** (6.5 g, 30 mmol) was dissolved in dry DMF (100 mL) under an N_2

atmosphere. To this solution was added PBr_3 (99.99%, 10 g, 37 mmol) and the mixture was warmed to 70 °C. After 3 h, the reaction was cooled to room temperature and poured into ice/H_2O (500 mL). The resulting precipitate was extracted into ethyl acetate (1 L), washed with saturated NaCl, dried ($MgSO_4$) and evaporated in vacuo. The residue was dissolved in a minimum of CH_2Cl_2 and applied to a silica gel column and eluted with 40% ethyl acetate/hexane. Isolated material was recrystallized from ethyl acetate/hexane, affording **12** as colorless needles (78%): mp 141–142 °C; NMR ($CDCl_3$) δ 4.10 (s, 3 H), 7.7–7.9 (m, 2 H), 8.2–8.4 (dd, 2 H), 8.49 (s, 1 H). Anal. ($C_{11}H_8BrNO_2$) C, H, N.

2-Carbomethoxy-4(*E*)-(2-carbomethoxyvinyl)quinoline (13). In a dry round-bottom flask, triphenylphosphine (262 mg, 1.0 mmol), tris(dibenzylideneacetone)dipalladium(0) (183 mg, 0.2 mmol), and bis(triphenylphosphine)palladium(II) chloride (150 g) were dissolved in a 4:1 mixture of triethylamine/acetonitrile (24 mL) under an atmosphere of N_2. After 10 min at room temperature, bromide **12** (500 mg, 1.87 mmol) in the above solvent mixture (10 mL) and methyl acrylate (5 mL, 60 mmol) were added to the yellow solution, and the reaction was heated to reflux. Starting bromide was consumed after ~20 h. The mixture was evaporated and the residue was chromatographed on silica gel, eluting with 35% ethyl acetate/hexane. Recrystallization from ethyl acetate/hexane afforded **13** as colorless needles (440 mg, 86%): mp 127–131 °C; NMR ($CDCl_3$) δ 3.90 (s, 3 H), 4.12 (s, 3 H), 6.79 (d, 1 H, *J* = 15 Hz), 7.76 (m, 1 H), 7.87 (m, 1 H), 8.23 (d, 1 H, *J* = 9 Hz), 8.36–8.50 (m, 3 H), includes a doublet, (1 H, *J* = 15 Hz). Anal. ($C_{15}H_{13}NO_4$) C, H, N.

2-Carbomethoxy-4-(2-carbomethoxyethyl)quinoline (14). Compound **13** (300 mg, 1.08 mmol) was added to a suspension of magnesium turnings (244 mg, 10 mmol) in dry methanol (12 mL). The reaction was stirred at room temperature under an N_2 atmosphere. After about 0.5 h, the reaction began to exotherm, and at about 1 h, TLC indicated that all of **13** was consumed. The reaction was quenched with 1 N HCl (20 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried ($MgSO_4$) and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with 30% ethyl acetate/hexane. Recrystallization from ether/hexane afforded **14** as a light yellow powder (40%): mp 84–85 °C; NMR ($CDCl_3$) δ 2.85 (t, 2 H, *J* = 8 Hz), 3.53 (t, 2 H, *J* = 8 Hz), 3.74 (s, 3 H), 4.10 (s, 3 H), 7.70 (m, 1 H), 7.80 (m, 1 H), 8.10 (m, 2 H), 8.33 (d, 1 H, *J* = 9 Hz). Anal. ($C_{15}H_{15}NO_4$) C, H, N.

2-Carboxy-4-(2-carboxyethyl)quinoline (15). The title compound was prepared from **14** using procedure B. The product was filtered directly from acidified water (86%: mp 230–236 °C; NMR (DMSO-d_6) 2.79 (t, 2 H, *J* = 7 Hz), 3.44 (t, 2 H, *J* = 7 Hz), 7.80 (m, 1 H), 7.91 (m, 1 H), 7.99 (s, 1 H), 8.25 (m, 2 H). Anal. ($C_{13}H_{11}NO_4 \cdot 0.3H_2O$) C, H, N.

