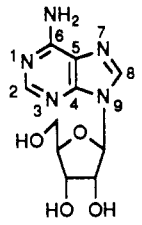
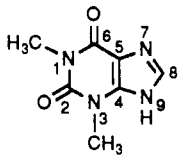
Tabel II. Absolute Charge Differences between Selected Adenosine and Theophylline Atoms



adenosine

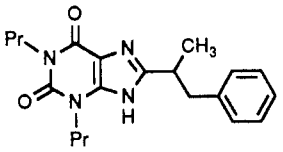


theophylline

binding mode	atoms: adenosine–theophylline	absolute charge differences[a] Gasteiger[b]	Mulliken[c]	AM1[d]
standard	N^1–N^1	0.00	0.13	0.08
standard	N^3–N^3	0.02	0.10	0.04
standard	N^7–N^7	0.07	0.10	0.01
standard	N^9–N^9	0.02	0.11	0.04
standard	C^6N–C^6=O	0.07	0.00	0.09
new	N^1–N^9	0.00	0.11	0.10
new	N^3–N^3	0.02	0.10	0.04
new	N^9–N^1	0.02	0.13	0.14
new	C^6NH–N^7H	0.01	0.08	0.07
new	N^7–C^6=O	0.04	0.14	0.14

[a] Charge calculations were performed with SYBYL (ref 18) software; the results were displayed on the Evans and Sutherland PS-390 Picture System. [b] Marsili, M.; Gasteiger, J. *Croat. Chem. Acta* 1981, *53*, 601; *Chem. Abstr.* 1981, *94*, 208209y. [c] Clementi, E. *J. Phys. Chem.* 1980, *84*, 2122. [d] References 18–20.

Table III. Binding Constants for 8-(Phenylisopropyl)xanthines at A_1 and A_2 Adenosine Receptors



compd	stereochem	A_1 receptor K_i,[a] nM	A_2 receptor K_i,[b] nM
6	*R*	6.9 ± 1.6	157 ± 27
3	racemic	32.6 ± 4.6	644 ± 209
7	*S*	60.7 ± 5.3	848 ± 99

[a] Binding of [^{3}H]CHA in whole rat brain membranes was measured at 25 °C. Values are geometric means ± standard error, n = 3 separate determinations. See: Goodman, R.; Cooper, M.; Gavish, M.; Snyder, S. *Mol. Pharmacol.* 1982, *21*, 329. [b] Binding of [^{3}H]NECA was measured in rat brain striatum at 25 °C. Values are geometric means ± standard error, n = 3 separate determinations. See: Bruns, R, R.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* 1986, *29*, 331.

were simultaneously fitted and energy minimized. It is clear from this overlay that the phenylisopropyl recognition units of each molecule can occupy the same space.

Several additional 8-substituted xanthines that are in preparation using the novel synthetic route described here will be the subject of a future report.

Supplementary Material Available: A listing of coordinates with AM1 charges for the fit versions of adenosine and theophylline and a figure showing compound 6 with assigned atom identification numbers (4 pages). Ordering information is given on any current masthead page.

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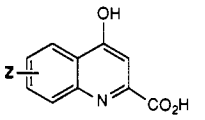
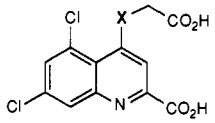
4-[(Carboxymethyl)oxy]- and 4-[(Carboxymethyl)amino]-5,7-dichloroquinoline-2-carboxylic Acid: New Antagonists of the Strychnine-Insensitive Glycine Binding Site on the *N*-Methyl-D-aspartate Receptor Complex

Recent research efforts have suggested that overstimulation of the *N*-methyl-D-aspartate (NMDA) receptor plays a critical role in the neuropathology of disease states including epilepsy, Huntington's chorea, and anoxic conditions, such as stroke.[1] A large body of work has shown that the NMDA receptor complex is composed of several distinct binding domains including the glutamate agonist site,[2] the strychnine-insensitive glycine site,[2] the receptor gated ion channel,[2] a zinc ion site,[2] and a recently described polyamine site.[3] The glycine site, which recognizes glycine and certain analogs[4] as agonists, was originally thought to be a modulatory site; however, recent work has proven that glycine is obligatory for L-glutamic acid to activate this receptor complex.[5] These findings suggest that potent, selective glycine site antagonists of the NMDA receptor complex would find utility in therapeutic areas, such as epilepsy and stroke.

The earliest reported glycine site antagonist, kynurenic acid (1), an endogenous product of the tryptophan metabolism pathway, has micromolar binding affinity for both the glycine site[6] and the L-glutamate site[7] (IC_{50} = 16 μM versus [^{3}H]glycine, IC_{50} = 71 μM versus [^{3}H]CPP,[8] respectively).[9] Subsequently, the chloro derivatives 7-chlorokynurenic acid[10] (2) (IC_{50} = 0.4 μM versus [^{3}H]-glycine; IC_{50} = 162 μM versus [^{3}H]CPP) and 5,7-dichlorokynurenic acid[11] (3) (IC_{50} = 0.08 μM versus [^{3}H]-glycine; IC_{50} = 37 μM versus [^{3}H]CPP) were found to be more potent and selective than kynurenic acid. In this communication we describe the synthesis and evaluation of two new potent, selective glycine site antagonists: 4-[(carboxymethyl)oxy]-5,7-dichloroquinoline-2-carboxylic acid (4) and 4-[(carboxymethyl)amino]-5,7-dichloroquinoline-2-carboxylic acid (5).



1: Z = H
2: Z = 7-Cl
3: Z = 5,7-di-Cl



4: X = O
5: X = NH

(1) (a) Olney, J. W. *Biol. Psychiatry* 1989, *26*, 505. (b) Lodge, D. *Drugs Today* 1989, *25*, 395. (c) Wood, P. L.; Rao, T. S.; Iyengar, S.; Lanthorn, T.; Monahan, J.; Cordi, A.; Sun, E.; Vazguez, M.; Gray, N.; Contreras, P. *Neurochem. Res.* 1990, *15*, 217.
(2) Lodge, D.; Jones, M.; Fletcher, E. *The NMDA Receptor*; Watkins, J. C., Collingridge, G. L., Eds.; Oxford University: Oxford, 1989; Chapter 3.
(3) Ransom, R. W.; Stec, N. L. *J. Neurochem.* 1988, *51*, 830.
(4) Johnson, J. W.; Ascher, P. *Nature* 1987, *325*, 529.
(5) (a) Verdorn, T. A.; Kleckner, N. W.; Dingledine, R. *Science* 1987, *238*, 1114. (b) 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. *Mol. Pharmacol.* 1990, *38*, 454.
(6) Kessler, M.; Terramani, T.; Lynch, G.; Baudry, M. *J. Neurochem.* 1989, *52*, 1319.
(7) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. *J. Pharmacol. Exp. Ther.* 1987, *240*, 778.
(8) CPP: [3-(2-carboxypiperazine-4-yl)propyl]phosphonic acid. Harris, E. W.; Ganong, A. H.; Monaghan, D. T.; Watkins, J. C.; Cotman, C. W. *Brain Res.* 1986, *382*, 169.
(9) Baron, B. M.; Cousino, D. M.; Harrison, B. L.; McCarty, D. R.; McDonald, I. A.; Palfreyman, M. G.; Schmidt, C. J.; Siegel, B. W.; Taylor, V. L. Unpublished results.
(10) Kemp, J. A.; Foster, P. D.; Leeson, P. D.; Priestly, R.; Tridgett, R.; Iversen, L. L. *Proc. Natl. Acad. Sci. U.S.A.* 1988, *85*, 6547.