2-Carboxy-4-methoxyquinoline (11). The title compound was prepared from **10** using procedure B. Recrystallization (ethyl acetate/hexane) afforded pure **11** as colorless crystals (70%): mp 195–200 °C; NMR (DMSO-d_6) δ 4.12 (s, 3 H), 7.55 (s, 1 H), 7.68 (m, 1 H), 7.85 (m, 1 H), 8.10 (d, 1 H, *J* = 9 Hz), 8.17 (d, 1 H, *J* = 7 Hz). Anal. ($C_{11}H_9NO_3$) C, H, N.

3-Bromo-2-carbethoxy-4,6-dichloroindole (17b). The starting indole ester 2-carbethoxy-4,6-dichloroindole (**16b**, 4.5 g, 17 mmol) was dissolved in pyridine (4.4 mL/mmol) and cooled in an ice/water bath under argon. Pyridinium bromide perbromide (1.05 equiv) in pyridine (5.5 mL/mmol) was added dropwise, the solution turned red, and a white precipitate appeared. After the addition was complete, ice water was added and the mixture was extracted with diethyl ether. The organic layer was dried over magnesium sulfate and concentrated to yield a white solid wherupon analytically pure **17b** (4.0 g) crystallized.

The mother liquor gave an additional material (1.91 g) as a white solid (total yield 5.91 g, 100%): mp 228–228.5 °C; ^1H NMR (DMSO-d_6, 300 Hz) δ 1.4 (t, 3 H, *J* = 7.1 Hz), 4.4 (q, 2 H, *J* = 7.1 Hz), 7.3 (s, 1 H), 7.5 (s, 1 H), 12.7 (b, 1 H).

3-[(Carbethoxylmethyl)thio]-2-carbethoxy-4,6-dichloroindole (18b). The starting bromo indole ester **17b** (3.0 g, 8.9 mmol), ethyl 2-mercaptoacetate (1.75 equiv), and potassium carbonate (1.75 equiv) were combined in acetone (20 mL/mmol) and refluxed under argon, until TLC indicated consumption of starting material. The reaction was allowed to cool to room temperature and the solvent was evaporated under vacuum. The resulting residue was taken up in diethyl ether and washed with water. The aqueous layer was extracted with diethyl ether. The combined organic layer was dried over magnesium sulfate and concentrated to give a white solid which was purified by silica gel chromatography (20% ethyl acetate/hexane) followed by recrystallization from hexane/ethyl acetate to afford (1.1 g, 33%) of **18b** (67% based on recovered starting material): mp 152.5 °C; NMR ($CDCl_3$, 300 MHz) δ 1.3 (t, 3 H, *J* = 7.1 Hz), 1.5 (t, 3 H, *J* = 7.1 Hz), 3.6 (s, 2 H), 4.2 (q, 2 H, *J* = 7.1 Hz), 4.4 (q, 2 H, *J* = 7.1 Hz), 6.8 (s, 1 H), 7.1 (s, 1 H), 10.1 (b, 1 H).

3-[(Carboxymethyl)thio]-2-carboxy-4,6-dichloroindole (19b). The starting diester **18b** (1.0 g, 2.7 mmol) was suspended in a 1:1 mixture of water/tetrahydrofuran (5 mL/mmol) and then treated overnight with $LiOH \cdot H_2O$ (3 equiv) under argon. The reaction was diluted with ethyl acetate and water, then the layers were separated, and the aqueous layer was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to yield a white solid which was recrystallized from ethyl acetate/hexane to furnish **19b** (0.52 g, 60%): mp 152.5–153 °C; NMR (DMSO-d_6, 300 MHz) δ 3.6 (s, 2 H), 7.3 (s, 1 H), 7.5 (s, 1 H), 12.6 (s, 1 H), 13.0 (b, 2 H).

3-Bromo-2-carbethoxyindole (17a). The above compound was prepared from 2-carbethoxyindole (1.16 g, 11.4 mmol) using the procedure described for **17b**. Recrystallization from ethyl acetate/hexane afforded **17a** as colorless needles (2.6 g, 80%): mp 150–152 °C; NMR ($CDCl_3$) δ 1.46 (t, 3 H, *J* = 7 Hz), 4.48 (q, 2 H, *J* = 7 Hz), 7.22 (m, 1 H), 7.38 (m, 2 H), 7.76 (m, 1 H), 9.15 (b, 1 H).

3-[(Carbethoxymethyl)thio]-2-carbethoxyindole (18a). The above material was prepared from 3-bromo-2-carbethoxyindole using the procedure described for **18b** (0.5 g, 17%): NMR ($CDCl_3$, 300 MHz) δ 1.1 (t, 3 H, *J* = 7.2 Hz), 1.5 (t, 3 H, *J* = 7.1 Hz), 3.6 (s, 2 H), 4.0 (q, 2 H, *J* = 7.2 Hz), 4.5 (q, 2 H, *J* = 7.1 Hz), 7.2–7.4 (m, 3 H), 7.9 (d, 1 H, *J* = 8 Hz), 9.2 (b, 1 H).

3-[(Carboxymethyl)thio]-2-carboxyindole (19a). The above material was prepared from **18a** (0.42 g, 1.39 mmol) using the procedure described for **19b** (0.25 g, 72%): mp 194–195 °C dec; NMR (DMSO-d_6, 300 MHz) δ 3.6 (s, 2 H), 7.2 (m, 1 H), 7.3 (m, 1 H), 7.5 (d, 1 H, *J* = 8.3 Hz), 7.7 (d, 1 H, *J* = 8 Hz), 12.1 (s, 1 H), 12.9 (b, 2 H).

Receptor Binding. Competition assays against [^3H]glycine and [^3H]CPP were performed as previously described using rat cortical and hippocampal membranes.[25] IC_{50} values represent the concentration of compound required to reduce the binding of the tritiated ligand by 50%. Numbers reported are the mean of two or more experiments. Standard errors are ±10–30% of the mean.

Quinolinic Acid Seizure.[35] Test compounds were dissolved or suspended in distilled water containing a few drops of Tween and administered in a volume of 1 mL/kg. Mice were given an appropriate dose of the test compound at a selected time prior to intracerebroventricularly (icv) administered quinolinic acid (7.7 μg in a volume of 5 μL of saline). This dose of quinolinic acid has been found to cause clonic–tonic seizures in 90–100% of otherwise-untreated mice. Immediately following quinolinic acid administration, animals were observed for the next 15 min for the occurrence of clonic–tonic seizures. Those mice not convulsing during the observation period were considered protected. The ED_{50} is defined as that dose protecting 50% of the mice. Animals treated with probenecid were pretreated, 30 min prior to administration of test compound, with 200 mg/kg (ip).

DBA Audiogenic Seizure.[36] Typically one group of six to eight male DBA/2J audiogenic susceptible mice were administered the test compound in a solution of methylcellulose. A second group of mice were administered an equal volume of a saline control by the same route. Five minutes later, the mice were placed individually into a glass jar and were exposed to a sound stimulus of 110 decibels (12 KHz) for 30 s. Each mouse was observed during the sound exposure for signs of seizure activity. Mice not displaying tonic hindlimb extension were considered protected. Animals treated with probenecid were coadministered probenecid (100 mg/kg, ip) and test compound 1 h prior to the seizure stimulus.