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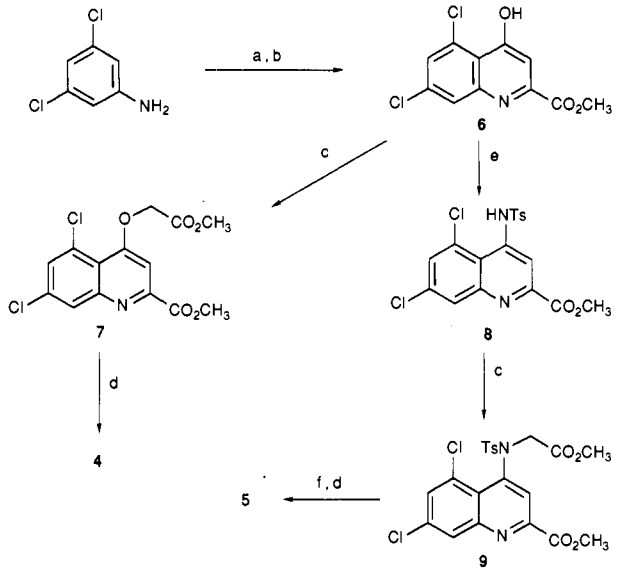
Scheme I[a]



[a] Reagents: (a) dimethyl acetylenedicarboxylate, MeOH; (b) diphenyl ether, 260 °C; (c) methyl bromoacetate, NaH, DMF; (d) 1.0 M LiOH, MeOH; (e) *p*-tolylsulfonyl isocyanate, CH_3CN, Δ; (f) 90% H_2SO_4, 0 °C.

Table I. Binding and Functional Activities of Compounds 1–5 on the NMDA Receptor Complex

compd	IC_{50},[a] μM [^{3}H]gly	[^{3}H]CPP	c-GMP	selectivity[b]
1	16.0	71.0		4.40
2	0.40 (0.60)[c]	162 (169)[c]	8.0	405 (282)[c]
3[d,e]	0.08	37.0	3.5	463
4[f]	9.0	893	225	99
5[f]	0.10	140	3.6	1400

[a] Methods, ref 15. Values shown are means ± SEM of 3–14 independent experiments. Standard errors in all cases were 20–30% of the mean value. [b] Selectivity is defined as the ratio of IC_{50} values for [^{3}H]CPP/[^{3}H]gly. [c] Numbers in parentheses refer to literature values, ref 10. [d] Method of preparation, ref 11b. [e] See ref 5b. [f] The structures of compounds 4 and 5 are consistent with all spectral and analytical data.

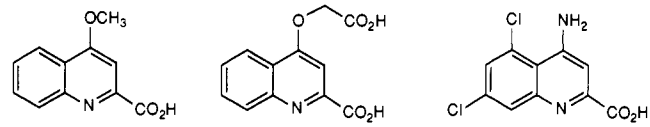
Chemistry

The quinoline-2-carboxylic acids **4** and **5** were synthesized as outlined in Scheme I in which the common intermediate **6** was prepared according to the method of Heindel et al.[12] O-Alkylation[13] with methyl bromoacetate followed by basic hydrolysis afforded **4** in good yield. Alternatively, treatment of **6** with *p*-tolylsulfonyl isocyanate in refluxing acetonitrile[14] gave the *N*-tosyl derivative **8** in 80% yield. Alkylation of **8** with methyl bromoacetate afforded diester **9**, which was hydrolyzed to the diacid **5**.

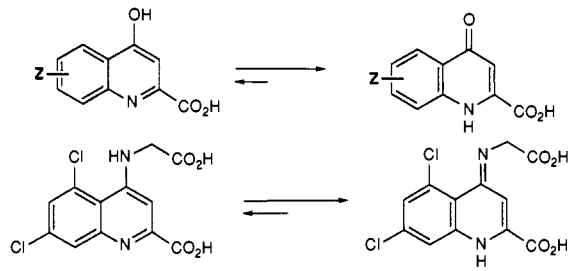
Results and Discussion

The binding potency and selectivity of compounds **1–5** for the NMDA receptor complex in rat brain homogenates was determined with [^{3}H]glycine and [^{3}H]CPP as competitive binding ligands for the glycine site and L-glutamate site, respectively[15] (Table I). These compounds were demonstrated to be antagonists at the glycine site by their ability to block NMDA-induced increases in cyclic GMP content in rat cerebellar slices (Table I).[16] Taken together, these results demonstrate that quinoline derivatives **4** and **5** are selective antagonists of the strychnine-insensitive glycine site. Whereas **4** was only slightly more potent than kynurenic acid (IC_{50} = 9.0 μM versus 16 μM) and approximately 25-fold more selective for the glycine site, the related amino derivative **5** (IC_{50} = 0.07 μM) was as potent as 5,7-dichlorokynurenic acid (**3**) (IC_{50} = 0.08 μM) and very selective for the glycine site (1400-fold, Table I).

When comparing **4** and **5** with the kynurenic acid derivatives **1–3**, the only structural difference, aside from ring substitution, is the heteroatom-substituted acetic acid side chain incorporated at C-4. There is indirect evidence that this side chain acid is binding to additional functional groups within the glycine binding site. Simple O-alkylation alone does not lead to increased potency; for example, 4-methoxyquinoline-2-carboxylic acid (**10**)[17] is essentially inactive (IC_{50} > 100 μM versus [^{3}H]glycine) in contrast to 4-[(carboxymethyl)oxy]quinoline-2-carboxylic acid (**11**)[18] (IC_{50} = 25 μM versus [^{3}H]glycine). Moreover, the 4-aminokynurenic acid analogue **12**[19] (IC_{50} = 4 μM versus [^{3}H]glycine) is approximately 60-fold less potent than **5**.



A key structural finding from this study is that replacement of the ether linkage in **4** with an amino group in **5** leads to the most potent antagonist yet described. It has been suggested that 4-hydroxyquinolines, such as the kynurenic acids, will exist in solution primarily as the 4(1*H*)-quinolone form.[20] Our own calculations[21] have confirmed this in that, regardless of ring substitution, the quinolone form is favored by approximately 12 kcal/mol. It is likely, therefore, that kynurenic acid and its derivatives preferentially bind to the glycine receptor in this tautomeric form and that the ability of **5**, in contrast to **4**, to tautomerize to **5a** is one of the keys to its remarkable potency.



(11) (a) Binding: Baron, B. M.; Siegel, B. W.; Slone, A. L.; Harrison, B. L.; Palfreyman, M. G.; Hurt, S. D. *Eur. J. Pharmacol.*, in press. (b) Synthesis: Surrey, A. R.; Hammer, H. F. *J. Am. Chem. Soc.* **1946**, *68*, 1244.

(12) Heindel, N. D.; Bechara, I. S.; Kennewell, J. M.; Ohnmacht, C. J.; Lemke, S. M.; Lemke, T. F. *J. Med. Chem.* **1968**, *11*, 1218.

(13) Proof that the alkylation of the bidentate anion from **6** occurred on oxygen was shown by a substantial NOE (Varian 300 MHz) observed between the $-CH_2-$ group of the side chain and the hydrogen at C-3.

(14) Wright, R. G. McR. *Synthesis* **1984**, 1058.

(15) Baron, B. M.; Dudley, M. W.; McCarty, D. R.; Miller, F. P.; Reynolds, I. J.; Schmidt, C. J. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 162.

(16) Wood, P. L.; Richard, J. W.; Pilapil, C.; Nair, N. P. V. *Neuropharmacology* **1982**, *21*, 1215.

(17) Prepared from **6** by O-methylation (MeI, NaH, DMF) and subsequent saponification (LiOH, THF, H_2O). Personal communication from F. G. Salituro.

(18) Prepared from **6** by an analogous procedure used in the preparation of **4** (see Scheme I).

(19) Prepared from **6** as described in ref 14.

(20) Tucker, G. F.; Irvin, J. L. *J. Am. Chem. Soc.* **1951**, *73*, 1923.

(21) The calculations were performed with AMPAC on the carboxylate anion of the kynurenic acid derivatives. In the protonated form, only minimal energy differences were noted between the tautomeric forms.

In conclusion, we have described a new class of compounds based on quinoline-2-carboxylic acid bearing an acetic acid side chain linked to the nucleus through a heteroatom at C-4. These compounds, especially **5**, have been shown to be potent and selective antagonists acting at the glycine binding site on the NMDA receptor complex. The potential utility of **5** for the treatment of neurological disorders characterized by excessive receptor activation (e.g., epilepsy, stroke) is currently under active investigation.