Registry No. 1, 13593-94-7; **2**, 133223-26-4; **4a**, 31529-28-9; **4b**, 130798-49-1; **4c**, 54904-18-6; **4d**, 130798-50-4; **4e**, 130942-03-9;

4f, 130798-51-5; 4g, 139896-76-7; 4h, 132004-34-3; 4i, 139896-77-8; 4j, 139896-78-9; 4k, 132004-30-9; 4l, 139896-79-0; 4m, 132004-35-4; 4n, 132004-36-5; 4o, 132004-32-1; 4p, 132004-33-2; 4q, 139896-80-3; 4r, 139896-81-4; 4s, 139896-82-5; 4t, 139896-83-6; 4u, 139896-84-7; 7b, 130798-57-1; 7d, 130798-58-2; 7f, 130829-27-5; 7g, 139896-86-9; 7m, 139896-87-0; 7n, 139896-88-1; 7r, 139896-89-2; 7s, 139896-90-5; 8, 101861-63-6; 10, 67976-94-7; 11, 15733-83-2; 12, 139896-91-6; 13, 139896-92-7; 14, 139896-93-8; 16b, 53995-82-7; 17a, 91348-45-7; 17b, 137836-40-9; 15, 139896-85-8; 18a, 137836-30-7; 18b, 137836-28-3; 19a, 137836-31-8; 19b, 137836-29-4; 20, 1477-50-5; 21, 123158-59-8; *m*-chloroaniline, 108-42-9; 4,6-dichloroaniline, 626-43-7; 3-nitroaniline, 99-09-2; 2-carboxymethoxy-4-hydroxyquinoline, 5965-59-3; methyl acrylate, 96-33-3; ethyl 2-mercaptoacetate, 623-51-8.

Synthesis and Antiviral Activity of 1-Cyclobutyl-5-(2-bromovinyl)uracil Nucleoside Analogues and Related Compounds

William A. Slusarchyk,* Gregory S. Bisacchi, A. Kirk Field, Deborah R. Hockstein, Glenn A. Jacobs, Bridget McGeever-Rubin, Joseph A. Tino, A. V. Tuomari, Gregory A. Yamanaka, Marian G. Young, and Robert Zahler

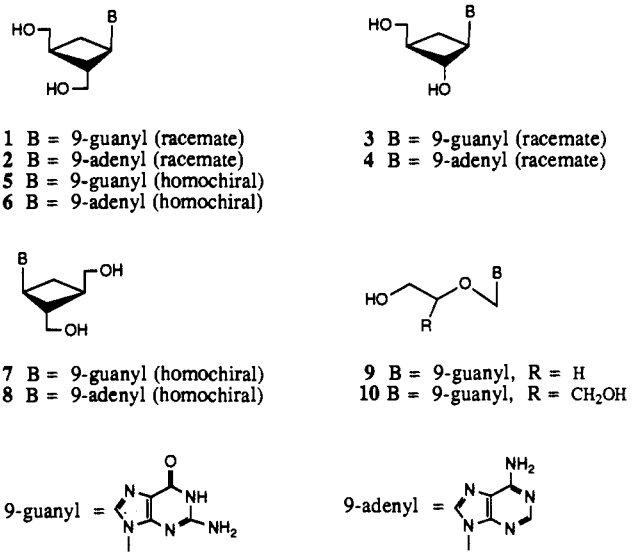
Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000.
Received June 24, 1991

A series of racemic (1α(*E*),2β,3α)-1-[2,3-bis(hydroxymethyl)cyclobutyl]-5-(2-halovinyl)uracils was synthesized and evaluated in cell culture. The bromovinyl, iodovinyl, and chlorovinyl analogues, **13**, **15**, and **16**, respectively, are all potent inhibitors of varicella zoster virus (VZV), but are less inhibitory to the replication of human cytomegalovirus (HCMV) and herpes simplex viruses 1 and 2 (HSV-1, HSV-2). The excellent anti-VZV activities of **13**, **15**, and **16** coupled with their virtual inability to inhibit WI-38 cell growth indicate high in vitro therapeutic indices. VZV thymidine kinase readily converts these compounds to their respective monophosphates but not to their corresponding diphosphates. Compound **13a**, the (1′*R*) enantiomer of the bromovinyl analogue **13**, was also synthesized, and its potency is comparable to that of the racemate. A lower homologue **14**, (1α(*E*),2β,3α)-1-[2-hydroxy-3-(hydroxymethyl)cyclobutyl]-5-(2-bromovinyl)uracil, was found to be inactive against VZV, HCMV, HSV-1, and HSV-2.