Acknowledgment. We thank Dr. Francesco G. Salituro for the preparation of compound **10** and for many informative discussions. We also thank Dr. Edward W. Huber for NMR structure analysis.

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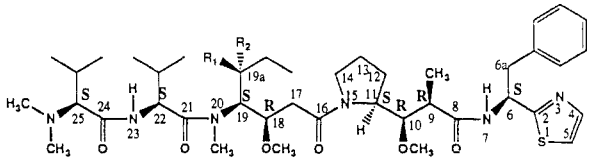
Chiral Modifications of Dolastatin 10: The Potent Cytostatic Peptide (19a*R*)-Isodolastatin 10[1]

When opisthobranch mollusca (sea hares) are threatened, they secrete complex mixtures of defensive substances from the genital pore, purple gland, and body surface. The latter secretion is believed to be the most potent.[2] Some estimation of consequences from higher than therapeutic levels can be obtained from the applications of Locusta,[3] who murdered[4] Caesar Agustus and Claudius Britonnicus, among others, with potions from a *Dolabella* sp. believed to be *auricularia*.[5] Our 17-year investigation of the potent antineoplastic constituents of this sea hare culminated in the isolation and total synthesis of the powerful dolastatin 10 (**1**).[6] In order to probe the chiral and other structural parameters for such remarkable antineoplastic activity, we have first explored the synthesis and biological effects of 18 (of 128 possible) chiral isomers of dolastatin 10 when one to five asymmetric carbons in the Dil–Dap sequence were reversed. We now report that isodolastatins **3–19** (Table I) are less cytostatic than the parent pentapeptide (**1**, P388 ED_{50} 10^{-4} μg/mL) against cell growth of the P388 lymphocytic leukemia (PS system) with ED_{50} values near the 10^{-3} μg/mL level and that (19a*R*)-isodolastatin 10 (**2**) was consistently found to be up to 10-fold more cell growth inhibitory, affording an ED_{50} of 4.9×10^{-5} μg/mL.



1. $R_1 = CH_3$, $R_2 = H$, DOLASTATIN 10
2. $R_1 = H$, $R_2 = CH_3$, 19aR-ISODOLASTATIN 10

Table I. Dolastatin 10 Chiral Isomers

no.	isodolastatin 10	mp,[a] °C	$[\alpha]^{28}_D$, deg (*c*, $CHCl_3$)	P388 ED_{50}, μg/mL
2	19a*R*	69–71	−57 (0.54)	4.9×10^{-5}
3	9*S*	73–75	−34 (0.85)	1.6×10^{-3}
4	10*S*	72–74	−49 (0.33)	3.9×10^{-3}
5	18*S*,19*R*	82–85	−47 (0.68)	3.2×10^{-3}
6	9*S*,19a*R*	85–89	−82 (0.5)	5.6×10^{-3}
7	18*S*,19*R*,19a*R*	78–80	−44 (0.34)	3.2×10^{-3}
8	9*S*,10*S*	75–77	−45 (0.40)	3.9×10^{-2}
9	18*S*	112–14	−97 (1.3)	6.0×10^{-1}
10	9*S*,18*S*	87–88	−115 (0.65)	6.2×10^{-1}
11	9*S*,18*S*,19*R*	82–84	−77 (0.5)	2.7×10^{-1}
12	9*S*,18*S*,19a*R*	88–91	−131 (0.26)	4.1×10^{-1}
13	9*S*,18*S*,19*R*,19a*R*	78–80	−93 (0.27)	1.5×10^{-1}
14	10*S*,18*S*,19*R*,19a*R*	65–69	−34 (0.18)	2.0×10^{-1}
15	9*S*,10*S*,18*S*,19*R*,19a*R*	76–79	−55 (0.48)	6.3×10^{-1}
16	9*S*,19*R*	108–12	−29 (0.34)	$>1.0 \times 10^{0}$
17	9*S*,19*R*,19a*R*	90–93	−16 (0.31)	$>1.0 \times 10^{0}$
18	9*S*,10*S*,18*S*	82–85	−55 (0.3)	$>1.0 \times 10^{0}$
19	9*S*,10*S*,19*R*,19a*R*	70–74	+19 (0.16)	$>1.0 \times 10^{0}$

[a] All recrystallized from acetone–hexane.