Introduction

We recently reported the synthesis and antiherpes activity of the 9-guanyl and 9-adenyl cyclobutyl nucleoside analogues **1**–**8** (Chart I).[1] The racemates **1** and **2**, and the corresponding lower homologue racemates **3** and **4**, are all potent inhibitors of a broad spectrum of herpesviruses, including herpes simplex virus 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), and human cytomegalovirus (HCMV). The guanine analogue **1** exhibits in vitro antiherpes activity superior to that of acyclovir (**9**)[2] and comparable to that of ganciclovir (**10**).[2c] The lower homologue **3** displays activity equivalent to that of acyclovir against HSV-1, HSV-2, and VZV, and is comparable to ganciclovir against HCMV. The enantiomers of "natural" configuration (**5** and **6**) have activity equal to or greater than that

Chart I



(1) (a) Slusarchyk, W. A.; Young, M. G.; Bisacchi, G. S.; Hockstein, D. R.; Zahler, R. Synthesis of SQ-33,054, a Novel Cyclobutane Nucleoside With Potent Antiviral Activity. *Tetrahedron Lett.* **1989**, *30*, 6453–6456. (b) Slusarchyk, W. A.; Bisacchi, G. S.; Hockstein, D. R.; Young, M. G.; Field, A. K.; McGeever-Rubin, B.; Tuomari, A. V.; Zahler, R. SQ-33,054: A Potent Member of a New Class of Nucleoside-Analog Antivirals. *29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989; Abstract 1330. (c) Field, A. K.; Tuomari, A. V.; McGeever-Rubin, B.; Terry, B. J.; Mazina, K. E.; Haffey, M. L.; Hagen, M. E.; Clark, J. M.; Braitman, A.; Slusarchyk, W. A.; Young, M. G.; Zahler, R. (±)-(1α,2β,3α)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine [(±)-BHCG or SQ33054]: a Potent and Selective Inhibitor of Herpesviruses. *Antiviral Res.* **1990**, *13*, 41–52. (d) Jacobs, G. A.; Tino, J. A.; Zahler, R. Synthesis of SQ-32,829, A New Nucleoside Antiviral Agent. *Tetrahedron Lett.* **1989**, *30*, 6955–6958. (e) Jacobs, G. A.; Slusarchyk, W. A.; Spergel, S. H.; Tino, J. A.; Field, A. K.; Tuomari, A. V.; Zahler, R. *In Vitro* Activity of SQ-32,829, a New Nucleoside-Analog Antiviral Agent. *29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989; Abstract 1328. (f) Zahler, R.; Jacobs, G. A. Purinyl and Pyrimidinyl Cyclobutanes and Their Use As Antiviral Agents. U.S. Patent 4,918,075, 1990. (g) Bisacchi, G. S.; Braitman, A.; Cianci, C. W.; Clark, J. M.; Field, A. K.; Hagen, M. E.; Hockstein, D. R.; Malley, M. R.; Mitt, T.; Slusarchyk, W. A.; Sundeen, J. E.; Terry, B. H.; Tuomari, A. V.; Weaver, E. R.; Young, M. G.; Zahler, R. Synthesis and Antiviral Activity of Enantiomeric Forms of Cyclobutyl Nucleoside Analogues. *J. Med. Chem.* **1991**, *34*, 1415–1421.

(2) (a) Thiers, B. H. Acyclovir in the Treatment of Herpesvirus Infections. *Dermatol. Clin.* **1990**, *8*, 583–587. (b) O'Brien, J. J.; Campoli-Richards, D. M. Acyclovir An Updated Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy. *Drugs* **1989**, *37*, 233–309. (c) Reines, E. D.; Gross, P. A. Antiviral Agents. *Med. Clin. North Am.* **1988**, *72*, 691–715.

0022-2623/92/1835-1799$03.00/0