Elucidation of the absolute configurations of dolastatin 10 (**1**) and its total synthesis[6] allowed us to begin evaluating subtle chiral changes that were expected to profoundly affect the conformation and thereby certain biological activities. That expectation was amply realized in the case of (19a*R*)-isodolastatin 10 (**2**). The total synthesis of (19a*R*)-isodolastatin 10 followed the general reaction sequence (Table II)[7] employed for obtaining dolastatin 10.[6] Physical and PS cytostatic properties of the isodolastatins (**2–19**) are given in Table I. Detailed syntheses of the isodolastatins, their synthetic precursors and minimum-energy conformations will be summarized in a future detailed report.

Presently PS cell line results indicate that epimerization at 19a (allo–iso–ile series) strongly increases the cytostatic effects and can compensate, in part, for more profound inversions of configuration elsewhere (cf. **6** and **7**). Inversion at C-19 does not markedly reduce the cytostatic effects (see **5** and **7**) while at C-18 alone and in general for C-9, inhibition of cell growth is greatly moderated to ED_{50} $10^{-1\text{ to }0}$ values. Furthermore, multiple inversions, as expected, greatly diminished potency. For example, (9*S*)-, (10*S*)-, (18*S*)-, (19*R*)-, (19a*R*)-isodolastatin 10 gave PS ED_{50} 6.3×10^{-1} μg/mL. Any change in the stereochemistry of one or more stereocenters at C-9, C-10, C-18, and C-19 reduced or eliminated the cytostatic activity in the series of isodolastatins (see **11–19**). More significantly, the diastereomeric 18*S*,19*R* pair (refer to **1–8**, **11**, **13**, **14**) was found to have better cytostatic activity than the 18*S*,19*S* pair (e.g., **16–19**). A 10-fold reduction of cytostatic activity was observed by inversion of C-9 or C-10 (**3**, **4**). Inversion of C-9 and C-10 in the same molecule diminished the activity 100-fold (**18**, **19**). A simple inversion of the chiral center at C-18 (**9**) reduced the in vitro activity by 1000-fold. Similar effects were observed by changing the chirality at C-9 and C-18 (**10**) or multiple inversions at C-9, C-10, C-18, and C-19a in any combination (**11–15**). Inversion of C-9 or C-10 with the C-18*S**,C-19*R** combination generally produced isodolastatins with relatively better cytostatic activity than using the diastereomeric pair 18*S*,19*S*. Comparison of the two sets (19a*S* and 19a*R*) of isodolastatins containing 18*S*,19*R* diastereomeric pairs revealed that the 18*S*,19*R* pair with 19a*S* (**5**) was 10 times less effective in inhibiting cell growth than the 18*R*,19*S* of dolastatin 10 (**1**). In the 18*S*,19*R* combination with 19a*R*

(1) Antineoplastic agents 205. For 204, see: McCrady, C. W.; Staniswalis, J.; Pettit, G. R.; Howe, C.; Grant, S. *Blood*, in press.
(2) Flury, F. *Arch. Exp. Pathol. Pharm.* **1915**, *79*, 250–263.
(3) Servant to Claudius Caesar Nero (37–68 A.D.) and his mother Agrippina Minor (16–59 A.D.).
(4) Donati, G. *La Conchiglia* **1984**, May issue.
(5) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482–485.
(6) Pettit, G. R.; Singh, S. B.; Hogan, F.; Lloyd-Williams, P.; Herald, D. L.; Burkett, D. D.; Clewlow, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 5463–5465.
(7) All the isodolastatins and reported intermediates gave satisfactory elemental analyses or HREIMS (or HRFABMS) and 400-MHz NMR spectra.

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